



# **Effects of dietary marula seed cake on indigenous boschveld chickens productive performance, health, gut metagenomes and meat quality**

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## Declaration

I, Zibukile Gcinile Mchunu, hereby declare that this dissertation with the title “Effects of Dietary Marula Seed cake on Indigenous Boschveld Chickens Productive Performance, Health, Gut Metagenomes and Meat Quality” is my work, conducted under the supervision of Dr. D.N.M. Mthiyane. The study has not been submitted to any other University’s degree programme. All sources used in this document have been properly cited and referenced.



27/11/2023

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## General Abstract

The rapidly growing human population particularly in developing countries demands sustainable production of poultry as a source of food to meet human protein requirements. As the most popular poultry species in rural areas of Southern Africa, indigenous chickens provide cheap animal protein in the form of meat and eggs and contribute significantly to the socio-economic wellbeing of rural communities. However, their contribution to food and nutrition security is suboptimal due poor nutrition ascribable to dependence mostly on scavengeable feed resources. Also, commercial feeds that could be used to feed these birds are unaffordable due to costly dietary protein sources especially due to environmentally and economically unsustainable soybean meal (SBM). Therefore, there is a need to seek cheaper locally available alternative protein ingredients for poultry feeds such as Marula seed cake (MSC) whose amino acid profile closely mirrors that of SBM. This study thus aimed to evaluate the effect of dietary replacement of SBM with MSC on Boschveld indigenous chickens' performance, carcass traits, viscera macro-morphometry, haematology and clinical biochemistry, gut metagenomes and meat and bone quality. Four hundred (400) 3-week-old Boschveld chicks were randomly allocated to and fed, for 9 weeks, 5 iso-energetic-nitrogenous maize and SBM-based grower diets with 0, 10, 15, 20, and 25% MSC replacing SBM, each with 5 replicate pens of 16 birds, in a completely randomised design (CRD. Feed intake (FI), weekly body weight gain (BWG) and feed conversion efficiency (FCE) were determined. A day before slaughter, blood was collected from 2 birds randomly selected per pen. At the end of the feeding trial, following a 12-hour fast but with access to drinking water, the chickens were slaughtered, dressed. Immediately thereafter viscera morphometry, carcass trait, meat and bone quality were determined. Caeca digesta samples were randomly collected from 2 birds per pen ( $n = 10$  and pooled for shotgun metagenomics analysis. All data were then statistically analysed using the

GLM procedure (PROC GLM) of SAS (SAS, 2012). Dietary MSC decreased FI (quadratic:  $P < 0.001$ ) and BWG (quadratic:  $P < 0.01$ ), as it linearly decreased their weights of the hot carcass (HCW;  $P < 0.05$ ), spleen ( $P < 0.05$ ), jejunum ( $P < 0.05$ ), ileum ( $P < 0.001$ ), and caecum ( $P < 0.001$ ). The MSC increased chickens' serum glucose (linear:  $P < 0.05$ ), cholesterol (linear:  $P = 0.001$ ) and phosphate (quadratic:  $P < 0.05$ ) concentrations but it decreased their plasma/serum amylase activity (linear:  $P < 0.01$ ). Dietary MSC decreased meat lightness at 45 min (linear:  $P < 0.05$ ) and yellowness at both 45 min (quadratic:  $P < 0.001$ ) and 24 h (quadratic:  $P < 0.001$ ). However, it increased the meat's redness both at 45 min (linear:  $P < 0.01$ ) and 24 h (linear:  $P < 0.05$ ) post-slaughter. MSC decreased the chickens' bone medial diaphysis ( $P < 0.05$ ). Dietary MSC had no effect ( $P > 0.05$ ) on feed conversion efficiency (FCE), gastrointestinal tract viscera morphometry, haematology, other carcass traits and meat quality and bone morphometry. Gut metagenomic analysis showed in chickens fed diets with 0%, 10%, 15%, 20% and 25% MSC inclusion the most predominant microbial communities in the chickens' ceaca bacteria with, 99.63, 99.66, 99.99, 98.31 and 99.99% frequency, respectively. In chickens fed the control (0% MSC) diet, Bacteroides (60.52%) and Firmicutes (34.15%) were predominant microbial phyla. The Firmicutes were abundant across treatment groups while the Bacteroidetes were abundant in birds fed diets with 10 and 20% MSC inclusion but their (Bacteroidetes) was lower in chickens fed diets with 15 and 25% MSC inclusion. In conclusion, indigenous chickens can be fed grower diets supplemented with MSC up to 15% inclusion level without detrimental effects on FCE, carcass characteristics, haematology parameters and bone size.

**Key words:** Chicken performance, gut metagenomes, haemo-biochemistry, meat quality, bone quality, Bacteroides, Firmicutes.

## **Dedication**

I dedicate this dissertation to my late parents Mr. Q.D. and Mrs. H.M. Shezi, my angels, Slindokuhle and Sinqumesihle Mchunu as well as the entire Mchunu family.

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## Abbreviations

|                 |                                     |
|-----------------|-------------------------------------|
| AA              | : Amino acids                       |
| ADF             | : Acid detergent fibre              |
| AWFI            | : Average weekly feed intake        |
| APS             | : Alternative protein source        |
| BWG             | : Body weight gain                  |
| Ca              | : Calcium                           |
| CP              | : Crude protein                     |
| CO <sub>2</sub> | : Carbon dioxide                    |
| CF              | : Crude fibre                       |
| CCW             | : Cold carcass weight               |
| CRD             | : Completely randomised design      |
| DM              | : Dry matter                        |
| DNA             | : Deoxyribonucleic acid             |
| FCE             | : Feed conversion efficiency        |
| FI              | : Feed intake                       |
| GIT             | : Gastrointestinal tract            |
| HCW             | : Hot carcass weight                |
| LIFDC           | : Low Income Food Deficit Countries |
| MDGs            | : Millennium development goals      |
| ME              | : Metabolizable energy              |
| MSC             | : Marula seed cake                  |
| MUFA            | : Monounsaturated fatty acid        |
| SFRB            | : Scavengeable feed resource base   |
| P               | : Phosphorus                        |
| PUFA            | : Polyunsaturated fatty acid        |

SBM : Soyabean meal

WHC : Water holding capacity

# Chapter One

## 1. General Introduction

### 1.1. Background

The current world human population of approximately 7.6 billion is expected to reach 8.6 billion in 2030 (Gerland et al., 2022). This rapid increase in human population has triggered massive livestock production to meet the demand of animal-derived protein for human consumption (Parolini et al., 2020). Currently about 927 million people are experiencing severe food insecurity and 323 million of them are in Southern Africa (WHO, 2021). Dietary proteins are an essential part of healthy human diet as they provide amino acids required for key biological functions in cells tissues and organs. Animal derived protein sources (meat, eggs and dairy products) provides nutritional benefits since in addition to providing proteins they are also sources of fats, carbohydrates and micronutrients (Colgrave et al., 2021). In recent years, the global consumption of poultry meat has significantly outpaced that of other meats (González et al., 2020). The major driver of increased consumption of chicken meat is its relative affordability per unit mass compared to other meats (Fehri et al., 2022). In most developing countries where malnutrition is a common phenomenon and demand for high quality protein is highest, small-scale extensive scavenging poultry production is the dominant system that provides food to feed the fast-growing human populations and provides income to rural poor households especially the unemployed youth and women (Mack et al., 2005). Scavenging family poultry production is found in almost all of Low Income Food Deficit Countries and is believed to make a vital contribution to the Millenium Development Goals (MDGs) to reduce poverty by 2025 (Erdaw and Beyene, 2022). The most important poultry species kept in the scavenging family poultry production system are chickens, guinea fowls, ducks, pigeons, geese and turkeys (Goromela et al., 2006).

Indigenous chickens (*Gallus domesticus*) are the most popular poultry species in rural areas of Southern Africa (Nyoni and Masika, 2012, Gunya et al., 2020). Improving the productivity of these chickens is of great significance to rural communities as the birds provide high quality, cheap and readily digestible animal protein in the form of white meat and eggs (Mapiye et al., 2008, Okoro et al., 2017). These chickens also play a major role through their contribution to

the socio-economic wellbeing of, as well by providing income to, rural women and youth (Melesse, 2014, Raphulu et al., 2015). They are excellent foragers and able to utilize non-edible feedstuffs and vegetation not suitable for human consumption into edible meat and eggs (Alders and Pym, 2009, Padhi, 2016). The birds are well adapted to harsh tropical environmental conditions and endemic diseases (Okoro et al., 2017). In addition, indigenous chickens have higher genetic potential when compared to exotic birds used in commercial poultry production (Alders and Pym, 2009). However, their contribution to food and nutrition security is currently suboptimal because of the production constraints (Wong et al., 2017). They are characterised by low productivity and high mortality due to Newcastle disease and predation (Kingori et al., 2010, Okoro et al., 2017). Their low performance is attributed to poor hygiene (Guèye, 2000) and low quality scavengeable feed resource base (Mtileni et al., 2012) which is inadequate in protein, Ca, and P to sustain both growth and egg production (Raphulu et al., 2015). A study by Alabi et al. (2013) revealed that a diet containing crude protein (CP) of 178 g/kg DM and ME of 14 MJ/kg DM could improve growth and egg production of indigenous Venda chickens between 1 and 6 weeks of age. The productivity of indigenous chickens can be improved through dietary supplementation with locally available feeds that have adequate nutrients such as conventional ingredients used in large poultry industry i.e. soya bean meal (SBM) and fishmeal (Khobondo et al., 2015, Malebana et al., 2018, Parolini et al., 2020). Use of such conventional feeds resources results in increased price of feed, hence the need for search and development of non-conventional feed resources, for example Marula nu meal.

Marula (*Sclerocarya birrea caffra*) seed cake (MSC) is the solid residue remaining after extraction of oil from marula kernels (Mdziniso et al., 2016) The marula tree belongs to the family *Anacardiaceae* and is widely distributed in the Southern African region including in South Africa, Zimbabwe, Botswana, Mozambique, Zambia and Namibia (Mariod and Abdelwahab, 2012, Komane et al., 2015). Recent studies reveal?? MSC as a good source of CP, fat, Ca, P, and amino acids except for lysine (Mlambo et al., 2011, Mdziniso et al., 2016, Mthiyane and Mhlanga, 2017). Therefore, MSC can be an alternative protein source in poultry nutrition. However, its use is currently limited by lipid peroxidation and the presence of anti-nutritional factors including phytates, oxalates, saponins, tannin, hydrocyanic acid, nitrates as well as mycotoxins(Mthiyane and Mhlanga, 2017).

## **1.2. Problem Statement**

The costs of chicken feed accounts for about 70 – 80% of the total production costs of raising poultry (Olaiya and Makinde, 2015, Saeed et al., 2017, Malebana et al., 2018, Nkukwana, 2018) which poses a significant threat for intensive production. In most intensive production systems, SBM is an expensive widely used dietary protein source (Alders and Pym, 2009, Khan, 2018, Mthiyane and Mhlanga, 2018). Apart from being expensive, SBM incorporation into poultry diets is considered to be in direct competition with human food requirements since SBM is also a human food (Mottet and Tempio, 2017). Another disadvantage of SBM is that its cultivation requires large areas of arable land (Alders and Pym, 2009). In addition, SBM production poses a threat to food security as it may contribute to a huge CO<sub>2</sub> footprint, global warming, deforestation, soil erosion, eutrophication and loss of biodiversity (Khan, 2018, Parolini et al., 2020). The lack of access to standard protein sources such as SBM, which are commonly used in intensive poultry production could possibly hinder the sustainable intensification of Boschveld indigenous chickens.

Marula seed cake (MSC) is underutilized protein source in poultry production. Based on evidence from a previous study, supplementing MSC into poultry diets is limited by the presence of anti-nutritional factors and lipid peroxidation (Mthiyane and Mhlanga, 2017). It has higher fibre content and residual oil (Muya et al., 2020). Therefore, supplementation of MSC into poultry diets can induce negative effects on poultry productive performance and carcass characteristics (Mthiyane and Mhlanga, 2017). Thus far, no studies have been conducted on indigenous Boschveld chickens fed MSC-supplemented diets. Therefore, in this study the effect of dietary replacement of SBM with MSC on Boschveld indigenous chickens was determined through measurements of performance, gut metagenomes, clinical haemobiochemistry, meat and bone quality.

## **1.3. Justification**

Indigenous chickens play a major role in rural communities (Guèye, 2000, Padhi, 2016). They are dual purpose producing both eggs and meat. Health-conscious consumers perceive their meat as having low levels of cholesterol and fat (Bett et al., 2012, Padhi, 2016). Their eggs are rich in digestible energy, choline, folate, selenium, and vitamins A, D, K, and B12 (Wong et

al., 2017). MSC is an abundant and affordable protein source in Sub-Saharan Africa (SSA), each tree can produce fruits that can weigh approximately 240 kg per annum (). After oil extraction, the oilcake contains high protein content (470 g CP/kg DM) and is a good source of Ca and P (Mthiyane and Mhlanga, 2017). Thus, it can be supplemented to indigenous chicken diets to satisfy the birds' nutritional requirements.

#### **1.4. Study aims and objectives**

This study sought to evaluate the effect of graded dietary replacement of SBM with MSC in Boschveld indigenous chicken's diets on growth performance, gut metagenomes, clinical haemo-biochemistry, meat and bone quality. To evaluate the potential of dietary MSC in Boschveld indigenous chickens, the study specifically determined its effects on:

- i. feed intake, body weight gain and feed conversion efficiency and viscera macro-morphometry.
- ii. clinical haemo-biochemistry.
- iii. carcass traits, meat, and bone quality.
- iv. gut microbial diversity and abundance.

#### **1.5. Hypotheses**

##### **a) $H_0$ Null hypothesis**

Dietary replacement of SBM with MSC does not improve growth performance, viscera macro-morphometry, clinical haemo-biochemistry, carcass traits, meat and bone quality and gut microbial diversity and abundance of indigenous Boschveld chickens.

##### **b) $H_1$ Alternative hypothesis**

Dietary replacements of SBM with MSC improves growth performance, viscera macro-morphometry, clinical haemo-biochemistry, carcass traits, meat and bone quality and gut microbial diversity and abundance of indigenous Boschveld chickens.

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## Chapter 2: Literature review

### 2.1. Indigenous chicken production in South Africa

Indigenous chicken farming can be categorised into free range, semi-intensive and intensive production systems (Mtileni et al., 2009). Almost every household in rural communities, and some in urban areas, extensively produces indigenous poultry of hardy native breeds (Nkukwana, 2018). In South Africa, dual-purpose indigenous chickens such as Venda, Naked Neck, Ovambo and Potchefstroom Koekoek breeds are reared under extensive scavenging production system (Mtileni et al., 2012). These chickens are known to produce low to moderate levels of both meat and eggs, about 40 – 60 eggs/hen/annum (Akinola and Essien, 2011, Mottet and Tempio, 2017). Historically, the Boschveld indigenous chicken (Fig. 2.1) is a cross-breed of Venda, Matabele and Ovambo breeds developed from deep rural areas of South Africa (Okoro et al., 2017). It was developed by Mike Bosch in the mid-1970s. Boschveld chickens are light-brown and white feathered dual purpose breeds. At 20 weeks of age, they reach sexual maturity and weigh between 1.7 and 2.6 kg (Maoba et al., 2022). They have high egg production and good mothering ability (Manyelo et al., 2020).



**Figure 2. 1:** Boschveld indigenous chickens.

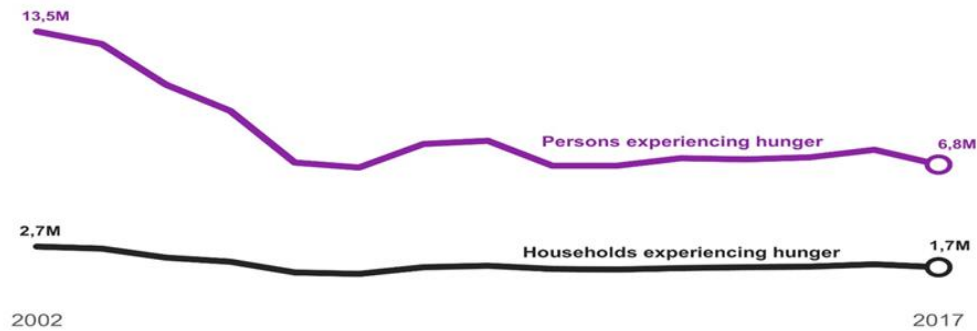
## **2.2. Indigenous chicken contribution to food security in South Africa**

In South Africa, approximately 6.8 million people are food insecure (Fig. 2.2). Yet the majority of these people rear indigenous chickens mainly as a source of food. Indeed, in most developing countries where feed shortage is a common phenomenon, rural poultry production is an integrated and complementary farming system playing a major role in food security (Okeno et al., 2012) and poverty alleviation (Hayat et al., 2016), providing income, and promotion of gender equality to disadvantaged groups in less favoured areas of Africa. In Africa, indigenous chicken production contributes 20% of animal protein intake and supply 70 – 90% of poultry products (Wong et al., 2017). They significantly contribute to food security of rural areas (Alders and Pym, 2009) by providing cheap animal-derived protein through meat and eggs (Tarwireyi and Fanadzo, 2013, Melesse, 2014, Mottet and Tempio, 2017). Each egg contain approximately 315 kJ of digestible energy and vitamins such as vitamins A, D, K, B<sub>12</sub>, choline, folate and selenium (Wong et al., 2017). Eggs have high biological value of 94% and this protein fraction is highly digestible to support growth and maintenance of the human body (Melesse, 2014).

