# DIETARY EFFECTS OF MARULA (*SCLEROCARYA BIRREA CAFFRA)* NUT MEAL ON THE GROWTH, HEALTH AND MEAT QUALITY OF BROILER GUINEA FOWL (*NUMIDA MELEAGRIS)*

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine

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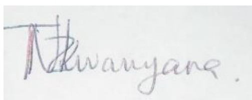
**Declaration**

I, **Thandanani Zola Nkwanyana**, declare that this Dissertation is my own unaided work. It is submitted for the degree of Master of Science in Medicine (Physiology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university. Where other sources have been used, they have been appropriately acknowledged.



**(Thandanani Zola Nkwanyana)**

24th day of September 2020 at University of Witwatersrand

## Dedication

*"Our deepest fear is not that we are inadequate. Our deepest fear is that we are powerful beyond measure. It is our light, not our darkness that most frightens us. As we let our own light shine, we unconsciously give other people permission to do the same. As we are liberated from our own fear, our presence automatically liberates others"*

**-Marianne Williamson.**

~ I wholeheartedly dedicate this dissertation to my YOUNG-SELF who doubted her worth, capabilities and potential. Look at you baby girl soaring to greater heights! Look at you!!! ~

**Presentation arising from this project**

Oral presentation

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**Abstract**

The sub-Saharan poultry feed industry depends on imported soyabean meal (SBM) as a dietary rotein (DP) source in feeds. This makes poultry production costly hence the need to develop local alternatives. Marula nut meal (MNM), a by-product of oil extraction from Marula kernels, has a high crude protein (CP) content and has been successfully used in quail feeds but has not been evaluated in Guinea fowl (GF) feeds. This study evaluated the potential of MNM to substitute SBM as a DP source in GF grower and finisher diets by determining its effects on the growth performance, health and meat quality of GF.

Five grower diets wherein MNM substituted SBM on a CP basis at 0, 25, 50, 75 and 100% were formulated. Thirty eight 4-week old keets were randomly assigned to the diets (n =7 to 8) and fed for 5 weeks after which they were transferred onto corresponding finisher diets and fed for 3 weeks. Weekly body weight, daily feed intake (FI) and terminal body weight (TBW) were determined. Body weight gain (BWG), average daily weight gain (ADG) and feed conversion ratio (FCR) were computed. On slaughter blood was collected. Haematocrit, blood glucose and triglyceride concentration were determined. Surrogate markers of liver and kidney function were determined and lipid peroxidation estimated. Viscera macro-morphometry, carcass weight and dressing percentage were determined and liver lipid content was also determined. Tibiae and femora mass, length and their mass to length ratio were measured. The meat's pH [initial (pHi) and ultimate (pHu)], colour, proximate and fatty acid composition were determined.

Dietary MNM had no effect ($P>0.05$) on the GF's haematocrit, blood glucose and triglyceride concentration, liver lipid content and TBW. During the grower phase, except for week 2 where GF fed diet 3 had the highest ($P<0.05$) BWG and the highest ADG ($P<0.05$), dietary MNM did not affect ($P>0.05$) the GF's BWG, ADG, FI and FCR. In week 5 of the grower phase GF fed diet 5 had the highest ($P<0.01$) FI. During the finisher phase, dietary MNM did not affect GF's weekly BWG, ADG, FI. However, in week 3 of the finisher phase, GF fed diet 3 had the highest ($P<0.05$) FCR. Overall (grower and finisher phases) dietary MNM had no effect on BWG, ADG and FI. However, GF reared on diet 3 had the highest ($P<0.05$) FCR. Dietary MNM did not affect ($P>0.05$) the tibiae and femora indices of the GF neither did it affect their viscera macro-morphometry, liver and kidney function and lipid peroxidation ($P>0.05$). Marula nut meal did not affect ($P>0.05$) the pH, colour and tenderness of the GF's meat. Breast meat of GF fed diet 3 and 4 had the highest ($P < 0.0001$) fat content. Meat from GF fed diet 1 and 3 had the highest ($P < 0.0001$) calcium content. Dietary MNM did not affect the lipid (total saturated fatty acids, total monounsaturated fatty acids and total polyunsaturated fatty acids) content of the meat but the oleic acid (OA) content of the meat increased non-significantly ($P>0.05$) with an increase in dietary MNM inclusion.

Dietary MNM at 25%, 75% and 100%  (CP basis) can potentially substitute SBM in grower and finisher GF feeds without compromising growth performance, liver and kidney function as well as meat quality. Importantly, it can be exploited to increase the OA content of the meat. However caution must be taken as its use at at 50% inclusion level compromised feed utilisation efficiency.

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James W. Frick once said “Don’t tell me where your priorities are, show me where you spend your money and I’ll tell you what they are.” I would like wholeheartedly thank the National Research Foundation (NRF) of South Africa as well as the Faculty of Health Science Research Committee (FRC) for the financial support. Without their financial support, I wouldn’t have been able to make ends meet during the duration of this study.

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*“Being a family means you are a part of something very wonderful. It means you will love and be loved for the rest of your life.”* My heart is full of gratitude boNtshosho kanye noZikode. Mom (B.H Mungwe), dad (S.H Nkwanyana) and siblings (Ntando, Nontobeko and Zandile). I thank you for trusting me and supporting me when I decided to embark on this journey. Yerrr! Ningithwalile Zithandwa zami. Ngibonga nje isineke senu, ukungeseka kanye nothando oluyisimanga. Amazwi ayangiphelela.

*“For I know the plans I have for you, declares the Lord, plans to prosper you and not to harm you, plans to give you hope and a future.”*(Jeremiah 29:10). I thank **GOD** Almighty and the Universe for

allowing my Journey in the Pursuit of Greatness to always amaze me on the heights I'm capable of reaching!

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**List of symbols**

| | |
|---|---|
| ADG | Average daily gain |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANFs | Anti-nutritional factors |
| ANOVA | Analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| ASF | Animal source foods |
| AST | Aspartate amino transferase |
| BWG | Body weight gain |
| CP | Crude protein |
| DAFF | Department of Agriculture, Forestry and Fisheries |
| FA | Fatty acid |
| FCR | Feed conversion ratio |
| FI | Feed intake |
| IFBTs | Indigenous fruit bearing trees |
| MNM | Marula nut meal |
| MUFA | Monounsaturated fatty acid |
| PSE | Pale soft and exudative |
| PUFA | Polyunsaturated fatty acid |
| SBM | Soyabean meal |
| SFA | Saturated fatty acid |

| | |
|---|---|
| SSA | sub-Saharan Africa |
| TBARS | Thiobarbituric acid reactive substances |
| TBW | Terminal body weight |
| TMUFA | Total mono-unsaturated fatty acid |
| TPUFA | Total polyunsaturated fatty acid |
| TSFA | Total saturated fatty acid |
| WBSF | Warner-Bratzler shear force |

## Nomenclature

| | |
|---|---|
| L* | Lightness |
| a* | Redness |
| b* | Yellowness |
| pHi | Initial pH |
| pHu | Ultimate pH |

# CHAPTER 1: INTRODUCTION AND JUSTIFICATION

## 1.0 Dissertation overview

This dissertation is made up of seven chapters. **Chapter 1** provides a concise study background, justification, aim and objectives of the study while **Chapter 2** provides a review of literature pertinent to the study. **Chapter 3** gives an account of the materials and methods, procedures and assays used in the determination of the effects of the Marula nut meal on the growth, health and meat quality of broiler guinea fowl. **Chapter 4** presents the results of the study while **Chapter 5** provides a detailed discourse on the discussion of the results obtained in the study. **Chapter 6** highlights the major findings and conclusions drawn from the study. It also highlights the limitations of the study and makes suggestions for potential areas of further study. **Chapter 7** contains a list of references cited in this dissertation as well as relevant appendices.

## 1.1 Introduction

The poultry industry is the largest contributor to the agricultural sector offering numerous social and economic benefits, globally. It is a prime employer directly and indirectly offering jobs to 1.3 billion individuals globally and to 245 000 employees in South Africa (Meissner *et al*., 2013). The livestock sector, including the poultry industry, have a massive direct influence on the nutrition security and diets of the 7 billion people occupying the world (Wong *et al.*, 2017). Poultry products such as eggs and meat form part of the prime sources of quality micronutrients, for example iron, zinc, vitamin A, vitamin $B_{12}$ and dietary protein required by the human body for good health and normal development (Miranda *et al.*, 2015), especially of the brain in early life (Prado and Dewey, 2014). These nutrient-rich products supply approximately 28% protein and 13% of the energy consumed worldwide (Steinfeld and Gerber, 2010). The consumption of poultry meat in sub-Saharan Africa (SSA) has reported a 99% increase since 2004 (Goodison, 2015). In the SSA region, the per capita consumption of chicken meat varies by country with, for example, per capita consumption levels were 1.41kg, 7.67kg and 32.98kg for Nigeria, Ghana and South Africa, respectively (Goodison, 2015). The high consumption of poultry products, especially chicken meat, stems from the relative affordability of broiler chicken meat when compared to red meats (Delport *et al.*, 2017). Additionally, the reported high consumption is also a result of the low fat and cholesterol content (Komprda *et al.*, 2003) and high protein content (Barroeta, 2015) of chicken meat which makes it a more healthful product. However, the production of improved broiler and pullet chicken breeds is associated with high housing, feed and veterinary cost (Matthews and Sumner, 2015; Gale and Arnade, 2015). The high production costs associated with these improved chicken breeds make it nearly impossible for rural communities, where animal-derived protein malnutrition is highest, to produce them (Kolahdooz *et al.*, 2013). There is, therefore, a need to consider alternative poultry species that can be produced in such areas at a relatively cheaper cost. uinea fowl (*Numida meleagris*) is one such potential alternative poultry species.

Guinea fowl (GF) are common wild birds inhabiting the grassland regions of Madagascar and South Africa and its production in organic agricultural systems has increased in the recent years (Eleroğlu *et al*., 2015; Abdul-Rahman and Adu, 2017). The growing interest in GF as a source of animal-derived dietary protein source for human consumption is driven by the meat's leanness, high vitamin and protein content (Olaoye, 2011) and low fat (4%) which makes it a healthier product compared to the more lipid-rich broiler chicken meat (Mir *et al.*, 2017). Meat with high cholesterol and saturated fatty acid content is known to contribute to the development of metabolic diseases inclusive of type II diabetes mellitus (Mari-Sanchis *et al.*, 2016), non-alcoholic fatty liver disease (Anania *et al.*, 2018) and atherosclerosis (Larsson and Orsini, 2014). Guinea fowl fertility and hatchability is 75-80% with their egg incubation period ranging from 26-28 days ( Eleroğlu *et al*., 2015; Khairunnesa *et al*., 2016). Importantly, Guinea fowl adapt better to climatic changes, have lower production costs as they can forage better than chicken and are resistant to common poultry diseases (Bernacki *et al*., 2013; Gono *et al*., 2013). These attributes make them a better-suited poultry species for production by resource-poor rural and or smallholder farmers (Saina, 2005).

A number of ingredients need to be considered when formulating poultry feed to ensure optimal growth performance, feed economy, bird health and product (eggs and meat) quality. Plant and animal protein sources are common ingredients used to formulate poultry diets (Denton *et al*., 2005). The dietary protein component in poultry diets, which is usually of plant origin, is one of the greatest contributors to the high cost of poultry feed (Beski *et al.*, 2015). Worldwide, soyabean meal (SBM) is the most commonly used dietary protein source in poultry feeds (Beski *et al.*, 2015). The global utilisation of SBM as a dietary protein source in poultry feeds is premised on its high protein content, high digestibility and a rich amino acid profile (Banaszkiewicz, 2011). The major problem faced by the South African Poultry Association (SAPA) is that the production of soyabean nationwide is insufficient to generate enough SBM to meet the human food and livestock feed industry requirements (Dlamini *et al*., 2016). Thus the livestock industry depends on costly SBM imports (Hasha, 2002). The failure of local soybean production to meet the SBM requirement of the country means the country survives on imports which are costly (DAAF, 2017). This dependency on imported SBM creates an urgent need to explore and develop alternative low-cost dietary protein sources for poultry feeds so as to ameliorate the shortage and importantly to facilitate the production of alternative poultry species such as Guinea fowl at rural household level. The SSA region is endowed with indigenous fruit-bearing trees (IFBTs) whose seeds and nuts could be exploited as feed ingredients including dietary protein sources for poultry feeds.

Marula *(Sclerocarya birrea caffra*) is a common fruit-bearing indigenous tree widely distributed throughout SSA (Mokgolodi *et al.*, 2011). Its seed is rich in vitamin C (Ojewole *et al.*, 2010) and has significant quantities of calcium and phosphorus, key nutrients in poultry nutrition and production (Abdulla *et al*., 2016). *Sclerocarya birrea caffra* fruit pulp contains 84% carbohydrate

(Wairagu *et al*., 2013) and the kernels of *S. birrea caffra* from Nigeria were reported to contain 36.7% crude protein (Mariod and Abdelwahab, 2012) while that from provenances from South Africa contains 28% crude protein (Wynberg *et al.*, 2012) and its partially defatted nut meal contains 33-39% crude protein (Malebana *et al.*, 2018). These nutritional attributes make *S. birrea caffra* a potential source of dietary protein and energy in poultry feeds. Previous studies have found Marula nut meal to be a potential dietary protein source in sheep (Muhammad *et al.*, 2016), cattle (Mdziniso *et al.*, 2016), broiler chicken feeds (Mdziniso *et al.*, 2016) and Japanese quail (Mazizi *et al.*, 2019).

### 1.2 Rationale

Despite previous research to assess the potential of *S. birrea* nut meal [Marula nut meal (MNM)] as a protein source in other livestock and poultry species, its nutritional value and effect in Guinea fowl needs to be interrogated although MNM has been demonstrated to effectively substitute SBM in broiler chicken feeds (Mdziniso *et al.*, 2016) and broiler quail feeds (Mazizi *et al.*, 2019), taking into account the differences in the gastrointestinal (GIT) microbiota composition which impact the digestion of feed in bird species, MNM might be differently processed by microbiota present in the Guinea fowl GIT.

### 1.3 Aim and objectives of the study

This study sought to evaluate the potential of dietary MNM to replace SBM in Guinea fowl grower and finisher diets by determining its effects on the growth performance, health and meat quality of broiler *Numida meleagris*. Specifically the study objectives involved formulating MNM-based Guinea fowl grower and finisher diets through a graded dietary substitution of SBM on a crude protein basis and determining their effects on:

a. growth performance using body weight [terminal body weight (TBW, body mass gain (BMG), average daily gain (ADG)] and long bone (tibiae and femora mass, length and bone mass to length ratio) based indices
b. feed intake (FI) and feed economy by computing the feed conversion ratio (FCR).
c. viscera macro-morphometry
d. packed cell volume

e. surrogate markers of liver [plasma Aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activity and plasma total protein, albumin and globulin concentration] and kidney (serum creatinine, bilirubin and uric acid concentration function.
f. metabolic substrate (blood glucose, cholesterol and total liver lipid) content.
g. physical (pH, colour, water holding capacity and tenderness) and the proximate and fatty acid content of the meat.

### 1.4 Hypothesis of the study

The hypothesis of the study was:

$H_0$: dietary substitution of SBM with MNM, on a crude protein basis, in broiler Guinea fowl grower and finisher diets does not affect the growth performance, feed utilisation efficiency, GIT viscera macro-morphometry, circulating and stored metabolic substrate content, liver and kidney health and meat quality of *Numida meleagris.*

$H_1$: dietary substitution of SBM with MNM, on a crude protein basis, in broiler Guinea fowl grower and finisher diets affects the growth performance, feed utilisation efficiency, GIT viscera macro-morphometry, circulating and stored metabolic substrate content, liver and kidney health and meat quality of *Numida meleagris.*

# CHAPTER 2: LITERATURE REVIEW

The SSA population continues to increase rapidly (Kabiru *et al*., 2012). According to the United Nations projections, the SSA human population, with an annual growth rate of above 2.3%, which stood at 969 million in 2015, is expected to reach 2168 million people by 2050 (United Nations, 2015). This rapid growth of the SSA human population projected for the next decades will create many social and economic strains in the region (Chauvin *et al*., 2012) inclusive of food shortage, especially animal-derived food products whose demand is on the increase (Henchion *et al*., 2017).

