

**Performance, Biochemico-physiological and Meat Quality
Responses to Dietary Aflatoxin-contaminated-Marula Seed Cake
with or without Wild Garlic in Piglets**

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DECLARATION

I, Shobande Monsuru Adio, hereby declare that this dissertation with the title “Performance, Biochemico-physiological and Meat Quality Responses to Dietary Mycotoxin-contaminated-Marula Seed Cake with or without Wild Garlic in Piglets” is my work, conducted under the supervision of Prof. M. Mwanza. This study is my work, and it has not been submitted to any other university's degree program. All additional sources utilized in this document have been properly mentioned in the text and referenced.

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DEDICATION

This study is dedicated to my late father, Mr F. Shobande. I pray to Almighty Allah to widen his grave, cleanse him of his shortcomings, give him an abode better than his home and household better than those he left behind and a company better than his previous company. I also pray for the Lord to admit him into Paradise and protect him from the punishment of the grave and that of hell-fire.

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ABBREVIATIONS

ADG:	Average Daily Gain
AFI:	Average Feed Intake
ALB:	Albumin
ALKP:	Alkaline phosphatase
ALT:	Alanine Aminotransferase
ANFs:	Antinutritional Factors
AWG:	Average Weight Gain
BUN:	Blood Urea Nitrogen
Ca:	Calcium
CCW:	Cold Carcass Weight
CHOL:	Cholesterol
CF:	Crude Fibre
CREA:	Creatinine
DM:	Dry Matter
EE:	Ether Extract
FCE:	Feed Conversion Efficiency
FI:	Feed intake
GGT:	Gamma-glutamyl transferase
GLU:	Glucose
HCW:	Hot Carcass Weight
MSC:	Marula Seed Cake
P:	Phosphorus
TBIL:	Total Bilirubin
TP:	Total Protein
WG:	Wild Garlic

ABSTRACT

The high cost of feed resulting from intensive pig production in developing countries highlights a crucial need to search for inexpensive alternatives to imported protein sources. Marula (*Sclerocarya birrea caffra*) seed cake (MSC) provides a noteworthy quantity of crude amino acids and protein, similar to soy bean meal (SBM) and can serve as a substitute dietary protein source for rearing piglets. However, MSC is inherently contaminated with mycotoxins due to its high level of residual oil, protein content, both of which offer favourable conditions for the establishment and propagation of toxigenic fungus. Mycotoxin is known to have the potential to induce deleterious effects, thereby causing economic losses (growth retardation, oxidative stress, immunosuppression and other effects) in pigs. Therefore, strategies to counter mycotoxins' deleterious effects in pigs fed MSC-supplemented diets are needed. This study was aimed at evaluating the effects of dietary inclusion of mycotoxin-contaminated MSC as an alternative to SBM on performance, carcass characteristics and serum biochemistry which could be ameliorated through wild garlic (*Tulbhagia violacea*) supplementation. A total of 48 four-week-old weaned Large White piglets were randomly allocated to four iso-caloric and iso-nitrogenous formulated diets, containing MSC substituted at 0%, 10% and 20% and 20% MSC inclusion supplemented with 2.5% wild garlic for six weeks, with each animal as an experimental unit. Thereafter, the experimental animals were sacrificed and the carcass characteristics and meat quality measurements were taken. Results showed that dietary MSC decreased body weight gain (BWG; $p \leq 0.01$), feed intake (FI; $P < 0.05$), liver weight ($P < 0.05$), foreleg weights ($P=0.001$), Hind leg weight ($p \leq 0.05$), carcass weight ($P = 0.001$), temperature ($p \leq 0.05$), Urea (mmol/L) ($p \leq 0.05$), Blood urea nitrogen/creatinine ($p \leq 0.05$), Albumin/Globulin ($p \leq 0.001$). Further, piglets offered 10% MSC without wild garlic had an increased ($p \leq 0.001$) BWG, whereas, the inclusion of wild garlic to the (20% MSC inclusion at 2.5 wild garlic) significantly decreased ($p \leq 0.001$) the overall BWG of piglets. The diet has a significant effect ($p \leq 0.001$) on the weekly body weight gain and weekly feed intake. There was no significant observable effect ($P > 0.05$) on the weekly feed conversion efficiency of weaned Large White piglets, while there were no significant effects ($P > 0.05$) on the weight of kidney, small intestine, large intestine, spleen, caecum and stomach. Moreover, the results on the biochemical indices showed that, there were significant ($p \leq 0.05$) interaction effects of diet x week on the symmetric dimethylarginine, creatinine, phosphorous, calcium, alanine aminotransferase and γ -Glutamyl transferase, while there were no significant interaction effects ($P > 0.05$) on the glucose, urea, blood urea, total protein, albumin, globulin, albumin/globulin

ratio, alkaline phosphate, total bilirubin, cholesterol, amylase and lipase. Conclusively, this study revealed that, dietary inclusion of MSC as an alternative protein source improve growth performance, carcass characteristic and some serum biochemistry in Large White piglets. However, inclusion of marula seed cake in pig ration should not exceed 10% as higher inclusion might pose a threat to the health of the animals. Also, the inclusion of wild garlic did not appear to have any beneficial effect on the measured parameters.

Keywords: Aflatoxin; marula seed cake; mycotoxin; piglets; proteins; soy bean meal and *Tulbaghia violacea*;

CHAPTER 1: INTRODUCTION

1.1 Background

Demand for animal protein for human consumption is on the increases due to the fast-growing human population, particularly in developing countries (World Bank Group, 2015). This necessitates the need for innovative approaches to that will ensure a sustainable-increase in livestock population to meet the ever rising demand. However, the cost of animal feeds (particularly the pigs feed) and inputs limits the production, world wide due to the shortage of protein-rich sources and other supplements required in formulating balanced pig rations (Adesehinwa, 2008).

Soybean meal (SBM) is the protein source mostly applied in the animal feed industry in the sub-Saharan African (SSA) region due to its high protein content (Malebana *et al.*, 2018). However, poor-performing variety, a lack of consistent rhizobial inoculants use, and a lack of fertilizer application have all contributed to sub-Saharan Africa's soybean production falling short of demand (Khojely *et al.*, 2018). The industrial and domestic utility of soybean as a means of human dietary protein and feed ingredients respectively limit its accessibility for other purposes (Biazatti & de Carvalho Miranda, 2021). This has made soybean an expensive feedstuff which has invariably led to a high cost of production in pigs (Schwarz *et al.*, 2021). Therefore, to meet the feed supply requirements for small holder swine farmers, there is a need to explore more affordable and secure protein sources (Lekule & Kyvsgaard, 2003) that would increase performance without any harmful effects on the animal. Of these cheap, and readily available alternatives sources is the Marula seed cake (MSC), which is naturally occurring and abundant feed resource (Thornton, 2010). The chaff or cake is a waste product generated after the oil is extracted from the dry seeds of ripe marula fruits (*Sclerocarya birrea*). It is very rich in quality dietary protein and other nutritive supplements required by livestock for healthy growth (Mdziniso *et al.*, 2016; Mlambo *et al.*, 2011). Marula seed cake's nutritional composition consist of crude protein (470.0 g/kg DM), ether extract (343.5 g/kg DM), crude fibre (58.2 g/kg DM), ash (54.3 g/kg DM), calcium (1.1 g/kg DM), phosphorous (11.0 g/kg DM), lysine (0.88%), methionine (0.79%), cysteine (1.18%), arginine (6.36%), glutamic acid (10.78%) (Banaszkiewicz, 2011). Marula seed cake is also rich in good fats such as oleic acid, palmitic acid and stearic acid (Banaszkiewicz, 2011). However, the linoleic and linolenic acids may be completely oxidized during the processing of MSC and therefore, leads to accumulation of high lipid peroxides in the MSC (Mthiyane & Mhlanga, 2017). In addition, Mthiyane and

Mhlanga (2017) reported that MSC is prone to mycotoxin contamination which may affect its use in pig production.

Mycotoxin is secondary metabolites of filamentous fungi that is toxic to humans and livestock (Eraslan *et al.*, 2005; Mavrommatis *et al.*, 2021). Common examples of mycotoxin in animal feed are deoxynivalenol, aflatoxins, zearalenone, fumonisins and ochratoxin. Contamination by these mycotoxins can take place in all agricultural products in the field, during transportation and/or during storage if the conditions favourable for fungal growth and development exists (Gruber-Dorninger *et al.*, 2019). Ingestion of mycotoxin through animal feeds leads to decreased growth rates, liver damage, immune suppression and oxidative stress; which indirectly result in impaired animal performance (Eraslan *et al.*, 2005; Mavrommatis *et al.*, 2021).

Additionally, it has been reported by Gruber-Dorninger *et al.* (2019) that more than 70% of animal feeds produced globally is contaminated with at least one form of mycotoxin or the other. This situation calls for serious attention, considering the health risks they may pose to humans and animals (Çelik, 2020; Dikhoba *et al.*, 2019).

Mycotoxin control remains a serious challenge for the agricultural industry. Chemical and microbiological techniques, among others, have been developed to regulate and prevent mycotoxins in food and feed (Adebisi *et al.*, 2019; Adebo *et al.*, 2017). Notwithstanding the effectiveness of these methods, their application may result in producing some side toxic effects on the product(s), health of the animals, the environment, and nutrients composition of the treated food and feeds. Alternative methods are therefore required to either stop the growth of fungi in feed or to detoxify or bio-transform mycotoxin residues into less- or non-toxic forms without any limitation (Haque *et al.*, 2020; Iram *et al.*, 2015; Powers *et al.*, 2019).

Wild garlic (*Tulbhagia violaceae*) is a plant rich in antioxidants, it belongs to the family of *Alliaceae* and it is used traditionally in South Africa for the management of different diseases (Olorunnisola *et al.*, 2011). The leaves and bulbs are recommended for various ailments (Bungu *et al.*, 2006) and many studies have also demonstrated the antioxidant activities of *T. violacea* against different diseases (Olorunnisola *et al.*, 2012a; Takaidza *et al.*, 2018). In the rat, for instance, wild garlic was reported to ameliorate oxidative stress markers (Kahalerras *et al.*, 2022).

Oxidative stress has been reported in several pathological conditions including fungal-linked diseases. *T. violacea* has shown its antifungal activity in both animal and human pathogens with no reported toxicity or side effects (Bansal & Priyadarsini, 2021). In earlier research (Makhuvele *et al.*, 2022), it was observed that the *T. violacea* extract contains some phytochemical compounds with antifungal and antioxidant activities. Determining the bioactivities of the *T. violacea* as a cocktail with Marula seed cake in the physiological and biochemical responses of the developing piglets is a step in the right path.

1.2 Problem Statement

Animal feed constituting seventy per cent of the total cost of animal production, is quite problematic for large scale production facilities (Delpont *et al.*, 2017). In pigs' formulated diet, protein ingredients are the most expensive which account for the high cost of the feed (Kim *et al.*, 2019; Rezaei *et al.*, 2013). Soybean meal is the most used conventional protein in pigs' rations due to its high protein value. However, SBM protein sources are not sustainable to ensure profitability in pig farming (Mottet *et al.*, 2017). Pigs demand high-quality dietary protein that is equivalent to that which is needed by humans due to the architecture of their digestive system (Stein *et al.*, 2016). In this case, where the high cost of and growing demand for SBM protein may prevent the sustainable intensification of pig production, a cheap and readily available alternative must be sought for to minimize cost pig production.

Marula seed cake contains sufficient qualities of dietary protein and other dietary supplements required in livestock production. However, there is little data on the effect of MSC as a protein supplement in pigs and there is also a paucity of data on its effect(s) on growing piglets. More so, marula seed cake and even SBM could contain some anti-nutritional factors such as mycotoxins and, studies exploring the use of antioxidative feed supplements to relieve the effects of these toxins are limited. Hence, it has become necessary to explore a cheaper and readily available means of relieving these negative health effects in pigs through the inclusion of antioxidant-rich natural products such as *Tulbhagia violaceae* bulbs, which is readily available in South Africa, in pig diets.

1.3 Justification

The utilization of MSC oil seed by-products as a protein source in pig production in this study would provide data on a cheaper nutrient-rich alternative to the expensive SBM. This study also provides data on the optimum dietary formulation which would be required to produce

quality pig products. The application of the findings from the present study on MSC as an alternative protein source if applied commercially and by smallholder farmers could increase the supply and quality of pig products especially dietary protein for human consumption thereby improving global food security. In addition, this study explored the use of *T. violaceae* in ameliorating the negative effect of aflatoxins on the health of pigs. The data provided in this study on the antioxidative properties of *T. violaceae* provides essential scientific evidence on alternative means for addressing the issue of oxidative stress induced by aflatoxins, data which is presently lacking.

1.4 Aims and Objectives

1.4.1 Aim

This study aims to improve the production of pigs using a cheap and readily available marula seed cake as an alternative protein source. Concurrently, *T. violacea* forage was explored as means to reduce the toxicity of mycotoxin-contaminated MSC diets in the piglets.

1.4.2 Main objective

The main objective of this study is to determine the ameliorative effects of *T. violacea* forage in piglets fed diets supplemented with mycotoxin-contaminated marula seed cake used as an alternative protein source.

1.4.3 Specific objectives

The specific objectives of this project are:

1. To determine body weight gain, feed intake and feed conversion efficiency in piglets fed diets supplemented with marula seed cake with or without wild garlic.
2. To determine serum-biochemical, physiological and meat quality parameters in piglets fed diets supplemented with marula seed cake with or without wild garlic.