In poor rural households where refrigerator facilities are not readily available prominent, indigenous chickens meat provide animal-derived protein for human consumption (Khubondo et al., 2015). The entire carcass and viscera is largely consumed in a single meal consumption, with no need for cold storage facilities (Wong et al., 2017). Some people prefer meat from indigenous chickens than meat from exotic birds due to its unique flavour, colour, tenderness and leanness (Bett et al., 2012, Padhi, 2016). Meat from indigenous chickens typically has low-cholesterol and fat content thus is healthy product as a source of nutrients in human diets (Mack et al., 2005, Akinola and Essien, 2011). The chickens may also contribute indirectly to food security of rural people by enhancing nutrient utilization and recycling of nutrients in the environment, enhancing food production by providing manure and helping in controlling pests and external parasites to other livestock (Mack et al., 2005, Alders and Pym, 2009, Wong et al., 2017). In addition, rural poultry production requires relatively less land compared to other livestock which leaves most of the land toward crop (cereal and pulses) production to meet human and livestock food and feed requirements , respectively (Sonaiya, 2007, Akinola and Essien, 2011)

**In 2017, 6,8 million South Africans experienced hunger.** While the number has dropped from 13,5 million in 2002, it still affects 1,7 million households across the country.

Number of households and persons experiencing hunger (2002 to 2017)



STATS SA  
STATISTICS SOUTH AFRICA

THE SOUTH AFRICA I KNOW, THE HOME I UNDERSTAND

**Figure 2.2:** The extent of food security in South Africa

### 2.3. Alternative protein sources for indigenous chickens

To alleviate inadequate supply of animal-derived protein for human consumption, especially in resource-limited rural and township communities, it is necessary to explore alternative, cheaper and locally available protein sources that will be tapped into to enhance livestock production and productivity (Yusuf et al., 2010; Olaiya and Makinde, 2015) (Table 2.1).

**Table 2.1:** Alternative protein sources used in small-scale poultry production

| Alternative protein source     | Composition (% DM) |       |       |                         |
|--------------------------------|--------------------|-------|-------|-------------------------|
|                                |                    | CP    | CF    | Reference               |
| Animal-derived protein sources | Earthworm          | 38.87 | -     | Babiker (2012)          |
|                                | Grasshopper        | 53.58 | 9.21  | Khan (2018)             |
|                                | Maggots            | 25.30 | -     | Khan et al. (2016)      |
| Plant-derived protein sources  | Cabbage            | 14.00 | 35.00 | Abdelnour et al. (2018) |
| 1. Leaf meals                  | Sweet potato       | 25.66 | 12.76 | Abdelnour et al. (2018) |

|                |          |       |       |                           |
|----------------|----------|-------|-------|---------------------------|
|                | Cassava  | 29.00 | 14.90 | Apata and Babalola (2012) |
|                | Coco Yam | 20.60 | 12.10 | Apata and Babalola (2012) |
|                | Moringa  | 22.50 | 4.38  | Olarotimi and Adu (2017)  |
|                | Pawpaw   | 26.30 | 14.80 | Abdelnour et al. (2018)   |
| 2. Seeds meals | Baobab   | 32.50 | 5.73  | Yusuf et al. (2010)       |
|                | Moringa  | 31.07 | 28.46 | Babiker (2012)            |

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### 2.3.1. Limitations of using alternative protein sources in poultry diets

In South Africa, using animal-derived dietary protein sources such as blood meal, meat and bone meal and hatchery waste is prohibited due to the occurrence of outbreaks related to use of these protein sources (Malebana et al., 2018). In Africa, there are native and under-exploited trees and shrubs that are potentially reliable sources of protein for both humans and animals (Yusuf et al., 2010, Olarotimi and Adu, 2017). During foraging for feed especially during the rainy season indigenous chickens usually get abundant animal protein by picking up worms, snails, and insects (Akinola and Essien, 2011). Insects belong to five groups namely Diptera (black soldier fly, housefly), Coleoptera (mealworms), Megadrilacea (earthworm), Lepidoptera (silkworm and *cirina forda*) and Orthoptera (grasshoppers, locust, and crickets) (Khan, 2018). They have an enormous potential for enhancing free-range poultry production as they have high protein content characterised by greater availability of essential amino acids (Saeed et al., 2017), fats, minerals, and vitamins (Khobondo et al., 2015). In addition, maggots, worms, and termites also can serve as alternative protein sources to free range chickens (Mtileni et al., 2012, Khobondo et al., 2015). However, alternative protein sources for indigenous chickens tend to fluctuate with season: with less insects, worms, and snails during the dry season (Akinola and Essien, 2011, Tarwireyi and Fanadzo, 2013) compared to during the rainy season. Therefore, there is a need to search for other alternative dietary protein sources that can be available for use throughout the year.

## 2.4. Seed cake meals and their value in poultry feed

Dietary protein is the most important nutrient in animal diet for maintenance, repair damages tissues and for proper growth and development (Manyeula et al., 2022). Seed cakes are residues from oil extraction from oilseeds and are potential protein supplements due to their rich in protein, minerals, and metabolizable energy content (Nigam and Pandey, 2009). There are two types of seed cakes, edible and non-edible. Edible seed cakes are used in animal feeds and non-edible are used in various agricultural applications (Siva Sankari et al., 2022). Conventional (soybean cake, rapeseed cake, sunflower cake, and groundnut cake) (Willis, 2003, Ramachandran et al., 2007) and non-conventional (baobab, macadamia, marula, castor, neem) seed cake can be used as plant-derived dietary protein sources in livestock feeds (Mlambo et al., 2011, Muya et al., 2020). Soyabean meal (SBM) is the main protein and energy rich ingredient used in most poultry production systems in Southern Africa (Manyeula et al., 2021, Abdelnour et al., 2018). Tables 2.2 and 2.3 show examples of conventional and the amino acid content of the cakes, respectively

**Table 2.2:** Conventional oilseed cake used and their values in poultry nutrition.

| Oil cake           | CP (%) | CF (%) | DM (%) | Ash (%) |
|--------------------|--------|--------|--------|---------|
| Soybean meal       | 48.0   | 6.2    | 84.6   | 6.4     |
| Sunflower oil cake | 34.1   | 25.5   | 91.0   | 6.6     |
| Sesame oil cake    | 35.6   | 7.6    | 83.2   | 11.8    |
| Groundnut oil cake | 49.5   | 5.3    | 92.6   | 4.5     |
| Canola oil cake    | 35.0   | 12.2   | 90     | 6.2     |
| Cotton seed cake   | 39.0   | 13.0   | 94.3   | 6.8     |

Sources: Wills (2003); Ramachandran *et al.* (2007).

**Table 2. 3:** Amino acids composition of conventional oilseed cakes

| Amino acid    | Composition (% CP) |      |      |      |      |      |
|---------------|--------------------|------|------|------|------|------|
|               | SBM                | SuOC | SOC  | GOC  | CaOC | COC  |
| Lysine        | 6.08               | 3.5  | 2.9  | 3.6  | 2.02 | 2.02 |
| Leucine       | 7.72               | 6.9  | 7.5  | 6.1  | 2.39 | 6.0  |
| Isoleucine    | 4.62               | 4.2  | 3.6  | 3.0  | 1.41 | 3.0  |
| Methionine    | 1.22               | 2.2  | 3.1  | 0.4  | 0.77 | 1.0  |
| Cysteine      | 1.70               | 1.8  | 2.1  | 0.9  | -    | 0.9  |
| Phenylalanine | 4.84               | 5.1  | 4.3  | 4.9  | 1.54 | 4.1  |
| Tyrosine      | 3.39               | 1.4  | 3.9  | 3.7  | 1.05 | 3.1  |
| Valine        | 4.59               | 5.8  | 4.0  | 3.7  | 1.71 | 5.8  |
| Histidine     | 2.50               | 2.8  | 2.9  | 2.5  | 1.13 | 2.1  |
| Threonine     | 3.76               | 3.4  | 3.2  | 2.8  | 1.50 | 3.0  |
| Glycine       | 4.01               | 5.6  | 5.3  | 4.5  | 1.75 | 4.2  |
| Arginine      | 7.13               | 9.1  | 12.8 | 11.0 | 2.12 | 11.0 |
| Tryptophan    | 1.24               | 1.4  | 1.4  | -    | 0.46 | -    |

**SBM**- soybean meal, **SuOC**- sunflower oil cake, **SOC**- Sesame oil cake, **GOC**- Groundnut oil cake, **CaOC**- Canola oil cake, **COC**- Coconut oil cake. **Sources:** Ramachandran et al. (2007); Saeed et al. (2017).

#### 2.4.1. Soya bean

Soya bean (*Glycine max*) is the primary oilseed, grown commercially in over 35 countries (Banaszkiewicz, 2011). It is a major global food, oil and protein source (Miransari, 2016). Soybean meal (SBM) is a global standard protein feed ingredient for livestock. This is due to its balanced amino acid content ((Dlamini et al., 2014). However SBM contains antinutritional

factors which are thermolabile thus are easily inactivated by heat during the processing (Dozier et al., 2011). The major producers of SBM are the United States, Brazil and Argentina, with very little being produced in Africa particularly in South Africa (Dlamini et al., 2014, Pagano and Miransari, 2016). In commercialised intensive poultry production SBM is a widely used expensive conventional dietary protein source (Alders and Pym, 2009, Khan et al., 2016, Malebana et al., 2018). It constitutes about 25 – 30% of poultry diets (Babiker et al., 2009, Khan et al., 2016). The SBM used in poultry feeds largely supplies both dietary protein and essential amino acids such as lysine, methionine, and threonine required by chickens for muscle growth and egg production (Tekeli, 2014).

#### 2.4.2. Marula tree

Marula, (*Sclerocarya birrea caffra*, is a fruit-bearing indigenous tree found in savannah areas of Sub-Saharan Africa (SSA). It belongs to the *Anacardiaceae* family and produces fruit which turns pale yellow when ripe (Mariod and Abdelwahab, 2012). Each Marula fruit which on average is 30 mm in diameter weighs 15 – 25g (Mariod and Abdelwahab, 2012, Malebana et al., 2018). These Maraula trees prefer well-drained soils at altitude of 1800 m and an annual rainfall of 200–1500 mm (Mariod and Abdelwahab, 2012). Marula fruits fall between January and April and are harvested from late December to mid-April, and then separated into nutritious pulp and nut, which is mostly sought for nutrition by humans and animals (Mlambo et al., 2011). Marula trees are distributed mostly from 17° 15' N in the air Mountain of Niger to 31° 00' S near Port Shepstone (Mthiyane and Mhlanga, 2017). Marula tree, fruit, kernel, and nut meal are illustrated in Figure 2.3.



**Figure 2. 3:** Marula tree, fruit, nuts, and seed cake

#### 2.4.2.1. Marula tree: importance in rural communities

Marula are highly appreciated by rural people (Hiwilepo-van Hal et al., 2014). Fruits, kernels, oil, bark, roots, leaves, and wood are used in various industries, including food, cooking, personal care, ethnomedicine, carving and utensils, and fuel wood (Wynberg et al., 2003). Local people eat fresh fruits or ferment it to make a traditional beer called buganu (Malebana et al., 2018). Commercially, Marula fruit pulp used as a raw material in the production of beverages, for example, Amarula cream liqueur and fruit juice as well as jam (Hiwilepo-van Hal et al., 2014, Malebana et al., 2018). Furthermore, oil used in the cosmeceutical and pharmaceutical industries is extracted from Marula seed kernels. The Marula seed cake is a by-product of Marula oil-kernel oil extraction.

#### 2.4.2.2. Nutritional value of Marula seed cake

Marula seed cake, a by-product from cold press oil extraction process in South African biodiesel industries, is used as an alternative cheap protein source for animals (Mlambo et al., 2011, Mdziniso et al., 2016, Mthiyane and Mhlanga, 2017). It is reported to contain 411g kg<sup>-1</sup> DM fats, 391 kg<sup>-1</sup> DM CP and 27.3 MJ metabolizable energy (Muya et al., 2020). Marula seed kernels are generally rich in Ca, P, K, Mg, Cu and Fe (Mariod and Abdelwahab, 2012). Based on its high nutrient (protein fat, and mineral ) content, MSC can potentially be used as a feed ingredient in poultry diets. Tables 2.4 and 2.5 below show the nutrient and amino acid content of MSC.

**Table 2. 4:** Nutritional and mineral composition (g/kg DM) of cold pressed extracted Marula seed cake.

| Component               | Amount |
|-------------------------|--------|
| Dy matter               | 946.60 |
| Crude protein           | 470.00 |
| Crude fibre             | 58.20  |
| Ash                     | 54.30  |
| Neutral detergent fibre | 207.80 |
| Acid detergent fibre    | 180.20 |

|            |      |
|------------|------|
| Calcium    | 1.10 |
| Phosphorus | 1.00 |

**Sources:** Mlambo et al. (2011); Mthiyane and Mhlanga (2017).

**Table 2. 5:** Amino acid composition (% DM) of Marula seed cake

| Amino acid    | Composition |
|---------------|-------------|
| Lysine        | 0.88        |
| Leucine       | 2.66        |
| Isoleucine    | 1.77        |
| Methionine    | 0.79        |
| Cysteine      | 1.18        |
| Phenylalanine | 1.91        |
| Valine        | 2.14        |
| Histidine     | 1.13        |
| Threonine     | 0.98        |
| Serine        | 1.19        |
| Glutamic acid | 10.78       |
| Aspartic acid | 3.51        |
| Proline       | 1.44        |
| Glycine       | 2.11        |
| Alanine       | 1.40        |
| Arginine      | 6.63        |

Source: Mthiyane and Mhlanga (2017)

#### **2.4.2.3. Marula seed cake: anti-nutritional factors content**

Anti-nutritional factors are natural secondary metabolites produced by plants that are highly biologically active and capable of precipitating deleterious effects in animals (Soetan, 2008, Gemedé and Ratta, 2014). They are known to disrupt metabolic processes, impairing nutrient digestion, absorption and bioavailability when plant seed cakes are used as feed ingredients (Sinha and Khare, 2017). Marula seed cake contains anti-nutritional factors that include phytates, oxalates, saponins, tannin, hydrocyanic acid, nitrates as well mycotoxins (Mthiyane and Mhlanga, 2017). For instance, tannins bind endogenous and exogenous protein compromising protein digestions (Wang et al., 2022), while oxalates are known for their toxicity to erythrocytes, and they also bind calcium (Ma et al., 2014).

### **2.5. Summary**

The nutrition of indigenous chickens is usually compromised by near sole dependence on scavengeable feed resources. The chickens usually get protein from scavenged snails, insects (Akinola and Essien, 2011), maggots, worms and termites (Mtileni et al., 2012, Khobondo et al., 2015). However, the availability and nutritional quality of these protein sources tend to fluctuate according to season as there is less insects, worms, and snails during the dry season (Akinola and Essien, 2011, Tarwireyi and Fanadzo, 2013). This seasonal availability compromises indigenous chickens' productivity during the "insect-snail-worm off-season." There is therefore a dire need to search and develop alternative dietary protein sources to make up for seasonal shortages such as MSC. . Few studies have studied the effect of dietary MSC supplementation in poultry production (Mthiyane and Mhlanga, 2017; Mthiyane and Mhlanga, 2018; Mazizi et al., 2019; Nkwanyana et al., 2022). So far, none of them have investigated the effect of substituting SBM with MSC in Boschveld indigenous chicken's diets and evaluating effects on performance, health, and product quality hence this study evaluated the effects of dietary Marula seed cake on Boschveld indigenous chickens' performance and viscera morphometry, carcass characteristics, clinical haemo-biochemistry, gut metagenomes and meat and bone quality.

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## Chapter 3

### **Dietary *Sclerocarya birrea caffra* seed cake: effect on growth performance and viscera morphometry, clinical haemp-biochemistry, carcass, meaty and bone quality.**

#### **Abstract**

The low productivity of indigenous chickens is mainly due to high feed costs fuelled by the high cost of soyabean meal (SBM) used as the primary dietary protein source in poultry diets. Marula seed cake (MSC), a by-product of oil extraction has a high content of protein which could be exploited in poultry production hence this study investigated its potential to substitute the expensive SBM in indigenous chicken diets. Four hundred 3-week-old Boschveld chicks were randomly allocated to and fed 5 iso-energetic-nitrogenous maize and SBM-based grower diets with 0, 10, 15, 20, and 25% MSC replacing SBM on crude protein basis, each with 5 replicate pens of 16 birds per (8 males and 8 females), in a completely randomised design for 9 weeks. Feed intake, body weight, feed conversion efficiency, haemato-biochemistry, viscera macro-morphometry, carcass characteristics, meat and bone quality parameters were measured. Dietary MSC decreased feed intake (FI;  $P < 0.001$ ) and body weight gain (BWG;  $P < 0.01$ ) with optimum responses at 15% inclusion level. It also decreased hot carcass ( $P < 0.05$ ), spleen ( $P < 0.05$ ), jejunum ( $P < 0.05$ ), ileum ( $P < 0.001$ ), and caecum ( $P < 0.001$ ) weights. However, dietary MSC increased the chickens' serum glucose (linear:  $P < 0.05$ ), cholesterol (linear:  $P = 0.001$ ) and phosphate (quadratic:  $P < 0.05$ ) concentration but decreased their amylase activity ( $P < 0.01$ ). Furthermore, dietary MSC decreased the chickens' meat lightness at 45 min (linear:  $P < 0.05$ ) and yellowness at both 45 min (quadratic:  $P < 0.001$ ) and 24 h (quadratic:  $P < 0.001$ ) but it increased its redness both at 45 min (linear:  $P < 0.01$ ) and 24 h (linear:  $P < 0.05$ ) post-slaughter. Dietary MSC decreased the chickens' bone medial diaphysis ( $P < 0.05$ ) but induced no effects ( $P > 0.05$ ) on feed conversion efficiency, carcass traits, viscera morphometry, haematology, meat quality and bone morphometry. In conclusion, Boschveld indigenous chickens can be fed grower diets wherein SBM is substituted with MSC up to 15% without detrimental effects on FCE, 2Carcass characteristics, haematology, and bone size of Boschveld chickens.