Currently, SSA has a high prevalence of malnutrition, with approximately half of the world's malnourished human population inhabiting this region (Bain *et al*., 2013). In developing countries, malnutrition and micronutrient deficiencies are a major health burden (Müller and Krawinkel, 2005; Zhang *et al*., 2016) and a number of interventions to prevent severe protein-energy malnutrition are required to mitigate malnutrition related health challenges (Volkert *et al*., 2019). To meet the growing demand for food, it has been estimated that agricultural output will need to increase by at least 70% (Hunter *et al*., 2017). The potential of animal-source foods (ASF) to alleviate micronutrient deficiencies as part of a food-based strategy is well recognised (Zhang *et al*., 2016; Thornton, 2010).

Poultry production, when compared to other farmed species, due to small size, short generation interval and relatively less space and feed requirements, could be used to mitigate the shortage of animal-derived protein for human consumption in SSA.

## 2.1 Poultry production

Poultry, characterised as domesticated birds kept either because of their meat, feathers or eggs, constitute a fascinating and diverse group of animals, which can be incorporated into many farming systems throughout the world (Vaarst *et al*., 2015). Their ability to convert numerous types of feed, such as residuals from agricultural activities, households and food processing industries into animal products and protein sources is more productive than most other animal species (Nkukwana, 2018). Indeed their contribution to food security, protein supply, and peoples' livelihoods makes poultry valuable globally (Vaarst *et al*., 2015; Henchion *et al*., 2017). The poultry industry has in recent years expanded, consolidated and globalised driven primarily by strong demand making it possibly the fastest developing of all livestock sectors (Upton, 2007; Nkukwana, 2018). The worldwide production of poultry meat and its products has been growing quicker than that of other species products in both developed and developing countries since the 1960s (Chang, 2007). Over the past three decades, the poultry sector has been expanding at more than 5% annually (Gerber, *et al*., 2015). Its share in global meat production increased from 15% three decades ago to 30% (Gerber, *et al*., 2015). Poultry production has been accepted as a significant way of increasing the consumption of animal-derived protein, particularly in developing countries (Sarven Bale *et al.*, 2013). The poultry industry is one of the most efficient food commodity groups that supply the rapidly growing human population and has a massive direct influence on the nutrition security and diets of the 7 billion people occupying the world (Wong *et al.*, 2017).

## 2.2 Global and local demand for poultry products

Global per capita consumption of poultry meat increased from 2.88 kg to 14.13 kg between 1961 and 2010, while global per capita consumption of eggs increased from 4.55 kg to 8.92 kg (Mottet and Tempio, 2017). The demand and consumption of poultry products in developed countries has expanded at a slow rate in comparison to that in developing countries (Kearney, 2010). In 2015, the demand of poultry meat in developed countries was put at 43.6 million tonnes with 67.5 million tonnes being consumed in developing countries (Mitchell, 2016). By 2024, the consumption of poultry meat in developed economies is expected to have accumulated approximately 5.2 million tonnes to a total of 48.8 million tonnes while for developing countries an increase of 16.7 million tonnes increase is expected as the total rises to 84.2 million tonnes (Mitchell, 2016). Since the mid-1990s, per capita meat consumption for veal and beef, pork, and poultry has grown around 3% annually in emerging economies, while growth has been only about 0.4% for developed countries (Nigatu and Seeley, 2015). However, the consumption of poultry meat in SSA has reported a 99% increase since 2004 (Goodison, 2015).

The fast-growth in human population, urbanisation and income (Wang, 2019) drive this massive increase in the demand for poultry meat. Compared to other SSA countries, South Africa is the largest chicken meat producer and had per capita consumption of 37.47kg in 2014 (Tan *et al.*, 2018). The most recent per capita consumption of broiler chicken meat in SA is 38.89kg (Brinkhuis, 2017). The South African poultry industry provides direct and indirect employment to 108 000 individuals throughout its value chain (Nkukwana, 2018). Due to the availability of constant incomes and economic growth, South Africa has seen sustained growth of animal-derived products and expansion of meat consumption (Esterhuizen, 2015). According to Esterhuizen (2015), 2.9 million tonnes of beef, poultry and pork meat are consumed in the country per annum. Out of this, poultry meat constitute more than 60% of total meat consumption (Esterhuizen, 2015). The predominance of the consumption of chicken meat in South Africa and elsewhere in the developing world results from its lower cost compared to other meats (Tan *et al.*, 2018). Additionally, it is proven that chicken meat (breast meat) contains more protein and less fat compared to red meat, thus deeming it a healthier product (Kralik *et al.*, 2018) hence its increased demand.

As much as broiler chicken meat and eggs may be the primary sources of protein consumed by humans, there is a broader scope of other avian species that the poultry industry can potentially produce.

## 2.3 Poultry species

### 2.3.1 Conventional poultry species

#### *2.3.1.1 Broiler and pullet chicken (Gallus gallus domesticus)*

Broiler and pullet chickens are the predominant poultry species in the rural areas of Africa (Melesse, 2014). They are reared for socio-economic and household food security purposes and thus form an integral part of the farming systems in rural areas. Chicken meat and eggs, respectively, dominate the global poultry meat and egg sector (Mbuza *et al.*, 2017). Through the use of intuitive skills, associated with preference and economic necessity, poultry breeders made progress in successfully exploiting the natural genetic variation available in chicken (Sanda *et al.*, 2012). The improved breeds of broiler and pullet chicken are bred for increased productive performance (Shao *et al.*, 2015). As such, they are efficient converters of feed into protein (meat and eggs) for human consumption (SAPA, 2017). However, because of their improved genetics, they consume more feed (Sell-kubiak *et al.*, 2017), require expensive housing and increased veterinary care demand (Ormandy *et al*., 2011), production is limited largely to commercial producers who are endowed with resources. Despite the high cost of producing these improved chicken breeds, when compared to other meats, broiler chicken meat is relatively cheaper hence the higher consumption (Delport *et al.*, 2017; Tan *et al.*, 2018), has a low fat and cholesterol content (Komprda *et al*., 2003; Marangoni *et al*., 2015) and its protein has a high biological value (Barroeta, 2015; da Silva *et al*., 2017) thus making it a more healthful product. Chicken meat is also appropriate for quick, simple preparation and it offers a variety of combinations with various foods making it a popular choice for modern consumers (Kralik *et al.*, 2018).

Chicken eggs, on the other hand, have been identified to represent the lowest-cost animal source for proteins, vitamins and the second-lowest-cost source for zinc and calcium (Réhault-Godbert *et al*., 2019). The avian (chicken) egg is a known excellent source of high-quality protein, essential vitamins and minerals, hence it is widely accepted for human consumption (Isidahomen *et al*., 2013). Chicken eggs are an inexpensive and low-calorie source of high-quality protein and other nutrients beneficial to human health. The protein content of eggs is high; 91% when cooked (Réhault-Godbert *et al*., 2019). Avian eggs contain about 70mg/dL of omega-3 (n-3) fatty acids and numerous essential fatty acids which are components of phospholipids that contribute to the flexibility of cells and reduced plasma cholesterol levels (Zaheer, 2015) and are (esential fatty acids) necessary for normal human development in early life (Osendarp, 2011). Despite all the merits associated with rearing broiler chicken and the supreme nutritional quality of broiler meat and eggs, there has been a growing interest in duck meat in various regions of the world.

#### *2.3.1.2 Muscovy duck (Cairina moschata)*

The Muscovy duck, is a large duck that belongs to the family *Anatidae* and native to Mexico, South and Central America (Adzitey and Adzitey, 2011). The global industry of duck production increased by 153% between 1993 to 2013 with meat production rising from 1.74 to 4.39 million tons (Molnár and Szőllősi, 2017). In terms of duck meat production, Asia leads other regions of the world (Gilbert *et al*., 2017) accounting for 82.6% of the total duck meat produced worldwide (Adzitey and Adzitey, 2011). In Africa, duck production is largely found in Madagascar and Egypt which produce 37 300 and 13 000 tons of duck meat per annum, respectively (Baéza *et al.*, 2018). Although broiler chickens tend to be dominant in the egg and meat sector, significant amounts of eggs and meat are also produced by ducks (FAO, 2009). Muscovy ducks are valued throughout the world for their unique high yield of breast meat (Smith *et al.*, 2015) of low caloric (Kameshpandian *et al*., 2018) and high polyunsaturated fatty acid content (Baéza *et al.*, 2018). Ducks are hardy, less demanding in terms of feed quality and are less vulnerable to common poultry diseases compared to chicken (Yakubu, 2013; Dai *et al.*, 2014).

Besides the conventional poultry species, in many areas represented by chicken and ducks, other non-conventional avian species are being made use of to increase the supply of poultry-derived protein for human consumption. Some such promising species include the Japanese quail (*Coturnix coturnix japonica*) and Guinea fowl (*Numida Meleagris)*.

### 2.3.2 Non-conventional poultry species

#### *2.3.2.1 Japanese quail (Coturnix coturnix japonica)*

Japanese quail are small hardy domesticated subspecies that belong to the family *Phasianidae* and order *Galliformes* (Altine *et al.*, 2016). They are mainly raised for their egg and meat which have become popular delicacy products among Asian (Jeke *et al.*, 2018) and European (Tolik *et al.*, 2014) people with Italy, France and Japan showing the highest consumption (Genchev *et al.*, 2008; Jeke *et al.*, 2018). Japanese quail stand out for their number of desirable qualities. They are relatively small birds thus they require small floor space and less demanding feed costs (20-25 g/adult bird/day) than broiler chicken (Filho *et al.*, 2012; Rahman *et al.*, 2016). They have rapid growth and early maturity (Vieira Filho *et al.*, 2016). Quail also have high egg productivity; laying an average of 300 eggs/bird/year (Santos *et al.*, 2013) and have high longevity at high production (Cruz *et al.*, 2019). These attributes place the Japanese quail as a potential poultry species for production by resource-limited rural communities who are at a higher risk of lacking animal-derived protein in their diets. The production and consumption of quail meat have increased in most countries due to its qualities that encourage consumer acceptance (Farrapo *et al.*, 2017). Japanese quail meat is gamey, tender, has a high essential amino acid, and is rich in vitamins, unsaturated fatty acids and phosphorus (Genchev *et al.*, 2008; Ayyub *et al.*, 2014). Additionally, quail meat has a low cholesterol, fat and caloric content making it an expedient product for health-conscious consumers (Maiorano *et al.*, 2011; Mussah, 2017).

#### *2.3.2.2 Guinea fowl* **(***Numida Meleagris)*

Guinea fowl are birds that form part of the *Numida* family and are thought to have originated from Africa (Yildrim, 2014). They live in dry areas in many African countries (Yildirim, 2012). Owing to the improvement in organic agriculture, their numbers have significantly increased in recent years (Yildirim, 2012). There are six Guinea fowl species that occur in Arabia and Africa (Weimann *et al.*, 2016): the white-breasted, black, plumed, vulturine, crested and helmeted Guinea fowl (Kumssa and Bekele, 2013). The helmeted Guinea fowl is the most distinct and agile of all known Guinea fowl species (Moghadam and Mohammadpour, 2017). Its conspicuous horny helmet on top of the bird's naked head is what distinguishes it from other Guinea fowl species (Fajemilehin, 2014). The helmeted Guinea fowl is widely distributed in South Africa (Ratcliffe and Crowe, 2001; Kumssa and Bekele, 2013; Faulkner *et al.*, 2017).

Due to their hardiness, Guinea fowl can be effectively reared under semi-insensitive conditions typical of rural areas (Kusina *et al.*, 2012) wherein communities are resource-limited. Despite being suitable to be produced by resource-limited farmers, Guinea fowl production now has a global presence with production also in North America, Europe and Australia (Moreki and Seabo, 2012). Additionally, the production of Guinea fowl in organic agricultural systems has recently increased (Baruwa and Sofoluwe, 2016) serving as an indicator to its growing importance in the poultry sector. These birds grow and mature rapidly, reproduce effectively, are well adapted to climatic change and a harsh production environment characterised by a low plane of nutrition and poor disease control (Bernacki *et al*., 2013; Gono *et al*., 2013). Guinea fowl are resistant to multiple poultry diseases such as gumboro, Newcastle disease and salmonellosis (van den Berg *et al*., 2010; Ayim-Akonor *et al*., 2018; Ebegbulem, 2018). These birds consume a large range of non-conventional feeds, including among others, leaves, fruits, seeds and insects that are not used by chicken (Adeyemo and Oyejola, 2004). This makes it the poultry species of choice for the poor. Their eggs and meat are recognised excellent sources of protein and income (Saina, 2005; Chepkemoi *et al*., 2015; Dzungwe and Gwaza, 2018). They can lay about 70 to 100 eggs per year ( Konlan and Avornyo, 2015). Incubation of Guinea fowl eggs takes ~26 to 28 days with a 68 to 80% hatchability rate (Khairunnesa *et al*., 2016; Houndonougbo *et al*., 2017). Guinea fowl eggs are distinct from eggs of other poultry species with a mass ranging from 35 to 52 g (Banaszweska *et al.*, 2015). The eggs have a better storage quality as they do not crack easily due to their thicker shells (Aminu *et al*., 2017;  Dzungwe and Gwaza, 2018). Guinea fowl meat is tastier but firmer than that of chicken (Abdul-Rahman and Adu, 2017; Amoah *et al.*, 2017). According to Ebegbulem, (2018), Guinea fowl meat has an 8% higher protein content compared to broiler chicken meat. The yield of consumable Guinea fowl meat is likewise higher than that of chicken because of its slim skeleton (Musundire *et al*., 2017; Ebegbulem, 2018). The interest in Guinea fowl meat and eggs has been on the increase over the years in light of their nutritional value (Amoah *et al.*, 2017). Guinea fowl meat has a high vitamin and protein content (Olaoye, 2011), it is leaner (4% vs 15% fat) compared to broiler chicken meat ( Griffin *et al*.,1992; Jiang *et al*., 2012) hence a more healthful product. These foregoing attributes qualify Guinea fowl as a probable alternative to broiler chicken in the resource-poor smallholder sector (Saina, 2005).

In order to optimise returns from these non-conventional poultry species, there is a need to formulate nutritionally balanced diets to meet their nutrient requirements. The feed ingredients, especially the dietary energy and protein sources (major components of poultry diets) must, as much as possible be of local origin in order to cut costs and must not present competition between human and animal requirements.

### 2.4 Poultry feed

Feed is a significant factor in poultry production and sustainability. Feed costs account for about 70% - 80% of the total poultry production costs (Thirumalaisamy *et al.*, 2016). Feed ingredients for poultry diets are selected for the nutrients they can provide, the absence of anti-nutritional or toxic factors, their palatability or effect on voluntary feed intake, and their cost. Basically, the nutritional value of diets fed to chicken can be evaluated by determining their digestibility *in vivo* and evaluating their effects on productivity. In order to optimise growth performance and ensure good health, intensively reared poultry species need a balanced array of nutrients in their diet. The nutrients required by birds vary according to species, age and production system, for example, broiler (meat) or pullet (egg) production. The key nutrients that need to be supplied by the dietary ingredients are amino acids contained in protein, vitamins and minerals. All physiological and biochemical processes that support life also require energy. Energy which is the pacesetter for production (Nkukwana, 2018) is obtained from carbohydrates, lipids and proteins. Protein is an expensive dietary ingredient but is central to meeting the essential amino acid requirements of the broiler and pullet poultry.

A number of feed ingredients are used to formulate poultry feed. These include cereals and or cereal by-products, plant-derived dietary protein sources, vitamin and mineral premixes, fats, animal by-product meals, synthetic essential amino acids and feed additives (Ravinadan, 2009; Schönfeldt *et al*., 2013; Richter *et al*., 2015). The high cost of feed ingredients, particularly dietary energy and protein sources, limits the capacity of resource-limited farmers to farm poultry including the less costly species such as Guinea fowl.

#### 2.4.1 Dietary energy sources

Most of the dietary energy for poultry feeds is derived from starch from cereal grains (Hossain *et al*., 2012). Wheat, maize, sorghum, rice bran and millet are some examples of the main cereal grains used predominantly as energy sources in poultry diets (Hossain, *et al*., 2013). The energy component provided by cereal grains accounts for 60 to 70% of the nutrient requirement of poultry making them one of the major feed ingredients required to formulate nutritionally adequate diets (Phosa, 2009; Hossain *et al*., 2013). Globally, maize is the predominant cereal grain used in poultry feeds (Guerre, 2016) because of its high digestibility (Dei, 2017). In addition to its high digestibility, it has high energy content (Indriani *et al.*, 2018), is highly palatable and generally free of anti-nutritional factors (Nkukwana, 2018). In Asian and African countries, maize production is insufficient to meet the needs of the constantly growing human population (Ravinadan, 2009) hence the competition between human requirements and the poultry feed industry requirements.