1.5 Research Hypothesis

- a. H₀: Marula seed cake inclusion diet with or without wild garlic does not affect body weight gain, feed intake and feed conversion of the growing piglet.
H₁: Marula seed cake inclusion diet with or without wild garlic affects body weight gain, feed intake and feed conversion of the growing piglet.

- b. H_0 : Marula seed cake inclusion in a diet with or without wild garlic does not affect serum-biochemical, physiological and meat quality parameters of the growing piglet.
 H_1 : Marula seed cake inclusion in a diet with or without wild garlic affects serum-biochemical, physiological and meat quality parameters of the growing piglet.

CHAPTER 2: LITERATURE REVIEW

Over the past 50 years, dependency on grains as food has been relieved by the increase in consumption of animal proteins worldwide (Ritchie & Roser, 2017). Livestock contributions are of importance to food and nutrition security, population growth, livelihood and earning income in different continents (Alders *et al.*, 2021; Osinubi & Apanisile, 2021). Despite these crucial roles, the Southern African Development Community (SADC), reported 345 million people, of which 30% suffer from severe food insecurity, 8% lack nutrition, and 50% depend on less than \$1 daily (Mapiye *et al.*, 2020). These challenges have prioritized livelihood, food, nutrition security and population as key priorities in this region. In response, sustainable livestock farming is of crucial by contributing to the agri-food systems of marginalized communities in the African region. Additionally, the growing population, urbanization and income of developing nations are creating a huge demand for animal products (Banda & Tanganyika, 2021).

Moreover, food security is a situation in which individuals have enough and can serve nutritious food at all times to meet their dietary demands and food preferences to maintain an active and healthy lifestyle (Mabe *et al.*, 2021). However, meeting substantial food security by world production remains a problem, as demand for food and nutritional needs keep rising at a greater rate faster than population growth (Calicioglu *et al.*, 2019). This, increasing pressure on food security and demand for high-quality nutritional diets such as pig products, has made it imperative for more attention to be focused on sustainable swine production.

2.1 Population growth and food security improvement in Africa

The increase in human population growth accompanied by increased demand for food, particularly proteins for human nutrition, is the major socio-economic challenge confronting emerging nations, particularly in sub-Saharan Africa (SSA) (OECD, 2016). The entire African population, according to FAO (2017) and FAOSTAT (2018), was estimated at 1.3 billion in 2017, with populations in rural area totaling 717 million and urban areas totaling 505 million. Also, a steady yearly increase of 3.59% and 1.74% was projected for the rural and urban communities respectively, hence, the eventual increase in the demand for animal products as a viable and reliable source of protein for human consumption. As part of sustainable intensification, agricultural productivity must increase to cope with the rising food demand.

Livestock products contribute significantly to the daily protein and energy consumed by the population (McDermott *et al.*, 2010). Livestock products like eggs, meat, and milk provide roughly 28% of the protein and 13% of the energy consumed globally (FAO, 2009). Meat is a good source of protein, minerals and vitamins; it contains essential amino acids and fatty acids (Marangoni *et al.*, 2015) needed in the human body for normal physiological development and function (Marangoni *et al.*, 2016). However, access to meat protein remains a persistent problem in developing countries because it is not readily available due to the high cost of production.

Moreover, the livestock sector is considered an important agricultural sub-sector with multidimensional purposes and socio-economic advantages (Rota & Urbani, 2021). The sector accounts for one-fifth of the global trade in agricultural products as it provides humans with nutrients for balanced diets (Perry & Sones, 2007). The impoverished dwell in rural regions and rural populations rely heavily on agriculture for a living (Mbatha *et al.*, 2021; Rahman & Westley, 2001), this gives significant opportunities to every community (Herrero *et al.*, 2010). The food and agriculture organization (FAO, 2009) estimated that over 80% of impoverished Africans and up to 66% of the poor people in India and Bangladesh raise livestock. Herrero *et al.* (2013) reported that livestock production and marketing in West Africa sustains 70 million people; dairy sustains 24 million people in East Africa; while small ruminants sustain 81 million people in West Africa and an additional 28 million in southern Africa (Staal *et al.*, 2009). Due to these multi purpose roles in terms of food security and the increase in the SSA population, livestock sustainability is a concern among researchers.

2.2 Pig dietary requirement

Pig demands a well-balanced diet with a specific amount of six general classes of nutrients (carbohydrates, water, fats, protein (amino acids), vitamins and minerals) to maintain, reproduce, lactate, grow, and perform other tasks. For pigs to grow to their full potential, the National Research Council (2012) advises a diet of 13.4 to 264g/kg CP and 3.3 to 3.4 MJ/kg ME for optimum growth (Table 1). Other dietary requirements for various swine weights are presented in Table 1. It is also noteworthy that factors such as feedstuff nutrient availability, environment, genetic variation and other challenges may raise the amount of some nutrients required for good function and fertility. In addition, Chemotherapeutic agents, antibiotics, enzymes, microbial supplements (prebiotics and probiotics), and other feed additives are routinely used in swine diets to speed up growth, enhance digestibility, and for other reasons;

nevertheless, they are not thought to be nutrient-rich. Pigs' nutritional needs are influenced by their stage of development, the amount of metabolizable energy (ME), and the substances used to make their feed. Due to the fact that it is a byproduct of the oxidation of carbohydrates and fats, energy is also a crucial dietary component although not in specified quantities.

Table 2. 1: Dietary Nutrient Requirements of Growing Pigs Allowed Ad Lib Feed (90% dry matter) according to NRC 2012

	Body weight (kg)						
	5–7	7–11	11–25	25–50	50–75	75–100	100–135
NE content of diet (kcal/kg) ^d	2,448	2,448	2,412	2,475	2,475	2,475	2,475
DE content of diet (kcal/kg) ^d	3,542	3,542	3,49	3,402	3,402	3,402	3,402
ME content of diet (kcal/kg) ^d	3,4	3,4	3,35	3,3	3,3	3,3	3,3
Estimated ME intake (kcal/day)	904	1,592	3,033	4,959	6,989	8,265	9,196
Estimated feed intake + wastage (g/day) ^e	280	493	953	1,582	2,229	2,636	2,933
Body wt gain (g/day)	210	335	585	758	900	917	867
Body protein deposition (g/day)	--	--	--	128	147	141	122
<i>Amino acids, total (%)^f</i>							
Arginine	0.75	0.68	0.62	0.50	0.44	0.38	0.32
Histidine	0.58	0.53	0.48	0.39	0.34	0.30	0.25
Isoleucine	0.88	0.79	0.73	0.59	0.52	0.45	0.39
Leucine	1.71	1.54	1.41	1.13	0.98	0.85	0.71
Lysine	1.70	1.53	1.40	1.12	0.97	0.84	0.71
Methionine	0.49	0.44	0.40	0.32	0.28	0.25	0.21
Methionine + cystine	0.96	0.87	0.79	0.65	0.57	0.50	0.43
Phenylalanine	1.01	0.91	0.83	0.68	0.59	0.51	0.43
Phenylalanine + tyrosine	1.60	1.44	1.32	1.08	0.94	0.82	0.70
Threonine	1.05	0.95	0.87	0.72	0.64	0.56	0.49
Tryptophan	0.28	0.25	0.23	0.19	0.17	0.15	0.13
Valine	1.10	1.00	0.91	0.75	0.65	0.57	0.49
Approximate crude protein ^g	24–26	22–24	21–23	19.3	17.1	15.2	13.4

2.2.1 Feeds commonly used for pigs

In the 1950s, it was documented that pigs thrive on a diet consisting of soybean meal (SBM) and maize when vitamins and minerals are provided (Kim, 2010). The most popular diet for pigs in America and many other nations has always included this combination of nutrients. Other cereal grains, including wheat, triticale, barley, sorghum, and rye, can be used in combination with SBM or other sources of protein in areas of the world where maize cultivation is uneconomical due to climatic circumstances (Stein *et al.*, 2016). Because of the enormous expansion in soybean production over the previous 50 years, SBM may now be used as the principal source of amino acids (AA) and crude protein (CP) in almost every country on the planet. Soybean production is presently the world's fastest-growing agricultural activity due to increased demand for SBM (Giraud, 2021). Despite the grain-SBM diet's superior nutritional content, a variety of additional feed ingredients are frequently employed in pig diets. The main reason for adding more ingredients is to reduce the price of diets by utilizing the vast amount of by-products produced by the food and other agro-allied industries (Stein *et al.*, 2016).

2.2.2 Conventional protein sources and associated problems

Conventional proteins such as soya bean meal (Molfetta *et al.*, 2022), groundnut seed cake and cotton seed cake (Aruwayo & Maigandi, 2013) and canola meal (Li *et al.*, 2013), are used intensively in monogastric (such as piggery, pullet, broiler, and aquaculture) raising in the sub-Saharan area (Chivandi *et al.*, 2012; Ssepuuya *et al.*, 2017). For example, according to estimates, soya bean meal (SBM) constitutes nearly 85% of the protein supplements provided to pigs worldwide (Cortamira *et al.*, 2000) making it one of the most important protein sources in livestock nutrition (Mabena *et al.*, 2022). Other conventional protein source includes fish meal, which however has its drawback by making the Pork products to become odor-tainted when incorporated in finishing diets (Meeker, 2009). Furthermore, decorticated cotton and sunflower seed cakes are generally low in levels of critical amino acid concentration (methionine and lysine), thus limiting their uses in non-ruminant feeds (McDonald *et al.*, 2002). As a result of commercial demand, commercial proteins sources are often unaffordable to smallholder farmers (Mdziniso *et al.*, 2016). To ensure the long-term viability of livestock production, the search for alternative sources of animal feed protein is crucial to serve as a cheaper alternative to the high costs of conventional protein sources (Parolini *et al.*, 2020).

2.2.3 Alternative protein sources and their limitations

Alternative sources of protein for both food and feed ingredients include insects, canola/rapeseed, micro-algae, and insects (Vigani *et al.*, 2015), as well as duckweed (van Huis, 2021). Insects have a high feed conversion rate, making them viable sources of animal protein (DiGiacomo & Leury, 2019). However, their uses for growing pigs and broilers are limited due to the lack of essential amino acids such as methionine or methionine + cystine and sometimes arginine (Veldkamp & Bosch, 2015). Meal made from canola/rapeseed is primarily used in the animal feed market, where its high protein content makes it a competitive feed ingredient. Canola protein offers high bioavailability and digestibility protein, and is economic, sustainable, and high in quality protein for human consumption (Campbell *et al.*, 2016). In spite decades of research, several technologies are being developed, and products are being mass produced on a large-scale. However, to other plant (seed) proteins, processing canola protein presents a number of special difficulties. For instance, when using aqueous processing, phenolic oxidation in the aqueous medium must be avoided because it can cause undesirable color and flavor changes in protein extracts and finished products.

2.2.4 Animal-derived alternative protein sources

Hydrolyzed hooves, horns, poultry feathers and hatchery waste are typically alternative protein sources derived from animals (Mozhiarasi & Natarajan, 2022). These are part of inedible animal by-products discarded (Kinyanjui & Noor, 2013) with a value less considered (Egbedi & Osibona, 2022). In contrast, these by-products tend to be rich in feed protein for livestock production, when processed appropriately (Shurson, 2020). For example, the chemical treatment method with soda ash (10% sodium ash for 60 hours at 20 °C) (AFRIS, 2012). These techniques can be used to produce high-quality animal protein from hoof keratin, which can then be used in livestock feeds to save feed costs, reduce environmental waste, and meet the nutritional demands of livestock (Falaye & Sule, 2020).

2.2.5 Plant-derived alternative protein sources

Nutritionists and livestock farmers now place a lot of emphasis on plant-based sources of protein. They are more affordable than protein generated from animals, conveniently accessible, sustainable, and environmentally safe. Also, Daniel (2018), reported high nutritional values and unique composition of plant-derived protein. In addition, its global market prediction is to reach 35.54 billion United States dollars by 2024, growing at 14% from 2020 to 2024 (Gomes & Sobral,

2021). However, the feed systems of today highlight a number of issues that put the capacity to deliver adequate high-quality protein globally in jeopardy. The term "phytobiotics" is given to the plant-based feed additives with unique phytochemical components that are involved in improving growth performance (Abdel-Latif *et al.*, 2020), enhancing immunity (Kuralkar & Kuralkar, 2021), fortifying antioxidant status (Mohammadi *et al.*, 2022), and promoting the healthy conditions of livestock (Ahmadifar *et al.*, 2021; Ali *et al.*, 2021; Hamed *et al.*, 2021).

2.3 Pig feeding and nutrition in Africa/South(ern) Africa

Despite increase in the quality of nutrition in recent years around the world, people within developing nations are still undernourished, especially in Africa. The world's greatest rate of undernourishment is seen in sub-Saharan Africa (FAO, 2015, 2017), which is aggravated by a competition as both component of human and pigs' diets, since typical African food is used as major ingredients in formulating pigs' rations.