**Keywords:** *Gallus domesticus*, Soybean meal, *Sclerocarya birrea caffra*, amino acids, alternative protein source.

### 3.1. Introduction

With the rapidly increasing demand for animal-derived protein-rich food emanating from the exponentially increasing world human population and urbanisation, there has been massive increase in livestock and poultry production in the last few decades (Mottet and Tempio, 2017, Parolini et al., 2020). Due to their relatively short production cycle and relative affordability of chicken meat and eggs, poultry production production wields enormous potential in the attainment of poverty alleviation and achievement sustainable food security a (Birhanu et al., 2023). Indigenous chickens (*Gallus domesticus*) are popular poultry species in rural areas of Southern Africa where they serve as a source of meat and eggs for human consumption and income (Okoro et al., 2017, Gunya et al., 2020). The indigenous chickens are genetically highly adapted to the stressful tropical environmental conditions and endemic diseases, are excellent foragers and users of poor-quality plant based feed resources compared to exotic genetically improved chickens (Alders and Pym, 2009, Okoro et al., 2017). These indigenous chickens concert the poor-quality plant-based feed resources into high protein quality meat and eggs for human consumption (Padhi, 2016).

Notwithstanding the indigenous chickens' potential to contribute to food and nutrition security in Southern Africa; their current contribution not optimal as a result of low productive performance (Okoro et al., 2017), and predation (Guèye, 2000, Mtileni et al., 2012). One of the main contributors to their low productivity and increased susceptibility to pathogenic infections is inadequate intake of dietary proteins, minerals, and energy to sustain growth performance and egg production (Raphulu et al., 2015). To resolve this dilemma, Alabi et al. (2013) proposed that feeding a commercial grower diet containing 178 g CP/kg DM and 14 MJ ME/kg DM could improve indigenous chickens' growth performance and egg production. This could be achieved through supplementation of chickens' diets with nutrient-rich conventional feed ingredients such as SBM (Khobondo et al., 2015, Selaledi et al., 2020). Unfortunately, SBM prices are prohibitively too high for most smallholder farmers thus limiting its use by resource-limited rural and urban backyard indigenous chicken producers resulting in compromised economic sustainability of chicken farming enterprises (Parolini et al., 2020). Since quality

dietary protein sources such as SBM are the major limiting factor to improved indigenous productivity, there is a dire need to explore and develop cheaper alternative protein sources (APS) for use in indigenous chicken's diets. Mural seed cake (MSC) a by-product of oil extraction has a high content of CP, minerals, residual oil and vitamins (Mlambo et al., 2011).

Marula, *Sclerocarya birrea caffra*, seed (kernel) cake is the solid residue remaining after extraction of oil from the kernels dislodged from the hard nutshells of the fruits of Marula trees (Mdziniso et al., 2016). The machines used to dislodge the kernels from the nutshells are unable to make a perfect separation of the kernels from the nutshells which results MSC containing significant remnants of the latter which contribute to the relatively high fibre content (58.2 g/kg DM) of MSC (Mthiyane and Mhlanga, 2017). Nutritionally MSC is a rich source of nutrients: it contains 470 g CP/kg DM characterised by a good profile of essential and non-essential amino acids which is similar to that of SBM, except for lysine. It also contains significant quantities of minerals particularly Ca, P and K and has a high residual oil content which has a preponderance (72-85%) of oleic acid, a monounsaturated fatty acid (Mlambo et al., 2011, Mdziniso et al., 2016, Mthiyane and Mhlanga, 2017, Malebana et al., 2018). Because of the remarkable preponderance of oleic acid, MSC residual oil's oxidative stability is 10 times more compared to that of olive oil (Mariod and Abdelwahab, 2012) which could be used as a source of anti-oxidant. In addition, Marula kernels from which MSC is derived are endowed with a kaleidoscope of bioactive substances mainly the polyphenolic compounds, flavonoids, tannins, phytic acid and oxalates (Mashau et al., 2022) that impart various health beneficial and anti-nutritional effects to chickens (Sun et al., 2019, Zhang et al., 2021).

Notwithstanding, MSC has hitherto not been investigated as a possible replacement for SBM in Boschveld indigenous chicken diets. Considering its favourable protein content, essential amino acid profile and functional secondary metabolites as well as relative widespread production in sub-Saharan African countries including South Africa, (Magaia, 2015, Mthiyane and Mhlanga, 2017, Malebana et al., 2018, Manyeula et al., 2021) which speaks to abundant local availability unlike the largely imported SBM, MSC would potentially be a perfect replacement for SBM in indigenous chicken diets. Therefore, this study investigated the effects graded incorporating MSC into indigenous Boschveld chicken diets in place of SBM on

chickens' growth performance, carcass traits, viscera morphometry, clinical haemato-biochemistry, meat and bone quality.

## **3.2. Materials and methods**

### **3.2.1. Study site and ingredient sources**

The study was conducted between June and September 2022 at North-West University's Molelwane Experimental Farm (25°86'00"S, 25°64'32"E) located ~ 10 km outside Mahikeng city in Ngaka Modiri Molema district, North West Province of South Africa. Marula seed cake (MSC) was supplied by The Marula Company in Phalaborwa, Limpopo Province, South Africa while the rest of ingredients were from Nutroteq in Centurion, Gauteng Province, South Africa.

### **3.2.2. Diet formulation**

Five iso-energetic and iso-nitrogenous maize and SBM-based diets, in mash form, were formulated to meet or exceed the nutritional requirements of grower birds as recommended by the National Research Council (NRC, 1994). The experimental diets were formulated such that the graded levels of MSC (0, 10, 15, 20, and 25%) were sequentially added in replacement of SBM on crude protein basis (Table 3.3).

### **3.2.3. Chemical analyses of MSC and experimental diets**

The experimental diets and MSC were milled to pass through a 2 mm-sieve (Polymix PX-MFC 90D) and subjected to proximate analysis using the Official Analytical Chemists International Methods (AOAC, 2005) and mineral assays using an ICP Mass Spectrometer, NexION® 2000, PerkinElmer (Pty) Ltd. (Table 3.1). For dry matter (DM) determination (method no.930.15), 1 g samples were added into pre-weighed crucibles and oven dried at 105 °C for 12 h followed by placing in the desiccator and then re-weighed. Thereafter, the DM was calculated as the difference between initial weight (W1) and sample weight after oven (W2) and expressed as g/kg. Organic matter (OM) content was determined (method no. 924.05) by burning the dried samples in a muffle furnace set at 550 °C for 6 h. The lost weight was measured as OM content and the remaining residue as ash content. Crude protein (CP) was determined following the

standard Kjeldahl method (method no. 984.13) whereby the nitrogen content was determined and then multiplied by a factor of 6.25.

**Table 3. 1:** Ingredient and chemical composition (g/kg as fed basis, unless otherwise stated) of experimental diets with graded levels of MSC.

| Ingredients                  | Diet  |        |        |        |        |        |
|------------------------------|-------|--------|--------|--------|--------|--------|
|                              | MSC   | MSC 0  | MSC 10 | MSC 15 | MSC 20 | MSC25  |
| Yellow maize                 |       | 618.6  | 626.2  | 628.8  | 633.6  | 586.6  |
| Full fat soya bean           |       | 48.5   | 31.7   | 30.2   | 0.0    | 10.0   |
| Soya bean meal               |       | 190.0  | 100.0  | 50.0   | 19.1   | 0.0    |
| MSC                          |       | 0.0    | 100.0  | 150.0  | 200.0  | 250.0  |
| Sunflower oilcake            |       | 59.8   | 55.6   | 52.8   | 53.8   | 15.9   |
| Soya bean oil                |       | 36.9   | 37.4   | 36.8   | 40.6   | 53.9   |
| Pellibond                    |       | 10.0   | 10.0   | 10.0   | 10.0   | 10.0   |
| Lysine HCL 78%               |       | 3.6    | 5.9    | 7.0    | 8.2    | 8.7    |
| Methionine DL 98%            |       | 2.9    | 2.7    | 2.6    | 2.5    | 2.6    |
| Threonine 98%                |       | 1.1    | 1.8    | 2.5    | 2.4    | 2.7    |
| Limestone                    |       | 13.0   | 13.5   | 13.8   | 14.0   | 43.6   |
| Mono-dicalcium phosphate     |       | 8.3    | 8.4    | 8.4    | 8.5    | 8.7    |
| Salt fine                    |       | 2.2    | 3.4    | 3.0    | 2.6    | 2.3    |
| Sodium bicarbonate           |       | 1.6    | 0.0    | 0.6    | 1.2    | 1.6    |
| Salinomycin 12%              |       | 0.5    | 0.5    | 0.5    | 0.5    | 0.5    |
| Zinc bacitracin              |       | 0.3    | 0.3    | 0.3    | 0.3    | 0.3    |
| AxtraPhy BF 1000 FTU         |       | 0.1    | 0.1    | 0.1    | 0.1    | 0.1    |
| BSFG Premix                  |       | 2.5    | 0.1    | 2.5    | 2.5    | 2.5    |
| Total                        |       | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| <b>Chemical composition</b>  |       |        |        |        |        |        |
| Dry Matter                   | 860   | 880    | 890    | 880    | 880    | 880    |
| Organic matter               | 952.4 | 955.5  | 959.1  | 959.8  | 955.6  | 937.9  |
| Ether extract                | 296.1 | 70.5   | 102.7  | 123.0  | 156.0  | 153.4  |
| Crude Protein                | 46.50 | 18.86  | 18.98  | 19.19  | 18.61  | 18.61  |
| Metabolizable energy (MJ/Kg) | 15.7  | 12.9   | 12.9   | 12.9   | 12.9   | 12.9   |
| Crude fibre                  | 66.1  | 36.4   | 40.8   | 40.2   | 42.3   | 37.4   |
| Calcium                      | 1.4   | 0.84   | 0.84   | 0.85   | 0.83   | 0.87   |

|                  |       |       |       |       |      |      |
|------------------|-------|-------|-------|-------|------|------|
| Phosphorus       | 11.2  | 0.714 | 0.757 | 0.778 | 0.80 | 0.82 |
| Potassium        | 8.4   | 0.81  | 0.80  | 0.80  | 0.79 | 0.80 |
| Sodium (mg/kg)   | 346.0 | 0.18  | 0.18  | 0.19  | 0.18 | 0.18 |
| Chloride (mg/kg) | -     | 0.24  | 0.35  | 0.35  | 0.35 | 0.34 |

### 3.2.4. Experimental design and bird management

A total of 400, 3-week-old Boschveld chicks ( $226.65 \pm 12.455$  g/bird) purchased from Boschveld Ranching PTY LTD (Bela-Bela, Limpopo, South Africa) were randomly allocated to 5 dietary treatments (MSC 0, MSC 10, MSC 15, MSC 20, and MSC 25) in a completely randomised design (CRD). Each treatment was replicated 5 times with 16 chicks (8 males and 8 females) per replicate pen (size: 1.8m long x 1.0m wide x 1.8m high) each with 1 feeder and 1 drinker. The feeding trial was conducted for 9 weeks. The pens had a wire mesh floor that did not require bedding; however, a polythene plastic was used to cover the pen floor and was changed on a regular basis. Upon arrival, the chicks were offered StressPack for 3 consecutive days. Infrared lighting was used as a source of supplementary heat for the first 2 weeks. Clean feed and water were offered everyday *ad libitum* throughout the feeding trial. At 4 weeks of age, the birds were vaccinated against coryza. Daily mortality was monitored and recorded.

### 3.2.5. Feed intake and growth performance measurements

The daily amount of FI per bird was calculated by subtracting the daily amount of feed leftovers from the feed offered and dividing by the total number of birds per pen. The daily feed intakes were then converted into weekly averages of FI (g/bird/week) by calculating the sum of feed intake pen averages over 7 days. The initial weights of the birds were measured on arrival and subsequently weekly using a digital scale. Thereafter, the BWG was calculated by subtracting current body weight from previous weight and dividing by the number of birds per pen. Then FCE per bird was calculated by dividing the weekly BWG by weekly FI.

### 3.2.6. Blood collection and analysis

A day before slaughter, 2 birds from each pen were randomly selected for blood collection. 5 mL of blood was drawn from the wing vein using a 21-gauge needle and 5 mL syringe into EDTA coated blood collection for haematological analyses. 1 mL of collected was placed into blood collection tubes with a clotting activator to generate serum biochemical analyses. The blood samples were analysed for haematocrit, haemoglobin concentration, red blood cells,

mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets using an automated IDEXX Laser-Cyte Haematology analyser (IDEXX Laboratories, Inc, Johannesburg, South Africa). Serum biochemical glucose, symmetric dimethylarginine (SDMA), urea, phosphate, calcium, total protein, albumin, globulin, total bilirubin and cholesterol concentrations, albumin/globulin ratio, alanine transaminase, alkaline phosphatase, amylase, and lipase activities were analysed using an automated IDEXX Vet Test Chemistry Analyzer (IDEXX Laboratories (Pty) Ltd., Johannesburg, South Africa) as per the manufacturer's instructions.

### **3.2.7. Slaughter procedures, measurement of carcass traits and viscera macromorphometry**

At the end of the feeding trial, the chickens were subjected to a 12 h fasting period but with access to clean drinking water to prevent dehydration. Terminal (final) fasted live weights were measured immediately following the fast. The chickens were then transported to Rooigrond Poultry Abattoir for slaughter. On arrival, the birds were electrically stunned and humanely slaughtered by cutting the jugular vein with a sharp knife and left hanging until bleeding stopped. The birds were de-feathered, and the visceral organs were dissected out and the mass of each measured using an electronic balance and length of small and large intestines, measured using a measuring tape attached to a dissection board. Hot carcasses were weighed without giblets using a digital weighing scale to obtain hot carcass weight (HCW). Carcasses were then transported to North-West University Animal Science laboratory for chilling at 4 °C for 24 h after which cold carcass weights (CCW) were measured. After 24 h of chilling, the breast, wing, thigh, and drumstick were removed from the left side of each carcass and weighed. Thereafter the yield of each commercial cut (breast, drumstick, thigh, and wing) was computed and expressed as percentages of cold carcass weight.

### **3.2.8. Meat quality measurements**

Meat pH and temperature were determined on the breast muscle of each bird using a portable waterproof fibre-optic pH and temperature meter (Hanna instruments, HI98163, Romania). It was measured at 45 min and 24 h post-mortem. After every 10 measurements, the pH meter was calibrated using pH 4, pH 7 and pH 10 standard solutions meant for this purpose. Breast meat colour coordinates L\* (lightness), a\* (redness) and b\* (yellowness) were measured at 45 min and 24 h post-mortem using a calibrated Konica Minolta colour guide [Narich (Pty) Ltd,

Cape Town, RSA] following the guidelines by the Commission International De l'Éclairage (CIE, 1978). The raw breast meat samples were weighed and broiled at 180 °C for 20 min (Honikel, 1998). Broiled samples were then allowed to cool at room temperature and then measured to obtain final weight. Cooking loss was then calculated using the following equation:

$$\text{Cooking loss} = \frac{\text{Weight before cooking (g)} - \text{Weight after cooking (g)}}{\text{Weight before cooking (g)}} \times 100\%$$

The water holding capacity (WHC) was determined using filter paper method as described by Honikel (1987). Briefly, ~ 10 g of breast meat samples were weighed and pressed onto an oven dried Whatman 125 mm filter paper under a 60 kg mass for 5 min. After pressing, the meat samples were weighed again and WHC was calculated. The drip loss was measured as described by Honikel (1998). Briefly, approximately 2 g of raw breast meat samples were weighed and individually hooked inside sample bottles and placed in a chiller (4 °C) for 72 h followed. The samples were hooked in a manner that they did not get in contact with the sides and surface of the bottles.

### **3.2.9. Bone morphometry assessment**

Left drumsticks were manually removed with a knife from 100 birds (4 birds /pen). The soft tissues were removed from the tibia bone using a scalpel and washed. After washing the tibia bones were kept in an identified plastic bag at -20 °C until analyses. Prior bone measurements the bones were thawed at room temperature. The bones were individually weighed using a digital scale to obtain their weights. Geometric parameters (seedor index and robusticity index) of each bone were determined and the medial and lateral walls were measured at the midpoint mark using a digital calliper. Seedor index was obtained as the ratio between the weight and the length of the bone (Seedor index = weight/length). The robusticity index of each bone was determined by dividing the bone length and the cubic of the bone weight (robustness index = length/  $\sqrt[3]{\text{weight}}$ ) (Evaris et al., 2021).

### **3.2.10. Statistical analysis**

The data measured weekly, that is, FI, BWG, and FCE, were subjected to repeated measures analysis using SAS (2012) statistical software. The data for overall FI, BWG, and FCE, clinical haemobiochemistry, carcass characteristics, meat quality and bone morphometry were

analysed using the GLM procedure (PROC GLM) of SAS (2012). Each replicate pen (5 per treatment) was the experimental unit, and the data were presented as Least Square Means (LSMs) with respective pooled Standard Errors of the Means (SEMs) ( $n = 25$ ). The LSMs were separated using the probability of difference (PDIFF) option in the LSMEANS statement and differences among them considered significant at  $P < 0.05$ . The polynomial contrasts (PROC RSREG) were used to assess data for linear and quadratic trends between diets and dependent variables using the following non-linear model:

$$y = ax^2 + bx + c$$

where  $y$  is the dependent variable;  $a$  and  $b$  are coefficients of the model;  $c$  is the intercept;  $x$  is dietary MSC inclusion level; and  $-b/2a$  is the  $x$  value that minimizes or maximizes a response parameter.