### 2.4.2 Dietary protein sources

#### *2.4.2.1 Conventional protein sources*

Animal-derived products such as fish, meat and bone and shrimp meals (Ayisi *et al.*, 2017) and plant-derived seed oil cakes and meals, for example, canola and soyabean are the commonly used dietary protein sources for poultry feeds (Tadele and Getachew, 2015). While animal-derived dietary protein sources can be used in poultry feeds, health concerns due to high cholesterol and saturated fat (Hoffman and Falvo, 2005) as well as potential zoonoses militate against their wholesale use (Mottet and Tempio, 2017). While fishmeal has an ideal amino acid profile to meet the essential amino acid requirements for poultry species (Furuya *et al*., 2010), its use is limited by high cost and by its odour tainting meat and eggs and thus cannot be used in finisher diets (Cockcroft, 2018). Due to the limitations associated with the use of animal-derived dietary protein sources, soyabean meal (SBM) is most commonly used as the dietary protein source in poultry feeds globally (Tangendjaja, 2012) in SSA (Cisse *et al.*, 2017) and in SA (Nkukwana, 2014).

Soyabean (*Glycine max*) is a commercial crop grown as the major oilseed in over 35 countries (Tidke *et al.*, 2015). The grain legume is not only a valuable oil crop but is also used as feed for aquaculture, livestock and poultry (Dei, 2011; Tangendjaja, 2012). The global utilisation of SBM as a dietary protein source in poultry feeds is premised on its high protein content, high digestibility and a rich amino acid profile (Banaszkiewicz, 2011). The major problem faced by the poultry and livestock feed industry in SSA, South Africa included, is that local production of soybean is insufficient to satisfy the demand of SBM by the human food and livestock feed industries (Mlambo *et al*., 2011). The domestic soybean meal production is reported to meet only 10% of the domestic SBM demand (Joubert, 2011). It is because of poor regional soyabean production that SSA countries, SA included, rely on imported SBM (Chianu *et al.*, 2009). At present, SA imports from

Argentina and Mexico (Joubert, 2011) an average of 72% of its high protein meal making it the largest importer of SBM in SSA (Sihlobo and Kapuya, 2016). In 2010, the total importation costs of soybean and soybean-related products amounted to R4, 35 billion and were estimated to increase by 9% annually (Joubert, 2011). The reliance on imported SBM is the major driver of increased feed costs which negatively impact poultry production, more so in resource-limited rural communities and in the process compromise household food security (Nkukwana, 2018). There is a need to explore and develop alternative low-cost locally available dietary protein sources for poultry feeds in order to facilitate cheaper poultry production and to enhance poultry farming in rural communities who have the highest risk of failing to get enough animal-derived protein in their diets. Previously conducted research on potential dietary protein sources for poultry feed has focused mostly on grain legumes (Tshovhote *et al.*, 2003; Jezierny *et al.*, 2010; Poel *et al.*, 2013) but not much work has evaluated the potential of tree from indigenous fruit-bearing trees (IFBTs). The fruits' seeds/kernels are often discarded and left to decay without further exploring their potential as animal feed sources. There is a need to interrogate the nutritive value of IFBT seeds and nuts to determine their potential use as sources of dietary protein in poultry feed.

#### *2.4.2.2 Potential of seeds from indigenous fruit-bearing trees*

Many countries in Africa boast an abundance of IFBTs that produce edible fruit (Awodoyin *et al.*, 2015) but whose seeds are discarded and never further exploited for their potential nutrient content (Fukushima *et al.*, 2010; Raihana *et al.*, 2015). Indigenous fruit-bearing trees are adapted to climatic conditions and soils of SSA thus permitting survival under conditions of environmental stresses and require less inputs compared to conventional agricultural crops (Cernansky, 2015). These IFBTs play a vital role in the livelihoods of most rural communities living in arid and semi-arid areas in the SSA region (Fukushima *et al.*, 2010; Simitu, 2011) as they yield edible fruit even under drought condition (Chivandi *et al.*, 2015). One such IFBT is the *Sclerocarya birrea caffra* (Marula) which is widely distributed in SSA (Sinthumule and Mashau, 2019).

### 2.4.3 *Sclerocarya birrea caffra*

*Sclerocarya birrea* (A. Rich.) Hochst. Subsp. *Caffra* (Sond.) Kokwaro (*Marula*) is an IFBT widely distributed throughout SSA (Maroyi, 2013) belonging to the Anacardiaceae family, which has 70 genera and 650 species, including common edible species like mango (*Mangifera indica*), pistachio (*Pistacia vera*) and cashew (*Anacardium occidentale*) (Mokgolodi *et al.*, 2011). Its distribution spans from Nigeria, through to Swaziland (Murye, 2017) and South Africa (Mokgolodi *et al.*, 2011; Maroyi, 2013).

#### *2.4.3.1 Botany*

In South Africa, *S. birrea* is common in the Savannah areas of the Northern Province, North West, Limpopo, Mpumalanga and Northern KwaZulu-Natal. *S. birrea* is a fast-growing IFBT reaching 7-17m in height (Shackleton *et al.*, 2002). The bark is grey and peels off round discs exposing the lighter, immature bark giving the tree its typically blotchy look. The tree usually flowers between September and December and releases fruits from January to March (Jama *et al.*, 2008). It's a prolific fruit-bearing tree producing up to 500kg of the Marula fruit per tree per annum (DAAF, 2010). Although the fruit sizes vary, they are roughly golf-ball- or plum-sized (Sinthumule and Mashau, 2019). Its fruit is plum-like pale yellow fruit with a juicy sticky flesh (Abdelwahab, 2012). When ripe, the fruit has light yellow skin with white succulent flesh and a strong distinctive turpentine flavour (Suarez and Buchwald-werner, 2012). In the centre of the Marula fruit pulp is a large stony seed containing two or three highly nutritious (Jøker and Erdey, 2003; Hundessa, 2014) kernels which are eaten raw and or roasted (Hal, 2013). Its leaves are 8-38cm long and oval-shaped with smooth margins (Mokgolodi *et al.*, 2011). The leaflets are dark green above and bluish-green below (Deutschländer *et al.*, 2009). Marula has a single round stem that generally branches at 3-4m high above the ground (Sinthumule and Mashau, 2019).

#### *2.4.3.2 Local uses*

The multi-faceted species has been previously identified as part of the top five IFBT species that requires integration into the domestication process in the African farming systems with the aim of supporting local nutritional health and income security (Gouwakinnou *et al.*, 2011). The fruit has multiple significant roles in the livelihoods, tradition and spirituality of various rural communities (Shackleton *et al.*, 2009). This multi-purpose commercial fruit crop is highly appreciated by locals for its fruit, cosmeceutical and medicinal properties from its seed and the bark, respectively (Hiwilepo-van Hal *et al.*, 2014). The leaves and the bark are harvested for traditional medicine. *S. birrea* tree also plays a crucial role in animal rearing as its leaves provide excellent forage for livestock (Gouwakinnou *et al.*, 2011) and wild herbivores (Wigley *et al.*, 2014). The fruit pulp can be consumed raw or boiled into a thick, black consistency and used for sweetening porridge. It is also used to make a delicious amber-coloured jelly (Ndlovu, 2016). Marula beer, a popular fermented alcoholic beverage, is also prepared from the ripe fruit. This beverage is reported to have twice as much ascorbic acid content as orange juice (Dlamini, and Dube, 2008) and thus is an excellent source of vitamin C. The seed kernels, described as a delicacy, are commonly used as diet supplements during winter and or in drought as the oil in the seed are rich in protein (Kugedera, 2015). The bark is used for panelling, furniture, flooring and household utensils such as spoons (Phiri, 2018). Drums and yokes are also made from the wood of this tree. A red-brown dye can be produced from the fresh inner bark (Hines and Eckman, 1993). The tree's gum is rich in tannins and is used as ink when mixed with soot (Massaud, 2007). A relatively good quality rope can be made using the inner bark. The tree's powdered bark is also valuable in traditional medicine for a wide variety of purposes such as treating dysentery, diarrhoea and haemorrhoids (Gathirwa *et al.*, 2007; Ojewole *et al.*, 2010). An applied infusion of the inner Marula bark is used to alleviate pain from snake bites and scorpion stings (Xaba, 2011). The leaves mitigate indigestion and heartburn when chewed (Mokgolodi *et al.*, 2011). An infusion of the leaves can also be used to treat gonorrhoea (Xaba, 2011).

#### *2.4.3.3 Commercial uses*

Given the pervasive distribution, significance and multiple uses of the Marula fruit amongst the rural communities, there have been numerous attempts to commercialise a variety of Marula-based products. The commercialisation of Marula products takes many forms, from household-level trade to international markets for Marula beverages (Wynberg *et al.*, 2012). The Marula fruit and seeds are extensively used for nutrition with the fruit pulp being processed into alcoholic beverages (Rampedi, 2010). The fruit is commercially harvested and the juice pressed for making delicate-flavoured jellies, juice, and jam (Bille *et al.*, 2013). Marula kernels tremendously benefit the cosmeceutical industry as they can be extracted and processed to cosmetic oil that is used in the treatment of numerous skin ailments. Oil extracted from Marula kernels can serve as a source of edible cooking oil and also be used as a meat preservative (Robinson.*et al*, 2012). The commercialisation of the Marula fruit brings a suite of opportunities for rural development and social upliftment through job creation and food security.

#### *2.4.3.4 Chemical nutrient composition of fruit pulp and seed*

The fruit pulp is rich in vitamin C (Ojewole *et al*., 2010) and the kernels are a good source of essential oils (Hiwilepo-van Hal *et al.*, 2014). Studies have reported kernels to be rich sources of protein ranging from 28-31% (Mokgolodi *et al.*, 2011), fats (56-61%), citric acid (2.02%), malic acid, sugars and macro- and micro-minerals (Abdulla *et al.*, 2016). The protein, fat and mineral content of Marula kernels can be exploited by using it to formulate poultry diets (Malebana *et al.*, 2018). When oil is extracted from the Marula kernels, the protein content of the resultant defatted meal has been reported to increase to about 54-70% (Hundessa, 2014) The oil yielded from Marula kernels has a fatty acid profile comparable to olive oil but with a stability that is ten times greater (Hundessa, 2014) thus can be used for culinary purposes with the advantage of a long shelf life.

#### *2.4.3.5 Marula nut meal*

Marula nut meal (MNM) is a by-product of oil extraction from the kernels from the ripe fruits of Marula. Different techniques of oil extraction are used during the production of Marula oil and the by-product of the oil extraction is MNM (Malebana *et al.*, 2018). One of these methods is cold pressing wherein dried kernels are heat-treated at 43°C for 2 hours before they are cold-pressed to extract oil while the other entails solvent extraction using a solvent such as hexane to extract the oil from a given kernel matrix (Taseski, 2011). Marula nut meal is a locally available potential alternative dietary protein source with a high crude protein content (470 g/kg DM) and an amino acid composition comparable to that of SBM with the exception of lysine (Mthiyane and Mhlanga, 2017). The nutritional attributes (high crude protein and a good amino acid profile) of MNM deem it a potential source of dietary protein for poultry feeds ideal for use by resource-poor smallholder farmers due to its wide availability. The use of MNM in poultry and livestock feed, compared to imported soybean meal, can potentially mitigate the imported SBM-induced high feed cost and facilitate the intensification of poultry production at household level, especially in rural communities. Compared to imported soybean meal, MNM is a locally available potential protein source for poultry and livestock especially to resource-poor rural farmers due to its local availability. Marula nut meal has been reported to be an ideal dietary protein source in broiler chicken feed (Mthiyane and Mhlanga, 2017), cattle (V. Mlambo *et al.*, 2011; Mdziniso *et al.*, 2016), goats (Mlambo *et al*., 2011) and sheep (Altine *et al.*, 2016). Despite the research that has been previously done to assess the potential of Marula kernel/seed meal as a protein source in the feeds of other poultry and livestock species, its nutritional value and effect in Guinea fowl needs to be interrogated. This study therefore sought to determine the potential of MNM to replace SBM as a dietary protein source in grower and finisher Guinea fowl diets on the growth performance and feed economy, GIT viscera macro-morphometry, circulating and stored metabolic substrates, liver and kidney health and meat quality of broiler Guinea fowl.

# CHAPTER 3: MATERIALS AND METHODS

### 3.1 Feed ingredients

#### 3.1.1 Source and processing of Marula nut meal

The Marula nut meal was procured from Mhlala Development Centre, a medium scale Marula oil extraction company in Bushbuckridge, Limpopo, South Africa. The MNM used in the study had a high (38%) residual oil (Malebana *et al*., 2018) and as such was further defatted through solvent extraction using hexane. Briefly, each 40kg batch of the partially defatted MNM in a cotton bag was steeped for 48 hours in 400 litres of 99.9% hexane in an 800-litre stainless steel tank. Immediately after 48 hours, the tap at the bottom side of the tank was opened and the oil-laden hexane was drained into 25L plastic containers. The solvent extracted MNM was then spread onto clean plastic sheets and dried at room temperature for 24 hours. The dry hexane-extracted (defatted) MNM was then packaged into jute bags and stored at room temperature till diet formulation.

#### 3.1.2 Other feed ingredients

Yellow maize, wheat bran and SBM were purchased from Obaro (Pty) Ltd, Pretoria, South Africa. Canola oil and iodated salt were purchased from Makro (Pty) Ltd, Johannesburg, South Africa. The vitamin-mineral premix was sourced from Trouw Nutrition, Edenvale, South Africa.

#### 3.1.3 Diet formulation and diets

Both the starter/grower and finisher diets were formulated such that MNM substituted SBM on a crude protein basis at 0, 25, 50, 75 and 100% for diet 1 through to 5, respectively. The grower and finisher diets were formulated to meet the nutritional requirements of growing and finis32hing Guinea fowl as recommended by Ensminger *et al*. (1990). The ingredient and chemical nutrient composition of the diets are shown in Table 3.1 and 3.2.

**Table 3.1: Ingredient and nutrient composition of the grower diets (1 – 5 weeks)**

| | Grower | | | | |
|---|---|---|---|---|---|
| **Ingredients (g/kg)** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** |
| Soyabean meal (45%) | 369.34 | 280.46 | 187.69 | 94.37 | 0.00 |
| Yellow maize meal | 491.85 | 562.57 | 518.42 | 572.57 | 623.01 |
| Marula nut meal | 0.00 | 65.57 | 131.60 | 198.54 | 272.52 |
| Wheat bran | 91.08 | 46.11 | 120.35 | 93.10 | 62.30 |
| Soyabean oil | 11.84 | 7.01 | 2.59 | 0.00 | 0.00 |
| Limestone | 20.95 | 21.21 | 21.29 | 21.41 | 22.04 |
| *DL*-Methionine, 99% | 2.19 | 1.84 | 1.30 | 0.93 | 0.48 |
| *L*-Lysine HCL 98.5% | 0.00 | 2.31 | 3.80 | 6.05 | 8.15 |
| Di-calcium phosphate | 3.64 | 3.69 | 3.70 | 3.72 | 1.92 |
| Salt | 4.55 | 4.61 | 4.63 | 4.66 | 4.79 |
| Vitamin/mineral premix* | 4.55 | 4.61 | 4.63 | 4.66 | 4.79 |
| **Total** | **1000.00** | **1000.00** | **1000.00** | **1000.00** | **1000.00** |
| ***Chemical composition (%)*** | | | | | |
| Dry matter | 90.00 | 89.15 | 88.88 | 88.64 | 86.37 |
| Crude protein | 21.94 | 21.93 | 22.80 | 22.95 | 22.85 |
| Ether extract | 3.67 | 3.63 | 3.63 | 3.84 | 4.21 |
| Calcium | 1.03 | 1.00 | 0.98 | 0.95 | 0.88 |
| Phosphorus | 0.41 | 0.46 | 0.53 | 0.58 | 0.59 |
| Gross energy | 16.89 | 16.61 | 16.51 | 16.40 | 16.01 |

*Vit A: 20,000,000.000 IU, Vit $B_1$ (Thiamine): 003.000 g, Vit $D_3$ (500 000): 3,000,000.000 IU, Vit E (500 iu):40,000.000 IU, Vit $K_3$ (43%): 003.000 g, Vit $B_2$ (80%): 010.000 g, Vit $B_6$ 98% (pyrod): 005.000 g, Vit $B_{12}$ 1 g/kg (m):100.000 mg, Niacine 99.5%: 060.000 g, Choline (Chloride 60): 606.060 g, Biotin-2%: 200.000 mg, Manganese (MnSO4–31%):160.000 g, Copper (CuSO4–25.2%): 005.000 g, Cobalt (CoSO4–20%): 100.000 mg, Selenium (Na2SeO3- 4.5%): 400:000 mg, Calcium pantothenate: 020.000 g, Folic acid (96% pure): 001.000 g, Anty ox Vit Dry: 100.000 g, Zinc (Zn SO4–35%): 090.000 g, Iodide (KI 76.45%): 001.000 g, Ferrous (FeSO4–30%): 035.000 g, Limestone powder: 2647.133 g; DL-Methionine with purity of 99%, L-Lysine HCL with purity of 98,5%; Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution.