In Africa, pigs are mainly fed a carbohydrate-based diet, with very little protein, and farmers report that access to quality feeds is a key constraint (Kagira *et al.*, 2010; Katongole *et al.*, 2012; Parmar *et al.*, 2017). Kitchen waste is available on many smallholder farms but rarely enough to feed an entire pig (Lekule & Kyvsgaard, 2003). Pigs are fed leftover food from restaurants and homes, vegetables, fruits, forages and weeds, cassava root and peel, banana peel, cattle blood and rumen contents, sweet potato vine and ground sun-dried fish, brewers and grist mill waste, maize bran (Carter, 2015). The amount of soybean meal (SBM) fed to pigs has been reported to fluctuate. However, the quantity is unlikely to offer enough protein in any scenario (Kagira *et al.*, 2010). Despite the fact that there are many different possible pig feedstuffs accessible, local feedstuffs are unfamiliar and have insufficient feeding value (Mutua *et al.*, 2012). These indigenous feedstuffs might be included into balanced diets to satisfy the nutritional needs of pigs and improve growth performance while lowering feed costs. It is necessary to create inexpensive diets utilizing locally accessible seasonal feedstuffs. These diets must be evaluated in order to identify their effectiveness and the development potential of African pigs.

2.3.1 Importance of pig production in food and nutrition security

Over a quarter of the worlds' population in 2017, was estimated for nutritional deficiency, which was largely a result of the lack of food security. Pigs are important in the processing of

high-quality meat into products that attract increasing interest in food and nutrition security. In 2014, the pork sector contributed 38% of the global meat supply and was also projected to increase by 3% annually (OECD/FAO, 2015; FAOSTAT, 2018). Thus, a pig is an important meat-based food comprising adequate dietary energy, protein and other micronutrients required in the human diet (Smith *et al.*, 2013). In addition, animal-based foods are richer in macro- and micronutrients which may be deficient in plant-based meals (Murphy & Allen, 2003; Randolph *et al.*, 2007).

2.4 Marula tree

Marula tree (*Sclerocarya birrea*), is a member of the Anacardiaceae family of savannah trees, and can reach heights of between 7 and 17 meters. It has a grey fissured bark, robust branchlets, and pale leaves (Mariod & Abdelwahab, 2012). Its native habitat extends from Namibia, Senegal, Ethiopia, Angola, South Africa, and the Democratic Republic of Congo (Chirwa & Akinnifesi, 2008). *Sclerocarya birrea* is one of the historically significant trees in southern Africa, and it is frequently used by rural people to meet their basic needs. It offers cultural goods and services that are crucial to society and the economy (Sinthumule & Mzamani, 2019). The root, stem, bark and leaf are used for ethnomedicine due to their rich medicinal compounds. Thus, various diseases are treated with it traditionally (Tapiwa, 2019)



Figure 2. 1: A picture of Marula Tree (Murye & Pelsler, 2018)

2.4.1 The Marula's fruit

The marula fruits are round and oval drupes and 3-5 cm in diameter when mature (Leakey, 2005). The fruit has valuable assets and is highly recognized as an important food source for rural communities (Mokgolodi *et al.*, 2011). All parts of the fruits are edible and its nutritional worth and product have both attracted a lot of attention (Tapiwa, 2019). The fruit has 180 mg of vitamin C per 100g, and it can be juiced or fermented to make alcoholic beverages, chewing

gum, jam, or wine (Leakey, 1999) . The seeds of the marula fruit are also possible sources of antioxidants and nutrients (including carbs, protein, fat, and vitamins and minerals).

Its kernels could serve as a source of protein for both humans and livestock because they contains oil (53.0%), protein (28.0%), and carbohydrate (8.0%) (Gouwakinnou *et al.*, 2011). The seeds can be crushed to produce oil, which is frequently used for cosmeceutical purposes (Vermaak *et al.*, 2011). The by-product of marula oil production is Marula seed cake meal (MSC).



Figure 2. 2: A picture of Marula Fruit (Murye & Pelsler, 2018)

2.4.2 Marula seed cake and other by-products

Marula seed cake (MSC) is a byproduct of marula kernel oil production (Shackleton *et al.*, 2014; Mthiyane & Mhlanga, 2017). It is rich in good dietary protein with high content of crude protein (CP) and ether extract (EE) with moderate crude fibre and ash (Mthiyane & Mhlanga, 2017). In addition, it shows high CP content when compared to groundnut meal (McDonald *et al.*, 2002), sunflower cake and cotton seed cake (Martens *et al.*, 2012; Ramachandran *et al.*, 2007), but similar to SBM (Banaszkiewicz, 2011) in terms of chemical composition. The flavonoids, polyphenols condensed tannins and polysaccharides are phytochemistry content in MSC (Mashau *et al.*, 2022).

The MSC, due to its protein content could be employed as a potential source of protein in animal feed, helping to lower the high cost of feed and increase pig production. Human nutrition intake improves as a result of pig production being intensified, and improving human nutrition is essential for enhancing food security (Thornton, 2010).

Hence increase in pig production has a corresponding impact on nutritional consumption in humans and food security, taking this into consideration, the nutrition of the MSC in pig production, is worth interrogating.

2.4.3 Marula seed cake as animal feed

The nutritional value of an ingredient is crucial for precise diet formulation and lower feed costs. Cereal grains and soybean meal are the most common conventional ingredients with the potential nutritional value used for swine nourishment. However, their increase in demand and supply by the world farmers would incur a high cost for swine producers. To overcome such high costs, alternative swine nutritional feed additives are sought for.

Marula seed cake (MSC) is a natural nutritionally-rich basis for crude protein and other nutritional requirements in animal feed. Its nutritional analysis is good with crude protein of 470 g/kg DM, ether extract of 343.5 g/kg DM, amino acids (except lysine) and other needed nutrients in animal feedstuff (Mariod & Abdelwahab, 2012; Mthiyane & Mhlanga, 2017). MSC is suitable for animal feed not only because of its large amounts of nutrients but also because it contains fewer anti-nutritional factors. Thus, it is used as an alternative nutritional source in ruminants and monogastric (Mdziniso *et al.*, 2016; Mthiyane & Mhlanga, 2017). Therefore, MSC incorporation as a nutritional source in the growing piglets would enhance good growth performance, physio-biological responses and the health status of the piglets. These excellent nutritional properties and stability advocate that MSC could be a more favourable potential substitute for soya bean meal in piglet diets in Southern Africa and elsewhere in SSA where marula trees occur in abundance.

The marula seed cake inclusion in diets does not have any harmful effect on the growth performance of pig, Japanese quail and poultry (Mabena *et al.*, 2022; Mazizi *et al.*, 2020; Mthiyane & Mhlanga, 2017). The high fat content in MSC may make diets sufficiently calorie-dense and enable pigs to boost feed efficiency and daily gains, which would lead to an increase in muscle accretion (De la Llata *et al.*, 2001). However, its potential use in animal diets has limitations due to extensive lipid peroxidation (Mthiyane & Mhlanga, 2017). It has been shown that feeding oxidized lipids reduces body weight gain (Cabel *et al.*, 1988; McGill *et al.*, 2011) and reduce meat quality (Delles *et al.*, 2014; Racanicci *et al.*, 2008) in broilers. Thus, the consumption of oxidized lipids in MSC could decrease growth performance productivity and

carcass characteristics. In addition, mycotoxins (DON and T-2 toxin) were detected at rather low levels in MSC (Mthiyane & Mhlanga, 2017).

Table 2. 2: Substitution of marula seed cake as an alternative protein source in feed formulation diet

Study	Species, inclusion level of MSC and study period	Comparative intervention	Origin of study	Study design	Findings
(Mthiyane & Mhlanga, 2017)	Cobb-500 broiler chicken-fed diets supplemented with varying levels of MSC for 16 days	SBM	Eswatini	CRD	Decreased BWG, FI and FCE, decreased live weight at slaughter, plucked weight and liver weight.
(Mthiyane & Mhlanga, 2018)	Two hundred and seventy 28-day-old (Cobb 500 broilers fed MSC at inclusion (0, 200 and 300 g/kg) for 28 days	SBM	Eswatini	CRD	MSC decreased BWG and FCE but shown increase in FI
(Mazizi <i>et al.</i> , 2019)	Two hundred Japanese quail chicks fed brooding and grower diet of supplement MSC at difference level (0%, 25%, 50% 75% and 100%) for 28 days	SBM	Central Animal Service (CAS) unit University of Witwatersrand	ANOVA	Showed positive effect on BMG, ADG, FI, and FCR during the brooding growth phase.
(Mabena <i>et al.</i> , 2022)	Thirty Large White × Landrace (LW × LR) pigs fed experimental diets that contained 0 (control), 50, 100, 150 and 200 g ANC/kg DM.	SBM	Agricultural Research Council (ARC)-Irene Campus, South Africa	RBD	No negative effect in ADFI but decreased ADG, BWG and FCR at 15 % dietary inclusion MSC
(Mlambo, Dlamini, Nkambule, <i>et al.</i> , 2011)	Twenty-four intact male Nguni goats	Hay, sunflower and SBM		CRD	
(Mlambo, Dlamini, Ngwenya, <i>et al.</i> , 2011)	Twelve dairy male weaners fed 50% MSC experimental diet for 58 days	Urea	Swaziland F	ANOVA	MSC had no negative effect on voluntary feed intake and growth rate of feedlot cattle.

2.5 Mycotoxins

Mycotoxins are secondary metabolites of fungal origin found to contaminate animal feeds resulting in deleterious effects on animal health and performance when consumed (Waithaka Ng'ang'a & Niyonshuti, 2022). Over 300 different distinct mycotoxins have been reported, although the most commonly found among them are zearalenone (ZEN), aflatoxin (Afs), ochratoxin A (OTA), deoxynivalenol (DON) and fumonisins (FBs) (Lee & Ryu, 2017). Greater than 70% of animal feeds and feedstuffs produced globally are contaminated with a minimum of one mycotoxin (Streit, Naehrer, *et al.*, 2013). Some studies have reported the concentrations of ochratoxin A, aflatoxin B₁, fumonisins, zearalenone, deoxynivalenol, and T-2 toxin in 74,821 samples of feed and feed ingredients (e.g., soybean, wheat, maize,) gathered between 2008 and 2017 from 100 countries. In all, 88% of the samples exhibited a minimum of one mycotoxin (Gruber-Dorninger *et al.*, 2019). Some groups of mycotoxins pose health threats to livestock including pigs. Ingestion of such mycotoxins could result in reduced feed intake, impaired growth performance, altered immune system and tissue damage (Sharma & Patial, 2021).

Generally, mycotoxin (especially DON) in swine feed decreases feed intake and weight gain and causes problems in livestock production processes (Lindemann *et al.*, 1993). EFSA (2004) reported long-term dietary exposure of animals to DON has been associated with decreased feed intake, weight gain and altered immune function (Hooft & Bureau, 2021). T-2 toxin causes broiler chickens to lose weight (Manafi *et al.*, 2015). T-2 toxin predominantly affects the immunological and hematopoietic systems (Adhikari *et al.*, 2017). Additionally, It may result in liver harm, weight loss, neurotoxicity and reproductive failure/toxicity (Schuhmacher-Wolz *et al.*, 2010)

DON and T-2 toxins promote free radical generation as the mechanism behind membrane and DNA damage during the oxidative stress brought by DON and T-2 toxins (Wu *et al.*, 2014). DON is another well-known protein synthesis inhibitor. It attaches to peptidyl transferase (Hooft & Bureau, 2021), inhibiting the synthesis of protein via binding to the ribosome. Cells and tissues with high rates of protein turnover, such as the liver, the immune system, and the small intestine, are most severely affected by DON intoxication (Azizi *et al.*, 2021). Therefore, these challenges urge for intensification of marula seed cake diets optimum utilization.

2.5.1 Aflatoxins (AFs)

Aflatoxins (AFs) are mainly produced by *Aspergillus* species and often contaminate feed diets (Thieu *et al.*, 2008), through fungal contamination of grains and nuts (Yu, 2012). The parent groups of aflatoxins include the B₁, B₂, G₁ and G₂, based on their fluorescence under ultraviolet light (Blue and Green) and their mobility during thin layer chromatography while aflatoxin B₁ (AFB₁) is the most toxic among the group. However, the European Commission recommends 0.01 mg/kg for young and 0.02 mg/kg for older pigs to reduce aflatoxin B₁ contamination in feedstuffs (Holanda *et al.*, 2020). Aflatoxins inhibit RNA polymerase transcription in the nucleus, reducing the production cell proteins (Yu, 1977), thus, influencing cell toxicity and death (Zimmermann *et al.*, 2015). Aflatoxin B₁ exhibits greater toxicity and carcinogenic effects than other aflatoxins (Rychlik *et al.*, 2014). Through the alteration of dendritic cell function by AFB₁, antigen-presenting cells can be suppressed and influence T-cell proliferation and differentiation (Mehrzhad *et al.*, 2014). Under chronic exposure, aflatoxin B₁ exhibits hepatic damage, immune suppression, growth impairment, and may interact with the DNA to cause neoplasia development (McCullough & Lloyd, 2019; Raisuddin *et al.*, 1993)

Aflatoxin B₁ has, in fact, had negative impacts on pigs' liver health and electrolyte balance (Schell *et al.*, 1993). Aflatoxins also inhibited animal growth due to cytokine release (Weaver *et al.*, 2014).