### 3.3.Results

The effect of dietary inclusion of MSC on weekly and overall, FI, BWG, and FCE of Boschveld chickens with associated regression equations are presented in Tables 3.2 and 3.3, respectively. Dietary MSC decreased FI in weeks 1 (linear:  $P < 0.01$ ), 2 (quadratic:  $P < 0.01$ ), 3 (quadratic:  $P < 0.01$ ), 4 (linear:  $P < 0.001$ ), 5 (linear:  $P < 0.05$ ), 7 (quadratic:  $P < 0.01$ ), 8 (quadratic:  $P < 0.001$ ) and 9 (quadratic:  $P < 0.001$ ), with no effect of diet on the FI in week 6 ( $P > 0.05$ ). Regarding BWG, dietary MSC quadratically decreased it in weeks 1 ( $P < 0.01$ ), 2 ( $P < 0.01$ ), 3 ( $P < 0.01$ ), 4 ( $P < 0.01$ ) and 6 ( $P < 0.05$ ) but caused neither linear nor quadratic effects in weeks 5, 7, 8 and 9 ( $P > 0.05$ ). On FCE, dietary MSC decreased it in weeks 4 (quadratic:  $P < 0.05$ ) and 5 (linear:  $P < 0.01$ ) but caused neither linear nor quadratic effects in weeks 1, 2, 3, 6, 7, 8 and 9 ( $P > 0.05$ ). Overall, dietary MSC quadratically decreased FI ( $P < 0.001$ ) and BWG ( $P < 0.01$ ), with optimum responses of both parameters at 15% inclusion level but the MSC did not induced any effects on FCE ( $P > 0.05$ ). FI, BWG and FCE increased with time ( $P < 0.001$ ) with FI particularly linearly increasing from week 1 to week 9 whilst both BWG and FCE quadratically reached an optimum around week 4 and week 2, respectively, showing decremental trends henceforth. Consequently, there was a significant diet by week interaction effect on FI ( $P < 0.001$ ) only.

**Table 3. 2:** Effect of dietary inclusion of MSC on feed intake, body weight gain, and feed conversion efficiency of indigenous Boschveld chickens

| Parameters           | Week | Dietary MSC (%)       |                       |                      |                       |                      | SEM   | P-value     |             |
|----------------------|------|-----------------------|-----------------------|----------------------|-----------------------|----------------------|-------|-------------|-------------|
|                      |      | 0                     | 10                    | 15                   | 20                    | 25                   |       | Linear      | Quadratic   |
| FI (g/bird)          | 1    | 233.50 <sup>a</sup>   | 194.00 <sup>c</sup>   | 237.38 <sup>a</sup>  | 203.75 <sup>b</sup>   | 180.88 <sup>d</sup>  | 2.84  | $P < 0.01$  | $P > 0.05$  |
|                      | 2    | 228.38 <sup>b</sup>   | 243.75 <sup>a</sup>   | 236.25 <sup>a</sup>  | 228.75 <sup>b</sup>   | 220.75 <sup>c</sup>  | 3.98  | $P > 0.05$  | $P < 0.01$  |
|                      | 3    | 311.25 <sup>b</sup>   | 325.50 <sup>a</sup>   | 323.00 <sup>a</sup>  | 312.00 <sup>b</sup>   | 299.50 <sup>b</sup>  | 4.50  | $P > 0.05$  | $P < 0.01$  |
|                      | 4    | 398.63 <sup>a</sup>   | 398.38 <sup>a</sup>   | 394.63 <sup>a</sup>  | 365.50 <sup>b</sup>   | 372.12 <sup>b</sup>  | 6.32  | $P < 0.001$ | $P > 0.05$  |
|                      | 5    | 428.70 <sup>a</sup>   | 418.00 <sup>b</sup>   | 438.13 <sup>a</sup>  | 376.22 <sup>d</sup>   | 407.27 <sup>c</sup>  | 3.37  | $P < 0.05$  | $P > 0.05$  |
|                      | 6    | 434.53                | 428.38                | 485.33               | 403.68                | 429.58               | 2.05  | $P > 0.05$  | $P > 0.05$  |
|                      | 7    | 483.00 <sup>b</sup>   | 492.15 <sup>b</sup>   | 586.63 <sup>a</sup>  | 491.97 <sup>b</sup>   | 453.95 <sup>c</sup>  | 3.34  | $P > 0.05$  | $P < 0.01$  |
|                      | 8    | 505.38 <sup>c</sup>   | 569.36 <sup>b</sup>   | 652.01 <sup>a</sup>  | 556.33 <sup>b</sup>   | 472.72 <sup>d</sup>  | 4.54  | $P > 0.05$  | $P < 0.001$ |
|                      | 9    | 863.63 <sup>c</sup>   | 935.00 <sup>b</sup>   | 1079.46 <sup>a</sup> | 907.75 <sup>b</sup>   | 761.85 <sup>d</sup>  | 12.46 | $P > 0.05$  | $P < 0.001$ |
| BWG (g/bird)         | 1    | 83.75 <sup>ab</sup>   | 89.50 <sup>a</sup>    | 90.00 <sup>a</sup>   | 89.75 <sup>a</sup>    | 79.00 <sup>b</sup>   | 3.52  | $P > 0.05$  | $P < 0.01$  |
|                      | 2    | 128.75 <sup>b</sup>   | 134.00 <sup>ab</sup>  | 145.00 <sup>a</sup>  | 130.25 <sup>b</sup>   | 121.00 <sup>b</sup>  | 4.33  | $P > 0.05$  | $P < 0.01$  |
|                      | 3    | 134.75 <sup>ab</sup>  | 142.25 <sup>a</sup>   | 136.50 <sup>a</sup>  | 138.50 <sup>a</sup>   | 131.50 <sup>b</sup>  | 6.21  | $P > 0.05$  | $P < 0.01$  |
|                      | 4    | 141.50 <sup>b</sup>   | 154.25 <sup>ab</sup>  | 157.50 <sup>ab</sup> | 174.50 <sup>a</sup>   | 134.50 <sup>b</sup>  | 8.33  | $P > 0.05$  | $P < 0.01$  |
|                      | 5    | 99.25                 | 119.25                | 116.25               | 86.25                 | 125.95               | 10.12 | $P > 0.05$  | $P > 0.05$  |
|                      | 6    | 128.00 <sup>ab</sup>  | 140.63 <sup>ab</sup>  | 142.00 <sup>ab</sup> | 146.75 <sup>a</sup>   | 107.80 <sup>b</sup>  | 11.84 | $P > 0.05$  | $P < 0.05$  |
|                      | 7    | 133.25                | 153.38                | 133.50               | 122.50                | 151.63               | 18.98 | $P > 0.05$  | $P > 0.05$  |
|                      | 8    | 124.75                | 124.04                | 123.13               | 120.75                | 99.15                | 17.10 | $P > 0.05$  | $P > 0.05$  |
|                      | 9    | 63.25                 | 113.08                | 180.38               | 72.64                 | 80.97                | 33.53 | $P > 0.05$  | $P > 0.05$  |
| FCE                  | 1    | 0.36                  | 0.46                  | 0.38                 | 0.44                  | 0.44                 | 0.02  | $P > 0.05$  | $P > 0.05$  |
|                      | 2    | 0.56                  | 0.55                  | 0.61                 | 0.57                  | 0.55                 | 0.02  | $P > 0.05$  | $P > 0.05$  |
|                      | 3    | 0.43                  | 0.44                  | 0.42                 | 0.44                  | 0.44                 | 0.02  | $P > 0.05$  | $P > 0.05$  |
|                      | 4    | 0.36 <sup>b</sup>     | 0.39 <sup>b</sup>     | 0.40 <sup>b</sup>    | 0.48 <sup>a</sup>     | 0.36 <sup>b</sup>    | 0.02  | $P > 0.05$  | $P < 0.05$  |
|                      | 5    | 0.23 <sup>b</sup>     | 0.29 <sup>ab</sup>    | 0.27 <sup>ab</sup>   | 0.23 <sup>b</sup>     | 0.31 <sup>a</sup>    | 0.03  | $P < 0.01$  | $P > 0.05$  |
|                      | 6    | 0.29                  | 0.29                  | 0.29                 | 0.36                  | 0.25                 | 0.03  | $P > 0.05$  | $P > 0.05$  |
|                      | 7    | 0.28                  | 0.31                  | 0.27                 | 0.25                  | 0.33                 | 0.03  | $P > 0.05$  | $P > 0.05$  |
|                      | 8    | 0.25                  | 0.22                  | 0.16                 | 0.22                  | 0.18                 | 0.03  | $P > 0.05$  | $P > 0.05$  |
|                      | 9    | 0.07                  | 0.12                  | 0.14                 | 0.05                  | 0.11                 | 0.04  | $P > 0.05$  | $P > 0.05$  |
| Overall FI (g/bird)  |      | 3886.98 <sup>c</sup>  | 4004.51 <sup>b</sup>  | 4432.80 <sup>a</sup> | 3845.93 <sup>c</sup>  | 3598.62 <sup>d</sup> | 22.66 | $P > 0.05$  | $P < 0.001$ |
| Overall BWG (g/bird) |      | 1037.25 <sup>ab</sup> | 1131.22 <sup>ab</sup> | 1177.55 <sup>a</sup> | 1051.37 <sup>ab</sup> | 1018.22 <sup>b</sup> | 54.43 | $P > 0.05$  | $P < 0.01$  |
| Overall FCE          |      | 0.27                  | 0.28                  | 0.27                 | 0.27                  | 0.28                 | 0.01  | $P > 0.05$  | $P > 0.05$  |

Means in the same row with different superscripts (<sup>abcd</sup>) are significantly different. BWG = body weight gain, FCE = feed conversion efficiency, FI = feed intake, MSC = marula seed cake, SEM = standard error of the mean.

**Table 3. 3:** Linear and quadric responses of FI, BWG, and FCE in indigenous Boschveld chickens fed diets supplemented with MSC.

| Parameters | Week | Regression equation                                                   | R <sup>2</sup> | P-value     |
|------------|------|-----------------------------------------------------------------------|----------------|-------------|
| FI         | 1    | $y = -0.055(\pm 0.065)x + 229.817(\pm 8.391)$                         | 0.4272         | $P < 0.01$  |
|            | 2    | $y = -0.097(\pm 0.030)x^2 + 2.126(\pm 0.739)x + 228.333(\pm 3.803)$   | 0.3887         | $P < 0.01$  |
|            | 3    | $y = -0.137(\pm 0.034)x^2 + 2.953(\pm 0.860)x + 310.698(\pm 4.426)$   | 0.4713         | $P < 0.01$  |
|            | 4    | $y = -0.061(\pm 0.048)x + 398.701(\pm 6.154)$                         | 0.4859         | $P < 0.001$ |
|            | 5    | $y = -0.009(\pm 0.069)x + 429.999(\pm 8.901)$                         | 0.3373         | $P < 0.05$  |
|            | 7    | $y = -0.426(\pm 0.114)x^2 + 9.801(\pm 2.855)x + 477.947(\pm 14.688)$  | 0.4611         | $P < 0.01$  |
|            | 8    | $y = -0.798(\pm 0.096)x^2 + 18.922(\pm 2.398)x + 500.744(\pm 12.338)$ | 0.8113         | $P < 0.001$ |
|            | 9    | $y = -1.276(\pm 0.176)x^2 + 28.271(\pm 4.401)x + 854.589(\pm 22.648)$ | 0.7318         | $P < 0.001$ |
|            |      |                                                                       |                |             |
| BWG        | 1    | $y = -0.087(\pm 0.025)x^2 + 1.940(\pm 0.628)x + 82.977(\pm 3.230)$    | 0.4197         | $P < 0.01$  |
|            | 2    | $y = -0.087(\pm 0.025)x^2 + 1.884(\pm 0.617)x + 128.021(\pm 3.174)$   | 0.4179         | $P < 0.01$  |
|            | 3    | $y = -0.094(\pm 0.025)x^2 + 2.144(\pm 0.628)x + 133.954(\pm 3.229)$   | 0.4616         | $P < 0.01$  |
|            | 4    | $y = -0.169(\pm 0.057)x^2 + 4.046(\pm 1.428)x + 139.471(\pm 7.347)$   | 0.3522         | $P < 0.01$  |
|            | 6    | $y = -0.221(\pm 0.098)x^2 + 5.064(\pm 2.446)x + 125.422(\pm 12.586)$  | 0.2392         | $P < 0.05$  |
| FCE        | 4    | $y = -0.0004(\pm 0.0002)x^2 + 0.010(\pm 0.004)x + 0.350(\pm 0.022)$   | 0.2174         | $P < 0.05$  |
|            | 5    | $y = 0.0001(\pm 0.0002)x + 0.237(\pm 0.021)$                          | 0.3402         | $P < 0.05$  |

|             |                                                                                     |             |
|-------------|-------------------------------------------------------------------------------------|-------------|
| Overall FI  | $y = -2.963(\pm 0.584)x^2 + 62.635(\pm 14.570)x + 0.5642$<br>$3865.067(\pm 74.974)$ | $P < 0.001$ |
| Overall BWG | $y = -1.126(\pm 0.345)x^2 + 27.865(\pm 8.618)x + 0.3972$<br>$1028.584(\pm 44.347)$  | $P < 0.01$  |

BWG - body weight gain, FCE - feed conversion efficiency, FI - feed intake.

Table 3.4 shows the effect of dietary inclusion of MSC on carcass characteristics and internal organs of Boschveld indigenous chickens. Whilst there were neither linear nor quadratic effects of diet on all carcass characteristics and internal organs ( $P > 0.05$ ), dietary MSC linearly decreased the birds' HCW [ $y = -0.303(\pm 0.162)x + 894.395(\pm 24.191)$ ;  $R^2 = 0.1550$ ;  $P < 0.05$ ] as well as their weights of the spleen [ $y = -0.00016(\pm 0.00015)x + 0.326(\pm 0.022)$ ;  $R^2 = 0.1605$ ;  $P < 0.05$ ], jejunum [ $y = -0.024(\pm 0.016)x + 1.419(\pm 0.093)$ ;  $R^2 = 0.2310$ ;  $P < 0.05$ ], ileum [ $y = -0.017(\pm 0.010)x + 1.127(\pm 0.059)$ ;  $R^2 = 0.4533$ ;  $P < 0.001$ ], and caecum [ $y = -0.010(\pm 0.006)x + 0.690(\pm 0.032)$ ;  $R^2 = 0.3931$ ;  $P < 0.001$ ].

**Table 3. 4:** Effect of dietary inclusion of MSC on carcass characteristics (%CCW) and viscera morphometry (%HCW) of indigenous Boschveld chickens.

| Parameter                 | Dietary MSC (%)     |                     |                     |                      |                     | SEM   | P-value    |            |
|---------------------------|---------------------|---------------------|---------------------|----------------------|---------------------|-------|------------|------------|
|                           | 0                   | 10                  | 15                  | 20                   | 25                  |       | Linear     | Quadratic  |
| Slaughter weight (g/bird) | 1263.50             | 1361.22             | 1391.55             | 1268.90              | 1244.22             | 62.07 | $P > 0.05$ | $P > 0.05$ |
| Hot carcass weight (g)    | 897.88 <sup>a</sup> | 897.25 <sup>a</sup> | 903.05 <sup>a</sup> | 878.03 <sup>ab</sup> | 815.15 <sup>b</sup> | 25.35 | $P < 0.05$ | $P > 0.05$ |
| Cold carcass weight (g)   | 885.64              | 886.03              | 887.10              | 867.50               | 837.22              | 20.48 | $P > 0.05$ | $P > 0.05$ |
| Dressing %                | 71.12               | 66.04               | 67.10               | 69.47                | 65.68               | 3.62  | $P > 0.05$ | $P > 0.05$ |
| Breast (%)                | 12.90               | 13.79               | 11.18               | 12.86                | 13.43               | 0.74  | $P > 0.05$ | $P > 0.05$ |
| Drumstick (%)             | 6.82                | 7.36                | 6.16                | 7.54                 | 7.49                | 0.46  | $P > 0.05$ | $P > 0.05$ |
| Thigh (%)                 | 8.32                | 8.22                | 6.91                | 8.75                 | 8.98                | 0.48  | $P > 0.05$ | $P > 0.05$ |
| Wing (%)                  | 6.34                | 6.47                | 5.45                | 6.29                 | 6.63                | 0.40  | $P > 0.05$ | $P > 0.05$ |
| Liver (%)                 | 2.03                | 1.87                | 1.89                | 2.08                 | 1.91                | 0.139 | $P > 0.05$ | $P > 0.05$ |
| Spleen (%)                | 0.32 <sup>ab</sup>  | 0.32 <sup>a</sup>   | 0.33 <sup>a</sup>   | 0.27 <sup>b</sup>    | 0.27 <sup>b</sup>   | 0.022 | $P < 0.05$ | $P > 0.05$ |
| Proventriculus (%)        | 0.58                | 0.49                | 0.51                | 0.57                 | 0.78                | 0.140 | $P > 0.05$ | $P > 0.05$ |
| Gizzard (%)               | 2.31                | 2.20                | 2.15                | 2.29                 | 2.31                | 0.153 | $P > 0.05$ | $P > 0.05$ |
| Duodenum (%)              | 1.04                | 0.97                | 0.89                | 0.99                 | 0.93                | 0.088 | $P > 0.05$ | $P > 0.05$ |

|                      |                   |                    |                    |                    |                   |       |             |            |
|----------------------|-------------------|--------------------|--------------------|--------------------|-------------------|-------|-------------|------------|
| Jejunum (%)          | 1.41 <sup>a</sup> | 1.26 <sup>ab</sup> | 1.10 <sup>ab</sup> | 1.15 <sup>b</sup>  | 1.10 <sup>b</sup> | 0.098 | $P < 0.05$  | $P > 0.05$ |
| Ileum (%)            | 1.12 <sup>a</sup> | 0.99 <sup>a</sup>  | 0.92 <sup>a</sup>  | 0.81 <sup>b</sup>  | 0.82 <sup>b</sup> | 0.062 | $P < 0.001$ | $P > 0.05$ |
| Caecum (%)           | 0.69 <sup>a</sup> | 0.60 <sup>a</sup>  | 0.58 <sup>a</sup>  | 0.55 <sup>ab</sup> | 0.53 <sup>b</sup> | 0.034 | $P < 0.001$ | $P > 0.05$ |
| Colon (%)            | 0.17              | 0.19               | 0.17               | 0.10               | 0.10              | 0.042 | $P > 0.05$  | $P > 0.05$ |
| Duodenum length (cm) | 25.03             | 24.01              | 24.11              | 24.21              | 25.84             | 1.00  | $P > 0.05$  | $P > 0.05$ |
| Jejunum length (cm)  | 52.38             | 50.11              | 48.12              | 48.60              | 48.22             | 2.31  | $P > 0.05$  | $P > 0.05$ |
| Ileum length (cm)    | 53.49             | 51.80              | 48.96              | 49.48              | 47.48             | 2.41  | $P > 0.05$  | $P > 0.05$ |
| Caecum length (cm)   | 16.54             | 18.88              | 22.50              | 14.61              | 14.89             | 2.47  | $P > 0.05$  | $P > 0.05$ |
| Colon length (cm)    | 3.97              | 4.22               | 5.72               | 4.15               | 3.82              | 0.623 | $P > 0.05$  | $P > 0.05$ |

Means in the same row with different superscripts (<sup>abc</sup>) are significantly different. MSC = marula seed cake, SEM = standard error of the mean.

