Response in growth performance, carcass traits, fatty acid profiles and health indices of pork from Windsnyer pigs supplemented with Amarula oil cake

By

Fortune Thabethe

A thesis submitted in fulfilment of the degree of

DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

Pietermaritzburg

South Africa

2023

Supervisor: Prof M. Chimonyo

Declaration

I, Fortune Thabethe, hereby declare that the work entitled "**Response in growth performance, carcass traits, fatty acid profiles and health indices of pork from Windsnyer pigs supplemented with Amarula oil cake**" is my own work, except indicated through citations or referencing. This thesis has not been submitted for any degree or examination at any public university other than the University of KwaZulu-Natal.

[redacted]

Candidate's signature: … Date: …06/02/23….

Supervisor's signature: … Date: …06/02/23….

List of Abbreviations

ADFI	Average daily feed intake
ADF	Acid detergent fibre
ADG	Average daily gain
ADL	Acid detergent lignin
AL	Atherogenic index
BW	Body weight
AK	Amarula kernels
ALP	Alkaline phosphatase
AST	Aspartate transaminase
ALT	Alanine aminotransferase
AOC	Amarula oil cake
AOAC	Association of Official Agricultural Chemists
ARC	Agricultural Research Council
DM	Dry matter
DP	Dressing percentage
CP	Crude protein
FA	Fatty acid
FAME	Fatty acid methyl esters
FCR	Feed conversion ratio
GLM	General linear model
MUFA	Monounsaturated fatty acids
NDF	Neutral detergent fibre
NRC	National Research Council
PUFA	Polyunsaturated fatty acids
RSREG	Response surface regression
SADFI	Scaled average daily feed intake
SADG	Scaled average daily gain
SFA	Saturated fatty acids
SEM	Standard error of the mean
SB	Soybean meal
SAS	Statistical Analysis Systems

TI	Thrombogenic index
UKZN	University of KwaZulu-Natal
DFT	Dorsal fat thickness
CL	Carcass length
SW	Slaughter weight
CCW	Cold carcass weight
BF	Backfat thickness
HIS	Hepatosomatic index

General Abstract

To conserve the slow-growing Windsnyer pigs, it is crucial to explore their efficiency in utilising locally available ingredients such as Amarula oil cake (AOC). The use of Amarula oil cake in pig diets can ease the pressure of relying on conventional feed sources and broaden the feed resource base for pigs. The broad objective of the study was to assess the relationship between feeding increasing levels of Amarula oil cake on the growth performance of Windsnyer pigs and selected pork quality traits. Twenty-five clinically healthy intact growing boars with an initial body weight of 19.9 ± 8.74 kg were used in the study which lasted for six weeks excluding the adaptation period of one week. Pigs were assigned to five experimental diets in a completely randomised design and diets were formulated to contain 0, 50, 100, 150, and 200 g/kg DM of AOC. The average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR), scaled average daily feed intake (SADFI), scaled average daily gain (SADG), and body weight (BW) were calculated weekly.

The diet affected ADFI, ADG, FCR, and SADG ($P < 0.05$). The scaled average daily feed intake was not affected by the diet ($P > 0.05$). There was a significant interaction between AOC inclusion and weeks of feeding on ADFI, ADG, and FCR ($P < 0.05$). A positive quadratic relationship between ADFI and increasing levels of AOC was observed ($P < 0.05$). Average daily gain, FCR, and SADG decreased linearly with increasing AOC levels ($P < 0.05$). Using the broken stick analyses, the maximum inclusion level of AOC was obtained at 102.17 g/kg DM with an optimum ADFI of 1.25 kg/day. Amarula oil cake can be incorporated in Windsnyer pig diets up to 100 g/kg DM without constraining growth performance of Windsnyer pigs.

The specific objective for experiment two was to determine the relationship between incremental levels of AOC, carcass characteristics, primal pork cuts, and visceral organ weights of South African Windsnyer pigs. There was a negative linear relationship between increasing AOC levels, carcass length, warm carcass weight, and cold carcass weight ($P < 0.05$). Stomach weight, backfat thickness, drip loss, and the hepatosomatic index increased linearly with increasing AOC levels ($P < 0.05$). The kidneys, small intestines, and large intestines weight of Windsnyer pigs had a quadratic response to AOC inclusion level ($P < 0.05$). The heart, lungs, and spleen were not related to increasing levels of Amarula oil cake inclusion ($P > 0.05$). Incremental AOC diets impaired carcass characteristics and the selected visceral organs of pigs. Windsnyer pigs can, therefore, be fed Amarula oil cake up to 100 g/kg dry matter.

The specific objective for experiment three was to assess the changes in nutritionally related metabolites and liver enzymes of Windsnyer pigs fed on increasing levels of AOC based diets. After subjecting the pigs to six weeks of feeding on the experimental diets, blood samples were collected. Serum was analysed for total protein (TP), albumin, globulin (G) iron, Uric acid (UA), albumin: globulin ratio (A: G), alkaline phosphatases (ALP), aspartate aminotransferase (AST), aminotransferase (ALT). The albumin concentration of pigs linearly decreased with incremental levels of AOC ($P < 0.05$). The concentration of TP and G decreased quadratically with incremental levels of AOC ($P < 0.05$). On the other hand, ALP increased quadratically with increasing levels of AOC ($P < 0.05$). The other blood metabolites and liver enzymes were not related to the inclusion level of AOC ($P > 0.05$).

The specific objective for experiment four was to determine the relation between fatty acid composition and health lipid indices of pork from Windsnyer pigs supplemented with different AOC levels. Increasing AOC inclusion levels, linearly increased C12:0, C14:1n9c, C18:1n9t and C18:3n6 of pork from Windsnyer pigs ($P < 0.05$). Increasing AOC levels linearly decreased SFA, PUFA/SFA ratio, C18:1n11c and C20:3n3 of pork from Windsnyer pigs ($P < 0.05$). There was a quadratic decrease in n-3 fatty acids, n-6/n-3 ratio, nutritive value, C22:0, C18:1n9c, C18:3n3, C18:2c911t, C20:4n6 and C22:5n3 of pork ($P < 0.05$). The total MUFA, PUFA, n-6 fatty acids, AI and TI were not related to AOC inclusion ($P > 0.05$). Due to the quadratic relation of n-3 PUFA, n-6/n-3 ratio of FA and nutritional value of pork, it is recommended that AOC based diets be fed up to 150 g/kg DM. Low levels of AOC of up to 100 g/kg DM improved growth performance, nutritionally related metabolites, carcass traits of pigs. High inclusion levels of AOC improved fatty composition of pork from Windsnyer pigs.

Key words: Body weight gain; carcass length: dietary fibres; feed intake; hepatosomatic index; organ weight; total protein; polyunsaturated fatty acids; saturated fatty acids

Dedication

This thesis is dedicated to my parents (*Jane Sambo and Lawrance Thabethe*)

To my daughter *Mihla Thabethe*

To my brother *Peaceful Thabethe*

To my fiancée *Nokubonga Nobuhle Funeka*

To my children "I hope you will continue working and surpass this level"

"*Good things come to those who believe, better things come to those who are patient and the best things come to those who don't give up*"

Acknowledgements

My sincere gratitude goes to my supervisor Prof. M. Chimonyo, for opening his door when I came knocking in 2016 and for that I will forever be grateful. I thank him for the opportunities and creating an environment that allowed me to grow as a researcher. I also thank my late Co-supervisor, Dr. R.S Thomas who could not live enough to see the fruits of this project, for his time, efforts, and the knowledge that he shared. I also acknowledge the sacrifices that he made to make sure that the trial ran smoothly. Prof A. M. de Almeida also assisted with chapter 3 and 5. I thank him for the inputs that he gave which improved the quality of the thesis. Special thanks also go to Prof C Mapiye for assisting with fatty composition analyses and interpretation of results.

Special thanks also go to Dr. V.A Hlatini, for constantly checking up on me, checking up on the progress of my work, for giving me hope when it seemed impossible and for teaching me some important aspects of statistical analyses. I also acknowledge the motivation and support from Dr. C.N Ncobela, Dr. M.V Mkwanazi, Dr. M.K Zuma, Dr. B.S Vilakazi, Mr. S.T Bhiya, Mr. S. Mungwe, Miss S.J Mnembe and Mr. K.K Khanyile. I could have not done it without your help guys, thank you. Dr. Obert Chikwanha, Dr. Katiyatiya, Dr. Farouk and Dr. Rebecca of Stellenbosch University also assisted with the analysis of fatty acids. I also thank my parents for their support, my fiancée, and my daughter for being patient and also for assisting with the logistics of Chapter 5. Above all, I thank God for his protection.

Special thanks goes to UKZN Animal and Poultry Science technicians especially Dr S. Ndlela. Agricultural Research Council Staff especially Bro Kgagi (O.G) and Bro killer, thank you for all the assistance that you offered me from 2019 to 2020. I appreciate students from UKZN, TUT and UNISA who assisted with data collection.

My appreciation goes to the following organisation who provided financial support during my PhD program at UKZN, the National Research Foundation (IT8114/98), Meat industry Trust (118221), FoodBev SETA, UKZN Post-graduate Cum-laude Scholarship, UKZN competitive grant and financial support received from my supervisor (Prof M. Chimonyo).

Thesis output

Publications

- Thabethe, F., Hlatini, V.A., de Almeida, A.M. and Chimonyo, M., 2022. Growth performance of South African Windsnyer pigs to the dietary inclusion of Amarula oil cake. *Tropical Animal Health and Production*, 54,1 - 9. https://doi.org/10.1007/s11250-022-03345-x
- Thabethe, F., Hlatini, V.A., de Almeida, A.M. and Chimonyo, M., 2022. Carcass traits and primal pork cuts of growing Windsnyer pigs fed on diets containing Amarula oil cake. *South African Journal of Animal Science*). (Accepted)

Conference proceedings

- F. Thabethe, V.A Hlatini, A.M de Almeida, M. Chimonyo., 2022. Growth performance of South African Windsnyer pigs to the dietary inclusion of Amarula oil cake. 53rd SASAS Congress (Ascot Conference Centre in Pietermaritzburg, September 2022). Virtual presentation

Under preparation

- F. Thabethe, C. Mapiye, V.A Hlatini, C.N Ncobela and M. Chimonyo., 2023. Nutritional related blood metabolites, liver enzymes and fatty acid composition of pork from Windsnyer pigs supplemented with Amarula oil cake diets.

Table of contents

List of Tables

List of Figures

Chapter 1: General introduction

1.1 Introduction

The expansion in the worldwide human population increases the demand and consumption of animal proteins, especially for pork and poultry (Townsend, 2015; Abdulkadir et al., 2016). Meat is a nutrient-dense food, which is a source of protein, vitamins, and minerals (Marangoni et al., 2015). It contains vital nutrients such as fatty acids, protein and amino acids that are required by the human body for the physiological perinatal human brain development (Marangoni et al., 2016) being essential for human nutrition and physiology. Pigs are a source of meat and also a source of livelihood in developing countries. Sustainable pig production, therefore, increases food security and welfare of communities (Mlambo and Mapiye, 2015).

Pig productivity largely depends on the availability of nutritious feed resources (Wanapat et al., 2013). Conventional feed sources are primarily derived from soybean and cereals such as corn, oats, or wheat (DAFF, 2013). The increasing human population puts a strain on the production of these feed sources that are also suitable for human nutrition. Soyabean is the main feedstuff used in pig diets, preferred for their outstanding nutritional characteristics such as high crude protein and essential amino acid contents, high digestibility, and palatability. South Africa depends on imports of soya bean (SB) since it produces less than a third of its SB requirements (Grain SA, 2016). This factor increases its cost which negatively affects pig production systems, especially for rural-based or small-holder farmers.

The interest in using non-conventional feedstuffs in pig diets is increasing. Apart from enhancing animal productivity, utilizing non-conventional feeds decreases the number of cereal grains that have to be imported at high costs. Potential non-conventional protein sources include *Vachellia* leaves, oil cakes from indigenous oil seeds, or nut plants such as sunflower, macadamia, and Amarula (Acheampong-Boateng et al., 2016). The Amarula tree (*Sclerocaya birrea subsp caffra*) is an indigenous fruit tree that is scattered in the savannah parts of the African continent lying from 17°15'N in Niger to 31°00'S to South Africa (Chirwa and Akinnifesi, 2008). The tree belongs to the *Anacardiaceae* family, having a plum-like pale fruit of about 3 - 4 cm in diameter with juicy mucilaginous flesh (Mariod and Abdelwahab, 2012). The tree is resistant to drought and does well in hot and warm areas when compared to other conventional plant sources (Shackleton and Shackleton, 2002). The oil cake from the seeds of Amarula contains about 470, 194, and 131 g/kg DM crude protein, neutral detergent fibre, and acid detergent fibre, respectively, which gives it a huge potential to be an alternative feed ingredient for pigs (Mlambo et al., 2011).

Windsnyer pigs are characterized by slow growth, a compact body conformation, a long nose, and their body is covered with a black coat. Their meat is tastier compared to commercial pigs (Chimonyo et al., 2005; Madzimure et al., 2015), making them a potential for consumers who prefer organically produced pork. Unlike commercial pig breeds, the slow-growing Windsnyer pigs have the potential of surviving on diets with lower protein content (Kanengoni et al., 2014). The exploration of Amarula based diets on indigenous pigs requires information on how these pigs respond to these diets in terms of growth, nutritional-related blood metabolites, and pork quality characteristics.

1.2 Justification

There is a need to focus on agricultural by-products that are regarded as waste and local plants as non-conventional feed sources such as Amarula, macadamia, *Vachellia* trees, and potato by-products to alleviate reliance on imported conventional feed ingredients (Khanyile et al., 2014; Ncobela et al., 2018). The use of non-conventional protein sources also addresses environmental challenges caused by these waste products.

In Southern Africa, measures of domesticating the *Sclerocarya* trees which are a source of Amarula fruits have been established to create orchards that provide fruits for the beverage and the canning industry (Mariod and Abdelwahab, 2012). An Amarula tree produces between 21 000 and 91 000 fruits per year (Mkwezalamba et al., 2015). The fruits are collected for human consumption, making beer and jam, or used as feed for ruminants. The surplus ripened fruits are left unattended which creates environmental hazards (Mthiyane and Mlanga, 2017). The exploration of Amarula fruits as a feed source for pig production systems could limit the environmental pollution caused by the Amarula processing companies. Amarula oil cake (AOC) is a residue after oil extraction of the dry seeds of the ripened fruits of Amarula (Shackleton, 2002), it is largely produced by different small-scale companies in South Africa and Eswatini. This also closes the gap between those who are employed and unemployed since some rural households collect and sell the fruits for processing (Malebana, 2018; Mthiyane et al., 2017; Mazizi et al., 2019).

The high residual oil of Amarula oil cake can be beneficial for fattening pigs in finishing operations. Moreover, the residual oil of Amarula kernels contains a high amount of oleic acid which protects muscle damage due to reactive oxygen species (ROS), thereby improving carcass traits and fatty acid composition of adipose tissues (Leheska et al., 2008; Carvalho et al., 2012; Pereira et al., 2012; Mthiyane and Mhlanga, 2018). On the other hand, secondary compounds found in plants improve meat quality by acting as natural anti-oxidants but when their concentration is above the recommended level, they can reduce the growth and welfare of animals. Determining the accurate inclusion levels of AOC enables pig nutritionists and farmers to appropriately incorporate AOC into pig diets without constraining growth performance and pork quality (Mas et al., 2010).

1.3 Objectives

The broad objective of the study was to assess the relationship between feeding increasing levels of Amarula oil cake with growth performance of Windsnyer pigs and pork quality. The specific objectives were to:

- Determine the maximum inclusion level of Amarula oil cake diets based on growth performance of Windsnyer pigs.
- Determine the relationship between carcass traits, primal pork cuts and visceral organ weights of Windsnyer pigs to AOC inclusion.
- Assess the changes in nutritionally related blood metabolites and liver enzymes of Windsnyer pigs fed on increasing levels of AOC based diets.
- Determine fatty acid composition and health lipid indices of pork from Windsnyer pigs fed on different levels of AOC.

1.4 Hypotheses

- The inclusion level of AOC beyond the maximum point would reduce growth performance of Windsnyer pigs;
- Inclusion level of AOC linearly improves carcass traits, primal pork cuts and visceral organ weight of pigs;
- Increasing levels of AOC decreases nutritionally related blood metabolites and liver enzymes of Windsnyer pigs; and
- Inclusion of AOC improves fatty acid composition and health lipid indices of pork.

1.5 References

Abdulkadir, A.R., Zawawi, D.D. and Jahan, M.S., 2016. Proximate and phytochemical screening of different parts of *Moringa oleifera*. *Russian Agricultural Sciences*, 42, 34 - 36.

Acheampong-Boateng, O., Bakare, A.G. and Mbatha, K.R., 2016. The potential of replacing soyabean oil cake with macadamia oil cake in broiler diets. *Tropical Animal Health and Production*, 48, 1283 - 1286.

Amarowicz, R., Pegg, R., Rahimi-Moghaddam, P., Barl, B. and Weil, J., 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, 84, 551 - 562.

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th edition. Washington DC, USA.

Benz, J.M., Linneen, S.K., Tokach, M.D., Dritz, S.S., Nelssen, J.L., DeRouchey, J.M., Goodband, R.D., Sulabo, R.C. and Prusa, K.J., 2010. Effects of dried distiller's grains with solubles on carcass fat quality of finishing pigs. *Journal of Animal Science,* 88, 3666 - 3682.

Carvalho, R.H.R., Galvão, E.L., Barros, J.A.C., Conceição, M.M. and Sousa, E.M.B.D., 2012. Extraction, fatty acid profile and antioxidant activity of sesame extract (*Sesamum Indicum L). Brazilian Journal of Chemical Engineering*, 29, 409 - 420.

Chimonyo, M., Bhebhe, E., Dzama, K., Halimani, K. and Kanengoni, A., 2005. Improving smallholder pig production for food security and livelihood of the poor in Southern Africa. *African Crop Science Conference Proceedings*, 7, 569 - 573.

Chimonyo, M., Dzama, K. and Mapiye, C., 2010. Growth performance and carcass characteristics of indigenous Mukota pigs of Zimbabwe. *Tropical Animal Health and Production*, 42, 1001 - 1007.

Chirwa, P.W. and Akinnifesi, F.K., 2008. Ecology and biology of *Uapaca kirkiana, Strychnos cocculoides* and *Sclerocarya birrea* in Southern Africa. In Indigenous fruit trees in the tropics: domestication, utilization and commercialization (pp. 322-340). Wallingford UK: CABI.

Department of Agriculture, Forestry and Fisheries (DAFF), (2013). Abstract of Agricultural Statistics. Annual report, pp. 1- 97.

Fernǻndez, M., Ordonez, J.A., Cambero, I., Santos, C., Pin, C., de la Hoz, L., 2007. Fatty acid composition of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chemistry,* 101, 107 - 112.

Folch, J., Lees, M. and Stanley, G.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*, 497 - 509.

Gous, R.M. and Morris, T.R., 1985. Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. *British Poultry Science*, 26, 147 - 161.

Grain SA, 2016. South Africa's soybean industry: A brief overview. https://www.grainsa.co.za/south-africa-s-soybean-industry:-a-brief-overview. (Accessed 23 October 2020).

Gudiso, X., Hlatini, V., Chimonyo, M. and Mafongoya, P., 2018. Response of broiler (*Gallus gallus domesticus*) performance and carcass traits to increasing levels of *Acacia angustissima* leaf meal as a partial replacement of standard protein sources. *The Journal of Applied Poultry Research*, 28, 13 - 22.

Halimani, T.E., Muchadeyi, F.C., Chimonyo, M. and Dzama, K., 2012. Opportunities for conservation and utilisation of local pig breeds in low-input production systems in Zimbabwe and South Africa. *Tropical Animal Health and Production*, 45, 81 - 90.

Jiang, J. and Xiong, Y.L., 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, 120, 107 - 117.

Jonfia-Essien, W.A., West, G., Alderson, P.G., Tucker, G., 2008. Phenolic content and antioxidant capacity of hybrid *variety cocoa* beans. *Food Chemistry*, 108, 1155 - 1159

Kamal-Eldin, A. and Moreau, R.A., 2009. Tree nut oils. In *Gourmet and health-promoting specialty oils* (pp. 127-149). AOCS Press.

Kanengoni, A.T., Chimonyo, M., Erlwanger, K.H., Ndimba, B.K. and Dzama, K., 2014. Growth performance, blood metabolic responses, and carcass characteristics of grower and finisher South African *Windsnyer*-type indigenous and Large White× Landrace crossbred pigs fed diets containing ensiled corncobs. *Journal of Animal Science*, *92*, 5739 - 5748.

Karioti, A., Hadjipavlou-Litina, D., Mensah, M., Fleischer, T. and Skaltsa, H. (2004). Composition and Antioxidant Activity of the Essential Oils of *Xylopia aethiopica* (Dun) *A. Rich.* (Annonaceae) Leaves, Stem Bark, Root Bark, and Fresh and Dried Fruits, Growing in Ghana. *Journal of Agricultural and Food Chemistry*, 52, 8094 - 8098.

Khanyile, M., Ndou, S.P. and Chimonyo, M., 2014. Influence of *Acacia tortilis* leaf meal-based diets on growth performance of pigs. *Livestock Science*, 167, 211 - 218.

Kuda, T., Tsunekawa, M., Goto, H. and Araki, Y., 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *Journal of Food Composition and Analysis*, 18, 625 - 633.

Leakey, O.C., Ajayi, G., Sileshi, Z., Tchoundjeu, P., Matakala, F.R. Kwesiga (ed.), Indigenous Fruit Trees in the Tropics: Domestication, Utilisation and Commercialisation. CABI International, London, UK, pp. 322 - 340.

Leheska, J.M., Thompson, L.D., Howe, J.C., Hentges, E., Boyce, J., Brooks, J.C., Shriver, B., Hoover, L. and Miller, M.F., 2008. Effects of conventional and grass-feeding systems on the nutrient composition of beef. *Journal of Animal Science*, 86, 3575 - 3585.

Liu, H.W. Gai, F., Gasco, L, Alberto Brugiapaglia, A., Carola Lussiana, C.,Guo, K.J., Tong, J.M., Zoccarato, I, 2009. Effects of chestnut tannins on carcass characteristics, meat quality, lipid oxidation and fatty acid composition of rabbits. *Meat Science*, 83, 678 - 683.

Madzimure, J., Bakare, A.G., Muchenje, V., Chimonyo, M., 2015. Sensory qualities of pork from Large White and *Windsnyer* gilts prepared by different cooking methods. *International Journal of Food and Nutritional Science*, 2,106 – 111.

Malebana, I. M. M. Dietary effects of *Sclerocarya birrea caffra* nut meal in growing fattening male dorper sheep. PhD Thesis, University of the Witwatersrand, South Africa, 2018.

Marangoni, F., Cetin, I., Verduci, E., Canzone, G., Giovannini, M., Scollo, P., Corsello, G. and Poli, A., 2016. Maternal diet and nutrient requirements in pregnancy and breastfeeding. An Italian consensus document. *Nutrients*, 8, 629.

Marangoni, F., Corsello, G., Cricelli, C., Ferrara, N., Ghiselli, A., Lucchin, L. and Poli, A., 2015. Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: an Italian consensus document. *Food and Nutrition Research*, 59, 27606.

Mariod, A.A. and Abdelwahab, S.I., 2012. *Sclerocarya birrea* (Marula), an African tree of nutritional and medicinal uses: a review. *Food Reviews International*, 28, 375 - 388.

Mas, G., Llavall, M., Coll, D., Roca, R., Diaz, I., Gispert, M., Oliver, M.A. and Realini, C.E., 2010. Carcass and meat quality characteristics and fatty acid composition of tissues from Pietrain-crossed barrows and gilts fed an elevated monounsaturated fat diet. *Meat Science*, 85, 707 - 714.

Mazizi, B.E., Moyo, D., Erlwanger, K.H. and Chivandi, E., 2019. Effects of dietary *Sclerocarya birrea caffra* (Marula) nut meal on the growth performance and viscera macromorphometry of broiler Japanese quail. *Journal of Applied Poultry Research*, 28, 1028 - 1038.

Mkwezalamba, I., Munthali, C.R. and Missanjo, E., 2015. Phenotypic Variation in Fruit Morphology among Provenances of *Sclerocarya birrea* (A. Rich.) *Hochst*. *International Journal of Forestry Research*, 2015.

Mlambo, V. and Mapiye, C., 2015. Towards household food and nutrition security in semi-arid areas: What role for condensed tannin-rich ruminant feedstuffs. *Food Research International*, 76, 953 - 961.

Mlambo, V., Dlamini, B.J., Nkambule, M.T., Mhazo, N. and Sikosana, J.L.N., 2011. Nutritional evaluation of marula (*Sclerocarya birrea*) seed cake as a protein supplement for goats fed grass hay. *Tropical Agriculture*, 41, 010035 - 09.

Mthiyane, D.M.N. and Mhlanga, B.S., 2017. The nutritive value of marula (*Sclerocarya birrea*) seed cake for broiler chickens: nutritional composition, performance, carcass characteristics and oxidative and mycotoxin status. *Tropical Animal Health and Production*, 49, 835 - 842.

Mthiyane, M.N. and Mhlanga, B.S., 2018. Effects of dietary replacement of soya bean meal with marula (*Sclerocarya birrea caffra*) seed cake with or without DL-methionine or phytase on productive performance and carcass characteristics in broiler chickens. *International Journal of Livestock Production*, 1 - 14.

Ncobela, C.N., Kanengoni, A.T., Thomas, R.S. and Chimonyo, M., 2018. Voluntary feed intake and growth performance of slow-growing pigs fed on increasing levels of ensiled potato hash meal. *Tropical Animal Health and Production*, 50, 113 - 120.

Pereira, A.S.C., Santos, M.V.D., Aferri, G., Corte, R.R.P.D.S., Silva, S.D.L., Freitas Júnior, J.E.D., Leme, P.R. and Rennó, F.P., 2012. Lipid and selenium sources on fatty acid composition of intramuscular fat and muscle selenium concentration of Nellore steers. *Revista Brasileira de Zootecnia*, 41, 2357 - 2363.

Reed, J.D., McDowell, R.T., Van Soest, P.J. and Horvath, P.R., 1982. Condensed tannins: a factor limiting the use of cassava forage. *Journal of the Science of Food and Agriculture*, 33, 213 - 220.

Shackleton, S., Shackleton, C., Wynberg, R., Sullivan, C., Leakey, R., Mander, M., McHardy, T., Den Adel, S., Botelle, A., du Plessis, P. and Lombard, C., 2009. Livelihood trade-offs in the commercialisation of multiple-use NTFP: lessons from Marula (*Sclerocarya birrea subsp. caffra*) in southern Africa. Ashoka Trust for Research in Ecology and the Environment (ATREE).

Townsend, R., 2015. Ending poverty and hunger by 2030: An agenda for the global food system (No. 95768, pp. 1 - 32). The World Bank.

Van Soest, P.V., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583 - 3597.

Wanapat, M., Kang, S. and Polyorach, S., 2013. Development of feeding systems and strategies of supplementation to enhance rumen fermentation and ruminant production in the tropics. *Journal of Animal Science and Biotechnology*, 4, 1 - 11.

Chapter 2: Literature review

2.1 Introduction

There is a growing concern with regards to the qualitative traits of pork (Nevrkla et al*.,* 2017). Pigs are mostly reared for their meat to meet the requirements for animal protein for human nutrition (FAOSTAT, 2012). Most of the pigs that are recorded in Southern Africa are those from commercial production kept for their meat, leaving out the indigenous type of pigs while estimates show that above 65 % of the global pig populations are located in developing countries and are kept by small scale farmers (Chiduwa et al., 2008).

Feed deficits remain a challenge for smallholders who are incapable of purchasing conventional feed sources for pigs hence resulting in low productivity (Stür et al., 2002). More research has focused on leguminous plant leaves as an alternative protein source for livestock and other agricultural by-products. To increase the spectrum of non-conventional feed sources in tropical areas for maximum productivity and the pork quality of indigenous pigs, it is important to examine the potential of nuts or pods of indigenous plants as alternative protein sources for pigs. The current review discusses the description of *Sclerocarya birrea caffra* tree (Marula), the production of Marula trees, uses of Marula trees, types of feed sources, the nutritional composition of Amarula kernels, the fatty acid composition of Amarula oil cake, the use of Amarula oil cake in pig diets, constraints in using Amarula oil cake in pig diets, anti-nutritional factors of Amarula, the potential of AOC in indigenous pigs, methods of inactivating secondary compounds in diets, the extraction methods of Amarula oil cake for processing, description of the Windsnyer pig and reasons for rearing Windsnyer pigs, growth performance of indigenous pigs (Windsnyer pigs), carcass characteristics and primal pork cuts of pigs, organs of pigs, nutritional related-blood metabolites, liver enzymes of pigs and fatty acid composition of pigs.

2.2 Description of *Sclerocarya birrea caffra* tree (Marula)

Sclerocarya birrea is a deciduous tree that is characterized by having a short stem of up to 4 m with a spreading crown (Nghitoolwa et al., 2003). It belongs to the *Anacardiaceae* family (the mango family), having close to 650 species, 70 genera of mostly tropical or subtropical deciduous trees, shrubs, and woody vines. *Sclerocarya birrea* tree is a multipurpose tree valued mainly for its fruits, seeds (kernels), leaves, and barks which are used for making medicine (Mutshinyalo and Tshisevhe 2003). The leaves are divided into 10 or more pairs of leaflets, each about 60 mm long and with a sharp point. The flowering of the tree occurs between September and December, female and male flowers grow in different trees. Ripening of Amarula fruits usually happens between January and March (Jama et al., 2008) and the number of fruits produced increases as the tree grows. The tree is known as umnganu (in Zulu and siSwati), marula (English), morula (Pedi and Tswana), mupfura (Shona), mura (Meru), mafula (Venda), maroela (Afrikaans), Nkanyi (Xitsonga) and is it found in the semi-arid and savannas of sub-Saharan Africa (Shackleton et al., 2002; DAFF, 2010). The distribution of Amarula trees is restricted by extreme frost, however, it can survive in arid areas with low rainfall of about 200 – 1500 mm per annum (Mokgolodi et al., 2011). Its distinct characteristic of enduring dry climatic conditions, makes it an ideal source of feed for small-scale farmers rearing pigs in rural-arid areas of southern Africa.