Aflatoxins result in altered functions of the kidney and liver, including their architecture (Eraslan *et al.*, 2017). The effects of mycotoxins in the liver and kidney exhibited significant metabolic activities, may have an impact on the production of cholesterol synthesis and later, vitamin D activation as well as phosphorus and calcium balance (Glahn *et al.*, 1991; Lawson *et al.*, 1971)). There is proof that mycotoxins affect metabolism of vitamin D. The toxic effects of aflatoxins have been demonstrated on the kidney, and against vitamin D and calcium levels in poultry (Marin *et al.*, 2002). In the case of consumption of aflatoxins, the liver play a crucial function in detoxification. The cytochrome P450 may either transform aflatoxins to their less hazardous M1 and M2 form or to their more dangerous epoxide form (Rychlik *et al.*, 2014).

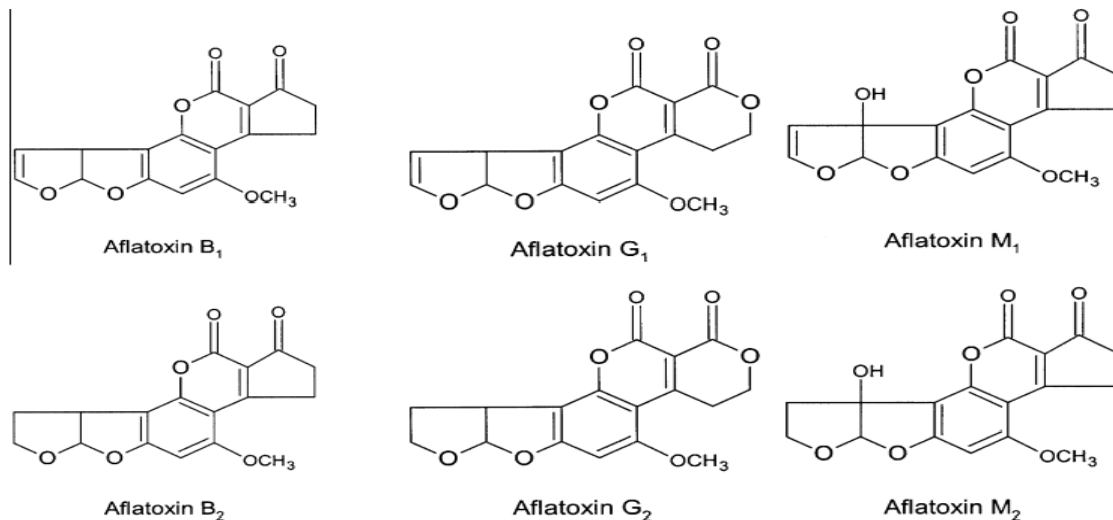


Figure 2. 3 Aflatoxins structures (Zain, 2011)

2.5.2 Ochratoxin (OT)

Ochratoxin (OT) is a toxic metabolite of *Aspergillus* species (Palabiyik *et al.*, 2013). Ochratoxins are grouped into A (OTA), B (OTB) and C (OTC). A survey of 74,821 feed samples from 100 countries found that 15% of the samples had OTA (Gruber-Dorninger *et al.*, 2019). This established more accumulation of OTA in animals' meat, human blood and breast milk due to dietary exposure (Erkekoğlu *et al.*, 2010). Approximately 41% and 35%, respectively, of chicken meat and eggs were contaminated (Al Khalaileh, 2018). However, pigs are more vulnerable to OTA exposure in comparison to other productive animals (Meucci *et al.*, 2019; Panel, 2020), Due to their heavy grain eating, they are exposed to such toxins and chronic diseases (Pierron *et al.*, 2016b). High OTA occurrence was also recorded in swine feed ingredients in many nations (Leiva *et al.*, 2019; Rosa *et al.*, 2009), indicating the pig exposure to the toxin. Battacone *et al.* (2010) and Hort *et al.* (2018) reported OTA accumulation in pig tissues, blood, kidneys and the liver, whereas lower concentrations have been found in muscles and fat. Ochratoxin majorly damages the kidney of all animal species, while it can also harm the liver in high amounts. The toxic effect and hazardous impact has been attributed to the inhibition of phenylalanine tRNA synthetase (Dirheimer & Creppy, 1991). It is also a teratogen, a carcinogen, and an immunological suppressant (Harwig *et al.*, 2020), and it is also implicated in the etiology of Balkan endemic nephropathy (Pfohl-Leszkowicz *et al.*, 2002). Studies have revealed that ochratoxin A-DNA

adducts are formed in the bladder and kidney tissues of Bulgarian patients after cancer surgery (Malir *et al.*, 2021; Pfohl-Leszkowicz & Manderville, 2007).

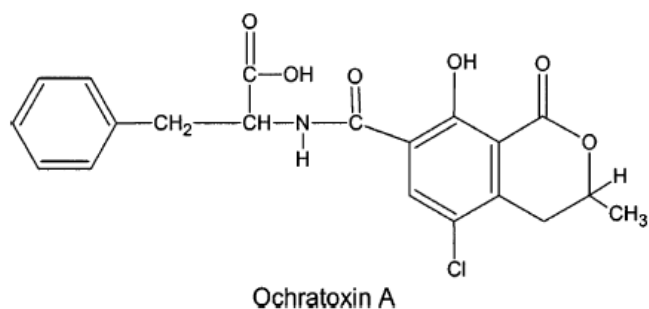


Figure 2. 4: Ochratoxin A structure (Zain, 2011)

2.5.3 Zearalenone (ZEN)

Zearalenone (ZEN) is majorly generated by *Fusarium* species and most often seen in maize and other grain crops (Al-Jaal *et al.*, 2019; Caglayan *et al.*, 2020). Exposure to ZEN is associated with lower levels of serum testosterone and progesterone in the bloodstream resulting in infertility and decreased instances of conception in animals such pigs, rats and cows, (Rai *et al.*, 2020; Yang *et al.*, 2007). ZEN promotes intracellular oxidative stress that causes oxidative apoptosis and DNA damage (Hassen *et al.*, 2007). The European Commission has set a limit of 2 mg·kg⁻¹ ZEN for feed ingredients, except for maize where the suggested maximum limit is 3 mg·kg⁻¹ ZEN.

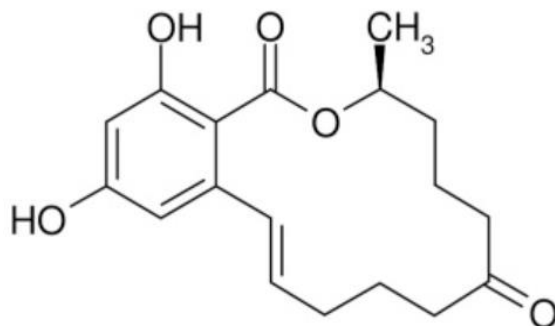


Figure 2. 5: Zearalenone structures (Rogowska *et al.*, 2019)

2.5.4 Deoxynivalenol (DON)

Deoxynivalenol is a type B trichothecene that has been produced naturally by fungus belonging to the *Fusarium* genus. Fusarium toxins are the most common mycotoxins worldwide over the past

few years (Gruber-Dorninger *et al.*, 2019). Deoxynivalenol was the most prevalent mycotoxin. More than two-thirds of samples of finished feed (maize and wheat) tested positive for it (Gruber-Dorninger *et al.*, 2019). Among feed ingredients that were positive for deoxynivalenol, wheat and corn were the most often contaminated (Gruber-Dorninger *et al.*, 2019). The DON contamination of crops was related with mild temperatures and more precipitation during the blossoming and maturity stages (Gruber-Dorninger *et al.*, 2019). As result of widespread prevalence and harmful consequences of DON, there exist-governmental controls in the European Union and the United States. The Food and Drug Administration's guideline values for deoxynivalenol in pig diets advise against exceeding 1 mg/kg (FDA, 2010). In contrast, the European Commission required 0.9 mg/kg of deoxynivalenol in pigs feed (Cast, 2003).

The dietary ingestion of deoxynivalenol is known to decrease feed intake and weight gain of pigs (Patience *et al.*, 2014). A study reported a reduction in growth performance at 1 to 3 mg/kg of DON fed to a pig (Marin *et al.*, 2013), in naturally contaminated diets, concentrations of 1-2 mg/kg of DON reduce feed intake and weight gain where each additional 1 mg/kg of DON further reduces weight gain by 8% in pigs (Dersjant-Li *et al.*, 2003). DON influences blood chemistry and the systemic immune response in growing pigs (Accensi *et al.*, 2006; Swamy *et al.*, 2002). DON contaminated diets increased total immunoglobulin (Ig)A titer in serum and interrupt the dendritic cells activities in pigs (Accensi *et al.*, 2006; Bimczok *et al.*, 2007).

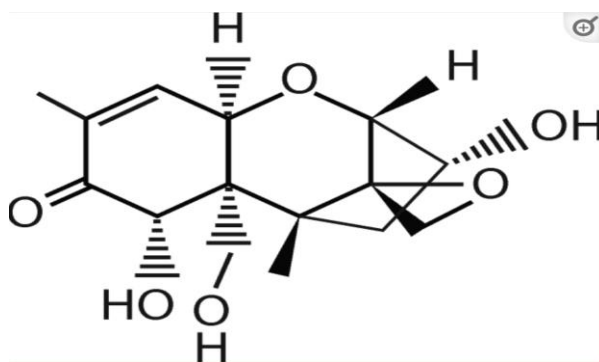


Figure 2. 6: Deoxynivalenol structure (Sobrova *et al.*, 2010)

2.5.5 Fumonisin (FM)

Fumonisin are produced by filamentous fungi. They are water-soluble secondary toxic metabolites and were first isolated in 1988 (Demir *et al.*, 2010). Fumonisin are notably contaminant of maize and maize-based products but can also be found in many other ingredients used in animal feed formulation (Stockmann-Juvala & Savolainen, 2008; Streit, Naehrer, *et al.*, 2013). Among many fumonisin analogues identified so far, the most often discovered are fumonisins B1 (FB1), B2 (FB2) and B3 (FB3). FB1 is the most widespread and is present in higher proportions (about 70%) in contaminated feed and food (Rheeder *et al.*, 1992; Streit, Schwab, *et al.*, 2013). The European Union has established fumonisins warning limits for pigs at 5 mg/kg of finished feed for fumonisins B1 and B2 (Holanda & Kim, 2021b), and the sum of fumonisins B1, B2, and B3 at 10 mg/kg of finished feed in the United States (FDA, 2019).

Fumonisin B1 and B2 have been shown to cause leukoencephalomalacia in the horse (Ji *et al.*, 2019), and pulmonary oedema and hydrothorax in pig species (Ross *et al.*, 1990). According to Riley and Merrill (2019), this toxin interferes with ceramide kinase, which disrupts the metabolism of sphingolipids. The high prevalence of oesophageal cancer in China, Italy, and South Africa has been linked with fumonisin B₁ (Braun & Wink, 2018). In a previous study, Wang *et al.* (2014) reported that fumonisin B₁ increased the protein expression of cyclin D1 and decreased that of cyclin E, p21, and p27 while promoting the proliferation of human oesophageal epithelial cells.

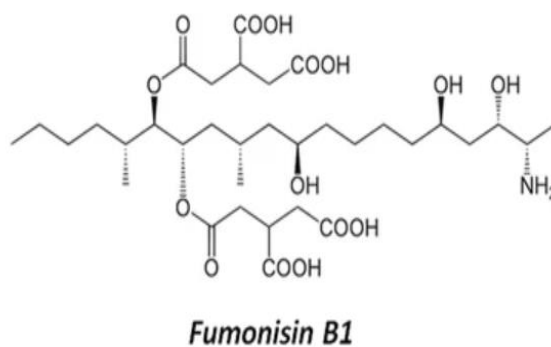


Figure 2. 7: Fumonisin structures (Pierron *et al.*, 2016a)

The prevalence of mycotoxins in agricultural commodities and animal feed is a cause for serious concern across the world, necessitating international measures to protect animals' health. However, mycotoxins are virtually universally present in food feeds. Hence, the assessment of the efficacy of feed additives as mycotoxin-detoxifying agents for the animal would be extremely valuable.

2.5.6 Dietary mitigation additives for mycotoxin toxicity

Mycotoxin-detoxifying or binding agents can be used as feed additives to allow for the utilization of contaminated feedstuffs and feeds through reducing or eliminating the toxic effects of mycotoxins (Colovic *et al.*, 2019). There are varieties of mycotoxin-detoxifying drugs available, each with its mode of action. Some compounds, for example, are utilized to reduce the harmful effects of mycotoxins in an animal by adsorption, immunological enhancement, and detoxification (such as microorganisms). Although, numerous mycotoxin-detoxifying agents are employed to detoxify their toxic effect in diets, the major problem sometimes encountered with their use, is their prohibitive cost. For example, in the USA, it is estimated that the cost of mycotoxins management and mitigation cause an estimated annual economic loss of USD 900 million (Ecker *et al.*, 2005). Consequently, there has been a heightened level of interest in developing a more cost-effective, safe and environmental friendly mycotoxin detoxifying agents from such locally available plant sources as *Tulbaghia violacea*.

2.6 *Tulbaghia violacea*

Tulbaghia violacea is a bulbous, fast-growing plant to a height of 0.5 m (Olorunnisola *et al.*, 2012b). When crushed, the leaves that are long, narrow, strap-like and a little meaty, smell strongly of garlic (Manning, 2013). The pinkish-mauve tubular blooms are carried above the leaves on a tall flower stalk and appear over a lengthy period in summer, grouped into umbels of up to 20 flowers (January to April). Triangular capsules form the fruit, which is gathered into a head and split when ripe to release the flattened, hard black seeds. This drought-resistant plant can be found from the Eastern Cape, KwaZulu-Natal, and Limpopo and all the way to Zimbabwe in the north (Mthethwa, 2009).