The effects of dietary MSC inclusion on clinical haemo-biochemistry parameters of Boschveld chickens is shown in Table 3.5. Whilst there were neither linear nor quadratic effects of diet on all haematological responses of birds ( $P > 0.05$ ). Dietary MSC increased serum glucose [linear:  $y = 0.042(\pm 0.061)x + 11.656(\pm 0.350)$ ;  $R^2 = 0.1683$ ;  $P < 0.05$ ], cholesterol [linear:  $y = 0.039(\pm 0.032)x + 2.430(\pm 0.182)$ ;  $R^2 = 0.3968$ ;  $P = 0.001$ ] and phosphate [quadratic:  $y = -0.002(\pm 0.0006)x^2 + 0.027(\pm 0.016)x + 2.642(\pm 0.095)$ ;  $R^2 = 0.1779$ ;  $P < 0.05$ ] concentrations but it linearly decreased its serum amylase activity [ $y = -2.518(\pm 4.949)x + 376.071(\pm 28.597)$ ;  $R^2 = 0.2807$ ;  $P < 0.01$ ] of the chickens.

**Table 3. 5:** Effect of dietary inclusion of MSC on clinical haemo-biochemistry of indigenous Boschveld chickens.

| Parameter                               | Dietary MSC (%) |       |       |       |       | SEM  | P-value         |                 |
|-----------------------------------------|-----------------|-------|-------|-------|-------|------|-----------------|-----------------|
|                                         | 0               | 10    | 15    | 20    | 25    |      | Linear          | Quadratic       |
| <i>Haematology</i>                      |                 |       |       |       |       |      |                 |                 |
| Red blood cells (×10 <sup>12</sup> /L)  | 1.28            | 1.16  | 1.15  | 1.17  | 1.17  | 0.10 | <i>P</i> > 0.05 | <i>P</i> > 0.05 |
| Haematocrit (L/L)                       | 8.59            | 8.27  | 9.05  | 7.76  | 8.15  | 0.38 | <i>P</i> > 0.05 | <i>P</i> > 0.05 |
| Haemoglobin (g/dL)                      | 8.60            | 9.73  | 9.64  | 9.92  | 10.16 | 0.70 | <i>P</i> > 0.05 | <i>P</i> > 0.05 |
| MCV (fL)                                | 56.35           | 71.56 | 67.13 | 66.29 | 67.45 | 5.64 | <i>P</i> > 0.05 | <i>P</i> > 0.05 |
| MCH (pg)                                | 64.60           | 81.90 | 72.80 | 81.84 | 78.58 | 4.79 | <i>P</i> > 0.05 | <i>P</i> > 0.05 |
| White blood cells (×10 <sup>9</sup> /L) | 27.60           | 19.89 | 11.84 | 9.86  | 9.34  | 5.13 | <i>P</i> > 0.05 | <i>P</i> > 0.05 |

|                                 |       |       |        |        |        |       |            |            |
|---------------------------------|-------|-------|--------|--------|--------|-------|------------|------------|
| Neutrophils ( $\times 10^9/L$ ) | 5.92  | 5.92  | 5.31   | 2.88   | 5.03   | 1.99  | $P > 0.05$ | $P > 0.05$ |
| Lymphocytes ( $\times 10^9/L$ ) | 7.32  | 7.61  | 2.73   | 2.47   | 1.80   | 0.72  | $P > 0.05$ | $P > 0.05$ |
| Monocytes ( $\times 10^9/L$ )   | 8.64  | 5.76  | 3.08   | 4.01   | 1.92   | 0.84  | $P > 0.05$ | $P > 0.05$ |
| Eosinophils ( $\times 10^9/L$ ) | 1.97  | 0.46  | 0.66   | 0.41   | 0.53   | 0.07  | $P > 0.05$ | $P > 0.05$ |
| Basophils ( $\times 10^9/L$ )   | 0.22  | 0.15  | 0.06   | 0.08   | 0.07   | 0.06  | $P > 0.05$ | $P > 0.05$ |
| Platelet (K/ $\mu L$ )          | 25.53 | 38.13 | 194.90 | 124.10 | 131.30 | 83.96 | $P > 0.05$ | $P > 0.05$ |

### **Clinical Biochemistry**

|                                 |                     |                     |                      |                      |                     |       |             |            |
|---------------------------------|---------------------|---------------------|----------------------|----------------------|---------------------|-------|-------------|------------|
| Glucose (mmol/L)                | 11.57 <sup>b</sup>  | 12.39 <sup>ab</sup> | 12.13 <sup>ab</sup>  | 12.15 <sup>ab</sup>  | 12.81 <sup>a</sup>  | 0.36  | $P < 0.05$  | $P > 0.05$ |
| SDMA ( $\mu g/dL$ )             | 21.90               | 22.50               | 18.80                | 24.10                | 19.50               | 1.89  | $P > 0.05$  | $P > 0.05$ |
| Urea (mmol/L)                   | 0.61                | 0.60                | 0.60                 | 0.60                 | 0.60                | 0.004 | $P > 0.05$  | $P > 0.05$ |
| Phosphate (mmol/L)              | 2.64 <sup>a</sup>   | 2.82 <sup>a</sup>   | 2.57 <sup>ab</sup>   | 2.70 <sup>a</sup>    | 2.34 <sup>b</sup>   | 0.02  | $P > 0.05$  | $P < 0.05$ |
| Calcium (mmol/L)                | 2.44                | 2.47                | 2.46                 | 2.50                 | 2.51                | 0.03  | $P > 0.05$  | $P > 0.05$ |
| Total protein (g/L)             | 52.30               | 57.10               | 51.40                | 58.40                | 53.20               | 2.50  | $P > 0.05$  | $P > 0.05$ |
| Albumin (g/L)                   | 17.80               | 19.30               | 17.70                | 20.80                | 19.00               | 0.77  | $P > 0.05$  | $P > 0.05$ |
| Globulin (g/L)                  | 34.80               | 37.50               | 33.80                | 37.60                | 34.30               | 1.73  | $P > 0.05$  | $P > 0.05$ |
| Albumin/globulin                | 0.52                | 0.51                | 0.55                 | 0.55                 | 0.57                | 0.02  | $P > 0.05$  | $P > 0.05$ |
| Alanine transaminase (U/L)      | 33.60               | 34.29               | 26.20                | 47.10                | 30.26               | 5.92  | $P > 0.05$  | $P > 0.05$ |
| Alkaline phosphatase (U/L)      | 237.20              | 183.10              | 156.30               | 219.30               | 169.40              | 42.95 | $P > 0.05$  | $P > 0.05$ |
| Total bilirubin ( $\mu mol/L$ ) | 24.00               | 32.00               | 22.70                | 38.40                | 26.00               | 5.97  | $P > 0.05$  | $P > 0.05$ |
| Cholesterol (mmol/L)            | 2.42 <sup>b</sup>   | 2.94 <sup>ab</sup>  | 2.77 <sup>b</sup>    | 3.32 <sup>a</sup>    | 3.31 <sup>a</sup>   | 0.18  | $P = 0.001$ | $P > 0.05$ |
| Amylase (U/L)                   | 374.34 <sup>a</sup> | 353.20 <sup>a</sup> | 307.70 <sup>ab</sup> | 298.85 <sup>ab</sup> | 264.60 <sup>b</sup> | 30.20 | $P < 0.01$  | $P > 0.05$ |
| Lipase (U/L)                    | 139.00              | 143.00              | 117.00               | 131.04               | 101.80              | 16.73 | $P > 0.05$  | $P > 0.05$ |

MCH = mean corpuscular haemoglobin, MCV = mean corpuscular volume, MSC = marula seed cake, SDMA = symmetric dimethylarginine, SEM = standard error of the mean.

The effect of dietary inclusion levels of MSC on breast meat quality of indigenous Boschveld chickens is shown in Table 3.6. Whilst the pH and temperature at both 45 min and 24 h post-slaughter, lightness at 24 h ( $L^*_{24\text{ h}}$ ) post-slaughter, WHC, drip loss, cooking loss and shear force were not influenced by treatment ( $P > 0.05$ ). Dietary MSC decreased meat lightness at 45 min ( $L^*_{45\text{ min}}$ ) [linear:  $y = -0.380(\pm 0.179)x + 46.459(\pm 1.034)$ ;  $R^2 = 0.1951$ ;  $P < 0.05$ ] as well as its yellowness ( $b^*$ ) at both 45 min [quadratic:  $y = -0.009(\pm 0.002)x^2 + 0.241(\pm 0.056)x + 20.074(\pm 0.323)$ ;  $R^2 = 0.3959$ ;  $P < 0.001$ ] and 24 h [quadratic:  $y = -0.008(\pm 0.002)x^2 + 0.220(\pm 0.044)x + 1.403(\pm 0.252)$ ;  $R^2 = 0.5149$ ;  $P < 0.001$ ] whilst it increased its redness ( $a^*$ )

both at 45 min [linear:  $y = 0.055(\pm 0.031)x + 0.386(\pm 0.176)$ ;  $R^2 = 0.3038$ ;  $P < 0.01$ ] and 24 h [ $y = 0.057(\pm 0.040)x + 0.949(\pm 0.232)$ ;  $R^2 = 0.1632$ ;  $P < 0.05$ ] post-slaughter.

**Table 3. 6:** Effect of dietary inclusion of MSC on breast meat quality of indigenous Boschveld chickens

| Parameter                          | Dietary MSC (%)    |                    |                     |                    |                    | SEM  | P-value    |             |
|------------------------------------|--------------------|--------------------|---------------------|--------------------|--------------------|------|------------|-------------|
|                                    | 0                  | 10                 | 15                  | 20                 | 25                 |      | Linear     | Quadratic   |
| pH <sub>45 min</sub>               | 5.87               | 5.91               | 5.75                | 5.88               | 5.81               | 0.05 | $P > 0.05$ | $P > 0.05$  |
| Temperature <sub>45 min</sub> (°C) | 26.32              | 27.16              | 26.36               | 26.10              | 26.21              | 0.43 | $P > 0.05$ | $P > 0.05$  |
| L* <sub>45 min</sub>               | 46.55 <sup>a</sup> | 42.85 <sup>b</sup> | 44.58 <sup>ab</sup> | 41.78 <sup>b</sup> | 43.54 <sup>b</sup> | 1.00 | $P < 0.05$ | $P > 0.05$  |
| a* <sub>45 min</sub>               | 0.36 <sup>b</sup>  | 0.98 <sup>a</sup>  | 0.74 <sup>ab</sup>  | 1.19 <sup>a</sup>  | 1.09 <sup>a</sup>  | 0.18 | $P < 0.01$ | $P > 0.05$  |
| b* <sub>45 min</sub>               | 0.19 <sup>c</sup>  | 1.01 <sup>bc</sup> | 2.35 <sup>a</sup>   | 1.42 <sup>b</sup>  | 0.55 <sup>c</sup>  | 0.28 | $P > 0.05$ | $P < 0.001$ |
| pH <sub>24 h</sub>                 | 6.08               | 5.99               | 6.13                | 6.49               | 6.35               | 0.16 | $P > 0.05$ | $P > 0.05$  |
| Temperature <sub>24 h</sub> (°C)   | 12.68              | 12.59              | 12.91               | 12.89              | 13.05              | 0.18 | $P > 0.05$ | $P > 0.05$  |
| L* <sub>24 h</sub>                 | 46.52              | 44.61              | 44.83               | 43.87              | 44.64              | 0.91 | $P > 0.05$ | $P > 0.05$  |
| a* <sub>24 h</sub>                 | 0.91 <sup>b</sup>  | 1.57 <sup>ab</sup> | 1.39 <sup>ab</sup>  | 1.51 <sup>ab</sup> | 1.64 <sup>a</sup>  | 0.24 | $P < 0.05$ | $P > 0.05$  |
| b* <sub>24 h</sub>                 | 1.47 <sup>c</sup>  | 2.39 <sup>b</sup>  | 3.29 <sup>a</sup>   | 2.34 <sup>b</sup>  | 1.66 <sup>c</sup>  | 0.22 | $P > 0.05$ | $P < 0.001$ |
| WHC (%)                            | 90.14              | 90.69              | 88.79               | 89.32              | 90.05              | 0.99 | $P > 0.05$ | $P > 0.05$  |
| Drip loss (%)                      | 16.51              | 16.37              | 19.87               | 17.47              | 13.87              | 1.67 | $P > 0.05$ | $P > 0.05$  |
| Cooking loss (%)                   | 24.16              | 25.09              | 27.14               | 25.01              | 26.45              | 1.57 | $P > 0.05$ | $P > 0.05$  |
| Shear force (N)                    | 8.14               | 7.51               | 6.79                | 9.85               | 8.32               | 0.85 | $P > 0.05$ | $P > 0.05$  |

Means in the same row with different superscripts (<sup>abc</sup>) are significantly different. a\* = redness, b\* = yellowness, C\* = chroma, L\* = lightness, MSC = marula seed cake, SEM = standard error of the mean.

Lastly, the bones results showed neither linear nor quadratic effects of treatment on all bone morphometric parameters, with the exception of the quadratic decrease in bone medial diaphysis [ $y = 0.0002(\pm 0.00009)x^2 - 0.006(\pm 0.002)x + 0.548(\pm 0.014)$ ;  $R^2 = 0.2166$ ;  $P < 0.05$ ] in response to dietary incremental levels of MSC (Table 3.7).

**Table 3. 7:** Effect of dietary inclusion of MSC on tibia morphometry of indigenous Boschveld chickens.

| Parameter              | Dietary MSC (%)   |                   |                    |                    |                    | SEM  | P-value    |            |
|------------------------|-------------------|-------------------|--------------------|--------------------|--------------------|------|------------|------------|
|                        | 0                 | 10                | 15                 | 20                 | 25                 |      | Linear     | Quadratic  |
| Tibia weight (g)       | 9.37              | 8.79              | 8.01               | 9.41               | 9.15               | 0.45 | $P > 0.05$ | $P > 0.05$ |
| Tibia length (cm)      | 11.28             | 11.39             | 11.17              | 10.96              | 11.34              | 0.20 | $P > 0.05$ | $P > 0.05$ |
| Diaphysis medial (mm)  | 0.55 <sup>a</sup> | 0.50 <sup>b</sup> | 0.52 <sup>ab</sup> | 0.51 <sup>ab</sup> | 0.54 <sup>ab</sup> | 0.01 | $P > 0.05$ | $P < 0.05$ |
| Diaphysis lateral (mm) | 0.67              | 0.64              | 0.65               | 0.63               | 0.66               | 0.02 | $P > 0.05$ | $P > 0.05$ |
| Proximal medial (mm)   | 1.02              | 1.17              | 1.29               | 1.32               | 1.32               | 0.16 | $P > 0.05$ | $P > 0.05$ |
| Proximal lateral (mm)  | 1.99              | 1.95              | 1.73               | 1.85               | 1.81               | 0.12 | $P > 0.05$ | $P > 0.05$ |
| Distal medial (mm)     | 0.89              | 0.88              | 0.94               | 0.98               | 0.96               | 0.06 | $P > 0.05$ | $P > 0.05$ |
| Distal lateral (mm)    | 1.20              | 1.21              | 1.10               | 1.18               | 1.16               | 0.07 | $P > 0.05$ | $P > 0.05$ |
| Seedor index (g/cm)    | 0.83              | 0.77              | 0.72               | 0.86               | 0.81               | 0.04 | $P > 0.05$ | $P > 0.05$ |
| Robusticity index      | 5.37              | 5.52              | 5.59               | 5.20               | 5.32               | 0.11 | $P > 0.05$ | $P > 0.05$ |

Means in the same row with different superscripts (<sup>ab</sup>) are significantly different. MSC = marula seed cake, SEM = standard error of the mean.