2.3 Production of Amarula trees

The trees are found in some parts of Limpopo, KwaZulu-Natal, the Eastern Cape, and Mpumalanga province. The Amarula tree is common in hot areas of the Saharan Africa and it's an important part of many people in rural areas (Shackleton et al., 2002). Each tree has the potential of producing an average of about 27 000 to 44 200 fruits per single tree with an average weight of 20 – 25 g per single fruit (Shackleton et al., 2003). DAFF, (2010) reported that a single tree has the potential to produce 500 kg of fruits each year or up to 70 000 fruits. Commercial products made from Amarula fruits such as liquors and cosmetics are available in South Africa and some parts of Swaziland, however, the exploration of Amarula as an ingredient for livestock is still lacking and if it is commercialized it can also benefit the livestock industry.

2.4 Uses of Amarula trees

In Africa, indigenous fruit types of trees are used as macro and micro-nutrient sources and health promoters because of the different types of nutritional properties and phytochemicals that the trees possess (Chivandi et al., 2015). The Amarula tree plays an important part in diet, culture, and tradition of the sub-Saharan rural communities and the commercial industry (Wynberg et al., 2002). The fruits are used for making juice, they can also be consumed when ripened. The fruits are reported to contain high content of ascorbic acid (as high as 194 mg) comparable to that of orange juice but also high as compared to other citrus types of fruit, hence it can also be used for treating scurvy (Shone, 1979). The seeds contain three or two kernels which are attained by means of breaking the nuts with a stone when they are dry and a narrow needle like object is used to remove the kernels (Mariod and Abdelwahab, 2012). Once the kernels are removed, they are readily consumed as snacks. The kerns are rich in oil which is used to make cosmetic ointments and as well as a meat preserver (Coates, 1990). Figure 2.1 shows the major uses of Amarula tree parts

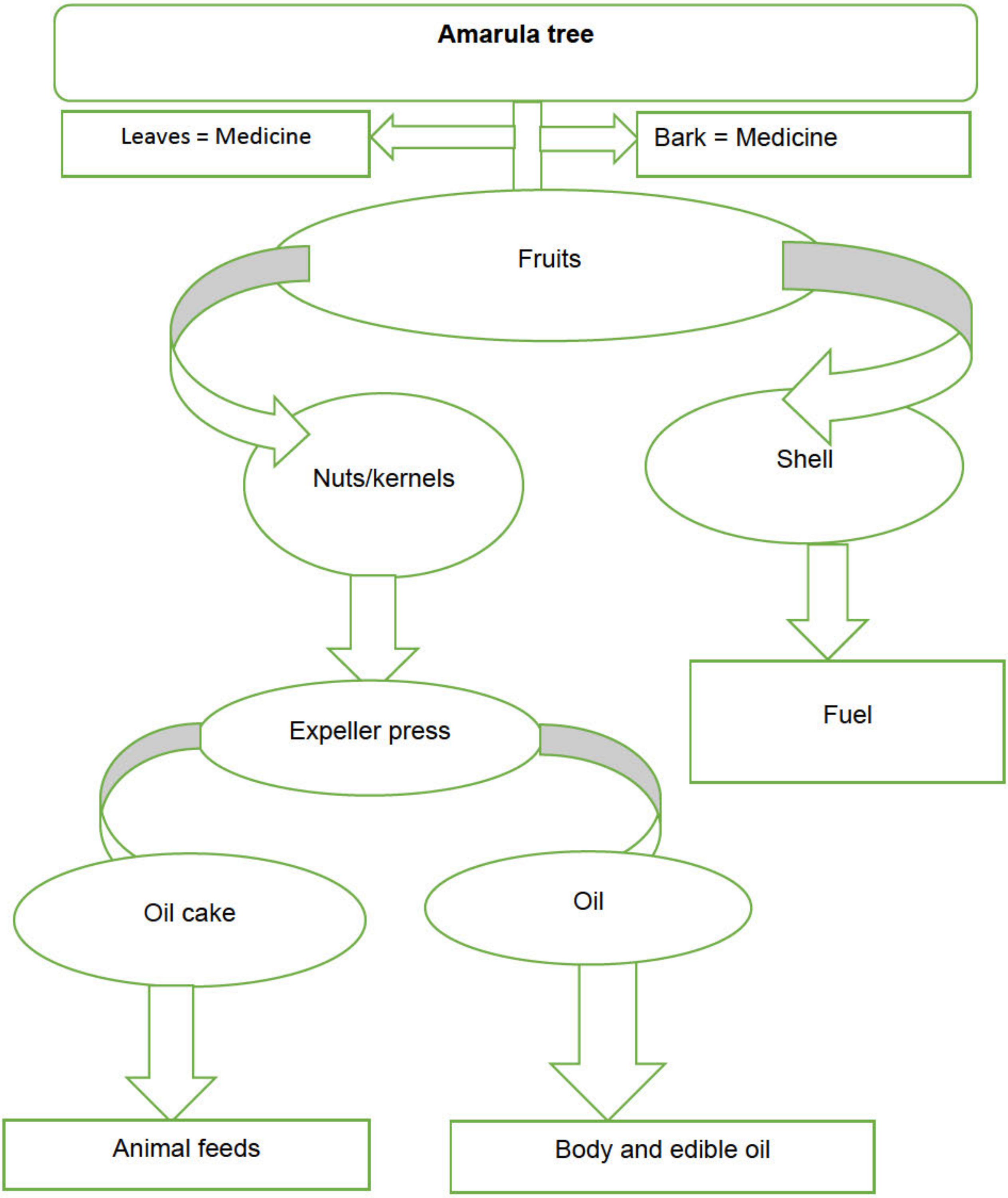


Figure 2.1: Uses of Amarula tree parts (Taseski, 2005)

2.5 Types of feed sources

Livestock feeds can be classified as conventional and non-conventional.

2.5.1 Conventional feed sources for pigs

Conventional feed sources are the primary highly nutritious ingredients used in pig diets. They are divided into plant-derived and animal-derived protein sources. Plant derived conventional feed sources include oil cakes or nut meals such as soybean meal, olive cake, sunflower and palm kernel oil cake. These ingredients are rich in protein and are intensively used in commercial farming systems (Kassahun et al., 2012). Animal derived protein sources include animal by-products such as fish meal, feather meal, bone meal and blood meal (Shipton and Hecht, 2005). Animal derived feed sources have, however, been rejected due to the presence of residues in meat products and their high costs (Brookes, 2001; Van Ryssen, 2001). The use of conventional feed sources is limited among small-holder farmers due to lack of knowledge, availability, and high prices.

2.5.2 non-conventional feed sources for pigs

Non-conventional feed sources are secondary ingredients that are mainly used by small-holder farmers. These feed ingredients include forages or leaf meals, kitchen waste, maize cobs, coarse maize meal, pumpkins, maize stovers and grasses (Ncobela et al., 2018; Thabethe et al., 2022). Most of these non-conventional feed sources are characterized by poor nutrient digestibility, therefore affecting growth of pigs. The nutritional contents of these ingredients match the requirements of livestock. Lack of research in the utilisation of these feed sources contribute significantly to the scarcity of highly nutritious feeds that can meet the demand for pig producers.

2.6 Nutritional composition of Amarula kernels

Amarula seeds or kernels contain high amount of protein, residual oil or ether extract (Mariod and Abdelwahab, 2012). Amarula oil cake (AOC) is defined as a residue of oil extraction of the dry seeds of Amarula kernels. The extracted oil is used by humans for consumption and for cosmetic functions. The nuts are taken after human use, sun dried, and mechanical decortication produces the kernels (Mlambo *et al*., 2011a). In addition, the seeds can be used as supplements to human diet in poverty-stricken areas due to their high nutritional value. The nutritional characteristics of AOC can meet the requirements of livestock if fully explored. For example, Amarula oil cake improved growth of quails, pigs, and crude protein content of Dorper mutton (Malebane, 2018; Mazizi et al., 2019; Thabethe et al., 2022). The proximate, mineral, and amino acid composition of Amarula oil cake is shown in Tables 2.1, 2.2 and 2.3, respectively.

Table 2.1: Proximate composition of Amarula oil cake

Chemical constituent (g/kg)	Composition	Source
Dry matter	901	Mlambo et al. (2011)
	938	Mdziniso et al. (2016)
	958.37	Malebane et al. (2017)
	963.50	Thabethe et al. (2022)
Ash	49.80	Thabethe et al. (2022)
	55.5	Mlambo et al. (2011b)
Crude protein	470	Mlambo et al. (2011)
	472.1	Mdziniso et al. (2016)
	362.20	Thabethe et al. (2022)
Ether extract	394	Mlambo et al. (2011)
	289.6	Mdziniso et al. (2016)
	411.32	Malebane et al. (2017)
	343.90	Thabethe et al. (2022)
Acid detergent fibre	134	Mlambo et al. (2011a)
	147.4	Mdziniso et al. (2016)
	245.60	Thabethe et al. (2022)
Neutral detergent fibre	194	Mlambo et al., (2011a)
	357.30	Thabethe et al. (2022)
Acid detergent lignin	114.30	Thabethe et al., (2022)
Metabolizable energy (MJ/kg DM)	15.44	Mlambo et al. (2011a)
Gross energy (MJ/kg DM)	25.34	Thabethe et al. (2022)
	28.49	Malebane et al. (2017)

Table 2.2: Mineral composition of Amarula oil cake

Minerals (g/kg)	Composition	Source
Calcium	1.08	Thabethe et al. (2022)
	1.43	Malebane et al. (2017)
	0.85	Mthiyane and Mhlanga, (2017)
Phosphorus	10.00	Malebane et al. (2017)
	9.40	Thabethe et al. (2022)
	9.40	Mthiyane and Mhlanga, (2017)
Magnesium	4.87	Malebane et al. (2017)
	5.70	Thabethe et al. (20122)
Potassium (mg/kg)	0.77	Mthiyane and Mhlanga, (2017)
	8.30	Thabethe et al. (2022)
	6.33	Malebane et al. (2017)
Iron (mg/kg)	95.50	Thabethe et al. (2022)
Iron (g/kg)	0.69	Malebane et al. (2017)
Zinc (mg/kg)	60.20	Thabethe et al. (2022)
Zinc (g/kg)	0.05	Malebane et al. (2017)
Sulphur	4.23	Malebane et al. (2017)

Table 2.3: Amino acid composition of Amarula oil cake

Amino acids	Composition	Source
Essential amino acids		
Lysine (g/kg DM)	7.73	Malebane et al. (2017)
Tryptophan (g/100 g DM)	0.68	Thabethe et al. (2022)
Methionine (g/kg DM)	6.30	Malebane et al. (2017)
Tryptophan (g/100 g DM)	0.44	Thabethe et al. (2022)
Tryptophan (g/kg DM)	3.36	Malebane et al. (2017)
Tryptophan (g/100 g DM)	0.44	Thabethe et al. (2022)
Threonine (g/kg DM)	6.92	Malebane et al. (2017)
Threonine (g/100 g DM)	0.81	Thabethe et al. (2022)
Arginine (g/kg DM)	50.92	Malebane et al. (2017)
Arginine (g/100 g DM)	5.51	Thabethe et al. (2022)
Leucine (g/kg DM)	16.90	Malebane et al. (2017)
Leucine (g/100 g DM)	1.23	Thabethe et al. (2022)
Isoleucine (g/kg DM)	12.30	Malebane et al. (2017)
Isoleucine (g/100 g DM)	1.29	Thabethe et al. (2022)
Non - essential amino acids		
Cysteine (g/kg DM)	2.75	Malebane et al. (2017)
Alanine (g/kg DM)	8.90	Malebane et al. (2017)
Alanine (g/100 g DM)	1.04	Thabethe et al. (2022)
Proline (g/kg DM)	13.00	Malebane et al. (2017)
Proline (g/100 g DM)	1.13	Thabethe et al. (2022)
Aspartic acid (g/kg DM)	19.82	Malebane et al. (2017)
Aspartic acid (g/100 g DM)	2.74	Thabethe et al. (2022)
Tyrosine (g/kg DM)	9.70	Malebane et al. (2017)
Tyrosine (g/100 g DM)	0.69	Thabethe et al. (2022)
Glycine (g/kg DM)	13.60	Malebane et al. (2017)
Glycine (g/100 g DM)	1.80	Thabethe et al. (2022)
Hydroxy-proline (g/kg DM)	0.63	Malebane et al. (2017)
Hydroxy-proline (g/100 g DM)	0.09	Thabethe et al. (2022)
Alanine (g/kg DM)	8.90	Malebane et al. (2017)
Alanine (g/100 g DM)	1.04	Thabethe et al. (2022)

2.7 Fatty acid composition of Amarula oil cake

Carbon atoms are the main components making up fatty acids (FA) and are in a form of an alkyl series with a carboxylic acid group and a methyl group attached at each end. The properties of fatty acids are affected by the number of carbon atoms in each fatty acid such as the carboxylic group, the position, and the number of double bonds (Belitz et al., 2009). Amarula oil cake is a good source of essential fatty acids such as 9-octadecanoic and 9,-12-octadecadienoic acids (Ogbobe, 1992).

The most dominant types of FA in AOC are oleic acid, hexadecanoic, octadecanoic, and arachidonic acids (Glew et al., 2004). Malebana et al. (2018) showed that AOC contain high amounts of monounsaturated fatty acids (MUFA) (773 g/kg-1) which is the largest component that make up the available fatty acids in AOC as compared to soybean meal (279 g/kg DM). The unsaturated fatty acids found in nut meals or oil cakes could prevent diseases (Wei and Shibamoto, 2010). Monounsaturated fatty acids are less susceptible to oxidation and could have a positive effect on human blood cholesterol levels. For example, the high oleic content (75 - 85 %) of Amarula oil cake could have some beneficial effects on human health since it is an anti-diabetic and anti-atherosclerotic monounsaturated fatty acids (Kuna and Achinna, 2013). Its high oxidative stability can benefit the meat industry by increasing shelf-life of meat which suggest that it could also be used as natural meat preserver. Fatty acids also form the main components of lipids and act as energy sources. Factors affecting fatty acid composition of Amarula seeds include genetic variation amongst trees, the harvesting time or stage of the tree and soil fertility. The composition of the fatty acids of Amarula nuts/kernels is shown in Table 2.4.

Table 2.4: Fatty acid composition of Amarula oil cake

Fatty acids (%)	Trivial name	Amount	Source
SFA			
C4:0	Butyric	-	Malebana et al. (2017)
		-	Mthiyane and Hugo, (2019)
C6:0	Caproic	-	Malebana et al. (2017)
		-	Mthiyane and Hugo, (2019)
C8:0	Caprylic	-	Malebana et al. (2017)
		-	Mthiyane and Hugo, (2019)
C10:0	Capric	0.02	Mthiyane and Hugo, (2019)
		-	Malebana et al. (2017)
C12:0 (g/kg)	Lauric	0.16	Malebana et al. (2017)
C14:0 (g/kg)	Myristic acid	1.28	Malebana et al. (2017)
C15:0	Pentadecyclic	0.29	Malebana et al. (2017)
C16:0	Palmitic acid	11.30	Mthiyane and Hugo, (2019)
C16:0 (g/100 g)		9.65	
C17:0	Margaric acid	0.13	Mthiyane and Hugo, (2019)
C18:0	Stearic	6.26	Mthiyane and Hugo, (2019)
C18:0 (g/100 g)		5.11	Mthiyane and Mhlanga, (2017)
C19:0	Nonadecanoic	0.04	Mthiyane and Hugo, (2019)
C20:0	Eicosanoic acid	2.49	Malebana et al. (2017)

C21:0	Heneicosylic acid	0.36	Malebana et al. (2017)
C22:0	Behenic acid	1.71	Malebana et al. (2017)
C23:0	Tricosylic acid	0.08	Malebana et al. (2017)
C24:0	Lignoceric	1.23	Malebana et al. (2017)
		0.13	Mthiyane and Hugo, (2019)
MUFA			
C14:1	Myristoleic acid	-	Malebana et al. (2017)
C16:1 (g/kg)	Palmitoleic acid	1.68	Malebana et al. (2017)
C17:1 (g/kg)	Heptadecanoic acid	0.63	Malebana et al. (2017)
C18:1n9c (g/kg)	Oleic acid	729.10	Malebana et al. (2017)
C18:1n9c (g/100 g)		85.24	
C18:1n9t (g/kg)	Elaidic acid	1.14	Malebana et al. (2017)
C20:1 (g/kg)	Gadoleic acid	3.33	Malebana et al. (2017)
C22:1n9 (g/kg)	Erucic acid	0.29	Malebana et al., 2017
C24:1 (g/kg)	Nervonic acid	-	Malebana et al. (2017)
Polyunsaturated			
C18:2n6c (g/kg)	Linoleic acid	69.05	Malebana et al. (2017)
C18:2n6t (g/kg)	Trans-linolenic acid	2.8	Malebana et al. (2017)
C20:2 (g/kg)	Eicosadienoic acid	-	Malebana et al. (2017)
C22:2 (g/kg)	Docosadienoic acid	-	Malebana et al. (2017)
C18:3n3 (g/kg)	α-linolenic acid	0.64	Malebana et al. (2017)

C18:3n6 (g/kg)	γ-linolenic acid	*ND*	Malebana et al. (2017)
C20:3n3 (g/kg)	Eicosatrienoic acid	1.19	Malebana et al. (2017)
C20:3n6 (g/kg)	Dihomo-gamma-linolenic acid	0.25	Malebana et al. (2017)
C20:4n6 (g/kg)	Arachidonic acid	-	Malebana et al. (2017)
C20:5n3 (g/kg)	Eicosapentaenoic acid	3.62	Malebana et al. (2017)
C22:6n3 (g/kg)	Docosahexaenoic acid	0.37	Malebana et al. (2017)

2.8 The use of Amarula oil cake in pig diets

The prevalence of diseases which are caused by climate change, weather changes and unreliable temperatures has increased the interest of animal nutritionists to increase the variety of feed sources required by livestock. Feed sources from trees that adapt well to local climatic conditions or seasonal changes could ease the reliance on imports, reduce greenhouse gases, reduce feed cost, and reduce competition between livestock farmers and humans for grain sources. Oil cakes are the second most vital protein-rich raw materials following cereal grains (Messad et al., 2016). Unlike soybean, the adaptability of Amarula trees in hot climatic conditions and the high protein content of Amarula kernels makes it an ideal protein source that can reduce the reliance on soybean.

Amarula oil cake inclusion reduces nitrogen excretion of Windsnyer pigs (Hlongwana et al., 2021). Mthiyane and Mhlanga (2017) reported a reduced growth performance of broiler chickens supplemented with Amarula oil cake diets. When feeding non-ruminants, high inclusion levels of Amarula oil cake is harmful due to the secondary compounds that are produced by Amarula kernels. Determining the correct inclusion level of AOC when used as an ingredient is, therefore, crucial. Besides growth performance, information about pork quality of pigs fed on Amarula based diets is lacking. Dose-response trials that assess the relationship between AOC and pork quality could increase the choice for consumers when selecting healthy foods.

2.9 Constraints in using AOC in pig diets

High amount of residual oil of Amarula meals could reduce of shelf life of feeds as a result of rancidity that develops from lipid peroxidation and thus decrease quality of diets that are made with Amarula kernels. The quality of nutrients from Amarula oil cake after oil extraction is influenced by the method of oil extraction, therefore, the high ether extract of Amarula oil cake as a result of screw pressing during oil extraction of Amarula kernels could decrease shelf-life and other quality aspects of pork (Mthiyane and Mhlanga, 2017; Nwabuebo, 2017). When processing Amarula kernels for the production of Amarula oil cake, the processing method should be considered because both extraction methods (mechanical and chemical) contribute to the high ether extract of the oil cake.

Anti-nutritional factors are plant secondary compounds that are naturally produced in plants. They include tannins, saponins, polyphenols, mycotoxins, phytates, nitrates, triterpenoids and cyanogens. Anti-nutritional factors act as defence mechanisms in plants against herbivores, while in animals, they restrict growth performance when consumed above optimal levels. Despite the increasing concern on the use of non-conventional feed sources in pig diets, the presence of secondary compounds remains a big challenge in livestock (Woyengo et al., 2017). Phytate, for example, chelates minerals rendering them unavailable for absorption in the gut. Similarly, tannins precipitate proteins hence reducing the daily requirements of animals which, in turn, compromise growth and pork quality (Jansman et al., 1993). To maximise returns and the productivity of pigs when Amarula-based diets are used in pig feeding systems, it is crucial for farmers to have a clear understanding of the challenges and toxicity of these plants secondary compounds.

2.10 Methods of inactivating secondary compounds in diets

Despite the high nutritional content of non-conventional oil cakes such as Amarula oil cake, olive oil cake and sunflower oil cake, the secondary compounds produced from these feed ingredients remain a big challenge in feed industries. The negative effects of secondary compounds in feeds can be deactivated using different techniques. The deactivation of secondary compounds in feed ingredients could improve growth performance of livestock by enhancing the availability of nutrients.

Methods of deactivating the toxic effects of feeds include heat treatment. Soaking and autoclaving, drying, solid state fermentation, the use of enzymes, the use of binding compounds, urea and wood ash (Silanikove et al., 2001; Makkar, 2003). The solid-state fermentation of ground nut oil cake diet and Pistia leaves increased crude protein, lipid, mineral, amino acids and fatty acids, while, reducing the amount anti-nutritional factors. Soaking and autoclaving decreased crude protein, lipid, minerals, amino acids, fatty acids and tannin content (Gosh, 2020). Even though some methods might reduce the anti-nutritional factors, they can still reduce the nutrient composition of ingredients.

2.10.1 Mechanical extraction technique

The removal of oil from kernels, seeds or nuts is referred to as oil extraction and the remaining product is called nut/seed/oil cake. The most common extraction methods of oil extraction of seeds used by commercial industries are mechanical and chemical extraction methods (Febrianto et al., 2012).

Mechanical oil extraction techniques of seeds/nuts/kernels involves the use of presses oil from the seeds using either a screw press or hydraulic press. This method does not require the use of chemicals; therefore, it is deemed to be user friendly. It, however, leaves a high amount of oil from the cake which indirectly affects the physico-chemical properties of the oil cake that is produced. Pressure is applied on seeds to break down cell membranes of the seed and it pushes the oil out of the seed. When using the screw press method, two apparatus are used, namely a feeder and horizontal screw which generate pressure on the seed. Oil is, therefore, released into a drum which is attached into the screw press. The size of the drum depends on the size of the screw press. When using the hydraulic press method, the seeds or nuts are loaded into a cage where pressure is applied. The end product which is the dry seed or oil cake is then removed manually. The difference between these two techniques is that the screw press requires a lot of energy and cannot function manually while the hydraulic press can function manually (Nwabuebo, 2017).

2.10.2 Chemical extraction method

The use of chemicals or organic solvents during the extraction of oil from seeds or nuts is mostly applied in commercial industries (Khoo et al., 2011). The main disadvantage about chemical extraction is that it is not environmentally friendly due to the use of toxic solvents that could negatively affect human welfare. These solvents include ethanol, hexane, benzene, methanol, acetone, chloroform and n-hexane being the commonly used solvents (Dunnuck, 1991; Bhattacharjee et al., 2007). Hexane is the most commonly used organic solvent.

Unlike the mechanical extraction method, the chemical extraction method produces a high content of oil. It requires less amount of energy and produces high quality of oil cake that contain low amounts of residual oil of about 0.5 % (Reverchon et al., 2006). Topare et al. (2021), also showed that the solvent extraction methods produce high concentrations of oil while leaving the oil cake with a smaller amount of the residual oil of about 0.7 %.

2.11 Attributes of Windsnyer pigs

A substantial amount of Windsnyer pigs is found in South Africa, Zimbabwe, Zambia and some parts of Mozambique (Lekule et al., 1990). The name Windsnyer (wind-cutter) originate from its body conformation (Halimani et al., 2010). The breed is characterised by a slim body, elongated nose, black coat, and a compact body (Ncobela et al., 2018). It is hard and adapted to harsh environments. Windsnyer sows are characterised by early sexual maturity, high fat deposition, low prolificacy, moderate body size and slow growth rates. These attributes make them suitable to survive droughts.

Windsnyer pigs are widely kept in resource-limited communities. The main reason for rearing these pigs, is the generation of income or as a form of investments. Farmers, usually integrate them with seasonal crops in the events of droughts where crops fail. In addition, the manure produced by these pigs is used as a fertilizer. The pigs are also slaughtered during special occasions such as traditional ceremonies or in the event of death of family members or relatives.

Their moderate body size enables them to require small space. They tolerate high fibrous diets, tannin-rich diets such as red sorghum and have a high survival instinct compared to fast-growing pig breeds (Ndindana et al., 2002; Chimonyo et al., 2010; Kanengoni et al., 2014). The use of balanced diets could improve their productivity and improve productivity of Windsnyer pigs.

2.11.1 Growth performance of Windsnyer pigs

Growth performance characteristics include the body weight gain, feed intake, lean body weight, feed conversion ratio and linear growth measurements (Yusuf et al., 2014; Lukuyu et al., 2016). Growth rate is a linear trait that depends on feed intake and is measured by an increase in body weight, daily gain and feed efficiency (Holness, 1991; Whittemore and Kyriazakis, 2006). The average daily gain of indigenous pigs was 0.41 kg /day for pigs reared for 32 weeks of age (Holness, 1991). Len et al. (2007) reported an average daily gain of 0.182 to 0.480 kg/day. Kanengoni et al. (2014) reported a gain feed ratio of 0.220 to 0.371.

Ndindana et al. (2002) indicated feed conversion ratio (FCR) values of between 4.1 and 4.3 in indigenous pigs fed on fibrous diets. Most indigenous pigs depend on inexpensive non-conventional or locally available feed resources. Most diets could be imbalanced and lack adequate amounts of protein, amino acids, and minerals. Fruits, maize husks, grasses, kitchen leftovers, vegetables and rotten maize are the common feed sources (Madzimure *et al*., 2012). Use of high-quality ingredients might improve the growth performance of indigenous pigs.

2.11.2 Carcass characteristics and primal pork cuts of pigs

The anti-nutritional factors of AOC are associated with a reduction in average daily feed intake and average daily gain when consumed in high amounts (Mthiyane and Mhlanga, 2018). A reduction in average daily gain and average daily feed intake of pigs has an indirect effect on the slaughter weight of pigs and pork quality. A reduction of cold dressed weight, dressing percentage and back fat thickness was reported in finishing Landrace x Large White pigs fed on fibrous diets (Kim et al., 2014; Khanyile et al., 2016).

A linear decrease in carcass length, shoulder fat and backfat thickness from indigenous pigs fed on fibrous diets was reported (Ncobela et al., 2018). A decrease in hindquarter length and hindquarter circumference was observed in pigs fed on fibrous diets (Ncobela et al., 2018). Amarula oil cake-based diet also decreased warm and cold carcass weights, hind quarter length and hind quarter weight of pigs (Mabena et al., 2021). Fibrous diets also reduced meat pH after slaughter which, in turn, affects pork quality (Li et al., 2015). Meat pH affects meat freshness, water-holding capacity, and colour of the muscle (Zhu et al., 2011). Inclusion of Amarula kernels might have a positive effect on carcass characteristics of Windsnyer pigs. Low levels of AOC of up to 100 g/kg DM improved the slaughter weight of Windsnyer pigs (Thabethe et al., 2022), which could also improve other carcass characteristics.

2.11.3 Internal organs of pigs

The size of internal organs such as the liver, kidney and stomach could be used to assess the physiological status and how pigs respond or adapt to diets (Ahamefule et al., 2006). The liver and kidneys are involved in the synthesis of the major protein and removing poisonous substances through excretion. The toxicity of feeds may cause lesions or damages to the liver which could, in turn, affect metabolism of nutrients, cause diseases and thus affect the overall performance of Windsnyer pigs.

Leguminous leaf meals inclusion increase the weight of kidneys in pigs (Fasuyi et al., 2013). A reduction in relative heart weight and small intestine weight from Chinese indigenous (Dahe pigs breeds) and their crossbreeds (Dawu breeds) was reported (Chen et al., 1999; Miao et al., 2009; Jiang et al., 2011). Khanyile et al. (2016), showed an increase in the hepatosomatic index, kidney weight and heart weight in pigs fed on *Vachellia* leaf meals. Agbede and Aletor, (2003) also indicated an increase in relative weight of the heart due to high fat deposition. The high residual oil or ether extract of Amarula oil cake could have similar effects on heart weight.

2.12 Nutritionally related metabolites and liver enzymes of pigs

Blood biochemistry characteristics are indicators of nutritional and health status of animals. They can also be used to assess feed quality during feed evaluation. Chief blood metabolites include total protein, iron, glucose, albumin, creatine, uric acid, globulin, and liver enzymes. The amount of anti-nutritional factors that are present in the diet affects the health of pigs (Akinmutimi, 2004). The effect of Amarula oil cake diets on nutritional related blood metabolites of Windsnyer pigs has not been assessed, yet these diets might contain some toxic effects that could affect the functioning of the liver, kidneys which turn could also affect growth of pigs.

Toxicity of feeds substances can be assessed by determining the concentration of liver enzymes. Liver damage may be indicated by the presence of haemorrhages

(Kudair and Al- Hussary, 2010). The inclusion of detoxified *Jatropha curcas* kernel meal (50 %) in diets of growing pigs affected the concentration of total protein and superoxide dismutase (Wang et al., 2011). Jatropha seed cake inclusion also affected glucose concentration in rabbits. An increase in albumin and globulin concentration of pigs fed on fibrous diets was reported (Ncobela et al., 2018). It is therefore important to quantify the concentration of blood metabolites and liver enzymes in pigs offered certain feeds in order to give clear advice to farmers using non-conventional oil cake diets for pigs. The values of metabolites of Windsnyer pigs are shown in Table. 2.5.

Table 2.5: Nutritional related blood metabolites and liver enzymes values of pigs

Variables	Ranges
Albumin (g/dL) (SAWIP)	1.8 - 3.3
Globulin (g/dL) (SAWIP)	3.9 - 6.0
Total protein (g/dL) (SAWIP)	6.0 - 8.0
Uric acid (mg/dL) (SAWIP)	N/F
Alkaline phosphatase (units/litre) (SAWIP)	92 – 294
Alanine aminotransferase (U/L) (SAWIP)	22 – 47
Aspartate aminotransferase (U/L) (SAWIP)	15 – 55
Alkaline phosphatases (U/L) (SAWIP)	41 – 176
Urea nitrogen (mg/ dL) (SAWIP)	8.2 – 25
Total cholesterol (mg/ dL) (SAWIP)	81 – 134

Latimer et al. (2003) and Kanengoni et al. (2014); SAWIP, South African Windsnyer Pigs; NF, Not found.

2.13 Fatty acid composition of pigs

Fat content and fatty acid composition are main characteristics that determines meat quality. Fatty acids are divided into three types, namely the saturated, monounsaturated and polyunsaturated fatty acids (Qwele, 2012). Health experts and nutritionists recommend a reduction of the intake of saturated fatty acids compared to unsaturated fatty acids (Jensen, 1998). The production of pork from organically reared pigs is gaining interest due to the safety of organic products. Fatty acid composition of pork tissues can be altered by feeding pigs diets that are high in fatty acids. Fatty acid composition is, however, more important compared to fatty acid composition of fat tissues due to health concerns of consumers (Wood et al., 2008). Unlike ruminants, saturated and monounsaturated fatty acids are synthesised *de novo* in pigs (Enser et al., 2000).

