THE POTENTIAL OF *SCLEROCARYA BIRREA CAFFRA* NUT MEAL AS A DIETARY PROTEIN SOURCE IN BROILER AND LAYER *CORTUNIX CORTUNIX JAPONICUM*

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, School of Physiology, in fulfilment of the requirements for the degree of Doctor of Philosophy. Johannesburg, 2020.

DECLARATION

I, Bulelani Elvis Mazizi declare that this thesis, hereby submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, is my own work and has not been submitted to any other institution for an award. Where other sources have been used; they have been appropriately cited and acknowledged.

_____

(Signature of candidate)

_____ Day of _____ 20_____ in _____

DEDICATION

~ I dedicate this master piece to my Master of Science supervisor who motivated me and pushed me to soar to greater heights. ~

Professor Voster Muchenje

21st June 1972 to 3rd July 2019

Lala ngoxolo Qhawe!

PUBLICATIONS

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PAPER(S) UNDER PREPARATION

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CONFERENCES

Bulelani E Mazizi; Davison Moyo; Kennedy H. Erlwanger; Eliton Chivandi. Effects of dietary *Sclerocarya birrea caffra* (Marula) nut meal on the growth performance and liver and kidney function of Japanese quail. 51st South African Society for Animal Sciences (SASAS) Congress, Bloemfontein 2019, South Africa.

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ABSTRACT

Despite its high crude protein (CP) content, Marula nut meal's (MNM) potential as a dietary protein source has not been evaluated in quail. Following determination of the nutrient composition of hexane-extracted MNM and comparing it with soyabean meal (SBM), its suitability as a substitute in broiler and pullet quail feeds was evaluated by determining its effects on productivity, health and product quality.

In the broiler study, 200 nine-day old broiler Japanese quail chicks, in a simple randomised interventional study, were allocated to five grower diets wherein MNM substituted SBM (CP basis) at 0%, 25%, 50%, 75% and 100% and fed for 4 weeks. The quail were then transferred to similarly constituted finisher diets and fed for 2 weeks. Weekly body weight and daily feed intake (FI) were measured. Bodyweight gain (BWG), average daily gain (ADG) and feed conversion ratio (FCR) were computed. At slaughter, blood was collected and carcasses eviscerated. Viscera macro-morphometry, biomarkers of liver and kidney function, carcass traits and the meat's pH_i and pH_u, colour, thawing and cooking loss (TL and CL), tenderness, proximate and fatty acid composition were determined.

In the pullet study, sixty 5-week old Japanese quail hens were, in a simple randomised design, allocated to five pullet diets wherein MNM substituted SBM (CP basis) at 0%, 25%, 50%, 75% and 100% and fed for 8 weeks. Egg production and quality, growth performance and feed utilisation efficiency, viscera macro-morphometry, plasma uric acid, calcium and phosphorus concentration were determined.

Proximate, fibre (aNDF, ADF and ADL), phosphorus, gross energy (GE), amino acid and TMUFA content of MNM was higher ($P < 0.05$) than that of SBM. Dietary MNM had no effect ($P > 0.05$) on terminal body mass, BMG, ADG, FI, FCR and carcass mass of broiler and pullet quail. Meat from broiler quail fed diet 1 had the lowest ($P < 0.05$) pH_i and pH_u and was lighter and less red ($P < 0.01$) compared to that from counterparts fed diet 5. MNM had no effect ($P > 0.05$) on TL, CL and tenderness of the meat. The meat's CP and fat content decreased with increasing dietary MNM but ash increased ($P < 0.05$). At 50 to 100% inclusion level dietary MNM increased ($P<0.05$) the oleic acid (OA) content of the meat. Liver and kidney function of broiler quail was not affected ($P > 0.05$) by MNM.

Pullet hens fed diets 3 to 5 commenced laying at 9-weeks of age. Counterparts fed diets 1 and 2 commenced at 10-weeks. Percent lay of hens fed diets 3 to 5 was similar at 9-weeks of age. At

10-weeks of age 50% of the hens fed diet 3 were laying. Their counterparts fed diet 5 were at 16.67%. The highest percentage of laying hens was at 13-weeks of age: hens fed diet 2 and 4 at 91.66% and those fed diets 1, 3 and 5 at 83.33%. The number of eggs per hen per week was similar ($P > 0.05$) across the dietary treatments at 9-weeks of age. At 10-weeks of age hens fed diet 4 had a higher ($P < 0.05$) number of eggs per hen compared to those fed diet 1. Hens fed diet 3 had a higher ($P < 0.05$) number of eggs per hen compared to those fed diet 1. At 12 and 13 weeks of age, the number of eggs per hen per week was similar ($P > 0.05$) across dietary treatments ($P > 0.05$). The number of eggs per laying hen was similar at 9-weeks of age but at 10-weeks of age hens fed diet 5 had more eggs ($P < 0.0001$) than from hens fed diets 1 to 4. At 11-weeks of age, the number of eggs per laying hen was lower ($P < 0.0001$) for birds fed diet 1 compared to those fed diets 3 to 5. Dietary MNM had no effect on egg mass (EM) at 9, 10, 11 and 13-weeks of age. At 12-weeks of age hens fed diet 2 produced lighter ($P > 0.05$) eggs compared to eggs from hens fed other diets. Dietary MNM had no effect ($P > 0.05$) on egg shape index and shell thickness. At 9-weeks of age the yolk index of the eggs from hens fed diet 4 was lower ($P > 0.05$) compared to that of eggs from birds fed diets 3. The albumen index of eggs from hens fed diet 4 was significantly lower ($P < 0.05$) compared to that of eggs from hens fed diets 2 and 5. The yolk ratio of eggs from hens fed diets diet 3 was significantly higher ($P < 0.05$) than that from counterparts fed diets 1 and 5. The yolks from eggs of birds fed diets 1 and 5 had lower ($P < 0.0001$) fat content compared to that of eggs from birds fed diets 2 and 3. The egg yolk cholesterol content from birds fed diets 2 and 3 was higher ($P < 0.01$) compared to that from hens fed diets 1, 4 and 5.

Marula nut meal can substitute SBM in Japanese quail broiler and pullet diets without compromising growth performance, feed utilisation efficiency, bird health, meat quality and the external egg quality. It can be used to improve the redness and OA content of quail meat but caution must be taken in its use in quail pullet diets as it compromised internal egg quality.

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LIST OF ABBREVIATIONS

AFMA: Animal feed manufacturers association

ADF: Acid detergent fibre

ADG: Average daily gain

ADL: Acid detergent lignin

ALP: Alkaline phosphatase

ALT: Alanine transaminase

ANFs: Anti-nutritional factors

ANOVA: Analysis of variance

AOAC: Association of Official Analytical Chemists

ARC: Agricultural Research Council

BWG: Body weight gain

BUN: Blood urea nitrogen

Ca: Calcium

CP: Crude protein

Cu: Copper

DAFF: Department of Agriculture, Forestry and Fisheries

DM: Dry matter

EAAs: Essential amino acids

EE: Ether extract

EFA: Essential fatty acid

EPA: Eicosapentaenoic acid

EU: European Union

FA: Fatty acid

FAO: Food and Agricultural Organisation

FCR: Feed conversion ratio

FI: Feed intake

GE: Gross energy

GIT: Gastrointestinal tract

IBW: Induction body weight

K: Potassium

MDA: Mineworkers Development Agency

Mg: Magnesium

MNM: Marula nut meal

MUFA: Monounsaturated fatty acid

n-3 fatty acid: Omega-3 fatty acid

n-6 fatty acid: Omega-6 fatty acid

n-9 fatty acid: Omega-9 fatty acid

NDF: Neutral detergent fibre

NRC: National Research Council

OA: Oleic acid

P: Phosphorus

pH_i: Initial pH

pH_u: Ultimate pH

PUFA: Polyunsaturated fatty acid

SA: South Africa

SFA: Saturated fatty acid

SBM: Soyabean meal

SSA: Sub-Saharan Africa

TBW: Terminal body weight

TMUFA: Total monounsaturated fatty acid

TPUFA: Total polyunsaturated fatty acid

TSFA: Total saturated fatty acid

USD: United States of America Dollar

WBSF: Warner Bratzler shear force

CHAPTER ONE - INTRODUCTION AND JUSTIFICATION

1.0 Thesis overview

The increasing demand for poultry products in sub-Saharan Africa (SSA) has triggered the need to intensify the production of poultry species (other than chicken and ducks) such as the Japanese quail. However, local farmers are faced with a challenge of costly poultry feeds. Over and above the competition for food/feed ingredients between humans and poultry, the high cost of poultry feeds in SSA is worsened by over reliance on imported feed ingredients which results in reduced productivity. Dietary protein for poultry feeds is SBM and it is largely imported and a major limiting factor to poultry production hence the need to explore locally available non-conventional dietary protein sources. Marula nut meal, a by-product of oil extraction from Marula kernels, is a potential dietary protein source that can be used in the poultry industry. This study evaluated, *in vivo*, the potential of MNM to substitute SBM as a source of dietary protein in broiler and pullet Japanese quail feeds.

This thesis is structured such that: Chapter 1 gives the background to the demand for livestock products worldwide, regionally and locally in South Africa. The Chapter gives a narration on the increased in the demand for poultry and other livestock products in SSA emphasising the role of the human population growth, expansion of urban settlements and an improvement in consumer incomes on poultry product demand. The Chapter also proposes and hypothesises that the use of a non-conventional dietary protein source, specifically MNM, in Japanese broiler and pullet quail feeds could alleviate the challenge imposed on the poultry industry by imported SBM and thus help meet the increasing demand for poultry products.

Chapter 2 critically reviews the literature pertinent to the study ultimately focussing on MNM as a potential dietary protein source. The discourse makes it clear that use of mechanically produced MNM in feeds faces the challenge of high residual oil in diets which could affect, not only the diets' quality and shelf life but palatability, nutrient content, feed intake, bird health and product quality.

Thus Chapter 3, the first of the experimental chapters, evaluates and reports on the effects of further defatting, by solvent (hexane) extraction, of mechanically produced MNM on its proximate, mineral, amino acid, fibre and fatty acid content and in comparison to SBM. Chapter 4, the second experimental chapter, focuses on the *in vivo* evaluation of the potential of MNM to replace SBM in broiler quail diets. Due to it being a non-conventional dietary protein source, the hexane-extracted MNM can potentially contain toxic anti-nutritional factors, thus the chapter, in addition to reporting on the meal's effects on growth performance, feed intake and utilisation efficiency, also narrated on its (MNM) effects on gastrointestinal viscera macro-morphometry, liver and kidney function and general health as well as product (meat) quality. The determination

of the meal's effect on GIT viscera morphometry sought to evaluate whether meal might improve and or compromise GIT digestive and absorptive function and hence nutrient supply to the birds. There is a huge emphasis and movement towards ensuring bird/animal welfare in the process of production hence the chapter focuses on the health status of the birds. Due to increased awareness of the effects of the consumption of animal products on human health, consumers now demand quality poultry (and animal) products hence the chapter also reports on the MNM's effects on the quality of broiler quail meat.

Apart from meat, poultry species also contribute eggs which are a cheap source of dietary protein for human consumption. Feed ingredients are known to impact external and internal egg quality parameters thus affecting shelf life and nutrient content, respectively. Thus Chapter 5, the third experimental chapter, evaluated and reports on the effects of substituting SBM in pullet quail diets with MNM on egg production and quality as well as bird health, growth performance, feed intake and feed utilisation efficiency. Chapter 6 summarises the major findings of the study, identifies study limitations and makes recommendations for future studies. References cited in the thesis and appendices (Animal Ethical Clearance Certificate and relevant supplementary date) are listed after chapter 7.

1.1 Introduction

The poultry industry plays a major role in the agricultural enterprises of developing countries because of numerous factors which include increased capacity for intensification, quick turn-around time and lower feed utilisation as compared to other livestock species (Wahyono and Utami, 2018; Thornton, 2010). In intensive poultry production, feed cost constitutes over 80% of the production cost (Burgos *et al.*, 2007). Protein and energy are the main ingredients of poultry feed (Ravindran, 2013). On average they make up 30 to 40% and 70 to 75% of poultry feed, respectively (He, 2004; Van Der Klis *et al.*, 2010). Although dietary energy is the pacesetter for production (Carré *et al.*, 2013), dietary protein is critical in the provision of essential amino acids that are required for productive performance (Beski *et al.*, 2015). Dietary protein contributes 45% of the cost of poultry feed (Ahmad *et al.*, 2006).

Fish meal and soyabean meal (SBM) are key sources of dietary protein for poultry feeds (Kanyinji and Zulu, 2014; Veldkamp and Bosch, 2015). In Sub-Saharan Africa SBM used in the livestock and poultry industry is largely imported (Chivandi *et al.*, 2018) since the region's soyabean production does not meet its SBM requirements (Malebana *et al.*, 2018). The SBM is renowned for its high digestibility, palatability and capacity to supply essential amino acids (Hoffman and Falvo, 2004). Fishmeal although it has an ideal amino acid profile, it can only be used in starter diets but not finisher diets because it causes odour tainting of products (Malebana

et al., 2018). Alternatively, cottonseed cake, groundnut meal and sunflower cake can be used as dietary protein sources in poultry feeds. However their use is beset by the presence of large amounts of anti-nutritional factors (ANFs) and mycotoxins (Akande *et al.*, 2010).

The ANFs and mycotoxins cause significant reductions in nutrient digestibility and absorption thus compromise feed intake and utilisation efficiency (Huisman and Jansman, 1991; Smith *et al.*, 2018). The failure by countries in SSA to meet the demand of SBM for the poultry and livestock industries makes it critical that locally available dietary protein sources, for example, seeds from indigenous fruit-bearing trees (IFBTs), be evaluated (Chivandi *et al.*, 2015).

In SSA, there are about 1200 species of IFBTs and 40% produce fruit and nuts that are consumed by humans and livestock (Sthapit *et al.*, 2012; Meregini, 2005; Shackleton and Shackleton, 2012). The seeds from IFBTs, which are potential sources of nutrients (Chivandi, 2012), are generally discarded despite their potential to be sources of feed ingredients, including dietary protein. *Sclerocarya birrea caffra* (Marula), a renowned IFBT (Francis and Tahir, 2016), is widely distributed in southern African countries (Shackleton and Shackleton, 2002). It grows well in many eco-zones (Zharare and Dlamini, 2000) and produces edible fruit (Hillman *et al.*, 2008; Zharare and Dlamini, 2000). Its fruit pulp is exploited in the production of juice, jam and alcoholic beverages (Wynberg *et al.*, 2002; Nyoka *et al.*, 2015). The fruit stone contains two or more oil- and protein-rich kernels (Hillman *et al.*, 2008; Zharare and Dhlamini, 2000). In South Africa, Mhala Development Centre in Bushbuckridge, Limpopo Province and Distell in Stellenbosch, Western Cape Province, process Marula fruit into beverages and kernels into oil. The Marula nut meal (MNM), a by-product of oil extraction is a potential dietary protein source in poultry and livestock feeds.

The CP content of the MNM ranges from 32 to 47% (Malebana *et al.*, 2018; Mdziniso *et al.*, 2016; Mlambo, Dlamini, Ngwenya, *et al.*, 2011). The MNM also contains calcium (1.10-1.43 g/kg DM) and phosphorus (10.0 to 10.9 g/kg DM) (Mthiyane and Mhlanga, 2017), which are key nutrients in poultry feeds (Ravindran, 2013). On the basis of its high protein content, MNM could potentially be used as a dietary protein source in broiler and layer poultry feeds. However, plant-derived seed meals and oil-seed cakes usually contain ANFs such as tannins, phytohaemagglutinins, saponins, oxalates and phytates (Francis *et al.*, 2001). These ANFs negatively impact the palatability and digestion of feed as well as nutrient absorption (Gemede and Ratta, 2014), and in some instances result in toxicity to poultry and livestock (Capuano *et al.*, 2009). It is therefore pertinent that in the evaluation of the potential of non-conventional plant-derived feed resources that focus also be applied on toxicity and animal welfare over and above determining their effects on production parameters.

1.2 Problem statement

Poor diets negatively impact the productive performance of broiler and pullet poultry due to compromised nutrient intake and feed utilisation efficiency (Mashaly *et al.*, 2004). The negative impacts usually manifest as low slaughter weights, poor meat quality, low egg count, small egg size and poor eggshell quality (Awad *et al.*, 2017; Bregendahl *et al.*, 2002; Powers and Angel, 2008). In addition to compromised nutrient intake and poor feed economy, poor diets also result in calcium deficiency which may negatively affect bone mass resulting in metabolic diseases such as femoral head necrosis and tibial dyschondroplasia (Cook, 2000). It is thus important to formulate nutritionally adequate diets to guard against poor productive performance and potential compromised health.

Feed costs constitute about 80% of the total cost of broiler and pullet chicken production with dietary protein being one of the most expensive dietary constituents (Mendieta-Araica *et al.*, 2011; Olugbemi *et al.*, 2010). The banning of the use of animal-derived dietary protein in the formulation of poultry and livestock feeds (European Commission Decisions No. 98/272/CE and 2000/374/CE) precipitated an increase in the demand for plant-derived dietary protein sources (Laudadio *et al.*, 2011). Globally, SBM is the dominant dietary protein source in poultry feeds (Laudadio *et al.*, 2011; Mendieta-Araica *et al.*, 2011) and its cost has increased tremendously due to its high demand. In SSA, the local production of soybean is inadequate to meet the demand of SBM (Malebana, 2018). South Africa depends on costly SBM imports to meet the requirements of the poultry and livestock industry (Department of Agriculture Forestry and Fisheries, 2017). South Africa produces about 742000 tonnes, imports about 270840 tonnes globally to meet the demand of about 1000000 tonnes and selling at R6202.86 per tonne locally (Department of Agriculture Forestry and Fisheries, 2017), thus finding an appropriate locally available substitute with minimal costs to harvest could reduce losses to the fiscus due to imports of SBM. Due to the dependence on SBM imports, the cost of broiler meat is increasing (Olugbemi *et al.*, 2010) such that some communities, especially in Southern Africa, are beginning to fail to access the "once cheaper' animal protein source (Department of Agriculture Forestry and Fisheries, 2017; Kanyinji and Zulu, 2014).

The high feed cost-driven failure to access poultry products for human consumption has dire consequences on the nutrition and health status of the physiologically vulnerable members of society (Olugbemi *et al.*, 2010). Furthermore, the heavy dependency of the Sub-Saharan African poultry industry on imported SBM militates against possible adoption and expansion of less costly non-traditional broiler and pullet species such as quail. In order to take full advantage of these non-traditional poultry species, there is a need to search and develop alternative dietary protein sources that, cost-wise, could be relatively better affordable to resource-limited farmers.

Such alternative dietary protein sources would stimulate increased adoption and the promotion and expansion of the non-traditional poultry species such as the Japanese quail. Locally available resources such as the MNM can potentially be used to supply dietary protein in broiler and pullet quail feeds. Thus, the potential of MNM as a dietary protein source in broiler and layer quail feeds needs interrogation.

1.3 Justification of the study

The cost of the protein component of poultry feeds is one of the common constraints in the production as it is the most expensive (Chakeredza *et al.*, 2008). Soyabean meal is the common dietary protein source in poultry feeds globally. In South Africa, there is a limited production of soyabean such that local production of SBM fails to meet the livestock and poultry feed industries requirements. The country thus depends on imported SBM to meet the deficit. In 2018 overall oilcake imports by South Africa grossed 519 071 tonnes at a cost of USD$ 143 522 400. 00 (Oilseeds focus, 2018). The high cost of SBM makes it very difficult for local farmers, especially smallholder farmers, to produce at their best potential. In order to reduce the dependency on costly SBM imports, there is a dire need to embrace alternative dietary protein sources for poultry and livestock feeds. The potential of Marula nut meal, a by-product of oil extraction in both South Africa and Swaziland, to substitute SBM in poultry feeds, particularly in quail feeds, needs to be evaluated. The use of MNM produced by mechanical oil extraction as a dietary protein source in broiler chicken feeds resulted in decreased feed intake as well as rancidity of the feed during storage (Mthiyane and Mhlanga, 2017). The observed rancidity suggests the need to reduce the oil content of the MNM to the lowest possible level prior to utilisation as a dietary protein source in poultry feeds.

In South Africa, broiler and pullet production industries are heavily dependent on imported SBM. This militates against possible adoption, production and expansion of cheaper non-traditional species such as broiler and layer quail production. In order to take full advantage of these non-traditional broiler and layer poultry production option, there is a need to explore an alternative dietary protein source which is cheaper, and could be relatively better affordable to resource-limited farmers. Such alternative dietary protein sources could stimulate increased adoption, promotion and expansion of the production of Japanese quail in Southern Africa. Locally, available potential dietary protein sources, such as the MNM can potentially supply dietary protein in poultry and including broiler and layer quail feeds. Thus, the potential of MNM as a dietary protein source in broiler and layer quail feeds needs interrogation. Despite the studies on the chemical nutrient of MNM, there is, to my knowledge, no study has evaluated the potential MNM as a dietary protein source in broiler and layer quail feeds in SSA. Thus, this

study aimed to interrogate the potential of MNM to substitute SBM as a dietary protein source in broiler and pullet quail feeds.

1.4 Aim of the study

This study determined the chemical nutrient composition of hexane-extracted MNM and evaluated, *in vivo*, it's potential to substitute SBM as a dietary protein source in broiler and pullet quail diets. The study was executed in three major experiments each; with its own specific objectives.

1.5 Objectives of the study

1.5.1 Experiment 1: chemical nutrient characterisation of solvent-extracted Marula nut meal

This experiment specifically sought to determine the proximate, energy, mineral, fibre, amino acid and fatty acid composition of the MNM for purposes of formulating MNM-based quail diets and test them *in vivo*.

1.5.2 Experiment 2: Evaluating the effects of dietary Marula nut meal in broiler quail

Specifically, this experiment sought to formulate MNM-based brooding/grower and finisher diets and to determine their effects in broiler quail on:

a) growth performance as measured by effects on terminal body mass, body mass gain and average daily gain and feed use economy as measured by feed intake and feed utilisation efficiency during the different growth phases.
b) meat quality by evaluating effects on meat colour, pH, tenderness, water-holding capacity, proximate, mineral, amino acid and fatty acid content. Bird health by evaluating effects on gastrointestinal viscera macro-morphometry, circulating and stored metabolic substrate content and surrogate markers of liver (aspartate aminotransferase and gamma-glutamyl transferase) and kidney (blood urea nitrogen, uric acid, total bilirubin) function and other markers (total protein, albumin and globulin) of health.