Figure 2. 8: *T. violacea* Plant (van Wyk *et al.*, 2013).

Tulbaghia violacea, also known as wild garlic in English, wildeknoffel in Afrikaans, Isihaqa in Zulu, and Moelela in Sotho, is used in traditional medicine to treat fevers, colds, asthma, tuberculosis, stomach aches, and oesophageal cancer. (Saibu *et al.*, 2015; Takaidza *et al.*, 2018).

2.6.1 Bioactive components of *Tulbaghia*

In the Alliaceae family, numerous chemical components have been found (Bastaki *et al.*, 2021; Bungu *et al.*, 2008). *Tulbaghia violacea* has been discovered to be high in sulphur compounds, which are responsible for both the *Tulbaghia* and *Allium* species' distinctive odour and medicinal capabilities (Lyantagaye, 2011). When the enzyme alliinase (EC.4.4.1.4) hydrolyzes the S-alka

(en)yl-L-Cys Sulfoxides (ACSOs) to create sulphur-containing volatiles, pyruvate, and ammonia, it produces the distinctive flavour of garlic (*Allium sativum*) and related alliums (Takaidza *et al.*, 2018). The enzyme C-lyase has been discovered in the *Tulbaghia* genus and is linked to the production of marasmicin (Danquah *et al.*, 2022).

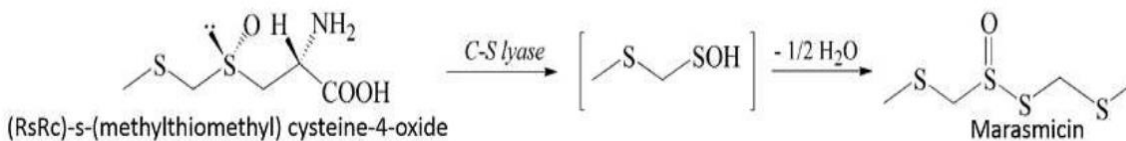


Figure 2. 9: Marasmicin synthesis in *Tulbaghia villoacea* Harv. (Kubec *et al.*, 2002).

Many of these chemicals have been demonstrated to have antibacterial, antifungal and anticancer activities, supporting the traditional use of various *Tulbaghia* species (Danquah *et al.*, 2022).

2.6.2 Antioxidant activity of *Tulbaghia*

T. violacea's antioxidant activity has been studied in a number of ways (Ncise *et al.*, 2021; Takaidza *et al.*, 2018). The antioxidant activity of *T. violacea* has been studied largely on the rhizomes, with mediocre results (Buwa & Afolayan, 2009; Olorunnisola *et al.*, 2012a). Given *Tulbaghia's* potential antioxidant effect, more research into different portions of the plant as well as undiscovered *Tulbaghia* species is needed.

2.6.3 Antimicrobial activity of *Tulbaghia*

Antibacterial functions of several extracts from *Tulbaghia violacea* have been investigated for a variety of microorganisms that damage both plants and humans. Varieties of antimicrobial activities of *T. violacea* have been reported by various researchers (Buwa & Afolayan, 2009; Ranglová *et al.*, 2015). Moreso, it has been demonstrated that crude extracts of *T. violacea* exhibit broad-spectrum antibacterial action against organisms like *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* (Aremu & Van Staden, 2013). *Tulbaghia violacea* and *T. alliacea* have also been shown to have antifungal properties (Olorunnisola *et al.*, 2012b). The presence of antimicrobial properties in *T. violacea* makes it incumbent to examine the other species in the genus for similar properties.

2.6.4 Immunomodulatory activity of Tulbaghia

Few studies demonstrate the ability of *Tulbaghia* species to modulate immune system (Gaidamashvili & Van Staden, 2006), this study shows weak anti-inflammatory actions of lectin-like proteins from *T. violacea*. Additionally, reaserch on the immunomodulatory properties of Tulbaghia species is required. A distinguishing characteristic of the species is the "garlic-like odor" (alliaceous odor) caused by the sulphur compounds obtained from cysteine released from injured or decomposing tissue and organs such as rhizomes and leaves (Kubec *et al.*, 2013).

2.6.5 Anticancer activity of Tulbaghia

Tulbaghia is traditionally used to treat oesophageal cancer (Bungu *et al.*, 2008; Bungu *et al.*, 2006). Globally, Oesophageal cancer makes up nine percent of cancers, and South African men have the highest incidence rate as it is the second most prevalent cancer among them. It also has one of the lowest chances of recovery, with an estimated 10% overall 5-year survival rate (Skerman *et al.*, 2011). According to research done thus far, *T. violacea* slows the development of cancer cells and triggers apoptosis (Bungu *et al.*, 2006; Saibu *et al.*, 2015). However, there have only been a few investigations into the species' anticancer properties. Therefore, additional research is necessary to determine the Tulbaghia species' ability to combat oesophageal cancer.

2.6.6 Toxicity of Tulbaghia

The consumption of traditional medicine made from *T. violacea* has been linked to side effects like gastro-enteritis, abdominal pain, and cessation of gastro-intestinal peristalsis, sloughing of the intestinal mucosa and contraction of the pupils (Aremu & Van Staden, 2013). Resende *et al.* (2012). In addition, research indicates that *T. violacea* may be toxic, despite claims to the contrary from other studies (Bamuamba *et al.*, 2008; Soyngibe *et al.*, 2013). Therefore, the safety and toxicity of species in the genus *Tulbaghia* need to be clarified via more research.

CHAPTER 3: MATERIALS AND METHODS

3.1 Ethical clearance

Ethical approval for this study was sought and it was issued by the Animal Care Ethic Committee of the Faculty of Natural and Agriculture Sciences (Ethics clearance number: NWU-00813-21-A5), North West University.

3.2 Study site

This study was conducted at Molewane research farm (25°40.459'S, 26°10.563'E), North-West University (North West, South Africa), at an altitude of 1 226 m above sea level. The vegetation is a semi-arid Savannah with an average annual rainfall of 450 mm. Temperatures ranged from 22 to 35 °C in summer and 2 to 20 °C during the winter months.

3.3 Source of Materials

A total of 48, weaned large white piglets (aged 4 weeks) were purchased from GHB Farms (Pty) Ltd and were weighed, ear-tagged and dewormed against internal parasites using 30 mg/kg Flubendazole and kept to adapt for 10 days. Marula seed cake was purchased from Magnum Production in KwaZulu-Natal Province, South Africa, whilst fresh *T. violacea* bulbs were harvested from the farm at the Mafikeng campus, North West University. The *T. violacea* bulbs were air dried at room temperature (15 to 25°C) with daily turning to minimize possible contamination by mould. The dried bulbs were afterwards, stored in an air-tight container until use. Soybean meal and other ingredients required for feed formulation were sourced from Simple Grow Agric Services (Pty) Ltd in Irene, Gauteng Province, South Africa. Preliminary laboratory tests were done to confirm their proximate nutritional composition.

3.4 Proximate determination of the formulated diet

The proximate analysis of the feed is crucial to rank the diet as an energy or protein source. Prior to the formulation of each set of diets, proximate composition was analyzed using the official analytical chemists' international procedures (AOAC, 2005).

Briefly, 1 g of each diet formulation was oven-dried at 105 °C for 4 h. Thereafter, each sample was taken out of the oven, and cooled in desiccators, and its dry weight was measured using a weighing scale. The dried samples were assayed for 12 hours at 550 °C in a muffle furnace to measure the amount of organic matter (OM). Ash content and OM content were used to determine the weight loss and residue, respectively.

3.4.1 Determination of the fibre content

To determine the neutral detergent fibre (NDF) and acid detergent fibre (ADF), 1g of each sample was refluxed for 60 minutes at 100 °C in 100 ml of neutral detergent solution (NDS) and acid detergent solution, respectively. Subsequently, the samples were filtered, rinsed, dried (100 °C overnight) and weighed after cooling. A Tecator Fiber System (Fibertec System, Tecator, Hoganas, Sweden) and an ANKOM2000 Fibre Analyser (ANKOM Technology, New York) were used to estimate NDF and ADF in accordance with what van Soest *et al.* (1991) reported. A similar procedure used to quantify NDF was also used to quantify acid detergent lignin (ADL) with slight modification. Each sample's residue was rinsed, and then 72% sulphuric acid was added, and the residue was heated for three hours at 700 °C . After boiling, the sulphuric acid was removed with hot water, and the residue was dried at 100 °C for 12 hours before being cooled and then weighed.

3.4.2 HPLC quantification of Aflatoxins in formulated diets

Aflatoxins were extracted from the feeds in accordance with the manufacturer's instructions, using the immunoaffinity extraction method using Easi-Extract® aflatoxin immunoaffinity columns (R-Biophram Rhome Limited, Glanglow G20 OXA, Scotland). Extraction was carried out according to the manufacturer's protocol with some modifications as follows; a portion of 50g of the sample was mixed with 5g sodium chloride (NaCl) in a laboratory blender, followed by 100 ml of methanol; water (80:20 v/v), and blended for 5 min. The mixture was filtered through a fluted filter paper (Whatman No.1) into a clean vessel. A volume of 2ml of the filtered solution was then diluted with 14ml phosphate buffer saline (PBS) solution and passed through an immunoaffinity column. The column was washed with 20ml of PBS and the aflatoxin extractions were finally eluted with 1ml method (LiChrosolv®, Merck, Germany) into a glass cuvette and diluted with 1ml of distilled water and stored at -20°C prior to analysis. Aflatoxin B₁, B₂, G₁ and G₂ standards (Trilogy Analytical Laboratory, Washington, USA) were diluted using acetonitrile (LiChrosolv®,

Merk Germany) to give the following concentrations; 5×10^{-6} , 5×10^{-5} , 5×10^{-4} , 5×10^{-3} , 5×10^{-2} mg/ml. Aflatoxin detection and quantification was done using HPLC (Shimadzu FCV-20H2) with operation conditions as given in the KOBRA® cell instruction manual as follows: derivatization using a KOBRA ® cell a 100 μ A setting with an analytical column Intersil ODS-3V 5 μ m, 4.6mmx150mm equipped with a C18 4 x 3 mm² i.d. Security Guard cartridge (Phenomenex, Torrance, CA, U.S.A.), The mobile phase was modified from the recommended water: methanol (60:40) to a working condition of (55:45) with 119mg/litre of potassium bromide (KBr) and 1 ml of 65% nitric acid added at a flow rate of 1.0ml/minute, and the fluorescence detector set at 362 nm for excitation and emission 425nm (AFB₁ and B₂) and 455 nm (AGB₁ and G₂). The injector was an autosampler that injected 100 μ l of sample, and the elution of the aflatoxins was in the order (AF) G₁, G₂, B₁, and B₂.

Calibration curves for each aflatoxin, a AF B₁, B₂, G₁ and G₂, were constructed using standard solutions that were diluted with acetonitrile to give the following concentrations: 0.005, 0.05, 0.5, 5, 50 μ g/kg. The limit of detection for all the standards was 0.005 μ g/kg. The linearity of the standard curves was determined using correlation regression (r^2). A curve with good linearity will have an r^2 value close to 1. The aflatoxin concentration of the sample was calculated by measuring the area of the peak and then interpolating from the standard curve.

3.5 Experimental design, diet, and their preparation

Naturally, ventilated pens equipped with stainless-steel feeders and nipple drinkers were used in this investigation. The present study applied a randomized block experimental design (RBD) with three iso-energetic and iso-nitrogenous experimental diet groups B, C, D, and a control group A. Feeds for each group comprised of; A = 0% MSC, B=10% MSC, C= 20% MSC and D = 20% MSC+2.5%WG (Table 3.1). A total number of forty eight (48) Large White weaned piglets of average weight ranging between 8 to 10 kg were used for this study. The piglets were randomly allocated on weight and sex equalization basis to the four dietary treatment. The Twelve (12) Large White weaned piglets (four weeks unisex) were randomly allocated to each treatment group. All groups were fed *ad libitum* with a starter diet comprising maize and soybean for 14 days. Thereafter, commencing 42 days feeding trial with 0% MSC (group A = control group), 10% MSC (group B), 20% MSC (group C), and 20% MSC supplemented with 2.5 WG (group D), replacing

the starter diet. Daily observations for clinical signs including body temperature were checked by a qualified veterinarian throughout the experimental period. The ingredient composition, proximate content and mycotoxin concentrations of experimental and control diets are presented in Tables 3.1, 3.2 and 3.3 respectively.