The consumption of red meat has been criticized due to its association with coronary heart disease and some cancers (Forman, 1999). The high amount of saturated fatty acids found in red meat are the main cause of the above-mentioned diseases (Wood and Enser, 1997). Pork from conventionally produced pigs is also related to a high amount of undesirable fatty acids. These fatty acids are rarely changed by the dietary fatty acids in pork muscle. Madzimure et al. (2017) reported high amounts of saturated fatty acids of pork tissues from Windsnyer gilts compared to Large White gilts. Indigenous pigs more especially sows reach puberty at an early stage and also accumulate more fat early leading to high fat deposition, which in turn, affect pork quality. To our knowledge, research about the effectiveness of oil cakes or seed cakes in fatty acid composition of Windsnyer pigs has never been conducted.

Amarula oil cake contain desirable fatty acids such as monounsaturated fatty acids that could improve the oxidative stability, thereby increasing meat shelf life and its nutritional value (Mthiyane and Mhlanga, 2017). For example, Khanyile et al. (2020) indicated an increase in monounsaturated fatty acids, the sum of polyunsaturated fatty acids and the sum of Omega – 3 polyunsaturated from pigs fed on *Vachellia* diets. The inclusion of olive cake influenced the content of monounsaturated and polyunsaturated fatty acids of pork muscle tissues (Liotta et al., 2019). The inclusion of olive cake in indigenous pig diets reduced the amount of saturated fatty acids while increasing the amount of monounsaturated fatty acids of muscle tissues (Hernández-Matamoros et al., 2011; Joven et al., 2014). Monounsaturated fatty acids are associated with the nutritional value index of pork, suggesting that increasing monounsaturated fatty acids also increases the nutritional value index of pork. The use of diets that are enriched with favourable fatty acids such as monounsaturated fatty acids, polyunsaturated fatty acids from oil cakes could improve pork quality and increase human health (Whetsell et al., 2003).

2.14 Summary

The interest of using non-conventional plant derived dietary sources is becoming popular due to their desirable fatty acids. Oil extraction companies are only interested in the oil of Amarula kernels due to its edible properties, therefore discarding the residue which is used when formulating Amarula oil cake diets. The use of non-conventional oil cakes from agricultural industries such as the biodiesel companies could benefit the pig sector and reduce reliance of imports, interdependence on farm enterprises, decrease the purchasing value of feeds and increases the spectrum of feed ingredients particularly for pigs. The use of Amarula oil cake is expected to improve growth, carcass characteristics and nutritional value of pork by altering the composition of fatty acids of muscle tissues. Therefore, the main objective of the study was to determine the optimum inclusion level of Amarula oil cake diets using growth indicators of Windsnyer pigs and determine the fatty acid profiles of Windsnyer pork.

2.15 References

Agbede, J.O. and Aletor, V.A., 2003. Evaluation of fish meal replaced with leaf protein concentrate from *Glyricidia* in diets for broiler-chicks: Effect on performance, muscle growth, haematology and serum metabolites. *International Journal of Poultry Science,* 2, 242 - 250.

Ahamefule, F.O., Eduok, G.O., Usman, A., Amaefule, K.U., Obua, B.E. and Oguike, S.A., 2006. Blood biochemistry and haematology of weaner rabbits fed sundried, ensiled and fermented cassava peel based diets. *Pakistan Journal of Nutrition* 5, 248 - 253.

Akinmutimi, A.H., 2004. Evaluation of sword bean (*Canavalia gladiata*) as an alternative feed resource for broiler chickens. Doctoral Thesis, Department of Non-ruminant Animal Production, Michael Okpara University of Agriculture, Nigeria.

Balasubramanian, S., Allen, J.D., Kanitkar, A., and Boldor, D., 2011. Oil extraction from *Scenedesmus obliquus* using a continuous microwave system – design, optimization, and quality characterization. *Bioresource Technology*, 102, 3396 - 3403.

Belitz, H.D., Grosch, W., and Schieberle, P., 2009. Edible fats and oils Food Chemistry, 4th Ed., Springer Verlag Berlin Heidelberg, 640 - 669.

Brookes, G., 2001. The EU animal feed sector: protein ingredient use and the implications of the ban on use of meat and bonemeal. Canterbury, UK: Brookes West.

Cesar, A.S.M., Silveira, A.C.P., Freitas, P.F.A., Guimaraes, E.C., Batista, D.F.A., Torido, L.C., Meirelles, F.V. and Antunes, R.C., 2010. Influence of Chinese breeds on pork quality of commercial pig lines. *Genetics and Molecular Research*, 9, 727 - 733.

Chen, H.Y,, Lewis, A.J,, Miller, P.S. and Yen, J.Y .,1999. The effect of excess protein on growth performance and protein metabolism of finishing barrows and gilts. *Journal of Animal Science*, 77, 3238 – 3247.

Chiduwa, G., Chimonyo, M., Halimani, T.E., Chisambara, S.R. and Dzama, K., 2008. Herd dynamics and contribution of indigenous pigs to the livelihoods of rural farmers in a semi-arid area of Zimbabwe. *Tropical Animal Health and Production*, 40, 125 - 136.

Chimonyo, M., Dzama, K. and Mapiye, C., 2010. Growth performance and carcass characteristics of indigenous Mukota pigs of Zimbambwe, *Tropical Animal Health and Production*, 42, 1001 - 1007.

Chivandi, E., Mukonowenzou, N., Nyakudya, T. and Erlwanger, K.H., 2015. Potential of indigenous fruit-bearing trees to curb malnutrition, improve household food security, income and community health in Sub-Saharan Africa: A review. *Food Research International*, 76, 980 - 985.

Coates, PK., 1990. Trees of South African.

Department of Agriculture, Forestry and Fisheries (DAFF), 2010. Marula Production.

Dunnuck, J. (1991). NTP technical report on the toxicity studies of n-Hexane in B6C3F1 Mice (Inhalation Studies)(CAS No. 110-54-3). Toxicity Report Series, 2, 1 - 32.

Enser, M., Richardson, R.I., Wood, J.D., Gill, B.P., Sheard, P.R., 2000. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Science*. 55, 201 – 212.

FAOSTAT, 2012. Food and Agricultural Organization of the United Nations, Rome, Italy. http://faostat.fao.org/site/569/default.aspx.

Febrianto, N., Abdullah, W. and Yang, T., 2012. Effect of fermentation time and roasting process on the antioxidant properties of rambutan (*Nephelium lappaceum*) seed fat. *Archives Des Sciences*, 65, 694 - 702.

Forman, D., 1999. Meat and Cancer: a relation in search of mechanism. Lancet, 353, 686 – 687.

Ghosh, S.M.K., 2020. Effect of different processing techniques on nutrient and anti-nutrient compositions of plant feedstuffs for their probable use as aqua-feed ingredients. *Journal of the Inland Fisheries Society of India*, 52, 173 - 182.

Glew, R., VanderJagt, D., Huang, Y., Chuang, L., Bosse, R., Glew, R., 2004. Nutritional analysis of the edible pit of *Sclerocarya birrea* in the Republic of Niger (*daniya, Hausa*). *Journal of Food Composition and Analyses*, 17, 99 – 111.

Halimani, T.E., Muchadeyi, F.C., Chimonyo, M. and Dzama, K., 2010. Pig genetic resource conservation, The Southern African perspective, *Ecology Economy*, 69, 944 - 951.

Hernández-Matamoros, A., Paniagua, B.M., Izquierdo, C.M., Tejeda, S.J., González-Sanchez, E. Use of olive cake and tomato peel in the Iberian pig feed. In Proceedings of the XIV Jornadas sobre Producción Animal, Zaragoza, Spain, 17–18 May 2011.

Hlongwana, F.Y., Thabethe, F., Thomas, R.S. and Chimonyo, M., 2021. Nitrogen balance in slow-growing Windsnyer pigs fed on incremental levels of Amarula (*Sclerocarya birrea subsp. caffra*) nut cake. *Tropical Animal Health and Production*, 53, 1 - 12.

Holness, D.H., 1991. The tropical agriculturist. Pigs. The tropical agriculturist. Pigs.

Jama, B.A., Mohamed, A.M., Mulatya, J. and Njui, A.N., 2008. Comparing the “Big Five”: A framework for the sustainable management of indigenous fruit trees in the drylands of East and Central Africa. *Ecological Indicators*, 8, 170 - 179.

Jansman, A.J.M., Verstegen, M.W.A. and Huisman, J., 1993. Effects of dietary inclusion of hulls of faba beans (*Vicia faba L*) with a low and high content of condensed tannins on digestion and some physiological parameters in piglets. *Animal Feed Science and Technology*, 43, 239 - 257.

Jensen, C., Lauridsen, C. and Bertelsen, G., 1998. Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends in Food Science and Technology,* 9, 62 - 72.I

Jiang, Y.Z., Zhu, L., Li, X.W. and Si, T., 2011. Evaluation of the Chinese indigenous pig breed Dahe and crossbred Dawu for growth and carcass characteristics, organ weight, meat quality and intramuscular fatty acid and amino acid composition. *Animal*, 5, 1485 - 1492.

Jones, G.F., 1998. Genetic aspects of domestication, common breeds and their origin. The Genetics of the Pig. 17 - 50.

Joven, M., Pintos, E., Latorre, M.A., Suárez-Belloch, J., Guada, J.A., Fondevilla, M., 2014. Effect of replacing barley by increasing levels of olive cake in the diet of finishing pigs: Growth performances, digestibility, carcass, meat and fat quality. *Animal Feed Science and Technology*, 197, 185 – 193.

Kanengoni, A.T., Chimonyo, M., Erlwanger, K.H., Ndimba, B.K. and Dzama, K., 2014. Growth performance, blood metabolic responses, and carcass characteristics of grower and finisher South African Windsnyer-type indigenous and Large

White × Landrace crossbred pigs fed diets containing ensiled corncobs, *Journal of Animal Science*, 92, 5739 – 5748.

Kassahun, A., Waidbacher, H. and Zollitsch, W., 2012. Proximate composition of selected potential feedstuffs for small-scale aquaculture in Ethiopia. *Livestock Research for Rural Development,* 24, 1 - 118.

Khoo, H., Sharratt, P., Das, P., Balasubramanian, R., Naraharisetti, P., & Shaik, S. (2011). Life cycle energy and CO^2 analysis of microalgae-to-biodiesel: preliminary results and comparisons. *Bioresource Technology*, 102, 5800 - 5807.

Kudair, I.M., and Al-Hussary, N.A. J., 2010. Effect of vaccination on some biochemical parameters in broiler chickens. *Iraqi Journal of Veterinary Sciences* 24, 59 - 64.

Kuna, A., Achinna, P., 2013. Monounsaturated fatty acids for CVD and diabetes: A healthy choice. A healthy choice. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 3, 236.

Latimer, K.S., Mahaffey, E.A., Prasse, K.W., 2003. Clinical Pathology, 4th ed. Duncan and Prasse’s veterinary laboratory medicine, Wiley-Blackwell. Lee, S.H., Shinde.

Lekule, F.P., Jørgensen, H., Fernández, J. and Just, A., 1990. Nutritive value of some tropical feedstuffs for pigs. Chemical composition, digestibility and metabolizable energy content. *Animal Feed Science and Technology*, 28, 91-101.

Len, N.T., Lindberg, J.E. and Ogle, B., 2007. Digestibility and nitrogen retention of diets containing different levels of fibre in local (Mong Cai), F1 (Mong Cai Yorkshire) and exotic (Landrace Yorkshire) growing pigs in Vietnam. *Journal of Animal Physiology and Animal Nutrition,* 91, 297 – 303.

Li, Y., Li, J., Zhang, L., et al. (2015). Effects of dietary energy sources on post mortem glycolysis, meat quality and muscle fibre type transformation of finishing pigs. *PloS one*, 10(6), e0131958.

Liotta, L., Chiofalo, V., Lo Presti, V. and Chiofalo, B., 2019. In vivo performances, carcass traits, and meat quality of pigs fed olive cake processing waste. *Animals*, 9, p.1155.

Mabena, P.M., Ratsaka, M.M., Nkukwana, T.T., Malebana, I.M.M, Nkosi, B.D., 2021. Growth performance, nutrient digestibility and carcass characteristics of pigs fed diets containing amarula (*Sclerocarya birrea A. Rich*) nut cake as replacement to soybean meal. *Tropical Animal Health and Production*, 54, 1 – 10.

Madzimure, J., Chimonyo, M., Hugo, A., Bakare, A.G., Katiyatiya, C.L.F. and Muchenje, V., 2017. Physico-chemical quality attributes and fatty acid profiles of pork from Windsnyer and Large White gilts. *South African Journal of Animal Science*, 47, 107 - 114.

Madzimure, J., Chimonyo, M., Zander, K.K. and Dzama, K., 2012. Potential for using indigenous pigs in subsistence-oriented and market-oriented small-scale farming systems of Southern Africa. *Tropical Animal Health and Production*, 45, 135 - 142.

Makkar, H.P.S., 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin rich feeds. *Small Ruminant Research*, 49, 241 - 256.

Malebana, I.M., Nkosi, B.D., Erlwanger, K.H. and Chivandi, E., 2018. A comparison of the proximate, fibre, mineral content, amino acid and the fatty acid profile of

Marula (*Sclerocarya birrea caffra*) nut and soyabean (Glycine max) meals. *Journal of the Science of Food and Agriculture*, *98*, 1381 - 1387.

Marghazani, I.B., Jabbar, M.A., Pasha, T.N. and Abdullah, M., 2013. Ruminal degradability characteristics in vegetable protein sources of Pakistan. *Journal of Animal and Plant Science*, *23*, 1578 - 1582.

Mariod, A.A. and Abdelwahab, S.I., 2012. *Sclerocarya birrea* (Marula), an African tree of nutritional and medicinal uses: a review. *Food Reviews International*, 28, 375 - 388.

Mazizi, B.E., Moyo, D., Erlwanger, K.H. and Chivandi, E., 2019. Effects of dietary *Sclerocarya birrea caffra* (Marula) nut meal on the growth performance and viscera macro morphometry of broiler Japanese quail. *Journal of Applied Poultry Research*, 28, 1028 - 1038.

Messad, F., Létourneau-Montminy, M.P., Charbonneau, E., Sauvant, D. and Guay, F., 2016. Metaanalysis of the amino acid digestibility of oilseed meal in growing pigs. *Animal*, 10, 1635 - 1644.

Miao, Z.G., Wang, L.J., Xu, Z.R., Huang, J.F. and Wang, Y.R., 2009. Developmental changes of carcass composition, meat quality and organs in the Jinhua pig and Landrace. *Animal,* 3, 468 – 473.

Mlambo, V., Dlamini, B.J., Ngwenya, M.D., Mhazo, N., Beyene, S.T. and Sikosana, J.L.N., 2011b. In *sacco* and in *vivo* evaluation of marula (*Sclerocarya birrea*) seed cake as a protein source in commercial cattle fattening diets. *Livestock Research for Rural Development,* 23, 1-10.

Mlambo, V., Dlamini, B.J., Nkambule, M.T., Mhazo, N. and Sikosana, J.L.N., 2011a. Nutritional evaluation of marula (*Sclerocarya birrea*) seed cake as a protein supplement for goats fed grass hay. *Tropical Agriculture*, 41, 010035 - 010009.

Mokgolodi, N.C., Ding, Y.F., Setshogo, M.P., Ma, C. and Liu, Y.J., 2011. The importance of an indigenous tree to southern African communities with specific relevance to its domestication and commercialization: a case of the marula tree. *Forestry Studies in China*, 13, 36 - 44.

Mthiyane, D.M.N. and Hugo, A., 2019. Comparative health-related fatty acid profiles, atherogenicity and desaturase indices of marula seed cake products from South Africa and Eswatini.

Mthiyane, D.M.N. and Mhlanga, B.S., 2017. The nutritive value of marula (*Sclerocarya birrea)* seed cake for broiler chickens: nutritional composition, performance, carcass characteristics and oxidative and mycotoxin status. *Tropical Animal Health and Production*, 49, 835 - 842.

Mutshinyalo T., Tshisevhe, J., 2003. *Sclerocarya birrea (A.Rich.) Hochst. Subsp. Caffra (Sond*.) Kokwaro. Pretoria National Botanical Garden.

Ncobela, C.N., Kanengoni, A.T. and Chimonyo, M., 2018. Response in nutritionally related blood metabolites, carcass traits and primal pork cuts of slow growing Windsnyer pigs fed on varying levels of potato hash silage. *South African Journal of Animal Science*, 48, 770 - 776.

Ndindana, W., Dzama, K., Ndiweni, P.N.B., Maswaure, S.M. and Chimonyo, M., 2002. Digestibility of high fibre diets and performance of growing Zimbabwean indigenous Mukota pigs and exotic Large White pigs fed maize based diets with graded levels of maize cobs. *Animal Feed Science and Technology*, 97, 199 - 208.

Nevrkla, P., Kapelański, W., Václavková, E., Hadaš, Z., Cebulska, A. and Horký, P., 2017. Meat quality and fatty acid profile of pork and backfat from an indigenous breed and a commercial hybrid of pigs. *Annals of Animal Science*, 17, 1215 - 1227.

Nghitoolwa, E., Hall, J.B. and Sinclair, F.L., 2003. Population status and gender imbalance of the marula tree, *Sclerocarya birrea subsp. caffra* in northern Namibia. *Agroforestry Systems*, 59, 289 - 294.

Nwabuebo, A.T., 2017. The effect of extraction methods on the oxidatives stability of marula and moringa seed oil (Doctoral dissertation).

Ogbobe, O., 1992. Physico-chemical composition and characterisation of the seed and seed oil of *Sclerocarya birrea*. *Plant Foods for Human Nutrition*, 42, 201 – 206.

Qwele, K. (2012). Antioxidant activity and the quality of meat from goats and broilers supplemented with Moringa (Moringa oleifera) leaves. M.Sc. Thesis. University of Fort Hare, Republic of South Africa.

Reverchon, E., and De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. *The Journal of Supercritical Fluids*, 38, 146 -166.

Shackleton, S.E., Shackleton, C.M., Cunningham, T., Lombard, C., Sullivan, C.A. and Netshiluvhi, T.R., 2002. Knowledge on *Sclerocarya Birrea subsp. caffra* with emphasis on its importance as a non-timber forest product in south and southern Mrica: a summary: part 1: taxonomy, ecology and role in rural livelihoods. *The Southern African Forestry Journal*, 194, 27 - 41.

Shipton, T. A. and Hecht, T. (2005). A review of the animal and aquafeed industries in South Africa. In: Moehl, J. and Halwart, M.A. ed. Synthesis of the formulated animal and aquafeed industry in Sub-Saharan Africa, Rome: Food and Agricultural Organization of the United States (FAO).

Shone A.K. 1979. Notes on the marula. Dept of Water Affairs & Forestry Bulletin 58: 1-89.

Silanikove, N., Perevolotsky, A. and Provenza, D. F., 2001. Use of tannin-binding chemicals to assay for tannins and their negative post-ingestive effects in ruminants. *Animal Feed Science and Technology*, 91, 69 - 81.

Stür, W.W., Gray, D. and Bastin, G., 2002. Review of the livestock sector in the Lao People's Democratic Republic.

Taseski, N., 2015. Supercritical fluid extraction of *Sclerocarya birrea* kernel oil (Doctoral dissertation).

Thabethe, F., Hlatini, V.A., de Almeida, A.M. and Chimonyo, M., 2022. Growth performance of South African *Windsnyer* pigs to the dietary inclusion of Amarula oil cake. *Tropical Animal Health and Production*, 54,1 - 9.

Van Ryssen JBJ., 2001. Poultry litter as a feedstuff for ruminants: A South African scene. *South African Journal of Animal Science*, 2, 1 – 8.

Wang, H., Chen, Y.I., Liu, H., Liu, J., Makkar, H.P. and Becker, K., 2011. Effects of replacing soybean meal by detoxified *Jatropha curcas* kernel meal in the diet of growing pigs on their growth, serum biochemical parameters and visceral organs. *Animal Feed Science and Technology*, 170, 141 - 146.

Wei, A. and Shibamoto, T., 2010. Medicinal activities of essential oils: role in disease prevention. In Bioactive foods in promoting health (pp. 59-70). Academic Press.

Whetsell, M., Raybun, E. and Dossier, J. D., 2003. Human health effects of fatty acids in beef. Pasture-Based Beef Systems for Appalachia Project, Extension Service. West Virginia University, Morgantown, Virginia, USA.

Whittemore, C. and Kyriazakis, I., 2006. Growth and body composition changes in pigs. Whittemore's Science and Practice of Pig Production, 65 - 103.

Widiyastuti, T., Sutardi, T.R. and Setyobudi, R.H., 2015. Evaluation of protein concentrate from Jatropha seed cake as a soybean meal substitution in the rabbit feed. *Energy Procedia,* 65, 362 - 367.

Wood, J. D. and Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, 78, 49 - 60.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I. and Whittington, F.M., 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343 - 358.

Woyengo, T.A., Beltranena, E. and Zijlstra, R.T., 2017. Effect of anti-nutritional factors of oilseed co-products on feed intake of pigs and poultry. *Animal Feed Science and Technology*, 233, 76 - 86.

Wynberg, R., Cribbins, J., Leakey, R., Lombard, C., Mander, M., Shackleton, S., and Sullivan, C., 2002. Knowledge on *Sclerocarya birrea subsp. caffra* with emphasis on its importance as a non-timber forest product in South and southern Africa: A Summary Part 2: Commercial use, tenure and policy, domestication, intellectual property rights and benefit-sharing. *Southern African Forestry Journal*, 196, 67 - 78.

Zhu, X., Ruusunen, M., Gusella, M., Zhou, G., Puolanne, E. 2011. High post-mortem temperature combined with rapid glycolysis induces phosphorylase denaturation and produces pale and exudative characteristics in broiler Pectoralis major muscles. *Meat Science*, 89, 181 - 188.

CHAPTER 3: Growth performance of South African Windsnyer pigs to the dietary inclusion of Amarula oil cake

(Published in Tropical Animal Health and Production: Appendix. 1)

Abstract

Dietary inclusion of Amarula oil cake (AOC) in pig diets can ease pressure of relying on non-native feed sources and benefit the pig industry. The study was conducted to determine the growth performance of Windsnyer pigs fed increasing levels of AOC. Twenty-five growing male boars with an initial body weight of 19.92 ± 8.74 kg were used in the study that lasted six weeks. All pigs were allocated to diets in a completely randomised design. Five experimental diets were formulated to contain 0, 50, 100, 150 and 200 g/kg DM of AOC. Average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR), scaled average daily feed intake (SADFI), scaled average daily gain (SADG) and body weight (BW) were calculated weekly. The diet affected ADFI, ADG, FCR and SADG ($P < 0.05$). Scaled average daily feed intake was not affected by the diet ($P > 0.05$). There was a significant interaction between AOC inclusion level and weeks of successive feeding on ADFI, ADG and FCR ($P < 0.05$). Age of pigs decreased FCR quadratically ($P < 0.001$). A quadratic relationship between ADFI and increasing levels of AOC was observed ($P < 0.05$). Average daily gain, FCR and SADG decreased linearly with increasing AOC levels ($P < 0.05$). Using the broken stick analyses, the maximum inclusion level of AOC was attained at 102.17 g/kg DM. Amarula oil cake can be incorporated in diets of Windsnyer pigs up to 100 g/kg DM without constraining growth performance.

Key words; Amarula oil cake; Windsnyer pigs; Feed intake; Neutral detergent fibre; Maximum inclusion level.

3.1 Introduction

The global production animal feed industry has experienced soaring prices of cereal grains and soybean meal commonly used in diets as competition with the ethanol industry and human consumption which is expected to double by 2050 (Van Huis 2013; Martens et al., 2014). Large-scale livestock producers, particularly pig and poultry, rely heavily on soybean meal as a protein source due to its high nutritional value (Kocher et al., 2002). The demand for soybean meal in South Africa exceeds its supply (DAFF, 2012), thus forcing importation. On the contrary, smallholder pig farmers in South Africa rely on cereal by-products and oil seed meals to improve the quality of imbalanced diets for pigs. This places the pig production sector under severe pressure in order to sustain current levels of demand for pork production. Furthermore, and with particular relevance to tropical countries, most of such protein sources have to be imported, something very costly and that uses hard currency. Finally, such imports increase substantially Greenhouse Gas Emissions and have high transport costs. Therefore, to reduce feed costs, alternative solutions through the use of alternative protein sources of local origin are required. These include microalgae, insects and by-products of the food industry (Kim et al., 2019).

Local pigs are prevalent in several African countries (Halimani et al., 2010). Examples are Busia pigs in Kenya, Mukota pigs in Zimbabwe and Mozambique, West African Dwarf pigs found in several countries of West Africa and Ashanti Dwarf pigs in Ghana (Mujibi et al., 2018). The Kolbroek and Windsnyer pigs are common in South Africa, Swaziland, Lesotho, Malawi and Zimbabwe with significant introgression of genes from imported fast-growing pig breeds into the local pigs has also been reported

(Halimani et al., 2010). Local pig breeds are closely related to Asian pig breeds (Chiduwa et al., 2008; Ramirez et al., 2009).

Small-scale farmers integrate indigenous pigs with cropping systems, increasing agricultural outputs with minimum input. Indigenous pigs are not fully characterised and are rarely used in structured breeding programmes or intensive pig production farms (Hoffmann, 2010). Indeed, slow growing indigenous pigs are rejected in mainstream economies in Africa, yet they have the potential of alleviating poverty in rural communities and also contribute positively to human nutrition, particularly in local populations in small scale subsistence farming systems (Halimani et al., 2012). Factors such as traditional biases, lack of structured breeding programs that focus on improving indigenous pigs, lack of organised markets and as well as research gap contribute to such rejection (Halimani et al., 2010; Madzimure et al., 2017). Nevertheless, it is noteworthy to mention that indigenous pigs have high thermotolerance, low nutritional requirements, and tolerance to parasites and overall may benefit resource-limited small-scale farmers (Wilson, 2009; Madzimure et al., 2017). They can meet their nutrient requirements from local available and unconventional feedstuffs such as kitchen waste, rotten maize, coarse maize meal, maize cobs and husks, pumpkins, watermelons, ground shells, fruits, grasses, and brewer’s waste (Ncobela et al., 2018).

Alternative cost-effective and locally available feedstuffs can easily meet their protein requirements. Amarula (*Sclerocarya birrea subsp. Caffr*) oil cake (AOC) is a by-product resulting from oil extraction of Amarula kernels (Shackleton, 2002; Mlambo et al., 2011a). Amarula fruits are collected for brewing and the fruit pulp is used to make traditional liquor. The kernel oil is also used by the cosmetic industry. The remaining residue is then used as an alternative feedstuff (Mlambo et al., 2011a). The AOC have high crude protein (390 g/kg DM), gross energy of 29 MJ/kg DM and amino acids contents, comparable to those of soybean meal, apart for lysine. In fact, and regarding such nutrients they match or exceed those required by monogastric animals (Mlambo et al., 2011a; Mthiyane and Mhlanga, 2017; Malebana et al., 2018). Additionally, AOC was reported to improve growth performance of Japanese quail (Mazizi et al., 2019). The exceptional energy content and good amino acid profile of AOC is likely to improve the production of local pigs like Windsnyer pigs.

Different parts of the Amarula tree are used by local people in treating against gastroenteritis, diabetes and high blood pressure (Ouédraogo et al., 2013), suggesting that the tree contains phytochemical that protect it against pathogenic microorganisms. These phytochemicals, however, may restrict voluntary feed intake and feed efficiency of indigenous pigs (Hlongwana et al., 2021; Mabena et al., 2022). The maximum inclusion level of AOC that does not restrict growth performances should be determined to assist pig nutritionists when formulating pig diets. The objective of this study was to determine the maximum inclusion level of Amarula oil cake diets based on growth performance parameters of South African Windsnyer pigs.

3.2 Materials and methods

3.2.1 Study site

The study was conducted at the Agricultural Research Council (ARC), Animal Production Institute (Pig Production Unit) in Pretoria (Gauteng, South Africa), located at 25°34′0″S and 28°22′0″E and 1526 m above sea level.

3.2.2 Collection and preparation of Amarula oil cake

The oil cake was purchased from African Exotic oils, Limpopo Province, South Africa. Before oil extraction from nuts or kernels at the processing plant, Amarula nuts were air dried to 3 % moisture content, cold-pressed at between 6.1 and 9.5 kg/h and 35 - 40 min rotations at 55 - 60 ^{0}C according to Mabena et al. (2022) and then transported at room temperature to the Agricultural Research Council pending diet formulation.

3.2.3 Pigs, experimental design and nutritional management

Twenty-five South African Windsnyer male pigs (SAWIP) aged about 67 days with an initial body weight of 19.9 ± 8.74 kg (mean ± standard deviation), were randomly selected from the Agricultural Research Council, Irene as growers and used for the study which lasted for six weeks excluding the adaptation period of one week. Prior to the arrival of the pigs to the research facility, the house was cleaned, disinfected and rested for a week to prevent any build-up of pathogenic micro-organisms. Pigs were reared in a 1.5 by 0.9 m pens in environmentally controlled houses with temperature ranging from 22 to 25°C. Pigs were housed individually in a completely randomized block design and were blocked by body weight with five replicates of pigs assigned to each experimental diet. Feeders were checked and adjusted at least twice a day to ensure constant access to feed, hence reducing possible wastage. Clean water was offered *ad libitum* to pigs through nipple drinkers which were also checked daily.

3.2.4 Experimental diets

Two diets, a control and bulky diet were formulated. The diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg, and 11.6 g lysine/kg, which met the nutritional requirements of growing pigs (National Research

Council 2012). The control diet was used as a summit diet and the bulky diet containing 200 g/kg of AOC was used as a diluent diet as shown in Table 3.1. The diets were formulated by mixing a summit diet (0 g/kg DM inclusion level of AOC) with a dilution diet (200 g/kg DM AOC) according to Gous and Morris (1985). A sequence and series of diets namely 50, 100 and 150 g/kg DM of AOC were derived from blending the summit with the dilution diet in different proportions. The blending ratios were as follows, 100:0, 75:25, 50:50, 25:75, and 0:100, respectively. Then five experimental diets were made which included the summit and dilution diets.