1.5.3 Experiment 3: Evaluating the effects of dietary Marula nut meal in pullet quail

Specifically, this experiment sought to formulate MNM-based layer diets to determine their effects in pullet quail on:

a) laying performance (percentage lay and egg production)
b) egg shell quality and egg quality and composition - egg mass, shell thickness, Haugh unit, albumen height, albumen diameter and cholesterol and fatty acid content of the yolk.
c) growth performance as measured by effects on terminal body mass, body mass gain and average daily gain and feed use economy as measured by feed intake and feed utilisation efficiency.

d) bird health by evaluating effects on gastrointestinal viscera macro-morphometry, circulating and stored metabolic substrate content and surrogate markers of kidney (uric acid) function and other markers (calcium and phosphate) of health.

1.6 Hypotheses of the study

The hypotheses of this study encompassed the three experiments as follows:

H_0: There are no significant differences in the proximate, mineral, amino acid, and fatty acid content of MNM and SBM and the dietary substitution of SBM with MNM as a dietary protein source in broiler and pullet quail diets has no effect on the growth and laying performance, feed utilisation economy, viscera macro-morphometry, health and meat and egg quality of broiler and pullet quail, respectively.

H_1: There are significant differences in the proximate, mineral, amino acid, and fatty acid content of MNM and SBM and the dietary substitution of SBM with MNM as a dietary protein source in broiler and pullet quail diets significantly affects the growth and laying performance, feed utilisation economy, viscera macro-morphometry, health and meat and egg quality of broiler and pullet quail, respectively.

CHAPTER TWO – LITERATURE REVIEW

2.0 Introduction

The poultry production sector is a component of the livestock production industry. The current production level of the poultry industry has increased fivefold compared to five decades ago (Mottet and Tempio, 2017). Globally, the poultry production industry is producing about 123 million tonnes of meat (WATT Poultry Trends, 2018) and 73 million tonnes of eggs (Mottet and Tempio, 2017). According to WATT Poultry Trends (2018) Asia, Africa, and North America contributed 43 000, 7000 and 23 000 metric tonnes, respectively, to the poultry meat production in the year 2018. In SSA, poultry production is one of the major sources of animal-derived dietary protein sources for human consumption (Mottet and Tempio, 2017). The SSA regional per capita consumption of poultry meat has increased by about 16% during the period 2008 to 2017. The level of consumption is expected to increase by 5.5% in the coming years (OECD-FAO, 2018). In South Africa, local broiler chicken meat production increased from 869 000 tonnes to 1 704 000 tonnes between the years 2001 to 2016 while consumption increased from 938 000 tonnes to 2 200 000 tonnes between the years 2001to 2017 (AFMA, 2018). The current global, SSA and SA per capita per year consumption of poultry meat is 38 kg, 8 to 9 kg and 41.2 kg, respectively (OECD-FAO, 2018; Statista, 2019).

While the genetically improved broiler and pullet chicken are the major sources of poultry-derived protein for human consumption in South Africa, these improved chicken breeds are costly in terms of feed, housing and veterinary requirements (Alders *et al.*, 2018). This is especially true in the context of resource-limited rural farming communities (Dana *et al.*, 2010) where protein malnutrition is highest (Asibey, 1974; Bain *et al.*, 2013). Therefore, there is an urgent need to consider alternative poultry species that are more amenable to production by resource-constrained rural farming communities in order to ensure household food security. Quail is a potential complement and or alternative to broiler and pullet chicken production by resource-limited rural farmers. The production of quail can significantly contribute to meeting the demand for animal-derived protein for human consumption (Nasr *et al.*, 2017). Quail have a short generation interval, mature early and require less feed and space when compared to improved chicken breeds (Oguz and Minvielle, 2001; Sakamoto *et al.*, 2018). Japanese quail meat provides the human body with essential nutrients such as essential amino acids (Genchev *et al.*, 2008; Nasr *et al.*, 2017), essential fatty acids, vitamins and antioxidants (Cavani *et al.*, 2009). These essential amino acids and fatty acids are vital for normal growth and development of the human brain (Bourre, 2004). Quail meat is low in calories but has a higher protein level when compared to other meats (Ribarski and Genchev, 2013). Additionally, quail meat is lean and possesses a lower cholesterol concentration compared to beef, pork and mutton meat(s) (Vali, 2008). In a world ravaged by many diet-induced metabolic diseases such as obesity, diabetes

mellitus and cardiovascular diseases, the quality of quail meat (leanness) allows for consumption to meet essential nutrient and protein requirements with a reduced risk of developing such diseases.

The protein content of the albumen for quail and chicken eggs is 10.4 and 10.6% respectively, while the yolk protein content is 16 and 16.6% respectively (Dudusola, 2010). The yolk fat content is 31.5% for quail and 32.6% for chicken (Dudusola, 2010). The quail egg contains saturated fatty acid (SFA), mono unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) at 29.9, 45.0 and 25.1% respectively (Nowaczewski *et al.*, 2013; Polat *et al.*, 2013). Compared to quail eggs, chicken eggs have similar SFA (29.7%), higher MUFA (39.1%) and lower PUFA (31.1%) (Nowaczewski *et al.*, 2013; Polat *et al.*, 2013). Thus, the chemical nutrient composition of quail egg is almost similar to that of chicken eggs.

Dietary manipulations can be used to improve the nutritional quality of eggs. In their study, Alvarez et al. (2004) reported an increase in long-chain omega-3 fatty acids yolk fat concentrations in eggs from hens fed feed supplemented with dietary fish oil. The PUFA in quail eggs has been reported to mitigate oxidative stress and to arrest the progression of tumours (Tunsaringkarn *et al.*, 2013). Moreover, apart from their contribution to human nutrition, quail eggs are utilised in ethnomedicine: in Nigeria, they are used in the management of sickle cell anaemia and diabetes mellitus (Nlekerem and Ibukun, 2016). Poultry meat and eggs are an important source of protein for human consumption (Kuang *et al.*, 2018; Pereira and Vicente, 2013). Their high protein content is important for the growth and development of the human body, especially during the early stages of life. They also contribute significant dietary amounts of lysine, threonine, methionine, cysteine and tryptophan, which are essential amino acids (Dowarah, 2013) as well as Omega-6 and Omega-3 fatty acids (Pereira and Vicente, 2013). Dietary Omega-6 and Omega-3 PUFAs have been observed to improve brain development and learning ability and importantly to decrease obesity and its associated metabolic diseases such as cardiovascular diseases and type 2 diabetes mellitus (Marangoni *et al.*, 2015).

In SSA the growth in human population, an expansion in urban settlement and an increase in incomes have seen an increase in the demand of animals, poultry included, derived foods (Thornton, 2010). The introduction of other poultry species to mainstream chicken production may not help SSA meet the demand of poultry products due to challenges besetting the region's poultry industry. The high cost of feed driven by a shortage of dietary energy and protein sources (Owen and Dike, 2013) is one of the major limiting factors to poultry production (Mnisi and Mlambo, 2018). Dietary protein constitutes 45% of the total feed costs (Ravindran, 2013) and the most costly poultry feed ingredient. Currently SSA's poultry feed industry depends on costly

imported SBM. Thus, it is important to search for and develop alternative dietary protein sources for poultry feeds which are locally available and cheaper compared to conventional dietary protein sources.

2.1 Dietary protein sources for poultry feeds

Plant and animal-derived dietary protein sources are largely used in poultry diets (Beski *et al.*, 2015). Fish meal is one of the major and commonly used dietary protein sources in poultry feeds while soybean meal, groundnut cake and cotton seed cake are the key plant-derived dietary protein sources (Al-Feky *et al.*, 2016; Odunsi *et al.*, 2007). Protein quality, as determined by availability of essential amino acids (EAAs), palatability and digestibility is of vital importance in any dietary protein source in poultry feeds (Liebert, 2017) as well as in any monogastric livestock feeds. The ban on the use of animal-derived products as feed ingredients by European Union (Jędrejek *et al.*, 2016) coupled with the odour tainting effect of fish meal on poultry and other animal products have led to an increase in the demand and use of plant-derived dietary protein sources, especially SBM (He, 2004) due to its high palatability and digestibility and its high essential amino content (Park *et al.*, 2002). The increased demand in the use of SBM has led to an increased price of SBM (Dalmasso *et al.*, 2004). Other plant-derived conventional dietary protein sources, such as cottonseed, linseed, sunflower meal and groundnut meal are poorly digestible and lack adequate quantities of EAAs, like lysine, cysteine and methionine, to meet nutritional requirements of poultry for meat and egg production (Iji *et al.*, 2017). The failure by such plant-derived dietary protein sources to meet the essential amino acid requirements of poultry means supplementary, but expensive synthetic EAAs, should also be included in the poultry diet. Additionally most of the poor quality plant-derived conventional dietary protein sources viz poultry nutritional requirements and digestive capability also contain ANFs (Iji et al., 2017). These ANFs compromise nutrient digestion, absorption and bioavailability and in some instances elicit toxic responses (Wang et al., 2016). There is a dire need to search and develop alternative dietary protein sources for poultry feeds in order to mitigate the dependency of costly SBM. Seeds from indigenous fruit-bearing trees (IFBTs) are potential sources of feed ingredients, including dietary protein for poultry feeds.

2.2 Non-conventional dietary protein sources

Indigenous fruit-bearing trees are adapted to the rainfall, edaphic and temperature regimen of SSA (Chivandi, 2012). Even under drought conditions, these trees produce a crop of fruit (Chivandi *et al.*, 2015). While the fruit pulp from the fruit of these IFTBs is consumed by animals and humans, the bulk of the seeds are lost yet they are potential sources of nutrients including protein (Chivandi *et al.*, 2015). Research on the potential contribution of indigenous trees to animal feeds has generally focused on browse (Katjiua and Ward, 2006) and limited to a

few trees such as the acacia species (Ahmed *et al.*, 2018). Most of these researches has interrogated the potential of browse in ruminants (Tsopito, 1998). Among the IFBTs that produce edible fruit, *Sclerocarya birrea caffra*, commonly known as the Marula (Nyoka *et al.*, 2015), is well known. The Marula is drought resistant (Hall *et al*, 2002) and is adapted to the agro-ecological conditions of SSA (Shackleton and Shackleton, 2002). Its fruit pulp is commercially exploited in the production of beverages, jams and tarts (Shackleton *et al.*, 2002) and the oil derived from its fruit kernels is an important raw material in industries (Mokgolodi *et al.*, 2011), especially the pharmaceutical and cosmeceutical industries.

2.3 *Sclerocarya birrea caffra*

2.3.1 Botany and distribution

The Marula, family *Anacardiaceae*, is indigenous to Africa. It is a deciduous and dioecious plant largely found in the savannah (Mariod and Abdelwahab, 2012). The tree thrives in well-drained, sandy and loamy soils (Chirwa and Akinnifesi, 2007) at altitudes ranging from sea level to 1800m and rainfall ranges of 200 to1500mm (Mariod and Abdelwahab, 2012). Its distribution in Southern Africa spans from Zambia through Zimbabwe, Botswana, Mozambique, Namibia, South Africa to Madagascar (Shackleton *et al.*, 2002). The Marula is a medium-sized tree reaching a height of 7 to 17 m (Mariod and Abdelwahab, 2012). It has a grey fissured bark, strong branchlets and light foliage (Coates Palgrave *et al.*, 2002). Its leaves are about 60 mm long with dark-green and sharp pointed tips (Mariod and Abdelwahab, 2012). The leaves are divided into 10 or more pairs of leaflets (Mariod and Abdelwahab, 2012). The tree's flowers are comprised of red sepals and yellow petals (Dube and Dlamini, 2012) which are borne in small oblong clusters with male and female flowers occurring separately (Mariod and Abdelwahab, 2012). Flowering occurs in proximal inflorescences of shoots (Nyoka et al., 2015). The Marula fruit, yellow in colour when ripe (Dube and Dlamini, 2012), has a diameter of 3 to 4cm and weighing between 15 to 25 g, ripens between March and late April (Nyoka *et al.*, 2015; Dube and Dlamini, 2012). Its fruit pulp has high ascorbic acid content (Hillman *et al.*, 2008). A single Marula tree can yield from 21,667 to 91,272 fruits per annum (Shackleton, 2002), weighing up to 240 kg (Muhammad *et al.*, 2016; Shokane *et al.*, 2016). The Marula is used as a multipurpose tree.

2.3.2 Uses of the Marula: common and commercial

The Marula fruit pulp is edible and is used as a source of nutrients (Moganedi *et al.*, 2007; Shackleton, 2002). Its seed contains two to three oil and protein-rich kernels which (kernels) are highly nutritious (Mariod *et al.*, 2004; Moganedi *et al.*, 2007). The kernels are eaten raw and or after roasting (Aganga and Mosase, 2001; Bille and Nambabi, M Shikongo-Cheikhyoussef,

2013). The tree is also used as fuelwood (Maroyi, 2014). A decoction of the Marula leaves is used to treat colds and flu (Maroyi, 2014), while its stem-bark extracts are used to treat high blood pressure and diarrhoea (Attakpa *et al.*, 2015; Eloff, 2001; Maroyi *et al.*, 2013). The tea from boiled Marula roots is used to treat sore eyes (Mariod and Abdelwahab, 2012). Commercially, the fruit is exploited in the production of an array of beverages and jam (Shackleton, 2002; Shackleton and Shackleton, 2012). The Marula-derived liqueurs produced by Distell are marketed to more than 100 countries regionally and internationally (Shokane *et al.*, 2016). In Zimbabwe, the fruits are also used to produce jelly with high vitamin C content (Odero, 2004). The oil extracted from Marula kernels is used for culinary, cosmeceutical and other industrial purposes including among others (Vermaak *et al.*, 2011) such as manufacturing of margarine, soap and candles (Bergfeld *et al.*, 2011). The Marula nut meal (MNM), a by-product of oil extraction, contains biomass and is a potential source of nutrients in monogastric animal and poultry feeds.

2.3.3 Marula nut meal: nutrient and anti-nutrient composition

Chemical characterisation of mechanically defatted MNM revealed a CP content ranging from 324.70 to 470 g/kg DM (Malebana *et al.*, 2018; Mlambo, Dlamini, Ngwenya, *et al.*, 2011). Such processed MNMs have been shown to contain 1.10 to 1.43 and 10.00 to 10.90 g/kg of calcium and phosphorus, respectively and 28.49 to 30.69 MJ/kg GE (Malebana et al., 2018). Malebana *et al.* (2018) also reported oxalate, phytate-phosphate, saponin and tannins contents of 15.03, 218.30, 21.21, and 0.07 mg/100 g DM respectively in hydraulic pressed MNM. Phytate compromises bioavailability of phosphorus, calcium, iron, zinc and manganese in livestock and poultry (Adesuyi *et al.*, 2012; Peisker, 2001) and in broiler chicken chicks saponins have been shown to reduce feed utilisation efficiency and growth performance and to compromise dietary lipid absorption (Jenkins and Atwal, 1994). Tannins precipitate iron and other metals reducing absorption (Adesuyi *et al.*, 2012). Additionally they form complexes with both dietary and endogenous protein (digestive enzymes) thus reducing the digestion and absorption of protein (Gemede, 2014). Deoxynivalenol and T-2 toxin have been noted to be present in MNM after cold press oil extraction (Mthiyane and Mhlanga, 2017). The toxin causes a reduction in feed intake, induces loss of body weight and causes liver, neuro and reproductive toxicity (Schuhmacher-Wolz *et al.*, 2017; van der Fels-Klerx and Stratakou, 2010). While there in data regarding the effect of mechanical extraction on the chemical nutrient composition of MNM (Malebana *et al.*, 2018; Mlambo *et al.*, 2011), there is none regarding the effect re-extraction of mechanically defatted MNM with hexane.

2.3.4 Marula nut meal as a feed ingredient: *in vivo* studies

In an effort to evaluate the potential of MNM to supply dietary protein in feeds, a number of *in vivo* studies have been carried out. Marula nut meal's potential as a dietary protein source in livestock and poultry feeds has been evaluated in dairy cattle (Mlambo *et al.,* 2011), goats (Mlambo *et al.*, 2011b), sheep (Malebana, 2018) and broiler chicken (Mthiyane and Mhlanga, 2017). The evaluation of the potential of MNM in ruminant animal feeds has confirmed the meal's huge potential as a dietary protein source (Malebana, 2018; Mlambo *et al.,* 2011; Mlambo *et al.,* 2011a). However in broiler feeds the MNM with a high residual oil, due to the development of possible rancidity and the presence of mycotoxins, resulted in decrease FI, live weight, plucked weight, dressed weight, liver weight and neck weight of broilers as the level of MNM increased in the diet (Mthiyane and Mhlanga, 2017). Further defatting of the screw press produced MNM by solvent extraction would reduce the fat and thus potentially mitigate the negative effect of high residual fat on the feeds and hence on the productive performance of poultry including broiler and pullet quail.

There are differences in the gastrointestinal (GIT) microbiota composition and GIT organ sizes between broiler chicken and broiler as well as pullet quail. The GIT of Japanese quail is populated by *Firmicutes, Lactobacillus, Faecal bacterium, Bacteroides, Ruminococcus* and *Clostridium* (Dahiya *et al.*, 2018; Wilkinson *et al.*, 2016) while that of chicken has a preponderance of *Bifidobacteria, Salmonella, Campylobacter*, and *E. coli* (Amit-Romach *et al.*, 2004; Wilkinson *et al.*, 2016). These differences in the GIT microbiota species could result in differences in the utilization of the MNM between the two species. In addition to differences in the GIT microbiota, there are also differences in the GIT organ sizes (Mabelebele *et al.*, 2014) which affect both nutrient digestive and absorptive capacity (de Verdal *et al.,* 2010). Importantly, further solvent-mediated defatting of the MNM is likely to improvement its sensory profile as well as improves its protein quality largely due to the formation of protein isolates and a reduction of the ANFs in the meal (Bader *et al.*, 2011; Saetae and Suntornsuk, 2011). These improvements in the sensory and nutrient profile of the meal could result in it becoming a suitable dietary protein source in quail feeds. Feed ingredient type and quality as well as diet composition are among the key factors that affect poultry productive performance, feed utilisation efficiency and product quality.

2.4 Ingredient and diet composition: effects on poultry meat and egg quality

Poultry meat and egg quality is a critical factor that affects product acceptability by consumers (Wideman *et al.*, 2016). Regarding meat quality, appearance and texture influence the consumers' decision to purchase (Jahan *et al.*, 2005; Resurreccion, 2004). In addition to appearance (colour) and texture, tenderness is a key determinant of the eating quality and hence

the acceptability of the meat (Fletcher, 2002; Hughes *et al.*, 2014). Egg quality is largely determined by eggshell characteristics (Caner, 2005) and cleanliness (Hernandez, 2005) as consumers consider safety and freshness of the egg (Hernandez, 2005). Among the multiplicity of factors that affect poultry products (meat and egg) quality (Dawson and Bouwkamp, 1969; Guan *et al.*, 2013), feed ingredients type and quality and diet composition are among the major determinants of meat and egg quality (Hasegawa *et al.*, 2014; Summers and Leeson, 1979). They affect meat colour, pH, tenderness and drip loss as well as egg quality parameters such as weight, size, shell thickness, albumen ratio, yolk ratio and Haugh unit (El-Tarabany, 2016; Kul and Seker, 2004; Mir *et al.*, 2017).

Dietary protein and energy sources contribute to the largest portion of poultry diets (Ravindran, 2013). There are conflicting results in the literature regarding the effect of dietary protein content on the growth performance and productive performance of broiler and pullet poultry species. Energy-dense diets (concentrate diets) have been reported to improve carcass and meat quality traits of broiler chicken (Hosseini-V *et al.*, 2010). The improvement in the quality of meat was mediated through the modification of the fatty acid composition of the muscle lipid (Kim *et al.*, 2015). Feeding quail a high-fat diet resulted in higher lipid content of the breast muscle of such fed birds when compared to the breast lipid content from quail fed a standard quail diet (Donaldson *et al.*, 2016). In laying chicken pullets, body weight gain was observed to increase with an increased in dietary CP from a baseline of 19% CP throughout the production period (Calderon and Jensen, 1990) and optimum body weight was recorded in layers fed diets with high CP content (Yakout, 2010). While the body weight gain of laying pullet chicken seemed to depend on dietary CP content (Calderon and Jansen, 1990; Yakout, 2010). Zeweil *et al.* (2011) reported that dietary CP content of 12%, 14% and 16% did not affect egg mass and egg production. However, low CP-layer diets in laying chicken were observed to compromise the internal egg quality parameters: dry and wet albumen percent, albumen solids, albumen protein content and yolk protein content (Novak *et al.*, 2006). Bertipaglia *et al.* (2016) reported that dietary supplementation with soybean oil, fish waste and grape seed resulted in an increase in polyunsaturated fatty acid content in quail eggs which shows that dietary manipulation can be used to influence egg nutrient profile. In quail, decreasing dietary CP from 26% through to 24%, 22% and 20% did not affect the body weight and feed conversion ratio of the broilers quail (Blake and Hess, 2013). Excess dietary protein, as well as a deficiency of dietary protein in poultry feeds has been observed to elicit metabolic derangements that compromise productive performance and carcass quality (Filho *et al.*, 2012). Excess dietary protein in poultry feeds stimulates increased catabolism of amino acids for energy and excretion of nitrogenous wastes which results in metabolic cost to productive performance while dietary protein deficiency

causes increased mobilisation of tissue protein and body fat which compromises carcass quality (Filho et al., 2012). Poultry feeds which are largely derived from plant material contain varying concentrations of phytochemicals. These phytochemicals are secondary plant metabolites that help protect plants against herbivory and insect pest attacks (War *et al.*, 2012). While these secondary plant metabolites are protective to plants, when ingested by poultry and livestock can elicit various negative reactions which manifest with compromised productive performance and product quality as well as health (Akande *et al.*, 2010). Due to their negative effects *in vivo,* these plant secondary metabolites are deemed to have an antinutritive effect in poultry and livestock. These phytochemicals that elicit antinutritive effects *in vivo* come in various chemical classes including among others, trypsin inhibitors, tannins and glucosinolates. Antinutritional factors (ANFs) found in poultry and livestock feeds elicit a multiplicity of physiological and pathophysiological effects *in vivo* which lead to compromised performance, health and product quality.