**Table 3.2: Ingredient and nutrient composition of the finisher diets (6 -8 weeks)**

| | Finisher | | | | |
|---|---|---|---|---|---|
| **Ingredients (g/kg)** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** |
| Soyabean meal (45%) | 235.32 | 176.98 | 117.70 | 58.66 | 0.00 |
| Yellow maize meal | 583.58 | 599.38 | 616.73 | 633.57 | 652.18 |
| Marula nut meal | 0.00 | 41.37 | 82.54 | 123.42 | 163.34 |
| Wheat bran | 141.19 | 141.59 | 141.23 | 140.79 | 139.75 |
| Soyabean oil | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Limestone | 21.65 | 23.13 | 23.54 | 24.40 | 24.69 |
| DL-Methionine, 99% | 3.20 | 2.93 | 2.73 | 2.44 | 2.24 |
| L-Lysine HCL 98.5% | 1.41 | 2.83 | 3.77 | 4.97 | 6.15 |
| Di-calcium phosphate | 5.65 | 3.78 | 3.77 | 3.75 | 3.73 |
| Salt | 3.29 | 3.30 | 3.30 | 3.29 | 3.26 |
| Vitamin/mineral premix* | 4.71 | 4.72 | 4.71 | 4.69 | 4.66 |
| **Total** | **1000.00** | **1000.00** | **1000.00** | **1000.00** | **1000.00** |
| ***Chemical composition (%)*** | | | | | |
| Dry matter | 87.27 | 87.17 | 87.54 | 87.96 | 88.77 |
| Crude protein | 16.97 | 17.19 | 17.41 | 17.64 | 17.92 |
| Ether extract | 2.74 | 3.02 | 3.31 | 3.60 | 3.91 |
| Calcium | 1.02 | 1.00 | 1.00 | 1.02 | 1.02 |
| Phosphorus | 0.41 | 0.41 | 0.44 | 0.48 | 0.51 |
| Gross energy | 15.72 | 15.71 | 15.76 | 15.82 | 15.96 |

∗Vit A: 20,000,000.000 IU, Vit $B_1$ (Thiamine): 003.000 g, Vit $D_3$ (500 000): 3,000,000.000 IU, Vit E (500 iu):40,000.000 IU, Vit $K_3$ (43%): 003.000 g, Vit $B_2$ (80%): 010.000 g, Vit $B_6$ 98% (pyrod): 005.000 g, Vit $B_{12}$ 1 g/kg (m):100.000 mg, Niacine 99.5%: 060.000 g, Choline (Chloride 60): 606.060 g, Biotin 2%: 200.000 mg, Manganese(MnSO4–31%):160.000 g, Copper (CuSO4–25.2%): 005.000 g, Cobalt (CoSO4–20%): 100.000 mg, Selenium (Na2SeO3- 4.5%): 400:000 mg, Calcium pantothenate: 020.000 g, Folic acid (96% pure): 001.000 g, Anty ox Vit Dry: 100.000 g, Zinc (Zn SO4 – 35%): 090.000 g, Iodide (KI 76.45%): 001.000 g, Ferrous (FeSO4 – 30%): 035.000 g, Limestone powder: 2647.133 g; DL-Methionine with purity of 99%, L-Lysine HCL with purity of 98,5%; Diet 1 – 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution.

### 3.2 Ethical approval and study site

This study was approved by the University of the Witwatersrand Animal Research Ethics Committee (Ethics clearance number: 2018/07/31/B) and was carried out at the University of the Witwatersrand's Central Animal Service unit (CAS) and the School of Physiology Laboratories.

### 3.3 Animals and animal management

The thirty-eight (38), day-old Guinea fowl (*Numida meleagris*) keets were sourced from Dominion Outfitters, Durban, KwaZulu-Natal, South Africa. The keets were grown to 4 weeks of age before commencement of the study. On arrival, the day-old keets were treated with 1ml/L Enrovet oral solution (Kyron Laboratories Pvt Ltd, Johannesburg, South Africa) in drinking water for 3 days. Prior to the beginning of the feeding trial, the keets were housed in a pen-under a deep litter system wherein clean wood shavings were used as bedding. The bedding was changed once weekly. During this 4-week preparatory period, a 12-hour light regimen was followed with lights on at 06h00 and lights off at 18h00. Infrared lamps were used to provide supplementary heat. Room temperature was maintained at 24℃ and the keets had *ad libitum* access to a commercial broiler chicken starter feed.

At 4 week old, the 38 Guinea fowl keets were moved from the pen housing and each keet was then individually housed in a cage (0.60m length x 0.60m width x 0.80m height) equipped with a feeding and watering trough. Each bird had *ad libitum* access to its respective dietary treatment and clean drinking water. During the grower phase and finishing phase, the room temperature was maintained at 24°C (Mushtaq *et al.*, 2013). A 12-hour light cycle (with lights on from 06h00 to 18h00) was maintained throughout the feeding trial. No mortatility was recorded during the course of the study.

### 3.4 Experimental design

Thirty eight 28-day old male and female Guinea fowl keets following a 2-day habituation period to individual housing in cages were randomly allocated to the grower diets wherein MNM replaced SBM on a CP basis at 0%, 25%, 50%, 75% and 100%, for diets 1 through to 5, respectively and fed for 5 weeks. Thereafter they were each transferred onto the corresponding finisher diet wherein the MNM again replaced SBM on CP basis at 0%, 25%, 50%, 75% and

100%, and fed for another 3 weeks. The random allocation was such that in each growth stage (grower and finisher) each dietary treatment was replicated 7 - 8 times as each individually housed bird acted as a replicate. At the end of the 8 weeks feeding trial, the birds were humanely slaughtered and tissue collected for various assays.

### 3.5 Measurements during the feeding trial

The induction body weight of each bird was measured prior to commencement of the feeding trial. Thereafter the body weight (BW) of the birds was measured twice every week using an electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan). The feed intake (FI) of the birds was recorded daily by subtracting the refusals from the total feed offered.

#### 3.5.1 Computations

At the end of the feeding trail the overall body weight gain (BWG), average daily gain (ADG) of each bird was computed from the induction body weight and terminal body weight data. BWG was computed using the equation: BWG = final body weight – induction body weight. Average daily gain was computed using the equation: ADG (g) = body weight gain / duration (days) of feeding trial. The BWG and ADG for the respective growth phases were computed using the same principle. The estimated total FI (g) was computed using the equation: FI = feed offered – feed refusals; with feed offered weighed in the morning and refusal weighed the following day in the morning. Feed conversion ratio (FCR) was computed using the equation: FCR = feed intake (g) / weight gain (g).

### 3.6 Terminal procedures

At the end of the feeding trial, the birds were fasted for 4 hours prior to slaughter but had access to clean drinking water. Immediatley after the fast, the terminal body weight (TBW) of each bird was determined by weighing each bird on an electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan). Thereafter each bird was humanely slaughtered via exsanguination using a guillotine. Blood from each bird was collected into 10 ml heparinised blood collection tubes (Becton Dickinson Vacutainer Systems, Meylan Cedex, France). A few drops of the heparinised blood were used to determine the packed cell volume (PCV) and haemoglobin concentration using a haemoglobin meter (URIT Digital HB Meter, Accurex

Biomedical Pty. Ltd) and the fasting blood glucose of each bird using glucometer (Accu-Check® Active, Roche Diagnostics, Mannheim, Germany). The rest of the blood was centrifuged at 5500g for 15-min. Plasma was harvested and stored at -20°C until the determination of plasma surrogate markers of kidney and liver function.

### 3.6.1 Determination of viscera macro-morphometry

Following plucking of the feathers from each carcass, viscera [heart, liver, proventriculus (stomach), ventriculus (gizzard), pancreas, small and large intestines, caeca, kidneys, visceral fat, and testis (in cocks)] were carefully dissected out. The weights and lengths (small and large intestines) of the visceral organs were measured using a calibrated Precisa 310M electronic balance (Precisa Instruments AG, Precisa, Dietikon, Switzerland) and a ruler fixed on a cooled dissection board. A sample of the liver was cut and stored in phosphate buffered formalin for possible histological analyses. The remainder of the liver was stored in a freezer (Haier Biomedical, China) at -20°C pending the determination of hepatic lipid content.

## 3.7-Determination of surrogate markers of liver and kidney function

The activity in plasma of aspartate aminotransferase (AST), alkaline phosphatase (ALP), plasma uric acid, creatinine concentration, cholesterol, total bilirubin, blood urea nitrogen and triglyceride concentrations were determined using a colorimetric-based IDEXX Clinical Veterinary System (IDEXX VetTest® Clinical Chemistry Analyser, Johannesburg, South Africa) according to manufacturer’s instructions. Briefly, plasma samples from each bird were thawed to room temperature.  Thereafter, 150 µL of each plasma sample was automatically pipetted. Immediately, thereafter 10 µL of the sample was loaded onto each pre-inserted disk and the assays then done by the clinical calorimeter. Results of the assay were then obtained from a print-out.

## 3.8 Determination of thiobarbituric-acid-reactive substances

The plasma thiobarbituric-acid-reactive substances (TBARS) activity-of each bird was determined using a commercial enzyme-based assay kit (OxiSelect™ TBARS Assay Kit-MDA Quantitation) as per the manufacturer’s instructions. The samples were thawed and acid-treated with TBARS acid reagent to precipitate interfering proteins and other substances. The samples were then incubated at room temperature for 15 minutes and then centrifuged in an microtube-centrifuge

(Eppendorf, Hamburg, Germany) at 12,000 x G for 4 minutes. The supernatant was then removed and used for the analysis. The optical density was pre-read at 532nm using a microplate reader (Bio-Tek Instruments, Vermont, USA). The samples were then incubated at 48ºC in an oven (Labcon, South Africa) for two and half hours and the optical densities were read again. The initial optical densities were then subtracted from the final readings. A standard curve was generated from the test standards and used to determine the TBARS concentrations of the test samples.

### 3.9 Determination of liver lipid content

Prior to the determination of liver lipid content, the frozen stored liver samples were allowed to defrost and thaw to room temperature. Thereafter, the fat content in the liver was determined using a solvent extraction procedure as described by Bligh and Dyer, (1959). Briefly, 5 g liver samples were steeped into a 150ml chloroform-methanol (2:1) solution overnight at 4°C. The next morning, each mixture was filtered using Whatman filter papers (Whatman®, No 1, size 185mm, pore size 7-11µm, England) into a separation funnel. Saline (30 ml, 0.9%) was added to each filtrate and mixture was allowed to stand at 4°C overnight by which time it separated into-two phases. The bottom phase was collected and reduced to dryness under vacuum at 37°C. An aliquot of 2ml of the extract was then placed into a dried, pre-weighed vial, and dried at 50°C for 30-min in an oven (Salvis ®, Salvis Lab, Switzerland) and then cooled in a desiccator and then reweighed to determine the lipid content. The liver lipid content was computed on the basis of the liver weight.

### 3.10 Determination of long bone indices

The tibia and femur from the left leg of each bird were dissected from each carcass, defleshed and all soft tissues were carefully removed. The bones were left to dry in an oven (Salvis®, Salvis Lab, Switzerland) at 50°C for 5 days to constant weight. The dried bones were allowed to cool at room temperature and the weight of each bone was measured on a digital scale (Snowrex, model: EQ: 1200, Taiwan, China) and the length of each bone was measured using an electronic digital Vernier calliper (SDP-S-ETP-1001, Major Tech, Johannesburg, South Africa). Tibia and femur length was determined by measuring the distance from the centre of the lateral condylar surface to the centre of the distal articular surface (Balakrishnan *et al*., 2018). The bone mass to length ratio was computed using the equation: Bone mass to length = dry mass of dry bone (mg)/ length of dry bone (mm).The tibiae breaking strength were determined using an Instron Materials tester (Model 4411, Instron Corp., Canton, MA) as per the manufacturer's instructions.

### 3.11 Determination of meat quality

#### 3.11.1 Determination of the meat's pH and colour

The initial pH (pHi) and ultimate pH (pHu) of the breast (*Pectoralis major*) and thigh muscles (*Fibular longus*) muscle of each carcass was measured 30 min and 24 hours post-slaughter, respectively, using a digital pH meter with a piercing electrode (CRISON pH25, CRISON instruments, SA, Spain) following a two-point (pH 4.0 and pH 7.0) calibration.

The colour [Lightness (L*), Redness (a*) and Yellowness (b*)] of the meat (*Pectoralis major* and *Fibular longus* muscles) was determined 30-min and 24-hours post-slaughter (following overnight storage of the carcasses at 4˚C) using a Lovibond colour meter (LC 100 Spectrophotometer, Lasec, SA, China) as per the manufacturer's instructions. Three points from each of the breast muscle were sampled in the determination of colour and the average of the points was recorded.

#### 3.11.2 Determination of the meat's drip loss

Drip loss was measured as described by Strydom *et al*. (2016). In brief, each 50g sub-sample (from the matured breast muscles) was sliced into 10 × 10 × 20mm strips. Each of the strips was weighed using an electronic balance to determine the initial weight of each strip. Thereafter, each strip was hung on a pin inside a 200ml sample bottle and stored at 4°C for 72 hours. Following the 72-hour storage at 4°C, the strips of meat were re-weighed to determine the final weight. Drip loss was then calculated as the difference between the initial weight and the final weight of the strip and expressed as a percentage of initial weight using the equation: percentage drip loss percentage = W1 –W2 W1 x 100; where W1 was the initial weight of the sub-sample and W2 the final weight of the sub-sample.

#### 3.11.3 Determination of the meat's tenderness

The Warner Bratzler shear force of the meat was determined as described by Raharjo *et al*., (1992). In summary, the fillet cuts obtained from the cooked breast muscles were sheared once in the centre. Shearing was done by using a Warner-Bratzler shear force machine with a Warner-

Bratzler Shear mounted on a Universal Instron Machine (Model 4301) (Instron Corporation, 1990). The shear force was determined at a cross speed of 200mm/min with a 1kN load cell as recommended by Stock and Board, (1995). The results of shear force were captured by a computer mounted to the Universal Instron machine (Model 4301, Instron Ltd, Buckinghamshire, England). The average shear force from the six cores per sub-sample was then recorded on a computer screen staged on the Universal Testing Machine.

### 3.12 Determination of the meat's proximate content

The proximate components (crude protein, ether extract and ash) of the breast meat were determined using standard AOAC procedures (AOAC2005: method numbers 930.15, 984.01, 920.39 and 942.15 respectively).

### 3.13 Determination of the meat's fatty acid profile

Prior to the determination of the fatty acid profile of the breast-meat oil was extracted from each meat sample using the Soxtet Apparatus as described by the Association of Analytical Chemists (AOAC, 2005; method number 920.39). The extracted oil was used to determine the fatty acid profile as described by Christopherson and Glass, (1960). In summary, the oil extracts were trans-methylated with 2mol/L of methanol sodium hydroxide and the resultant fatty acid methyl esters were extracted in heptane, filtered and dried under nitrogen after which they were separated by a temperature gradient over 45 minutes on a gas chromatograph with nitrogen as carrier gas using a DB-23 capillary column (90cm × 250μm × 0.25μm) (Supelco, Sigma-Aldrich). The detector and injector temperatures were set at 300°C. A PC equipped with Chemstation software (Agilent Technologies Inc., Santa Clara, CA, USA) was used for fatty acid quantification. Nonadecanoic (C19:0) acid was used as an internal standard.