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

Ingredient	Summit diet	Dilution diet
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 oil cake	-	200
Soybean oil cake	88.2	-
#Mineral and vitamin premix	2	2
Total	1000	1000

Provides (/kg DM of diet): vitamin A Acetate, 2 000 000 IU; vitamin D3, 400 000 IU; vitamin E, 1 000 mg; vitamin B1, 200 mg; vitamin B12, 0.5 mg; vitamin C, 1 000 mg; vitamin B2, 250 mg; vitamin B6, 200 mg; vitamin K3, 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

3.2.5 Chemical analyses of diets

The chemical composition of the diets was examined in triplicate at UKZN, Animal and Poultry Science laboratory. Prior to analysis, samples from each diet were milled to pass through a 1 mm sieve. By using the oven drying method, diets were dried for 24 hours at 60 ^{0}C to measure their dry matter (DM) content. Bomb calorimetry was used to determine the gross energy (GE) (MS-1000 modular calorimeter, Energy Instrumentation, Centurion, South Africa). The Soxhlet equipment was used to calculate the ether extract (EE) in accordance with Method 920.39 (AOAC, 1990). After the samples were burned at 550 °C for 4 hours, the amount of ash was calculated using method 990.05 of AOAC, (1990).

Nitrogen content was measured using the Dumas Combustion method in a Leco Truspec Nitrogen Analyzer (St. Joseph, MI, USA) by method 990.3 (AOAC, 1990). Crude protein (CP) content was estimated using the formula: N x 6.25, (AOAC, 1990). According to a method developed by Van Soest et al. (1991), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured. According to Folch et al. (1957), the fatty acids in Amarula kernels and diets were identified. According to the water displacement method outlined by Kyriazakis and Emmans (1995), Canibe and Bach Knudsen (2002), and Whittemore et al. (2003), respectively, the bulk density, swelling, and water holding capacity of the diets were assessed. Samples were ashed overnight at 550 ^{0}C, dissolved in 1M HCL standard solution, and then analysed using Varian 720 Inductively Coupled Plasma Emission Spectrometer (ICP- OES, Frankfurt, Germany) with an atomic absorption detector to determine the minerals (Table 3.2). According to AOAC (1990) method 982.30, amino acids were hydrolysed in acid and then analysed using an amino acid analyser (SY-KAM, Erising, Germany) (Table 3.3).

Table 3.2: Nutrient composition of AOC and experimental diets

Component	AOC	AOC inclusion level (g/kg DM)				
		0	50	100	150	200
Dry matter (g/kg)	963.50	956.80	958.90	954.40	953.80	956.30
Ash (g/kg)	49.80	51.20	50.60	46.90	47.20	47.00
Crude protein (g/kg)	362.20	194.90	176.80	162.60	157.00	140.30
Ether extracts (g/kg)	343.90	44.40	56.00	72.40	78.10	96.80
GE (MJ/kg DM)	25.34	17.65	17.67	17.77	18.09	18.50
NDF (g/kg)	357.30	305.90	312.30	327.50	341.30	353.00
ADF (g/kg)	245.60	85.80	93.40	99.50	111.20	121.10
ADL (g/kg)	114.30	19.40	25.80	34.70	42.90	48.60
Bulk density (g/ml)	1.66	1.45	1.49	1.55	1.64	1.71
SC (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
Minerals						
Calcium (g/kg)	1.80	7.1	7.9	8.3	8.3	8.2
Magnesium (g/kg)	5.70	2.4	2.2	2.1	2.1	2.0
Phosphorus (g/kg)	9.40	6.3	5.5	5.5	5.5	5.1
Potassium (g/kg)	8.30	7.90	6.6	6.2	5.8	5.1
Sodium (mg/kg)	345.70	243.2	255.4	239.8	233.8	227.3
Iron (mg/kg)	95.50	161.5	169.8	149.5	143.3	135.5
Copper (mg/kg)	27.90	73.3	58.4	58.2	60.2	59.8
Manganese (mg/kg)	10.20	84.9	73.8	70.2	84.6	63.0
Zinc (mg/kg)	60.20	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
Calculated nutrients						
Dry matter (g/kg)	-	860.4	-	-	-	896.6
Crude protein (g/kg)	-	180.0	-	-	-	180.0
Ether extracts (g/kg)	-	63.2	-	-	-	87.6
Crude fibre (g/kg)	-	36.8	-	-	-	62.5
DE (MJ/kg DM)	-	13.80	-	-	-	13.80
Calcium (g/kg)	-	8.00	-	-	-	10.0
Phosphorus (g/kg)	-	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, GE- gross energy, SC-swelling capacity

Table 3.3: Amino acid composition of AOC and experimental diets

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

3.2.6 Growth performance measurements

Pigs were weighed before the experiment to determine their starting weight, and once per week throughout the experiment to determine their weekly weight. Prior to feeding, the pigs were weighed every Monday at 0700 h. The average daily feed intake was calculated by deducting leftovers and spillages from the offered feed. Pigs were fed *ad libitum* every morning between 0800 and 0900 h. In order to account for any potential spillages of feed and reduce feed intake variance, trays were placed beneath each feeder. The average daily gain (ADG), which was determined using weekly body weights, was calculated as final weekly weight minus initial weight divided by seven. The FCR was calculated by dividing ADG with ADFI. Body weight was used to scale the ADFI and ADG. Scaled average daily feed intake (SADFI) and scaled average daily growth (SADG) were computed as grams of feed consumed per kilogram of body weight per day and grams of weight gained per kilogram of body weight per day, respectively.

3.2.7 Statistical analyses

Data for the effect of AOC inclusion level, week and their interaction on ADFI, ADG, FCR, SADFI, SADG and BW were analysed using the PROC MIXED procedure of SAS, (2008) which accounted for repeated measures and the pig was used as an experimental unit. The LS means statement in SAS, (2008) was used to compare the least square means using the probability difference (PDIFF) option. The first-order autoregressive correlation (AR [1]) was fitted to a model. The initial body weight of pigs was included in the model as a covariate. The dose related responses to inclusion level of AOC against ADFI, ADG, FCR, BW, SADFI and SADG were modelled by the following quadratic equation from the response surface model (PROC RSREG) of SAS (2008).

The model used was:

$Y = a + \beta_1 x + \beta_2 x^2$

Where: Y is the response variables (ADFI, ADG, FCR, SADFI, SADG and BW)

β_1, β_2 regression coefficients

x is the inclusion level of AOC

a is the intercept

$-\beta_1/2\beta_2$ = AOC inclusion level for optimal response

The quadratic model was inserted to the experimental data using the piecewise regression (broken stick) of the NLIN procedure of SAS (SAS 2008). The broken-stick model of regression was only fitted on the average daily feed intake since it responded quadratically to incremental levels of AOC and other growth parameters were omitted due to the linear response shown. The level of statistical significance was set at $P < 0.05$.

3.3 Results

3.3.1 Significance levels for fixed factors and their interactions

The significance levels of the effect of AOC inclusion level on growth performance parameters of Windsnyer pigs is shown in Table 3.4. The diet affected the ADFI, ADG, FCR and SADG of pigs ($P < 0.05$). Scaled average daily feed intake and BW of pigs were not affected by the diet ($P > 0.05$). There was an interaction between AOC inclusion level and week of feeding ADFI, ADG and FCR ($P < 0.05$).

3.3.2 Growth performance of pigs to increasing levels of Amarula oil cake inclusion

Table 3.5 shows the response in growth performance of Windsnyer pigs fed on incremental levels of AOC based diets. The relationship between ADFI and increasing levels of AOC in pigs was quadratic ($P < 0.05$). As the inclusion of Amarula oil cake increased in pig diet, the ADFI increased at a decreasing rate ($P < 0.05$). Average daily gain, FCR and scaled average daily gain decreased linearly with increasing levels of AOC ($P < 0.05$). The linear equations of ADG, FCR and SADG were as follows, $y = -13.31x + 15.82$, $y = -5.10x + 13.39$, $y = -0.11x + 13.39$, respectively. There was no relationship between incremental inclusion level of AOC with BW and SADFI of pigs ($P > 0.05$). Figure 3.1 illustrates the ADFI of pigs fed on increasing levels of AOC.

3.2.3 Relationship between week of feeding and growth performance of pigs

The relationship between the successive week of feeding and growth performance of pigs fed on AOC based diet is shown in Table 3.6. Average daily feed intake and SADFI of pigs fed on AOC based diet were not affected by weeks of feeding ($P > 0.05$). Average daily gain and SADG increased linearly with successive weeks feeding in pigs ($P < 0.001$). As successive weeks feeding progressed, ADG and SADG continued to increase until the last week of feeding. Weeks of feeding decreased FCR of pigs quadratically ($P < 0.001$). The FCR was 0.22 on week 1 of feeding and 0.61 on week 5 and 6, respectively. The quadratic relationship suggests that further increase in weeks of feeding decreases FCR of pigs. There was a linear increase between successive weeks of feeding and body weight of pigs fed on incremental levels of AOC diets (P < 0.001). Average daily feed intake was not related to successive weeks of feeding (P > 0.05).

Table 3.4: Levels of significance of average daily feed intake, average daily gain, feed conversion ratio, scaled average daily feed intake, scaled average daily gain and body weight of growing pigs fed on AOC

	Amarula oil cake level	Week	Amarula oil cake level x Week
Variable			
Average daily feed intake	**	***	**
Average daily gain	***	***	**
Feed conversion ratio	**	***	**
Scaled average daily feed intake	NS	***	NS
Scaled average daily gain	**	***	NS
Body weight	NS	***	NS

***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; NS, not significant ($p > 0.05$)

Table 3.5: Dietary influence of AOC inclusion level on growth performance responses of Windsnyer pigs

	Inclusion levels of Amarula oil cake (g/kg DM)						Sig-level		
Variables	0	50	100	150	200	SEM	Linear	Quadratic	R^2
ADFI (kg/day)	1.01	1.16	1.17	1.13	1.02	0.06	NS	**	0.06
ADG (kg/day)	0.51	0.64	0.50	0.41	0.31	0.04	***	NS	0.15
FCR	0.56	0.58	0.44	0.40	0.32	0.04	***	NS	0.14
SADFI	66.85	59.29	62.12	72.24	57.17	4.68	NS	NS	0.01
SADG	38.04	33.61	28.22	26.61	17.57	3.45	***	NS	0.11
BW (kg)	27.04	28.15	27.49	27.32	27.06	71.28	NS	NS	0.04

BW, body weight; ADFI, average daily feed intake; SADFI, scaled daily feed intake; ADG, average daily gain; SADG, scaled average daily gain; FCR, Feed conversion ratio; SEM, standard error of the mean; R^2, the coefficient of determination; NS, not significant; Sig.- Level of significance.

Table 3.6: Least square means for pig performance fed on Amarula based diets in a 6-week period

Variables	Weeks of successive feeding							Significance level		
	1	2	3	4	5	6	SEM	Linear	Quadratic	R^2
ADFI (kg/day)	1.01	1.29	1.09	1.11	1.09	1.11	0.063	0.889	0.985	0.00
ADG (kg/day)	0.19	0.41	0.45	0.54	0.64	0.66	0.029	<0.0001	0.0068	0.40
FCR	0.22	0.33	0.46	0.51	0.61	0.61	0.035	<0.0001	0.0002	0.36
SADFI (g/kg BW per day)	58.14	73.27	60.42	62.81	64.50	62.08	5.42	0.9224	0.8019	0.00
SADG (g/kg BW per day)	11.20	24.78	27.13	32.64	38.40	38.73	3.25	<0.0001	0.0104	0.24
BW (kg)	23.04	25.48	27.07	28.46	29.54	30.90	2.24	0.002	0.274	0.07

BW, body weight; ADFI, average daily feed intake; SADFI, scaled daily feed intake; ADG, average daily gain; SADG, scaled average daily gain; FCR, feed conversion ratio, R^2 ,coefficient of determination.

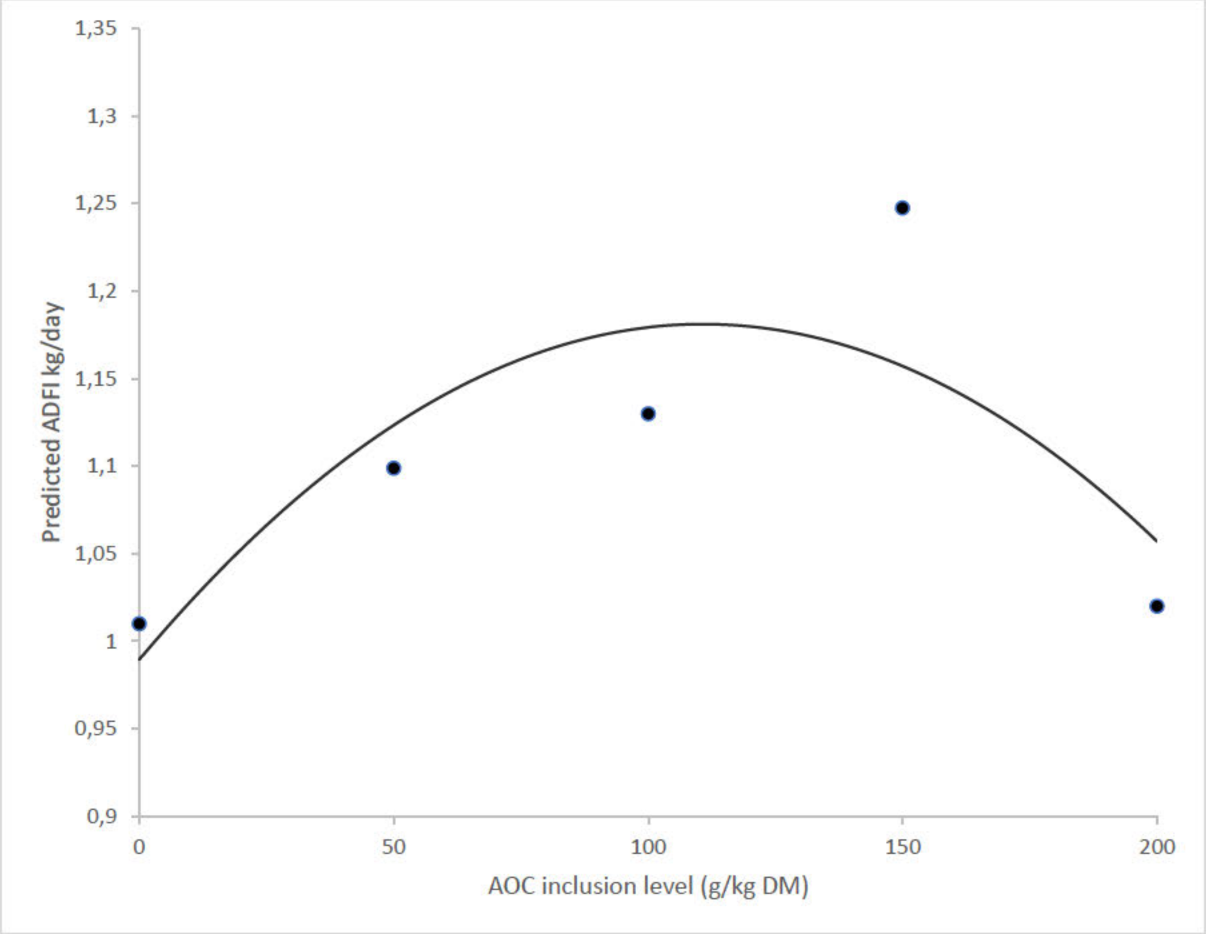


Figure 3.1: Predicted average daily feed intake of pigs fed on increasing levels of Amarula oil cake.

The broken-stick model of regression was only fitted on the ADFI since it responded quadratically to incremental levels of AOC and other growth parameters such as ADG, FCR, SADFI, SADG and BW were omitted from the broken stick model due to the linear response or relationship. By using the NLIN procedure (piecewise regression), the maximum level of AOC inclusion was obtained at X_0 = 102.17 g/kg DM and the optimum ADFI (plateau) was achieved at 1.25 kg/day ($P < 0.05$). Increasing the inclusion level of AOC beyond 102.2 g/kg caused no significant improvement in ADFI. The quadratic equation is $ADFI = 1.01 + 0.00235x – 0.000015x^2$.

3.4 Discussion

Slow-growing Windsnyer pigs are under the threat of extinction, and it is important to preserve them through sustainable use (Hlatini et al., 2020). The rather small sample size used in the current study resulted from the challenge of acquiring purebred male Windsnyer pigs at ARC breeding unit that were of the same age. For dose-response experiments, however, reliable inferences can be made from a reduced number of replicates (Kaps and Lamberson 2017).

Increasing inclusion levels of AOC increased the dry matter content, ether extract, gross energy, fibre characteristics such as NDF, ADF and ADL and the bulk properties of the diets. Crude protein content decreased with the inclusion level of AOC. The first limiting amino acids, phenylalanine, and other amino acids decreased with the inclusion level of AOC. The differences between crude protein values amongst the authors might be due to geographical areas where Amarula was harvested before oil extraction and also the techniques used by different companies during the extraction process of AOC (Mlambo et al., 2011a; Mdziniso et al., 2016). Even though the diets were formulated to be isocaloric and isoproteic, the crude protein content of diets decreased with the inclusion level of AOC. The reduction of crude protein with increasing AOC might be due to increasing fibre content of AOC which might have diluted the presence of other nutrients in the diets, therefore affecting growth performance. The ether extract of Amarula was high and it increased on the other diets with its inclusion level. Amarula kernels are reported to have high content of ether extract (Mlambo et al., 2011). The quadratic response in ADFI of Windsnyer pigs gives a clear indication that the oil cake has the potential of supplying energy that is required by pigs. The fibre characteristics and bulk properties of the oil cake also increased with the inclusion level of AOC. Both fibre characteristic and bulk properties are reported to negatively affect feed intake in mono-gastric animals (Lindberg, 2014). The decremental protein and lysine content of AOC might have contributed to the linear reduction of ADG of Windsnyer pigs.

The quadratic increase in ADFI with response to increasing levels of AOC agrees with earlier reports (Ndou et al., 2013; Joven et al., 2014; Ncobela et al., 2018; Hlongwana et al., 2021). Feed bulk also affect feed intake, the bulky characteristics of the diet also increased with the inclusion level of AOC, suggesting that Amarula is bulkier. The quadratic increase in ADFI suggest that feed intake increases up to a certain point and decrease afterwards. From the piecewise regression data, highest ADFI was reached at 102.07 g/kg DM of AOC and after that the ADFI started to decrease with increasing levels of AOC. The increase in feed intake of pigs before it started to decrease with increasing levels of AOC can be explained by the fact that pigs were eating to fulfil their requirements at low levels of the oil cake. When nutrient requirements are met, feed intake decreases. The increase in feed intake may also be related to the gross energy content of the experimental diets which were almost similar to those of Joven et al. (2014). When pigs are fed *ad libitum*, they eat to preserve a constant energy intake which controls the degree of feeding hence affecting feed intake of pigs as it has long been established (Cole et al., 1967). Mariod et al. (2004) showed a remarkable high oxidative stability of Marula kernels (43.7 h, expressed as induction period) compared to other common oil cakes such as linseed and groundnut (0.3 h and 10 h). The oxidation of AOC due to high fat content might have also resulted in the reduction of average daily feed intake at higher levels of the oil cake, which might have affected the palatability of pigs when eating and this also supports the two diets

(0 and 50 g/kg DM) that were preferred or mostly eaten at a high rate during data collection compared to the other diets. Another factor that could have contributed to poor palatability of the oil cake under *ad libitum* conditions could be the development of moulds on feed which is supported by the high amount of feed left recorded at high levels of AOC during data collection. Some pigs tend to refuse eating until fresh feed was introduced which can be assumed to have increased their motivation to eat. This suggests that constant checking of feed troughs is required during *ad libitum* feeding so that pigs can be fed according to the rate at which they eat.

A linear increase in average daily gain of Windsnyer pigs fed increasing levels of AOC was expected due to the fact that Windsnyer pigs are proven to have the ability of utilising high fibrous diets (Kanengoni et al., 2014). However, increasing levels of AOC reduced the average daily gain linearly, which also reduced the scaled average daily gain. This suggests that low inclusion level of the oil cake may improve the growth potential of pigs, but further increases may constrain it as shown by the linear decrease. Our results contradict those of Hlongwana et al. (2021) who reported a quadratic increase in average daily gain of Windsnyer pigs fed increasing level of AOC. The difference between the two studies can be explained by body weight differences as well as the large variation in body weight of pigs. Our pigs had a low initial body weight compared to those of Hlongwana et al. (2020) and this might have affected the ADFI of Windsnyer pigs. Pigs with high body weight consume more feed compared to those with low body weight. Our results corroborate those of Ncobela et al. (2018) who also reported a linear decrease in ADG of Windsnyer pigs fed fibrous rich diets. The decrease in ADG of pigs might also be explained by the fact that Windsnyer pigs accumulate more fat at an early stage of growth when fed energy rich diets (Chimonyo et al., 2010). The dietary fibre in feeds is known to reduce the supply of nutrients for growth and maintenance (Ndou et al., 2013). As AOC increased, the NDF, ADF and ADL also increased thus constraining the average daily gain of pigs at higher levels. Partly indigestible cell wall materials (lignin, non-starch polysaccharides) influence the digestion and absorption processes of nutrients along the gastrointestinal tract of pigs. Successive weeks of feeding linearly increased growth performance parameters of pigs. As the week of feeding increased, the body weight of Windsnyer pigs fed on AOC diets also increased due to the fact that the pigs were still growing. Animals increase their body weight with increasing time as they grow to achieve a mature size (Kyriazakis and Whittemore, 2008).

The response in FCR to increasing levels of AOC was similar to that of the ADG. The linear reduction in FCR to increasing levels of AOC was related to the decreasing ADG of pigs. Our results agree with those available in the literature (Ncobela et al., 2018; Khanyile et al., 2020; Mabena et al., 2022) who also reported a linear reduction in FCR of pigs. However, our results again contradicted Hlongwana et al. (2021) who reported a positive linear response of Windsnyer pigs fed increasing levels of AOC. The discrepancies between the two studies may be explained by age difference which is correlated to body weight of pigs and the size of gastro-intestinal tract which is responsible for digesting and absorption of nutrients. Younger pigs have lower feed intake and limited digestive capacity, hence affecting the FCR (Sloat et al., 1985). The efficacy of converting feed into body muscle depend on the body weight and age of the pig (Cromwell et al., 1993).

3.5 Conclusions

High levels of the oil cake linearly reduced ADG, FCR and SADG while increasing ADFI quadratically. The quadratic response between increasing level of AOC and ADFI was however negative suggesting that high inclusion levels are detrimental to growth performance of Windsnyer pigs which is also supported by linear reduction in ADG, FCR and SADG. Amarula oil cake inclusion level was optimal at 102.17 g/kg DM, suggesting that AOC can be included up to 100 g/kg DM without affecting growth parameters of indigenous pigs. When formulating AOC based diets for pigs, feed bulk, fat content and secondary compounds of Amarula kernels should be considered. Therefore, it is important to determine the response in nutritional-related metabolites and liver enzymes of Windsnyer pigs fed Amarula based diets.

3.6 References

Association of Officials Agricultural Chemists (AOAC), 1990. Official Methods of Analysis, 15th edition, AOAC International.

Canibe, N., and Bach Knudsen, K.E., 2002. Degradation and physicochemical changes of barley and pea fibre along the gastrointestinal tract of pigs. *Journal of the Science of Food and Agriculture*, 82, 27 - 39

Chimonyo, M. and Dzama, K., 2007. Estimation of genetic parameters for growth performance and carcass traits in Mukota pigs. *Animal*, 1, 317 - 323.

Chimonyo, M., Dzama, K. and Mapiye, C., 2010. Growth performance and carcass characteristics of indigenous Mukota pigs of Zimbabwe. *Tropical Animal Health and Production*, 42, 1001 - 1007.

Chiduwa, G., Chimonyo, M., Halimani, T.E., Chisambara, S.R. and Dzama, K., 2008. Herd dynamics and contribution of indigenous pigs to the livelihoods of rural farmers in a semi-arid area of Zimbabwe. *Tropical Animal Health and Production*, 40, 125 - 136.

Cole, D.J.A., Duckworth, J.E. and Holmes, W., 1967. Factors affecting voluntary feed intake in pigs. The effect of digestible energy content of the diet on the intake of castrated male pigs housed in holding pens and in metabolism crates. *Animal Science*, 9, 141 - 148.

Cromwell, G.L., Cline, T.R., Crenshaw, J.D., Crenshaw, T.D., Ewan, R.C., Hamilton, C.R., Lewis, A.J., Mahan, D.C., Miller, E.R., Pettigrew, J.E. and Tribble, L.F., 1993. The dietary protein and (or) lysine requirements of barrows and gilts. *Journal of Animal Science*, 71, 1510 - 1519.

Department of Agriculture, Forestry and Fisheries (DAFF), 2012. Economic review of the South African Agriculture, Pretoria, South Africa. Annual Report. pp. 1 - 17.

Gous, R.M., and Morris, T.R., 1985. Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. *British Poultry Science*, 26, 141 - 161

Hlatini, V.A., Ncobela, C.N. and Chimonyo, M., 2020. Nitrogen balance response to varying levels of dietary protein in slow-growing Windsnyer pigs. *South African Journal of Animal Science*, 50.

Halimani, T.E., Muchadeyi, F.C., Chimonyo, M. and Dzama, K., 2010. Pig genetic resource conservation: The Southern African perspective. *Ecological Economics*. 69, 944 - 951.

Halimani, T.E., Muchadeyi, F.C., Chimonyo, M. and Dzama, K., 2012. Some insights into the phenotypic and genetic diversity of indigenous pigs in southern Africa. *South African Journal of Animal Science*, 42, 507 - 510.

Hlongwana, F.Y., Thabethe, F., Thomas, R.S. and Chimonyo, M., 2021. Nitrogen balance in slow-growing Windsnyer pigs fed on incremental levels of amarula (*Sclerocarya birrea subsp. caffra*) nut cake. *Tropical Animal Health and Production*, 53, 1 - 12.

Hoffmann, I., 2010. Climate change and the characterization, breeding and conservation of animal genetic resources. *Animal Genetics*, 41, 32 - 46.

Joven, M., Pintos, E., Latorre, M.A., Suárez-Belloch, J., Guada, J.A. and Fondevila, M., 2014. Effect of replacing barley by increasing levels of olive cake in the diet of finishing pigs: Growth performances, digestibility, carcass, meat and fat quality. *Animal Feed Science and Technology*, 197, 185 - 193.

Kanengoni, A.T., Chimonyo, M., Erlwanger, K.H., Ndimba, B.K. and Dzama, K., 2014. Growth performance, blood metabolic responses, and carcass characteristics of grower and finisher South African *Windsnyer*-type indigenous and Large

White× Landrace crossbred pigs fed diets containing ensiled corncobs. *Journal of Animal Science*, 92, 5739 - 5748.

Kaps, M. and Lamberson, W.R. eds., 2017. Biostatistics for animal science. Cabi.

Khanyile, M., Mapiye, C., Thabethe, F., Ncobela, C.N. and Chimonyo, M., 2020. Growth performance, carcass characteristics and fatty acid composition of finishing pigs fed on graded levels of *Vachellia tortilis* leaf meal. *Livestock Science*, 241, 104 - 259.

Kim, S.W., Less, J.F., Wang, L., Yan, T,. Kiron, V., Kaushik, S.J., Lei, X.G., 2019. Meeting global feed protein demand: challenge, opportunity, and strategy. *Annual Reviews of Animal Biosciences*, 15, 221 - 243.

Kocher, A., Choct, M., Porter, M.D. and Broz, J., 2002. Effects of feed enzymes on nutritive value of soyabean meal fed to broilers. *British Poultry Science*, 43, 54 - 63.

Kyriazakis, I and Emmans, G. C., 1995. The voluntary feed intake of pigs given feeds based on wheat bran, dried citrus pulp and grass meal, in relation to measurements of feed bulk. *British Journal of Nutrition*, 73, 191 - 207.

Kyriazakis, I. and Whittemore, C.T., 2008. Whittemore's science and practice of pig production. John Wiley & Sons.

Mabena, P.M., Ratsaka, M.M., Nkukwana, T.T., Malebana, I.M.M. and Nkosi, B.D., 2022. Growth performance, nutrient digestibility and carcass characteristi cs of pigs fed diets containing amarula (*Sclerocarya birrea A. Rich*) nut cake as replacement to soybean meal. *Tropical Animal Health and Production*, 54, 1 - 10.

Malebana, I. M., Nkosi, B. D., Erlwanger, K. H. and Chivandi, E., 2018. A comparison of the proximate, fibre, mineral content, amino acid and the fatty acid profle of

Marula (*Sclerocarya birrea cafra)* nut and soyabean (Glycine max) meals. *Journal of the Science of Food and Agriculture* 98, 1381 - 1387.

Madzimure, J., Chimonyo, M., Hugo, A., Bakare, A.G., Katiyatiya, C.L.F. and Muchenje, V., 2017. Physico-chemical quality attributes and fatty acid profiles of pork from *Windsnyer* and Large White gilts. *South African Journal of Animal Science*, 47, 107 - 114.

Mariod, A., Matthäus, B. and Eichner, K., 2004. Fatty acid, tocopherol and sterol composition as well as oxidative stability of three unusual Sudanese oils. *Journal of Food Lipids*, 11, 179 - 189.

Martens, S.D., Hoedtke, S., Avila, P., Heinritz, S.N. and Zeyner, A., 2014. Effect of ensiling treatment on secondary compounds and amino acids profile of tropical forage legumes, and implication for their pig feeding potential. *Journal of the Science of Food and Agriculture*, 94, 1107 - 1115.

Mazizi, B.E., Moyo, D., Erlwanger, K.H. and Chivandi, E., 2019. Effects of dietary *Sclerocarya birrea caffra* (Marula) nut meal on the growth performance and viscera macro morphometry of broiler Japanese quail. *Journal of Applied Poultry Research*, 28, 1028 - 1038.

Mdziniso, P.M., Dlamini, A.M., Khumalo, G.Z. and Mupangwa, J.F., 2016. Nutritional evaluation of marula (*Sclerocarya birrea*) seed cake as a protein supplement in dairy meal. *Journal of Applied Life Sciences International*, 4, 1 - 11.

Mlambo, V., Dlamini, B.J., Ngwenya, M.D., Mhazo, N., Beyene, S.T. and Sikosana, J.L.N., 2011. In sacco and in vivo evaluation of marula (*Sclerocarya birrea*) seed cake as a protein source in commercial cattle fattening diets. *Livestock Research for Rural Development*, 23, 1 - 10.

Mthiyane, D.M.N and Mhlanga, B., 2017. The nutritive value of marula (*Sclerocarya birrea*) seed cake for broiler chickens: nutritional composition, performance, carcass characteristics and oxidative and mycotoxin status. *Tropical Animal Health and Production*, 49, 835 - 842

Mujibi, F.D., Okoth, E., Cheruiyot, E.K., Onzere, C., Bishop, R.P., Fèvre, E.M., Thomas, L., Masembe, C., Plastow, G. and Rothschild, M., 2018. Genetic diversity, breed composition and admixture of Kenyan domestic pigs. *PLoS One*, 13, p.e0190080.