2.5 Effect of antinutritional factors on quail health

The phytochemicals in feed ingredients and diets which elicit antinutritive effects compromise poultry, quail included, at the level of the gastrointestinal tract (Hetland *et al.*, 2004), with regards to metabolic substrate homeostasis (Butler, 2016). They also cause derangement and damage of vital organs, for example, the liver and kidney (Zaefarian *et al.*, 2019). Phytohaemagluttins have been shown to erode the ileal mucosa leading to reduced nutrient absorption capacity (Gemede and Ratta, 2014). The carbohydrate moieties of these phytohaemagluttins have also been shown to react with erythrocyte cell membranes leading to haemolysis (Akande et al., 2010) and a reduction in the haematocrit. Trypsin inhibitors that prevent the activity of the enzymes trypsin and chymotrypsin in the small intestine consequently preventing protein digestion are found in many plant species (Hill, 2004). Tannins may bind and inhibit the endogenous protein (Kumar and Singh, 1984) including digestive enzymes resulting in compromised protein digestion and absorption which manifest in stunting of growing quail. Also, tannins have the ability to interfere with the absorption of important vitamins, particularly vitamin B_{12} and minerals such as iron (Butler, 1989; Liener, 1980). It has been reported that saponins affect animal performance in various ways including erythrocyte haemolysis, hindering smooth muscle and inhibiting enzyme activity, reducing nutrient absorption and growth rate (Cheeke, 1971). Mineral chelates such as oxalates and phytic acid in feedstuffs are known to affect calcium, magnesium, phosphorus and zinc metabolism and react with proteins to form complexes which that refractory to peptic digestion (Akande *et al.*, 2010; Nwokolo and Bragg, 2010). These mineral chelate-derived effects compromise animal growth and reproductive performance as well as their health. Glucosinolates have been shown to reduce feed intake

resulting in iodine deficiency, increased mortality and compromise egg production and egg weight (Tripathi and Mishra, 2007). Terpenoids, for example, cicutoxin, atractyloside, daphnetoxin, digoxin and gibberellic acid have toxic effects that elicit gastrointestinal problems and derangements (Mbaveng *et al.*, 2014) resulting in poor nutrient digestion and absorption.

Before non-conventional feeds and additives can be used, it is important to characterise their nutritional content. In the next chapter, the characterisation of MNM is reported.

CHAPTER THREE – CHEMICAL CHARACTERISATION OF HEXANE-EXTRACTED MARULA NUT MEAL

3.0 Introduction

Oilseeds, for example, groundnut, soyabean, sunflower, cotton, palm, coconut, sesame and canola by virtue of their high lipid and protein content are not only nutrient-dense (Oilseeds focus, 2018; Sonawane and Pokharkar, 2019) but are also important sources of essential nutrients such as essential EAAs and essential fatty acids (Bernard, 2016; González-Pérez and Arellano, 2009). Rarely are these oilseeds used prior to processing. Oil is usually extracted leaving either a cake or a seed meal as a by-product depending on the oil extraction method. Mechanical extraction is associated with the production of seed cakes while solvent extraction results in seed meals (Banat *et al.*, 2013). Seed cakes and or seed meals derived from conventionally farmed crops are largely used as feed ingredients in livestock and poultry diets (Sivaramakrishnan and Gangadharan, 2009). Their use as feed ingredients is based on their high CP content ranging from 15% to 50% (Ramachandran *et al.*, 2007) as well as their significant contribution to dietary energy due to the residual fat they contain. Solvent extraction is a more efficient oil extraction method as it results in higher oil yields and seed meals with higher CP content compared to when the oil seed is mechanically extracted (Domínguez *et al.*, 1995). Importantly, extraction with hexane is associated with the improvement of the protein content and removal of ANFs in the resultant seed meal (Mosenthin *et al.*, 2016). Higher residual oils associated with mechanical oil extraction from oilseeds lead to poor feed ingredient and feed shelf life due to the problem of rancidity (Wallace *et al.*, 2010). Thus, the oil extraction method has a bearing on the quality of feed produced using the seed cake and or seed meal. The MNM evaluated using sheep had a high residual oil (38%) content (Malebana, 2018) and that used by Mthiyane and Mhlanga (2017) as a dietary protein source in broiler chicken feed also had a high residual oil content which caused some degree of rancidity. The latter was thought to be the cause of the depressed feed intake and growth performance by the broiler chicken (Mthiyane and Mhlanga, 2017). In order to reduce the residual oil content in the MNM produced through mechanical oil extraction, it became necessary to re-extract the high residual oil in the meal using hexane and then to determine its chemical nutrient composition before evaluating its potential *in vivo* using quail.

3.1 Study objectives

This study sought to determine the chemical nutrient composition of solvent extracted MNM by specifically quantifying the meal's proximate (dry matter, crude protein, ash, and fat), fibre (acid detergent fibre, neutral detergent fibre and acid detergent lignin), minerals (calcium and phosphorus), GE, amino acid and fatty acid content and compare it to that of solvent-extracted SBM.

3.2 Hypothesis

H_0: There are no differences in the proximate, GE, mineral, amino acid, and fatty acid content of MNM and SBM.

H_1: There are differences in the proximate, GE, mineral, amino acid, and fatty acid content of MNM and SBM.

3.3 Materials and methods

The proximate, gross energy (GE), mineral amino acid and fatty acid content of the defatted Marual nut meal and SBM were determined with 3 replicates for each assay.

3.3.1 Marula nut meal: source and processing

Marula nut meal was obtained from Home of Phadima Marula Oil, Phalaborwa, Limpopo, South Africa. The MNM had been partially defatted using the expeller method (Malebana *et al.*, 2018). Prior to evaluating its potential as a dietary protein source in quail diets, the MNM was further defatted using hexane. Briefly, each batch of the partially defatted 40 kg of MNM was placed in a cotton bag and steeped for 48 hour (h) in 400 litres of 99% hexane in a stainless steel 800-litre tank equipped with the drainage tap. Immediately after 48 h, the tank tap was opened and the oil-laden hexane was drained out. The hexane extracted MNM was then spread onto clean plastic sheets and dried at room temperature for 24 h. While samples of the hexane extracted meal were taken for chemical nutrient analyses, the bulk of the hexane extracted and dry MNM was packaged into jute bags and stored at room temperature until use to formulate diets used to evaluate its potential as a dietary protein source. The SBM used was produced from dehulled soyabeans. The dehulled beans were crushed and the meal was subjected to two oil extraction processes: firstly to mechanical oil extraction which produced a partially defatted meal which was then solvent extracted with hexane to produce the SBM.

3.3.2 Determination of the chemical nutrient composition of the meals

Subsamples from bulk samples of SBM (bought from Obaro, Pretoria, SA) and the dried hexane-extracted MNM had their chemical nutrient evaluation done at the Agricultural Research Council-Animal Production Institute laboratory, in Irene, Pretoria, South Africa. The chemicals and reagents used during the assays were of analytical grade. Each of the assays was done in triplicate.

3.3.2.1 Determination of the proximate and gross energy content

The DM, CP, ash and EE of the SBM and MNM were determined as described by the Association of Official Analytical Chemists (AOAC, 2006 method numbers 934.01, 942.05, 954.01, and 920.39, respectively). The organic matter (OM) content of each meal was computed using the equation: OM = DM-ash.

The gross energy (GE) content of each of the meals (MNM and SBM) was determined using an MC-1000 Modular Calorimeter (Energy Instrumentation, Centurion, South Africa) equipped with a PC and MC1000 software according to the manufacturer's instructions.

3.3.2.2 Determination of the calcium and phosphorus content

The calcium and phosphorus content of the seed meals was determined as described by Zasoski and Burau, (1977). Briefly, 0.5 g of seed meal sample was digested in 25 ml of 65% nitric acid and 5 ml of perchloric acid at 200 °C. The digest solution was then used to spectrophotometrically determine the mineral content of the seed meal using inductively coupled plasma-optical emission spectrometry (ICP-OES) on a Varian Liberty 200 spectrometer (Varian, Perth, Australia) as described by Huang and Schulte (1985).

3.3.2.3 Determination of the amino acid content

The amino acid concentration of each of the seed meals was determined as described by Einarsson *et al.* (1983). Briefly, the seed meal was hydrolysed in an acid (6M HCl) at a temperature of 110°C for 24 h followed by pre-column fluorescence derivatisation of amino acids with 9-flourenylmethyl chloroformate. The amino acids were then extracted with pentane and separated by gradient elution on a chromatograph. The chromatograph consisted of a SpectraSystem P4000 Quaternary high-performance liquid chromatography system (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a SpectraSystem FL3000 fluorescence detector (Thermo Fisher Scientific Inc.) and a Rheodyne 7125 valve (IDEX Corp., Rohnert Park, CA, USA) with a 20-μL injection loop. The amino acids were separated using an OmniSper 5 C18 150 × 4.6 analytical column and guard-column (Varian Australia Pty Ltd, Perth, Australia). The identification of the amino acids was done at an excitation wavelength of 264nm and an emission wavelength of 340 nm. A PC equipped with TSP software was used for quantification.

3.3.2.4 Determination of the fibre content

The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) components of MNM and SBM were determined as described by Van Soest *et al.* (1991). Briefly, the NDF of each meal was determined by adding 0.5 g of the meal sample in 100 ml of the neutral detergent solution (sodium lauryl sulphate and ethylenediamine-tetraacetic acid) which contained heat-stable alpha-amylase (20 350 IU/ml) and refluxed at 100 ºC for 1 h. Thereafter the mixture was filtered and the residue dried and then weighed. To determine the ADF of each meal, 0.5 g of the meal sample was refluxed in the acid detergent (20 g cetyl-trimethyl ammonium bromide dissolved in 1L N H_2SO_4) solution for 1 h. Immediately, thereafter the mixture was filtered and the residue was dried and then weighed. The ADL was determined

using a similar procedure to that of the determination of NDF except that after rinsing each sample residue, 72% of concentrated sulphuric acid was added to the residue and boiled at 700 ºC for 3 h. After boiling, the sulphuric acid was washed off with hot water and the residue was dried in an oven at 100 ºC for 12 h, cooled and then weighed

3.3.2.5 Determination of the fatty acid content

The Soxhlet method was used to extract the fat from each of the meal samples as described by the Association of Official Analytical Chemists (AOAC, 2006, method number 920.39). The methyl ethers were analyzed using gas chromatography as described by Christopherson and Glass, (1969). Briefly, the fat extracts were trans-methylated with 2M methanol-sodium hydroxide. Heptane was used to extract the fatty acid methyl esters which were then filtered and dried under nitrogen. The fatty acids were separated by a temperature gradient over 45 min on gas chromatography with nitrogen as carrier gas on a DB-23 capillary column (90 cm × 250 μm × 0.25 μm) (Supelco, Sigma-Aldrich). The gas chromatograph consisted of HP6890 GC (Hewlett Packard, Bristol, United Kingdom) with a flame ionization detector (FID). Both the detector and injector temperatures were set at 300 °C. A PC equipped with Chemstation software was used for quantification. Nonadecanoic acid (C19:0) was used as an internal standard.

3.4 Statistical analysis

Data are presented as means ± SD. GraphPad Prism version 5 was used to analyse data and a t-test was used to compare the means. Statistical significance was set at $P < 0.05$.

3.5 Results

3.5.1 Chemical nutrient composition of the meals

The chemical nutrient content of MNM and SBM are shown in Table 3.1. The proximate, GE and fibre content of MNM was higher than that of SBM but its calcium content was lower than that of SBM ($P < 0.0001$).

Table 3.1: Proximate, fibre, mineral and energy content of Marula nut meal and soyabean meal

Analyte	MNM	SBM	Significance level
Proximate (g/kg DM)			
Dry matter	906.40 ± 0.07[a]	883.00 ± 0.03[b]	***
Crude protein	648.20 ± 0.18[a]	454.30 ± 0.15[b]	***
Organic matter	830.00 ± 0.05[a]	822.60 ± 0.07[b]	*
Ash	73.40 ± 0.04[a]	60.30 ± 0.04[b]	***
Ether extract	73.50 ± 0.01[a]	11.50 ± 0.03[b]	***
Energy (MJ/kg DM)			
Gross energy	22.89 ± 0.01[a]	21.45 ± 0.01[b]	*
Fibre(g/kg)			
aNDF	88.30 ± 0.09[a]	11.63 ± 0.34[b]	**
ADF	66.90 ± 0.28[a]	44.80±0.05[b]	**
ADL	45.50 ± 0.17[a]	8.90 ± 0.69[b]	**
Mineral content (mg/100g)			
Calcium	2.70 ± 0.01[a]	4.80 ± 0.09[b]	***
Phosphorus	15.0 ± 0.01[a]	5.20 ± 0.11[b]	***

P >0.05, ** P <0.01, *** P <0.0001, [ab] within row means with different superscripts are significantly different at P < 0.05. The DM, CP, OM, EE, GE, ash, fibre content of MNM was significantly different compared to that of SBM. MNM = solvent extracted; SBM = solvent extracted soyabean meal; Data presented as mean ± SD; n = 3.

The amino acid profile of MNM and SBM is shown in Table 3.2. The MNM had a higher ($P < 0.0001$) alanine, arginine, glumatic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine and valine concentration per unit mass than SBM. However, SBM had a higher concentration ($P < 0.0001$) of aspartic acid, lysine, and tyrosine than their concentration in MNM. The concentration of threonine and hydroxyproline was similar ($P > 0.05$) in the MNM and SBM.

Table 3.2: Amino acid content of the Marula nut meal and soyabean meal

Amino acid (g/100g DM)	**MNM**	**SBM**	**Significance level**
Alanine	2.44 ± 0.01^{a}	2.23 ± 0.01^{b}	***
Arginine	8.93 ± 0.30^{a}	4.06 ± 0.06^{b}	***
Aspartic acid	2.47 ± 0.11^{a}	4.81 ± 0.28^{b}	**
Glumatic acid	10.84 ± 0.05^{a}	6.16 ± 0.09^{b}	***
Glycine	3.93 ± 0.06^{a}	2.03 ± 0.03^{b}	***
Histidine	3.46 ± 0.01^{a}	1.95 ± 0.06^{b}	***
Hydroxy-proline	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	ns
Isoleucine	3.61 ± 0.18^{a}	2.21 ± 0.03^{b}	**
Leucine	4.97 ± 0.03^{a}	3.62 ± 0.09^{b}	***
Lysine	2.29 ± 0.04^{a}	3.28 ± 1.02^{b}	*
Methionine	1.44 ± 0.02^{a}	0.60 ± 0.04^{b}	***
Phenylalanine	3.83 ± 0.05^{a}	2.47 ± 0.01^{b}	***
Proline	2.80 ± 0.08^{a}	2.48 ± 0.01^{b}	*
Serine	3.92 ± 0.20^{a}	2.65 ± 0.01^{b}	**
Threonine	1.92 ± 0.03^{a}	1.99 ± 0.03^{a}	ns
Tyrosine	2.12 ± 0.02^{a}	2.94 ± 0.06^{b}	***
Valine	3.78 ± 0.00^{a}	2.10 ± 0.01^{b}	***
Total	**62.84 ± 1.2**	**46.47 ± 1.87**	***

ns = not significant, P >0.05, *P <0.05, ** P <0.01, *** P <0.0001, ^{ab} Within row means with different superscripts are significantly different at p <0.05. The amino acid content of MNM was significantly different as compared to that of SBM except for threonine and hydroxyproline which were similar (P >0.05). MNM = solvent extracted marula nut meal; SBM = solvent extracted soyabean meal; data presented as mean ± SD; n = 3.

The fatty acid profile of MNM and SBM is shown in Table 3.3. The TSFA percentage of MNM and SBM were similar ($P > 0.05$). However, MNM had higher percentage ($P < 0.0001$) of TMUFA than SBM. Soyabean meal had a higher percentage ($P < 0.0001$) of TPUFA than MNM. However, the content of TSFA and TMUFA of MNM was higher ($P < 0.0001$) than that of SBM but the content of TPUFA was higher ($P < 0.0001$) in SBM compared to that of MNM (See supplementary data in Appendix 1).

Table 3.3: Fatty acid profile of Marula nut meal and soyabean meal lipids

Fatty acid (%)	MNM	SBM	Significance level
Saturated			
C20:0 (arachidic acid)	nd	0.41 ± 0.06	-
C22:0 (behenic acid)	0.77 ± 0.05[a]	1.36 ± 0.63[a]	ns
C12:0 (lauric acid)	0.15 ± 0.00[a]	0.12 ± 0.03[a]	ns
C24:0 (lignoceric acid)	0.20 ± 0.01[a]	0.23 ± 0.03[a]	ns
C17:0 (margaric acid)	0.18 ± 0.01[a]	0.20 ± 0.03[a]	ns
C14:0 (myristic acid)	0.16 ± 0.01[a]	0.14 ± 0.03[a]	ns
C16:0 (palmitic acid)	14.16 ± 0.09[a]	13.71 ± 1.13[a]	ns
C15:0 (pentadecanoic acid)	nd	0.26 ± 0.05	-
C18:0 (stearic acid)	7.68 ± 0.96[a]	5.16 ± 0.36[a]	ns
C23:0(tricosanoic acid)	nd	0.15 ± 0.06	-
TSFA	**25.06 ± 1.13**	**23.48 ± 2.41**	**ns**
Monounsaturated			
C18:1n9 (oleic acid)	65.93 ± 1.34[a]	21.86 ± 2.99[b]	***
C22:1n9 (erucic acid)	0.32 ± 0.09[a]	0.15 ± 0.06[a]	ns
TMUFA	**67.39 ± 1.43**	**19.83 ± 3.05**	****
Polyunsaturated			
C18:3n3 (α-linolenic acid)	0.16 ± 0.01[a]	5.22 ± 2.19[b]	**
C20:5n3 (Eicosapentaenoic acid)	0.32 ± 0.06[a]	4.93 ± 1.76[b]	***
C18:2n6t (linolelaidic acid)	nd	0.15 ± 0.03	-
C18:2n6c (linoleic acid)	6.68 ± 0.35[a]	51.77 ± 0.88[b]	***
C18:3n6 (γ-linolenic acid)	nd	0.20 ± 0.08	-
TPUFA	**7.14 ± 0.42**	**58.35 ± 4.94**	****
Trans fatty acids	nd	0.15 ± 0.03	-
Cis fatty acids	75.01 ± 1.23[a]	25.62 ± 3.52[b]	***
Omega-3	0.71 ± 0.05[a]	0.14 ± 0.03[b]	***

Omega-6	7.28 ± 0.73[a]	47.22 ± 4.08[b]	***
Omega-9	67.21 ± 0.30[a]	24.75 ± 3.23[b]	***
EPA (Eicosapentaenoic acid)	0.23 ± 0.01[a]	0.11 ± 0.03[b]	*
DHA (Docosahexaenoic acid)	0.05 ± 0.01	nd	-

ns = not significant, P >0.05, *P <0.05 ** P <0.01, *** P <0.0001, [ab] Within row means with different superscripts are significantly different at P <0.05. There were significant differences in monounsaturated, polyunsaturated and Cis Fats, Omega-3, Omega-6, Omega-9 as well as the EPA of MNM and SBM. MNM = solvent extracted Marula nut meal; SBM = solvent extracted soyabean meal; data presented as mean ± SD; n = 3.

3.6 Discussion

3.6.1 Proximate, mineral and fibre composition

In this study, the higher DM, CP, OM and EE of the MNM show that per unit mass, the MNM has a higher nutrient density than SBM. The MNM produced by mechanical extraction of oil had 32% CP content (Malebana *et al.,* 2018) which is comparatively lower than that of the hexane-extracted MNM (64% CP) evaluated in the current study. The high CP, EE and GE content of hexane-extracted MNM compared to SBM, may translate to less inclusion of the meal (MNM) in poultry diets that may lead to reduced feed costs. Monogastric animals and poultry can tolerate a dietary fibre as follows: NDF from 7 to 18.6% (Nepomuceno *et al.,* 2018), ADF from 2.5% to 21% ADF (Hollister, 1991; Miao *et al.*, 2003) and 15% ADL (Benkeblia, 2014). While the fibre content (NDF – 8.8%, ADF – 6.6% and ADL – 4.5%) of the MNM was higher than that of SBM (Table 3.1), its concentration is within the range of dietary NDF, ADF and ADL tolerated by monogastric animals and poultry (Hollister, 1991). The higher energy concentration in the MNM compared to SBM could be exploited to reduce the inclusion level of the dietary energy source during feed formulation which positively impacts feed costs. Thus, apart from its potential to contribute dietary protein in poultry feeds, the MNM could also be exploited to supply part of the dietary energy component. With regards to the calcium and phosphorus content of the meals, due to its lower calcium concentration, use of the MNM in diet formulation might require additional and probably more supplementation with calcium than when compared to when SBM is used. However, the use of MNM in place of SBM would entail less, if any requirement to supplement the diet with phosphorus as the phosphorus content is about three times higher (Table 3.1). However, the calcium to phosphorus ratio of both meals is less than the recommended for quail feeds. For laying quails recommendations are about 3.0% Ca with 0.25% available phosphorus (Pav) in their diets (Amoah *et al.*, 2012) while broiler quails require about 0.8% Ca and 0.45% Pav (Shrivastav, 2000).

The results of the current study confirm that solvent extraction is more efficient oil extraction method compared to the mechanical extraction of oil from oilseed meals. High residual oil can result in rancidity of feeds (Bochkarev *et al.*, 2016). High dietary lipids can also compromise nutrient digestion and absorption (Wang *et al.*, 2016) especially in growing poultry (Turner *et al.*, 1999). Thus, re-extraction of mechanically extracted MNM with hexane can potentially reduce the risk of high residual lipid-induced feed rancidity and negative effects on nutrient digestion and absorption.

3.6.2 Amino acid content

The higher concentration of alanine, arginine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine and valine (Table 3.2) in MNM than SBM denotes that MNM can supply the animals with more essential amino acids that are needed by the body to produce proteins required for growth and reproduction. However, MNM has a lower concentration of other essential amino acids such as lysine as compared to SBM. In terms of the amount there were no differences (P >0.05) in lysine content of MNM and SBM (see supplementary data in Appendix 2). Although the lysine content of MNM is lower than that of SBM, the other feed ingredients used in formulation may add some lysine which may end up reaching the required value for broiler and layer production. Altine *et al.* (2016) reported that broiler and layer quail require of about 1.20% and 0.80% respectively. The concentration of threonine and hydroxyproline was similar (P >0.05) in the MNM and SBM. However, the amounts of threonine and hydroxyproline were higher in MNM as compared to SBM (see supplementary data in Appendix II). These findings could imply that the threonine and hydroxyproline requirements of poultry species can be also met by utilizing higher volume of MNM instead of SBM.