### 3.14 Determination of the meat's calcium, magnesium, phosphorus and iron content

The meat's Ca, Mg, P and F was determined as described by Huang and Schulte, (1985). Briefly, 0.5g of the meat sample was digested into 25ml of 65% nitric acid and 5ml of perchloric acid at 200°C. The digest solution was then used to spectrophotometrically determine the mineral content of the meat using inductively coupled plasma-optical emission spectrometry (ICP-OES) on a Varian Liberty 200 spectrometer (Varian, Perth, Australia).

### 3.15 Data analysis

All parametric data are expressed as mean ± SD and non-parametric data expressed as median and range. The data were analysed using GraphPad Prism 5 software (Graph-Pad Software Inc., San Diego, CA, USA). The weekly body masses and feed intake of birds within groups were analysed using repeated measures analysis of variance (ANOVA). A one-way ANOVA was used to analyse all other multiple group parametric data. Tukey's *post-hoc* test was used to compare means. Statistical significance was set at $P < 0.05$.

# CHAPTER 4: RESULTS

## 4.1. Growth performance

Table 4.1 shows the effect of graded dietary substitution of Soyabean meal with Marula nut meal on the growth performance, feed intake and feed utilisation efficiency in broiler Guinea fowl. During the grower phase, except for in week 2 where GF fed diet 3 (50% SBM substitution with MNM) had the highest ($P < 0.05$) BWG as well as the highest ADG ($P < 0.05$)-dietary MNM did not affect ($P > 0.05$) the GF's BWG, ADG, FI (weeks 1 to 4) and FCR. However in week 5 of the grower phase GF fed diet 5 (100% substitution of SBM with MNM) had the highest ($P < 0.01$) FI.

During the finisher phase, dietary MNM did not affect weekly (week 6 to 8) BWG, ADG and FI of the GF. However, in week 3 (week 8 of the feeding trial) of the finisher phase, GF fed diets 2 and 3 had the similar ($P > 0.05$) but highest ($P < 0.05$) FI. Overall (grower and finisher phases combined), dietary MNM had no effect on BWG, ADG and FI however GF reared on diet 3 had the highest ($P < 0.05$) FCR.

**Table 4.1: Effect of graded dietary substitution of Soyabean meal with Marula nut meal on the growth performance and feed utilisation efficiency, empty carcass mass and dressing percentage of broiler Guinea fowl**

| | | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Week** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| Induction weight (g) | | 241 ± 50.5[a] | 288 ± 62.9 [a] | 291 ± 68.1 [a] | 212 ± 95.6 [a] | 241 ± 95.9 [a] | ns |
| Terminal body weight (g) | | 869 ± 88.1[a] | 954 ± 115[a] | 968 ± 233[a] | 771 ± 222[a] | 809 ± 185[a] | ns |
| **Grower phase** | | | | | | | |
| BWG (g) | 1 | 35.0 ± 11.4[a] | 52.9 ± 13.8 [a] | 50.0 ± 15.7 [a] | 29.1 ± 15.3[a] | 47.8 ± 23.3[a] | ns |
| | 2 | 80.4 ± 19.4[a] | 74.4 ± 27.4[a] | 107 ± 38.7[b] | 63.3 ± 20.9[a] | 67.3 ± 31.1[a] | * |
| | 3 | 58.9 ± 26.4 [a] | 89.0 ± 49.4 [a] | 93.8 ± 39.4 [a] | 65.1 ± 33.8 [a] | 43.4 ± 29.6 [a] | ns |
| | 4 | 119 ± 26.8 [a] | 87.0 ± 30.6 [a] | 83.5 ± 40.5 [a] | 96.0 ± 37.8 [a] | 127 ± 62.5 [a] | ns |
| | 5 | 77.0 ± 25.5 [a] | 104 ± 52.4 [a] | 97.6 ± 36.4 [a] | 78.4 ± 45.3 [a] | 90.8 ± 26.2 [a] | ns |
| ADG (g/d) | 1 | 5.00 ± 1.63[a] | 7.55 ± 1.97[a] | 7.14 ± 2.25[a] | 4.16 ± 2.16[a] | 6.82 ± 3.33[a] | ns |
| | 2 | 11.5 ± 2.77[a] | 10.6 ± 3.91[a] | 15.2 ± 3.91[b] | 9.04 ± 2.99[a] | 9.61 ± 4.44[a] | * |
| | 3 | 8.41 ± 3.77[a] | 12.7 ± 7.05[a] | 13.4 ± 5.63 [a] | 9.31 ± 4.82[a] | 6.95 ± 3.67[a] | ns |
| | 4 | 16.9 ± 3.82[a] | 12.4 ± 4.37[a] | 11.9 ± 5.78[a] | 13.7 ± 5.41[a] | 18.0 ± 8.63[a] | ns |
| | 5 | 11.0 ± 3.624[a] | 14.8 ± 7.48[a] | 13.9 ± 5.20[a] | 11.2 ± 6.47[a] | 13.0 ± 3.74[a] | ns |
| FI (g) | 1 | 192.0 ± 23.2[a] | 215.0 ± 39.5[a] | 212.0 ± 41.8[a] | 181.0 ± 62.6[a] | 203.0 ± 39.2[a] | ns |
| | 2 | 145.0 ± 21.1[a] | 172.0 ± 25.4[a] | 178.0 ± 28.1[a] | 133.0 ± 42.4[a] | 142.0 ± 27.4[a] | ns |
| | 3 | 181.0 ± 27.7[a] | 190.0 ± 29.7[a] | 204.0 ± 39.7[a] | 164.0 ± 40.5[a] | 174.0 ± 34.3[a] | ns |
| | 4 | 200.0 ± 25.2[a] | 219.0 ± 28.2[a] | 224.0 ± 43.2[a] | 178.0 ± 50.0[a] | 206.0 ± 34.7[a] | ns |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 202.0 ± 17.7[a] | 253.0 ± 46.4[b] | 265.0 ± 52.5[b] | 182.0 ± 54.1[a] | 205.0 ± 43.9[a] | ** |
| FCR | 1 | 6.15 ± 2.58[a] | 4.22 ± 0.84[a] | 4.63 ± 1.87[a] | 7.72 ± 4.15[a] | 5.64 ± 3.95[a] | ns |
| | 2 | 1.89 ± 0.47[a] | 2.82 ± 1.83[a] | 1.92 ± 0.85[a] | 2.31 ± 1.08[a] | 2.53 ± 1.14[a] | ns |
| | 3 | 3.52 ± 1.31[a] | 2.44 ± 0.67[a] | 3.03 ± 2.57[a] | 3.25 ± 2.39[a] | 7.44 ± 7.26[a] | ns |
| | 4 | 1.77 ± 0.46[a] | 3.18 ± 2.38[a] | 7.35 ± 14.0[a] | 2.28 ± 1.59[a] | 1.98 ± 0.95[a] | ns |
| | 5 | 2.49 ± 1.03[a] | 2.60 ± 0.91[a] | 2.99 ± 0.935[a] | 3.05 ± 2.90[a] | 2.41 ± 0.74[a] | ns |
| **Finisher phase** | | | | | | | |
| BWG (g) | 6 | 70.7 ± 26.7[a] | 74.5 ± 45.5[a] | 103.0 ± 34.3[a] | 63.9 ± 34.9[a] | 77.9 ± 21.8[a] | ns |
| | 7 | 73.7 ± 37.2[a] | 71.5 ± 28.3[a] | 62.9 ± 34.0[a] | 80.0 ± 49.4[a] | 54.6 ± 40.4[a] | ns |
| | 8 | 68.9 ± 35.2[a] | 99.2 ± 65.4[a] | 45.6 ± 42.0[a] | 78.5 ± 54.6[a] | 77.6 ± 64.4[a] | ns |
| ADG (g/d) | 6 | 10.1 ± 3.82[a] | 10.6 ± 6.50[a] | 14.7 ± 4.90[a] | 9.12 ± 4.99[a] | 11.1 ± 3.12[a] | ns |
| | 7 | 10.5 ± 5.32[a] | 10.2 ± 4.04[a] | 8.98 ± 4.85[a] | 11.4 ± 7.05[a] | 7.80 ± 5.77[a] | ns |
| | 8 | 9.08 ± 4.91[a] | 12.3 ± 8.07[a] | 5.47 ± 5.47[a] | 10.8 ± 8.34[a] | 11.0 ± 9.83[a] | ns |
| FI | 6 | 245.0 ± 20.1[a] | 271.0 ± 43.7[a] | 293.0 ± 51.1[a] | 235.0 ± 52.3[a] | 248.0 ± 49.2[a] | ns |
| | 7 | 266.0 ± 24.6[a] | 291.0 ± 47.8[a] | 320.0 ± 59.6[a] | 264.0 ± 56.7[a] | 278.0 ± 47.8[a] | ns |
| | 8 | 296.0 ± 32.8[a] | 311.0 ± 38.5[a] | 344.0 ± 65.2[a] | 293.0 ± 62.2[a] | 303.0 ± 40.7[a] | ns |
| FCR | 6 | 3.86 ± 1.30[a] | -1.85 ± 15.2[a] | 3.03 ± 0.69[a] | 7.44 ± 9.98[a] | 3.31 ± 0.86[a] | ns |
| | 7 | 3.71 ± 1.62[a] | 5.52 ± 4.87[a] | 7.12 ± 5.38[a] | 4.92 ± 4.18[a] | 2.71 ± 5.18[a] | ns |
| | 8 | 6.64 ± 5.01[a] | 3.92 ± 1.63[a] | 29.8 ± 32.6[b] | 9.46 ± 13.1[a] | 1.48 ± 5.15[a] | * |
| **Overall (week 1-8)** | | | | | | | |
| BWG (g) | | 578.0 ± 101[a] | 639.0 ± 67.5[a] | 635.0 ± 152[a] | 552.0 ± 205[a] | 585.0 ± 139[a] | ns |
| ADG | | 10.3 ± 1.80[a] | 11.4 ± 1.21[a] | 11.3 ± 2.72[a] | 9.85 ± 3.67[a] | 10.4 ± 2.48[a] | ns |

| | | | | | | |
|---|---|---|---|---|---|---|
| FI | 1726.0 ± 99.2[a] | 1921.0 ± 270[a] | 2039.0 ± 370[a] | 1631.0 ± 388[a] | 1758.0±288[a] | ns |
| FCR | 30.0 ± 8.34[a] | 22.8 ± 17.7[a] | 59.9 ± 37.2[b] | 40.4 ± 24.9[a] | 27.5 ± 9.87[a] | * |

ns = not significant, *P < 0.05, **P < 0.01, [a, b]Within row means with the same superscripts are not significantly different (P > 0.05). BWG- body weight gain, ADG - average daily gain, FI - feed intake, FCR - feed conversion ratio Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8.

### 4.2 Femora and tibiae length, mass and mass to length ratio

Table 4.2 shows the effect of graded dietary substitution of Soyabean meal with Marula nut meal on the lengths, masses and densities of tibiae and femora of broiler Guinea fowl. Dietary MNM had no effect ($P>0.05$) on the tibiae and femora lengths, masses and mass to length ratio of the Guinea fowl.

**Table 4.2: Effect of graded dietary substitution of Soyabean meal with Marula nut meal on tibiae and femora length, mass and bone mass to length ratio of broiler Guinea fowl**

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| **Tibiae** | | | | | | |
| Length (mm) | 87.7 ± 22.3[a] | 98.7 ± 7.60[a] | 101 ± 11.5[a] | 93.4 ± 10.5[a] | 100 ± 5.41[a] | ns |
| Mass (mg) | 3528.0 ± 461[a] | 3624.0 ± 908[a] | 3999.0 ± 1320[a] | 3025.0 ± 1085[a] | 3538.0 ± 891[a] | ns |
| Mass:length ($mg.mm^{-1}$) | 45.8 ± 22.1[a] | 36.4 ± 7.45[a] | 38.7 ± 10.1[a] | 31.9 ± 8.49[a] | 35.2 ± 8.00[a] | ns |
| **Femora** | | | | | | |
| Length (mm) | 67.6 ± 2.37[a] | 74.2 ± 6.69[a] | 74.7 ± 7.02[a] | 65.9 ± 7.85[a] | 70.7 ± 9.21[a] | ns |
| Mass (mg) | 2613.0 ± 418[a] | 2873.0 ± 889[a] | 3225.0 ± 903[a] | 2340.0 ± 989[a] | 2755.0 ± 836[a] | ns |
| Mass:length ($mg.mm^{-1}$) | 38.6 ± 5.57[a] | 38.6 ± 10.8[a] | 42.5 ± 9.53[a] | 34.6 ± 11.0[a] | 38.2 ± 8.60[a] | ns |

ns = not significant, $P > 0.05$. Femora lengths, masses and mass to length ratio of the birds across dietary treatments were similar ($P > 0.05$). Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8.

### 4.3 Viscera morphometry

Table 4.3 shows the effect of graded dietary substitution of Soyabean meal with Marula nut meal, on a CP basis, on the macro-morphometry of the GIT and GIT accessory viscera of broiler Guinea fowl. Dietary MNM had no effect ($P>0.05$) on the masses and lengths (where relevant) of GIT and GIT accessory visceral organs.

**Table 4.3: Effect of graded dietary substitution of Soyabean meal with Marula nut meal on viscera macro morphometry of broiler Guinea fowl**

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| **Liver (g)** | 13.6 ± 2.78[a] | 14.5 ± 2.98[a] | 14.8 ± 2.56[a] | 11.1 ± 3.56[a] | 11.6 ± 3.42[a] | ns |
| (% body mass) | 1.57 ± 0.23[a] | 1.52 ± 0.21[a] | 1.60 ± 0.41[a] | 1.44 ± 0.48[a] | 1.51 ± 0.48[a] | ns |
| **Pancreas (g)** | 1.43 ± 0.53[a] | 1.44 ± 0.32[a] | 1.56 ± 0.17[a] | 1.36 ± 0.63[a] | 1.25 ± 0.46[a] | ns |
| (% body mass) | 0.16 ± 0.05[a] | 0.15 ± 0.03[a] | 0.17 ± 0.06[a] | 0.17 ± 0.05[a] | 0.16 ± 0.06[a] | ns |
| **Proventriculus (g)** | 2.29 ± 0.75[a] | 2.13 ± 0.954[a] | 5.44 ± 9.55[a] | 2.14 ± 1.07[a] | 2.19 ± 0.70[a] | ns |
| (% body mass) | 0.26 ± 0.07[a] | 0.23 ± 0.10[a] | 0.56 ± 0.92[a] | 0.26 ± 0.08 | 0.28 ± 0.12[a] | ns |
| **Ventriculus (g)** | 23.7 ± 6.29[a] | 26.4 ± 4.66[a] | 26.8 ± 9.40[a] | 20.4 ± 10.5[a] | 23.0 ± 9.86[a] | ns |
| (% body mass) | 2.71 ± 0.52[a] | 2.76 ± 0.23[a] | 2.95 ± 1.33[a] | 2.55 ± 1.13[a] | 2.89 ± 1.24[a] | ns |
| **Small intestines (g)** | 12.1 ± 1.95[a] | 14.6 ± 2.91[a] | 14.6 ± 3.49[a] | 11.9 ± 3.08[a] | 13.9 ± 3.10[a] | ns |
| (% body mass) | 1.39 ± 0.13[a] | 1.52 ± 0.16[a] | 1.61 ± 0.62[a] | 1.59 ± 0.43[a] | 1.82 ± 0.66[a] | ns |
| Small intestines length (mm) | 711 ± 57.9[a] | 783 ± 55.5[a] | 764 ± 74.6[a] | 703 ± 35.8[a] | 736 ± 91.0[a] | ns |
| **Large intestines (g)** | 1.29 ± 0.39[a] | 1.38 ± 0.35[a] | 1.44 ± 0.17[a] | 1.29 ± 0.75[a] | 1.38 ± 0.35[a] | ns |
| (% body mass) | 0.14 ± 0.03[a] | 0.14 ± 0.03[a] | 0.16 ± 0.06[a] | 0.15 ± 0.05[a] | 0.17 ± 0.17[a] | ns |
| Large intestines length (mm) | 85.0 ± 7.07[a] | 86.9 ± 6.51[a] | 83.1 ± 9.98[a] | 78.6 ± 15.7[a] | 80.0 ± 8.86[a] | ns |
| **Caecum (g)** | 2.07 ± 0.45[a] | 1.88 ± 0.74[a] | 1.88 ± 0.64[a] | 1.64 ± 0.55[a] | 1.69 ± 0.25[a] | ns |
| (% body mass) | 0.23 ± 0.03[a] | 0.20 ± 0.09[a] | 0.20 ± 0.08[a] | 0.21 ± 0.06[a] | 0.21 ± 0.07[a] | ns |

n.s = not significant, $P > 0.05$. [a] Within row means with the same superscripts are not significantly different ($P > 0.05$). Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of

SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8

## 4.4 General health profile

Table 4.4 shows the effect of graded dietary substitution of SBM with MNM, on CP basis, on circulating metabolic substrate (glucose, triglyceride and cholesterol) and stored (liver lipid) content, haematocrit, plasma surrogate markers of liver and kidney function and other markers of general health of broiler Guinea fowl. Dietary MNM had no effect ($P>0.05$) on the circulating metabolic substrate (circulating and stored) content, haematocrit and plasma surrogate markers of liver and kidney function of the Guinea fowl.