Ncobela, C.N., Kanengoni, A.T., Thomas, R.S. and Chimonyo, M., 2018. Voluntary feed intake and growth performance of slow-growing pigs fed on increasing levels of ensiled potato hash meal. *Tropical Animal Health and Production*, 50, 113 - 120.

Ndou, S.P., Gous, R.M. and Chimonyo, M., 2013. Prediction of scaled feed intake in weaner pigs using physico-chemical properties of fibrous feeds. *British Journal of Nutrition*, 110, 774 - 780.

National Research Council (NRC) 2012. Nutrient Requirements of Swine, 11th revised edition. National Academy Press, Washington, DC, USA.

Lindberg, J.E., 2014. Fiber effects in nutrition and gut health in pigs. *Journal of Animal Science and Biotechnology*, 5, 1 - 7.

Ouédraogo, A., Lykke, A.M., Lankoandé, B. and Korbéogo, G., 2013. Potentials for promoting oil products identified from traditional knowledge of native trees in Burkina Faso. *Ethnobotany Research and Applications,* 11, 071 - 083.

Ramirez, O., Ojeda, A., Tomas, A., Gallardo, D., Huang, L.S., Folch, J.M., Clop, A., Sánchez, A., Badaoui, B., Hanotte, O. and Galman-Omitogun, O., 2009.

Integrating Y-chromosome, mitochondrial, and autosomal data to analyse the origin of pig breeds. *Molecular Biology and Evolution*, 26, 2061 - 2072.

SAS., 2008. SAS User's Guide: Statistics, Version9.1. SAS Institute, Cary.

Shackleton, S.E., Shackleton, C.M., Cunningham, T., Lombard, C., Sullivan, C.A. and Netshiluvhi, T.R., 2002. Knowledge on *Sclerocarya birrea subsp. caffra* with emphasis on its importance as a non-timber forest product in South and southern Africa: a summary: Part 1: Taxonomy, ecology and role in rural livelihoods. *Southern African Forestry Journal*, 2002, 27 - 42.

Sloat, D.A., Mahan, D.C. and Roehrig, K.L., 1985. Effect of pig weaning weight on postweaning body composition and digestive enzyme development. *Nutrition Reports International*, 31, 627 - 634.

Van Huis, A., 2013. Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, 58, 563 - 583.

Van Soest, P.J., Robertson, J.B., and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Diary Science*,74, 3583 - 3597.

Wilson, R.T., 2009. Fit for purpose – the right animal in the right place. *Tropical Animal Health and Production*, 41, 1081 - 1090.

Whittemore, E.C., Emmans, G.C. and Kyriazakis, I., 2003. The relationship between live weight and the intake of bulky foods in pigs. *Animal Science*, 76, 89 - 10.

CHAPTER 4: Carcass traits and primal pork cuts of growing Windsnyer pigs fed diets containing Amarula oil cake

(Accepted: South African Journal of Animal Science)

Abstract

Carcass characteristics and primal pork cuts of local pig breeds are rarely documented, therefore, the current study was conducted to establish the relation between incremental levels of Amarula oil cake (AOC), carcass traits, primal pork cuts and relative internal organ weight of Windsnyer pigs. Twenty-five clinically healthy growing male Windsnyer pigs with an initial body weight of 19.9 ± 8.74 kg (mean ± standard deviation) were used. Pigs were used in their growing period when they were about 67 days old. The study lasted for six weeks excluding one week of adaptation period. Pigs were allotted to pens in a completely randomized design and assigned to each of the five experimental diets, which contained 0, 50, 100, 150 and 200 g/ kg dry matter of Amarula oil cake, respectively. Feed and water were offered *ad libitum*. Post slaughter, data for carcass characteristics, primal pork cuts and relative organ weights of Windsnyer pigs were analyzed using polynomial regression. There was a negative linear relationship between increasing levels of Amarula oil cake, carcass length, warm carcass weight and cold carcass weight. Stomach weight, backfat thickness, drip loss and the hepatosomatic index increased linearly with increasing levels of Amarula oil cake. The kidneys, small intestines, and large intestines weight of Windsnyer pigs had a quadratic response to Amarula oil cake inclusion level. The heart, lungs and spleen were not related to increasing levels of Amarula oil cake inclusion. Incremental levels of Amarula oil cake diets impaired carcass characteristics and the selected visceral organs, therefore Windsnyer pigs can be fed Amarula oil cake up to100 g/kg DM.

Keywords: backfat thickness; carcass length; drip loss; hepatosomatic index; slow-growing pigs; warm carcass.

4.1 Introduction

Indigenous pigs are the main source of livelihood for most people globally more especially for resource limited farmers found in many developing countries (Halimani *et al*., 2010). Their production can therefore contribute to alleviating poverty and improving food security. Globally, the production of pork from indigenous pigs is very low, suggesting that most indigenous pig breeds found in rural communities are kept for home consumption and as cash investments (Madzimure *et al*., 2012). Indigenous pigs have a higher feed conversion ratio. They, however, produce pork of good organoleptic properties and can be raised under low - cost conditions (Leterme *et al*., 2005; Halimani *et al*., 2012). The exploitation of indigenous pig breeds and providing knowledge about them is crucial and it contributes markedly to preserving their existence because of their distinct roles and functions in sustainable agricultural development (Chimonyo *et al.,* 2006).

Pig breeds in Africa are closely related to international pig breeds (Ramírez *et al*., 2009). The indigenous pig breeds of South Africa, such as the African type pig, the Kolbroek and the Windsnyer pig are all classified as *Sus scrofa*. They exhibit similar characteristics to the strongly introgressed Chinese and British pig breeds (Ramírez *et al*., 2009), suggesting that these pig breeds can thrive in other countries due to their genetic similarities. A commonly found type of indigenous pig breed in South Africa is the Windsnyer pig. Its resilience to diseases and superior ability to utilise fibrous agricultural by-products has long been established (Kanengoni *et al*., 2014; Ncobela *et al*., 2018).

Amarula tree (*Sclerocarya birrea* subspecies caffra) is an African indigenous tree that is mostly found in the northern parts of Eswatini, Limpopo, Mpumalanga, and KwaZulu-Natal. The kernels are used for making traditional beverages, to make protein supplement for poultry and as a source of food by rural households (Mlambo *et al*., 2011; Mthiyane and Mhlanga, 2017). Amarula oil cake, is a by-product of oil extraction from Amarula kernels. The nutritional characteristics of Amarula oil cake such as high crude protein, and fibre content may influence carcass quality of pigs positively when used intensively. Kerr et al. (1995), reported poor carcass yield grades of pigs fed on reduced protein diets compared to carcass yield of pigs which were fed high crude protein diets, therefore, the high crude protein content of Amarula might positively affect carcass grades of Windsnyer pigs. The ability of Windsnyer pigs to tolerate fibrous feeds might improve carcass characteristics of pigs. Fibrous diets negatively affected warm carcass weight of pigs due to increasing gut contents in Mong Cai pig breed (Len *et al*., 2008). Jha *et al*. (2013), reported that incremental fibre levels in pig diets reduced carcass weight of pigs. Most fibrous feeds do not meet nutrient requirements of pigs, therefore, balanced diets such as Amarula oil cake that provides both fibre and protein to Windsnyer pigs might increase slaughter weight of Windsnyer pigs hence improving carcass quality. Visceral organ weight account for about 15 % or less of the entire weight of animals (Yen, 1992) and they respond to inclusion of fibrous or tanniferous diets (Agyekum *et al*., 2012). Heavier relative weight of heart, liver and kidneys were reported in pigs on high fibre diets compared to low fibre diets (Len *et al*., 2009). Dietary interventions that improve carcass traits of indigenous pigs are therefore, required.

Carcass length is a trait of economic importance that affect the weight of many pork cuts (Poto *et al*., 2007). Carcass dressed weights, carcass length, and carcass conformation are direct determinants of carcass grades and thereby revenue (Chimonyo *et al*., 2010). Understanding these carcass characteristics of Windsnyer pigs could provide knowledge that enhance the choices for health-conscious consumers. The relationships between carcass characteristics, primal pork cuts and relative internal organs of Windsnyer pigs fed on Amarula oil cake (AOC) diets are poorly understood. Such knowledge might change the current negative perspective that policymakers and pork scientists have about indigenous pig breeds. The objective of the study was, therefore, to determine the relationship between incremental levels of AOC, carcass characteristics, primal pork cuts and visceral organ weights of South African Windsnyer pigs. It was hypothesized that incremental levels of AOC would linearly increase carcass characteristics, primal pork cuts and visceral organ weight of South African Windsnyer pigs.

4.2 Materials and methods

4.2.1 Study site

The study site has been briefly described in chapter 3.

4.2.3 Collection, preparation of Amarula oil cake and diets

The collection and preparation of the experimental diets has been described in chapter 3.

4.2.4 Experimental design and management of pigs

The experimental design and management of Windsnyer pigs has been briefly described previously in chapter 3.

4.2.5 Chemical analyses of experimental diets

The chemical composition of the experimental diets has been described previously in chapter 3.

4.2.6 Slaughtering, carcass, primal pork cuts and internal organs

After the experimental feeding period, pigs were fasted for 24 hours and weighed to determine the slaughter weight. Pigs were transported to the ARC abattoir located about 1.5 km from the trial facility for slaughter. All pigs were handled based on routine abattoir procedures of ARC, Irene, before slaughter and it included the ante-mortem inspection by the ARC veterinarian. The pigs were then stunned with an electrical stunner set at 220V and 1.8A with a current flow for 6s and exsanguinated within 10s of stunning. Exsanguination, de-hairing, evisceration and cutting were done according to Kanengoni *et al*. (2014).

Carcass length (CL) was measured using a measuring tape from the first rib to the pubic bone and warm carcass weight was measured with a scale after dressing. The

ham length was measured from the cranial edge of the pubic symphysis to the medio-distal point where the hind trotter was removed. Thereafter, carcasses were stored at the abattoir cold room and kept at 0 ^{0}C for 24 hours. The dressing percentage was determined by considering warm carcass weight as a percentage of slaughter weight (SW). The cooler shrink (CS) was calculated from the carcasses under chill storage in a cold room (0°C for 24 h) using the following formula:
(1 – (cold carcass weight / warm carcass weight) x100.

The initial pH and temperature reading were taken right after slaughter from the longissimus thoracis muscle (eye muscle) using a pH meter. Prior the recording of pH, the pH meter electrode was calibrated at pH_4 and pH_7 buffers. Distilled water was used to rinse the electrodes after each measurement to avoid contamination amongst treatments. Carcasses were kept in a cold room and stored at about 0°C for 24 h, thereafter, measurements for cold carcass weights (CCW) were then taken. The final pH and temperature were recorded 24h post slaughter.

Backfat measurements were taken at first rib (dorsal fat thickness at first rib (DFT1)), last rib (dorsal fat thickness at last rib (DFT2)), and last lumbar vertebra (dorsal fat thickness at last lumbar vertebra (DFT3)) off the median plane cut surface (Kanengoni *et al*., 2014). All other carcass measurements were taken from the left side. The backfat depth was measured at P2 position of each carcass with a vernier calliper over the eye muscle, 60 mm from the carcass midline. From the same cut where P2 measurements were taken, a sample joint measuring 2.5 cm thick and 16 cm long measured along the surface of the back of the eye muscle was cut out and weighed (chop mass). This sample joint was placed in a nylon mesh and sealed in plastic bags, which was then tied in such a way as to prevent the sample joint from touching the bottom of the plastic bag or air coming into the bag. The sample was then stored in a refrigerator between 0 and 5 °C for 24 h, after which the mass of the water lost was calculated from the weight of the water in the bag and used to calculate the drip loss (Kanengoni *et al*., 2014).

Upon slaughter, the heart, liver, lungs, spleen, kidney, stomach, small and large intestines for each pig were removed and weighed separately with a digital scale. The contents of stomach, small intestines and large intestines were emptied before recording their weight. The weight of visceral organs was scaled by dividing the weights of the heart, lungs, spleen, kidney, stomach, small and large intestines with slaughter weight. The hepatosomatic index (HSI) was calculated by dividing the liver weight with slaughter weight and expressed as a percentage (Liu et al., 2009).

4.2.7 Statistical analyses

The normality of the data was determined using the PROC UNIVARIATE procedure of SAS, (2009). The LS means statement in SAS, (2008) was used to compare the least square means using the probability difference (PDIFF) option. Relationships between inclusion levels of Amarula oil cake and carcass characteristics, primal pork cuts and relative organ weights were determined with the polynomial regression procedure of (SAS, 2008).

4.3 Results

Table 4.1 shows the relationship between incremental levels of Amarula oil cake diets, carcass traits and primal pork cuts of South African Windsnyer pigs. Increasing inclusion levels of Amarula oil cake linearly reduced the slaughter weight, carcass length, warm carcass weight ($y = -5.54(32.12)x + 648,53$), and cold carcass weight ($y = -8,17(30,41)x + 134.61$) of pigs ($P < 0.05$). Increasing inclusion levels of AOC led to a linear increase in backfat thickness and drip loss ($P < 0.05$). Other carcass traits such as pH_{45}, pH_{24}, $temperature_{45}$ and $temperature_{24}$, cooler shrink, dressing percentage and shoulder fat showed no relationship with increasing inclusion level of AOC diets ($P > 0.05$). The Dorsal fat thickness (DFT3) ($y = -26.42 (26.89)x + 473.81$) decreased linearly with increasing inclusion levels of AOC ($P < 0.05$). Other Primal pork cuts traits such as ham length, ham diameter, backfat depth at P2 position, dorsal fat thickness 1, dorsal fat thickness 2, hind quarter weight proportion, shoulder weight proportion and rib weight proportion were not affected by increasing inclusion levels of Amarula oil cake ($P > 0.05$). The relationship between AOC inclusion level with slaughter weight, carcass length, warm carcass weight cold carcass weight, back fat thickness, driploss and dorsal fat thickness of pigs is shown in Figure 4.1 (A-D).

Table 4.2 shows the relationship between incremental levels of Amarula oil cake and visceral organ weights of pigs. Increasing inclusion levels of Amarula oil cake diets increased the hepatosomatic index (HIS) ($y = 330.33 (157.66)x – 243,01$) and stomach weight ($y = 13.80(6.75)x – 69.25$) of pigs linearly ($P < 0.05$). The kidneys, small intestines, and large intestines weight of Windsnyer pigs decreased quadratically with increasing inclusion levels of Amarula oil cake diets ($P < 0.05$). The heart, lungs and spleen showed no relationship with increasing inclusion levels of Amarula oil cake ($P > 0.05$). The relationship between AOC inclusion level with hepatosomatic index, stomach weight, kidney weight, small intestine (SIW) and large intestine (LIW) weight of Windsnyer pigs is shown in Figure 4.2 (A).

Table 4.1: Relationship between increasing levels of Amarula oil cake, carcass traits and primal pork cuts of Windsnyer pigs

Measurements	Inclusion level of Amarula oil cake (g/kg DM)					SEM	Significance		R^2
	0	50	100	150	200		Linear	Quadratic	
Dressing percentage (%)	65.50	65.56	72.18	61.82	72.53	6.73	NS	NS	0.03
Slaughter weight (kg)	36.80	34.70	32.24	30.80	28.00	3.86	*	*	0.33
Carcass length (cm)	69.00	65.60	63.80	62.80	61.00	2.76	*	NS	0.19
WCW (kg)	19.96	19.50	20.30	17.06	15.66	1.57	*	NS	0.23
Cold carcass weight (kg)	24.82	22.62	20.26	19.04	19.00	1.49	***	NS	0.34
pH_{45}	6.50	6.57	6.36	6.58	6.60	0.09	NS	NS	0.03
pH_{24}	5.37	5.70	5.31	5.79	5.24	0.13	NS	NS	0.01
$Temperature_{45}$	36.57	33.18	37.04	35.24	36.64	0.73	NS	NS	0.03
$Temperature_{24}$	5.32	2.56	4.42	6.92	5.88	1.19	NS	NS	0.01
Cooler shrink (%)	4.19	3.07	3.66	3.72	3.13	0.74	NS	NS	0.03
Backfat thickness (mm)	19.00	19.40	19.45	19.79	20.00	0.60	**	NS	0.26
Shoulder fat (mm)	27.00	25.00	29.80	31.00	26.20	3.25	NS	NS	0.11
Chop (kg)	0.14	0.13	0.12	0.10	0.10	0.02	NS	NS	0.11
Drip loss (%)	0.22	0.14	0.27	0.38	0.62	0.16	*	NS	0.06
Primal pork cuts									
Ham length (cm)	26.80	28.00	27.60	25.20	27.60	1.40	NS	NS	0.11
Ham diameter (mm)	28.60	27.40	30.60	29.40	31.00	2.60	NS	NS	0.09
Backfat depth at P2	90.00	76.40	93.00	70.00	68.80	9.04	NS	NS	0.13
DFT1 (mm)	26.40	30.80	26.40	30.80	26.40	4.03	NS	NS	0.00
DFT2 (mm)	15.80	21.20	17.60	18.60	17.60	1.76	NS	NS	0.07
DFT3 (mm)	23.00	22.20	19.80	18.80	17.20	1.62	**	NS	0.31
HQWP %	22.55	24.63	22.86	26.62	21.62	4.40	NS	NS	0.04
SHDWP %	26.18	14.06	14.12	15.17	12.31	5.96	NS	NS	0.09
RWP %	13.13	13.59	12.22	13.12	11.68	2.60	NS	NS	0.01

Dorsal fat thickness (DFT), Warm carcass weight (WCW), Hind quatre weight proportion (HQWP), Shoulder weight proportion, Rib weight proportion (RWP), Coefficient of determination (R^2), Standard error of the mean (SEM). *P<0.05; **P<0.01; ***P<0.001; NS: not significant (P >0.05).

Table 4.2: Relationship between inclusion levels of Amarula oil cake and visceral organ weights of Windsnyer pigs

	Inclusion level of Amarula oil cake (g/kg DM)						Significance		
Visceral organs (g/bw)	0	50	100	150	200	SEM	Linear	Quadratic	R^2
Heart	2.93	3.16	3.85	4.14	3.82	0.72	NS	NS	0.07
Hepatosomatic index (%)	1.37	1.61	1.74	1.82	1.93	0.23	*	NS	0.25
Lungs	9.81	7.81	9.35	11.50	12.87	1.67	NS	NS	0.14
Spleen	1.40	1.58	1.97	2.47	2.25	0.48	NS	NS	0.11
Empty stomach	11.81	15.48	18.30	22.11	23.08	3.38	**	NS	0.33
Kidney	3.14	3.60	4.22	4.20	3.91	0.55	*	*	0.36
Small intestine	23.19	23.76	24.15	24.96	23.59	2.98	NS	*	0.19
Large intestine	33.88	34.70	34.98	35.00	33.00	6.20	NS	*	0.23

Standard error of the mean (SEM), Coefficient of determination (R^2), *$P<0.05$; **$P<0.01$; NS: not significant ($P > 0.05$), g/kg DM (grams/Kilograms dry matter).

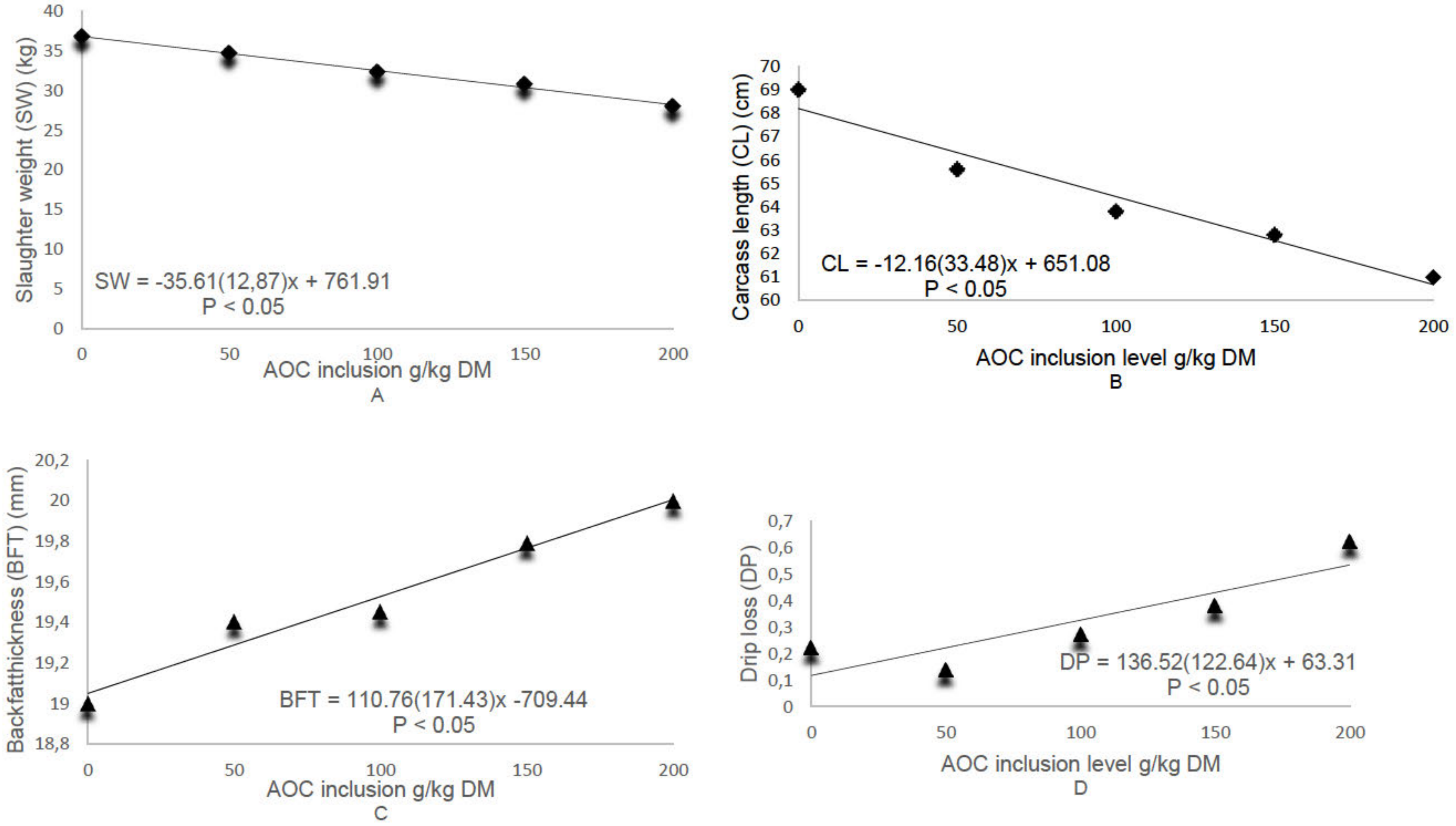


Figure 4.1: (A-D) Relationship between AOC inclusion level with slaughter weight, carcass length, back fat thickness and driploss.

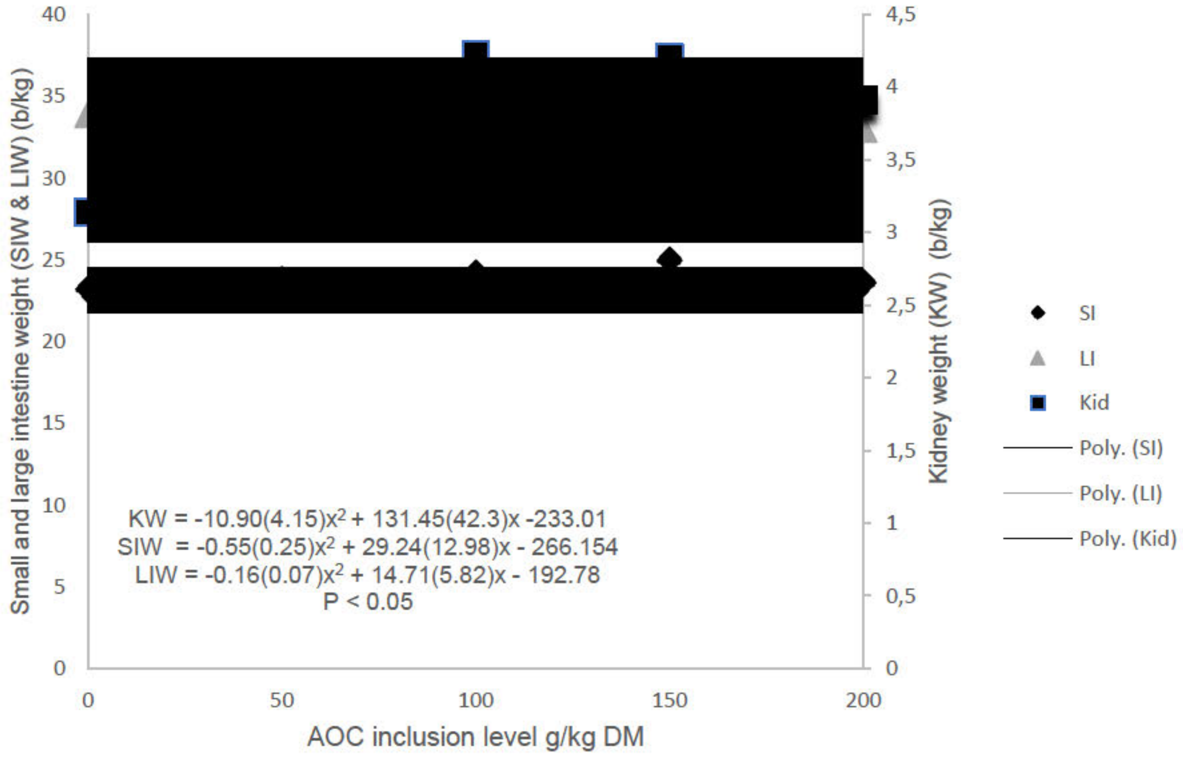


Figure 4.2: Relationship between AOC inclusion level with kidney wight, small intestine (SIW) and large intestine (LIW) weight of Windsnyer pig. Body weight/kilogram (b/kg).

4.4 Discussion

Indigenous pigs such as the Windsnyer pig are rejected by the carcass classification systems used in abattoirs. Strategies that improve the production of indigenous pig genotypes, selection standards that include Windsnyer pigs in breeding programs and the conservation of indigenous pigs are of the utmost importance in tropical countries. There is limited research about the relationship between increasing levels of Amarula oil cake diets and carcass characteristics of Windsnyer pigs.

The observation that the slaughter weight of Windsnyer pigs decreased with increasing levels of Amarula oil cake suggests that including higher amount of the oil cake in the diet compromises pig performance. Miya et al. (2019), reported a similar linear reduction in slaughter weight of broilers that were fed on increasing levels of *Vachellia tortilis* leaf meal diets. Khanyile et al. (2016), however, reported a quadratic decrease in slaughter weight of pigs fed on increasing levels of *Vachellia tortilis* leaf meal. Nevertheless, such a quadratic decrease concurs with the fact that high inclusion levels of *Vachellia* diets reduced the overall slaughter weight of pigs.

The linear decrease in carcass length with increasing inclusion levels of Amarula oil cake concurs with Ncobela et al. (2018). The decreasing carcass length of South African Windsnyer pigs can be related to the linear reduction in average daily gain. Windsnyer pigs grow up to a point, after which they essentially deposit fat, hence affecting its weight. Several African indigenous pig genotypes are reported to have high fat deposition when attaining puberty (Chimonyo et al., 2010).

As expected, increasing levels of Amarula oil cake linearly decreased both the warm and cold carcass weights of pigs. Len et al. (2008) reported a similar reduction in warm carcass weight of Mong Cai pigs fed on high fibrous diet. The reduction in warm carcass was related to increased weight of gastro-intestinal tract of pigs which was a result of the high fibre content of the diet. Fibrous feeds increased the size of internal organs of pigs (Qin et al., 2002). Increasing AOC levels increased the fibre level in the diet. Similarly, Ćirić et al. (2017), showed strong correlations between the slaughter weight, warm carcass weight and cold carcass weight of pigs.

Carcass quality of pigs can also be assessed by measuring backfat thickness, which estimates the thickness of the subcutaneous fat. Both the backfat and intramuscular fat alter pork quality and should be considered (Chimonyo et al., 2001). As expected, increasing levels of AOC linearly increased backfat thickness of Windsnyer pigs (Liu et al., 2015; Khanyile et al., 2016). Our results were, however, in contradiction with those of Ncobela et al. (2018) who reported a linear reduction in backfat thickness of Windsnyer pigs fed on increasing levels of potato hash silage. The discrepancies between the two studies can be explained by differences in ether extract concentration of the diets which, in turn, alters the energy content of the diets. Fats are used as energy sources in monogastric animals. The consumption of energy-rich diets triggers lipogenesis and, consequently, visceral fat deposition. The ether extract and gross energy were directly proportional to the inclusion level of AOC.

Drip loss is an important characteristic that affects palatability and acceptability of meat (Forrest et al., 2000). Increasing the inclusion level of AOC linearly increased drip loss in Windsnyer pigs. Our results were consistent with those of Mushandu et al. (2005), who reported a linear increase in drip loss of Mukota pigs fed on increasing levels of sorghum-based diets. On the contrary, diets with the incorporation of avocado, had no effect on drip loss of Large White x Landrace pigs (Seshoka et al., 2020). Apart from the diet, drip loss is also affected by pig genotype and pH of meat (Mushandu et al., 2005; Kanengoni et al*.,* 2014). Both the initial and final pH in the current study showed no response to inclusion levels of AOC.

Increasing AOC inclusion level in Windsnyer pig diets was expected to increase the backfat measurement (DFT1, DFT2 and DFT3) of Windsnyer pigs due to the high fat content of AOC. However, that was not the case in the current study. Increasing levels of AOC diets linearly reduced the dorsal fat thickness (DFT3), as also reported by Ncobela et al. (2018). The reduction in DFT3 might be assumed to have also been caused by the linear reduction of carcass length, which affects most pork cuts (Poto et al., 2007).

The hepatosomatic index is the scaled liver weight expressed as a percentage. A linear increase in the hepatosomatic index (HSI) with increasing levels of AOC suggest that higher levels of the oil cake increase liver weight. On the contrary, Ma et al. (2002) and Bakare et al. (2017) showed a reduction in liver weights of pigs fed on maize cob and straw-based diets, respectively. The present results were nevertheless consistent with those of Khanyile et al. (2016), who reported a linear increase in liver weight of pigs fed on *Vachellia* diets. The increasing HSI might be related to increasing weight of the stomach of pigs, which might have resulted to an increase in the secretion of liver enzymes that detoxify toxic substances in the diets. A linear increase in stomach weight of Windsnyer pigs was indeed observed. The results are consistent with Kaensombath et al. (2013) and Bakare et al. (2017), who reported an increase in stomach weight of Moo Lath pigs fed Stylo silage and Landrace pigs fed on maize cob-based diets. The fibre content of the diet influences the weight of visceral organs (Nyachoti et al., 2000). Similar observations were also confirmed by Zhao et al. (1995).