Table 3. 1: Composition of formulated diets with and without MSC

Ingredient (%)	Diet Group			
	A	B	C	D
	0%MSC	10%MSC	20%MSC	20%MSC+2.5WG
Maize 7.50 % CP	68.62	64.45	64.45	62.99
Wheat bran	4.87	10.11	10.11	10.10
Soya Oil cake 47% CP	22.32	11.30	1.35	-
Marula Seed Cake	-	9.95	19.90	19.89
Wild Garlic	-	-	-	2.5
L-lysine HCl	12.181	12.612	12.612	12.860
DL-Methionine	7.730	7.657	7.657	7.545
L-Threonine	4.892	5.159	5.159	9.243
L-Tryptophane	2.182	2.158	2.158	2.195
Optiphos Plus 5000	0.263	0.263	0.263	0.244
Hostazym X	0.263	0.263	0.263	0.244
Limestone	28.484	30.523	32.627	29.907
MDCP 21 %	22.484	19.706	17.604	17.667
Salt	15.511	15.758	15.757	14.627
Std Swine Weaner Px 2.2kg	5.789	5.789	0.5789	5.366
Endox Dry	0.111	0.111	0.111	0.102

Table 3.2. 1: Proximate composition of formulated feeds with or without MSC and wild garlic

Proximate	Diet				
	0%MSC	10%MSC	20%MSC	20%MSC+2.5WG	MSC
Dry matter (DM%)	90.92	91.46	91.32	91.42	94.8
Crude protein (DM%)	28.10	28.64	28.64	31.34	47.0
Ash (DM%)	5.23	5.21	4.38	4.03	5.3
Fat (DM%)	3.63	5.77	6.74	7.82	37.1
NDF	10.87	14.07	15.17	18.31	2.7
ADF	7.53	9.92	12.41	13.66	7.2
ADL	1.27	2.24	3.10	3.53	5.6

Key: NDF (neutral detergent fibre), ADF (acid detergent fibre) and ADL (acid detergent fibre)

Table 3.2. 2: Mycotoxin contamination in diet MSC with or without wild garlic

Mycotoxin status (ppb)	Diet				
	0%MSC	10%MS C	20%MS C	20%MSC+2.5W G	MSC
AFB1	65.57	2297.66	312.54	1419.29	104.41
AFB2	11584.53	2297.66	5336.53	16293.33	5750.67
AFG1	422.13	9171.47	2419.73	2934.93	6094.67
AFG2	307.4	1653.8	407.0	1225.8	14226.35
Total	12379.6	15420.6	8475.8	21873.4	26176.1

3.6 Measurement

3.6.1 Growth performance

Average weekly feed intake (AWFI) per piglet was determined by subtracting the weight of the feed refused from feed offered weekly and dividing the difference by the total number of piglets in the treatment. After two weeks of adaptation, all piglets per experimental unit were weighed to determine the initial body weights, and thereafter body weight gain was weighed weekly by subtracting the weight of the previous week from the weight of the current week until six weeks of the experimental period. The live weights obtained weekly were used to calculate the average weekly body weight gain (ADG) per piglet as follows:

1. Average daily gain (ADG) = $\frac{\text{final weight gain} - \text{initial weight gain}}{\text{Total period of experiment}}$

2. Average daily feed intake (ADFI) = $\frac{\text{feed offered} - \text{leftover feed}}{\text{Total period of experiment}}$

3. Average Feed conversion efficiency (FCE) = $\frac{\text{weight gain}}{\text{feed intake}}$

3.6.2 Carcass, internal organs and meat quality

At six weeks of the experimental period, the pigs were transported to an abattoir (Benade Abattoir, Mafikeng, North-West province, South Africa) for slaughter. After 12 hours of feed restriction, the pigs were stunned and exsanguinated (using electric) while suspended. Carcasses were placed in a dehairer at 62 °C for 5 minutes, and the remaining hair was removed manually using a knife and flame. Thereafter, the carcasses were eviscerated, split and individually measured to determine the hot carcass measurements and the internal organs of each piglet (spleen, liver, kidney, small and large intestines) were collected to determine their weight before the carcasses were stored in a chiller cold room set at -80 °C for 24 hours. After chilling, cold carcass measurements were performed and all the *Longissimus dorsi* muscles were collected from the back of individual piglets for meat quality analysis. The *L. dorsi* muscle was then transferred into a refrigerator and examined

for meat quality traits. For preservation purposes, muscle samples were vacuum-packed and deep-frozen at -80 °C.

3.6.3 Internal organs and carcass measurement

After slaughter, the weight of the collected internal organs (spleen, liver, kidney, large intestine and small intestine) was weighed using a digital balance (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA),

Then the carcasses were manually eviscerated and weighed to determine hot carcass weights (HCW). The carcasses were then chilled in a cold room at -80 °C for 24 hours and reweighed to determine cold carcass weight (CCW). Carcass yield was calculated as the proportion of HCW on slaughter weight (final body weight). Weights of carcass cut (ribs length, back length, shoulder, fore leg, hind leg, ham and belly) were measured using a digital balance (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA), while rib and back lengths were measured using a tape.

3.6.4 Meat Quality

a. Meat pH, temperature measurements and colour

Meat pH after 45 minutes and 24 hours post-slaughter including the temperature were measured on the longissimus muscle (central area of the longissimus) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) fitted with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland). After every 10 measurements, the pH meter was re-calibrated with pH standard solutions (Ingold Messtechnik AG, Udorf, Switzerland).

Meat colour coordinates: L* (lightness), a* (redness) and b* (yellowness) were measured from the longissimus meat samples after 45 minutes and 24 hours post-slaughter using a Minolta colour-guide 45/0 BYK- Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-daylight, 10° observation angle after 30 minutes blooming time. The colour meter was calibrated before measurements and after every 10 samples using the zero and white calibration as recommended by the manufacturer. Colour recording was done on the surface of a freshly cut slice of all the *L. dorsi* after being allowed to bloom for 1 hour on a polystyrene tray at 4 °C.

b. Thawing

Thawing loss was measured and calculated from the *L. dorsi* for a percentage of weight loss before and after thawing. Thawed samples (1 cm thick and 70 ± 5 g) were cut perpendicularly to muscle direction. Then all samples were hung in a vertical chiller for 12 hours as described by to Ali *et al.* (2016) and the liquid was collected in cups placed under the hangers. Thawing loss was calculated as follows:

$$\text{Thawing (\%)} = \frac{\text{frozen sample weight (g)} - \text{final sample weight}}{\text{frozen sample weight}} \times 100$$

c. Cooking loss

Raw longissimus meat samples were individually weighed to obtain initial weight and followed by cooking the samples in an oven set at 100 °C for 1 hour. They were pre-heated to 72 °C for 45 min and cooked, after which they were cooled to room temperature (± 20 °C) for 30 minutes. The internal temperature was measured with an analogue thermometer (75 °C). The samples were allowed to cool before measuring the final weight. The following formula was employed to measure the cooking loss:

$$\text{Cooking loss (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

d. Water holding capacity (WHC)

Water holding capacity was measured by pre-weighing (~ 2 to 3 g) freshly cut longissimus muscle samples, which were placed in-between a pre-weighed No. 18 Whatman filter paper and compressed for five minutes at a pressure of 60-kg with dumbbell weights (Grau & Hamm, 1957). The water expressed from fresh meat was absorbed using filter bags and thereby use to calculate water holding capacity expressed in proportion to the initial weight as shown in formula below:

$$\text{WHC (\%)} = \frac{\text{initial weight} - \text{weight after pressing}}{\text{initial weight}} \times 100$$

e. Drip loss

Drip loss was measured based on the methods of Zhang *et al.* (2009), duplicate samples from each pen weighing about 2 g (w_1) were kept hanging within an airtight container. The cold chamber was set up with the temperature at 4 °C, and the suspended samples were kept there for 72 hours. Thereafter, the meat samples were weighed again to determine their weight after drip (w_2). Each sample's weight both before and after a drip was determined using the following percentage drip loss formula:

$$Drip\ loss\ (\%) = \frac{w_1 - w_2}{w_1} \times 100$$

3.7 Blood collection for serum analysis

Blood samples (7mL) were collected by a technician aseptically from the jugular vein of pigs at weeks 1, 3, and 6 of the experimental periods into collection tubes without anticoagulant for serum biochemical and measurements. Serum was obtained based on the guidelines stipulated by Washington and van Hoosier (2012). Briefly, clotted blood was centrifuged at 1000 g for 15 minutes. The acquired serum was analysed for glucose (GLU), symmetric dimethylarginine (SDMA), creatinine (CREA), alkaline phosphate (ALKP), alanine transaminase (ALT), albumin (ALB), calcium (Ca), cholesterol (CHOL), globulin (GLOB), phosphorus (P), Blood urea nitrogen and creatinine (BUN/CREA), gamma-glutamyl Transferase (GGT), total protein (TP), urea (UREA), amylase (AMLY), and lipid pathway analysis (LIPA) using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories S.A.PTY, Gauteng, South Africa) and albumin-to-globulin ratio (ALB/GLOB) was calculated. After six weeks of the experimental period, the whole piglets were taken to the Benade pigs abattoir where they were electrically stunned and humanely slaughtered by cutting the jugular vein with a very sharp knife and left hanging until the bleeding stopped.

3.8 Statistical analysis

The whole data with repeated measurements (body weight gain, feed intake and feed conversion efficiency) were analysed using the general linear model procedure of Minitab (2000) version 13 with the following model:

$$Y_{ij} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ij}$$

The statistical model was interpreted below:

Y_{ij} = dependable variable

μ = overall mean

D_i = main effect of diet

W_j = main effect of week

$(D \times W)_{ij}$ = interaction effect of diet and week

E_{ij} = random error associated with observation ij , assumed to be normally and independently distributed.

Otherwise, data on overall growth performance, serum-biochemical indices, and meat quality parameters were analysed using the GLM procedure of Minitab (2000) version 13 using the following linear statistical model:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where Y_{ij} = dependable variable, μ = overall mean, D_i = main effect of diet, and E_{ij} = random error associated with observation ij , assumed to be normally and independently distributed.

For all statistical tests, significance was declared at $P < 0.05$. Where statistically different, least square means were compared using Tukey's test.

CHAPTER 4: RESULTS

4.1 Growth performance

The effects of dietary inclusion of mycotoxin contaminated marula seed cake (MSC) with or without wild garlic (WG) on overall feed intake (FI), body weight gain (BWG) and feed conversion efficiency (FCE) of weaned large white piglets are presented in Table 4.1. The results showed that there were significant ($P < 0.001$) effects of MSC with or without wild garlic on the feed intake and body weight gain, but not on feed conversion efficiency ($P > 0.05$). The inclusion of MSC with wild garlic (20% MSC + 2.5% WG) significantly decreased ($P < 0.001$) FI in the piglets. Further, piglets offered 10% MSC only without wild garlic had an increased ($P < 0.001$) BWG. Meanwhile, a further increase in the inclusion level of MSC to 20% without wild garlic showed a significant reduction ($P < 0.001$) in BWG. Dietary inclusion of mixtures of MSC and WG wild garlic (20%MSC + 2.5 WG) recorded the least ($P < 0.001$) body weight gain of piglets.

The results of diet and week on weekly body weight gain, feed intake and feed conversion efficiency of weaned large white piglets are presented in Table 4.2. The interaction effect of diet and week was seen to be significant for body weight gain and feed intake ($P < 0.05$), but not for weekly feed conversion efficiency ($P > 0.05$). The main effect of diet was highly significant on the weekly body weight gain and weekly feed intake ($P < 0.001$) with no observable effect on the weekly feed conversion efficiency ($P > 0.05$) of weaned large white piglets. Also, the results showed that the body weight, feed intake and feed conversion efficiency were significantly ($P < 0.001$) influenced by week. The body weight gain of the piglets was seen to be higher at week 3 than at weeks 1 and 2. Piglets fed 0% MSC, 10% MSC and 20% MSC at week 3; and 0% MSC at week 6 had higher body weight gain while those fed 20% MSC at week 1, 20% MSC +2.5% WG at week 1 and 2, 0% MSC at week 5 had the lowest body weight gain. As the weeks progressed, the BWG of piglets fed 20% + 2.5 WG significantly decreased. Also, the feed intake of piglets at week 6 was higher than the feed intake of piglets at week 1. Piglets fed 0% MSC, 10% MSC, 20% MSC and 20% MSC + 2.5% WG had the highest feed intake at week 6 compared with piglets fed 0% MSC, 10% MSC, 20% MSC and 20% MSC + 2.5% WG at week 1. On the other hand, the feed conversion efficiency of piglets fed 20% MSC at week 1 and 0% MSC at week 5 were better than the feed conversion efficiency of piglets fed 0% MSC, 10% MSC and 20% MSC at week 3.

Table 4. 1: Effect of MSC with or without wild garlic on overall body weight gain, feed intake and feed conversion efficiency of weaned large white piglets.

Parameter	Diets				SEM	P-value
	0% MSC	10% MSC	20% MSC	20% MSC+2.5WG		
Feed intake (g/day)	827.21	825.97	821.28	699.92	2.15	P < 0.001
Body weight gain (g/day)	2578.03	2591.67	2041.67	1481.97	64.96	P = 0.001
Feed conversion efficiency (g/g)	3.03	3.14	2.51	2.13	0.09	P > 0.05

MSC =Marula Seed Cake; WG =Wild Garlic; SEM =standard error of the mean; n = 12 piglets.