**Table 4.4: Effect of graded dietary substitution of Soyabean meal with Marula nut meal on circulating stored metabolic substrate content haematocrit, plasma surrogate markers of liver and kidney function, urea nitrogen, uric acid and thiobarbituric acid reactive substances of Guinea fowl**

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| Glucose (mg/dL) | 13.9 ± 1.25 | 14.0 ± 1.14 | 14.6 ± 1.39 | 14.4 ± 2.41 | 14.7 ± 1.83 | ns |
| Triglyceride (mg/dL) | 0.72 ± 0.33 | 0.57 ± 0.31 | 0.62 ± 0.55 | 0.51 ± 0.36 | 0.72 ± 0.35 | ns |
| Cholesterol (mmol/L) | 2.81 ± 0.67 | 2.39 ± 0.63 | 2.54 ± 1.08 | 2.57 ± 0.76 | 3.14 ± 1.41 | ns |
| Liver lipid content (%) | 27.2 ± 28.9 | 23.7 ± 21.4 | 12.2 ± 10.7 | 25.2 ± 29.0 | 22.6 ± 15.2 | ns |
| Haematocrit (%) | 27.7 ± 10.7 | 30.9 ± 5.67 | 27.5 ± 6.70 | 28.0 ± 8.02 | 26.1 ± 5.19 | ns |
| Total bilirubin (mg/dL) | 1.71 ± 0.00 | 1.71 ± 0.0 | 1.71 ± 0.00 | 1.71 ± 0.00 | 1.71 ± 0.0 | ns |
| AST (U/L) | 298 ± 55.1 | 302 ± 93.9 | 280 ± 80.1 | 322 ± 142 | 307 ± 64.8 | ns |
| ALP (U/L) | 289 ± 131 | 251 ± 126 | 235 ± 110 | 219 ± 47.3 | 229 ± 44.0 | ns |
| BUN (mg/dL) | 0.71 ± 0.00 | 0.83 ± 0.29 | 0.71 ± 0.00 | 0.71 ± 0.00 | 0.71 ± 0.0 | ns |
| Uric acid (µmol/L) | 174 ± 63.9 | 203 ± 41.1 | 236 ± 44.5 | 179 ± 38.8 | 217 ± 83.8 | ns |
| TBARS (Nm/mL) | 0.04 ± 0.03 | 0.02 ± 0.09 | 0.02 ± 0.07 | 0.015 ± 0.04 | 0.03 ± 0.20 | ns |

n.s = not significant, P > 0.05. [a]Within row means with the same superscripts are not significantly different (P > 0.05). Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8.

## 4.5 Meat quality

### 4.5.1 Physical characteristics of breast meat

Table 4.5 and 4.6 shows the effect of graded dietary substitution of SBM with MNM on meat yield, the meat's pH, colour, moisture characteristics and tenderness. Dietary MNM had no effect (P>0.05) on the carcass yield (empty carcass mass and dressing percentage), pH, colour and tenderness of the GF thigh and breast meat.

**Table 4.5: Effect of graded substitution of Soyabean meal with Marula nut meal on broiler Guinea fowl carcass yield, breast meat pH, colour, drip and cooking loss and tenderness**

| | | | Dietary treatments | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| **Carcass yield** | | | | | | |
| Empty carcass mass (g) | 648 ± 61.6 | 717 ± 85.8 | 733 ± 182 | 589 ± 179 | 620 ± 155 | ns |
| Dressing percentage | 74.7 ± 1.09 | 75.2 ± 1.05 | 75.6 ± 8.01 | 76.0 ± 2.26 | 76.6 ± 9.78 | ns |
| **30 mins post slaughter** | | | | | | |
| Muscle $pH_i$ | 5.96 ± 0.23 | 5.91 ± 0.21 | 6.12 ± 0.27 | 5.87 ± 0.33 | 5.88 ± 0.29 | ns |
| Lightness (L*) | 44.0 ± 2.92 | 42.8 ± 3.07 | 43.6 ± 1.15 | 43.8 ± 3.66 | 51.4 ± 13.0 | ns |
| Redness (a*) | 2.91 ± 1.12 | 2.58 ± 1.27 | 2.68 ± 1.12 | 3.17 ± 0.79 | 2.97 ± 2.92 | ns |
| Yellowness (b*) | 14.2 ± 1.62 | 13.5 ± 2.35 | 14.1 ± 2.52 | 13.9 ± 2.91 | 12.5 ± 5.14 | ns |
| Chroma (C*) | 14.8 ± 1.68 | 13.8 ± 2.43 | 14.5 ± 2.73 | 14.4 ± 2.90 | 12.7 ± 5.29 | ns |
| Hue (H*) | 78.4 ± 4.37 | 79.2 ± 4.21 | 79.6 ± 3.72 | 76.2 ± 4.36 | 86.3 ± 12.3 | ns |
| **24 hrs. post-slaughter** | | | | | | |
| Muscle $pH_u$ | 5.80 ± 0.305 | 5.87 ± 0.38 | 6.01 ± 0.29 | 6.01 ± 0.20 | 5.99 ± 0.22 | ns |
| Lightness (L*) | 48.2 ± 2.63 | 52.2 ± 18.2 | 48.8 ± 3.26 | 52.8 ± 24.1 | 52.8 ± 17.8 | ns |
| Redness (a*) | 3.94 ± 3.29 | 2.83 ± 1.92 | 2.84 ± 1.67 | 1.94 ± 1.95 | 3.00 ± 2.43 | ns |
| Yellowness (b*) | 16.6 ± 2.46 | 15.3 ± 6.05 | 17.1 ± 2.73 | 12.9 ± 8.26 | 13.8 ± 8.01 | ns |
| Chroma (C*) | 17.0 ± 2.54 | 15.7 ± 5.92 | 17.4 ± 2.91 | 13.2 ± 8.35 | 14.8 ± 7.28 | ns |
| Hue (H*) | 78.9 ± 4.38 | 87.0 ± 23.1 | 81.1 ± 3.91 | 90.7 ± 18.8 | 85.4 ± 15.6 | ns |
| **Moisture characteristics** | | | | | | |
| Drip loss % | 3.90 ± 1.09 | 4.03 ± 1.22 | 4.03 ± 1.47 | 3.36 ± 2.41 | 2.70 ± 4.40 | ns |

| | | | | | | |
|---|---|---|---|---|---|---|
| Cooking loss % | 14.0± 2.11 | 13.8 ± 2.34 | 15.0 ± 2.87 | 13.7 ± 1.80 | 15.2± 5.31 | ns |
| **Tenderness** | | | | | | |
| Shear force (N/cm$^2$) | 8.45 ± 2.69 | 8.14 ± 3.57 | 9.30 ± 2.62 | 7.31 ± 1.61 | 7.36 ± 1.51 | ns |

ns = not significant, [a]Within row means with the same superscripts are not significantly different (P > 0.05). Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8.

**Table 4.6: Effect of graded substitution of Soyabean meal with Marula nut meal on broiler Guinea fowl carcass yield and thigh meat pH and colour**

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| **30 mins post slaughter** | | | | | | |
| Muscle $pH_i$ | 6.13 ± 0.17 | 6.12 ± 0.07 | 6.10 ± 0.09 | 6.22±0.14 | 6.14 ± 0.13 | ns |
| Lightness (L*) | 39.2 ± 2.91 | 40.6 ± 4.48 | 40.5 ± 1.02 | 39.0 ± 2.85 | 48.0 ± 15.4 | ns |
| Redness (a*) | 9.30 ± 1.47 | 8.05 ± 2.43 | 9.86 ± 1.93 | 9.88 ± 1.76 | 7.35 ± 3.84 | ns |
| Yellowness (b*) | 14.1 ± 2.13 | 13.9 ± 3.17 | 14.9 ± 2.33 | 14.4 ± 2.02 | 11.1 ± 4.39 | ns |
| Chroma (C*) | 16.8 ± 2.13 | 16.3 ± 3.20 | 18.0 ± 2.64 | 17.8 ± 2.59 | 13.3 ± 5.41 | ns |
| Hue (H*) | 55.4 ±5.46 | 59.7 ± 8.58 | 56.8 ± 5.08 | 54.7 ± 3.11 | 95.1 ± 90.6 | ns |
| **24 hrs. post-slaughter** | | | | | | |
| Muscle $pH_u$ | 6.14 ± 0.29 | 6.16 ± 0.41 | 6.22 ± 0.33 | 6.17 ± 0.33 | 6.23 ± 0.35 | ns |
| Lightness (L*) | 45.3 ± 6.09 | 52.7 ± 18.7 | 43.7 ± 4.47 | 58.7 ± 27.2 | 48.7± 10.1 | ns |
| Redness (a*) | 8.22 ± 2.47 | 7.36±4.03 | 8.30 ± 2.74 | 7.06 ± 5.60 | 7.06 ± 3.91 | ns |
| Yellowness (b*) | 13.8 ± 4.21 | 11.0 ± 6.56 | 12.9 ± 6.22 | 11.4 ± 7.54 | 11.4 ± 5.95 | ns |
| Chroma (C*) | 16.1 ± 4.29 | 13.6 ± 7.04 | 15.8 ± 6.04 | 13.7 ± 8.74 | 18.8 ± 16.2 | ns |
| Hue (H*) | 55.4 ± 5.46 | 59.7 ± 8.58 | 56.8 ± 5.08 | 54.7 ± 3.11 | 95.1 ± 90.6 | ns |

ns = not significant, [a]Within row means with the same superscripts are not significantly different (P > 0.05). Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8.

### 4.5.2 Chemical composition of breast meat

#### *4.5.2.1 Proximate composition*

Table 4.7 shows the effect of graded dietary substitution of SBM with MNM as a dietary protein source on the proximate content of broiler Guinea fowl breast meat. Breast meat of GF fed diet 3 and 4 had the highest ($P < 0.001$) fat content compared to those fed other diets. However, dietary MNM did not affect ($P > 0.05$) the crude protein and ash content of the breast meat.

**Table 4.7: Effect of graded substitution of Soyabean meal with Marula nut meal on the proximate content of Guinea fowl breast meat**

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| Protein (% DM) | 81.6 ± 2.00[a] | 82.42±0.08[a] | 82.47 ± 0.41[a] | 82.81± 0.46[a] | 82.13 ± 0.62[a] | ns |
| Fat (% DM) | 1.82 ± 0.09[a] | 3.09 ± 0.02[b] | 9.60 ± 0.13[c] | 5.63 ± 0.17[c] | 2.66 ± 0.17[b] | *** |
| Ash (% DM) | 4.43 ± 0.16[a] | 4.14 ± 0.03[a] | 4.26 ± 0.09[a] | 4.25 ± 0.06[a] | 4.29 ± 0.01[a] | ns |

ns = not significant, P> 0.05, *** P < 0.001. [abc]Within row means with different superscripts are significantly different at P ≤ 0.05. Crude protein and ash components of the breast muscles were similar (P > 0.05) across dietary treatments. Breast meat of GF fed diet 3 and 4 had the highest (P < 0.001) fat content compared to those fed other diets. Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8.

#### *4.5.2.2 Mineral composition*

Table 4.8 shows the effect of graded dietary substitution of SBM with MNM as a dietary protein source on the mineral omposition of broiler Guinea fowl breast meat. Breast meat of GF fed diet 1 and 3 had the highest ($P < 0.0001$) Ca content compared to those fed other diets. However, dietary MNM did not affect ($P > 0.05$) the Mg, P and Fe content of the GF breast meat.

**Table 4.8: Effect of graded substitution of Soyabean meal with Marula nut meal on the Mineral composition of Guinea fowl breast meat**

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| Calcium (mg/kg) | 441 ± 103[c] | 166 ± 40.4[a] | 205 ± 22.2[b] | 144 ± 16.8[a] | 140 ± 54.6[a] | *** |
| Magnesium (mg/kg) | 1028 ± 16.8[a] | 1033 ±27.1[a] | 1011 ± 42.9[a] | 1051 ± 59.5[a] | 1039 ± 15.5[a] | ns |
| Phosphorus (mg/kg) | 9149 ± 214[a] | 8968 ± 265[a] | 8928 ± 136[a] | 9109 ± 440[a] | 9145 ± 90.0[a] | ns |
| Iron (mg/kg) | 37.3 ± 3.91[a] | 29.9 ± 1.70[a] | 33.9 ± 9.76[a] | 32.9 ± 1.65[a] | 38.2 ± 6.58[a] | ns |

ns = not significant, P> 0.05, *** P < 0.001. [a,b,c] Within row means with different superscripts are significantly different at P ≤ 0.001. Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8.

#### *4.5.2.3 Fatty acid profile*

Table 4.9 shows the effect of graded dietary substitution of SBM with MNM as a dietary protein source on the fatty acid profile of the broiler Guinea fowl breast meat. Breast meat total saturated fatty acid (TSFA), total mono-unsaturated fatty acid (TMUFA) and total poly-unsaturated fatty acid (TPUFA) was similar (P>0.05) across all dietary treatments.