A quadratic decrease in kidney, small and large intestine weight of pigs with increasing levels of Amarula oil cake diets was observed in the study. Miya et al. (2019), reported a linear increase in relative liver weights of broilers fed on increasing levels of *Vachellia* diets. It is possible that high inclusion levels of AOC and the time of exposure of the pigs to the diets affected the relative kidney weight of pigs in our study. As such, the duration of feeding also needs to be considered when AOC based diets are used. Kidneys are the main sites for clearance of nitrogen and detoxify toxic compounds in the body. Increasing dietary protein level increase the weight of liver and kidneys of pigs (Chen *et al*., 1996). The presence of mycotoxins in Amarula diets were detected by Mthiyane and Mhlanga. (2017). Mycotoxins reduce pig performance by reducing protein digestibility and absorption. Increasing AOC levels in the diet might also increase the amounts of mycotoxins that result in mould development in the diet.

A linear increase in both small and large intestines of Windsnyer to dietary inclusion level of AOC pigs was expected. Indeed, this would be due to the increased bulk properties and the cell wall constituent contents of AOC diets. In addition, the hind gut of Windsnyer pigs can ferment fibrous feeds effectively, as demonstrated earlier (Kanengoni et al., 2014). The observed quadratic reduction in both the small and large intestines could have been related to the quadratic reduction in ADFI, suggesting that Windsnyer pigs can tolerate AOC based diets up to 150 g/kg DM. The small intestines and large intestines of pigs are sites for nutrient and water absorption. Dietary factors that affect the normal functioning of these organs may, therefore, interfere with nutrient absorption, hence reducing growth and health of pigs. Incorporating Amarula oil cake increased feed bulk. An increase in water holding capacity and bulk density, reduces energy availability and consequently reduces pig growth (Linares and Huang, 2010).

The absorption of nutrients is largely influenced by feed intake, which in turn, is affected by dietary properties of the feeds (Nyachoti et al., 2004). The decrease in both small and large intestines weights at higher levels of AOC diets could be a clear indication that the intestinal epithelium was compromised, suggesting that gut health and morphology of Windsnyer pigs to AOC-based diets need to be investigated.

4.5 Conclusions

The was a negative linear relationship between slaughter weight, carcass length, warm carcass weight, cold carcass weight and dorsal fat thickness with increasing levels of Amarula oil cake. Backfat thickness and drip loss showed a positive linear relationship with increasing levels of Amarula oil cake. Other selected carcass characteristics and primal pork cuts had no clear relationship. The HSI and stomach weight showed a negative linear relationship with increasing levels of Amarula oil cake. The kidneys, small and large intestine weight showed a negative quadratic relationship with increasing levels of Amarula oil cake, suggesting that high levels of Amarula also constrained some of the selected visceral organs of Windsnyer pigs. However, some parameters of visceral organs such as the heart, lungs and spleen were not affected by the inclusion level of Amarula. Incremental levels of Amarula oil cake diets impaired carcass characteristics and selected visceral organs of pigs, therefore Windsnyer pigs can be fed AOC diets up to100 g/kg DM. When carcass traits and visceral organs of Windsnyer pigs are selected, low levels of Amarula oil cake needs to be considered. The gut health of Windsnyer pigs also need to be considered when feeding pigs with Amarula based diets. Besides carcass traits and primal pork cuts, it is important to determine serum related metabolites and liver enzymes of pigs to strengthen knowledge about effects of AOC diets on the health of pigs.

4.6 References

A0AC, 2005. Official methods of analysis. Eighteenth edition. AOAC International, Gaithersburg, Maryland, USA.

Agyekum, A.K., Slominski, B.A, and Nyachoti, C.M., 2012. Organ weight, intestinal morphology, and fasting whole-body oxygen consumption in growing pigs fed diets containing distillers dried grains with solubles alone or in combination with a multienzyme supplement. *Journal of Animal Science*. 90, 3032 - 3040.

AOAC, 1990. Official methods of analysis. 15th edition. Association of Official Analytical Chemists, Arlington, VA.

Bakare, A.G. and Chimonyo, M., 2017. Relationship between feed characteristics and histomorphometry of small intestines of growing pigs. *South African Journal of Animal Science*, 47, 7 - 15.

Canibe, N. and Bach Knudsen, K.E., 2002. Degradation and physicochemical changes of barley and pea fibre along the gastrointestinal tract of pigs. *Journal of Science Food and Agriculture*, 82, 27 - 39.

Carcass Traits of Mong Cai, F1 Crossbred (Mong Cai Yorkshire) and Landrace X Yorkshire Pigs. *Asian-australasian Journal of Animal Science*, 21, 245 - 251.

Carter, N.A., Dewey, C.E., Thomas, L.F., Lukuyu, B., Grace, D. and de Lange, C., 2016. Nutrient requirements and low-cost balanced diets, based on seasonally available local feedstuffs, for local pigs on smallholder farms in Western Kenya. *Tropical Animal Health and Production*, 48, 337 - 347.

Chen, H.Y., Lewis, A.J. and Miller, P.S., 1996. The effects of dietary protein concentration on performance and visceral organ mass in finishing barrows and gilts. 1996 Nebraska Swine Report.

Chimonyo, M., Dzama, K. and Bhebhe, E., 2006. Genetic determination of individual birth weight, litter weight and litter size in Mukota pigs. *Livestock Science*, 105, 69 - 77.

Chimonyo, M., Dzama, K. and Mapiye, C., 2010. Growth performance and carcass characteristics of indigenous Mukota pigs of Zimbabwe. *Tropical Animal Health and Production*, 42, 1001 - 1007.

Chimonyo, M., Kanengoni, A.T. and Dzama, K., 2001. Influence of maize cob inclusion level in pig diets on growth performance and carcass traits of Mukota x Large White F1 crossbred male pigs. *Asian-australasian Journal of Animal Science*, 14, 1724 - 1727.

Ćirić, J., Lukić, M., Radulović, S., Janjić, J., Glamočlija, N., Marković, R. and Baltić, M.Ž., 2017. The relationship between the carcass characteristics and meat composition of young Simmental beef cattle. In IOP Conference Series: *Earth and Environmental Science* (Vol. 85, No. 1, p. 012061). IOP Publishing.

Forrest, J.C., Morgan, M.T., Borggaard, C., Rasmussen, A.J., Jespersen, B.L. and Andersen, J.R., 2000. Development of technology for the early post-mortem prediction of water holding capacity and drip loss in fresh pork. *Meat Science*, 55, 115 - 122.

Gous, R.M. and Morris, T.R., 1985. Evaluation of a diet dilution technique for measuring the response of broiler chickens to increasing concentrations of lysine. *British Journal of Poultry Science*, 26, 147 - 161.

Halimani, T.E., Muchadeyi, F.C., Chimonyo, M. and Dzama, K., 2010. Pig genetic resource conservation: The Southern African perspective. *Ecological Economics,* 69, 944 - 951.

Halimani, T.E., Muchadeyi, F.C., Chimonyo, M. and Dzama, K., 2012. Opportunities for conservation and utilisation of local pig breeds in low-input production systems in Zimbabwe and South Africa. *Tropical Animal Health and Production*, 45, 81 - 90.

Hlongwana, F.Y., Thabethe, F., Thomas, R.S. and Chimonyo, M., 2021. Nitrogen balance in slow-growing Windsnyer pigs fed on incremental levels of amarula (*Sclerocarya birrea subsp. caffra*) nut cake. *Tropical Animal Health and Production,* 53, 1 - 12.

Jha, R., Htoo, J.K., Young, M.G., Beltranena, E. and Zijlstra, R.T., 2013. Effects of increasing co-product inclusion and reducing dietary protein on growth performance, carcass characteristics, and jowl fatty acid profile of growing–finishing pigs. *Journal of Animal Science*, 9, 2178 - 2191.

Kaensombath, L., Neil, M. and Lindberg, J.E., 2013. Effect of replacing soybean protein with protein from ensiled stylo (*Stylosanthes guianensis (Aubl.) Sw. var. guianensis*) on growth performance, carcass traits and organ weights of exotic (Landrace X Yorkshire) and native (Moo Lath) Lao pigs. *Tropical Animal Health and Production*, 45, 865 - 871.

Kanengoni, A. T., Chimonyo, M., Erlwanger, K. H., Ndimba, B. K., Dzama, K. 2014. Growth performance, blood metabolic responses, and carcass characteristics of grower and finisher South African *Windsnyer*-type indigenous and Large White× Landrace crossbred pigs fed diets containing ensiled corncobs. *Animal Science Journal*, 92, 5739 - 5748.

Kanengoni, A.T., Chimonyo, M., Ndimba, B.K. and Dzama, K., 2015. Feed preference, nutrient digestibility and colon volatile fatty acid production in growing South

African *Windsnyer*-type indigenous pigs and Large White× Landrace crosses fed diets containing ensiled maize cobs. *Livestock Science*, 171, 28 - 35.

Kerr, B.J., McKeith, F.K. and Easter, R.A., 1995. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. *Animal Science Journal*, 73, 433 - 440.

Khanyile, M., Ndou, S.P. and Chimonyo, M., 2016. Influence of *Acacia tortilis* leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science*, *57*, 675 - 682.

Kyriazakis, I. and Emmans, G.C., 1995. The voluntary feed intake of pigs given feeds based on wheat bran, dried citrus pulp and grass meal, in relation to measurements of feed bulk. *British Journal of Nutrition*, 73, 191 - 207.

Len, N.T., Hong, T.T.T., Ogle, B. and Lindberg, J.E., 2009. Comparison of total tract digestibility, development of visceral organs and digestive tract of Mong cai and Yorkshire × Landrace piglets fed diets with different fibre sources. *Journal of Animal Physiology and Animal Nutrition*, 93, 181 - 191.

Len, N.T., Lindberg, J.E. and Ogle, B., 2008. Effect of Dietary Fiber Level on the Performance and Carcass Traits of Mong Cai, F1 Crossbred (Mong Cai Yorkshire) and Landrace Yorkshire Pigs. *Asian-Australasian Journal of Animal Sciences*, 21, 245 - 251.

Leterme, P., Londoño, A.M., Estrada, F., Souffrant, W.B. and Buldgen, A., 2005. Chemical composition, nutritive value and voluntary intake of tropical tree foliage and cocoyam in pigs. *Journal of Science Food and Agriculture*, 85, 1725 - 1732.

Linares, L. and Huang, K., 2010, August. Effects of energy levels and different feed processing methods on performance of broilers. In XIIIth Eur. Poult. Conf., August, 24-26. https://doi.org/10.1002/jsfa.2177

Liu, H.W., Gai, F., Gasco, L., Brugiapaglia, A., Lussiana, C., Guo, K.J., Tong, J.M. and Zoccarato, I., 2009. Effects of chestnut tannins on carcass characteristics, meat quality, lipid oxidation and fatty acid composition of rabbits. *Meat Science*, *83*, 678 - 683.

Liu, Y., Kong, X., Jiang, G., Tan, B.E., Deng, J., Yang, X., Li, F., Xiong, X. & Yin, Y., 2015. Effects of dietary protein/energy ratio on growth performance, carcass trait, meat quality, and plasma metabolites in pigs of different genotypes. *Journal of Animal Science and Biotechnology*, 6, 1 - 10.

Ma, Y., Li, D., Qiao, S.Y., Huang, C.H. and Han, I.K., 2002. The effects of fiber source on organ weight, digesta pH, specific activities of digestive enzymes and bacterial activity in the gastrointestinal tract of piglets. *Asian-australasian Journal of Animal Science*, 15, 1482 - 1488.

Mabena, P.M., Ratsaka, M.M., Nkukwana, T.T., Malebana, I.M.M. and Nkosi, B.D., 2022. Growth performance, nutrient digestibility and carcass characteristics of pigs fed diets containing amarula (*Sclerocarya birrea A. Rich*) nut cake as replacement to soybean meal. *Tropical Animal Health and Production*, *54*, 1-10.

Madzimure, J., Chimonyo, M., Zander, K.K. and Dzama, K., 2012. Potential for using indigenous pigs in subsistence-oriented and market-oriented small-scale farming systems of Southern Africa. *Tropical Animal Health and Production*, *45*, 135 - 142.

Miya, A., Sithole, A.N., Mthethwa, N., Khanyile, M. and Chimonyo, M., 2019. Response in carcass yield, organ weights, and gut morphology of broiler chickens to incremental levels of *Vachellia tortilis* leaf meal. *Canadian Journal of Animal Science*, 100, 282 - 291.

Mlambo, V., Dlamini, B.J., Ngwenya, M.D., Mhazo, N., Beyene, S.T. and Sikosana, J.L.N., 2011. In sacco and in vivo evaluation of marula (*Sclerocarya birrea*) seed cake as a protein source in commercial cattle fattening diets. *Livestock Research and Rural Development*, 23, 1 - 10.

Mthiyane, D.M.N. and Mhlanga, B.S., 2017. The nutritive value of marula (*Sclerocarya birrea*) seed cake for broiler chickens: nutritional composition, performance, carcass characteristics and oxidative and mycotoxin status. *Tropical Animal Health and Production*, 49, 835 - 842.

Mushandu, J., Chimonyo, M., Dzama, K., Makuza, S.M. and Mhlanga, F.N., 2005. Influence of sorghum inclusion level on performance of growing local Mukota, Large White and their F1 crossbred pigs in Zimbabwe. *Animal Feed Science and Technology*, 122, 1 - 329.

Ncobela, C.N., Kanengoni, A.T. and Chimonyo, M., 2018. Response in nutritionally related blood metabolites, carcass traits and primal pork cuts of slow growing Windsnyer pigs fed on varying levels of potato hash silage. *South African Journal of Animal Science*, 48, 77 - 77.

Nyachoti, C.M., De Lange, C.F.M., McBride, B.W., Leeson, S. and Schulze, H., 2000. Dietary influence on organ size and in vitro oxygen consumption by visceral organs of growing pigs. *Livestock Production Science*, 65, 229 - 237.

Nyachoti, C.M., Zijlstra, R.T., De Lange, C.F.M. and Patience, J.F., 2004. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. *Canadian Journal of Animal Science*, 84, 549 - 566.

Poto, A., Galián, M. and Peinado, B., 2007. Chato Murciano pig and its crosses with Iberian and Large White pigs, reared outdoors. Comparative study of the carcass and meat characteristics. *Livestock Science*, 111, 96 - 103.

Qin, G.X., Xu, L.M., Jiang, H.L., Van der Poel, A.F.B. and Bosch, M.W., Verstegen, M.W.A., 2002. The effects of Chinese and Argentine soybeans on nutrient digestibility and organ morphology in Landrace and Chinese Min pigs. *Asian-Australasian Journal of Animal Science*, 15, 555 - 564.

Ramirez, O., Ojeda, A., Tomas, A., Gallardo, D., Huang, L.S., Folch, J.M., Clop, A., Sánchez, A., Badaoui, B., Hanotte, O. and Galman-Omitogun, O., 2009. Integrating Y-chromosome, mitochondrial, and autosomal data to analyze the origin of pig breeds. *Molecular Biology and Evolution*, *26*, 2061 - 2072.

SAS, (2009). SAS User’s Guide: Statistics, Version9.1. SAS Institute, Cary.

Seshoka, M.L., Fourie, P.J., Kanengoni, A.T., Malebana, I.M.M., Thomas, R.S. and Nkosi, B.D., 2020. Dietary inclusion of ensiled avocado oil cake affects growth, nutrient digestion, and carcass characteristics of pigs. *South African Journal of Animal Science*, 50, 2221 - 4062.

Van Soest, P.V., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583 - 3597.

Whittemore, E.C., Emmans, G.C. and Kyriazakis, I., 2003. The relationship between live weight and the intake of bulky foods in pigs. *Animal Science Journal*, *76*, 89 - 100.

Yen, J.T. and Nienaber, J.A., 1992. Influence of carbadox on fasting oxygen consumption by portal vein-drained organs and by the whole animal in growing pigs. *Journal of Animal Science*, 70, 478 - 483.

Zhao, X., Jørgensen, H. and Eggum, B.O., 1995. The influence of dietary fibre on body composition, visceral organ weight, digestibility and energy balance in rats housed in different thermal environments. *British Journal of Nutrition*, 73, 687 - 699.

CHAPTER 5: Nutritionally related blood metabolites in growing Windsnyer pigs fed on Amarula oil cake

Abstract

The study was conducted to assess changes between increasing levels of Amarula oil cake (AOC), nutritionally related blood metabolites and liver enzymes of South African Windsnyer pigs. A total of 25 Windsnyer male grower pigs were used. Pigs were assigned to dietary treatments in a completely randomized designed and allocated to diets which were formulated to contain 0, 50, 100, 150 and 200 g/kg DM of AOC. Feed and water were provided *ad libitum* to pigs for six weeks. On the day of slaughter, blood samples were collected from each pig and kept in a cooler box during the collection period and stored at - 20 ^{0}C pending analyses. Data were analysed using the response surface regression of SAS. The albumin concentration of pigs linearly decreased with incremental levels of AOC, $Y = -11.268x + 10.405$, $R^2 = 0.33$, ($P < 0.05$). The concentration of total protein (TP) and globulin (G) decreased quadratically with incremental levels of AOC and their equations are as follows $TP = -0.062x^2 + 3.291x – 30.957$, $R^2 = 0.20$ ($P < 0.05$) and $G = - 0.068 x^2 + 2.945x - 19.494$, $R^2 = 0.20$ ($P < 0.05$). On the other hand, Alkaline phosphatase (ALP) increased quadratically with increasing levels of AOC, $ALP = 0.467x^2 – 4.591x + 19.347$, $R^2 = 0.27$, ($P < 0.05$). The other blood metabolites and liver enzymes were not related to the inclusion level of AOC ($P > 0.05$). The negative relationship shows that high levels of the oil cake compromised nutrient utilisation and bioavailability in Windsnyer pigs.

Key words; Serum; secondary compounds; total protein; albumin; diets

5.1 Introduction

Livestock animals form an integral element of livelihoods of about 70 % of the global poor population and the main breeds kept by resource limited farmers in rural areas are mainly indigenous breeds which are adapted to local conditions and surroundings (Livestock in Development, 1999). The sustainable conservation of indigenous pig genotypes needs characterisation of their characteristics and proper market channels for resource-limited farmers. Indigenous pig genotypes are dispersed in Southern Africa and are kept for many reasons by local people (Anderson, 2003).

The Windsnyer pig is a South African indigenous pig with very little research that has been conducted on it (Kanengoni et al., 2014; Ncobela et al., 2018). The merits of this breed include adaptability to extreme environmental conditions, tolerance to gastro-internal parasites, the ability to efficiently utilize fibrous based feed stuff, thriving in nutritional challenges, small body frame size and having strong feet (Chiduwa et al., 2008; Halimani et al., 2010; Ncobela et al., 2018). These slow-growing pigs are kept extensively due to feed shortages. They rely on scavenging during the day and minimal attention is given to these pigs. Their small body size, which is correlated to its poor growth rate, makes them require small spaces when kept intensively and fed on balanced diets.

The number of pigs that farmers can keep is largely affected by the availability of feed. Resource-poor farmers who are rearing indigenous pigs keep a small number of pigs due to insufficient feeds sources. Factors such as lack of high-quality feeds, low revenue and lack of markets contribute to the low herd sizes (Mashatise, 2002). The small herd sizes are likely to lead to extinction due to inbreeding and possible natural disasters. It is important to conserve these Windsnyer pigs. The use of local non-conventional feed sources or agricultural by-products such as Amarula oil cake (AOC) in Windsnyer pig diets might solve feed-related challenges that smallholder farmers face. Windsnyer pigs responded well to feeding on ensiled maize cobs (Kanengoni et al., 2015).

Low levels of Amarula oil cake diets increased the average daily feed intake of Windsnyer pigs; however, high levels of AOC restricted growth performance of Windsnyer pigs (Thabethe et al., 2022). Besides proteins, fibre and fats, AOC also contains secondary compounds that may interfere with nutrient metabolism of pigs. Phytic and oxalic acids are some of the main secondary compounds which may reduce the bioavailability of nutrients, therefore reducing growth performance (Khajali and Slominski 2012).

Nutritionally related metabolites and liver enzymes play a vital role as indicators of the nutritional status of pigs. These metabolites are used to detect nutritional discrepancies and toxicity of diets. Therefore, dose-response studies that investigate the relation between incremental levels of AOC, blood serum metabolites and liver enzymes of Windsnyer pigs are necessary. The objective of the study was, therefore, to assess the relationship between increasing levels of AOC, nutritionally related metabolites, and liver enzymes of Windsnyer pigs. It was hypothesized that nutritionally related metabolites of pigs increase in response to AOC inclusion level.

5.2 Materials and methods

5.2.1 Study site

The study site has been described in Chapter 3.

5.2.2 Collection, preparation of Amarula oil cake and diets

The collection and preparation of the experimental diets has been described in Chapter 3.

5.2.3 Experimental design and management of pigs

The experimental design and management of Windsnyer pigs has been described in Chapter 3.

5.2.4 Chemical analyses of experimental diets

The chemical composition of the experimental diets has been described in Chapter 3.

5.2.5 Blood collection

All pigs were fasted for 24 hours at the end of the growth performance trial, which lasted for six weeks. Then, prior to weighing, a blood sample of 10 ml was taken from each pig via jugular venipuncture and transferred to non-coagulated vacutainer tubes (Becton Dickinson, Franklin, NJ) that contained sodium heparin. On the final day of the trial, all blood samples were taken in the morning prior to the weighing of the pigs. Each blood sample was kept in an ice-filled cooler box throughout blood collection. Within two hours of collection, samples were allowed to coagulate before being centrifuged. They underwent a 10-minute, 1000 x g centrifugation at 25 °C. The serum was centrifuged, separated, and kept in polypropylene tubes that were stored at - 20 °C, until they were analysed.

5.2.6 Laboratory analyses of serum samples

Total protein (TP) concentration was analysed using a technique outlined by Doumas and Biggs, (1972). Following the description of the colometric method by Tietz et al. 1993, alkaline phosphatase (ALP) was analysed. According to Bergmeyer et al. (1986), the concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analysed using the ultraviolet technique. The enzymatic method was used to analyse uric acid (Tietz et al., 1993). According to Tietz (1976) and Lowry et al. (1951) the concentration of iron and albumin were analysed spectrophotometrically. Albumin and total protein were subtracted to determine globulin, and albumin, globulin was divided to determine the albumin to globulin ratio (A:G ratio).

5.2.7 Statistical analyses

The serum nutritionally related blood metabolites data was subjected to the PROC UNIVARIATE procedure of SAS (2008) to check for its normality and thereafter, the response surface regression of SAS (2008) was used to determine the relationship between inclusion level of AOC with TP, ALP, AST, ALT, uric acid, iron, albumin, globulin, and the albumin:globulin ratio. The least square means were computed using the probability difference (PDIFF) option of the General liner model of SAS (2008).

5.3 Results

5.3.1 Nutritional related blood metabolites of Windsnyer pigs

Table 5.1 shows the relationship between blood nutritionally related metabolites of South African Windsnyer pigs to increasing levels of Amarula oil cake diets (AOC). The albumin concentration of pigs linearly decreased with incremental levels of AOC $Y = -11{,}268x + 10{,}405$, $R^2 = 0{,}325$, ($P < 0{,}05$). Feeding incremental levels of Amarula oil cake diets negatively affected albumin concentrations of Windsnyer pigs. The concentration of total protein and globulin decreased quadratically with incremental levels of AOC and their equations are as follows $TP = -0.062x^2 + 3.291x – 30.957$, $R^2 = 0.1977$ ($P < 0.05$) and $G = - 0.068x^2 + 2.945x – 19.494$, $R^2 - 0.195$ ($P < 0.05$). Feeding low levels of AOC improved the concentration of total protein and globulin while higher levels of AOC decreased the concentration of total protein and globulin. Other blood metabolites such as uric acid, Iron and A:G ratio showed no relationship with increasing levels of Amarula oil cake ($P > 0.05$). The was no relationship between AOC inclusion level and the concentration of uric acid, Iron and A:G ratio of Windsnyer pigs.

5.3.2 Liver enzymes of Windsnyer pigs

Table 5.2 shows the relationship between increasing inclusion levels of Amarula oil cake and liver enzymes of Windsnyer pigs. Increasing levels of Amarula oil cake diets quadratically increased Alkaline phosphatase concentrations, $ALP = 0.467x^2 – 4.591x + 19.347$, $R^2 = 0.268$, ($P < 0.05$). The concentration of alkaline phosphatase decreased at low levels of AOC and further increment of the diet also increased alkaline phosphatase. The was no relationship between increasing levels of AOC with aspartate transaminase and alanine transaminase ($P >0.05$). Amarula oil cake inclusion levels did not cause any effect on aspartate transaminase and alanine transaminase.

Table 5.1: Relationship between increasing levels of AOC and serum related metabolites of Windsnyer pigs

	AOC inclusion level g/kg					SEM	Regression coefficient	
Traits	0	50	100	150	200		Linear	Quadratic
Albumin	4.28	4.33	4.58	4.78	4.90	0.17	-11.29^{**}	2.42^{NS}
TP	29.36	24.72	28.29	26.89	27.64	3.01	3.29^{NS}	-0.06*
Uric acid	38.39	34.41	39.30	38.37	40.04	5.99	1.24^{NS}	-0.02^{NS}
Globulin	25.08	20.33	23.71	22.11	22.75	2.90	2.95^{NS}	-0.07^{*}
Iron	381.60	379.60	339.20	379.40	363.20	31.46	0.18^{NS}	-0.002^{NS}
A:G ratio	0.19	0.22	0.23	0.22	0.22	0.03	192.57^{NS}	-341.8^{NS}

SEM: Standard error of the mean; NF: Not found NR: Normal ranges; NS: Non-significant; Sig: significant; AOC: Amarula oil cake; ALP; Alkaline phosphatase (U/L); AST; Aspartate transaminase (U/L); ALT, Alanine transaminase (U/L); Albumin (g/dl); Albumin: Globulin ratio (A: G) ratio; TP, Total protein (g/dl); Uric acid (mg/dL); Iron (ug/dl). Normal ranges: Albumin (85 – 160 g/dL); TP (6.0 - 8.0 g/dL); Uric acid (NF); Globulin (3.9 – 6 mg/dL); Iron (NF); A:G ratio (NF) (Kanengoni et al., 2014).

Table 5.2: Relationship between increasing levels of AOC and liver enzymes of Windsnyer pigs

	AOC inclusion level (g/kg DM)					SEM	Regression coefficients	
Traits	0	50	100	150	200		Linear	Quadratic
ALP	4.60	4.20	4.40	4.80	6.80	0.90	-4.59^{NS}	0.47*
AST	182.00	146.80	131.00	201.40	211.00	38.58	-0.04^{NS}	0.001^{NS}
ALT	78.80	123.20	86.20	109.60	87.40	25.08	-0.05^{NS}	0.002^{NS}

SEM: Standard error of the mean; NS: Non-significant; Sig: significant; AOC: Amarula oil cake; ALP; Alkaline phosphatase (U/L); AST; Aspartate transaminase (U/L); ALT, Alanine transaminase (U/L); Albumin (g/dl).

5.4 Discussion

Blood metabolites are important parameters that represent the physiological state of the animal (Khan and Zafar, 2005). Apart from determining growth response of Windsnyer pigs to AOC based diets, nutritionally related blood metabolites and liver enzymes can also be used to assess the physiological status of pigs, the bioavailability of nutrients and the toxicity level of AOC when fed to Windsnyer pigs. Lowrey et al. (1962), stated that the concentration of total serum protein might be a good indicator of dietary protein in pigs. On the other hand, Esonu et al. (2002), reported that activities of AST, ALP and ALT can be used to assess the liver damage of animals caused by the toxic effects of different diets.

A negative relationship between increasing levels of AOC and albumin was observed. The linear decrease in the concentration of albumin could be related to decremental levels of crude protein in the experimental diets. The concentration of albumin shows the protein status of pigs, and it is also an important indicator of the capacity of the liver to synthesize nutrients mainly proteins which are precursors of amino acids (Mahdavi et al., 2012; Hlatini et al., 2016). The decreasing average daily gain with increasing levels of AOC reported in the previous chapter also supports the decreasing concentration of albumin in the current study. A reduction in serum albumin of pigs fed diets containing insufficient crude protein was reported suggesting that low levels of protein in diets reduces serum albumin and results in hypoalbuminemia (Yang et al., 2008; Kamalakar et al., 2009). Studies that will investigate the gut morphology or the health of Windsnyer pigs to AOC diets are required.

A decrease in the concentration of total protein was expected due to the observed decrease in the concentration of albumin and globulin in the current study. Increasing levels of AOC quadratically decreased concentration of total protein as expected, however, our results for serum protein were above the ranges of the results reported by Kanengoni et al. (2014) which requires further investigation. The quadratic response suggest that total protein increased at low inclusion level of AOC but further increasing the oil cake in the diet decreased the concentration of serum protein in the blood. The quadratic decrease is related to the quadratic decrease in average daily feed intake of pigs fed increasing levels of AOC as previously reported in the previous chapter (Thabethe et al., 2022). When nutrient requirements are met, pigs stop eating and when nutrients such as protein are lacking in diets pigs increase feed intake to compensate for unavailable nutrients in feeds. Our results contradicted those of Khanyile et al. (2016) who observed no relationship between incremental levels of *Vachellia tortilis* leaf meal with total protein. Surprisingly, Hlatini et al. (2016) reported an increase in serum protein of pigs fed increasing levels of *Vachellia tortilis* leaf meal treated with polyethylene glycol.

Globulins are defined as globular proteins which are insoluble in water which dissolves in salts concentrations (Ncobela et al., 2017). A quadratic decrease in the concentration of globulin was observed. The quadratic decrease in globulin is related to the concentration of total protein which also decreased quadratically in the current study. Globulin values were extremely high and above the ranges of those reported by Kanengoni et al. (2014) and Ncobela et al. (2018). The discrepancies between our results and the above-mentioned authors might be due to variation in composition of the diets used, age and weight differences of pigs which warrants further investigation.

In addition, high environmental temperatures were also reported to increase globulin concentrations in ruminants, therefore, the effect of environmental temperature on pigs fed AOC based diets also needs to be investigated (Qokweni et al., 2022).