The lysine content of MNM found in the study was 2.29 ± 0.04% which was higher compared to the 0.79% from mechanically extracted meal reported by Mthiyane and Mhlanga (2017) which is also lower than the 3.28 ± 1.02% of SBM reported in the current study. Therefore, implying that there would be less supplementation of lysine if solvent-extracted MNM is used instead of screw-pressed oil extracted MNM, although the use of SBM would be more ideal. However, Malebana *et al.* (2018)reported that generally the MNM contained less amino acid concentration than SBM. However, findings from this study suggest that hexane-extracted MNM generally contained more amino acids concentration than SBM, implying that hexane defatted MNM may have the capacity to supply sufficient amino acids in the diets thus cutting cost of using SBM. Findings from the current study confirm that solvent extraction of oilseeds and or their meals yields meals with less residual oil containing a higher CP and amino acid concentration

compared to mechanically produced meals. It can therefore be inferred that solvent extraction of oil from the mechanical produced MNM resulted in nutritionally higher quality protein meal.

3.6.3 Fatty-acid profile

The fatty acids play an important role in the body as they are a good source of energy, major components of cellular membranes (Nagy and Tiuca, 2017) as well as for the efficient digestion and absorption of fat-soluble vitamins (Di Pasquale, 2009). It is common practice in modern poultry production to include fats and/or oils not just to boost the calorific content of the diets but to improve the absorption of fat-soluble vitamins, diminish the pulverulence, increase the palatability of the rations as well as to increase the efficiency of utilisation of energy (Ayed *et al.*, 2015). Findings from the current study show that the percent TSFA, TMUFA and TPUFA in MNM was 25.06%, 67.39% and 7.14%, respectively while that of SBM was 23.48%, 19.83% and 58.35% for TSFA, TMUFA and TPUFA, respectively. While the percentage of the TSFA of the MNM and that of SBM were similar, content-wise MNM had a higher ($P < 0.0001$) amount of TSFA compared to that of SBM (Appendix 1). Saturated fatty acids are more reduced and hence energy dense (German and Dillard, 2004). Intensive poultry and livestock production hinges on feeding energy-dense feeds (Ahiwe *et al.*, 2018; Leng, 2004). The high residual TSFA content in the MNM most likely means the meal has a high energy content which could potentially be exploited in formulating energy-dense feeds for poultry and livestock and most probably allow for the reduction of the maize meal proportion in the feeds. Importantly, saturated fatty acids are more stable compared to mono- and poly-unsaturated fatty acids which are more prone to oxidation resulting in rancidity (Peplow *et al.*, 1990; Imahara *et al.*, 2008). Due to its high residual TSFA fatty acid content, the MNM could be utilised in formulating poultry and livestock diets with a longer shelf life. Findings from the current study show that the MNM had both a higher TMUFA percentage (Table 3.3) and content (Appendix 1) when compared to SBM and the dominant monounsaturated fatty acid in the meal is shown to be oleic acid (Appendix 1). It is important to note that oleic acid was the dominant monounsaturated fatty acid in the MNM. Olive oil which is rich in OA has been shown to have beneficial effects on cancer, autoimmune and inflammatory diseases (Sales-Campos *et al.*, 2013).

By its nature, intensive poultry and livestock production causes some degree of stress within the birds and or animals (Curtis, 1987) and generally synthetic antioxidants are incorporated in feeds to mitigate stress (Salami *et al.*, 2015). Due to increased consumer awareness on the importance of clean poultry and livestock production, there is now a preference to eliminate the use of synthetic agents from the food production chain. The MNM over and above being a potential

dietary protein source could also be exploited as a source of natural antioxidants in poultry and livestock feeds due to its high OA content (Hernandez, 2015).

The fatty acids composition of oils used in poultry rations are reflected in the birds' meat and eggs because dietary fatty acids are incorporated with little change into the bird body fats (Scaife *et al.*, 1994). The high OA content of the MNM could therefore be potentially exploited to formulate poultry diets that will positively impact on the OA content of the meat and eggs. Additionally, the high TSFA and OA content of the MNM could positively impact feed intake since supplementation of poultry rations with fats and oils has been shown to improve palatability (Ayed *et al.*, 2015).

3.7 Conclusion

Hexane-extraction of the mechanically produced MNM reduced the residual oil content and resulted in a meal with a high CP and phosphorus content which can potentially be used as a dietary protein source in poultry and livestock feeds. Due to its high phosphorus and oleic acid content, the hexane-extracted MNM can potentially be exploited in pullet diets with minimal phosphorus supplementation as well as in manipulating (increasing) product (meat and eggs) oleic acid content. Defatting the meal could yield feed that is more stable against oxidation thus improving the shelf life due to reduced chances of developing rancidity.

Premised on the positive outcomes hexane-extraction had on the chemical nutrient composition of the MNM, the next chapter of this thesis focuses on the *in vivo* effects of replacing SBM with the MNM on broiler quail growth performance, feed utilisation efficiency, health and meat quality.

CHAPTER FOUR- EFFECT OF DIETARY MARULA NUT MEAL ON BROILER QUAIL GROWTH PERFORMANCE, FEED CONVERSION EFFICIENCY, HEALTH AND MEAT QUALITY

4.0 Introduction

In chapter 2, under heading 2.0 of this thesis the increase in the demand for livestock and poultry products in Sub-Saharan Africa (SSA) was discussed. This increased demand is against a background of dwindling off-take of ruminant livestock products from the natural veld due to the loss of natural pasture to crop cultivation and urban settlements (Thornton, 2010). An increase in the human population, the growth of urban settlements and an increase in incomes are the major drivers of the observed increase in the demand for animal products. The SSA human population is expected to more than double by 2050 (FAO, 2013; van Huis, 2013) resulting in a sustained increase in the demand of foods of animal origin (Ssepuuya *et al.*, 2017). Due to the decline in the animal product yield from the natural veld, there is a need to intensify production to meet the increasing demand. The poultry industry, which comparatively requires less land, is a potential alternative to pasture-dependent animal product production. The industry is generally on a growth trajectory in SSA (Roberts, 2017). In the SSA region, South Africa is the highest producer and consumer of chicken and chicken products (Roberts, 2017). Compared to red meats, chicken meat is a cheaper source of animal-derived protein (Jaturasitha *et al.*, 2004) and has a lower lipid content and moderately high concentration of polyunsaturated fatty acids (Nkukwana *et al.*, 2016). It is therefore a healthier product when compared to beef, pork and mutton (Vukasovič, 2010). While chicken meat is cheaper compared red meats, resource-limited smallholder rural communities find commercial chicken production expensive as they cannot afford the housing, feed and veterinary requirements of the genetically improved broiler and pullet chicken (Etal, 2014; Moreki, 2010). This, therefore, limits chicken production in rural communities of SSA where protein malnutrition is endemic and the requirement of foods of animal origin is greatest. In such communities, while there are indigenous chicken; their growth rate is slow (Norris *et al.*, 2007) such that production does not meet the dietary protein requirements for adequate human nutrition and nourishment. Poultry species that require less investment viz land, housing and feed and veterinary requirements are best suited for production in rural communities of SSA (Nkukwana, 2019). Such species would help address the challenges associated with the production of both indigenous and genetically improved chicken breeds. Such species could possibly be utilised to complement and in some instances replace chicken.

Japanese quail (*Cortunix cortunix japonicum*) are small and are characterised by having a shorter generation interval, rapid growth and require less land area (Minvielle, 2004). These characteristics make it relatively cheaper to breed and produce quail (Shanaway, 1994; Minvielle, 2004) even by resource-limited rural communities. Besides the relatively cheaper investment required for producing quail, their meat when compared to broiler chicken meat has lower fat and cholesterol but a higher iron and potassium content (Aminzade *et al.*, 2012; Raji *et*

al., 2015). This makes Japanese quail meat a healthier product in view of the metabolic derangements and diseases that result from the consumption of unhealthy products such as fat-laden meat.

Despite the potential availed by quail, smallholder farmers generally do not have enough resources to buy SBM, an essential dietary protein source in poultry feeds. While SBM is the major dietary protein source for the SSA poultry industry, regional production of soyabean does not meet the human and poultry requirements for SBM. The shortfall in SBM is made up through costly imports which translate into increased feed cost thus making poultry meat and eggs more expensive. In order to tap into the potential of quail as a source of animal-derived protein for human consumption, there is a need to search and develop alternative dietary protein sources. The development of alternatives allows for greater choice translating into less costly poultry feeds. Marula nut meal, a by-product of oil extraction Marula kernels by local small to medium scale Marula fruit processors, is a potential dietary protein source in quail feeds. Recent *in vitro* characterisation (Malebana, 2018) and *in vivo* studies with broiler chicken (Mthiyane and Mhlanga, 2017) have demonstrated the potential of MNM to replace SBM in broiler chicken feeds. Importantly, the re-extraction of the MNM with hexane (Chapter 3) produced a meal with a higher CP content than that of SBM. Although MNM has been demonstrated to viably substitute SBM in broiler chicken feeds (Mthiyane and Mhlanga, 2017), due to differences in the gastrointestinal (GIT) microbiota composition which impact digestion of feed in avian species (Wilkinson *et al.*, 2016), MNM might be differently processed by microbiota resident in the quail GIT. The *firmicutes, lactobacillus, faecal bacterium, bacteroides, ruminococcus* and *clostridium* species dominates the GIT of Japanese quail (Dahiya *et al.*, 2018; Wilkinson *et al.*, 2016) which could result in differences in the utilisation of the MNM as chicken has a preponderance of *bifidobacteria, salmonella, campylobacter* and *E. coli* (Amit-Romach *et al.*, 2004; Wilkinson *et al.*, 2016). Additionally, both nutrient digestive and absorptive capacity (de Verdal et al., 2010) can also be affected by the GIT organ sizes (Mabelebele *et al.*, 2014). Importantly, phytochemicals in some of these plant-derived non-conventional feed ingredients either positively and or negatively impact the macro- and micro-morphometry of the GIT viscera (Arsi *et al.*, 2014) which in turn affects digestive and absorptive function (Abdel-Mohsein *et al.*, 2014). Based on the aforesaid, despite the potential MNM demonstrated in broiler chicken feeds to supply dietary protein, it is pertinent that its potential to replace SBM in broiler quail feeds be interrogated.

4.1. Study aim

This study thus evaluated the effects of a graded substitution of soyabean meal with hexane-extracted Marula nut meal as a dietary protein source in quail starter and finisher diets on growth performance, feed utilisation economy, viscera macro-morphometry, liver and kidney function and meat quality of broiler Japanese quail.

4.1.1 Study objectives

The specific objectives of this study were to formulate MNM-based brooding/grower and finisher diets and to determine their effects on growth performance, feed utilisation efficiency, viscera macro-morphometry, health, carcass characteristics and meat quality of broiler quail.

4.1.2 Study hypothesis

H_0: The graded substitution of SBM with dietary MNM has no effect on the growth performance (body mass), feed intake and utilisation efficiency, viscera macro-morphometry, hepatic lipid content, liver and kidney function and general health, carcass traits, and meat quality of broiler Japanese quail.

H_1: The graded substitution of SBM with dietary MNM has an effect on the growth performance (body mass), feed intake and utilisation efficiency, viscera macro-morphometry, hepatic lipid content, liver and kidney function and general health, carcass traits, and meat quality of broiler Japanese quail.

4.2 Materials and methods

4.2.1 Study site and ethical clearance

The study was carried out at the Central Animal Service (CAS) unit of the University of the Witwatersrand (Wits) and the Wits School of Physiology Laboratories. It was approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, South Africa (AESC approval number: 2017/08/54B).

4.2.2 Feed ingredients

4.2.2.1 Marula nut meal - source and processing

The partially defatted MNM was obtained from Home of Phadima Marula Oil, Phalaborwa, Limpopo, South Africa and processed as described earlier in Chapter 3; under subheading 3.3.1 above. Prior to use in feed formulation, samples of the solvent-extract MNM were chemically (proximate, mineral, fibre, amino acid and fatty acid content) characterised.

4.2.2.2 Other feed ingredients

Soyabean meal, yellow maize, limestone and wheat bran were purchased from Obaro, Pretoria, South Africa. The vitamin and mineral premix, synthetic lysine and methionine were purchased from Trouw Nutrition (Isando, Johannesburg, South Africa).

4.2.3 Diet formulation and dietary treatments

Iso-caloric and iso-nitrogenous diets that met the nutritional requirements of growing and finishing quail were formulated based on the National Research Council (NRC, 1994) recommendations. The diets were formulated such that MNM replaced SBM, on a CP basis, at 0%, 25%, 50%, 75% and 100% for diets 1 through to 5, respectively, for both the grower and finisher diets. Tables 4.1 and 4.2, show the ingredient and nutrient composition of the brooding/grower and finisher diets, respectively.

4.2.4 Animals, housing and feeding

Two hundred (200) 7-day old Japanese quail chicks used in the study were sourced from SA Quail Breeders, East London, South Africa. The chicks were habituated for two days before the commencement of the trial. During this period (habituation), they were dewormed with piperazine (Kyron Laboratories Pvt Ltd, Johannesburg, South Africa). The piperazine was added to their drinking water at 90 mg/L. A deep litter system was used to house the chicks. Feeders and drinkers were provided. Feed and clean drinking water were provided *ad libitum*. Since the study was done indoors, lights were switched on at 07h00 and off at 19h00. Infra-red lights were used to provide supplementary heat. Bedding, made from clean dry wheat straw, was changed twice a week. The quail were fed the brooder/grower diets for 4 weeks and then transferred onto corresponding finisher diets for 2 weeks.

Table 4.1: Ingredient and chemical composition of brooding/grower dietary treatment

Ingredients (g/kg)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Maize meal	422.00	402.00	417.00	423.00	379.00
Soyabean meal	406.00	318.00	212.00	108.00	0.00
Marula nut meal	0.00	74.00	149.00	228.00	315.00
	(0%)	(25%)	(50%)	(75%)	(100%)
Wheat bran	135.00	140.00	141.00	144.00	180.00
Limestone	9.00	9.00	9.00	10.00	10.00
Vegetable oil	0.00	33.00	47.00	63.00	90.00
Dl-methionine (99%)	2.00	2.00	2.00	2.00	2.00
L-lysine HCL (98.5%)	1.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	17.00	12.00	12.00	13.00	13.00
Salt (NaCl)	3.00	2.00	2.00	2.00	3.00
*Vit/mineral premix	5.00	5.00	5.00	5.00	5.00
Total	**1000**	**1000**	**1000**	**1000**	**1000**

Calculated chemical composition

DM (%)	89.95	88.36	87.82	87.30	87.43
Crude protein (% DM)	24.05	24.05	24.05	24.03	24.04
Crude fibre (% DM)	3.76	3.64	3.62	3.57	3.65
Ash (% DM)	3.69	3.71	3.63	3.55	3.62
Fat (% DM)	2.46	5.92	7.72	9.48	12.04
Neutral detergent fibre (% DM)	14.37	13.53	13.10	12.56	12.58
Acid detergent fibre (%DM)	4.75	4.17	3.69	3.19	2.86
Calcium (%DM)	0.90	0.89	0.91	0.90	0.91
Phosphorus (%DM)	0.54	0.56	0.57	0.57	0.57
Sodium (%DM)	0.16	0.16	0.16	0.16	0.16
Chloride (%DM)	0.27	0.17	0.17	0.27	0.27
Methionine (%DM)	0.54	0.52	0.54	0.52	0.54
Lysine (%DM)	1.37	1.37	1.37	1.37	1.37
Metabolisable energy (MJ/kg DM)	16.27	16.62	16.88	16.97	17.03

*Vit A: 20000000.000 IU, Vit B_1 (Thiamine): 003.000g, Vit D_3 (500 000): 3000000.000 IU, Vit E (500 iu):40000.000 IU, Vit K_3 (43%): 003.000g, Vit B_2 (80%): 010.000g, Vit B_6 98% (pyrod): 005.000g, Vit B_{12} 1g/kg (m):100.000mg, Niacine 99.5%: 060.000g, Choline (Chloride 60): 606.060g, Biotine 2%: 200.000mg, Manganese(MnSO4-31%):160.000g, Copper ($CuSO_4$-25.2%): 005.000g, Cobalt ($CoSO_4$-20%): 100.000mg, Selenium (Na_2SeO_3- 4.5%): 400:000mg, Calcium pantothenate: 020.000g, Folic acid (96% pure): 001.000g, Anty-ox Vit Dry: 100.000g, Zinc (Zn SO_4-35%): 090.000g, Iodide (KI 76.45%): 001.000g, Ferrous ($FeSO_4$-30%): 035.000g, Limestone powder: 2647.133g; DL-Methionine with purity of 99%, L-Lysine HCL with purity of 98,5%; Diet 1 – 0% MNM meal 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 4.2: Ingredient and chemical composition of finisher dietary treatments

Ingredients (g/kg)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Maize meal	560.60	554.80	566.00	606.20	607.80
Soyabean meal	327.00	246.50	165.00	82.90	0.00
Marula nut meal	0.00	57.80	115.00	173.80	233.00
	(0%)	(25%)	(50%)	(75%)	(100%)
Wheat bran	79.40	103.60	113.10	94.70	114.00
Limestone	18.70	20.30	20.30	20.8	20.90
Dl-methionine (99%)	2.10	2.80	3.00	3.50	4.00
L-lysine HCL (98.5%)	2.00	4.70	6.00	9.5	11.70
Dicalcium phosphate	1.90	9.00	9.00	0.00	0.00
Salt (NaCl)	3.60	3.80	3.80	3.80	3.80
*Vit/mineral premix	4.70	4.80	4.80	4.80	4.80
Total	**1000**	**1000**	**1000**	**1000**	**1000**
Calculated chemical composition					
Dry matter (% DM)	88.27	87.61	87.50	87.28	87.09
Crude protein(% DM)	20.04	20.57	21.02	21.25	21.79
Crude fibre(% DM)	3.15	3.30	3.38	3.26	3.39
Ash (% DM)	3.33	3.31	3.30	3.19	3.22
Fat (% DM)	2.59	2.94	3.33	3.71	4.09
Neutral detergent fibre (% DM)	12.20	12.59	12.66	11.89	12.22
Acid detergent fibre(% DM)	3.93	3.78	3.53	3.03	2.86
Calcium (%DM)	0.81	0.83	0.82	0.80	0.80
Phosphorus (%DM)	0.38	0.41	0.45	0.48	0.52
Sodium (%DM)	0.14	0.14	0.14	0.14	0.14

Chloride (%DM)	0.25	0.26	0.26	0.25	0.25
Methionine (%DM)	0.52	0.53	0.50	0.50	0.50
Lysine (%DM)	1.28	1.32	1.30	1.32	1.30
Metabolisable energy (MJ/kg DM)	15.67	15.62	15.66	15.68	15.72

*Vit A: 20000000.000 IU, Vit B_1 (Thiamine): 003.000g, Vit D_3 (500 000): 3000000.000 IU, Vit E (500 iu):40000.000 IU, Vit K_3 (43%): 003.000g, Vit B_2 (80%): 010.000g, Vit B_6 98% (pyrod): 005.000g, Vit B_{12} 1g/kg (m):100.000mg, Niacine 99.5%: 060.000g, Choline (Chloride 60): 606.060g, Biotine 2%: 200.000mg, Manganese(MnSO4-31%):160.000g, Copper ($CuSO_4$-25.2%): 005.000g, Cobalt ($CoSO_4$-20%): 100.000mg, Selenium (Na_2SeO_3- 4.5%): 400:000mg, Calcium pantothenate: 020.000g, Folic acid (96% pure): 001.000g, Anty-ox Vit Dry: 100.000g, Zinc (Zn SO_4-35%): 090.000g, Iodide (KI 76.45%): 001.000g, Ferrous ($FeSO_4$-30%): 035.000g, Limestone powder: 2647.133g; DL-Methionine with purity of 99%, L-Lysine HCL with purity of 98,5%; Diet 1 – 0% MNM meal 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.

4.2.5 Study design

The two hundred (200) 9-day old Japanese quail chicks, in a simple randomised interventional study, with four replicates of 10 birds each, were randomly allocated to and fed brooder/grower diets wherein MNM replaced SBM on a CP basis for four weeks as follows: diet 1 - 0% MNM + 100% SBM, diet 2 - 25% MNM + 75% SBM, diet 3 - 50% MNM + 50% SBM, diet 4 - 75% MNM + 25% SBM and diet 5 - 100% MNM + 0% SBM. Immediately thereafter they were transferred to corresponding finisher diets and fed for two weeks.

4.2.6 Measurements during the feeding trial

Body mass (BM) at induction and thereafter once weekly of the birds was measured using an electronic balance (Snowrex, model: EQ: 1200, Taiwan, China). Feed intake (FI) was measured daily by subtracting the refusals from the total feed offered.

4.2.7 Pre-termination computations

Body mass gain (BMG), average daily gain (ADG) and feed conversion ratio (FCR) were computed from data on body mass and feed intake. Body mass gain, ADG and FCR computations were done using equations as described by Varkoohi *et al.* (2010). These computations were made for the grower and finisher phases as well as overall.

4.2.8 Terminal procedures and measurements

At the end of the six-week feeding trial, in preparation for slaughter, the birds were fasted for 4 h but with access to clean drinking water. Twenty-four birds (n = 12 males and n = 12 females) were randomly selected from each treatment group and were humanely slaughtered by decapitation using a small animal guillotine (Harvard Apparatus, Holliston, Massachusetts, United States). Blood was collected into heparinised collection tubes. The collected blood was then centrifuged in a Thermo Sorvall® MC 12V centrifuge (Du Pont Instruments, Connecticut, USA) at room temperature for 15 min at a force of 5000 × g. A pipette (20-200µL Pipetman, Gilson, France) was used to transfer the plasma from each tube into 1.5 ml microtubes (Greiner Bio-One GmbH, Frickenhausen, Germany). The plasma samples were immediately frozen and stored in a freezer at -20 ºC pending determination of the surrogate markers of health. After slaughter, feathers were hand-plugged and viscera were dissected out. The length of the small and large intestines was measured by gently stretching them on a ruler fixed on a dissection board. Prior to determining the mass of each GIT visceral organ, digesta in the organ was removed by gently squeezing the digesta out. The weight of the liver, pancreas and visceral fat

were measured using an electronic balance (Snowrex, model: EQ: 1200, Taiwan, China). Visceral organ masses were also computed relative to body mass. Samples of the livers were stored in a freezer at -20 ºC pending the determination of liver lipid content. The hot carcass mass was measured using a Snowrex electronic balance (Snowrex, model: EQ: 1200, Taiwan, China) soon after the removal of the viscera and the cold carcass mass was measured following carcass storage at 4 ºC for 24 h. The left breast and thigh meat were used to measure both pH and colour 30 min after slaughter and 24 h post-slaughter after storage at 4 ºC. The breast and thigh meat were used in the determination of meat quality. The left fleshed tibiae were dissected and stored at -18 ºC for later analysis of bone mass, length and bone density.