**Table 4.9: Effect of graded substitution of Soyabean meal with Marula nut meal on the Mineral composition of Guinea fowl breast meat**

| | Dietary treatments | | | | | |
|---|---|---|---|---|---|---|
| **Fatty acids (%)** | **Diet 1** | **Diet 2** | **Diet 3** | **Diet 4** | **Diet 5** | **Significance** |
| Saturated fatty acids | | | | | | |
| C14:0 (myristic acid) | 0.36 ± 0.00[a] | 0.37 ± 0.01[a] | 0.39 ± 0.00[a] | 0.43 ± 0.00[a] | 0.35 ± 0.01[a] | ns |
| C16:0 (palmitic acid) | 20.5 ± 0.04[a] | 21.1 ± 0.11[a] | 20.9 ± 0.05[a] | 20.7 ± 0.03[a] | 20.1 ± 0.06[a] | ns |
| C17:0 (heptadeconoic acid) | 0.15 ± 0.00[a] | 0.15 ± 0.00[a] | 0.16 ± 0.00[a] | 0.17 ± 0.00[a] | 0.16 ± 0.00[a] | ns |
| C18:0 (stearic acid) | 17.6 ± 0.09[a] | 17.3 ± 0.09[a] | 17.0 ± 0.03[a] | 16.7 ± 0.09[a] | 17.1 ± 0.15[a] | ns |
| C20:0 (arachidic acid) | 0.25 ± 0.00[a] | 0.25 ± 0.00[a] | 0.26 ± 0.00[a] | 0.30 ± 0.02[a] | 0.32 ± 0.01[a] | ns |
| C22:0 (behenic acid) | 0.29 ± 0.00[a] | 0.26 ± 0.00[a] | 0.26 ± 0.00[a] | 0.23 ± 0.00[a] | 0.28 ± 0.00[a] | ns |
| C23:0 (tricosylic acid) | 10.3 ± 0.05[a] | 10.3 ± 0.13[a] | 9.45 ± 0.05[a] | 8.59 ± 0.05[b] | 10.7± 0.08[a] | * |
| C24:0 (ligonoceric acid) | 0.22 ± 0.01[a] | 0.18 ± 0.00[a] | 0.20 ± 0.01[a] | 0.16 ± 0.06[a] | 0.19 ± 0.05[a] | ns |
| **Total saturated fatty acid** | **49.67 ± 0.18[a]** | **49.91 ± 0.35[a]** | **48.62 ± 0.14[a]** | **53.58 ± 0.25[a]** | **49.2 ± 0.32[a]** | **ns** |
| Mono-unsaturated fatty acids | | | | | | |
| C16:1 (palmitoleic acid) | 1.53 ± 0.01[b] | 1.44 ± 0.02[a] | 1.36 ± 0.06[a] | 1.29 ± 0.02[a] | 0.96[a] ± 0.01 | * |
| C18:1n9t (elaidic acid) | 0.17 ± 0.00[a] | 0.18 ± 0.00[a] | 0.18 ± 0.00[a] | 0.17 ± 0.00[a] | 0.17 ± 0.00[a] | ns |
| C18:1n9c (oleic acid) | 18.0 ± 0.09[a] | 18.7 ± 0.18[a] | 20.2 ± 0.06[a] | 22.3 ± 0.05[a] | 22.9± 0.07[a] | ns |
| C20:1 (11-eicosenoic acid) | 0.16 ± 0.00[a] | 0.18 ± 0.00[a] | 0.20 ±0.00[a] | 0.25 ± 0.00[a] | 0.21 ± 0.00[a] | ns |
| C24:1 (nervonic acid) | 2.32 ± 0.02[a] | 2.19 ± 0.04[a] | 1.82 ± 0.02[a] | 1.42 ± 0.00[a] | 1.38 ± 0.01[b] | * |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Total mono-unsaturated FA** | **22.2 ± 0.11[a]** | **22.70 ± 0.26[a]** | **23.78 ± 0.14[a]** | **26.00 ± 0.07[a]** | **25.02 ± 0.10[a]** | **ns** |
| Poly-unsaturated FA | | | | | | |
| C18:2n6c (linoleic acid) | 23.1 ± 0.05[a] | 22.2 ± 0.08[a] | 22.8 ± 0.01[a] | 22.2 ± 0.14[a] | 21.5 ± 0.16[a] | ns |
| C18:3n3 (α-linolenic acid) | 0.36 ± 0.00[a] | 0.36 ± 0.00[a] | 0.34 ± 0.00[a] | 0.30 ± 0.01[a] | 0.18 ± 0.00[b] | * |
| C20:2 (eicosadienoic acid) | 0.72 ± 0.01[a] | 0.74 ± 0.01[a] | 0.69 ± 0.00[a] | 0.73 ± 0.00[a] | 0.66 ± 0.00[a] | ns |
| C20:3n6 (dihomo-y-linolenic acid) | 0.93 ± 0.00[a] | 0.96 ± 0.02[a] | 0.94 ± 0.01[a] | 0.86 ± 0.01[a] | 0.87 ± 0.01[a] | ns |
| C20:5n3 (eicosapentaenoic acid) | 0.32 ± 0.05[a] | 0.27 ± 0.00[a] | 0.24 ± 0.05[a] | 0.15 ± 0.00[b] | 0.12 ± 0.00[b] | * |
| C22:6n3(docosahexaenoic acid) | 2.32 ± 0.02[b] | 2.19 ± 0.04[b] | 1.82 ± 0.08[a] | 1.42 ± 0.01[a] | 1.38 ± 0.01[a] | ** |
| **Total poly-unsaturated FA** | **27.7 ± 0.11[a]** | **26.7 ± 0.28[a]** | **26.8 ± 0.85[a]** | **25.7 ± 0.17[a]** | **24.7 ± 0.18[a]** | ns |

ns = not significant, P > 0.05,* P < 0.05 ** P < 0.01; [a,b]Within row means with different superscripts are significantly different at P < 0.05. TSFA, TMUFA and TPUFA were significantly similar (P>0.05) across dietary treatments. TSFA= total saturated fatty acids, TMUFA= total mono-unsaturated fatty acids, TPUFA= total poly-unsaturated fatty acids, nd= not detected. Diet 1– 0% MNM CP substitution of SBM CP contribution, Diet 2 – 25% MNM meal CP substitution of SBM CP contribution, Diet 3 – 50% MNM meal CP substitution of SBM CP contribution, Diet 4 – 75% MNM meal CP substitution of SBM CP contribution, Diet 5 – 100% MNM meal CP substitution of SBM CP contribution. Data presented as mean ± SD; n = 7 to 8

# CHAPTER 5: DISCUSSION

The current study evaluated the dietary effects of MNM on growth performance, feed economy, metabolic substrate (circulating and stored) content, aspects of the general health profile and meat quality of broiler Guinea fowl. The main study findings show dietary MNM did not affect growth performance, health profile and meat quality of Guinea fowl.

**5.1 Growth performance**

Poultry growth performance is influenced chiefly by genetic make-up and the environmental factors surrounding the birds (Zaghari *et al*., 2011; Gerber *et al*., 2015). Of the environmental factors, nutrition plays a critical role in the determination of growth performance (Nkukwana, 2018). Inadequate nutrient supply results in depressed growth performance and also affects the skeletal system resulting in poorly mineralised and weak bones that are susceptible to structural deformities (Filipowska *et al.*, 2017). Excess nutrient supply, especially excess dietary energy, results in excessive fat deposition, for example in the liver leading to the development of fatty liver disease (Cohen *et al*., 2011). The excess dietary energy also leads to excessive adiposity and higher than normal amounts of intramuscular fat which impact the nutritional quality of the product (Park *et al.*, 2018), as well as the physical properties of the meat (Drahansky *et al.*, 2016). Body weight-based indices of growth, for example, TBW, BWG and ADG have been and continue to be used to evaluate the effects of dietary interventions on poultry growth performance (Drahansky *et al*., 2016).

In the current study, in week 2 of the 5-week grower phase, Guinea fowl fed diet 3 had higher weekly BWG and ADG compared to counterparts fed other diets. During the fifth week of the grower phase, Guinea fowl fed diets 2 and 3, respectively had higher feed intakes compared to the rest but there were no differences on the FCRs of the birds across dietary treatments. No differences in weekly BWG, ADG and FI were observed during the finisher phase but in week 3 of this phase of the trial (week 8 of the feeding trial) birds fed diet 3 had a higher FCR compared to the rest. Overall (grower and finisher phases) BWG, ADG and FI were similar across dietary treatments but overall FCR of Guinea fowl fed diet 3 was highest. It can be inferred from the findings of the current study that Guinea fed diets 1, 2, 4 and 5 lagged in growth performance during the second week of the grower phase. They however compensated for this initial lag as demonstrated by similarity in BWG and ADG during weeks 4 and 5 and overall indicating compensatory growth.

Findings from the current study also suggest that in the last week of the growth phase, dietary substitution of SBM with MNM at 25 and 50% (on a CP basis) caused inefficient feed utilisation as Guinea fed these diets consumed more feed but with similar BWG and ADG to counterparts fed diets 1, 4 and 5. The similarities in the overall BWG, ADG and FI suggests that MNM can be used to substitute SBM as a dietary protein source in broiler Guinea fowl starter and finisher diets without compromising growth performance. That said, the higher overall FCR for Guinea fowl fed diet 3 suggests that substitution of SBM with MNM at 50% on a CP basis reduced the efficiency of feed utilisation. While it is difficult to provide a solid reason for this finding, it can be speculated that the poor feed utilisation efficiency might have resulted from negative associative effects from feed ingredients at this level of substitution. At the end of the 5-week grower phase, the mean body weight gain of the Guinea fowl across dietary treatment ranged from 77 g to 104 g, while at study termination (12 weeks of age) the Guinea fowl slaughter body weight ranged from 771 g to 968 g.

The observed grower body weight gain of Guinea in the current study (77 to 104 g) is not in agreement with that reported by Nahashon *et al*. (2006) which ranged from 277 to 299 g, while their 12-week body weight is higher than the 650 to 780 g mean reported by Houndonougbo *et al*. (2017) but in agreement with that observed by Bernacki *et al*. (2013) as well as that by Ebegbulem and Asuquo. (2018). Research has reported slaughter weight ranges of 950 to 975 g (Ebegbulem and Asuquo, 2018) and dressing percentages ranging from 70 to 77% ( Hughes and Jones, 1980; Nahashon *et al*., 2005). It is evident that the reported literature values for dressing percentage are comparable to the findings of this study where the dressing percentage recorded ranged from 74 to 76%. Taken together, findings from the current study suggest that MNM can potentially replace SBM in Guinea fowl grower and finisher diets without compromising growth performance and carcass (meat) yield.

While these body weight-based indices of growth performance are still of value, it is important to note that they are affected by factors such as gut fill (Steyn *et al*., 2012) and hydration status (Popkin and Rosenberg, 2011). Thus, they are not accurate measures of growth performance. However the mass, length and bone length to mass ratio of tibiae and femora are a better proxy for the evaluation of growth performance as these bones respond to growth hormone in a dose-dependent manner (Melin, 2005). Thus, bone indices measured from such anti-gravity bones can be used as better and more-accurate determinants of growth performance (Lourenço *et al.*, 2012). In the current study, dietary MNM had no effect on the length, mass and length to mass ratio (Table 4.2) of the Guinea fowl's femora and tibiae suggesting that MNM can be used as a dietary protein source in starter and finisher Guinea fowl diets without compromising growth performance.

## 5.2 Viscera morphometry

The gastrointestinal tract (GIT) comprises of organs and accessory GIT organs whose main function is to digest the ingested food into monomeric absorbable units which are then used to support maintenance and productive functions by the animal/bird (Yıldırım *et al.*, 2014). Feed ingredients and diet composition are among a host of factors affecting the development and function of the digestive system (Celi *et al.*, 2017). Moderate amounts of fibre in poultry feed have been reported to be beneficial for GIT development, health and function which manifest with increased growth performance (de Vries, 2015). On the other hand, lipid-rich feeds have been reported to have a positive influence on the composition and consistency of broiler's adipose tissue (Zollitsch *et al*., 1997; Wang *et al*., 2017). Non-conventional plant-derived dietary protein sources, for example, *Moringa oleifera* leaf meal, Baobab seed meal and *Eisenia foetida* which contain structural components in the form of coarse fibre (Svihus, 2014) have been shown to improve nutrient flow retention, yield a lower pH and better grinding in digestive sites of the GIT in poultry ( Nkukwana *et al*., 2014; Gunya, 2016; Alagbe, 2017). In the current study dietary MNM had no effect on the macro-morphometry of the proventriculus, ventriculus, small and large intestines, caeca, liver and pancreas of the Guinea fowl. The similarities in the macro-morphometry of the GIT and GIT accessory organs suggest that dietary MNM neither compromised nor improved the development and function of the gastrointestinal tract. Based on the observed similarity in the TBW and overall ADG, FI and FCR of the birds across dietary treatments, it can also be speculated that dietary MNM did not compromise the digestive and absorptive function of the GIT.

Poultry rely on the ventriculus (gizzard) to mechanically grind and mix ingested feed (Rodrigues and Choct, 2018). The size of the ventriculus is of importance in digestion of birds: increased ventriculus size increases the grinding action resulting in improved digestion as well as the incidence of gastric reflexes that serve to re-expose the digesta to pepsin in the pro-ventriculus (Wu and Ravindran, 2004). In addition the re-exposure of digesta to pepsin in the pro-ventriculus, it reduces the risk of microbial proliferation that would usually result in competition for nutrients and may cause disease. In the current study, the mass of the ventriculi ranged from 20.4 to 26.1 g across dietary treatments. The weight of the ventriculi reported in the current study is lower and thus contradicts the 24 to 39g ventriculi weight reported in 12-week old Guinea fowl (Aire *et al.*, 1980). Aguzey *et al*. (2018) explains that chickens fed pellets have reduced gizzard weights compared to those fed mesh diets thus justifying the low ventriculi weight reported in this study. Additionally, a decrease in ventriculi weight was noted with increase in dietary MNM inclusion in a diet and this pattern is in agreement with (Wu and Ravindran, 2004) who found that increase in inclusion level of protein source in a diet reduced gizzard weight. In the current study the observed lack of effect of dietary MNM on key GIT (proventriculus, ventriculus, small and large intestines) is similar to its lack of effect on GIT organs of quail (Mazizi *et al.*, 2019). While there was a decrease in the weight of the ventriculi from the Guinea fowl with an increase in dietary MNM, it can be inferred that since the gizzard weight ranged reported in the current study fell within some reported in literature, dietary MNM did not negatively impact the ventriculi weight thus suggesting that it (MNM) can be used as a dietary protein source without the risk of compromising GIT development and function.

## 5.3 Health profile

### 5.3.1 Metabolic substrate content and peroxidation status

Lipid peroxidation is one of the most commonly reported indices of oxidative stress (Moselhy *et al.*, 2013) and is a process wherein oxidants attack lipids, especially PUFAs (Ayala *et al*., 2014). Malondialdehyde (MDA) concentration is one of the indices used to estimate the degree of lipid peroxidation (Siddique *et al*., 2012). In the current study, across dietary treatments, the plasma MDA concentration and the circulating metabolic substrate (glucose, triglyceride and total cholesterol) concentrations of the Guinea fowl was similar. The plasma TBARS assay is employed as a proxy for estimating the degree oxidative stress damage. In the current study, across dietary treatments, the plasma TBARS concentration of the Guinea fowl were similar suggesting that dietary MNM did not trigger oxidative stress. Additionally the similarity in the plasma glucose, total cholesterol and triglyceride concentration in the Guinea fowl across dietary treatments-suggests that dietary MNM did not alter the metabolism of these metabolites. The blood glucose and triglyceride concentration of the birds ranged from 13.9 to 14.7 mg/dL and 0.51 to 0.72 mg/dL, respectively and are in agreement with those reported by Wallace *et al*. (2017) in 12-week old Guinea fowl.

Fatty liver disease in poultry can potentially reduce egg production and compromise health (Navarro-Villa *et al.*, 2019). The predisposing factors of fatty liver disease in birds range from nutritional imbalances, endogenous hormones (laying hens) and presence of toxins in the feed (Navarro-Villa *et al.*, 2019). Excessive dietary energy especially from carbohydrates or providing a low-fat level in the feed has been associated with the development of fatty liver (Fouad and El-Senousey, 2014). In the present study, there were no differences in the liver lipid content across dietary treatments. These findings suggest that dietary MNM did not alter lipid metabolism in the birds' livers thus can potentially be used in Guinea fowl grower and finisher diets without the risk of triggering the development of fatty liver disease.

Cholesterol can be obtained directly from the diet or it can be synthesised *de novo* from acetyl-CoA (Arnold and Kwiterovichjr, 2003). Even when dietary cholesterol intake is low, *de novo* synthesis ensures adequate production to meet the needs of various biological processes (Soliman, 2018). The blood cholesterol concentration in this study ranged from 2.3 to 3.1 mmol/L. These findings complement those reported by Uko and Ataja, (1996) in Guinea fowl. It can therefore be inferred that MNM can be used as a dietary protein source in Guinea grower and finisher diets without the risk of causing derangements in the plasma concentration of these circulating (glucose, total cholesterol and triglycerides) metabolites

### 5.3.2 Surrogate markers of liver and kidney function

The liver is the main organ involved in the biotransformation and metabolism of xenobiotics in the body (Gu and Manautou, 2008). Although measurement of liver enzyme activities in plasma is a very useful diagnostic tool of bird disease, the wide range of activity makes it difficult to interpret (Lumeij, 1997). Hepatic cell dysfunction and damage can be tracked by evaluating cytosolic enzyme aspartate aminotransferase (AST) and alkaline phosphatase (ALP) concentrations in the blood ( Giannini *et al.*, 2005; McGill, 2016) . Plasma uric acid is one of the reliable surrogate physiological biomarkers of oxidative stress (Pasalic *et al.*, 2012). In birds, uric acid is produced as the major end-product of protein metabolism in the liver and excreted by the kidney (Van der Most and Lake, 2013). Results of the current study showed similarities in the Guinea fowl’s plasma activities of AST and ALP and plasma uric acid concentration across dietary treatments. These findings suggest that the dietary inclusion of MNM did not compromise liver and kidney function thus it can be speculated that MNM can potentially be used as a dietary protein source in Guinea with no risk of triggering liver and kidney damage.

### 5.4 Meat quality

#### 5.4.1 Physical characteristics of the meat

Consumers consider meat colour to be an important quality to look out for at the point of purchase (Xazela *et al.*, 2017). Broiler meat quality is influenced by numerous factors such as production, pre-slaughter handling and lairage conditions, slaughter process, post-slaughter handling and storage as well as and packaging (Baracho *et al.*, 2006). The colour of meat is also dependent on the concentration and chemical form of myoglobin (Mb), the haem pigment found in muscle as well as the physical characteristics of the meat (Kim *et al.*, 2010). Not only the amount of myoglobin but also the amount and distribution of water in muscle tissue affect meat colour, firmness and drip loss (Ponnampalam *et al.*, 2017). Meat colour parameters are quantified by the Hunter L* (lightness/darkness), a* (redness/greenness) and b* (yellowness/blueness) system.