Table 4. 2: Weekly body weight gain, feed intake, and feed conversion efficiency in weaned large white piglets fed diets supplemented with MSC with or without Wild garlic

Week	Diet	BWG (g/day)	FI (g/day)	FCE
1	0%MSC	1466.7	484.4	3.1
	10%MSC	1033.3	476.5	2.2
	20%MSC	433.3	485.8	0.9
	20%MSC+2.5% WG	472.7	407.0	1.2
2	0%MSC	2066.7	554.5	3.8
	10%MSC	1583.3	542.9	3.0
	20%MSC	1416.7	548.4	2.6
	20%MSC+2.5% WG	818.2	429.45	1.9
3	0%MSC	4983.3	809.2	6.1
	10%MSC	4391.7	810.3	5.4
	20%MSC	4300.0	819.4	5.2
	20%MSC+2.5% WG	2600.0	674.6	3.9
4	0%MSC	2833.3	991.7	2.9
	10%MSC	2325.0	983.8	2.4
	20%MSC	2216.7	985.5	2.3
	20%MSC+2.5% WG	2410.0	787.9	3.1
5	0%MSC	300.0	902.7	-0.8
	10%MSC	3155.0	950.1	3.3
	20%MSC	1983.3	942.5	2.1
	20%MSC+2.5% WG	1227.3	816.1	1.5
6	0%MSC	3818.2	1220.7	3.1
	10%MSC	3066.7	1192.3	2.6
	20%MSC	1900.0	1146.1	1.9
	20%MSC+2.5% WG	1363.6	1084.5	1.3
	SEM	159.0	5.0	0.2
Effects	Diet	P = 0.001	P < 0.001	P > 0.05
	Week	P < 0.001	P < 0.001	P < 0.001
	Diet x week	P < 0.05	P < 0.05	P > 0.05

BWG =body weight gain; FI =Feed intake; FCE=Feed conversion efficiency (BWG/FI); MSC =Marula Seed Cake; WG =Wild Garlic; SEM =standard error of the mean (SEM); n = 12 piglets

4.2 Internal organ weight

Table 4.3 shows the internal organs weight of weaned Large White piglets fed mycotoxin contaminated MSC with or without wild garlic. The result of the study showed that there were significant ($P < 0.05$) effects of the diet only on the liver weight, while there were no significant effects ($P > 0.05$) on the weight of kidney, small intestine, large intestine, spleen, caecum and stomach. The liver weight reduced ($P < 0.05$) with increasing inclusion levels of MSC, while the least ($P < 0.05$) liver weight was observed with piglets offered 20% MSC + 2.5% WG. Hence, piglets fed 0% MSC had a higher liver weight value.

Table 4. 3: Effect of dietary MSC inclusion with or without wild garlic on weights of internal organs of weaned large white piglets.

Parameter (g)	Diet				SEM	P value
	0%MSC	10%MSC	20%MSC	20% MSC&2.5% WG		
Kidney	42.8	56.7	44.0	37.6	2.0	P > 0.05
Liver	1190.6	985.5	893.7	747.6	29.9	P < 0.05
Small intestine	782.9	939.1	838.3	791.8	22.5	P > 0.05
Large intestine	402.8	401.8	374.0	382.2	14.0	P > 0.05
Spleen	47.4	48.7	40.2	40.6	1.6	P > 0.05
Caecum	49.9	64.7	52.7	58.4	2.3	P > 0.05
Stomach	333.1	307.1	298.0	300.8	8.3	P > 0,05

SEM =standard error of the mean; n = 12 piglets

4.3 Carcass and Meat Quality

4.3.1 Carcass Parameters

The effect of mycotoxin contaminated MSC with or without garlic on rib length, shoulder weight, fore-leg weight, ham weight, hind-leg weight, belly weight and back length of weaned large white piglets are presented in Table 4.4. The results of the study showed that the inclusion of MSC with or without wild garlic significantly ($P = 0.001$) influenced the foreleg weight and hindleg weight ($P < 0.05$), while the rib length, shoulder weight, ham weight, hindleg weight, belly weight and back length were not significantly ($P > 0.05$) influenced. The foreleg weight of the piglets was significantly ($P = 0.001$) increased with the inclusion of 10% MSC, while further increase in the inclusion of MSC to 20% and inclusion of mixtures of MSC and WG (20% MSC + 2.5 WG) significantly ($P = 0.001$) decreased the foreleg weight. On the other hand, increasing inclusion levels of MSC from 0% (at control) to 20% lead to significantly ($P < 0.05$) increasing weight of the hind leg from 706.6 to 869.4g. However, dietary inclusion of 20% MSC + 2.5 WG significantly ($P < 0.05$) decreased the weight of the hindleg.

Table 4. 4: Effect of dietary inclusion of MSC with or without wild garlic on weights of carcass cuts of weaned large white piglets

Parameter (g)	Diet				SEM	P value
	0%MSC	10%MSC	20%MSC	20%MSC+2.5%WG		
Rib length	29.8	32.2	30.0	29.3	0.3	P > 0.05
Shoulder weight	749.8	791.2	679.1	632.8	14.4	P > 0.05
Foreleg weight	719.2	938.1	654.5	564.0	17.3	P = 0.001
Ham weight	1443.3	1507.6	1331.4	1142.4	29.2	P > 0.05
Hindleg weight	706.6	803.5	869.4	621.5	18.1	P < 0.05
Belly weight	550.3	536.3	536.0	433.6	10.4	P > 0.05
Back length	50.6	52.7	51.1	46.6	0.5	P > 0.05

SEM =standard error of the mean; n = 12 piglets

4.3.2 Carcass weight, temperature, pH and colour

The result of the effect of time (45min and 24hr) x diet on carcass weight, temperature, pH and colour L* lightness, a* redness and b* yellowness of weaned large white piglets are presented in Table 4.5. There were no significant ($P > 0.05$) interaction effects of time and diet for the carcass weight, temperature, pH, L*, a*, and b*. Time significantly ($P < 0.05$) influenced temperature, pH, L*, a*, and b* but did not have any effect ($P > 0.05$) on the carcass weight of the experimental animals. After a 45 mins time interval, the temperature and pH were higher than those of 24 hrs. Subsequently, the L*, a*, and b* of animals after 24 hrs were higher than those of the 45 mins. On the other hand, results showed that carcass weight and temperature were significantly ($P < .05$) influenced by the diet while pH, L*, a*, and b* were not significantly ($P > 0.05$) affected by the diet. Animals fed 0% MSC had the highest carcass weights while those on the 20% MSC+2.5% WG produced the least carcass weights. Also, the highest ($P < 0.05$) temperature values were observed in animals fed the 0%MSC while that of the 10%MSC was the lowest.

Table 4. 5: Effect of time x diet on the inclusion of MSC with or without wild garlic on carcass weight, temperature, pH and colour of weaned large white piglet

		Parameter					
	Diet	Carcass weight (kg)	Temp °C	pH	L*(lightness)	a*(redness)	b*(yellowness)
45min	0%MSC	20.6	28.0	5.9	38.9	3.8	2.9
	10%MSC	20.4	25.9	5.8	42.0	4.1	2.9
	20%MSC	18.9	25.8	5.9	43.0	4.1	3.0
	20%MSC+2.5%WG	15.6	27.1	6.0	42.4	4.3	2.6
24hr	0%MSC	19.3	10.0	5.7	46.0	5.7	4.8
	10%MSC	18.0	9.3	5.6	47.5	10.1	4.8
	20%MSC	18.6	9.9	5.7	44.4	5.6	4.2
	20%MSC+2.5%WG	14.2	10.6	5.7	46.1	6.0	4.5
	SEM	0.3	0.2	0.02	0.4	0.4	0.1
Effects	Diet	P < 0.001	P < 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05
	Time	P > 0.05	P < 0.001	P < 0.001	P < 0.001	P = 0.003	P < 0.001
	Diet & Time	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05

SEM =standard error of the mean; n = 12 piglets

4.4.3 Water holding capacity, drip loss, thawing loss and cooking loss

The result of water holding capacity (WHC), drip loss, thawing loss and cooking loss are presented in Table 4.6. Results showed that there were no significant ($P > 0.05$) effects of the inclusion of MSC with or without wild garlic on the WCH, drip loss, thawing loss and cooking loss.

Table 4. 5: Meat quality parameters of weaned Large White piglets fed diets supplemented with graded levels of marula seed cake with or without wild garlic

Parameter (%)	Diet				SEM	P value
	0%MSC	10%MSC	20%MSC	20%MSC+2.5%WG		
WHC	24.0	28.1	24.4	28.2	0.6	P > 0.05
Drip loss	3.4	4.7	3.9	3.7	0.1	P > 0.05
Thawing loss	6.7	6.9	7.7	7.5	0.2	P > 0.05
Cooking loss	58.3	58,6	57.2	59.4	0.3	P > 0.05

WHC = Water Holding Capacity; SEM=standard error of the mean; n = 12 piglet

4.3.4 Serum Biochemical Parameters

The effect of dietary inclusion of mycotoxin contaminated MSC with or without wild garlic on serum biochemical parameters of Large White piglets is presented in Table 4.7. The results showed that there were significant ($P < 0.05$) interaction effects of diet x week on the symmetric dimethylarginine, creatinine, phosphorous, calcium, alanine aminotransferase and γ -Glutamyl transferase, while there were no interaction effects ($P > 0.05$) of diet x week on the glucose, urea, blood urea, total protein, albumin, globulin, albumin/globulin, alkaline phosphate, total bilirubin, cholesterol, amylase and lipase. Diet significantly ($P = 0.001$) influenced symmetric dimethylarginine, Urea ($P < 0.05$), blood urea ($P < 0.05$), and albumin/globulin ($P < 0.001$), but did not affect ($P > 0.05$) glucose, creatine, phosphorus, calcium, total protein, albumin, globulin, alanine aminotransferase, alkaline phosphate, γ -glutamyl transferase, total bilirubin, cholesterol, amylase and lipase. On the other hand, there was a significant effect of a week on symmetric dimethylarginine ($P = 0.001$), creatinine ($P < 0.001$), phosphorus ($P < 0.001$), calcium ($P < 0.001$), total protein ($P = 0.002$), albumin ($P < 0.001$), γ -glutamyl transferase ($P = 0.001$), total bilirubin ($P < 0.05$), and lipase ($P < 0.05$), whereas, glucose, urea, blood urea, globulin. Albumin/globulin, alanine aminotransferase, alkaline phosphate, cholesterol, and amylase were not significantly ($P > 0.05$) influenced.

The symmetric dimethylarginine of piglets fed 20% MSC and 20% MSC + 2.5 WG at week 6 were higher than those that were fed 0% MSC at week 1. Piglets fed 0% MSC at week 6 had the highest creatine while those on 0% MSC at week 1 produced the lowest creatinine level. In addition, the urea level of piglets that were fed 20% MSC at week 3 and 20% MSC at week 6 were increased compared to other groups. Experimental animals that received 20% MSC and 20% MSC + 2.5 WG at week 3 had an increased blood urea nitrogen/creatinine than others. The phosphorus and glucose of piglets at week 6 were higher than ($P < 0.05$) at week 3. Piglets' calcium levels at weeks 3 and 6 were statistically increased ($P < 0.05$) compared to piglets that were fed 0% MSC and 20% MSC + 2.5 WG had higher albumin at week 6 while piglets fed 20% + 2.5 WG had the lowest albumin value at week 3. The globulin level of piglets fed 20% MSC + 2.5 WG increased with increasing weeks. The albumin /globulin of piglets across the treatment levels and weeks were statistically similar. Again, piglets fed 20% + 2.5 WG at week 1, 10% MSC at week 3 and 0% MSC and 20% MSC + 2.5 WG at week 6 had higher ALT than those that were fed 0% MSC at week 1 and 20% MSC at week 3. There was an observed

increase in the γ -glutamyl transferase, total bilirubin, amylase and lipase at week 6. The cholesterol of piglets fed 20% MSC + 2.5 WG at week 1 was highly increased compared to other groups. Finally, Large White piglets in weeks 3 and 6 had slightly increased ALP than at week 1.