### 3.4. Discussion

This study investigated the potential MSC to replace SBM as a dietary protein source in indigenous Boschveld chicken diets by evaluating effects of dietary MSC on growth performance, carcass characteristics, viscera morphometry, clinical haemo-biochemistry, meat quality, and bone morphometry. Showing a decrease in FI, BWG and HCW in response to incremental dietary MSC inclusion levels, with an optimum at 15%, our data congruently mirrored previous observations in broiler chickens (Mthana et al., 2023) and pigs (Mabena et al., 2022, Thabethe et al., 2022) fed diets supplemented with high levels of MSC. The putative mechanism underlying these untoward effects of high dietary inclusion levels of MSC may be related to the presence of high fibre and residual oil (see Table 1) and ANFs such as condensed tannins (Mthana et al., 2023). Due to its relatively high fibre content, MSC inclusion in pig diets has been shown to increase their fibre content (Mabena et al., 2022). Notwithstanding its beneficial effects in terms of stimulating commensal and beneficial bacteria, enhancement of volatile fatty acids production, and immuno-enhancement (Singh and Kim, 2021) *inter alia*, high dietary fibre can induce antinutritional effects in broiler chickens by decreasing metabolizable energy and nutrient digestibility whilst increasing the viscosity of their diets, resulting to compromised bird growth performance (Choct and Annison, 1992) Otherwise, the residual oil in MSC has previously been shown to contain some lipid peroxidation (Mthiyane and Mhlanga, 2017), which might have led to unpleasant odour of experimental diets with

concomitantly decreased FI. Indeed, dietary consumption of high levels of peroxidised oil is reported to induced oxidative stress and decreased growth performance in the birds (Oliveira et al., 2010, Zhang et al., 2020).

It is however not known how the detrimental effects of high dietary MSC affect the FCE of the birds, and why BWG and FCE quadratically decreased with time in week 4 and week 2, respectively. The significant diet  $\times$  week interaction effect on FI implies that feed intake of birds depends on their age. This is possibly due to the ability of older bird's gut to digest solid feeds. Indeed, bird's taste buds and thus feed palatability regulation in broiler chicks develop with age post-hatch (Rajapaksha et al., 2016), which explains the significant diet  $\times$  week interaction effect on FI in the current study. Also, the anatomy and functionality of the gastrointestinal system matures and develops with advancing age of birds (Ravindran and Abdollahi, 2021).

The intriguingly linearly decreased weights of the spleen, jejunum, ileum, and caecum in response to dietary incremental levels of MSC would appear to be related to the high content of oleic acid in the MSC as well as the concomitantly decreased FI. A corroborative study reported decreased spleen weight in broiler chickens fed a diet supplemented with brown rice (Nanto-Hara et al., 2021) whose fatty acid has a preponderance of oleic acid (Munarko et al., 2021, Naveed et al., 2023). It is speculated that this may occur through the mechanism of oleic acid dose-dependently suppressing spleen lymphocyte proliferation and natural killer cell activity (Yaqoob et al., 1994, Jeffery et al., 1996, Jeffery et al., 1997). Otherwise, the decreased weights of the small intestinal segments and caecum may be due to supposedly low digestive activity of the gut consequent to decreased FI in response to dietary MSC. Indeed, studies have previously demonstrated decreased absolute weights of GIT viscera (Palo et al., 1995) as well as the length of the small intestine (Duarte et al., 2014) in feed-restricted birds. Otherwise, oleic acid in MSC-supplemented diets might have modified the gut microbiota thus contributing to decreased gut weight. Indeed, a high oleic acid diet was shown to alter gut microbiota diversity towards increased richness in *Prevotella* (López-García et al., 2021), the bacterial genus associated with decreased villus height-to-crypt depth ratio (Recharla et al., 2021) and therefore decreased gut weight.

Unlike previous studies with broiler chickens whereby increasing dietary inclusion of MSC decreased white blood cell (Manyeula et al., 2022, Mthana et al., 2023) and lymphocyte (Mthana et al., 2023) counts, this study found neither linear nor quadratic effects of the alternative protein source on all haematological responses of indigenous Boschveld chickens. This is probably an indication of better adaptability of indigenous chickens to utilising diets fraught with anti-nutritional compounds without ill effects on their health compared to genetically improved broiler chickens (Manyeula et al., 2020). Furthermore, haematological findings from the current study were within the normal range for healthy chickens (Bounous et al., 2000) suggesting MSC containing diets does not negatively impact the health status of indigenous Boschveld chickens. The observed linear increase in serum glucose levels in response to dietary MSC is unexpected. Studies involving dietary consumption of oleic acid-rich oils (85.24% of total fats), like the residual oil of MSC, have demonstrated decreased blood and plasma glucose levels in humans and rodents (Mokuda et al., 1993, Rasmussen et al., 1993). However, in agreement with our results, Ibrahim et al. (2019) observed increased fasting blood glucose levels in mice following oleic acid administration (Ibrahim et al., 2019). Corroborating our previous observation in broiler chickens fed MSC-supplemented diets Mthana et al. (2023), the linearly increased serum cholesterol levels in response to dietary MSC seem to be related to the presence of oleic acid in MSC.

The observed quadratic increase in serum phosphate content in response to dietary MSC is not unexpected considering the significantly high P content in the MSC. MSC from Southern Africa is reported to contain 9.27 to 11.0 g P/kg DM (Mthiyane and Mhlanga, 2017, Nkosi et al., 2019) which is 1.6 x higher than the P content of SBM (6.9 g/kg DM) (Ramachandran et al., 2007). The MSC from the Mozambican Marula provenance contains 15.2 g P/kg DM (Magaia, 2015). Despite the relatively high content of phytic acid in Marula kernels (3.4%) (Magaia, 2015) which would otherwise chelate the minerals (mainly phosphorus) and make it unavailable, it is apparent from the MSC-induced quadratic increase in serum phosphate levels of broiler chickens in the current study that the birds efficiently utilized the dietary phytic acid-bound P. Evidently, this may be due to the incorporated phytase enzyme in the form of AxtaPhy BF 1000 FTU (0.1 g/kg feed) in the experimental diets, though the birds fed the diet with 25% inclusion level of MSC indicated compromised P utilization.

Aside salivary glands, amylase is one of digestive enzymes secreted by the pancreas (de-Madaria et al., 2021) for the digestion of starch (Pieper-Bigelow et al., 1990). Its increased serum activity is indicative of acute pancreatitis (Regasa et al., 2022, Beiriger et al., 2023). Indeed, marula kernels from which MSC is derived are naturally endowed with a myriad of bioactive compounds including flavonoids, phenolic acid, tannins, proanthocyanidins and others (Mashau et al., 2022). Many of these compounds particularly the flavonoids and polyphenols are well-known as  $\alpha$ -amylase inhibitors in the GIT where they decrease starch hydrolysis to generate glucose which would be absorbed resulting possible hyperglycaemia (Proença et al., 2019, Sun et al., 2019, Zhao et al., 2021).

The lack of effect of dietary MSC on most meat quality attributes is of great interest as it suggests that the use of MSC as protein source in indigenous chicken diets would not affect the marketability of the meat and purchasing decision making processes at the level of the consumer. These findings concur with those of Mazizi et al. (2020) who also found no effect of dietary MSC on pH, colour, lightness, thawing and cooking losses, and tenderness of breast and thigh meat from Japanese quails (Mazizi et al., 2020). Notwithstanding, our study showed decreased meat lightness at 45 min and yellowness at both 45 min and 24 h in contrast to increased meat redness both at 45 min and 24 h post-slaughter in response to dietary MSC and these data suggest diet-induced heme-ion containing myoglobin protein deposition into muscle (meat). Since a higher muscle myoglobin content induces darker (redder) meat colour (Wideman et al., 2016) the high crude protein content, balanced amino acid composition and iron (Fe) content of MSC (Phenya, 2018) and its general lack of pigments (carotenoids), suggest that its incorporation into chicken diets may have mediated increased redness of the meat at the expense of its (meat) lightness and yellowness.

The demonstrated lack of effect of dietary MSC on most tibia morphometric traits in this study indicates benign effects of the alternative protein source on tibia quality of indigenous chickens which shows that it neither improved nor compromised tibiae quality of the Boschveld chickens. This observed lack of effect on tibia quality also shows that despite the reported presence of ANFs in MSC, that would negatively affect mineral supply (Mthana et al., 2023) that they (ANFs) did not interfere with bone mineralisation and growth. However, the observed quadratic decrease in bone medial diaphysis in response to dietary MSC suggests presence of

some bioactive compound that compromised bone shaft elongated by MSC. This suggests a need for further studies to elucidate the mechanisms underpinning negative effects of MSC on bone medial diaphysis.

### **3.5.Conclusion**

The data showed that dietary MSC decreased indigenous chicken FI and BWG, hot carcass, spleen, jejunum, ileum, and caecum masses and that optimal dietary MSC inclusion was shown to be 15%. However, MSC increased the serum glucose, cholesterol, and phosphate concentrations but amylase activity of the Boschveld chickens. Furthermore, dietary MSC decreased meat lightness at 45 min as well as its yellowness at both 45 min and 24 h as it increased its redness both at 45 min and 24 h post-slaughter and it also tibia medial diaphysis. Dietary substitution of SBM with MSC had no effects on FCE, carcass characteristics, viscera morphometry, and haematology of the native birds. Therefore, MSC can be used to substitute SBM up to 15% in indigenous Boschveld chicken diets without detrimental effects on the birds.

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## Chapter 4

### Caecal microbiota abundance and diversity in Boschveld indigenous chickens fed grower diets with graded substitution of soyabean meal with Marula seed cake

#### Abstract

In chickens, caeca harbour a diverse and complex microbiota that plays an important role in nutrient digestion and absorption, immune system development and pathogen exclusion. Research on MSC has largely evaluated its potential on the productive performance, health and meat quality of genetically broiler chicken strains but not in indigenous chickens. Importantly, its effect on caecal microbiota abundance and diversity in indigenous Boschveld chicken has not been evaluated hence. this study evaluated the effects of dietary MSC on the diversity and abundance of caeca microbial communities of indigenous Boschveld chickens fed diets supplemented with MSC at 0, 10, 15, 20, and 25% inclusion levels. After 90 days of feeding, the chickens were slaughtered, and caecal samples collected from 2 birds per replicate pen hence  $n = 10$  per treatment. The pooled samples per treatment were subjected to DNA extraction, sequencing and metagenomic analysis. The most predominant microbial were bacteria with, 99.63, 99.66, 99.99, 98.31 and 99.99% frequencies in chickens feed diets with 0%, 15%, 20% and 25% MSC inclusion, respectively. In chickens fed the control (0% MSC) diet, bacteroides (60.52%) and firmicutes (34.15%) were predominant bacterial phylla. The Firmicutes phylum were abundant in all treatment groups while Bacteroidetes phylum was abundant in birds fed diets supplemented with 10 and 20% but lower in chickens fed diets with 15 and 25% MSC. Chickens fed diets with MSC inclusion level above 10% were obese due to their high *Firmicutes/Bacteroidetes* ratios.

**Keywords:** Marula seed cake, Boschveld indigenous chickens, cecal microbiota, shotgun metagenomics sequencing.

#### 4.1. Introduction

In most developing countries where hunger and malnutrition are common, small-scale scavenging poultry production dominated by indigenous chickens provides food to the human population (Mack et al., 2005). Scavenging chickens are found in almost all of the Low Income Food Deficit Countries (Sonaiya, 2007). However, their productivity is low due to lack of

adequate nutrient and are characterised by high mortality (Kingori et al., 2010). Indigenous chicken are usually fed on poor scavengeable feed resource base (SFRB) which is inadequate in protein, Ca, and P to sustain both growth and egg production (Raphulu et al., 2015). Improving the productivity of these chickens is of great importance to rural communities as they contribute significantly to livelihoods (Mapiye et al., 2008). Dietary supplementation of these chickens' feed resources base with quality feed ingredients such as SBM would promote their productivity (Khobondo et al., 2015, Malebana et al., 2018). Gut microbiota through dietary fibre supplementation have been shown to improve the productive performance of broiler chickens (Yadav & Jha, 2019). This is due to the production of short chain fatty acid as fermentation metabolites by caecal microbiota (Singh & Kim, 2021). Despite this demonstrated positive effects of gut microbiota in poultry productivity, no studies have evaluated the effect MSC-fortified grower diets on the caeca microbiota abundance and diversity and their ability to improve the productivity of indigenous Boschveld chickens (Sergeant et al., 2014).

The gastrointestinal tract (GIT) is the primary site for nutrient absorption and digestion (Liu et al., 2011). In particular the caecum plays a major role in vitamin and amino acid production (Du et al., 2020) due to its dense population of microbial community. The caeca microbial community impact nutrition and nutrient utilization, health, physiology and productivity of chickens (Choi et al., 2015, Siegerstetter et al., 2017). These microorganism metabolize nutrients and modulate immune system development resulting in increased capacity to fight intestinal colonization by infectious pathogens (Shang et al., 2018, Ocejo et al., 2019). The GIT microbial community of chickens differs in terms of species with GIT segment (Diaz Carrasco et al., 2019, Ocejo et al., 2019). The microbial community have an important role in the metabolism of a wide variety of dietary substrates (Liu et al., 2011). Through their metabolism of nutrients, caecal microbiota improve the feed use efficiency by chickens (Yan et al., 2017) in addition to supporting and enhancing biochemical transformation in the chickens' GIT (Qu et al., 2008, Du et al., 2020). Most sugars even at low concentration are absorbed in the cecum (Yan et al., 2017). Therefore, this study assessed caecal microbial communities of Boschveld indigenous chickens fed diets containing graded level (0, 10, 15, 20, and 25%) of MSC.

## **4.2. Materials and methods**

The study site, diet formulation and chemical analysis, experimental design and slaughter procedure were as described in Chapter 3.

### **4.2.1. Evaluation of gut metagenomes**

#### **4.2.1.1. Sample collection**

With sanitized gloves on hands, caecal digesta samples were collected from 2 carcasses per pen (i.e., 10 per treatment), smashed and pooled per dietary treatment [0 (control), 10, 15, 20 and, 25% MSC] to obtain 5 samples (MSC0%, MSC10%, MSC15%, MSC20% and MSC25%) of 5g each. The collected caecal digesta samples were then poured into 50 ml sterile falcon tubes, snap-frozen in liquid N and then stored in a freezer at -80 °C until analysis.

#### **4.2.1.2. Metagenomic DNA extraction, library preparation, and sequencing**

The caecal samples (denoted as: MSC0%, MSC10%, MSC15%, MSC20% and MSC25%) were thawed at room temperature for 30 min before the extraction of DNA. In summary, total metagenomic DNA was extracted from the collected samples using a QIAmp DNeasy PowerFecal DNA kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. NanoDrop spectrometer (Thermo Fisher Scientific) was used to check quality of extracted DNA. A DNA sample with a ratio of 1.8 to 2.0 (260/280) or 2.0 to 2.2 (260/230) was deemed pure and suitable for sequencing (Lucena-Aguilar et al., 2016).

#### **4.2.1.3. Metagenomic analysis**

Shotgun metagenomic sequencing was performed at Inqaba Biotec (Pretoria, South Africa). Following the manufacturer's instructions, DNA libraries (1 ng input) were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, Sa Diego CA, United States) and IDT Unique Dual indexes. Nextera XT fragmentation enzyme (illumine) was used to fragment genomic DNA. After adding unique dual indexes, samples went through 12 cycles of PCR to build libraries. Then, the DNA libraries were purified with AMPure magnetic beads (Beckman Coulter, Brea CA, United States) followed by elution in Qiagen EB buffer. A Qubit 4 fluorometer and the Qubit<sup>TM</sup> dsDNA HS Assay kit were used to quantify the final DNA

libraries. Libraries were then sequenced on an Illumina HiSeq 4,000 platform at 2150 bp with paired reads (Montso et al., 2022).

#### **4.2.1.4. Bioinformatics analysis**

The raw reads were uploaded into the Kbase platform version 2.1 (<https://kbase.us/>). The sequence quality of the raw reads was checked using FastQC version 0.11.5 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Trimmomatic 0.36 was used to filter and trim the adaptors and ambiguous nucleotide sequences (Bolger et al., 2014). *De novo* assembly of the trimmed (good quality) reads was performed using MEGAHIT version 1.2.9. The prokaryotic genome annotation pipeline (PGAP) was used to annotate the drafts of the assembly genome on the Kbase platform (<https://kbase.us/>). The taxonomic classification of the shotgun metagenomic reads data was performed using Kaiji version 1.9.0 and GOTTCHA2 version 2.1.7 for comparisons.

#### **4.2.2. Statistical analysis**

After metagenomic analyses of the raw data using KBase online portal, the analysed data were exported to Microsoft Excel to compute the abundance and distribution of the microbiota at the phyla and genera levels. The abundance of microbial communities (phylum and genus level) among the treatment groups was determined using the linear discriminant analysis (LDA) effect size (LEfSe) on galaxy platform (version1.0), with a threshold score set at 2.03.

### **4.3. Results**

#### **4.3.1. Domain**

In this study, genomic contents of 198457, 55886, 15225, 100091 and 23296 were found in the caecal contents of chickens fed different dietary levels of MSC (0, 10, 15, 20 and 25%, respectively). The taxonomic classification of the sequenced samples revealed that two main domains; the bacteria and viruses, were dominant in the microbial community of all sequenced samples (Fig. 4.1). The most predominant domain in the microbial community of MSC0%, MSC10%, MSC15%, MSC20% and MSC25% samples were the bacteria with 99.63, 99.66, 99.99, 98.31 and 99.99%, respectively. The results reveal that the bacterial domain of treatments increased as incorporation level of MSC increased from 0% to 15% (MSC0%)

(99.66%), MSC10% (99.99%) and MSC15% (99.99%). However, MSC20% had a low bacterial domain (98.31%) when compared to the control.