4.2.9 Determination of the general health profile

The plasma albumin, globulin, total protein, calcium, phosphorus cholesterol, total bilirubin, and uric acid concentration as well as aspartate transaminase, gamma-glutamyl transferase and α-amylase activities of the quail were determined using the IDEXX colorimetric-based clinical chemistry analyzer (IDEXX VetTest® Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) as per the manufacturer's instructions. Briefly, plasma samples from each bird were thawed and allowed to warm to room temperature. The microtube containing each thawed plasma sample was gently inverted to mix the contents prior to analysis. Thereafter the analyzer automatically drew up 150 µL of the plasma and then loaded 10 µL of plasma onto each of the pre-loaded disks after which each sample was then analysed. A print out of the results was obtained for each sample.

4.2.10 Determination of liver lipid content

The liver lipid content of the frozen-stored liver samples was determined as described by Bligh and Dyer (1959). Briefly, approximately 5 g of the liver sample was steeped in 150 ml of 2:1 chloroform: methanol solution overnight in a refrigerator at 4 °C. Following the overnight steeping, each mixture was then filtered through Whatmann filter paper (Whatmann®, No 1, size 185mm, pore size 7 to11µm, England) into a 250 ml separation funnel. Thirty (30) millilitres of 0.9 % saline was then added to each filtrate, mixed and allowed to stand in a refrigerator at 4°C overnight during which time the mixture separated into two phases. The bottom (chloroform) phase was then collected and reduced to dryness under vacuum at 37 °C using a rota-evaporator (Labex®, Krugersdorp, South Africa) and then made up to 20 ml with chloroform. An aliquot of 2 ml of the extract was then placed into a dried, pre-weighed vial, and then dried at 50 °C for 30-min in an oven (Salvis®, Salvis Lab, Switzerland), cooled in a desiccator and reweighed to determine the lipid content.

The liver lipid content was computed on the basis of the dry liver weight using the following equation:

$$\text{lipid content \%} = \frac{\text{weight of oil (g)}}{\text{weight of oil and dry liver (g)}} \times 100$$

4.3 Determination of the physical attributes of the meat

The meat colour and pH were determined from the breast (*Pectoralis major*) and thigh (*Iliotibialis* and *femerotibialis*), while the TL, CL and tenderness were determined from the breast meat only of the carcasses from each treatment group. Samples were randomly taken from carcasses of 12 male and 12 female birds per dietary treatment.

4.3.1 Determination of colour and pH

Thirty mins post-slaughter, meat colour [lightness (L*), redness (a*), yellowness (b*), chroma (C) and hue angle (H)] was determined using a Lovibond Colour Meter (LC 100 Spectrophotometer, Lasec, SA, China) as recommended by the Commission International De I' Eclairage, (1976). The initial pH (pH_i) of the meat was also measured 30 mins post-slaughter on the breast and thigh muscles of each carcass using a digital pH meter (following a two-point calibration at pH 4.0 and pH 7.0) with a piercing electrode set at 24 °C (Crison pH25, CRISON instruments, SA, Spain) as per the manufacturer's instructions. Following storage at 4 °C for 24 h the ultimate meat pH (pH_u) and meat colour were determined as described above. The meat samples were put into ziplock plastic bags and stored at -18 °C until the determination of CL and TL.

4.3.2 Determination of thawing and cooking loss

A total of twenty-four breast meat samples were randomly selected per dietary treatment group to determine the TL and CL as described by De Marchi *et al.* (2011). Briefly, the whole breast stored samples (-18 °C) were weighed, defrosted at room temperature for approximately 15 h then blotted dry using a paper towel and weighed again using an electronic balance. The TL was computed using the equation:

$$\text{TL} = \frac{Weight\ of\ frozen\ meat\ sample\ (g) - Weight\ of\ thawed\ meat\ sample\ (g)}{Weight\ of\ frozen\ meat\ sample\ (g)} \times 100$$

Thereafter the thawed meat samples were cooked in self-seal plastic bags in a water bath (Julabo PURA a30, Gerhard-Juchheim-Strasse, Seelbach, Germany) until an internal temperature of 75 °C was reached for 60 mins.

The CL was determined using the equation:

$$\text{CL} = \frac{Weight\ of\ thawed\ meat\ sample\ (g) - Weight\ of\ cooked\ meat\ sample\ (g)}{Weight\ of\ thawed\ meat\ sample\ (g)} \times 100$$

4.3.3 Determination of tenderness

Immediately after determining the CL, the samples were used to determine tenderness after they had been cooled to room temperature (23 to 25 °C). 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 (WBSF) machine with a Warner-Bratzler Shear mounted on a Universal Instron Machine (Model 4301, Instron Corporation, Massachusetts, United States). The shear force was determined at a cross speed of 200mm/min with a 1 kN load cell as recommended by the Stock and Board (1995). The results of shear force were captured by a personal computer linked to the Universal Instron machine.

4.4 Determination of the proximate chemical composition of the meat

The proximate components: CP, ash and EE of the meat and fatty acid profile of the breast meat (only) were determined on a dry matter basis. The chemical assays were determined from twenty-four breast meat samples from twenty-four carcasses randomly selected from each dietary treatment group.

4.4.1 Determination of the proximate composition

The proximate content (CP, ash and EE, respectively) of the breast muscle was determined as described by the Association of Official Analytical Chemists (AOAC, 2006) method numbers: 942.05, 988.05 and 920.39 respectively. Each assay was done in triplicate.

4.4.2 Determination of the fatty acid profile

The Soxhlet Apparatus was used to extract the oil from the breast muscle sample as described by the Association of Analytical Chemists (AOAC, 2006; method number: 920.39). The fatty acid profiles of the oil extracted from the meat were determined as described by Christopherson and Glass (1969). Briefly, the oil extracts were trans-methylated with 2 mol/L methanol sodium hydroxide. The resulting fatty acid methyl esters were extracted in heptane, filtered (Nylon syringe filters, pore size: 0.45 µm, diameter: 13 mm with a glass fibre prefilter, Membrane Solutions) and dried under nitrogen after which they were separated by a temperature gradient over 45-min on a gas chromatograph with nitrogen as carrier gas on a DB-23 capillary column (90cm × 250μm × 0.25μm;Supelco, Sigma-Aldrich). The gas chromatograph consisted of an

HP6890 GC (Hewlett Packard, Bristol, UK) with a flame ionisation detector. Both the detector and injector temperatures were set at 300 °C. A personal computer equipped with Chemstation software (Agilent Technologies Inc., Santa Clara, CA, USA) was used for quantification. Nonadecanoic acid (C19:0) was used as an internal standard.

4.5 Statistical analyses

Results are presented as mean ± SD. GraphPad Prism, version 5 (GraphPad Software, San Diego, California, USA) was used to analyse the data. Data on weekly body weight and weekly feed intake-were analysed using the repeated measures of ANOVA. Treatment means were compared using Tukey's *post hoc* test. Significance was set at $P < 0.05$. The following statistical linear model was employed:

$$Y_{ij} = \mu+\beta_i+W_j+E_{ij}$$

where: Y_{ij} = dependent variable (weekly body weight and weekly feed intake)

μ = population mean

β_i = effect of diets (1,2….,5)

W_j = effect of week (1,2…..5)

E_{ij} = random error

Data on terminal body mass, body mass gain, average daily gain, feed conversion ratio and total feed intake by phase and overall, empty carcass mass, dressing percentage, surrogate markers of health, liver lipid content and physicochemical characteristics of meat were analysed using a one-way ANOVA. Treatment means were compared using Tukey's *post hoc* test. Significance was set at $P < 0.05$. The linear statistical model employed was as follows:

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

where: Y_{ij} = dependent variable (terminal body mass, body mass gain, average daily gain, feed conversion ratio and total feed intake by phase and overall, empty carcass mass, dressing percentage, cold carcass mass, surrogate markers of health, liver lipid content and physicochemical characteristics of meat)

μ= population mean

β_i = effect of diets (1,2….,5)

E_{ij} = random error

4.6 Results

4.6.1 Effect of dietary Marula nut meal on growth performance and feed economy

Table 4.3 shows the effects of dietary MNM on growth performance and feed utilisation efficiency during the grower (day 10 to 37), finisher (38 to 51) phases and overall study period (day 10 to 51) of the broiler quail. Dietary MNM had no effect ($P > 0.05$) on the birds' BMG, ADG, FI and FCR during the brooding/growth and finisher phase phases as well as overall. There was no interaction between diet and week of feeding observed. While the dressing percentage of the quail carcasses was similar across dietary treatments, carcasses from quail fed diet 4 (75% substitution of SBM CP with MNM CP) were heavier $P < 0.01$ compared to those from quail fed diet 1.

Table 4.3: Effect of graded dietary substitution of soybean meal with Marula nut meal on the growth performance and feed utilization efficiency of broiler Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Induction mass (g)	39.65 ± 1.51[a]	37.92 ± 1.06[a]	38.98 ± 1.20[a]	39.54 ± 1.65[a]	40.85 ± 1.49[a]	ns
Terminal body mass (g)	216.90 ± 5.80[a]	214.80 ± 5.37[a]	211.20 ± 5.65[a]	212.00 ± 5.24[a]	219.80 ± 5.81[a]	ns
Grower phase (day 10 to 37)						
BWG (g)	145.90 ± 4.64[a]	155.50 ± 3.91[a]	152.30 ± 4.90[a]	140.40 ± 5.26[a]	145.50 ± 4.78[a]	ns
ADG (g/d)	5.23 ± 0.13[a]	5.59 ± 0.40[a]	5.44 ± 0.49[a]	4.99 ± 0.72[a]	5.22 ± 0.56[a]	ns
FI (g)	1059.00 ± 61.23[a]	1117.00 ± 108.10[a]	1051.00 ± 46.60[a]	1194.00 ± 250.30[a]	1058.00 ± 186.30[a]	ns
FCR	7.24 ± 0.59[a]	7.20 ± 0.70[a]	6.96 ± 0. 0.84[a]	8.65 ± 1.88[a]	7.20 ± 0.65[a]	ns
Finisher phase (day 38 to 51)						
BWG (g)	31.35 ± 4.04[a]	21.36 ± 4.08[a]	19.90 ± 4.70[a]	32.13 ± 4.55[a]	33.46 ± 4.76[a]	ns
ADG (g/d)	2.12 ± 1.24[a]	1.53 ± 0.38[a]	1.42 ± 0.69[a]	2.32 ± 0.91[a]	2.36 ± 1.10[a]	ns
FI (g)	1170.00 ± 55.23[a]	1176.00 ± 73.51[a]	1181.00 ± 2.27[a]	1251.00 ± 371.40[a]	1711.00 ± 283.20[a]	ns
FCR	37.32 ± 12.49[a]	55.05 ± 6.88[a]	59.35 ± 18.75[a]	38.89 ± 18.43[a]	55.22 ± 12.05[a]	ns
Overall (day 10 to 51)						
BWG (g)	177.10 ± 13.33[a]	176.20 ± 17.82[a]	172.20 ± 22.18[a]	173.00 ± 14.98[a]	179.10 ± 7.49[a]	ns

ADG (g/d)	4.22 ± 0.32[a]	4.20 ± 0.42[a]	4.10 ± 0.53[a]	4.12 ± 0.36[a]	4.26 ± 0.18[a]	ns
FI (g)	1827.00 ± 67.58[a]	1859.00 ± 40.84[a]	1824.00 ± 52.70[a]	1877.00 ± 170.90[a]	1848.00 ± 366.0[a]	ns
FCR	10.34 ± 0.55[a]	10.62 ± 0.88[a]	10.74 ± 1.54[a]	10.94 ± 1.65[a]	10.36 ± 2.28[a]	ns
Empty carcass mass (g)	124.90 ± 2.99[a]	130.50 ± 3.73[ab]	135.80 ± 3.59[ab]	144.30 ± 4.47[b]	139.00 ± 4.06[ab]	**
Dressing (%)	60.77 ± 2.21[a]	62.70 ± 2.71[a]	64.93 ± 1.69[a]	63.82 ± 1.72[a]	64.73 ± 2.61[a]	ns

ns = not significant, P >0.05, ** P <0.01; [ab] Within row means with different superscripts are significantly different at P <0.05. There were no significant differences (P >0.05) in the growth performance of quail birds across dietary treatments. The empty carcass mass from quail fed diet 4 was significantly heavier (P <0.0001) compared to that from counterpart fed diet 1. BWG = body weight gain, ADG = average daily gain, FI = total feed intake, FCR =feed conversion ratio. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 40 birds.

4.6.2 Effect of dietary Marula nut meal on macro-morphometry of the viscera

The effect of dietary MNM on the macro-morphometry of GIT viscera from the broiler Japanese quail is presented in Table 4.4. Dietary MNM had no effect ($P > 0.05$) on the absolute masses and lengths (where relevant) of the proventriculi, ventriculi, small and large intestines. However relative to body mass, caeca from quail fed diet 4 (75% substitution of SBM dietary CP contribution) were lighter ($P < 0.001$) compared to caeca from quail fed diet 3 (Table 4.4).

Table 4. 4: Effect of graded dietary substitution of soybean meal with Marula nut meal on GIT viscera of broiler Japanese quail

Visceral organ	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Proventriculus						
Absolute (g)	0.82 ± 0.07[a]	0.79 ± 0.04[a]	0.87 ± 0.07[a]	0.85 ± 0.04[a]	0.83 ± 0.04[a]	ns
Relative to body mass (%)	0.40 ± 0.03[a]	0.38 ± 0.02[a]	0.41 ± 0.03[a]	0.38 ± 0.02[a]	0.39 ± 0.02[a]	ns
Ventriculus						
Absolute (g)	3.85 ± 0.16[a]	4.11 ± 0.18[a]	4.31 ± 0.21[a]	4.36 ± 0.24[a]	4.36 ± 0.22[a]	ns
Relative to body mass (%)	1.87 ± 0.09[a]	1.96 ± 0.10[a]	2.05 ± 0.09[a]	1.93 ± 0.10[a]	2.05 ± 0.12[a]	ns
Small intestines						
Absolute (g)	5.49 ± 0.36[a]	5.05 ± 0.24[a]	5.97 ± 0.35[a]	5.42 ± 0.39[a]	5.63 ± 0.32[a]	ns
Relative to body mass (%)	2.68 ± 0.20[a]	2.41 ± 0.12[a]	2.82 ± 0.13[a]	2.35 ± 0.12[a]	2.62 ± 0.17[a]	ns
Length (mm)	631.50 ± 15.00[a]	618.50 ± 11.48[a]	667.20 ± 17.30[a]	639.40 ± 19.70[a]	637.90 ± 15.04[a]	ns
Large intestines						
Absolute (g)	0.56 ± 0.05[a]	0.55 ± 0.03[a]	0.60 ± 0.05[a]	0.53 ± 0.04[a]	0.49 ± 0.03[a]	ns
Relative to body mass (%)	0.27 ± 0.02[a]	0.27 ± 0.02[a]	0.28 ± 0.02[a]	0.23 ± 0.02[a]	0.23 ± 0.02[a]	ns
Length (mm)	67.08 ± 2.62[a]	67.92 ± 1.78[a]	68.75 ± 3.20[a]	68.54 ± 3.23[a]	64.17 ± 2.23[a]	ns
Caecum						
Absolute (g)	0.87 ±0.05[a]	0.87 ± 0.07[a]	0.96 ± 0.06[a]	0.78 ± 0.05[a]	0.81 ± 0.04[a]	ns
Relative to body mass (%)	0.42 ± 0.02[ab]	0.41 ± 0.03[ab]	0.46 ± 0.03[a]	0.34 ± 0.02[b]	0.36 ± 0.02[ab]	**

ns = not significant, ** P <0.01, [ab] Within row means with different superscripts are significantly different at P <0.05. No significant differences (P >0.05) in the macro-morphometry of the GIT viscera except the caeca. Relative to body mass caeca from carcasses of quail fed diet 3 were significantly different (P <0.01) compared to those from quail fed diet 4. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis,

Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; expressed as mean ± SD; n = 24 birds (n = 12 males and n = 12 females).

The effect of dietary MNM on the macro-morphometry of accessory GIT viscera and visceral fat pad of broiler Japanese quail is presented in Table 4.5. Quails fed diet 4 had heavier ($P < 0.05$) absolute visceral fat mass compared to those fed diet 1.

Table 4.5: Effect of graded dietary substitution of soybean meal with Marula nut meal on the mass (absolute and relative to body weight) of the liver, pancreas and visceral fat of broiler Japanese quail

Visceral organ	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Liver						
Absolute (g)	4.62 ± 0.33[a]	4.37 ± 0.27[a]	5.11 ± 0.38[a]	5.37 ± 0.62[a]	4.89 ±0.33[a]	ns
Relative to body mass (%)	2.26 ± 0.18[a]	2.12 ± 0.16[a]	2.41 ± 0.17[a]	2.33 ± 0.25[a]	2.25 ± 0.15[a]	ns
Pancreas						
Absolute (g)	0.55 ± 0.03[a]	0.69 ± 0.16[a]	0.57 ± 0.04[a]	0.51 ± 0.03[a]	0.53 ± 0.03[a]	ns
Relative to body mass (%)	0.27 ± 0.02[a]	0.31 ± 0.05[a]	0.27 ± 0.02[a]	0.22 ± 0.01[a]	0.25 ± 0.02[a]	ns
Visceral fat						
Absolute (g)	1.23 ± 0.26[a]	1.63 ± 0.31[ab]	1.55 ±0.23[ab]	2.57 ± 0.39[b]	2.54 ± 0.44[ab]	*
Relative to body mass (%)	0.58 ± 0.11[a]	1.05 ± 0.22[a]	0.73 ± 0.11[a]	1.15 ± 0.89[a]	1.18 ± 0.21[a]	ns

ns = not significant,* P <0.05, [ab] Within row means with different superscripts are significantly different at P >0.05. The visceral fat (absolute) of quails fed diet 4 was significantly different (P <0.05) compared to that of quail fed diet 1. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; expressed as mean ± SD; n = 24 birds (n = 12 males and n = 12 females).

4.6.3 Effect of MNM on meat quality of Japanese quail

4.6.3.1 Effect on the physical quality of the meat

The effect of dietary MNM on the colour and pH of breast meat from female broiler Japanese quail are presented in Table 4.6. The breast meat from carcasses of female quail fed diet 1 was lighter ($P < 0.01$) compared to that from carcasses of quail fed diets 2 to 5 at both 30 min and 24 h post-slaughter. The breast meat from carcasses of female quail fed diet 1 and 3 was less ($P < 0.05$) red compared to those fed diet 2 and 5 at both 30 min and 24 h post-slaughter. The H* of the breast meat from carcasses of quail fed diet 1 was higher ($P < 0.05$) than those fed diet 5 at 30 min post-slaughter. The breast meat colour from carcasses of male quail was similar ($P > 0.05$) across dietary treatments at 30 min post-slaughter. However, at 24 h post-slaughter, the breast meat from carcasses of male quail fed diet 1 was lighter and less red ($P < 0.05$) compared to that from counterparts fed diet 5. The pH of breast meat from carcasses of quail fed diet 1 was lower ($P < 0.05$) compared to that carcasses of quail fed diets 2 to 5 both at 30-min and 24 h post-slaughter. The breast meat from carcasses of male quail fed diet 2 had a higher pH decline compared to of counterparts fed diet 3.