The concentration of ALP, AST and ALT are indicators of liver function (Ndou et al., 2015). For example, ALP is a membrane-bound enzyme that can be used to evaluate the integrity of the plasma membrane and endoplasmic reticulum and high concentration of ALP indicates membrane damage to tissues. Furthermore, increasing in the concentration of ALP may also be a good indicator of stress which impairs pig welfare (Akanji et al.,1993). As expected, the concentration of ALP increased quadratically, and it contradicted Ncobela et al. (2018) who reported a linear increase in his findings. Even though a significant increase in ALP was observed, the values of ALP were far below the normal ranges of Kanengoni et al. (2014) and Ncobela et al. (2018). At low levels of AOC, ALP decreased but as AOC was increased, ALP also increased. This suggest that further increasing AOC also increases the secondary compounds of AOC. Phenolic compounds such as tannins are reported to cause acute toxicosis and hepatocellular damage in pigs (Ndou et al., 2015). In our case, it is quite difficult to relate the quadratic increase of ALP to acute toxicosis and hepatocellular damage of the liver of pigs since the increment of ALP was very low and below the range of De et al. (2020).

5.5 Conclusions

The concentration AST, ALT, Iron, uric acid and A: G ratio were not related to the increasing levels of AOC. Albumin had a negative relationship, total protein and globulin also had a negative relationship which was quadratic. Alkaline phosphatase increased linearly. The negative relationship shows that high levels of the oil cake compromised nutrient utilisation of Windsnyer pigs, and it is also important to assess the fatty acid composition of pigs fed AOC based diets.

5.6 References

Akanji, M.A., Olagoke, O.A. and Oloyede, O.B., 1993. Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. *Toxicology*, 81, 173 - 179.

Anderson, S., 2003. Animal genetic resources and sustainable livelihoods. *Ecological Economics*, 45, 331 - 339.

Bergmeyer, H.U., Herder, M. and Ref, R., 1986. International federation of clinical chemistry (IFCC). *Clinical Journal of Biochemistry*, *24*, 497 - 510.

Chiduwa, G., Chimonyo, M., Halimani, T.E., Chisambara, S.R. and Dzama, K., 2008. Herd dynamics and contribution of indigenous pigs to the livelihoods of rural farmers in a semi-arid area of Zimbabwe. *Tropical Animal Health and Production*, 40, 125 - 136.

De, A.K., Sawhney, S., Mondal, S., Ponraj, P., Ravi, S.K., Sarkar, G., Banik, S., Malakar, D., Muniswamy, K., Kumar, A. and Tripathi, A.K., 2020. Host-parasite interaction in *Sarcoptes scabiei* infestation in porcine model with a preliminary note on its genetic lineage from India. *Animals*, 10, 23 - 12.

Doumas, B.T., and Biggs, H.G. 1972. Determination of serum albumin. Pages 175 in G.A. Cooper, ed. Standard methods of clinical chemistry, Academic Press, Inc., New York 5.

Esonu, B.O., Iheukwumere, F.C., Emenalom, O.O., Uchegbu, M.C., and Etuk, E.B. 2002. Performance, nutrient utilisation and organ characteristics of broilers fed

Microdesmis puberula leaf meal. *Livestock Research and Rural Development*. 14(6).

Halimani, T.E., Muchadeyi, F.C., Chimonyo, M. and Dzama, K., 2010. Pig genetic resource conservation: The Southern African perspective. *Ecological Economics,* 69, 944 - 951.

Hlatini, V.A. and Chimonyo, M., 2016. Nutritionally related blood metabolites and liver enzymes in growing pigs fed on *Acacia tortilis* treated with polyethylene glycol. *Livestock Science*, 187, 158 -161.

Holness, D.H., 1991. The tropical agriculturist. Pigs. The tropical agriculturist. Pigs.

Kamalakar, R.B., Chiba, L.I., Divakala, K.C., Rodning, S.P., Welles, E.G., Bergen, W.G., Kerth, C.R., Kuhlers, D.L. and Nadarajah, N.K., 2009. Effect of the degree and duration of early dietary amino acid restrictions on subsequent and overall pig performance and physical and sensory characteristics of pork. *Journal of Animal Science*, 87, 3596 - 3606.

Kanengoni, A. T., Chimonyo, M., Erlwanger, K. H., Ndimba, B. K., Dzama, K. 2014. Growth performance, blood metabolic responses, and carcass characteristics of grower and finisher South African Windsnyer-type indigenous and Large White × Landrace crossbred pigs fed diets containing ensiled corncobs. *Journal of Animal Science*, 92, 5739 - 5748.

Khajali, F. and Slominski, B.A., 2012. Factors that affect the nutritive value of canola meal for poultry. *Poultry Science*, 91, 2564 - 2575.

Khan, T.A., and Zafar, F. 2005. Haematological study in response to varying doses of estrogen in broiler chicken. *International Journal of Poultry Science*, 4, 748 – 751.

Khanyile, M., Ndou, S.P. and Chimonyo, M., 2016. Influence of *Acacia tortilis* leaf meal-based diet on serum biochemistry, carcass characteristics and internal organs of finishing pigs. *Animal Production Science*, 57, 675 - 682.

Livestock in Development (LID) (1999). Livestock in poverty focused development. Livestock in Development, Crewekerne, UK, 94 pp.

Lowrey, R.S., Pond, W.G., Barnes, R.H., Krook, L. and Loosli, J.K., 1962. Influence of caloric level and protein quality on the manifestations of protein deficiency in the young pig. *The Journal of Nutrition*, *78*, 245 - 253.

Lowry, O.H., Rosebrough, N.J., Farr, A.I. and Randall, R.J., 1951. Protein measurement with folin-phenol reagent. *Journal of Biological Chemistry*, 193, 75 - 265.

Mahdavi, A., Shivazad, M., Alemi, F., Zaghari, M., Moravej, H., Darabighane, B., 2012. Digestible lysine requirement of broilers based on practical diet. *Italian Journal of Animal Science*, 11, 68 – 76.

Ncobela, C.N., Kanengoni, A.T. and Chimonyo, M., 2018. Response in nutritionally related blood metabolites, carcass traits and primal pork cuts of slow growing Windsnyer pigs fed on varying levels of potato hash silage. *South African Journal of Animal Science*, 48, 770 - 776.

Ndou, S.P., Khanyile, M. and Chimonyo, M., 2015. Growth performance and nutrition-related serum metabolites in growing pigs fed on *Acacia tortilis* leaf meal. *Livestock Science*, 182, 22 - 27.

SAS, (2008). SAS User’s Guide: Statistics, Version9.1. SAS Institute.

Thabethe, F., Hlatini, V.A., de Almeida, A.M. and Chimonyo, M., 2022. Growth performance of South African Windsnyer pigs to the dietary inclusion of Amarula oil cake. *Tropical Animal Health and Production*, 54,1 - 9.

Tietz, N.W., 1976. Fundamentals of clinical chemistry WB Saunders Co. Philadelphia PA, 47 - 51.

Tietz, N.W., Rinkerand, D., and Shaw, L.M. 1993. IFCC method for alkaline phosphatase. Journal of Clinical Biochemistry, 21, 1731 – 1748.

Yang, Y.X., Jin, Z., Yoon, S.Y., Choi, J.Y., Shinde, P.L., Piao, X.S., Kim, B.W., Ohh, S.J. and Chae, B.J., 2008. Lysine restriction during grower phase on growth performance, blood metabolites, carcass traits and pork quality in grower finisher pigs. *Acta Agriculturae Scandinavica section A-Animal Science*, 58, 14 - 22.

CHAPTER 6: Fatty acid composition and health indices of pork from pigs fed diets containing Amarula oil cake

Abstract

The study was conducted to determine the relationship between inclusion level of Amarula oil cake, fatty acid composition and health lipid indices of pork tissues. A total of 25 male Windsnyer boar pigs were used. Pigs were assigned to separate pens in a completely randomised design and allocated to the five experimental diets which were formulated to contain 0, 50, 100, 150 and 200 g/kg DM of Amarula oil cake (AOC). After the growth performance study, all pigs were slaughtered, and pork samples were taken from the Longissimus dorsi muscles (from the 5th and 13th rib) and kept at -20 ^{0}C pending analyses of fatty acids. Increasing AOC inclusion level, linearly increased C12:0, C14:1n9c, C18:1n9t and C18:3n6 ($P < 0.05$). Increasing levels of Amarula oil cake linearly decreased the sum of SFA, PUFA/SFA ratio, C14:0, C16:0, C18:0, C20:0, C18:1n11c and C20:3n3 ($P < 0.05$). There was a quadratic decrease in n-3 fatty acids, n-6/n-3 ratio, nutritive value, C22:0, C18:1n9c, C18:3n3, C18:2c911t, C20:4n6 and C22:5n3 of pork ($P < 0.05$). The total MUFA, PUFA, n-6 fatty acids, AI and TI were not related to Amarula oil cake inclusion level ($P > 0.05$). Due to the quadratic relation of n-3 PUFA, n-6/n-3 ratio of FA and nutritional value of pork, it is recommended that AOC based diets be fed up to 150 g/kg DM

Key words: Lipids, Saturated fatty acids, Polysaturated fatty acids, Nutritive value, thrombogenic index.

6.1 Introduction

Meat is an essential dietary constituent that forms the main part of consumer nutritional requirements such as fatty acids, proteins, and amino acids (Costa et al., 2011). The development of people's lifestyles has changed the focus of meat consumption from quantity to quality (Henchion et al., 2014), which requires meat consumers to demand tastier and healthier meat of local origin due to the effects of non-organic products. The promotion of organically produced indigenous pork on the market enhances the choices for the increasing health-conscious population (Madzimure et al., 2017).

Costa et al. (2011), reported that morden and hybrid pigs are advantages compared to indigenous pigs due to their high demand in markets and rapid economic returns, however, they are reported to contain an increased amount of undesirable fatty acids such as the saturated fatty acids (SFA), the n-6 fatty acids and are also related with the unsuitable n-6/n-3 fatty acids ratios (Nuernberg et al., 2015). The replacement of saturated fatty acids with unsaturated fatty acids in monogastric products and human nutrition through feeding strategies is increasing (Bou et al., 2004; Vahmani et al., 2015). On the other hand, superior taste and nutritional value of meat from non-intensively reared indigenous animals was reported (Muchenje et al., 2009; Mapiye et al., 2011). Csapó et al. (2002), also reported good meat quality characteristics of Mangalitsa pigs.

The type of feed consumed by pigs mainly affects the nutritional quality of pork meat. Therefore, changing the diet of pigs and poultry might also change the composition of fatty acids and meat quality relative to the diet (Bee and Wenk, 1994; Dugan et al., 2004). Incremental levels of olive oil cake in pig diets were reported to reduce saturated fatty acid (SFA) and increased monosaturated fatty acids (MUFA), hence enhancing fatty acid composition of pork (Joven et al., 2014). The demand for healthy meat that contains less amount of saturated fatty acids is increasing because saturated fatty acids are associated with increasing the risk of heart diseases and some cancers (Walker et al., 2005). The use of agro-industrial by-products such as oil cakes from oil extraction industries such as the biofuel and food processing industries could reduce environmental pollution, improve the nutritional quality and fatty acids of pork, therefore, benefit the pig industry.

Amarula oil cake, an agro industrial by product of Amarula dry kernels is produced commercially in Eswatini by small-scale farmers and also by the Mine Workers Development Agency Marula Project in South Africa (Malebana et al., 2018). The kernels of Amarula are high in ether extract, with high oxidative stability as compared to other oil cakes, high oleic acid, a hypo-cholesterolemic and also anti-diabetic monounsaturated fatty acid (Griffin et al., 1996; Mariod and Abdelwahab, 2012; Malebana et al., 2018). Unlike ruminants, in porcine species, fats are synthesized *de novo* and the favourable fatty acids of Amarula such as monounsaturated fatty acids could improve the fatty composition of Windsnyer pigs. Pork quality characteristics such as fatty acid composition was reported for Large White x Landrace, Duroc and Yorkshire breeds (Li et al., 2013; Khanyile et al., 2020). Apart from Madzimure et al. (2017), there are no studies that have been conducted that assess fatty acid composition and lipid health indices of Windsnyer pigs objectively. The relationships between increasing levels of Amarula oil cake and fatty acid composition of pork from Windsnyer pigs are unknown and the information about these relationships could give clear recommendations about the suitable inclusion level when focusing on pork quality. The objective of the study was to determine the relation between increasing levels of Amarula oil cake, fatty acid composition and health lipid indices of pork. It was hypothesized that the inclusion of Amarula oil cake would improve fatty acid composition and nutritional value index of pork.

6.2 Materials and methods

6.2.1 Study site

The study site has been briefly described in chapter 3.

6.2.2 Collection, preparation of Amarula oil cake and diets

The collection of Amarula oil cake and preparation of the experimental diets has been described in chapter 3.

6.2.3 Experimental design and management of pigs

The experimental design and management of Windsnyer pigs has been briefly described previously in chapter 3.

6.2.4 Chemical analyses of experimental diets

The chemical composition and fatty acid composition of the experimental diets has been described previously in chapter 3 and the fatty acid composition of experimental diets is shown in Table 6.1.

6.2.5 Fatty acid composition of experimental diets and pork

Total lipids of pork from Windsnyer pigs were extracted according to a method described by Folch et al. (1957). Briefly, about 1 g of pork sample from the longissimus dorsi muscle (LD) was weighed on a balance scale. A 2: 1 chloroform–methanol solution, containing 0.001 % butylated hydroxytoluene was used for the extraction of lipids. The sample was homogenised (WiggenHauser D-500 Homogenizer) for 30 s. About 250 µL of the extract was then transmethylated at 70 ºC for about two hours with 2 mL of methanol: sulphuric acid solution. Thereafter, fatty acid methyl esters (FAMEs) were extracted with deionised water and hexane solution at room temperature. After separating the FAMEs that had hexane solution and water, the top hexane layer was then transferred to a tube and then dried with nitrogen in a water bath at 45°C. After drying, about 50 µL of hexane solution was added to the FAMEs and 1 µL of hexane was injected into the gas-chromatograph. The FAMEs were analysed with a gas-chromatograph (Thermo Electron Corporation, Milan, Italy) that was equipped with a flame-ionization detector, a 30 m TR-Fame capillary column with an internal diameter of 0.25 mm and a 0.25 µL film, and a run time of 40 mins. The analyses were conducted with an initial isothermic period (50 ºC for 1 min) and a final temperature of 240 ºC that was reached after three ramps and the temperature was increased at a rate of 25 ºC/min until reaching 175 ºC. Followed by an immediate increase of temperature at a rate of 1.5 ºC/min to reach 200 °C and the temperature was maintained for a minimum of 2 minutes. The injector temperature and detector temperature were as follows 240 ºC and 250 ºC, respectively with a hydrogen gas flow rate of 40 mL/min. Fatty acid methyl esters were identified by comparing the retention times of FAME peaks from samples with those of standard mixtures from Supelco (Supelco 37 Component Fame Mix, 47885-U,Sulpeco, USA).

Data for fatty acids were used to calculate the following ratios of fatty acids.

The saturation (saturated FA: unsaturated FA ratio, S:P), atherogenic index (AI), and thrombogenic index (TI) were calculated as follows: $AI = (C12{:}0 + 4 \times C14{:}0 + C16{:}0)/[\Sigma MUFA + \Sigma(n\text{-}6) + \Sigma(n\text{-}3)]$; $TI = (C14{:}0 + C16{:}0 + C18{:}0)/[0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n\text{-}6) + 3 \times \Sigma(n\text{-}3) + \Sigma(n\text{-}3)/\Sigma(n\text{-}6)]$ (Ulbricht and Southgate, 1991).

Where:

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. The nutritive value of pork was calculated using the formulae: $[(C18{:}0+C18{:}1n9c)/C16{:}0]$ (Fernåndez et al., 2007).

Table 6.1: Fatty acid composition (% of total FAs) of Amarula kernels (Ak) and the experimental diets

	Inclusion level of Amarula oil cake (g/kg DM)					
SFA	AK	0	5	10	15	20
C12:0	0.012	0.063	0.057	0.015	0.0223	0.016
C14:0	0.142	0.197	0.162	0.155	0.159	0.159
C16:0	15.907	14.042	14.949	15.769	16.795	17.751
C18:0	5.360	4.141	4.328	4.634	4.841	5.018
C20:0	0.207	0.522	0.532	0.381	0.318	0.402
MUFA						
C16:1	0. 298	0.23	0.246	0.205	0.243	0.264
C18:1n9c	0. 238	0.134	0.395	0.489	0.455	0.521
PUFA						
C18:2n6	9.228	32.211	32. 012	30.506	30.901	28.899
C18:3n3	0.209	0.982	1.057	1.241	1.285	1.295
C20:2	0.025	0.604	0.387	0.163	0.121	0.104
Sum (SFA)	21.629	18.966	20.027	20.954	22.036	23.345
Sum (MUFA)	0.536	0.371	0. 640	0.694	0.698	0.785
Sum (PUFA)	9.463	33.798	34.455	31.911	32.308	30.298
PUFA: SFA	0.437	1.782	1.720	1.523	1.466	1.298

AK = Amarula kernels, FAs = Fatty acids, AOC = Amarula oil cake, SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids, c = *cis*

6.2.6 Statistical analyses

The response surface regression procedure of SAS, (2008) was used to determine relationships between increasing levels of AOC with fatty acid composition, atherogenic index, thrombogenic indices and nutritive value of pork. The LS means statement of SAS, (2008) was used to compute the least square means using the probability difference (PDIFF) option. The regression model used was as follows:

$Y = D_o + D_1A + D_2A^2 + e$, where

Y = is the response variable (fatty acids, atherogenic index, thrombogenic indices and nutritive value)

D_o= is the intercept

D_1A = is the linear regression coefficient

D_2A^2 = is the quadratic regression coefficient and

e = is the error

6.3 Results

6.3.1 Fatty acid composition of pork muscle tissues from Windsnyer pigs

The relationship between the fatty acid composition of pork from Windsnyer pigs fed diets containing AOC is shown in Table 6.2. Individual fatty acids of pork from Windsnyer pigs such as C12:0, C14:1n9c, C18:1n9t and C18:3n6 increased linearly with increasing levels of AOC ($P < 0.05$). Increasing levels of Amarula oil cake in Windsnyer pig diet linearly decreased individual fatty acids such as C14:0, C16:0, C18:0, C20:0, C18:1n11c and C20:3n3 ($P < 0.05$). Incremental levels of AOC in Windsnyer pig diets quadratically increased Pentadecyclic acid (C15:0) in pork muscle tissues ($P < 0.05$). Feeding Amarula oil diets to Windsnyer pigs quadratically decreased C22:0, C18:1n9c, C18:3n3, C18:2c911t, C20:4n6 and C22:5n3 ($P < 0.05$) of pork muscle tissues. The following individual fatty acids C16:1n9c, C18:1n11t, C20:1n5c, C22:1n13c, C18:2n6 and C20:2n6 of pork were not related to AOC inclusion level in Windsnyer pigs ($P > 0.05$).

6.3.2 Sum of fatty acids, fatty acid ratios and lipid health indices of pork

The sum of fatty acids, fatty acids ratios and lipid health indicators of pork from Windsnyer pigs fed diets containing Amarula oil cake is shown in Table 6.3. Dietary inclusion of AOC decreased SFA and PUFA/SFA ratio of pork from Windsnyer pigs linearly ($P < 0.05$). The omega n-3 fatty acids, n-6/n-3 ratio and nutritive value of pork decreased quadratically with inclusion level of AOC ($P < 0.05$). The sum of MUFA, PUFA, n-6 FA, AI and TI were not related to the inclusion level of Amarula oil cake diets ($P > 0.05$).

Table 6.2: Relationship between increasing levels of Amarula oil cake and fatty acid (% of total FA) composition of pork

	AOC inclusion level						Sig. level			
Fatty acids (% of total FA)	0	50	100	150	200	SEM	Linear	Quadratic	Equations	R^2
C12:0	0.18	0.20	0.23	0.34	0.44	0.09	**	NS	$Y = 37.41x + 1.24$	0.32
C14:0	2.33	1.93	1.53	1.33	1.16	0.42	**	NS	$Y = - 10.30x + 22.43$	0.31
C15:0	0.29	0.27	0.22	0.15	0.12	0.07	**	**	$Y = 125.81x^2 – 115.67x + 26.14$	0.45
C16:0	22.72	20.60	18.94	17.67	17.67	1.21	***	NS	$Y = - 0.05x + 28.07$	0.45
C17:0	0.61	0.56	0.42	0.35	0.25	0.16	**	NS	$Y = - 34.178x + 20.12$	0.28
C18:0	18,73	15.73	14.12	13.00	11.57	1.02	***	NS	$Y = - 6.07x + 70.41$	0.74
C20:0	0.35	0.22	0.22	0.16	0.11	0.09	*	NS	$Y = - 46.63 x +16.40$	0.27
C22:0	2.02	2.00	1.67	10.79	1.28	3.32	NS	**	$Y = - 0.39x^2 – 12.02x +28.70$	0.45
C14:1n9c	0.05	0.04	0.03	0.06	0.11	0.02	*	NS	$Y = 11.98x + 6.58$	0.25
C16:1n9c	2.65	2.53	2.62	2.94	2.39	0.60	NS	NS		
C18:1n9t	0.51	0.53	0.66	0.78	0.84	0.12	**	NS	$Y = 33.84x – 7.09$	0.36
C18:1n11t	0.11	0.19	0.11	0.16	0.34	0.13	NS	NS		
C18:1n9c	32.76	38.95	43.38	45.52	49.28	5.53	*	*	$Y = - 0.02x^2 + 2.46x – 49.16$	0.47
C18:1n11c	20.08	23.43	22.21	19.01	14.22	2.01	*	NS	$Y = - 3.50x + 52.01$	0.37
C20:1n5c	0.86	0.21	0.12	0.75	0.02	0.49	NS	NS		
C22:1n13c	0.15	0.04	0.02	0.01	0.05	0.06	NS	NS		
C18:2n6	16.89	16.48	16.00	15.94	15.82	1.08	NS	NS		
C18:3n6	0.49	0.45	0.41	0.35	0.28	0.08	*	NS	$Y = 13.69x +13.62$	0.29
C18:3n3	0.53	0.64	0.79	0.66	0.57	0.09	NS	*	$Y = - 110.85x^2 + 134.17x -27.56$	0.34
C18:2c911t	0.53	0.55	0.70	0.72	0.74	0.24	NS	***	$Y = - 28.641x^2 +50,57x – 5.90$	0.79
C20:2n6	0.82	0.85	0.84	0.86	0.94	0.20	NS	NS		
C20:3n3	0.76	0.79	0.84	0.86	0.89	0.05	*	NS	$Y = - 18.57x – 2.70$	0.29
C20:4n6	2.15	2.18	2.28	2.39	2.04	0.59	NS	*	$Y = - 3.121x^2 +16.86x – 9.08$	0.29
C22:5n3	0.46	0.17	0.29	0.48	0.30	0.17	NS	*	$Y = - 31.908x^2 + 42.03x + 2.15$	0.29

FA = Fatty acids, AOC = Amarula oil cake, SEM = Standard error of the mean, R^2 = Coefficient of determination, Sig = Significance.

Table 6.3: Relationship between Amarula oil cake inclusion with sum of fatty acids, fatty acid ratios and lipid health indices of pork

	AOC inclusion level						Sig. level			
Fatty acids	0	50	100	150	200	SEM	Linear	Quadratic	Equations	R^2
SFA	47.21	41.50	37.45	44.49	32.60	2.60	***	NS	$Y = - 8.551x + 201.624$	0.808
MUFA	57.16	65.92	69.16	68.25	67.24	4.88	NS	NS		
PUFA	22.62	22.10	22.15	22.25	21.57	1.70	NS	NS		
PUFA/SFA ratio	0.48	0.53	0.59	0.52	0.66	0.05	*	NS	$Y = -212.43x + 53.89$	0.41
Omega n-3	1.75	1.60	1.92	2.00	1.76	0.27	NS	**	$Y = -14.18x^2 + 61.31X – 51.34$	0.48
Omega n-6	20.87	20.50	20.23	20.25	19.81	1.65	NS	NS		
n-6/n-3 ratio	13.90	13.42	10.76	10.20	11.37	1.94	NS	*	$Y = -0.33x^2 + 8.01X – 34.43$	0.37
AI	0.40	0.33	0.28	0.27	0.26	0.03	NS	NS		
IT	0.12	0.11	0.09	0.08	0.09	0.01	NS	NS		
NV	2.27	2.86	3.03	3.22	3.46	0.44	*	*	$Y = -4.20x^2 + 32.00 – 45.97$	0.51

Sig = significant, AI = atherogenic index, IT = thrombogenic index, NV = Nutritional value, R^2 = Coefficient of determination.

6.4 Discussion

Meat is an essential dietary component which provides essential nutrients such as fatty acids, proteins, and amino acids. Research on indigenous pigs such as the Windsnyer pigs focuses on growth performance and digestibility of nutrients (Ncobela et al., 2018, 2022; Hlatini et al., 2021; Hlongwana et al., 2021; Thabethe et al., 2022) while ignoring the fatty acid composition of pork meat which forms an integral part of meat quality. Apart from Madzimure et al. (2017), information about the fatty acid composition of Windsnyer pork is scantly documented. The objective of the study was to assess the relation between increasing levels of Amarula oil cake, fatty acid composition and health lipid indicators of pork from Windsnyer pigs.

A decrease in total amount of saturated fatty acids and individual saturated fatty acids was observed with increasing levels of Amarula oil cake. Arjin et al. (2021), also observed a reduction in the sum of saturated fatty acids of pork from pigs fed diets containing perilla cake. A linear decrease in total amount of saturated fatty acids of pigs to leaf meal diets was previously reported (Khanyile et al., 2020). The reduction of the total saturated fatty acid might be due to the *de novo* synthesis of lipids in adipose tissue of pigs since these fatty acids are less readily influenced by dietary changes like polyunsaturated fatty acids. It is also quite difficult to relate the linear reduction in total saturated fatty acids to the inclusion level of AOC due to the *in vivo* synthesis of saturated fatty acids in monogastric animals as previously reported by Enser et al. (2000).

Pork from conventionally reared pigs such as the Large White, Landrace and Duroc pigs is associated with high levels of unfavourable fatty acids such as the saturated fatty acids. The reduction in total amount of saturated fatty acids suggest that pork from indigenous pigs might improve the health status of meat, therefore improving the health of consumers relying on organic food products. Increasing Amarula oil cake diets also increased the dietary fibres which might have also contributed to the reduction of the total saturated fatty acids. Nutrient absorption varies depending on physico-chemical characteristics of fibres. Fibres are reported to affect nutrient utilization in monogastrics, they adsorb other dietary compounds such as lipids during transit in the gastrointestinal tract of animals (Ndou et al., 2019). Individual saturated fatty acids such as Lauric (C12:0) and Pentadecylic acid (C15:0), increased with the inclusion level of Amarula oil cake. Teye et al. (2006) also reported an increase in Lauric acid of pork from pigs supplemented with palm kernel oil.

An increase in the amount of total monounsaturated fatty acids of pork was expected. However, the total amount of monounsaturated fatty acids and polyunsaturated fatty acids were not related to the inclusion level of Amarula oil cake. Similarly, increasing levels of olive cake-based diets had no effect on the sum of monounsaturated fatty acid composition of pork from Bísaro pigs (Leite et al., 2022). Most of the monounsaturated fatty acids found in pork were not detected in our diets which also support the *de no* synthesis of lipids in monogastric animals.

Another possible factor which might have contributed to the diminishing of monounsaturated fatty acids in the experimental diets might be due to oxidation of lipids during the collection of dietary samples after the formulation of experimental diets. Our observation contradicted that of Joven et al. (2014) who indicated an increase in the sum of monounsaturated fatty acids from pigs fed olive-based diets. A quadratic increase in the sum of monosaturated fatty acids of pork from pig fed on *Vachellia* tortilis leaf meal was also indicated by Khanyile et al. (2020). The discrepancies between the authors might be attributed to breed differences and the age at which pigs were slaughtered. Some individual monounsaturated fatty acids of pork such as C14:1n9c and C18:1n9t increased linearly as expected. These fatty acids were not detected in Amarula kernels, and the experimental diets as previously indicated by Mthiyane and Hugo, (2019).

Oleic acid (C18:1n9c) is known as the main monounsaturated fatty acid found in Amarula kernels and it decreased quadratically with AOC inclusion level on pork muscle tissues. The numerical values for Oleic acid were high and were closely similar to the values that were previously reported by Malebane et al. (2018). Remarkably high numerical values of Oleic acid from Amarula kernels were also indicated (Mthiyane and Mhlanga, 2017; Mthiyane and Hugo 2019). Oleic acid has been reported to have high oxidative stability as compared to other olive oils therefore suggesting that it might have the potential to increase shelf-life of pork of Windsnyer pigs (Mariod and Abdelwahab, 2012; Burger et al., 1987). Oleic acid is also reported to play a role in lowering blood fat, having anti-diabetic properties and it prevents many inflammatory processes in the blood (Burger et al., 1987; Mariod and Abdelwahab, 2012; Miura et al., 2013).

Increasing levels of AOC linearly increased PUFA/SFA ratio of pork of Windsnyer pigs. This observation contradicted with Khanyile et al. (2020) and Leite et al. (2022).

Similarly, our observation was similar to Sringarm et al. (2022) who also observed an increased PUFA/SFA ratio of pigs supplemented with Perilla cake meal. Besides, nutrition, the genetics of the animal is another main factor that influences the PUFA/SFA ratio of pigs (De Smet et al., 2004). The increasing content of PUFA/SFA ratio of pork was between the recommended ratio which is 0,4 to 0,7 therefore suggesting that pork from Windsnyer pigs might be beneficial (Wood et al., 2003; Teye et al., 2006). The linear increase also suggests that further increasing AOC in pig diet will continue to increase the PUFA/SFA ratio of pork.

A linear increase in Omega 3 fatty acids and the ratio between n - 6 and n - 3 fatty acids was expected due to the high residual oil of Amarula oil cake which is proven to alter the fatty acid composition of pork tissues (Arjin et al., 2021). However, that was not the case in the current study, a quadratic decrease in n - 3 and n - 6/n - 3 fatty acids was observed. The numerical values for n - 6/n - 3 ratio of fatty acids were far beyond the standard values recommended in foods which is less than 4, however, a high n - 6/n - 3 ratio of 7.22 % of total fatty acids was reported in fast growing pigs (Wood et al., 2003). The increasing ratio of n-6:n-3 PUFA increases the incident of heart diseases and some cancers (Enser, 2001). The quadratic decrease in n-6/n-3 PUFA indicates that high levels of AOC have the potential of decreasing n-6/n-3 PUFA in Windsnyer pigs. Mthiyane and Hugo, (2019) also indicated highly significant values (23 and 27 %) of n - 6/n - 3 PUFA from Amarula kernels cultivated in South African and Eswatini.