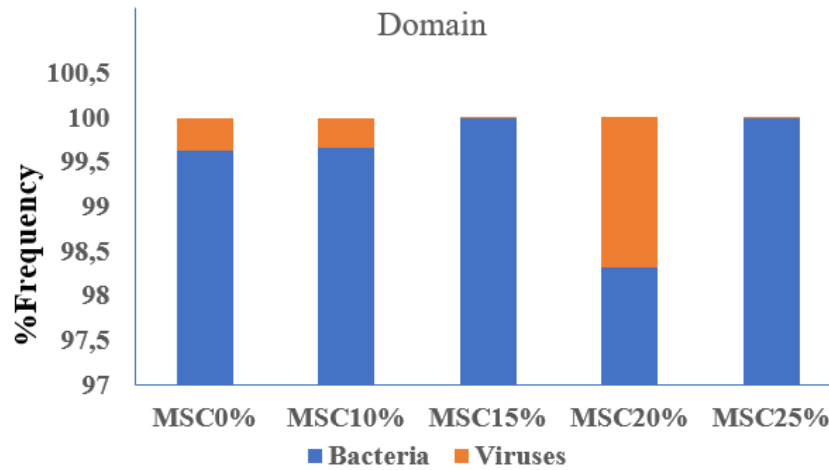


**Figure 4. 1:** Stacked bar chart showing the major domain of the microbial communities in the caecum digesta sample.

#### 4.3.2. Phylum

The frequency of phyla for samples MSC0%, MSC10%, MSC15%, MSC20%, and MSC25% were 6, 7, 6, 6, and 7 respectively. As shown in Figure 4. 2, Firmicutes, Bacteroidetes, Proteobacteria, and Verrucomicrobia were present. In the microbial community of chickens fed the Control diet (MSC0%), Bacteroidetes (60.52%), Firmicutes (34.15%), and Proteobacteria (4.16) were the predominant bacterial phyla whilst the Adenoviridae (0.35%) were the predominant virus phylum (figure 4.2). The Bacteroidetes phyla in (MSC15%) and (MSC25%) were lower (15.95% and 16.27%) compared to the Control diet (60.52%). The Firmicutes phyla increased as the dietary incorporation of MSC increased with the highest percentage (37.04%) observed in sample (MSC25%). All caecal contents of chickens fed MSC-supplemented diets (MSC10%), (MSC15%), (MSC20%) and (MSC25%) had lower bacterial phyla (Proteobacteria) 2.05, 1.56, 1.60, and 1.49% respectively compared to those from chickens fed the Control diet (4.16%). Also, the Verrucomicrobia were predominant in caecal content of chickens fed 15 and 25% MSC (45.77 and 45.01%) respectively.

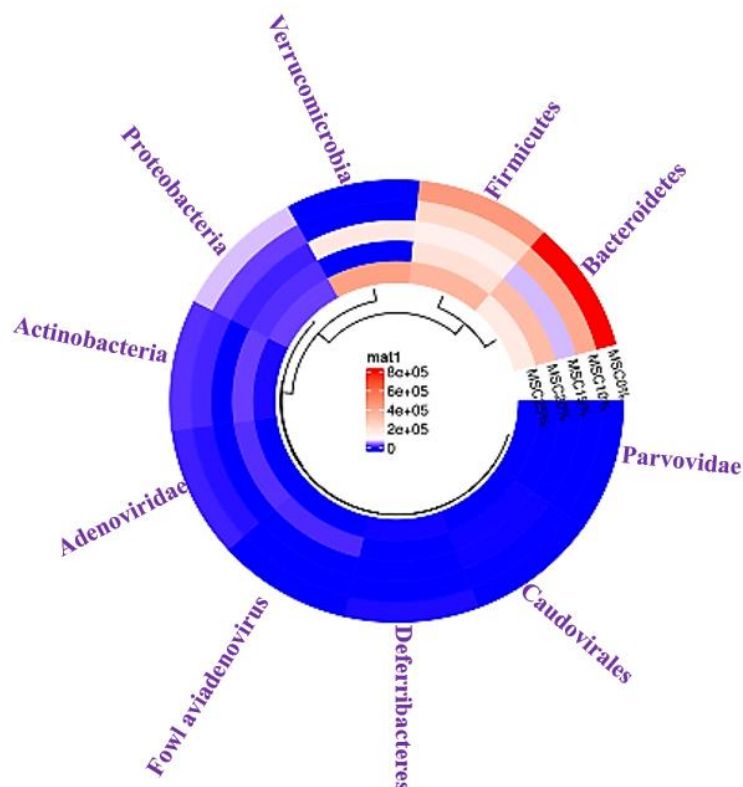


**Figure 4. 2:** Circular heatmap showing the major phyla of the microbial communities in the caecum digesta sample.

#### 4.3.3. Class

Bacteroidia and Clostridia were the most predominant microbial bacterial classes in all sequenced samples MSC0%, MSC10%, MSC15%, MSC20%, and MSC25%, with Verrucomicrobiae found only in caecal samples from birds fed the diets with 15% MSC (MSC15%) and 25% MSC (MSC25%). In caecal contents of chickens fed the control diet (MSC0%), a total of 11 microbial classes were observed, with the Bacteroidia (60.52%), Clostridia (20.05%), and Negativicutes (7.83%) being the mostly predominant bacterial classes, whereas the Adenoviridae (0.35%) were the predominant virus class. In caecal contents of chickens fed diets with 10 % MSC, the Bacteroidia (60.58%), Clostridia (17.53%), and Bacilli (14.01%) were the most predominant bacterial class. However, dietary inclusion of 15% MSC suddenly made the Verrucomicrobiae to be the most predominant bacterial class (45.77%). Then, in the caecal contents from birds fed the diet with 20% MSC the Bacteroidia (59.41%), Bacilli (16.91%) and Clostridia (13.62%) were the predominant bacterial class. Lastly, the caecal contents of birds fed the diet with 25% MSC had 4 predominant bacteria

classes, the Verrucomicrobiaea (45.01%), Bacilli (24.54%), Bacteroidia (16.27%), and Clostridia (11.77%). The percentage frequency of the class in the microbial community of MSC0%, MSC10%, MSC15%, MSC20%, and MSC25% samples are shown in Figure 4.3.

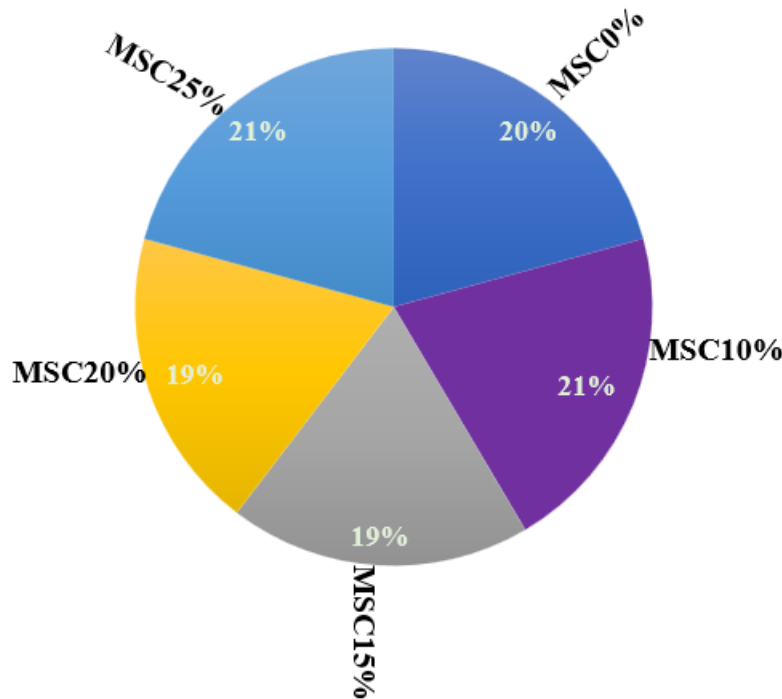


**Figure 4. 3:** Pie chart showing the percentage frequency of the class among the microbial communities in the caecum digesta sample.

#### 4.3.4. Order

A total of 56 microbial orders were detected in caecal contents of chickens fed diets supplemented with graded levels of MSC. The frequency of order of the microbial community of the MSC0%, MSC10%, MSC15%, MSC20%, and MSC25% samples were 12, 12, 10, 11 and 11, respectively. As shown in Figure 4.4, Eubacteriales and Bacteroidales were the most predominant bacterial orders in all sequenced samples. In caecal contents from birds fed the Control diet (MSC0%), Bacteroidales (60.52%), Clostridiales (20.05%), and Senomonadales (7.83%) were the predominant bacterial orders. On the other hand, the Bacteroidales were the predominant bacterial order in the caeca of birds fed the diets with 10% MSC (MSC10%; 62.18%) and 20% MSC (MSC20%; 59.41%). In the caeca of birds from both dietary treatments with 15% MSC (MSC15%) and 25% MSC (MSC25%), Verrucomicrobiales were the predominant bacterial order (45.01 and 45.77%, respectively) whilst the Adenoviridae were the viral order predominant in the caeca of birds fed diets 0% MSC (MSC0%), 10% MSC

(MSC10%) and 20% MSC (MSC20%) and the Caudovirales were present in caecal contents from birds in diets with 0, 10, 15, 20, and 25% MSC.

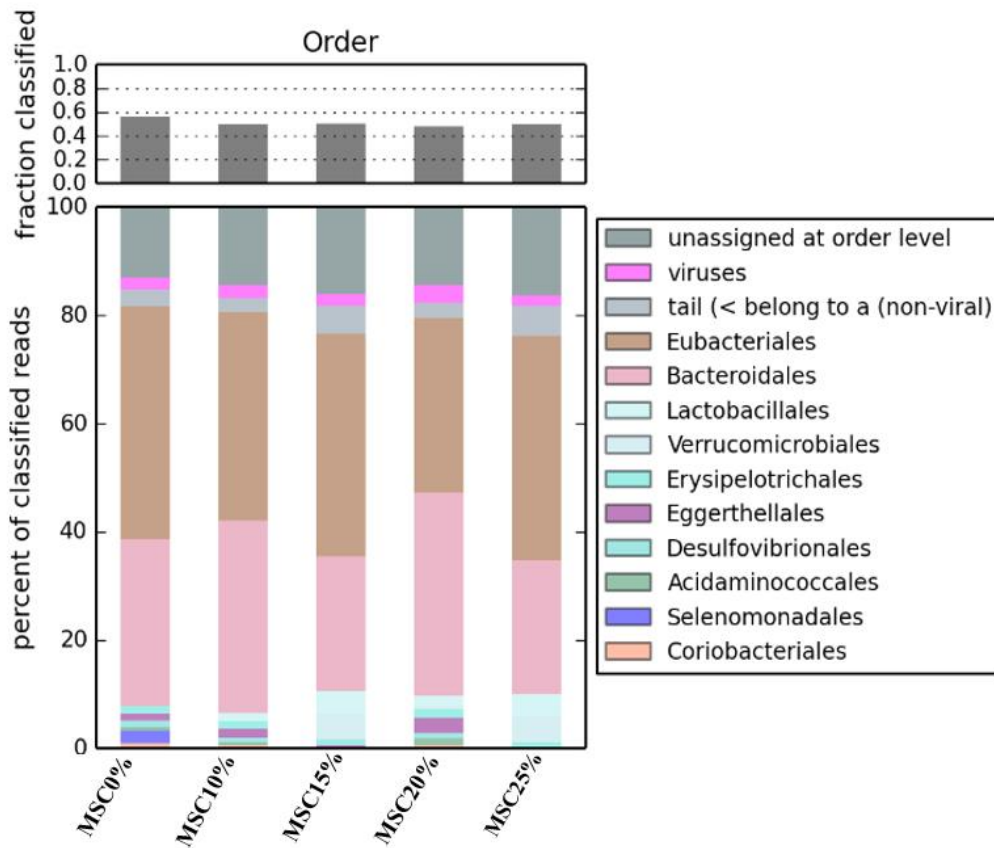


**Figure 4. 4:** Stacked bar chart showing the major order of the microbial communities in the caecum digesta sample.

#### 4.3.5. Family

A total of 117 families were observed in the genomes of caecal microbiota from chickens fed diets with 0, 10, 15, 20, and 25% of MSC. As shown in Figure 4.5, Bacteroidaceae and Lachnospiraceae bacterial families were present in sequenced caecal samples from all dietary treatments. The most predominant bacterial families in the caeca of birds fed the diet with 0% MSC were the Bacteroidaceae (57.79%), Lachnospiraceae (11.04%), and Selenomonadaceae (7.83%). Also, the leading bacterial family in the caeca of birds fed diet incorporated with 10% MSC was the Bacteroidaceae (55.96%) while that of birds fed 15% MSC, Akkermansiaceae (45.77%) were predominant. In diet containing 20% MSC, the Bacteroidaceae (44.29%) and Lachnospiraceae (31.06%) were the leading bacterial families, whilst the Akkermansiaceae

(45.77%) and Lactobacillaceae (24.11%) were predominated in caecal content of chickens fed diet incorporated with 25% MSC.

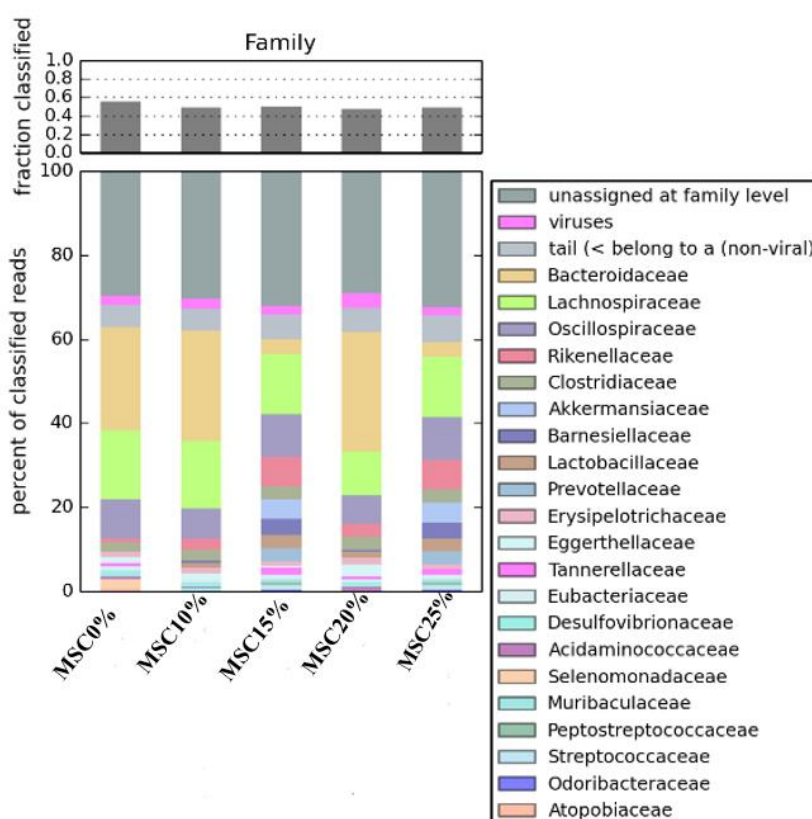


**Figure 4. 5:** Stacked bar chart showing the major family of the microbial communities in the caecum digesta sample.

#### 4.3.6. Genus

The frequency of genera in the microbial community of chickens fed 0, 10, 15, 20, and 25% of MSC were 54, 47, 35, 49, and 40 respectively. The genus *Akkermansia*, *Lactobacillus*, *Mordavella*, *Bacteroides*, *Barnesiella*, *Helicobacter*, *Lachnoclostridium*, *Massiliomicrobiota*, *Mediterranea*, *Culturomica* and *Odoribacter* were present in all sequenced samples. As shown in Table 4.1, *Bacteroides* were predominant in the caecal content of birds fed diets with 0% MSC (MSC0%; 65.27%), 10% MSC (MSC10%; 52.80%), and 20% MSC (MSC20%; 47.19%) while the *Akkermansia* predominated in birds fed diets with 15% MSC (MSC15%; 29.29%) and 25% MSC (MSC25%; 45.09%).