Table 4.6: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the colour and pH of breast muscle from female and male broiler Japanese quail

Female	**Parameter**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
30-min post-slaughter	pH_i	5.36 ± 0.11^{a}	6.37 ± 0.05^{b}	6.29 ± 0.08^{b}	6.37 ± 0.06^{b}	6.33 ± 0.08^{b}	*
	L^*	49.99 ± 0.68^{a}	44.24 ± 0.75^{b}	46.09 ± 0.90^{b}	44.13 ± 1.53^{b}	44.20 ± 0.60^{b}	**
	a^*	1.58 ± 0.44^{a}	3.78 ± 0.80^{b}	1.63 ± 0.34^{a}	2.14 ± 0.42^{ab}	4.16 ± 0.89^{b}	*
	b^*	9.76 ± 0.37^{a}	9.71 ± 0.31^{a}	8.93 ± 0.53^{a}	9.331 ± 0.73^{a}	9.84 ± 0.51^{a}	ns
	C^*	10.66 ± 0.78^{a}	10.76 ± 0.49^{a}	9.20 ± 0.56^{a}	11.31 ± 1.53^{a}	11.03 ± 0.75^{a}	ns
	H*	68.37 ± 3.68^{a}	69.18 ± 3.75^{ab}	82.95 ± 3.16^{ab}	76.64 ± 3.03^{ab}	84.68 ± 5.90^{b}	*
24-hours post-slaughter	pH_u	5.14 ± 0.04^{a}	6.31 ± 0.06^{b}	6.28 ± 0.18^{b}	6.26 ± 0.04^{b}	6.22 ± 0.08^{b}	*
	pH decline	0.22 ± 0.46^{a}	0.06 ± 0.31^{a}	0.00 ± 0.73^{a}	0.11 ± 0.21^{a}	0.11 ± 0.28^{a}	ns
	L^*	48.57 ± 0.93^{a}	46.20 ± 1.12^{b}	46.05 ± 1.29^{b}	46.14 ± 1.12^{b}	46.29 ± 0.80^{b}	*
	a^*	2.92 ± 0.41^{a}	4.75 ± 0.68^{b}	2.52 ± 0.45^{a}	3.60 ± 0.54^{a}	6.06 ± 0.84^{b}	*
	b^*	12.05 ± 0.81^{a}	12.73 ± 0.58^{a}	12.20 ± 0.59^{a}	12.04 ± 0.96^{a}	14.30 ± 0.76^{a}	ns
	C^*	13.21 ± 0.90^{a}	18.66 ± 4.71^{a}	12.60 ± 0.55^{a}	13.83 ± 0.56^{a}	15.71 ± 0.93^{a}	ns
	H*	93.50 ± 17.28^{a}	72.53 ± 4.08^{a}	78.01 ± 2.21^{a}	68.65 ± 5.95^{a}	67.38 ± 2.29^{a}	ns
Male							
30-min post-slaughter	pH_i	6.47 ± 0.13^{a}	6.50 ± 0.07^{a}	6.26 ± 0.09^{a}	6.37 ± 0.09^{a}	6.40 ± 0.07^{a}	ns

	L^*	42.67 ± 1.40^a	42.41 ± 0.73^a	43.20 ± 0.77^a	43.72 ± 0.36^a	42.04 ± 0.53^a	ns
	a^*	4.14 ± 0.93^a	5.00 ± 0.72^a	4.38 ± 0.39^a	5.45 ± 0.33^a	5.82 ±0.74^a	ns
	b^*	8.76 ± 1.14^a	10.11 ± 0.69^a	10.11 ± 0.54^a	9.63 ± 0.65^a	10.00 ± 0.66^a	ns
	C^*	14.27 ± 2.55^a	11.54 ± 0.75^a	11.13 ± 0.59^a	11.57 ±0.60^a	12.10 ± 0.67^a	ns
	H*	70.50 ± 8.81^a	65.97 ± 3.28^a	66.49 ± 1.67^a	59.83 ± 1.76^a	59.40 ± 3.75^a	ns
24-hours post-slaughter	pH_u	6.27 ± 0.08^a	6.14 ± 0.19^a	6.52 ± 0.08^a	6.23 ± 0.07^a	6.16 ± 0.10^a	ns
	pH decline	0.21 ± 0.43^{ab}	0.37 ± 0.72^a	-0.27 ± 0.45^b	0.14 ± 0.42^{ab}	0.24 ± 0.28^{ab}	*
	L^*	47.72 ± 1.50^a	48.67 ± 4.56^{ab}	45.72 ± 1.49^{ab}	49.17 ± 4.53^{ab}	44.77 ± 1.00^b	*
	a^*	4.00 ± 0.93^a	4.74 ± 1.13^{ab}	4.94 ± 0.59^{ab}	6.10 ± 0.78^{ab}	8.11 ± 1.16^b	*
	b^*	12.38 ± 1.34^a	10.37 ± 1.18^a	8.81 ± 0.92^a	9.22 ± 0.58^a	10.80 ± 0.87^a	ns
	C^*	12.35 ± 1.11^a	13.83 ± 1.39^a	12.81 ± 0.91^a	13.49 ± 1.36^a	16.41 ± 1.05^a	ns
	H*	72.25 ± 3.94^a	73.96 ± 5.26^a	68.11 ± 2.62^a	66.96 ± 5.25^a	62.86 ± 2.14^a	ns

ns = not significant, P >0.05, * P <0.05, ** P <0.01, L*-lightness, a*- redness , b*-yellowness, C*-chroma, H*-Hue angle, pH_u-ultimate pH, pH_i-initial pH, ^{ab} Within row means with different superscripts are significantly different at P <0.05. Thirty minutes post-slaughter; the L* of the female breast meat of the birds fed diet 1 was higher (P <0.01) compared to other the breast of the birds fed on other dietary treatment groups. The a* of the breast meat from female birds fed diets 2, 4 and 5 were higher (P <0.05) compared to those fed diets 1 and 3 at 30 min post-slaughter. The H* if the breast meat from birds fed diet 1 was lower (P <0.05) compared to those fed diet 5. The pH_i of the breast meat from birds fed diet 1 was lower (P <0.05) compared to the other groups. Twenty four hours post-slaughter, the L*of the breast meat from female birds fed diet 1 was lower (P <0.05) compared to the rest of the birds fed on other dietary treatment groups. The a* of the breast meat from female birds fed diets 2, 4 and 5 were lower (P <0.05) compared to those fed diet 1 and 3 at 24 h post-slaughter. The pH_u of the breast meat from female birds diet 1 was lower (P <0.05) than the rest of the birds fed on other dietary treatment groups. Thirty min post-slaughter, the colour and pH were similar (P >0.05) across all dietary treatment groups. However, at 24 h post-slaughter, the

L* of the breast meat from birds fed diet 1 was higher ($P < 0.05$) than those from birds fed diet 5. The a* of the breast meat from birds fed diet 1 was lower than those from birds fed diet 5. The pH decline of the breast meat from birds fed diet 2 was lower ($P < 0.05$) compared to those fed diet 3. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; values expressed as mean ± SD; n = 24 birds (n = 12 males and n = 12 females).

The effect of dietary MNM on the colour and pH of thigh muscles from female and male broiler Japanese quail are presented in Table 4.7. The pH_i of the thigh meat from carcasses of female quail fed diets 2 was higher ($P < 0.05$) compared to that of thigh meat from counterparts fed diet 3 at 30 min post-slaughter. Similarly the pH_i of the thigh meat from carcasses of male quail fed diet 1 was higher ($P < 0.05$) compared to that of thigh meat from counterparts fed diet 3 at 30 min post-slaughter. The thigh meat from male quail fed diet 1 had the highest pH decline ($P < 0.05$). The thigh meat from carcasses of male quail fed diet 1, 2, 4 and 5 was more yellow compared to that from counterparts fed diet 3 at 24 h after slaughter.

Table 4.7: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the colour and pH of thigh muscles from female and male broiler Japanese quail

Female	**Parameter**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
30-min post-slaughter	pH_i	6.86 ± 0.07^{ab}	6.92 ± 0.06^{a}	6.69 ± 0.06^{b}	6.83 ± 0.04^{ab}	6.81 ± 0.03^{ab}	*
	L^*	46.57 ± 0.94^{a}	46.20 ± 1.12^{a}	49.05 ± 1.29^{a}	49.44 ± 0.93^{a}	47.88 ± 0.98^{a}	ns
	a^*	0.64 ± 0.31^{a}	2.63 ± 0.67^{a}	0.89 ± 0.76^{a}	2.18 ± 0.62^{a}	3.00 ± 0.79^{a}	ns
	b^*	8.83 ± 0.64^{a}	10.33 ± 1.29^{a}	8.63 ± 0.69^{a}	10.88 ± 0.89^{a}	11.44 ± 1.16^{a}	ns
	C^*	9.06 ± 0.63^{a}	11.01 ± 1.35^{a}	8.98 ± 0.78^{a}	11.36 ± 0.92^{a}	12.09 ± 1.28^{a}	ns
	H*	90.17 ± 3.23^{a}	80.99 ± 3.48^{a}	84.42 ± 4.08^{a}	78.36 ± 3.91^{a}	78.39 ± 3.49^{a}	ns
24-hours post-slaughter	pH_u	6.88 ± 0.04^{a}	6.94 ± 0.09^{a}	6.84 ± 0.09^{a}	$6.67 \pm 0.13^{a\#}$	6.82 ± 0.06^{a}	ns
	pH decline	0.35 ± 1.47^{a}	-0.01 ± 0.30^{a}	-0.14 ± 0.31^{a}	0.15 ± 0.47^{a}	-0.00 ± 0.24^{a}	ns
	L^*	46.20 ± 0.91^{a}	44.65 ± 1.64^{a}	43.72 ± 1.85^{a}	42.81 ± 3.55^{a}	47.15 ± 1.02^{a}	ns
	a^*	4.06 ± 0.62^{a}	5.00 ± 0.53^{a}	3.05 ± 0.95^{a}	3.87 ± 0.64^{a}	3.19 ± 0.43^{a}	ns
	b^*	11.36 ± 0.96^{a}	13.00 ± 0.64^{a}	10.68 ± 1.09^{a}	11.06 ± 0.97^{a}	11.32 ± 1.15^{a}	ns
	C^*	12.09 ± 1.02^{a}	14.49 ± 0.77^{a}	11.41 ± 1.11^{a}	11.80 ± 1.11^{a}	11.98 ± 1.19^{a}	ns
	H*	73.03 ± 1.81^{a}	72.24 ± 4.05^{a}	74.17 ± 4.34^{a}	80.96 ± 8.05^{a}	74.80 ± 2.08^{a}	ns

Male

30-min post-slaughter	pH_i	7.03 ± 0.06[a]	6.86 ± 0.08[ab]	6.88 ± 0.03[b]	6.81 ± 0.07[ab]	6.88 ± 0.08[ab]	**
	L^*	48.16 ± 0.99[a]	45.55 ± 1.42[a]	47.30 ± 0.80[a]	45.69 ± 1.13[a]	46.49 ± 1.21[a]	ns
	a^*	3.83 ± 0.79[a]	4.58 ± 1.09[a]	2.61 ± 0.50[a]	3.26 ± 0.80[a]	3.43 ± 0.45[a]	ns
	b^*	12.38 ± 1.34[a]	10.37 ± 1.17[a]	8.81 ± 0.92[a]	10.26 ± 0.87[a]	11.33 ± 0.73[a]	ns
	C^*	12.27 ± 0.91[a]	11.74 ± 1.54[a]	9.47 ± 0.99[a]	11.15 ± 1.03[a]	12.12 ± 0.80[a]	ns
	H*	73.30 ± 4.36[a]	69.02 ± 3.45[a]	71.13 ± 3.43[a]	73.10 ± 3.40[a]	75.82 ± 3.89[a]	ns
24-hours post-slaughter	pH_u	6.84 ± 0.10[a]	6.77 ± 0.10[a]	6.63 ±0.09[a]	6.55 ± 0.16[a]	6.65 ± 0.08[a]	ns
	pH decline	0.93 ± 0.68[a]	0.09 ± 0.31[b]	-0.25 ± 0.38[c]	0.26 ± 0.71[b]	0.23 ± 0.41[b]	***
	L^*	44.50 ± 1.37[a]	47.81 ± 1.70[a]	42.70 ± 1.17[a]	45.69 ± 1.13[a]	44.34 ± 1.21[a]	ns
	a^*	5.55 ± 0.83[a]	5.77 ± 0.79[a]	4.15 ± 0.66[a]	6.84 ± 1.00[a]	6.69 ± 1.37[a]	ns
	b^*	11.07 ± 0.92[a]	13.37 ± 1.21[a]	7.07 ± 0.90[b]	10.83 ± 1.25[a]	10.84 ± 0.88[a]	***
	C^*	14.36 ± 2.17[a]	15.13 ± 1.38[a]	11.17 ± 2.44[a]	13.36 ± 1.31[a]	12.88 ± 0.95[a]	ns
	H*	62.80 ± 3.56[a]	68.80 ± 2.46[a]	69.09 ± 6.31[a]	62.80 ± 6.28[a]	64.35 ± 4.17[a]	ns

ns = not significant, P >0.05, * P <0.05, ** P <0.01, *** P <0.0001, [ab] Within row means with different superscripts are significantly different at P <0.05. L*-lightness, a*-redness, b*-yellowness, C*-chroma, H*-Hue angle, pHu-ultimate pH, pHi-initial pH. The b* of birds fed diet 3 was lower (P <0.0001) than the rest of the dietary treatment groups at 24 h after slaughter. However, the rest of the colour components (L*, a*, b*, C* and H*) of breast muscle from carcasses of birds were similar (P >0.05) across dietary treatments. The pH at 30 min after the slaughter of diet 2 was higher (P <0.05) compared to those fed diet 3. The pHi of the thigh meat from the carcasses if birds fed

diet 1 was higher (P <0.01) compared to that from those diet 3. The birds fed diet 1 had higher (P <0.0001) pH decline compared to the rest of the birds in other dietary treatment groups. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; values expressed as mean ± SD; n = 24 birds (n = 12 males and n = 12 females).

The effect of graded dietary substitution of SBM with MNM on the moisture characteristics and tenderness of Japanese quail breast meat is presented in Table 4.8. In analysing the finding for TL, CL and tenderness, the data for the males and females was combined as there were no significant differences when they were separated. The TL, CL and tenderness of the breast meat from the carcasses of Japanese quail was similar ($P > 0.05$) across dietary treatments.

Table 4.8: Effect of graded dietary substitution of soyabean meal with Marula nut meal on thawing loss, cooking loss and tenderness of Japanese quail breast meat

Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
Thawing loss (%)	3.83 ± 4.71[a]	2.23 ± 2.32[a]	2.59 ± 4.13[a]	3.66 ± 6.12[a]	3.79 ± 6.06[a]	ns
Cooking loss (%)	16.25 ± 6.31[a]	17.59 ± 4.79[a]	16.48 ± 5.71[a]	16.46 ± 4.91[a]	15.70 ± 9.76[a]	ns
Tenderness (N)	8.03 ± 2.08[a]	7.51 ± 1.75[a]	7.28 ± 1.91[a]	7.60 ± 1.57[a]	7.39 ± 2.16[a]	ns

ns = not significant, P >0.05. There were no significant differences (P >0.05) in the thawing loss, cooking loss and tenderness of the quail breast muscles. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 24 birds (n = 12 males and n = 12 females).

4.6.3.2 Effect on the proximate content of the meat

The effect of graded dietary substitution of SBM with MNM on the proximate composition of Japanese quail breast muscles is presented in table 4.9. The CP content of the breast meat from carcasses of quail fed diets 1 and 2 was higher ($P < 0.0001$) compared to that from carcasses of birds fed on diet 5. The ash content of the breast meat from the carcasses of quail fed diet 1 was lower ($P < 0.0001$) compared to that from carcasses of birds fed diet 5. Carcasses from quail fed diet 1 had the highest ($P < 0.0001$) EE content.

Table 4.9: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the proximate composition of Japanese quail breast muscles

Proximate (% DM)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
CP	84.79 ± 0.37^{a}	83.38 ± 0.35^{c}	83.87 ± 0.24^{ac}	83.86 ± 0.32^{ac}	78.91 ± 0.72^{b}	***
Ash	6.96 ± 0.42^{a}	7.27 ± 0.42^{ab}	7.25 ± 0.71^{ab}	7.92 ± 0.18^{ab}	8.29 ± 0.27^{b}	***
EE	13.84 ± 0.14^{a}	9.38 ± 0.01^{b}	8.77 ± 0.31^{cd}	8.71 ± 0.18^{d}	7.54 ± 0.21^{e}	***

ns = not significant, P >0.05, *** P <0.0001; [ab] Within row means with different superscripts are significantly different at P <0.05. The crude protein content of the breast muscles from birds fed diet 1 was higher (P <0.0001) compared to those fed diets 2 and 5. The ash content of the breast muscles of birds fed diet 1 was lower (P <0.0001) compared to those fed diet 5. The EE content of the breast muscles of birds fed diet 1 was higher (P <0.0001) than those fed diets 2, 3, 4 and 5. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 24 birds (n = 12 males and n = 12 females).

The effect of graded dietary substitution of SBM with MNM on the fatty acid profile of Japanese quail breast meat is presented in Table 4.10. The TSFA percentage of the breast meat from carcasses of quail fed diets 1, 2 and 5 were higher compared to that from quail fed diet 4 ($P < 0.01$). The TMUFA percentage of the breast meat from carcasses of quail fed diets 1, 2 and 3 were lower compared to that from counterparts those fed 4 and 5 ($P < 0.0001$). The TPUFA percentage of the breast meat from carcasses of quail was similar across all dietary treatments ($P > 0.05$). The Omega-3 percentage of the breast meat from carcasses of quail fed diets 1, 2, 3 and 5 was lower compared to that from quail fed diet 4 ($P < 0.01$). The Omega-6 percentage of the breast meat from carcasses of quail fed diets 1, 3, 4, 5 were lower compared to of quail fed diet 2 ($P < 0.0001$).

Table 4.10: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the fatty acid profile of Japanese quail breast muscles

Fatty acid (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Saturated						
C10:0 (capric acid)	0.06 ± 0.01^{a}	0.06 ± 0.00^{a}	0.05 ± 0.02^{a}	nd	0.03 ± 0.04^{a}	ns
C12:0 (lauric acid)	0.07 ± 0.00^{a}	0.07 ± 0.01^{a}	0.05 ± 0.01^{a}	nd	0.03 ± 0.04^{a}	ns
C14:0 (myristic acid)	0.57 ± 0.07^{a}	0.51 ± 0.02^{a}	0.49 ± 0.10^{a}	0.38 ± 0.07^{a}	0.39 ± 0.01^{a}	ns
C15:0 (pentadecanoic acid)	0.03 ± 0.04^{a}	0.61 ± 0.03^{b}	0.32 ± 0.03^{c}	nd	nd	**
C16:0 (palmitic acid)	19.77 ± 0.18^{a}	19.74 ± 0.03^{a}	18.39 ± 0.57^{a}	15.88 ± 0.84^{b}	19.53 ± 0.67^{a}	ns
C17:0 (margaric acid)	0.14 ± 0.01^{a}	0.17 ± 0.01^{a}	0.18 ± 0.04^{a}	0.16 ± 0.06^{a}	0.14 ± 0.05	ns
C18:0 (stearic acid)	6.73 ± 0.01^{ab}	7.31 ± 0.05^{a}	6.24 ± 0.02^{b}	7.75 ± 0.45^{c}	7.50 ± 0.19^{ac}	**
C20:0 (arachidic acid)	0.14 ± 0.03^{a}	0.27 ± 0.23^{a}	0.23 ± 0.08^{a}	0.70 ± 0.36^{a}	0.08 ± 0.12^{a}	ns
C20:2 (eicosadienoic acid)	0.03 ± 0.04^{a}	nd	nd	0.25 ± 0.35^{a}	nd	ns

C21:0 (henicosanoic acid)	0.09 ± 0.06[a]	0.23 ± 0.33[b]	nd	nd	nd	*
C22:0 (behenic acid)	0.01 ± 0.01	nd	nd	nd	nd	-
C24:0 (lignoceric acid)	0.18 ± 0.07[a]	0.43 ± 0.35[a]	0.10 ± 0.08[a]	0.87 ± 0.86[a]	0.19 ± 0.24[a]	ns
TSFA	27.77 ± 0.25[a]	29.16 ± 0.61[a]	26.16 ± 0.33[ab]	25.03 ± 0.51[b]	27.91 ± 0.43[a]	**
Monounsaturated						
C18:1n9c (oleic acid)	46.50 ± 0.51[a]	46.76 ± 0.21[a]	50.54 ± 0.78[ab]	52.32 ± 3.39[ab]	53.68 ± 0.81[b]	*
C18:1n9t (elaidic acid)	0.06 ± 0.09[a]	0.15 ± 0.02[a]	0.07 ± 0.02[a]	0.22 ± 0.30[a]	nd	ns
C14:1(myristoleic acid)	0.13 ± 0.00[a]	0.17 ± 0.04[a]	0.12 ± 0.05[a]	0.14 ± 0.20[a]	0.05 ± 0.08[a]	ns
C16:1(palmitoleic acid)	7.54 ± 0.26[a]	6.46 ± 0.02[ab]	5.90 ± 0.44[b]	4.78 ± 0.83[b]	5.38 ± 0.42[b]	*
C17:1(cis-10-heptadecanoic acid)	0.08 ± 0.03[a]	0.18 ± 0.07[a]	0.12 ± 0.04[a]	nd	0.12 ± 0.04[a]	ns
C20:1 (eicosenoic acid)	0.25 ± 0.27[a]	0.32 ± 0.20[a]	0.35 ± 0.02[a]	0.16 ± 0.22[a]	3.98 ± 5.12[a]	ns
C15:1 (pentadecenoic acid)	nd	nd	0.31 ± 0.03[a]	2.06 ± 2.52[a]	0.34 ± 0.16[a]	ns
TMUFA	54.52 ± 0.01[a]	53.88 ± 0.52[a]	57.35 ± 0.26[b]	59.52 ± 0.56[c]	60.27 ± 0.07[c]	***

Polyunsaturated						
C18:2n6t(linolelaidic acid)	0.04 ± 0.05[a]	nd	0.05 ± 0.01[a]	0.29 ± 0.24[a]	0.30 ± 0.34[a]	ns
C18:2n6c (linoleic acid)	17.64 ± 0.08[a]	nd	16.55 ± 0.16[ab]	16.59 ± 0.38[ab]	15.89 ± 0.67[b]	*
C18:3n3 (α-linolenic acid)	1.14 ± 0.02[a]	0.96 ± 0.06[b]	1.44 ± 0.02[a]	1.41 ± 0.09[a]	0.93 ± 0.09[b]	**
C18:3n6 (γ-linolenic acid)	0.09 ± 0.03	nd	nd	nd	nd	-
C20:3n3 (eicosatrienoic acid)	2.22 ± 0.09[a]	2.34 ± 0.05[a]	1.53 ± 0.01[c]	3.27 ± 0.55[b]	1.97 ± 0.11[c]	**
C20:3n6 (eicosatrienoic acid)	0.12 ± 0.05[a]	nd	0.09 ± 0.03[a]	nd	nd	ns
C22:6n3 (docosahexaenoic acid)	0.21 ± 0.01[a]	0.11 ± 0.15[a]	0.14 ± 0.08[a]	nd	nd	ns
TPUFA	21.45 ± 0.09[a]	22.30 ± 0.08[a]	19.77 ± 0.09[a]	41.06 ± 28.11[a]	18.99 ± 0.79[a]	ns
Trans fatty acids	0.10 ± 0.14[a]	0.15 ± 0.02[a]	0.13 ± 0.03[a]	0.50 ± 0.53[a]	0.35 ± 0.28[a]	ns
Cis fatty acids	64.14 ± 0.42[a]	65.65 ± 0.13[a]	67.08 ± 0.92[a]	68.92 ± 3.77[a]	69.57 ± 1.48[a]	ns
Omega-3	3.58 ± 0.05[a]	3.40 ± 0.18[a]	3.10 ± 0.04[a]	4.67 ± 0.45[b]	3.10 ± 0.11[a]	**
Omega-6	17.88 ± 0.06[a]	18.90 ± 0.09[b]	16.71 ± 0.13[c]	16.88 ± 0.14[c]	16.19 ± 0.33[c]	***

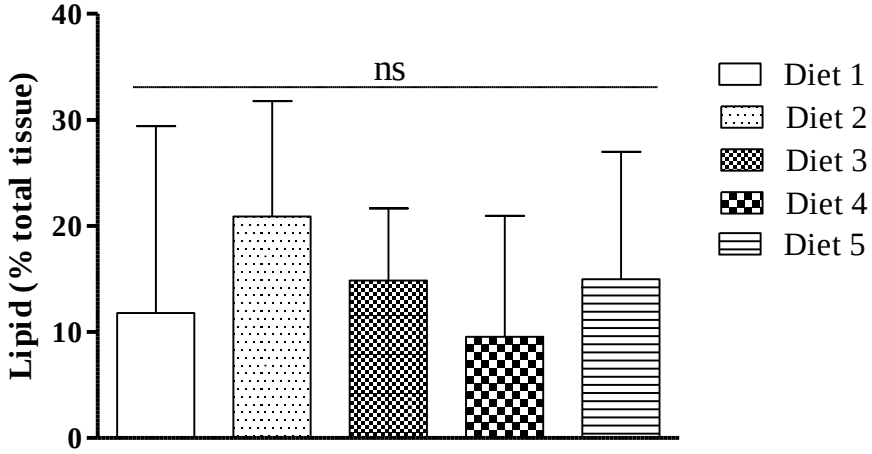
Omega-9	46.58 ± 0.62[a]	46.90 ± 0.22[a]	50.63 ± 0.79[ab]	52.54 ± 3.09[ab]	53.81 ± 0.82[b]	*
DHA (Docosahexaenoic acid)	0.21 ± 0.01[a]	0.11 ± 0.15[a]	0.14 ± 0.08[a]	nd	0.06 ± 0.09[a]	ns

ns = not significant, P >0.05, *P <0.05, ** P <0.01, *** P <0.0001; [ab] Within row means with different superscripts are significantly different at P <0.05. TSFA = total saturated fatty acids, TMUFA = total monounsaturated fatty acids, TPUFA = total poly-unsaturated fatty acids, nd = not detected. Pentadecanoic acid percentage of the breast muscle of birds fed diet 1 was lower (P <0.01) compared to that of quail fed diet 2 and those fed diet 3. The stearic acid of the breast muscle of birds fed diet 2 was higher (P <0.01) than that from quail fed diet 3. However, the breast muscles of birds fed diets 2 and 3 had lower (P <0.01) stearic acid compared to those fed diet 4. The TSFA percentage of the breast meat from carcasses of quail fed diets 1, 2 and 5 were higher compared to diet 4 (P <0.01). The oleic acid of the breast muscle of birds fed diets 1 and 2 had lower (P <0.05) percentage compared to those fed diet 5. The palmitoleic acid percentage of the breast muscle of birds fed diet 1 was higher (P <0.05) compared to fed diet 3 and diet 5. The TMUFA percentage of the breast meat from carcasses of quail fed diets 1, 2 and 3 were lower (P <0.0001) than those fed 4 and 5. The linoleic acid of the breast muscle of birds fed diet 1 was higher (P <0.05) compared to those fed diet 5. The α-linolenic acid content of the breast muscle of birds fed diets 1, 3, 4 and 5 were higher (P <0.01) compared to those fed diet 2. Eicosatrienoic acid content of the breast muscle of birds fed diets 1, 2 and 3 were lower (P <0.01) compared to those fed diet 4 and diet 5. The Omega-3 percentage of the breast meat from carcasses of quail fed diets 1, 2, 3 and 5 was lower compared to those fed diet 4 (P <0.01). The Omega-6 percentage of the breast meat from carcasses of quail fed diets 1, 3, 4, 5 were lower compared to those fed diet 2 (P <0.0001). Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 24 birds (n = 12 males and n = 12 females).