The quadratic relation of Omega 3 fatty acids suggests that pork from indigenous pigs fed low levels of AOC might have the potential of reducing the risk of cardiovascular diseases through their mode of action such as decreasing the rate of growth of the atherosclerotic plaque and reducing the risk of arrhythmias (Kris-Etherton et al., 2003). Our results however, contradicted those of Khanyile et al. (2020), who observed an increase in Omega 3 fatty acids of pigs fed *Vachellia* leaf meal diets. High amount of Omega 3 fatty acids were also reported in Large White gilts compared to Windsnyer gilts (Madzimure et al., 2017). The inconsistencies between the studies might be due to breed, dietary and age differences.

The atherogenic index was not related to the inclusion level of Amarula oil cake, however, the numerical least square mean values attained were less than 0.5 as previously recommended by the World Health Organisation (WHO, 2003). The quadratic decrease in nutritional value of pork could be related to the linear reduction in saturated fatty acids of pork tissues which in turn reduces the n-6/n-3 ratio of pork.

6.5 Conclusions

The result of this study showed that the dietary inclusion of Amarula oil cake had positive consequences on pork by reducing the total unsaturated fatty acids and PUFA/SFA ratio of pork. Amarula oil cake inclusion had a quadratic effect on omega 3 fatty acids, n - 6/n - 3 ratio and the nutritional value of pork meat. Due to the quadratic relation of n - 3 PUFA, n - 6/n - 3 ratio of FA and nutritional value of pork, it is recommended that AOC based diets be fed up to 150 g/kg DM. The high oxidation rate of Amarula oil and high proportions of oleic acid in pork muscles, makes it important for future studies to investigate the oxidative stability and shelf-life of pork from Windsnyer pigs fed on different levels of Amarula oil cake-based diets.

6.6 References

Arjin, C., Souphannavong, C., Norkeaw, R., Chaiwang, N., Mekchay, S., Sartsook, A., Thongkham, M., Yosen, T., Ruksiriwanich, W., Sommano, S.R. and Sringarm, K., 2021. Effects of dietary perilla cake supplementation in growing pig on productive performance, meat quality, and fatty acid profiles. *Animals*, 11, 3213.

Bee, G. and Wenk, C., 1994. Effect of soybean-oil and beef-tallow supplementation to pig diets on the fatty-acid profile of body lipids. *Journal of Animal Physiology and Animal Nutrition (Germany)*.

Burger, A.E.C., De Villiers, J.B.M., Du Plessis, L.M., 1987. Composition of the kernel oil and protein of the marula seed. *South African Journal of Science*, 83, 733 – 735.

Bou, R., Guardiola, F., Tres, A., Barroeta, A.C. and Codony, R., 2004. Effect of dietary fish oil, α-tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poultry Science*, *83*, 282 - 292.

Cameron, N.D., Enser, M., Nute, G.R., Whittington, F.M., Penman, J.C., Fisken, A.C., Perry, A.M. and Wood, J.D., 2000. Genotype with nutrition interaction on fatty acid composition of intramuscular fat and the relationship with flavour of pig meat. *Meat Science*, 55, 187 - 195.

Csapó, J., Vargá-Visi, É., Csapó-Kiss, Z.S., and Csokona, É., 2002. Fatty acid composition and cholesterol content of the fat of pigs of various genotypes. *Acta Agraria Kaposvariensis,* 6, 107 - 113.

Costa, P.A.F., Costa, P.A., Lopes, C.M., Alfia, R.J.B., Bessa, A.A.M., Roseiro, and J. A.M., Prates. 2011. Fatty acid composition, cholesterol and a- Tocopherol of barossa- PDO veal produced in farms located in lowlands, ridges and mountains. *Journal of Food Composition and Analyses*. 24, 987 - 994.

De Smet, S., Raes, K. and Demeyer, D., 2004. Meat fatty acid composition as affected by fatness and genetic factors: a review. *Animal Research*, *53*, 81 - 98.

Dugan, M.E.R., Aalhus, J.L. and Uttaro, B., 2004. Nutritional manipulation of pork quality: Current opportunities. *Advances in Pork Production*, 15, 237 - 243.

Enser, M. (2001). The role of fats in human nutrition. In B. Rossell (Ed.), Oils and fats, Vol. 2. Animal carcass fats (pp. 77–122). Leatherhead, Surrey, UK: Leatherhead Publishing.

Enser, M., Richardson, R.I., Wood, J.D., Gill, B.P. and Sheard, P.R., 2000. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat science*, 55, 201 - 212.

Fernåndez, M., Ordonez, J.A., Cambero, I, Santos, C., Pin, C., de la Hoz, L. 2007. Fatty acid composition of selected varieties of Spanish dry ham related to their nutritional implications. *Food Chemistry*, 101, 107 - 112.

Folch, J., Lees, M., Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biology and Chemistry*, 226, 497 - 509.

Griffin, M.E., Dimitriadis, E., Lenehan, K., Owens, D., Collins, P., Johnson, A., Tomkin, G.H., 1996. Non-insulin-dependent diabetes mellitus: dietary monounsaturated fatty acids and low-density lipoprotein composition and function. QJM: *An International Journal of Medicine*, 89, 211 - 216.

Henchion, M., McCarthy, M., Resconi, V.C. and Troy, D., 2014. Meat consumption: Trends and quality matters. *Meat Science*, 98, 561 - 568.

Joven, M., Pintos, E., Latorre, M.A., Suárez-Belloch, J., Guada, J.A. and Fondevila, M., 2014. Effect of replacing barley by increasing levels of olive cake in the diet of finishing pigs: Growth performances, digestibility, carcass, meat and fat quality. *Animal Feed Science and Technology*, *197*, 185 - 193.

Kris-Etherton, P.M., Harris, W.S. and Appel, L.J., 2003. Omega - 3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 23, 151 - 152.

Nuernberg, K., Nuernberg, G., Priepke, A. and Dannenberger, D., 2015. Sea buckthorn pomace supplementation in the finishing diets of pigs – are there effects on meat quality and muscle fatty acids?. *Archives Animal Breeding*, *58*, 107 - 113.

Malebana, I.M.M., 2018. Dietary effects of *Sclerocarya birrea caffra* nut meal in growing-fattening male dorper sheep. PhD Thesis, University of the Witwatersrand, South Africa.

Malebana IMM, Nkosi BD, Erlwanger KH, Chivandi E (2018). A comparison of the proximate, fibre, mineral content, amino acid and the fatty acid profile of marula (Sclerocarya birrea caffra) nut and soyabean (Glycine max) meals. *Journal of Science and Food Agriculture*, 98, 1381 - 1387.

Mapiye C., Chimonyo M., Dzama K., Hugo A., Strydom, P.E. & Muchenje, V., 2011. Fatty acid composition of beef from Nguni steers supplemented with Acacia karroo leaf-meal. *Journal of Food Composition and Analyses*, 24, 523 - 528.

Mariod, A.A., Abdelwahab, S.I., 2012. *Sclerocarya birrea* (Marula), an African tree of nutritional and medicinal uses: a review. *Food Reviews International*, 28, 375 - 388.

Miura, K., Stamler, J., Brown, I.J., Ueshima, H., Nakagawa, H., Sakurai, M., Chan, Q., Appel, L.J., Okayama, A., Okuda, N., Curb, J.D., Rodriguez, B.L., Robertson, C., Zhao, L., Elliot, P., 2013. Relationship of dietary monounsaturated fatty acids to blood pressure: the international study of macro/ micronutrients and blood pressure. *Journal of Hypertension*, 31, 1144 - 1150.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P.E., Hugo, A. and Raats, J.G., 2009a. Some biochemical aspects pertaining to beef eating quality and consumer health: a review. *Food Chemistry*. 112, 279 - 289

Mthiyane, D.M.N., and Hugo, A., 2019. Comparative health-related fatty acid profiles, atherogenicity and desaturase indices of marula seed cake products from South Africa and Eswatini. doi:10.20944/preprints201911.0132.v1

Mthiyane, D.M.N., Mhlanga, B.S., 2017. The nutritive value of marula (*Sclerocarya birrea*) seed cake for broiler chickens: nutritional composition, performance, carcass characteristics and oxidative and mycotoxin status. *Tropical Animal Health and Production*, 49, 835 – 842.

Leite, A., Domínguez, R., Vasconcelos, L., Ferreira, I., Pereira, E., Pinheiro, V., Outor-Monteiro, D., Rodrigues, S., Lorenzo, J.M., Santos, E.M. and Andrés, S.C., 2022. Can the Introduction of Different Olive Cakes Affect the Carcass, Meat and Fat Quality of Bísaro Pork? *Foods*, 11, 1650.

SAS, (2008). SAS User's Guide: Statistics, Version9.1. SAS Institute.

Sringarm, K., Chaiwang, N., Wattanakul, W., Mahinchai, P., Satsook, A., Norkeaw, R., Seel-Audom, M., Moonmanee, T., Mekchay, S., Sommano, S.R. and Ruksiriwanich, W., 2022. Improvement of Intramuscular Fat in longissimus Muscle of Finishing Thai Crossbred Black Pigs by Perilla Cake Supplementation in a Low-Lysine Diet. *Foods*, *11*, 907.

Teye, G.A., Sheard, P.R., Whittington, F.M., Nute, G.R., Stewart, A. and Wood, J.D., 2006. Influence of dietary oils and protein level on pork quality. 1. Effects on muscle fatty acid composition, carcass, meat and eating quality. *Meat Science*, 73, 157 - 165.

Thabethe, F., Mkwanazi, M.V., Khanyile, M. and Chimonyo, M., 2018. Adaptation of finishing pigs to graded levels of *Vachellia tortilis* leaf meal diet. *Livestock Science*, 218, 20 - 25.

Ulbricht, T.L.V., Southgate, D.A.T. 1991. Coronary heart disease seven dietary factors. *Lancet*, 338, 985 - 992.

Vahmani, P., Mapiye, C., Prieto, N., Rolland, D.C., McAllister, T.A., Aalhus, J.L., Dugan, M.E.R. 2015. The scope for the manipulating polyunsaturated fatty acid content of beef: A review, *Journal of Animal Science and Biotechnology, 6, 29.*

Walker, P., Rhubart-Berg, P., McKenzie, S., Kelling, K. and Lawrence, R. S. 2005. Public health implications of meat production and consumption. *Public Health Nutrition*, 8, 348 – 356.

Wang, L., Wang, A., Wang, L., Li, K., Yang, G., He, R., Qian, L., Xu, N.Y., Huang, R.H., Peng, Z.Z. and Zeng, Y., 2011. Animal genetic resources in China: pigs. China National Commission of Animal Genetic Resources; China Agriculture Press: Beijing, China.

WHO 2003. Diet, nutrition and the prevention of chronic diseases, Report of a joint WHO/FAO expert consultation, World Health Organization, Geneva, Switzerland, WHO technical report series 916, 2003.

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R. and Enser, M., 2004. Effects of fatty acids on meat quality: a review. *Meat Science*, 66, 21 - 32.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., and Enser, M., 2003. Effects of fatty acids on meat quality: a review, *Meat Science*, 66, 21 – 32.

Chapter 7: General discussion, Conclusions and Recommendations

7.1 General discussion

The broad objective of the study was to assess the relationship between feeding increasing levels Amarula oil cake diets with growth performance of South African Windsnyer pigs and selected pork quality parameters of Windsnyer pigs. The use of dose response trials to determine the relationship between increasing levels of Amarula oil cake, growth indicators of Indigenous pigs and pork quality would provide a cost-effective strategy that predicts the optimum point of inclusion level of AOC that does not affect growth and welfare of pigs. This will benefit farmers more especially resource limited farmers based in rural communities who can't afford to buy expensive chemicals and use laboratory resources to deactivate the toxic effects of Amarula based diets. Additionally, relationship between pork quality parameters of pork such as the fatty acid composition and health lipid indicators to increasing levels of AOC would also give the correct level of AOC that positively affects fatty acids of pork meat.

In chapter 3, the objective of the study was to determine the maximum inclusion level of Amarula oil cake diets based on growth performance parameters of Windsnyer pigs. It was hypothesized that the inclusion level of AOC beyond the maximum point would elicit a quadratic response in terms of average daily gain of Windsnyer pigs. The results for chapter 3 indicated that the diet had a significant effect on ADFI, ADG, FCR and SADG of Windsnyer pigs. However, the SADFI of Windsnyer pigs was not significantly affected by Amarula oil cake diet. Feed conversion ratio of Windsnyer pigs was also affected by the age of the pig. Increasing levels of Amarula oil cake diets quadratically decreased ADFI of Windsnyer pigs. Low levels of the oil cake showed an increase in ADFI, as AOC was increasing the ADFI of pigs also decreased after attaining the maximum ADFI. This suggests that Windsnyer pigs were efficient at low levels of the oil cake that was not beyond 100 g/kg DM. Increasing levels of AOC had a negative relationship on ADG, FCR and SADG. The negative relationship of ADG, FCR and SADG is also supported by the negative quadratic relationship of ADFI of pigs which suggest that high incremental levels of Amarula oil cake diets compromised growth performance indicators of pigs.

In chapter 4, the objective of the study was to determine the relationship between carcass traits, primal pork cuts and visceral organ weights of Windsnyer pigs to AOC inclusion level. It was hypothesized that feeding incremental levels of Amarula oil cake diets would linearly increase carcass traits, primal pork cuts and visceral organ weight of Windsnyer pigs. There was a negative linear relationship between increasing levels of Amarula oil cake, carcass length, warm carcass weight and cold carcass weight. This suggests that high inclusion levels of Amarula oil diets negatively affect the above-mentioned parameters of Windsnyer pigs. Stomach weight, backfat thickness, drip loss and the hepatosomatic index increased linearly with increasing levels of Amarula oil cake. The high fibre characteristics, high ether extract and anti-nutritional compounds of Amarula kernels, increased the stomach weight, backfat thickness, drip loss and the hepatosomatic index of pigs. The kidneys, small intestines, and large intestines weight of Windsnyer pigs had a quadratic response to Amarula oil cake inclusion level. When carcass traits and visceral organ weight are of particular interest in Windsnyer pigs, Amarula oil cake diets can be included in pig diets up to100 g/kg DM.

In chapter 5, the objective of the study was to assess the changes in nutritionally related blood metabolites and liver enzymes of Windsnyer pigs fed on increasing levels of AOC based diets. It was hypothesized that increasing levels of AOC will linearly decrease nutritional related blood metabolites and liver enzymes of Windsnyer pigs. Increasing levels of AOC had a negative linear relationship on the concentration of albumin. As AOC increased in the diets, albumin concentration was observed to be decreasing. Albumin indicates the protein status of animals, the decreasing albumin concentration of pigs could also indicate the unavailability of nutrients in AOC diets which in turn negatively affected growth performance of Windsnyer pigs. The concentration of total protein and globulin decreased quadratically with incremental levels of AOC. High levels of AOC negatively affect the concentration total protein and globulin of Windsnyer pigs. The protein content of the experimental diets was formulated to meet or exceed the requirements of pigs, however, the results indicated that the diet had a significant effect on serum protein of pigs which make it more important to investigate the mechanism at which the secondary compounds of Amarula kernels affects the protein function of indigenous pigs. Liver enzymes such as ALP, AST and ALT are some of the important parameters used in monogastric nutrition to assess the nutritional quality, toxic effects and hepatic diseases in pigs. Alkaline phosphatase (ALP) increased quadratically with increasing levels of AOC. The quadratic increase shows that low levels of AOC decreased ALP but high levels of AOC increased it. Our values of ALP were below the values of those who were reported by other researchers. Increasing ALP is associated with acute toxicosis and hepatocellular damage of the liver in pigs, therefore high inclusion levels of Amarula oil cake must be avoided.

In chapter 6, the objective of the study was to determine the fatty acid composition and health lipid indices of pork from Windsnyer pigs fed different levels of AOC. It was hypothesized that high inclusion level of AOC would improve the fatty acid composition and health lipid indices of pork. Feeding incremental levels of AOC to Windsnyer pigs increased the following individual fatty acids linearly namely, C12:0, C14:1n9c, C18:1n9t and C18:3n6. The sum of SFA, PUFA/SFA ratio and some individual fatty acids such as C14:0, C16:0, C18:0, C20:0, C18:1n11c and C20:3n3 linearly decreased with the inclusion level of Amarula oil cake diets in Windsnyer pigs. This indicates that pork from indigenous pigs fed with Amarula based diets contains less amount of SFA which are deemed to be undesirable for human consumption. Secondary compounds of plants act as natural antioxidants in meat, thereby improving meat quality and shelf-life and tannins for example, are reported to reduce nutrient availability by precipitating protein, minerals and saturated fatty acids in muscle tissues of pork. There was a quadratic decrease in n - 3 fatty acids, n - 6/n - 3 ratio, nutritive value, C22:0, C18:1n9c, C18:3n3, C18:2c911t, C20:4n6 and C22:5n3 of pork. Feeding low levels of AOC improved the composition of n - 3 fatty acids, n - 6/n - 3 ratio and the nutritional value of pork from indigenous Windsnyer pigs. The n - 6/n - 3 ratio observed in the study was far beyond the recommended values for foods which could have been attributed to the high proportion of some of the monounsaturated and polyunsaturated fatty acids.

7.2 Conclusions

The inclusion of Amarula oil cake in Windsnyer pig diets negatively affected growth performance of slow growing Windsnyer pigs by reducing ADFI, ADG, FCR and SADG. By using the broken stick model of regression, the optimum inclusion level of

AOC was attained at 102,17 g/kg DM and further increment of AOC beyond the optimum point compromised ADFI of Windsnyer pigs. Therefore, when feeding Windsnyer pigs AOC diets it is recommended to use appropriate inclusions that are not above 100 g/kg DM. Increasing levels of Amarula oil cake negatively affected the slaughter weight, carcass length, warm carcass weight, cold carcass weight, dorsal fat thickness, stomach weight, kidney weight, small and large intestine weight of Windsnyer pigs, however, increasing levels of AOC showed a positive influence on backfat thickness and drip loss of Windsnyer pigs. Incremental levels of Amarula oil cake diets impaired carcass characteristics and selected visceral organs of pigs. Feeding incremental levels of AOC diets to Windsnyer pigs negatively affected the concentration of albumin, total protein, and globulin. Alkaline phosphatase concentration of the serum of Windsnyer pigs was positively affected by AOC. Dietary inclusion of Amarula oil cake had positive consequences on pork of Windsnyer pigs by reducing the sum of saturated fatty acid and PUFA/SFA ratio of pork. However, high inclusion of the oil cake negatively affected Omega 3 fatty acids, n - 6/n - 3 ratio and the nutritional value of pork. Due to the quadratic relation of n - 3 PUFA, n - 6/n - 3 ratio of FA and nutritional value of pork, it is recommended that AOC based diets be fed up to 150 g/kg DM when pork quality is of particular interest.

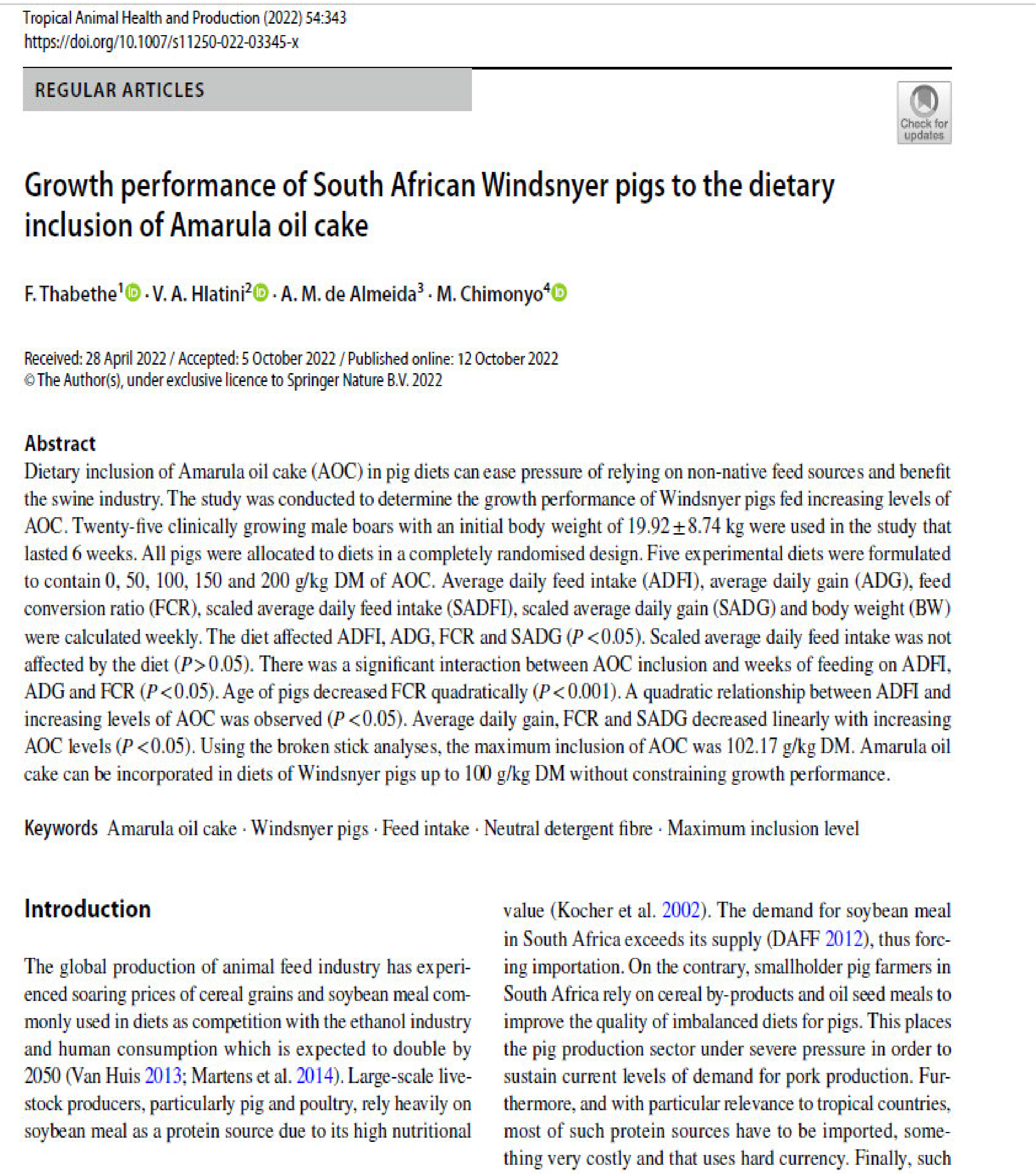
7.3 Recommendations

The results of the study indicated that AOC can be used in pig diets as an alternative protein source. The appropriate recommendations for AOC inclusion in pigs diets are 100 g/kg DM for optimum growth responses of Windsnyer pigs while for pork quality characteristics, inclusion levels of up to 150 g/kg DM can be used. However, the effectiveness of AOC diets in pigs is also affected by the presence of anti-nutritional factors, therefore ways of deactivating these compounds prior to feeding pigs are important hence, worth investigating.

Studies that require further investigation include the following:

- Determining the effect of AOC based diets on shelf-life of pork from Windsnyer pigs.
- Determining the oxidative stability of pork from indigenous pigs fed increasing levels of AOC diets.
- Determining the antioxidant potential of pork from indigenous pigs fed increasing levels of AOC diets.
- Determining the effect of season on nutritional related blood metabolites and liver enzymes of indigenous pigs fed on increasing levels of AOC diets.
- Determining the effect of AOC diets on gut morphology of Windsnyer pigs.
- Determining the mineral composition of pork from indigenous pigs fed increasing levels of AOC diets.

Appendix 1: Growth performance of South African Windsnyer pigs to the dietary inclusion of Amarula oil cake



Tropical Animal Health and Production (2022) 54:343
https://doi.org/10.1007/s11250-022-03345-x

REGULAR ARTICLES

Growth performance of South African Windsnyer pigs to the dietary inclusion of Amarula oil cake

F. Thabethe[1] · V. A. Hlatini[2] · A. M. de Almeida[3] · M. Chimonyo[4]

Received: 28 April 2022 / Accepted: 5 October 2022 / Published online: 12 October 2022

Abstract

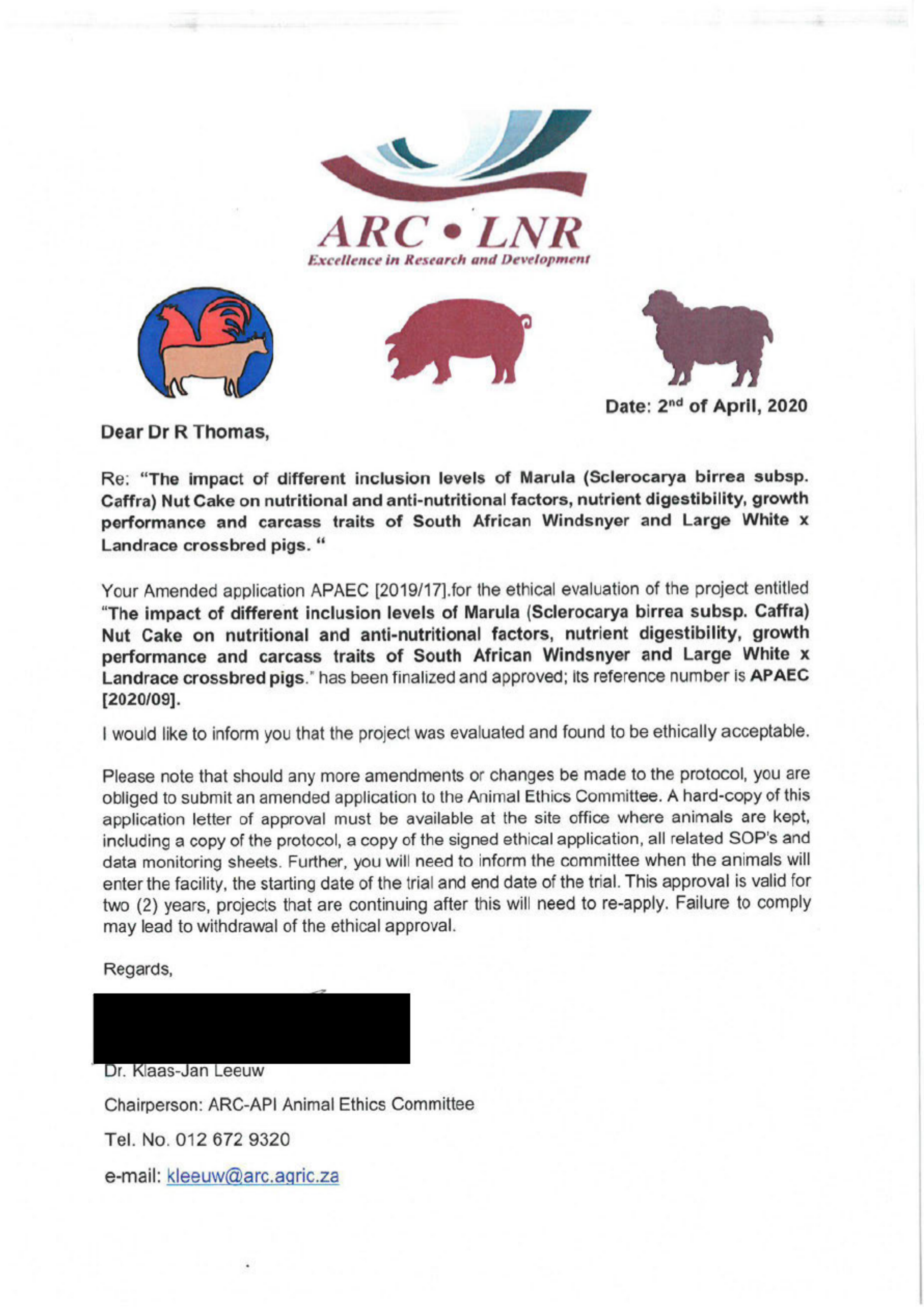
Dietary inclusion of Amarula oil cake (AOC) in pig diets can ease pressure of relying on non-native feed sources and benefit the swine industry. The study was conducted to determine the growth performance of Windsnyer pigs fed increasing levels of AOC. Twenty-five clinically growing male boars with an initial body weight of 19.92 ± 8.74 kg were used in the study that lasted 6 weeks. All pigs were allocated to diets in a completely randomised design. Five experimental diets were formulated to contain 0, 50, 100, 150 and 200 g/kg DM of AOC. Average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR), scaled average daily feed intake (SADFI), scaled average daily gain (SADG) and body weight (BW) were calculated weekly. The diet affected ADFI, ADG, FCR and SADG ($P < 0.05$). Scaled average daily feed intake was not affected by the diet ($P > 0.05$). There was a significant interaction between AOC inclusion and weeks of feeding on ADFI, ADG and FCR ($P < 0.05$). Age of pigs decreased FCR quadratically ($P < 0.001$). A quadratic relationship between ADFI and increasing levels of AOC was observed ($P < 0.05$). Average daily gain, FCR and SADG decreased linearly with increasing AOC levels ($P < 0.05$). Using the broken stick analyses, the maximum inclusion of AOC was 102.17 g/kg DM. Amarula oil cake can be incorporated in diets of Windsnyer pigs up to 100 g/kg DM without constraining growth performance.

Keywords Amarula oil cake · Windsnyer pigs · Feed intake · Neutral detergent fibre · Maximum inclusion level

Introduction

The global production of animal feed industry has experienced soaring prices of cereal grains and soybean meal commonly used in diets as competition with the ethanol industry and human consumption which is expected to double by 2050 (Van Huis 2013; Martens et al. 2014). Large-scale livestock producers, particularly pig and poultry, rely heavily on soybean meal as a protein source due to its high nutritional value (Kocher et al. 2002). The demand for soybean meal in South Africa exceeds its supply (DAFF 2012), thus forcing importation. On the contrary, smallholder pig farmers in South Africa rely on cereal by-products and oil seed meals to improve the quality of imbalanced diets for pigs. This places the pig production sector under severe pressure in order to sustain current levels of demand for pork production. Furthermore, and with particular relevance to tropical countries, most of such protein sources have to be imported, something very costly and that uses hard currency. Finally, such

Appendix 2: Ethical clearance certificate



ARC • LNR
Excellence in Research and Development

Date: 2nd of April, 2020

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 Amended application APAEC [2019/17].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 [2020/09]**.

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, a copy of the signed ethical application, all related SOP's and data monitoring sheets. Further, you will need to inform the committee when the animals will enter the facility, the starting date of the trial and end date of the trial. This approval is valid for two (2) years, projects that are continuing after this will need to re-apply. Failure to comply may lead to withdrawal of the ethical approval.

Regards,

[redacted]

Dr. Klaas-Jan Leeuw

Chairperson: ARC-API Animal Ethics Committee

Tel. No. 012 672 9320

e-mail: kleeuw@arc.agric.za