In the present study, results showed similarities in the Guinea fowl's breast and thigh meat colour indexes across dietary treatments. Normal broiler chicken meat is reported to have a lightness (L*) of $48<L^*<53$ with meat of an $L^*<48$ being abnormally dark (Qiao *et al.*, 2001). In the current study, 30-minutes post-slaughter, the Guinea fowl breast meat lightness ranged from 43 to 48. Laudadio *et al*. (2012) reported an L* of 43 to 47 from 12-week old broiler Guinea fowl meat 30-minutes post-slaughter thus findings from the current study are in agreement with those reported in literature. Barbut, (1997) contends that raw breast and thigh meat with an L* value ≥54 is characterised as pale soft and exudative (PSE). Based on Barbut's characterisation, results from the current study indicate the dietary MNM did not cause PSE.

Carcass weight and dressing percentage are elements of product quality as they speak to product yield. Dressing percentage is both a value and yield-determining factor and is therefore an important yardstick in assessing performance of meat producing animals (Yusef *et al*., 2012). In the present study, dietary MNM did not affect ($P>0.05$) the carcass weight and dressing percentage thus dietary MNM can potentially be used in Guinea fowl grower and finisher diets without compromising meat yield. Importantly, the observed dressing percentage in the current study is in agreement with that by Yamak *et al*. (2018) for Guinea fowl of the same age.

The redness of meat is an important trait as it is associated with the degree of freshness of the meat (Sen *et al*., 2014). Several factors, among them, pH affect redness (a*) of meat. In addition an increase in hue (H*) angle results in less red meat while an increase in chroma (C*) results in redder meat (Hernández *et al.*, 2016). In the current study, while there were no differences in redness of the meat, its value (redness) ranged from 2.58 to 3.17 for breast and 7.35 to 9.88 for thigh meat and is in agreement with that reported by Musundire *et al*. (2017). The similarity in the redness of the breast and thigh meat from Guinea fowl carcasses across dietary treatments suggests that dietary MNM can potentially be used in Guinea fowl grower and finisher diets without affecting the "freshness" and hence acceptability of the meat by consumers. It can also be speculated that dietary MNM did not impact the Hue and Chroma of the meat which would have otherwise affected the meat's redness

Among the several factors that impact meat quality, the pH of meat is one of the critical determinants of quality (Guerrero *et al.*, 2013). There is a relatable linear relationship between the colour of raw meat and its pH (Fletcher *et al.*, 2000). Meat with an abnormally low pH (pH<5.8) tends to have poor water holding capacity such that water exudes from it resulting in PSE meat (Woelfel *et al.*, 2002). The abnormal pH which induces the development of PSE results from rapid metabolism of muscle glycogen into lactic acid (El Rammouz *et al*., 2004). Poultry meat with a pH 6.0 and or above is generally classified as dark, firm and dry (DFD) due to its increased water holding capacity which results in the development of meat with a firm texture (Ponnampalam *et al.*, 2017). Importantly, high meat pH values shorten the product shelf life as it results in the creation of a micro-environment that is favourable to bacterial multiplication (Chen *et al.*, 2015).

According to Sarica *et al*. (2019), Guinea fowl breast meat generally has a pHi of 6.50 - 6.80 while Kokoszynski *et al*. (2011) states that meat from adult Guinea fowl has a pH of 6.1 to 6.4. In the current study, the pHu of breast meat from the Guinea fowl carcasses ranged from 5.80 to 6.01 and this is in agreement with that reported in the literature (Kokoszynski *et al.*, 2011) but in disagreement with pHu reported by Sarica *et al*. (2019). Importantly, findings from the current study show that dietary MNM had no effect on both the pHi and pHu of Guinea fowl meat across dietary treatments. Taken together, it can be inferred that MNM can potentially be used in Guinea fowl grower and finisher diets without the risk of inducing the formation of PSE and or DFD meat and without interfering with the shelf life of the meat.

The ability of meat muscle to retain moisture is key to many meat quality factors taken into high regard by consumers and industry (Torres Filho *et al.*, 2017). Drip loss, an ongoing process involving the exchange of water from myofibrils to the extracellular space (Huff-lonergan and Sosnicki, 2010), is critical to the meat industry since it impacts palatability and profitability (Warner, 2017). Results from the current study show similarities in the drip loss of the meat from the carcasses of the Guinea fowl across dietary treatments. Importantly, findings from the current study show drip loss values ranging from 2.70 to 4.03% which are in tandem with the 1.59 to 4.96% for Guinea fowl as reported by Sarica *et al*. (2019). Similarities in the drip loss of the meat across dietary treatments suggest that dietary MNM did not compromise the ability of the meat to retain moisture thus can potentially be utilised as a dietary protein source in Guinea fowl grower and finisher feeds with no negative impact on product palatability and profitability.

Tenderness, a complex trait, is the most important sensory attribute that affects consumer acceptability and eating satisfaction of meat products and also impacts the future possibility of repeated purchase (Drey and O'Quinn, 2017). Shear force is a force required to shear a cooked standardized piece of meat to measure its tenderness score (Devine and North, 2005). In the present study, the force necessary to slice through h breast fillets was similar across dietary treatments. This observation shows that dietary MNM neither improved nor compromised the tenderness of the meat and as such can be used as a dietary protein source in Guinea fowl feeds without the risk of negatively impacting this key meat quality attribute which affects acceptability and eating satisfaction.

### 5.4.2 Chemical composition of the meat

#### *5.4.2.1 Proximate content*

The value of poultry meat is determined by its nutrient content (Marangoni *et al.*, 2015). The chemical composition of guinea fowl meat,which is considered nutritionally superior because of its lower fat content and higher protein content compared to broiler chicken meat (Penkov *et al.*, 2017). Meat from Guinea fowl breast muscle on average contains 20-28% protein and 4 % fat (Moreki and Seabo, 2012). Findings of the proximate composition of Guinea fowl meat in the current study show that, across dietary treatments, the breast meat of giunea fowl, on a dry matter basis, had a CP, EE and ash content ranging from 81.6 to 82.8%, 1.8 to 9.6 % and 4.1 to 4.4 %, respectively. Comparative studies report breast meat from Guinea fowl to contain 29.18% CP, 4.7 % EE and 2.41% ash content (Chepkemoi *et al.*, 2015; Musundire *et al.*, 2017; Penkov *et al.*, 2017).The seemingly different and higher CP, lipid and ash content of Ginea fowl meat is due to the fact that dry and not fresh Guinea fowl meat was used in the chemical assays of the nutrient content of the meat while most studies make use of fresh meat. Thus the values reported by Chepkemoi *et al*. (2015) and other researchers were converted to dry matter basis, there is a high likelihood that there would be no differences in the proximate content reported in the current study.

#### *5.4.2.2 Mineral composition*

Calcium, phosphorus, iron and magnesium are known to be vital for proper function and growth of the many systems in the human body and are reported to be the most lacking in the nutrition diet of citizens of developing countries (FAO, 1998). Calcium and phosphorus are necessary for bone development whilst a severe deficiency of iron causes iron defieciency anaemia (Abbaspour *et al.*, 2014). Results of the current study showed similarities in Mg, P and Fe content in Guinea fowl breast meat. However, breast meat of Guinea fowl fed diet 1 had the highest ($P<0.001$) Ca content compared to other counterparts. In previous work, it was shown the SBM contained higher concentrations of Ca compared to MNM (Malebana *et al*., 2018).

In poultry and livestock production, nutrient composition closely mirrors dietary and feed ingredient composition (Rochell, 2018). Thus it can be speculated that the higher Ca content of the breast meat from Guinea fowl fed diet 1 stems from the high Ca in the SBM. It is also noteworthy that, though not significantly different ($P>0.5$) accross dietary treatments, Fe content in this study was higher (30 – 38 kg/mg) than those reported by (Bernacki, Bawej and Kokoszy, 2012) ranging from 15–19 kg/mg and this can be ascribed to the high myglobin levels in breast muscles (Askew and Marsh, 2002) which in turn can be related to the flight behaviour of the guinea fowl. Taken together it can be inferred that MNM can be used as a dietary protein source in Guinea fowl feeds without compromising the meat’s Mg, Fe and P content.

#### *5.4.2.3 Fatty acid profile*

Nutritional properties of meat are largely related to its fat content and fatty acid composition (Fajardo *et al.*, 2016a). The fatty acid content of animal muscles depends on breed, content of internal and external fat, climate, nutrition, and housing conditions (De Almeida *et al.*, 2006). The high level of poly-unsaturated fatty acids (PUFAs) in poultry meat make its nutritive value higher than that of pork or beef (Fajardo *et al.*, 2016b). The World Health Organisation recommends the daily fat intake in adults to be reduced to 30% of the total energy intake and the saturated fats (SFA) be limited to 10 % of this caloric intake (WHO, 2008). It is also advised that, mono-unsatutated fatty acids (MUFAs), PUFAs be limited to the recommended range of 15-20% and 6-11%, respectively (WHO, 2008).

Gurikar *et al*. (2014) reported the oxidation of SFA to induce volatile substances that in turn provide aroma and flavour thus improving meat quality. Breast meat from Guinea fowl is reported to contain 42.8 to 43.5% total saturated fatty acids (TSFA), 20 to 20.3% total monosaturated fatty acids (TMUFA) and 36.2 to 37.2% total polyunsaturated fatty acids (TPUFA) (Bernacki *et al.*, 2012). However, (Tufarelli *et al.*, 2015) reported 24.99 to 26.78% TSFA, 39.06 to 40.30% TMUFA and 32.92 to 35.39% TPUFA in 12 week old Guinea fowl. Findings from the current study showed that the Guinea fowl breast meat had a TSFA, TMUFA and TPUFA composition of 48.62 to 53.58%, 22.20 to 26.00% and 24.71 to 27.74%, respectively. The TPUFA content of the breast meat in the current study aligns with the range reported by (Chepkemoi *et al.*, 2017) whilst (Mazizi *et al.*, 2020) reported higher ranges of TMUFA (53.88 to 60.27%) and TPUFA (22.30 to 41.06%) content in Japanese quail fed dietary MNM.

Fatty acid (FA) composition in poultry meat is a direct reflection of the FA composition of the diet (Skřivan *et al.*, 2018). The proximate analysis data (unpublished) of the MNM used in this study has a 14.16% palmitic acid, 7.86% stearic acid and 65.93% oleic acid content. Palmitic acid (20.1 to 21.%) and stearic acid (16.7 to 17.6%) were the most abundant SFAs while oleic acid (18.02 to 22.9%) and linoleic acid (21.5 to 23.1%) were the dominant MUFAs and PUFA, respectively thus the MUFA and PUFA content of the meat in the current mirrors the ingredients (MNM) fatty acid profile. Oleic acid is a major fatty acid (FA) in all meats and has high antioxidant capacity which enhances oxidative stability and shelf life of the meat (Wood *et al.*, 2007; Dragoev *et al.*, 2014). In the current study, there is a notable increase in the meat's oleic acid content ($P>0.05$) with an increase in dietary MNM. From these fundings, we can speculate that dietary MNM does not negatively impact the meats' fatty acid composition and can be potentially used in Guinea fowl grower and finisher diets without posing health risks on consumers.

# CHAPTER 6: CONCLUSIONS, LIMITATIONS AND FUTURE RECOMMENDATIONS

## 6.1 Conclusions

The current study aimed to investigate whether the graded substitution of SBM in broiler Guinea fowl grower and finisher diets with Marula nut meal, on a crude protein basis, would have any effect on the growth performance, feed utilisation efficiency, viscera macro-morphometry , health and meat quality of Guinea fowl. Based on the findings of this study, dietary MNM could be potentially utilised as a substitute of SBM on a crude protein basis at 25%, 75% and 100% in Guinea fowl diets without compromising the growth performance of the birds. However, caution needs to be taken at 50% MNM inclusion, as it evidently reduced feed economy.

In poultry production, the overall health and wellbeing of the animals should be given focus so as to ensure maximal product yield and quality. Dietary MNM did not compromise the circulating and stored metabolic substrates, kidney and liver function of Guinea fowl. Additionally, MNM can be used as a dietary protein source in Guinea diets without the risk of compromising GIT development and function. Dietary MNM had no negative effect on meat yield and quality as determined by the carcass weight, dressing percentage and the breast meat physical attributes; pHu, colour, moisture characteristics and tenderness. Marula nut meal could potentially substitute SBM in Guinea fowl grower and finisher diets without compromising the proximate (ash and crude protein), mineral composition (P, Mg, Fe) and fatty acid profile ( TSFA, TMUFA and TPUFA) of Guinea fowl meat.

## 6.2 Limitations and recommendations

The current study did not assess all factors that affect meat colour (i.e myoglobin and white fibre content) and intramuscular fat. Additionally, indirect markers were used to measure the oxidative stress status of the birds but markers of antioxidant status, such as, catalase, SOD and GPx activities were not determined. These together with the aroma, flavour and taste of the meat should be considered for future studies

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# APPENDICES

**Appendix 1**

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2018/07/31/B

| | |
|---|---|
| APPLICANT: | Ms TZ Nkwanyana |
| SCHOOL:<br>DEPARTMENT:<br>LOCATION: | Biochemistry |
| PROJECT TITLE: | Dietary effects of Sclerocarya birrea caffra (Marula) nut on the growth, viscera morphometry and health of broiler Numidia meleagris |

**Number and Species**

40X 4-week old male and female Coronata Broiler Guinea fowl (Numididae meleagris coronata)

Approval was given for the use of animals for the project described above at an AESC meeting held on 2018/07/31. This approval remains valid until 2020/09/12.
Unreported changes to the application may invalidate the clearance given by the AESC
An annual progress report must be provided

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and is subject to any additional conditions listed below:

**Applicant to supply the necessary permits**

Signed: ____________ (Chairperson, AESC) Date: 14th September 2018.

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: ____________ (Registered Veterinarian) Date: 13 October 2018

cc: Supervisor: Prof E Chivandi and Dr B Lembede
Director: CAS

Works 2000/lain0015/AESCCert.wps

**Appendix 2**

agriculture and
rural development
Department: Agriculture and Rural Development
**GAUTENG PROVINCE**

**Diamond Corner Building, 68 Eloff & Market Street, Johannesburg**
**P O Box 8769, Johannesburg, 2000**
**Telephone: (011) 355-1900**
**Fax: (011) 355-1000**
**Email: gdard@gauteng.gov.za**
**Website: http://www.gdard.gpg.gov.za**

**31/01/2019**

Dear Miss Thandanani Zola Nkwanyana

**RE: Support for undertaking of feeding research in Guinea fowl at the University of the Witwatersrand**

Your enquiry about the State veterinarian letters refers. The completed Section 20 application was received today the **31st of January 2019**

I have no objection to the movement of the guinea fowl into Gauteng should the disease situation in the KZN warrant the movement. There should there for be no movement restrictions due to disease outbreaks **at the time of movement.**

To safeguard all parties please request the SV at origin to issue an ordinary movement permit for the consignment of healthy birds. A copy of the permit can be emailed to the SV Germiston. (duma.mpofu@gauteng.gov.za)

Kind regards

DR. J WALTERS
DEPUTY DIRECTOR: ANIMAL HEALTH GAUTENG
Cell: 082 373 7726

## Appendix 3

agriculture
& rural development

Department
agriculture
& rural development
PROVINCE OF KWAZULU-NATAL

KZN Department of Agriculture & Rural Development
Private Bag X577, Eshowe, 3815

Enquiries: Dr. V. J. Meyer
Tel: 035 473 0400
Toll-Free: 0800 000996
Email:
Website:

**MOVEMENT OF GUINEA FOWL**

This letter serves to confirm that there are currently no restrictions on the movement of guinea fowl from Esikhawini, Umhlatuze municipality in KwaZulu Natal, to Gauteng Province.

The birds originate from an area that has not experienced any recent Poultry disease outbreaks, and is not under any quarantine restrictions.

REPUBLIC OF SOUTH AFRICA
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OFFICIAL VETERINARIAN
2018 -11- 07
DR V.J. MEYER
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