Table 4. 6: Weekly serum biochemical parameters of weaned Large White piglets fed diets supplemented with MSC with or without wild garlic

Week	Diet	Parameter					
		Glucose (mmol/L)	Symmetric dimethylarginine ($\mu\text{g/dL}$)	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/L)	Blood urea nitrogen/creatinine	Phosphorus (mmol/L)
1	0%MSC	2.0	26.0	72.0	1.0	3.5	3.4
	10%MSC	1.8	25.8	69.4	1.1	3.8	4.8
	20%MSC	0.7	35.0	93.3	2.8	7.7	5.1
	20%+2.5%WG	1.5	34.5	91.5	2.5	6.5	4.9
3	0%MSC	2.9	31.4	88.2	0.9	2.8	4.1
	10%MSC	3.4	31.9	89.9	2.3	5.6	3.4
	20%MSC	1.6	34.7	93.5	3.7	9.8	4.4
	20%+2.5%WG	2.2	31.5	74.8	2.5	8.5	2.6
6	0%MSC	3.1	32.0	107.0	1.5	3.4	5.3
	10%MSC	21.4	35.8	91.4	1.7	5.1	4.7
	20%MSC	1.3	46.8	99.6	3.1	7.9	5.3
	20%+2.5%WG	0.6	61.0	134.7	2.6	4.7	5.3
	SEM	2.9	1.1	2.5	0.2	0.5	0.1
Effect	Diet	P>0.05	P=0.001	P>0.05	P<0.05	P<0.05	P>0.05
	Week	P>0.05	P<0.001	P<0.001	P>0.05	P>0.05	P<0.001
	Diet x Week	P>0.05	P<0.05	P<0.05	P>0.05	P>0.05	P<0.05

Table 4. 7: Weekly serum biochemical parameters of weaned large white piglets fed diets supplemented with MSC with or without wild garlic

Week	Diet	Parameter					Alanine aminotransferase (U/L)
		Calcium (mmol/L)	Total Protein (g/L)	Albumin (g/L)	Globulin (g/L)	Albumin/Globulin	
1	0%MSC	1.6	31.5	17.5	14.0	1.6	42.0
	10%MSC	1.9	54.6	20.6	33.8	0.7	79.8
	20%MSC	2.0	65.3	23.0	42.7	0.6	74.0
	20%+2.5% WG	2.1	56.0	24.3	32.0	0.8	97.5
3	0%MSC	2.1	52.6	24.4	28.0	0.9	72.8
	10%MSC	2.0	64.1	24.4	40.4	0.6	81.9
	20%MSC	1.9	52.5	21.2	40.8	0.7	63.7
	20%+2.5% WG	1.6	52.0	17.8	33.8	0.6	41.0
6	0%MSC	2.3	71.0	34.4	36.6	1.3	85.6
	10%MSC	2.2	61.0	28.5	32.5	0.9	79.5
	20%MSC	2.3	67.6	26.9	40.8	0.7	73.8
	20%+2.5% WG	2.8	82.7	31.3	51.3	0.6	85.7
	SEM	0.0	2.2	0.7	1.9	0.0	2.9
Effect	Diet	P>0.05	P>0.05	P>0.05	P>0.05	P<0.001	P>0.05
	Week	P<0.001	P=0.002	P<0.001	P>0.05	P>0.05	P>0.05
	Diet x Week	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05	P<0.05

Standard error of mean (SEM); n = 12 pigs

Table 4.7 Weekly serum biochemical parameters of weaned large white piglets fed diets supplemented with MSC with or without wild garlic

Week	Diet	Parameter					
		Alkaline phosphatase (U/L)	γ -Glutamyl transferase	Total bilirubin (mmol/L)	Cholesterol (mmol/L)	Amylase (U/L)	Lipase (U/L)
1	0%MSC	85.0	5.0	1.9	0.7	473.0	14.0
	10%MSC	126.2	35.6	1.9	1.8	674.8	22.8
	20%MSC	96.3	19.7	1.9	2.0	931.0	23.7
	20%+2.5% WG	108.3	29.0	5.7	31.8	602.0	15.0
3	0%MSC	111.0	17.4	6.3	1.8	831.2	29.0
	10%MSC	130.7	4.9	3.9	2.0	654.7	36.7
	20%MSC	128.5	10.0	5.12	1.8	613.2	25.3
	20%+2.5% WG	50.8	19.5	8.0	0.9	431.3	13.0
6	0%MSC	132.8	25.8	20.4	2.3	1093.0	26.4
	10%MSC	125.1	28.1	14.1	1.9	836.3	50.5
	20%MSC	115.1	31.6	5.0	2.3	651.5	28.5
	20%+2.5% WG	174.7	25.3	6.7	3.21	702.0	53.7
	SEM	6.6	1.3	1.2	2.0	36.0	2.8
Effect	Diet	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
	Week	P>0.05	P=0.001	P<0.05	P>0.05	P>0.05	P<0.05
	Diet x Week	P>0.05	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05

Standard error of mean (SEM); n = 12 pig

CHAPTER 5: DISCUSSION

5.1 Discussion

5.1.1 Proximate composition and mycotoxin status of marula seed cake

The proximate composition of marula seed cake (MSC) showed that MSC contains a high amount of crude protein (470.0 g/kg DM) and ether extract (371.0 g/kg DM), with average ash (53.0 g/kg DM), neutral detergent fibre (27.0 g/kg DM) and acid detergent fibre (72.0 g/kg DM) contents. This result is in line with the findings of Mdziniso *et al.* (2016) and Mthiyane and Mhlanga (2017), who reported similar values for crude protein (470.0-470.21 g/kg DM), ether extract (289.6-394 g/kg DM) and ash (55.5-66.1 g/kg DM). As noted by Mthiyane and Mhlanga (2017), the crude protein of MSC is higher than most alternative sources of protein for monogastric animals such as cotton seed cake (403 g/kg DM), canola oilcake (339 g/kg DM) and oilcake (341 g/kg DM), hence, it provides an important source of protein for substituting SBM in these animals (Banaszkiewicz, 2011). On the other hand, MSC has been noted to have high levels of aflatoxin (AFG₂ 14226.35) which is an anti-nutritional factor (ANFs) that could affect nutrient absorption, digestion and utilization by the animals (Chen *et al.*, 2016).

5.1.2 Growth performance and carcass characteristics

The dietary inclusion of MSC with or without wild garlic significantly affected the body weight gain ($P < 0.001$) and feed intake ($P < 0.001$). In this study, the negative effects of MSC on performance were also seen in some of the carcass characteristics of the slaughtered piglets. A significant decrease was observed in the foreleg weight ($P = 0.001$) and hindleg weight ($P < 0.05$) of piglets as the level of MSC increased in the diet. The results suggested that MSC at low inclusion could replace SBM in pig's diet for improved growth performance and better cut yield. This is in line with the report of Sigudla (2016) and Simelane (2017) who reported that the dietary supplementation with MSC (0-200 g/kg feed) depressed growth performance and carcass characteristics in weaned Large White piglets. Similarly, other studies (Magagula, 2013; Mamba, 2013; Mncina, 2018; Mthiyane & Mhlanga, 2017) observed a significant decrease in the growth performance and carcass characteristics of broilers fed dietary inclusion

of MSC. The noted negative effect of high inclusion level of MSC might be because of the presence of aflatoxin which impairs protein synthesis and affects intestinal microbial and nutrient digestibility (Santos *et al.*, 2021). The United State reported 300ppb as a maximum tolerance level of aflatoxin in pigs diets (Marin *et al.*, 2002). The high inclusion level of MSC in pig ration can lead to lipid peroxidation which results in stale, rancid flavour in feed and feeding of oxidized lipids which had been shown to decrease body weight and cause poor feed conversion efficiency (Mthiyane & Mhlanga, 2017). Hence, the negative effect might likely not be linked to poor palatability of MSC as it is generally consumed by people of rural areas across the African continent with neither bitterness nor unpleasant odour being reported (Gouwakinnou *et al.*, 2011; Mthiyane and Mhlanga, 2017; (Holanda & Kim, 2021a) Nevertheless, MSC is a plant-derived protein source with anti-nutritional factors (ANFs) such as tannins, saponins, protease inhibitors, phytohaemagglutinins and chelating agents which might have interfered with the digestibility of crude protein by the piglets and thus could have affected the efficiency (Mabena *et al.*, 2022).

The contamination of MSC which is a protein-rich product by mycotoxin is another plausible mechanism behind the deleterious effects. These results demonstrated an interesting perspective about the concern of MSC and showed that there is no need for an ameliorative strategy in the form of supplementary wild garlic as the inclusion of MSC and wild garlic increased aflatoxin as shown in (Table 3.2). The prolonged exposure of animals to mycotoxin-contaminated feed generally reduced feed intake, decreased weight gain and as well altered immune function (Mthiyane & Mhlanga, 2017). A diet containing 20% MSC + 2.5% wild garlic showed higher amounts of aflatoxin (104.41ppb AFB₁, 5750.67ppb AFB₂, 6094.67ppb AFG₁ and 14226.35 ppb AFG₂) and this affected performance and carcass characteristics of piglets. The lower and single inclusion level gave a better result than the combination of MSC and WG, it could be that some bio content in MSC may have been antagonistic to WG that made them not actually be compatible. It is also possible that a combination of both interfered with each others' mechanism of action in a negative way.

5.1.3 Internal organ characteristics

The result of this study showed that dietary inclusion of MSC with or without wild garlic had a significant ($P < 0.05$) effect on the liver but no significant ($P > 0.05$) effect on the kidney,

small intestine, large intestine, spleen, caecum and stomach. The inclusion of 20% MSC and 20% MSC + 2.5 WG tended to decrease the liver weight. The results are in contrast to the study on broiler Japanese quail whereby 5%, 25%, 50%, 75% and 100% of MSC inclusion did not affect liver weight (Mazizi *et al.*, 2020). The species variation may be the reason for differences, even though, both pigs and quails are monogastric, still, their digestive capacity may vary (Robeson *et al.*, 2018). Also, these variable results may be attributed to the available contents of the MSC and the processing method. The non-adverse effect of the diet on the liver suggests that MSC and wild garlic improved bile production as well biotransformation of xenobiotics from the body of the animal (Mazizi *et al.*, 2020). However, the significant effect on the liver weight organs tends to suggest that increasing inclusion level of MSC had toxic effect on this organ, even with the inclusion of WG. This report is in an agreement with report of (Owumi *et al.*, 2022) who reported decreased in liver weight of rats exposed to Aflatoxin,

5.1.3 Meat quality

Carcass traits and meat quality such as tenderness and colour, are critical for consumer acceptance. In this study, dietary inclusion of 20% MSC and 20% MSC + 2.5% wild garlic tended to decrease carcass weight and temperature. This result is in agreement with the report of (Mabena *et al.*, 2022) who reported a decrease in meat quality parameters of pigs as dietary inclusion of MSC increased which could be due to the present of aflatoxin in the diet. The inclusion of MSC with or without WG has no adverse effect on the pH and colour, if not the colour would have exceeded the normal range which shows the poor quality of the meat. The average values of the pH and meat colour from the result by (Temperan *et al.*, 2014) is within the normal range (L^* , a^* and b^*) ranges of 34–58, 3–10 and 2–9, pH (5.5- 5.2), respectively. The redness (a^*) value is related to the concentration of pigments, while the lightness (L^*) is related to the moisture and fat content of the carcass and is also affected by the pH of the carcass. The inclusion of MSC with or without wild garlic revealed that there was enough glycogen and lactic acid available that resulted to better colour cutting. The water holding capacity and meat colour observed in the study showed that the pH was not negatively affected by diet, as low meat pH is often associated with low water holding capacity and pale meat (Tatiany *et al.*, 2019; Mabena *et al.*, 2022). Dark-coloured meats are unattractive to consumers and also have a metallic off-taste. To maintain the quality of meat that can be attractive to consumers and also safe for consumption, the inclusion of MSC at 10% or lower is advised.

5.1.3 Serum biochemistry

The blood biochemical traits usually reflect the physiological response of the animal to changes in its external and internal environment and thus serve as a viable tool for monitoring animal health. The inclusion of MSC with or without wild garlic had significant ($P < 0.05$) effects on symmetric dimethylarginine, creatinine, phosphorus, calcium, ALT and γ -glutamyl transferase, while the diet significantly ($P < 0.05$) influenced urea, blood urea nitrogen/creatinine the albumin/globulin decreased. There is a paucity of information and limited data on serum biochemistry of monogastric animals such as a pig fed MSC. However, the results of the present study contradict the result of Jeong *et al.* (2022) who revealed that dietary inclusion of 25%, 50%, 75% and 100% MSC did not affect serum biochemistry parameters of quails. The variation in the results may be attributed to the genetic makeup and variation in the digestive system of the two species could contribute to the variation. The biochemical parameters reported in the present study were within the normal range of a healthy pig; urea (1.21 to 6.71 mmol/L), symmetric dimethylarginine (20- 70 $\mu\text{g.dL}$) (Li *et al.*, 2021); blood urea nitrogen/creatinine (3.92 to 26.79) (Yeom *et al.*, 2012) and albumin/globulin (0.52 to 3.47) (Klem *et al.*, 2010; Yeom *et al.*, 2012).

In the study, a lower inclusion level of MSC reduced the concentration of urea, symmetric dimethylarginine and creatinine. Some authors Relford *et al.* (2016); Choi *et al.* (2022); Jeong *et al.* (2022) reported that a lower concentration of urea in the system is an indication of reduced abnormal absorption, reduced protein malnutrition and reduced advanced liver diseases. The inclusion of MSC at 10% may have improved the capacity of the liver in removing toxic substances from the body of the animal.-The observed decrease in cholesterol levels in the 10% MSC inclusion groups could be a result of increased breakdown of cholesterol by bile acids and the inhibiting effect of MSC on micelle formation in the low pH-digesta content (Jeong *et al.*, 2022). The high level of symmetric dimethylarginine and creatinine in the blood of these animals at 20% MSC and 20% MSC + 2.5 WG may indicate the presence of aflatoxin in the feed. However, it is expected that the addition of wild garlic to mycotoxin-contaminated MSC could have possibly ameliorated the harmful effect of the toxins which was not the case. In other words, the presence of mycotoxin in the feed may cause immunosuppression which may pave way for invading microorganisms such as fungi, virus and bacteria to invade the host, hence, resulting in pathogenic conditions. The non-significant results obtained for glucose,

globulin, ALT and total protein showed that piglets fed any of the treatments used in the study did not have any deleterious effects on the physiological status of the animals.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the outcome of this research, we, therefore, recommend that the inclusion of marula seed cake in pig ration should not exceed 10% as higher inclusion may pose a threat to the health of the animals. The inclusion of 10% can be used as a pig ration for improved growth performance and better cut yield. It also improved meat quality and biochemical traits of piglets, the no significant effect of some of the biochemical parameters indicates that the inclusion of 10% MSC does not have any negative effect on the vital organs such as; the liver and kidney. Also, the inclusion of WG did not appear to have any beneficial effect on the measured parameters. Therefore, there is no benefit from supplementing marula seed cake with WG.

6.2 Recommendations

Future research should focus on ways to eliminate or reduce the presence of mycotoxin in marula seed cake. We also recommend that inclusion levels lower the 10% in the pig's diet should further be researched.

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