4.6.5 Effect of dietary Marula nut meal on the liver lipid content

The effect of graded dietary MNM on the liver lipid content of male and female broiler Japanese quail fed MNM-based diets is presented in figure 4.1. Dietary MNM had no effect (P >0.05) on the liver lipid content of male and female Japanese quail.

A.



B.

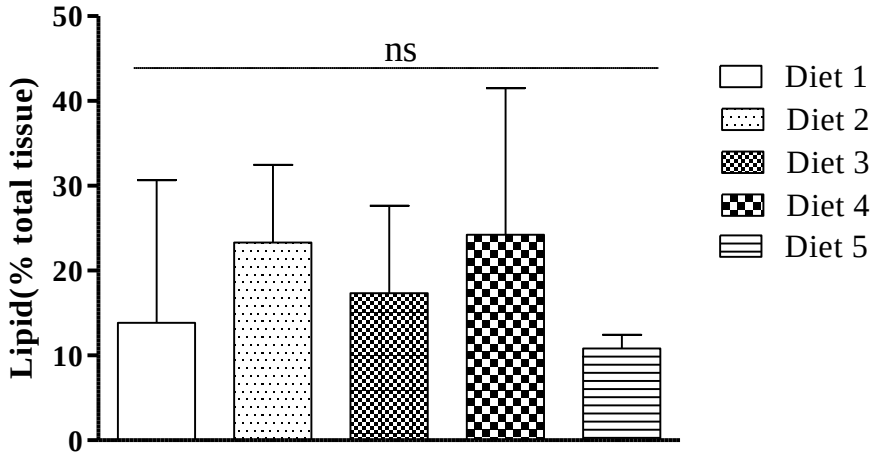


Figure 4. 1: Effect of dietary Marula nut meal on the liver lipid content of male (A) and female (B) Japanese quail

ns = not significant. There were no significant differences (P >0.05) the liver lipid content of male and female quail across dietary treatments. A = male, B = female. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; values expressed as mean ± SD; n = 12 males and n = 12 females.

4.6.6 Effect of MNM on surrogate markers of liver and kidney function

The effect of graded dietary MNM on surrogate markers of liver and kidney function of broiler Japanese quail is presented in Table 4.11. The data for the males and females was combined as there were no differences when they analysed separately. There were no significant differences in the plasma activities of aspartate aminotransferase and gamma-glutamyl transpeptidase as well as the plasma urea, total bilirubin, cholesterol, total protein, albumin and globulin concentration of the Japanese quail across dietary treatments ($P > 0.05$).

Table 4.11: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the general health profile of broiler Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Urea (mmol/L)	0.89 ± 0.05[a]	0.92 ± 0.05[a]	0.97 ± 0.09[a]	0.89 ± 0.05[a]	0.86 ± 0.05[a]	ns
Total bilirubin (µmol/L)	55.30 ± 20.19[a]	60.31 ± 19.49[a]	40.24 ± 17.52[a]	42.01 ± 17.52[a]	44.15 ± 17.59[a]	ns
Cholesterol (mmol/L)	5.02 ± 1.26[a]	4.29 ± 0.55[a]	5.19 ± 0.50[a]	5.28 ± 0.53[a]	5.42 ± 0.55[a]	ns
Total protein (g/L)	44.00 ± 3.44[a]	44.92 ± 5.59[a]	38.91 ± 3.82[a]	40.58 ± 2.11[a]	40.33 ±3.63[a]	ns
Albumin (g/L)	15.75 ± 0.97[a]	15.50 ± 1.67[a]	14.45 ± 1.12[a]	14.17 ± 0.47[a]	15.50 ± 1.08[a]	ns
Globulin (g/L)	30.25 ± 2.50[a]	29.42 ± 4.06[a]	25.64 ± 2.94[a]	26.33 ± 1.78[a]	24.83 ± 2.70[a]	ns
Albumin:globulin ratio	0.54 ± 0.02[a]	0.60 ± 0.07[a]	0.60 ± 0.04[a]	0.56 ± 0.02[a]	0.69 ± 0.08[a]	ns
AST (U/L)	365.40 ± 32.85[a]	292.80 ± 33.46[a]	293.50 ± 16.70[a]	337.20 ± 22.35[a]	355.80 ± 43.91[a]	ns
GGT (U/L)	1.67 ± 0.73[a]	4.05 ± 2.08[a]	3.55 ± 1.62[a]	2.67 ± 1.07[a]	3.42 ± 1.26[a]	ns

[a] Within row means with the same superscripts are statistically similar (P >0.05), ns –not significantly different. AST = Aspartate aminotransferase and GGT = gamma-glutamyl transpeptidase. No significant differences the surrogate markers of liver and kidney function as wells as the general health of the birds across dietary treatments. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; values expressed as mean ± SD; n = 12 birds (n = 6 males and n = 6 females).

4.7 Discussion

4.7.1 Effect of MNM on growth performance and feed economy

In the current study, dietary MNM did not affect terminal body mass (TBM), BMG, ADG, FI and FCR of the quail across dietary treatments and importantly, across dietary treatments, the birds grew significantly (Table 4.3). These findings suggest that dietary MNM, at all dietary inclusion levels neither compromised growth performance nor feed intake and or feed utilisation efficiency. Plant-derived dietary protein sources for poultry feeds contain ANFs such as lectins, tannins, saponins, phytic acid, oxalates, protease inhibitors and phytohemagglutinins (Woyengo *et al.*, 2017) that interfere with nutrient digestion, absorption and utilisation (Akande *et al.*, 2010). The similarities in the TBM, BMG, ADG, and FCR for the grower and finisher growth phases and overall, can be speculated to mean that either the defatting of MNM with hexane reduced the ANFs concentration to levels that did not elicit physico-biochemical derangements that would otherwise result in interference with nutrient digestion and absorption or that the MNM did not contain ANFs in concentrations high enough to interfere with digestion, absorption and utilisation of nutrients. The similarity in the FCR (grower, finisher and overall) suggests that the efficiency with which MNM was utilised as a dietary protein source for broiler quail grower and finisher diets was similar to that of SBM utilisation. Bader *et al.* (2011) reported that using hexane to solvent extract seed meals results in an improvement in the meal's sensory profile, as well an improvement in protein quality due to the formation of protein isolates. It can, therefore, be speculated that defatting the MNM with hexane might have improved its aroma and flavour thus probably positively contributed to feed intake and efficiency of nutrient utilisation hence the observed similarity in the FI and feed utilisation efficiency. In their study, Mathiyane and Mhlanga (2017) reported that broiler chicken fed dietary MNM had decreased growth performance and FI. They inferred that the decreased growth performance and poor intake and utilisation efficiency emanated from the high residual oil in the MNM which caused rancidity in the MNM-based diets. In the current study the similarities in the growth performance and feed utilisation efficiency of quail across dietary treatments (Table 4.3) suggests that residual fat in the MNM following hexane extraction was at a level that neither influenced the energy density of the diets negatively nor caused rancidity. It can therefore be inferred that the hexane-extraction of the MNM effectively reduced the meal's residual fat content.

4.7.2 Effect on viscera macro-morphometry

The gastrointestinal tract of the Japanese quail becomes morphologically and functionally mature between 7 and 14 days of age (Mikhajlov *et al.*, 2008). Importantly, the weight, morphology, and morphometry of internal organs of quail are influenced by type and composition of feed (Tarasewicz *et al.*, 2007). Additionally it has been established that high energy feeds are associated with increased visceral adiposity (Fouad and El-Senousey, 2014). Findings from the current study show that dietary MNM did not affect ($P > 0.05$) the macro-morphometry (absolute and relative to body) of the proventriculi, ventriculi, small and large intestines but relative to body mass only caeca from quail fed diet 4 (75% substitution of SBM dietary CP contribution) were lighter compared to those from quail fed diet 3 (Table 4.4). Except for the caeca (relative to body mass) it can be inferred that dietary MNM did not improve and or compromise GIT viscera development suggesting that it can be used as a dietary protein source possibly without risking the functional efficiency of the GIT. Toxins from plant-derived dietary ingredients (Makkar and Becker, 1999; Rahma *et al.*, 2013) as well as mycotoxins produced from fungal activity in dietary ingredients and or feed with high fat content (Mthiyane and Mhlanga, 2017) can damage GIT organs and other viscera. Findings from the current study show that there were no differences in the liver and pancreata masses of the quail across dietary treatments (Table 4.6). However quail fed diet 4 had heavier (absolute) visceral fat mass compared to that of quail fed diet 1. The heavier visceral fat mass from birds fed diet 4 could be ascribed to the higher dietary fat content of brooder/grower diet 4 compared to that of diet 1 (Table 4.5). The findings from the current study contradict those from a study by Mthiyane and Mhlanga (2017) who reported a decrease in liver mass and similarities in the ventriculus and visceral fat mass from quail fed diets comprising of 5 to 20% MNM on CP basis. It is imported to note that Mthiyane and Mhlanga (2017) observed that the high residual fat in the MNM they used caused rancidity of the diets which affected feed intake and feed utilisation efficiency and hence growth performance. Reduced feed intake affects key organ, for example, liver and pancreata mass (Carew *et al.*, 2003). The similarities in the liver and pancreata mass of the birds across dietary treatments in the current study could have been due to similarities in FI and utilisation efficiency. Additionally, further defatting of the MNM with hexane may have reduced the possibility of the feed developing rancidity which reduces FI resulting in malnutrition affecting liver and pancreas mass.

4.7.3 Effect on physical properties of meat

Irregular muscle pH has been associated with effects on meat quality parameters such as colour, tenderness and water-holding capacity (Qiao *et al.*, 2001). High muscle pH is associated with dark, less tender meat and dry meat referred to as dry firm dry (DFD), while pale soft and exudative (PSE) meat is associated with lower than normal muscle pH (Fletcher, 2002). The pH_u of the breast meat from Japanese quail has been reported to range from 5.4 to 6.62 (Boni *et al.*, 2010; Genchev and Mihaylov, 2008; Gevrekçi *et al.*, 2009; Mnisi and Mlambo, 2018; Narinc *et al.*, 2013; Nasr *et al.*, 2017; Ribarski and Genchev, 2013). Studies by Genchev *et al.* (2008) and Nasr *et al.* (2017) reported the pH_u of meat from Japanese quail to range from 6.24 to 6.37. In the current study, the pH_u of the breast meat from the carcasses of the quail which ranged from 6.14 to 6.52 fell within the reported range from previous studies but that of meat from the thighs, which ranged from 6.55 to 6.94, was higher (Table 4.6). While dietary MNM significantly impacted the pH_u of the breast meat from carcasses of the Japanese quail, the observed values fall within the range reported in previous studies suggesting that dietary MNM neither altered nor negatively impacted the biochemical processes that occur when breast muscle is converted to meat.

In the process of converting muscle to meat, muscle glycogen content is a critical determinant of the ultimate pH (Mir *et al.*, 2017) as it impacts the production of lactic acid. Pre-slaughter stress has been shown to deplete muscle glycogen reserves leading to reduced lactic acid production (Matarneh *et al.*, 2017). While all efforts were put in place to keep stress to a minimum during the feeding trial and the pre-slaughter period, the higher than previously reported pH_u of meat derived from the thigh muscles reported in the current study may be related to differences in muscle activity. The housing conditions during the feeding trial and pre-slaughter meant the quail mostly used their leg muscles when compared to breast muscle (flight muscles). This might have led to low glycogen reserves in the thigh muscles hence the observed higher than normal pH_u values.

Consumers tend to be concerned about the colour of the meat as they consider it as an indicator of freshness (Çelen *et al.*, 2016; Fletcher, 2002; Kralik *et al.*, 2018; Ribarski and Genchev, 2013). Despite the decreasing lightness values with increasing MNM, at 24 h post-slaughter, the L* values of breast meat from both male and female quail reported in this study ranged from 45.72 ± 1.49 to 49.17 ± 4.53. Various researchers have reported L* values ranging from 42.92 to 54.87 for quail breast meat (Gevrekçi *et al.*, 2009; Mnisi and Mlambo, 2018; Ribarski and Genchev, 2013). However, Narinc *et al.* (2013) and Genchev *et al.* (2008) reported average L*

values of 43.09 and 40.81, respectively for breast meat in Japanese breast. While the L* values of the quail breast meat of the current study are within the range reported by other researchers (Mnisi and Mlambo, 2018; Ribarski and Genchev, 2013; Gevrekçi *et al.*, 2009), they are higher compared to those reported by Narinc *et al.* (2013) and Genchev *et al.* (2008). In comparison to the findings by Mnisi and Mlambo (2018), Ribarski and Gencheve (2013) and, Gevrekçi *et al.* (2009), results from the current study show that dietary MNM did not negatively affect the pH and colour of both the breast and thigh meat. However, when compared to the findings of Narinc *et al.* (2013) and Genchev *et al.* (2008) it would appear that dietary MNM resulted in "whiter" quail breast meat.

The redness of the meat is a major determinant of the acceptability of meat by consumers. The content of myoglobin in muscle (meat) is the major factor impacting redness (a*) of the meat (Çelen *et al.*, 2016). Higher muscle (meat) myoglobin content is associated with increased redness and increased acceptability (Neethling *et al.*, 2017; Viljoen *et al.*, 2002). In the current study at 24 h post-slaughter following storage at 4 °C, redness (a*) the meat (breast) increased with increasing dietary MNM with a range of 2.52 ± 0.45 to 8.11 ± 1.16 (Table 4.6). Gevrekçi *et al.* (2009) and Mnisi and Mlambo (2018) reported a* values of 3.23 and 3.09 to 6.79, respectively. These reports show that generally, the redness of the quail breast was within the range (considering the variance) reported by other researchers suggesting that MNM could be used as a dietary protein source without the risk of compromising acceptability of quail meat by consumers.

The composition of the finisher diet has been shown to greatly impact yellowness (b*) of meat (Ribarski and Genchev, 2013). In the current study, only the yellowness of the thigh meat from the male quail was influenced by dietary MNM, with quail fed diet 3 having the least yellow meat (Table 4.7). Although, the thigh meat from birds fed diet 3 had the lowest b* value, the range (7.07 ± 0.90 to 14.30 ± 0.76) of the b* values of the meat from the thigh muscles from the carcasses of male quail in the current study was within the range (7.74 to 12.72) reported by Ribarski and Genchev (2013), Genchev *et al.* (2008) and Narinc *et al.* (2013).

A low pH of meat during post mortem affects water retention, juiciness and tenderness (Barbut, 1997; Fletcher, 2002). Despite the discernible decline in pH of the breast meat 24 h post-slaughter, its pH_u was within the range of that reported by other researchers (Gevrekçi *et al.*, 2009; Mnisi and Mlambo, 2018; Narinc *et al.*, 2013; Nasr *et al.*, 2017). The meat's within previously reported range pH_u potentially accounts for the lack of effect on TL, CL and

tenderness (see Table 4.8) thus it can be inferred that MNM can be used as a dietary protein source without compromising the meat's water-holding capacity and tenderness as well as its acceptability (based on colour) by consumers.

4.7.4 Effect on the chemical composition of meat

4.7.4.1 Effect on the proximate analysis

The nutrient content in foodstuffs and feedstuffs is better compared on a dry matter basis (Hall *et al.*, 2009). Therefore, for the purposes of comparing findings on components of the proximate composition of meat from the current study, results from comparative studies reported in the literature have been converted to a dry matter basis (see supplementary table of data in appendix 3). Findings on the determined proximate components of the meat in the current study show that across dietary treatments, the breast meat from the quail carcasses, on a dry matter basis, had a CP, EE and ash content which ranged between 78.90 ± 0.72 to 83.86 ± 0.32%, 7.54 ± 0.21 to 9.38 ± 0.01% and 7.27 ± 0.42 to 8.29 ± 0.27%, respectively (Table 4.9). My findings show that substitution of SBM with MNM at 25% and 100% significantly decreased the meat's CP but at 100% substitution of SBM its (meat's) ash content was significantly increased. Dietary MNM decreased the meat's lipid content. It can be speculated that at 25% and 100% dietary the associative effects of accretion of protein where negatively impacted hence the lower CP content of the meat. The higher ash content of the meat at 100% replacement of SBM with MNM could due to a mirroring of the ash content of MNM which was higher compared to that of SBM (Table 3.1).It can be speculated that in its metabolism when compared to that SBM, the MNM reduced the deposition of fat in the meat. Breast meat from Japanese quail has previously been reported to contain 80.81 to 96.14% CP, 2.85 to 13.91% EE and 2.60 to 6.21% ash (Fakolade, 2015; Gecgel *et al.*, 2015; Genchev *et al.*, 2008; Lukanov *et al.*, 2018; Raji *et al.*, 2015). With regards to CP, EE and ash content of the breast meat of Japanese quail from the current study, it is evident that these fall within the ranges reported in literature. It can, therefore, be inferred that MNM can be used a dietary protein source in Japanese quail grower and finisher diets without the risk of altering its (meat's) CP, EE and ash content suggesting that dietary MNM does not compromise the nutrient (CP, EE and ash) content of the meat. Importantly, dietary fat, particularly the consumption of diets rich in saturated fatty acids is associated with increased risk of developing obesity (Lai *et al.*, 2018) leading to a host of metabolic derangements and diseases (Yan *et al.*, 2015). The within previously reported range of EE content of the meat suggests that MNM can potentially be used as a dietary protein source in quail without the risk of producing

fat-laden meat which would otherwise increase the risk of developing obesity and its associated metabolic disease in consumers.

4.7.4.2 Effect on fatty acid profile

The World Health Organisation (WHO) recommends a daily dietary fat intake of less than 30% of the daily energy needed, in which saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) should not exceed 10% and <1%, respectively (WHO, 2018). The SFA content of meat plays an important role in meat quality with higher content known to improve juiciness, flavour and tenderness (De Smet *et al.*, 2004). The extent of fatty acid content of the meat has been associated with the composition of the diet (Raes *et al.*, 2004). There are differing reports on the lipid profile of breast meat from Japanese quail. It was previously reported to contain 34.13 to 34.65% total saturated fatty acids (TSFA), 40.70 to 49.70% total monounsaturated fatty acids (TMUFA) and 13.81 to 24.98 % total polyunsaturated fatty acids (TPUFA) in studies by Gecgel *et al.* (2015) and Genchev *et al.* (2008). However Boni *et al.* (2010) reported 25.84 to 29.07% TSFA, 41.90 to 42.76% TMUFA and 28.40 to 32.59% TPUFA, respectively. Findings from the current study showed that quail breast meat contained 25.03 ± 0.51 to 29.16 ± 0.61%, 53.88 ± 0.52 to 60.27 ± 0.07% and 41.06 ± 28.11 to 22.30 ± 0.08% TSFA, TMUFA and TPUFA, respectively (Table 4.10). In the breast meat, stearic acid (6.73 ± 0.01 to 7.75 ± 0.45%) was the dominant SFA while oleic acid (46.50 ± 0.51 to 53.68 ± 0.81%) and linoleic acid (15.89 ± 0.67 to 17.64 ± 0.08%) were the dominant MUFA and PUFA, respectively. While the TSFA and TPUFA content of the quail breast meat were within the range reported by Boni *et al.* (2010), Gecgel *et al.* (2015) and Genchev *et al.* (2008) its TMUFA was lower compared to that reported by Boni *et al.* (2010), Gecgel *et al.* (2015) and Genchev *et al.* (2008). These differences may be due to the feed composition. The MNM used to formulate the experimental diets whose residual lipid content was 17.5% had an oleic acid content of 65.93%. Since ingredient and diet composition impact the fatty acid profile of meat (Sheard *et al.*, 2000; Bryhni *et al.*, 2002), the high oleic acid content in the breast meat from carcasses of quail fed MNM-based diets could be attributed to the high oleic acid content of the MNM.