**Table 4. 1:** Major genera of the microbial communities in the caecum digesta sample.

| <b>Genus</b>           | <b>MSC0%</b> | <b>MSC10%</b> | <b>MSC15%</b> | <b>MSC20%</b> | <b>MSC25%</b> |
|------------------------|--------------|---------------|---------------|---------------|---------------|
| Akkermansia            | 0            | 0             | 149983        | 0             | 410178        |
| Lactobacillus          | 30 248       | 85 086        | 791 20        | 77 549        | 219 761       |
| Millionella            | 525          | 0             | 14443         | 1207          | 40588         |
| Alistipes              | 3066         | 0             | 9838          | 5635          | 29526         |
| Merdimonas             | 0            | 0             | 10289         | 0             | 28745         |
| Mordavella             | 45162        | 18989         | 8124          | 36346         | 22131         |
| Bacteroides            | 721 149      | 325 222       | 14 575        | 241 268       | 40 030        |
| Barnesiella            | 6 518        | 11 702        | 8 863         | 15 701        | 25 669        |
| Streptococcus          | 42           | 18            | 143           | 38            | 656           |
| Helicobacter           | 3104         | 3406          | 2452          | 525           | 6576          |
| Lachnoclostridium      | 70 293       | 16 860        | 4 333         | 6 946         | 11 588        |
| Romboutsia             | 3123         | 0             | 1455          | 806           | 4267          |
| Massiliomicrobiota     | 39 804       | 24 428        | 1 270         | 20 240        | 3 482         |
| Anaerotignum           | 8072         | 3838          | 1705          | 1855          | 4774          |
| Mediterranea           | 23954        | 19465         | 1931          | 30151         | 5357          |
| Fournierella           | 6922         | 3298          | 1918          | 2072          | 5264          |
| Pseudoflavonifractor   | 6109         | 2632          | 1855          | 2009          | 5501          |
| Bilophila              | 19131        | 0             | 2243          | 1414          | 5695          |
| Angelakisella          | 8293         | 3261          | 1890          | 2815          | 5474          |
| Enterococcus           | 1 321        | 1 394         | 815           | 8 714         | 3 230         |
| V5virus                | 0            | 0             | 18            | 0             | 69            |
| Subdoligranulum        | 9 808        | 2 530         | 1 121         | 1 630         | 3 015         |
| Erysipelatoclostridium | 3518         | 425           | 540           | 0             | 1588          |
| Culturomica            | 12 634       | 6 550         | 1 369         | 7 337         | 3 936         |
| Agathobaculum          | 969          | 0             | 912           | 0             | 2569          |
| Oscillibacter          | 2304         | 972           | 1018          | 576           | 2690          |
| Odoribacter            | 8712         | 4887          | 1249          | 7417          | 3161          |
| Gordonibacter          | 2051         | 1521          | 441           | 10439         | 1162          |
| [Clostridium] leptum   | 3178         | 896           | 831           | 1096          | 1983          |
| Provencibacterium      | 4788         | 947           | 681           | 733           | 1729          |
| Merdibacter            | 3853         | 1614          | 558           | 816           | 1620          |

|                     |       |       |        |      |      |
|---------------------|-------|-------|--------|------|------|
| Clostridiales       | 2161  | 1017  | 651    | 1055 | 1828 |
| bacterium           |       |       |        |      |      |
| Anaerotruncus       | 0     | 0     | 625    | 0    | 1871 |
| Desulfovibrio       | 13135 | 7082  | 410    | 6205 | 1254 |
| Escherichia         | 0     | 0     | 4      | 297  | 23   |
| Aviadenovirus       | 0     | 1910  | 4551   | 0    | 0    |
| Megamonas           | 0     | 1253  | 100915 | 0    | 0    |
| Campylobacter       | 0     | 2187  | 18237  | 0    | 0    |
| Merdimonas          | 0     | 48683 | 60703  | 9400 | 0    |
| Rtpvirus            | 110   | 77    | 0      | 9    | 0    |
| Faecalibacterium    | 10003 | 784   | 0      | 0    | 0    |
| Olsenella           | 3098  | 1392  | 0      | 1054 | 0    |
| Sellimonas          | 3295  | 0     | 0      | 838  | 0    |
| Clostridium         | 2844  | 1012  | 0      | 671  | 0    |
| Eggerthella         | 2900  | 1530  | 0      | 1439 | 0    |
| Butyricicoccus      | 2923  | 971   | 0      | 768  | 1619 |
| Parabacteroides     | 3692  | 4804  | 0      | 0    | 0    |
| T7virus             | 4     | 0     | 15     | 0    | 0    |
| Negativibacillus    | 1949  | 745   | 0      | 0    | 0    |
| Mucispirillum       | 1241  | 0     | 0      | 0    | 496  |
| Traorella           | 1194  | 0     | 0      | 690  | 0    |
| Enorma              | 1073  | 1152  | 0      | 0    | 0    |
| Flavonifractor      | 2071  | 770   | 0      | 685  | 0    |
| Faecalitalea        | 721   | 0     | 0      | 0    | 0    |
| Ruthenibacterium    | 1700  | 0     | 0      | 0    | 0    |
| Bittarella          | 845   | 0     | 0      | 482  | 0    |
| Erysipelotrichaceae | 245   | 0     | 0      | 271  | 0    |
| bacterium           |       |       |        |      |      |
| Clostridioides      | 458   | 0     | 0      | 0    | 511  |
| Massilimaliae       | 533   | 0     | 0      | 0    | 0    |
| Dependoparvovirus   | 0     | 8     | 0      | 0    | 0    |
| K1gvirus            | 0     | 10    | 0      | 11   | 0    |
| Jerseyvirus         | 0     | 7     | 0      | 0    | 0    |

|                        |   |     |   |      |   |
|------------------------|---|-----|---|------|---|
| G7cvirus               | 0 | 4   | 0 | 3    | 0 |
| Traorella              | 0 | 588 | 0 | 0    | 0 |
| Rogue1virus            | 0 | 7   | 0 | 0    | 0 |
| Escherichia phage      | 0 | 20  | 0 | 268  | 0 |
| Sp18virus              | 0 | 3   | 0 | 0    | 0 |
| Escherichia phage      | 0 | 17  | 0 | 0    | 0 |
| Enterobacteria phage   | 0 | 0   | 0 | 25   | 0 |
| Enterobacteria phage   | 0 | 0   | 0 | 9    | 0 |
| Escherichia phage      | 0 | 0   | 0 | 94   | 0 |
| Erysipelatoclostridium | 0 | 0   | 0 | 1698 | 0 |

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#### 4.3.7. Species

A total of 335 microbial species were observed in caecal contents of indigenous Boschveld chickens fed MSC-supplemented diets. The frequency of species in microbial community of the chickens fed different level of MSC (0, 10, 15, 20, and 25%) were 77, 72, 49, 72, and 65 respectively. As shown in Figure 4.4.7.1, the *Bacteroides plebeius* (15.57%), *Bacteroides salanitronis* (14.42%) and *Bacteroides coprophilus* (13.75%) were the most predominant bacterial species in birds fed the Control diet with 0% MSC (MSC0%) whereas the *Bacteroides barnesiae* predominated in birds fed diet with 10% MSC (MSC10%; 34.63%) and 15% MSC (MSC15%; 30.13%). On the other hand, the *Bacteroides plebeius* predominated in birds fed diets with 0% MSC (MSC0%; 15.57%) and 20% MSC (MSC20%; 21.15%) whilst the *Akkermansia muciniphila* bacterial species were predominant in caecal content of chickens fed 15 and 25% MSC (45.12 and 31.96%) respectively.

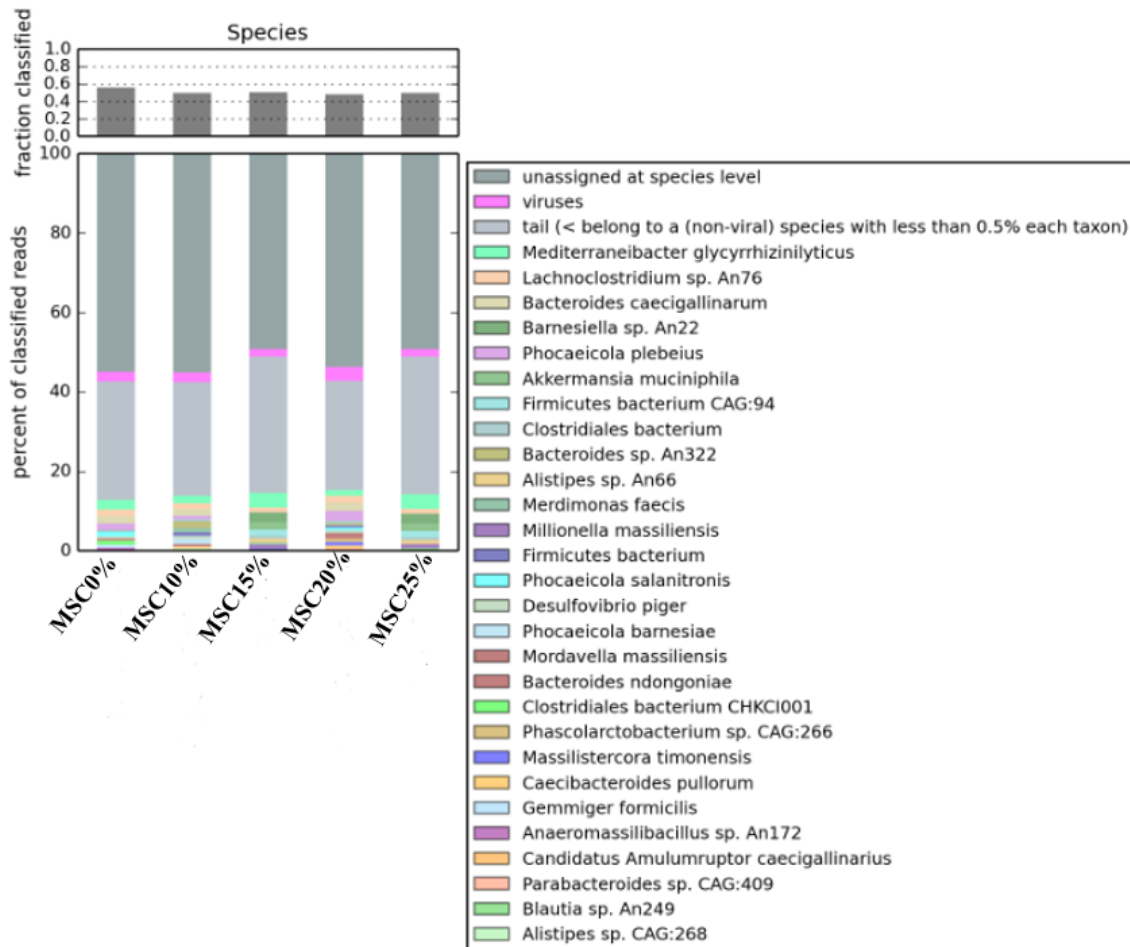


**Figure 4. 6:** Stacked bar chart showing the major species of the microbial communities in the caecum digesta sample.

#### 4.4. Discussion

The gut microbiota significantly enhances the host's intestinal health, enhancing nutrient digestibility, growth performance and overall bird health (Xiao et al., 2017, Shang et al., 2018). Understanding and predicting changes in normal chicken intestinal microbial communities is crucial for predicting changes related to physiological state of the animal. However, no study has been conducted on the caecal microbiota of Boschveld chickens fed MSC-supplemented diets as a protein alternative to SBM. In this study, the bacteria domains, and phyla *Bacteroides*, *Firmicutes* and *Proteobacteria* dominated the caeca birds fed diets with control diet. These findings align with prior research that examined the microbial communities of chickens based on their sex and breed (Lee et al., 2017), and the study of Montso et al. (2022) in Ross 308 broiler chickens fed marama bean meal. The bacteria phyla such as *Bacteroidetes*, *Firmicutes* and *Proteobacteria* are responsible for protecting the host against harmful microbes (Thiam et

al., 2022). This study found that the *Firmicutes* phyla were abundant in all treatment groups, while *Bacteroidetes* phyla abundant in birds fed diets incorporated with 10 and 20% MSC, and lower in birds fed diets incorporated with 15 and 25% of MSC. *Firmicutes* and *Bacteroidetes* have impact on chicken performance traits i.e. body weight gain, feed conversion ratio and residual feed intake (Diaz Carrasco et al., 2019). These bacterial phyla are associated with high feed conversion ratio thus positively impact body weight gain (Bae et al., 2017). The efficiency of energy harvesting in various animals is linked to the abundance ratio of *Firmicutes* to *Bacteroidetes* in the gut microbiota (Zhu et al., 2019). Based on these findings, chickens fed diets with MSC inclusion levels above 10% would be expected to be obese as they had high *Firmicutes/Bacteroidetes* ratios, thus more energy would be absorbed as abdominal fat (Xiang et al., 2021). *Bacteroides* are linked to the breakdown of polysaccharides, particularly starch and glucans and the formation of short-chain fatty acids (Pandit et al., 2018). In addition, *Bacteroides* are associated with high body weight gain and residual feed intake (Yan et al., 2017, Johnson et al., 2018), and are also responsible for preventing enteric diseases in chickens (Montso et al., 2022). This therefore explains the increase in FI and BWG of birds when fed diets containing MSC at <15 as shown in chapter 3.

MSC is rich in dietary fiber and fat (Mthiyane and Mhlanga, 2017) thus it may alter gut microbiota to favour growth of bacteria associated with fiber degradation (Pandit et al., 2018) and fat-loving such as *Verrucomicrobia* and *Bacteroidaceae* (Haberecht et al., 2020). *Akkermansia*, a gram-negative bacterium, that promotes immunomodulatory and anti-inflammatory responses to protect animals against obesity, inflammatory bowel disease, and type 2 diabetes (Yan et al., 2017, Rashid et al., 2021) was dominant in caecal samples of birds fed diet containing 15% MSC. In chickens *Akkermansia* is associated with low body weight gain (Han et al., 2016, Diaz Carrasco et al., 2019). This suggest that *Akkermansia muciniphila* species colonized gut of chicken fed diet incorporated with higher MSC content (15- 25%) to aid in reducing body fat deposition in chicken (Wei et al., 2022).

#### **4.5. Conclusion**

The results showed that MSC can be incorporated into indigenous Boschveld chicken diets up to 10% inclusion level without causing negative effect gut microbiota.

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## Chapter 5: General discussion, conclusion, and recommendations

### 5.1. Discussion

This study investigated the potential of MSC to replace SBM as a dietary protein in the grower diets of indigenous Boschveld chickens. Due to high crude protein and favourable essential amino acid SBM is a widely used conventional protein source ingredient in poultry production (Dozier et al., 2011, Muya et al., 2020). Previous studies that evaluated MSC as an alternative to SBM in the diets genetically improved chickens showed that it could be used as feed ingredient as its use resulted in comparable nutrient utilization and growth performance, health status, and meat quality (Sola-Ojo et al., 2013, Mnisi and Mlambo, 2018). However, due to lack of studies on MSC's impact in indigenous Boschveld chickens, this study sought to address that gap in information.

The results in chapter three demonstrated a decrease in FI, BWG and HCW in response to incremental dietary MSC inclusion levels, with an optimum at 15%. These findings were in line with those reported in previous observations in broiler chickens (Mthana et al., 2023) and pigs (Mabena et al., 2022, Thabethe et al., 2022) fed diets supplemented with varying levels of MSC. These were probably due to high fibre and residual oil which might have induced the anti-nutritional effects on the bird. It is however not known how the detrimental effects of high dietary MSC affect the FCE of the birds. The linearly decreased weights of the spleen, jejunum, ileum, and caecum in response to dietary incremental levels of MSC would appear to be related to the concomitantly decreased FI. Otherwise, the decreased weights of the small intestinal segments and caecum may be due to supposedly low digestive activity of the gut consequent to decreased FI in response to dietary MSC.

This study found no effects of the alternative protein source on all haematological responses of indigenous Boschveld chickens unlike previous studies with broiler chickens whereby increasing dietary inclusion of MSC decreased white blood cell (Manyeula et al., 2022, Mthana et al., 2023) and lymphocyte (Mthana et al., 2023) counts. This is probably an indication of better adaptability of indigenous Boschveld chickens to utilising diets fraught with ANFs

without detrimental effects on their health compared to genetically improved broiler chickens. Validating our previous observation in broiler chickens fed MSC-supplemented diets (Mthana et al. (2023), the linearly increased serum cholesterol levels in response to dietary MSC seem to be related to the presence of oleic acid in MSC. The observed increase in serum phosphate content in response to dietary MSC was not unexpected considering the significantly high P content in the MSC 9.27 to 11.0 g P/kg DM (Mthiyane and Mhlanga, 2017, Nkosi et al., 2019) which is higher than the P content of SBM (6.9 g/kg DM) (Ramachandran et al., 2007). Most meat quality attributes were not affected by dietary MSC. This is of great interest as it suggests that the use of MSC as protein source in indigenous Boschveld chicken diets would not affect the marketability and purchasing decision of the consumer on meat. These findings coincide with those of Mazizi et al. (2020) who also found no effect of dietary MSC on pH, colour, lightness, thawing and cooking losses, and tenderness of breast and thigh meat from Japanese quails (Mazizi et al., 2020). The demonstrated lack of effect of dietary MSC on most tibia morphometric traits in this study indicates benign effects of the alternative protein source on tibia quality of indigenous chickens which shows that it neither improved nor compromised tibiae quality of the Boschveld chickens. However, the observed decrease in bone medial diaphysis in response to dietary MSC suggests presence of some bioactive compound that compromised bone shaft elongated by MSC.

In chapter four, the effect of dietary MSC on microbial metagenomes of caecal digesta samples was explored. In this experiment, the bacteria domains, and phyla *Bacteroides*, *Firmicutes* and *Proteobacteria* dominated the caeca of birds fed diets with control diet. These findings were in line with previous research that examined the microbial communities of chickens fed maramba bean meal (Montso et al., 2022). This study showed that the *Firmicutes* phyla were abundant in all treatment groups, while *Bacteroidetes* phyla abundant in birds fed diets incorporated with 10 and 20% MSC, and lower in birds fed diets incorporated with 15 and 25% of MSC. These bacteria phyla are responsible for protecting the host against harmful microbes (Thiam et al., 2022). Furthermore, *Firmicutes* and *Bacteroidetes* have impact on chicken performance traits i.e. body weight gain, feed conversion ratio and residual feed intake (Diaz Carrasco et al., 2019). In addition, *Bacteroides* are associated with high body weight gain and residual feed intake (Yan et al., 2017, Johnson et al., 2018), and are also responsible for preventing enteric diseases in chickens (Montso et al., 2022).

## **5.2. General Conclusion and Recommendations**

Based on the obtained data, it was concluded that MSC can be successfully included in the diets of indigenous Boschveld chickens up to 15% without negatively affecting performance, carcass characteristics and viscera morphometry, physiology, meat and tibiae quality. However, further research is necessary to investigate valorisation strategies to further increase MSC inclusion in poultry diets to fully replace SBM. Also, there is a need for further research to elucidate the mechanisms underlying the depressive effects of MSC on spleen weight particularly as it suggests possible compromising of the immunity of birds. Additionally, there is a need for elucidation of mechanisms that mediate the observed MSC-induced linear increase of serum cholesterol levels and the cholesterol types. The inclusion of MSC above 10% of the diet has been shown to increase the preponderance of *Firmicutes* over *Bacteroidetes* in chickens' caeca, which is most likely to result in increased susceptibility to obesity.

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