In general, the consumption of high dietary saturated fatty acids induces metabolic derangements and diseases (Dupont, 2003). Findings from the current study point to high but similar concentration of palmitic and stearic acid in the breast meat (Table 4.10). While high intake of dietary palmitic acid is associated with the development of metabolic diseases (Calle and Kaaks, 2004), the similarity in its concentration in the meat across dietary treatments suggests that

MNM can potentially be used as a dietary protein source in quail grower and finisher diets without decreasing and or increasing the palmitic acid content of the meat. Stearic acid (C18:0) has been reported to reduce total and low-density lipoprotein cholesterol (Romero *et al.*, 2013) which makes it an important nutrient in human diets. Oleic acid has several health beneficial activities/properties including among other antioxidant (Hernandez, 2015) and hypocholesterolaemic activities (Kris-Etherton, 1999) thus is vital to mitigating coronary heart disease. In the current study, there is a general increase in the meat's stearic acid content with an increase in dietary MNM (Table 4.10). Similarly, there is also an increase in oleic acid content of the meat with an increase in dietary MNM (Table 4.10). These findings suggest that dietary MNM positively impacted the fatty acid profile of the meat. It can, therefore, be speculated that dietary MNM can be used to manipulate the fatty acid profile of poultry meat to increase the desirable and health beneficial stearic and oleic acid.

4.7.5 Effect on liver lipid content

Diets are composed of different components which are mainly carbohydrates and proteins and have been reported to have a direct effect on the liver lipid content as these are partially converted into energy stored as fat if in excess (jaya Putra *et al.*, 2015). Excessive lipid accumulation in the liver is associated with metabolic conditions such as fatty liver disease (Fong *et al.*, 2000; Parry and Hodson, 2017). Shini and Bryden (2009) reported that hens with liver fat content ranging from 40 to 70% are affected with fatty liver disease. Diets have been reported to have a direct effect on the liver lipid content (Velu *et al.*, 1971; Enes *et al.*, 2011). In poultry excessive deposition of fat in the liver results in liver dysfunction leading to conditions such as steatosis and steatohepatitis (Ayala *et al.*, 2009) which may lead to liver failure.

In the current study, there were no differences in the liver lipid content of the broiler quail across dietary treatments. The lipid content in the male quail liver ranged from 9.54 to 20.90% while that of females ranged from 10.82 to 24.24%. The lipid content in both male and female livers is respectively below the 29% and 37% previously reported in quail (Maeda *et al.*, 1986). Despite the MNM having high residual oil content, the diets fed to the birds did not cause an accumulation of hepatic lipids to the extent of causing fatty liver disease [>40% hepatic lipid (Shini and Bryden, 2009)] which can negatively impact the health of the birds.

Findings from the current study suggest that hexane extracted dietary MNM can be used in place of SBM without risk to mediating increased liver lipid content in the quail. It can be speculated that the MNM could be used without posing a risk to the development of fatty liver disease in

quail. Laying hens are more susceptible to the development of fatty liver (Maurice *et al.,* 1979), thus dietary MNM, which reduced liver lipid content in female quail (Figure 4.3 B), could have health beneficial effects when used as a protein source in pullet quail feeds.

4.7.6 Effect on health

4.7.6.1 Effect on surrogate markers of liver function

The liver is the metabolic centre of the body (Rui, 2014) where macronutrients are metabolised (Fong *et al.,* 2000; Guan and He, 2015). Importantly, endogenous metabolites such as hormones and exogenous compounds, for example, toxins and pharmacological agents are inactivated in the liver (Guan and He, 2015; Rui, 2014). The multiple metabolic reactions that occur in the liver are catalysed by hepatocyte-resident enzymes, including among others, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) (Lala and Minter, 2019). Damage to hepatocytes and bile duct and bile canaliculi cells is manifested by increased plasma ALT, AST, ALP and GGT activities as these enzymes leak into the circulatory system.

Anti-nutritional factors in some plant-derived feed ingredients, for example, tannins, phytates, oxalates, saponins, lectins and alkaloids are known to poison and damage liver cells (Gemede and Ratta, 2014). In the current study dietary MNM had no effect on plasma cholesterol and urea concentration as well as plasma AST and GGT activities of the quail across dietary treatments. However the plasma activities of AST reported in the current study (range: 292.80 ± 33.46 to 365.40 ± 32.85 IU/L) are much higher than that reported (59.99 ± 1.42 IU/L) for 5 to 6 week old Japanese quail (Agina *et al.*, 2017) but lower than the 402±13.00 to 422 ± 9.50 IU/L range reported by Scholtz *et al.* (2009). Importantly, the observed high plasma AST activities reported in the current findings may not be a true indication of liver damage. Myocytes and erythrocytes are known to contribute to plasma AST activity (Bovera *et al.*, 2015; Sokół *et al.*, 2015). In the current study, the assay for AST did not differentiate between the different AST isoforms, thus it cannot be categorically stated that the observed AST activity is of liver origin only. Results from the current study suggest that MNM can be used in quail grower and finisher diets without causing liver damage and also without affecting the metabolism of cholesterol. Importantly, it can be inferred that the use of dietary MNM did not compromise the liver's capacity to metabolise protein and to synthesise urea.

Scholtz *et al.* (2009) reported plasma GGT activities of 1.7 ± 0.2 U/L and 1.9 ± 0.1U/L in adult male and female quail, respectively. The plasma GGT activities of both male and female quail in

the current study fall within the range of that reported by Scholtz *et al.* (2009), thus my results are in agreement with other published research. Gamma-glutamyltransferase is found in the cell membranes of bile duct cells and it is a more specific indicator of liver damage in birds (Ognik and Krauze, 2016). Thus, similarities in the GGT activity found in the plasma of quails used in this study suggest that no liver damage was elicited by MNM.

Agina *et al.* (2017) and Ali *et al.* (2012) reported plasma cholesterol concentrations of 8.14 mmol/L and 11.81 mmol/L, respectively in adult quail. These values are much higher than those (range: 4.29 ± 0.55 to 5.42 ± 0.55 mmol/L; Table 4.11), cholesterol concentration reported in the current study. The lower plasma cholesterol content may be due to the low-fat content of the MNM due to further defatting with hexane. Walzem *et al.* (2018) reported that laying birds generally have higher levels of cholesterol as hepatic synthesis of triglycerides, phospholipids, and cholesterol is increased. The lower plasma cholesterol of quail across dietary treatments can be inferred to mean that dietary MNM did not elicit cholesterol increase which could lead to heart failure and liver malfunction. Thus, it can be speculated that MNM did not result in elevated cholesterol concentration in the plasma of Japanese quail of which higher than normal concentrations may lead to fat accumulation that may affect animal health and meat quality.

4.7.6.2 Effect on surrogate markers of kidney function

The kidneys play a major role in the excretion of metabolic waste from the body (Onopiuk *et al.*, 2015). They synthesise key messengers such as erythropoietin and renin (Kurtz, 2017) and also activate calcitriol (Vitamin D_3) (Murabito and Hallmark, 2018). Additionally, the kidneys help regulate body fluid and electrolyte balance (Reece *et al.*, 2015). Kidney function can be compromised by toxins, infectious agents, stones and crystals and or tumours. The toxins, ANFs and infectious agents may be feed borne (Echols, 2006; Siller, 1981). These various aetiological factors can lead to severe damage to the kidneys and or cause kidney failure. The gold standard to evaluating kidney health is by determining the glomerular filtration rate. However kidney damage or malfunction can also be assessed by determining plasma surrogate markers of kidney function which include, among others, blood urea and uric acid concentration (Giordano *et al.*, 2015).

Findings from this study depicted no differences in the plasma concentration of surrogate markers (blood urea, uric and bilirubin) of kidney function of the birds across dietary treatments. Bovera *et al.* (2007) reported that levels of blood urea increase with increased dietary CP. Diets used in the current study were so formulated to be iso-caloric and iso-nitrogenous. This fact

combined with the lack of differences in the plasma urea and uric acid concentration of the quail suggests that dietary MNM did not compromise the detoxification capacity of the liver, which includes among others, the conversion of metabolic ammonia to urea. The plasma uric acid concentration reported in this study is lower than the 16.02 ± 1.01 mg/dl and 13.25 ± 1.93 mg/dl reported by Agina *et al.* (2017) and Ali *et al.* (2012), respectively. Scholtz *et al.* (2009) reported total plasma bilirubin concentrations of 20.4 ± 1.0 and 8.9 ± 0.7 μmol/L in male and female quail, respectively. These reference total bilirubin values as reported by Scholtz *et al.* (2009) are much lower than those observed in the current study suggesting that in the current study the kidneys of Japanese quail might not have efficiently filtered and excreted the bilirubin as compared to those of counterparts used by Scholtz *et al.* (2009).

The levels of uric acid can be affected by the physiological state of the birds, as laying birds tend to have high values of bilirubin and uric acid (Scholtz *et al.*, 2009). These findings on uric acid concentration suggest that inclusion of MNM in place of soybean in quail diets does not negatively affect the kidney function of the birds suggesting that hexane-extracted MNM can potentially be used as a dietary protein source in broiler quail without any risk of eliciting kidney damage.

4.7.6.3 Effect on general markers of health

Factors that induce alterations in blood parameters include stress, physical activity, quality and availability of food resources as well as environmental contaminants (Sturkie and Griminger, 1986; Vleck, 2006). Diets can alter the total blood protein, bilirubin and globulin concentration depending on the formulation (Otter, 2013). Dehydration can cause an increase in plasma protein concentration (Sakas, 2002) as well as the haematocrit. In this study, the quails had similar blood total protein concentrations ranging from 38.91 ± 3.82 to 44.92 ± 5.59 g/L across the treatment groups. Agina *et al.* (2017) reported a plasma total protein, albumin and globulin of 5.19 ± 0.17 g/dl, 3.25 ± 0.15 g/dl and 1.94 ± 0.04 g/dl, respectively while Ali *et al.* (2012) reported plasma total protein concentration of 4.78 ± 0.39 g/dl and albumin of 1.54 ± 0.23 g/dl in 6-week old Japanese quail. Agina *et al.* (2017) and Ali *et al.* (2012) reported plasma protein and albumin concentrations that are higher compared to those from this study. However, the globulin concentrations found in plasma of quail used in this study was lower than those reported by Agina *et al.* (2017) and Ali *et al.* (2012). It is noteworthy that the quail in this study did not exhibit any signs of dehydration, kidney failure nor liver dysfunction which could have impacted these parameters. In view of the similarities in the plasma protein concentration of the quail across dietary treatments, it can be inferred that dietary MNM nut meal did not alter plasma

protein metabolism. Differences in some plasma metabolite concentrations in my results compared to those reported by other researchers could be due to differences in bird management and production system. It is therefore necessary to establish normal references values of plasma metabolites for quail under different production environments in order to account for environment-induced differences.

4.8 Conclusion

Dietary MNM, at all levels of SBM substitution met the nutritional requirements of broiler Japanese quail as confirmed by similarities in growth performance, feed intake, feed utilisation efficiency and GIT viscera macro-morphometry across dietary treatments. Similarities in feed intake and efficiency of utilisation as wells GIT viscera macro-morphometry indicates that the MNM did not compromise palatability and possibly digestion and absorption of nutrients. Marula nut meal can be used as a dietary protein source in quail grower and finisher diets in place of SBM without compromising broiler quail meat's water-holding properties (TL and CL) but it can be used to improve redness and the stearic and oleic acid content of the meat. Importantly, similarities in plasma surrogate markers of liver and kidney function and other plasma metabolites of the quail across dietary treatments show that MNM can be used as a dietary protein source in broiler quail starter and finisher diets without risk of compromising bird health. Hexane-extracted MNM can therefore be used as a source of dietary protein in grower and finisher Japanese broiler quail diets without compromising the growth performance, feed utilisation efficiency, meat quality and health status.

Poultry are raised not only for their meat but also for eggs. Egg production also ensures successful continuation of the species. The process of producing eggs places a heavy metabolic demand on the layers. It is under conditions of severe metabolic demand that nutritional deficiencies of diets may translate into compromised health and productivity of livestock. Thus, the next chapter explored the effect of dietary Marula nut meal on egg production and egg quality in pullet quail.

CHAPTER FIVE - EFFECT OF DIETARY MARULA NUT MEAL MEAL ON PULLET QUAIL EGG PRODUCTION AND EGG QUALITY

5.0 Introduction

In terms of poultry products eggs are regarded as the cheapest source of dietary protein for human consumption (Etal, 2014; Memon *et al.*, 2015; Surai and Sparks, 2001). Eggs are a rich source of protein and an important component of many diets (Larsson *et al.*, 2015). Egg proteins, which are rich in essential amino acids required for normal human development, are highly digestible (Surai and Sparks, 2001). Eggs are also a source of macro- and micro-minerals such as iron and zinc, respectively (Johnson *et al.*, 2019). They are also a major source of the fat-soluble vitamins A, D and E as well as water-soluble vitamins B_6 and B_{12} (Surai and Sparks, 2001). Even though eggs are regarded as a cheaper source of animal protein compared to meat, their production is beset with challenges.

Resource-limited smallholder rural communities find commercial chicken production expensive as they cannot afford housing requirements, feed and veterinary costs of the genetically improved broiler and pullet chicken (Etal, 2014; Moreki, 2010). The high cost associated with the farming of improved breeds of pullet chicken translates into protein malnutrition especially among the vulnerable members of society (growing children, pregnant and nursing mothers as well as the sick and old) in rural communities. Alternative and or complementary egg-producing poultry species, such as the Japanese quail, which are small thus, require less space and less feed and are characterised by rapid growth, a shorter generation interval, early maturity and high rate of lay (Minvielle, 2004; Panda and Singh, 1990), could be farmed to increase options for the production of eggs for human consumption.

Quail eggs have been reported to contain approximately 12.7 kcal/100g CP and GE value of approximately 156.50 kcal/100g (Tunsaringkarn *et al.*, 2013). While the proximate composition of quail eggs is reported to be comparable to that of chicken eggs (Panda and Singh, 1990), when compared to Guinea fowl eggs, quail egg albumen and yolk have been shown to have a lower fat but higher protein content (Song *et al.*, 2000) hence are nutritionally a more healthful product. When compared to Guinea fowl eggs, quail eggs have a larger yolk index (YI) and Haugh unit (HU) (Song *et al.*, 2000) and importantly their albumen:yolk ratio of 65:35 (Singh and Panda, 1987) is comparable to the 64:36 ratio of chicken eggs (Powrie, 1977). Egg quality is critical both in terms of nutrient supply to the consumer, for shelf life as well as for amenability to processing (Newberry, 2017; Song and Kerver, 2000). Several factors interplay and are considered in the determination of egg quality (De Ketelaere *et al.*, 2004; Icken *et al.*, 2006; Minelli *et al.*, 2007). Chief among them are exterior egg quality parameters which include egg

mass, shape index and shell thickness as wells as interior egg quality parameters that encompass albumen index, yolk index, yolk ratio, yolk/albumen ratio and Haugh unit (Icken *et al.*, 2006; De Ketelaere *et al.*, 2004; Minelli *et al.*, 2007). Among the many egg quality characteristics, external quality parameters are important determinants for egg acceptability by consumers. Interior quality parameters are important-particularly for the egg production industry (Song et al., 2000) because they give an indication of the nutrient composition and quality of the egg.

Sub-Saharan Africa is characterised by a rapidly growing human population, expansion of urban settlements and a general improvement in consumer incomes (Mutunga *et al.*, 2012). This scenario has created an increase in the demand for animal-derived products, including poultry eggs for human consumption (Thornton, 2010). While the production and introduction of quail eggs can help alleviate the shortage of animal-derived dietary protein for human consumption in SSA, rural resource-limited farmers, whose communities would benefit the most from egg production from pullet quail, are faced with challenges of high feed cost. The cost of SBM, the major dietary protein source in poultry feeds, has been on the increase, resulting in increased poultry feed costs (Morgan and Choct, 2016) making it costly to produce even promising poultry species such as pullet quail. As a consequence of the high cost of the largely imported SBM (Skoufos *et al.*, 2016), there is a great need to introduce locally available, easily accessible, alternative dietary protein sources for poultry feeds in order to lower the cost of production (Morgan and Choct, 2016), including that of pullet quail. While the chemical nutrients and *in vivo* potential of Marula nut meal as a potential dietary protein source in poultry feeds has been described in the previous chapter (see Chapter 2, subheading 2.3.4, page 15 and Chapter 3, subheading 3.3.1, page 21), its potential to supply dietary protein in pullet quail feeds has not been evaluated.

5.1 Study aim

This study sought to determine the potential of hexane-extracted MNM to substitute SBM as a dietary protein source in layer (pullet) Japanese quail diets by specifically determining its effects on the laying performance, egg and eggshell quality and composition, growth performance (body mass and long bone based indices of growth), feed utilisation economy, viscera macro-morphometry and liver and kidney function of layer Japanese quail.

5.1.1 Study objectives

The specific objectives of this study were to formulate hexane-extracted MNM-based Japanese quail layer diets and to determine their effects on the laying performance, egg and eggshell

quality and egg nutrient composition as well as on the growth performance (body mass and long bone indices), feed utilisation economy, viscera macro-morphometry and liver and kidney function of layer Japanese quail.

5.1.2 Hypothesis

H_0: The graded substitution of SBM with dietary MNM has no effect on the laying performance, egg and eggshell quality, egg nutrient composition, growth performance, feed utilisation economy, viscera macro-morphometry and liver and kidney function of layer Japanese quail.

H_1: The graded substitution of SBM with dietary MNM affects the laying performance, egg and eggshell quality, egg nutrient composition, growth performance, feed utilisation economy, viscera macro-morphometry and liver and kidney function of layer Japanese quail.

5.2 Materials and methods

5.2.1 Study site and ethical clearance

The study site and ethical clearance are as described in Chapter 4, subheading 4.2.1.

5.2.2 Feed ingredients

The sourcing of feed ingredients and the hexane extraction of partially defatted MNM are as described in Chapter 4; under subheading 4.2.2.

5.2.3 Diet formulation and diets

The diets were formulated to be iso-caloric and iso-nitrogenous and met the nutritional requirements of laying quail according to the National Research Council (NRC, 1994) guidelines. The diets were formulated such that MNM replaced SBM on a CP basis at 0%, 25%, 50%, 75% and 100% for diets 1 through to 5, respectively. The ingredient and chemical nutrient composition of the layer diets are shown in Table 5.1.

5.2.4 Animals, housing and feeding

Sixty 5-week old point of lay Japanese quail hens were sourced from South African Quail breeders, East London, South Africa. The quail hens were procured from the supplier at 5 weeks of age because that is the optimum age at which quail can be sexed. The hens were each housed in a cage (0.60 × 0.60 × 0.80 m) equipped with a feed and water trough, respectively. Fresh dry wheat straw was used as bedding and was changed twice a week. The study was done indoors with lights switched on at 07h00 and switched off 19h00 daily. The birds were habituated for two days prior to the start of the feeding trial. During the habituation period, the hens had *ad libitum* access to the control diet and drinking water. During the habituation period, piperazine

(Kyron Laboratories Pvt Ltd, Johannesburg, South Africa) was used to deworm the hens at 90 mg/L in their drinking water. Thereafter the hens were fed the layer diet for 8 weeks. The hens had *ad libitum* access to feed and clean drinking water throughout the 8-week feeding trial period.

Table 5. 1:Ingredient and chemical composition of Japanese quail layer dietary treatment

Ingredients (g/kg)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Maize Meal	411.40	428.60	457.70	475.30	548.40
SBM (45%)	391.80	299.00	199.00	101.10	0.00
Marula Nut Meal	0.00	69.90	139.60	212.70	279.60
	(0%)	(25%)	(50%)	(75%)	(100%)
Wheat Bran	88.10	89.70	89.60	91.00	49.90
Limestone	93.00	94.70	94.50	96.10	94.70
DL-Methionine, 99%	2.00	2.60	3.30	4.00	4.50
L-Lysine HCL, 98.5%	1.00	3.10	5.00	8.10	11.00
Dicalcium Phosphate	2.90	2.50	1.40	1.5	2.00
Salt (NaCl)	4.90	5.00	5.00	5.10	5.00
*Vit/Mineral premix	4.90	5.00	5.00	5.10	5.00
Total	**1000**	**1000**	**1000**	**1000**	**1000**
Calculated chemical composition					
Dry matter (DM %)	84.55	83.16	83.36	82.12	83.53
Crude protein (DM %)	20.82	21.10	21.51	21.87	22.16
Crude fibre (DM %)	2.99	2.97	2.99	2.98	2.77

Ash (DM %)	3.51	3.40	3.36	3.29	3.13
Fat (DM %)	2.07	2.46	2.92	3.31	3.82
Neutral detergent fibre (DM %)	11.24	10.87	10.69	10.32	9.15
Acid detergent fibre (DM %)	3.76	3.35	2.98	2.56	1.89
Calcium (DM %)	3.23	3.21	3.17	3.16	3.16
Phosphorus (DM %)	0.39	0.42	0.45	0.50	0.56
Sodium (DM %)	0.18	0.18	0.18	0.18	0.17
Chloride (DM %)	0.30	0.30	0.30	0.30	0.30
Methionine (DM %)	0.50	0.49	0.50	0.51	0.50
Lysine (DM %)	1.28	1.21	1.12	1.13	1.13
Metabolisable energy (MJ/kg DM)	13.87	13.71	13.83	13.68	13.95

*Vit A: 20000000.000 IU, Vit B_1 (Thiamine): 003.000g, Vit D_3 (500 000): 3000000.000 IU, Vit E (500 iu):40000.000 IU, Vit K_3 (43%): 003.000g, Vit B_2 (80%): 010.000g, Vit B_6 98% (pyrod): 005.000g, Vit B_{12} 1g/kg (m):100.000mg, Niacine 99.5%: 060.000g, Choline (Chloride 60): 606.060g, Biotine 2%: 200.000mg, Manganese($MnSO_4$-31%):160.000g, Copper ($CuSO_4$-25.2%): 005.000g, Cobalt ($CoSO_4$-20%): 100.000mg, Selenium (Na_2SeO_3- 4.5%): 400:000mg, Calcium pantothenate: 020.000g, Folic acid (96% pure): 001.000g, Anty-ox Vit Dry: 100.000g, Zinc (Zn SO_4-35%): 090.000g, Iodide (KI 76.45%): 001.000g, Ferrous ($FeSO_4$-30%): 035.000g, Limestone powder: 2647.133g; DL-Methionine with purity of 99%, L-Lysine HCL with purity of 98,5%; Diet 1 – 0% MNM meal 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.

5.2.5 Study design

The quail hens were randomly allocated to individual cages. Following the 2-day habituation period, the sixty 5-week old point-of-lay Japanese quail hens n = 12) were, in an interventional study randomly allocated to five-layer dietary treatments wherein MNM replaced SBM on a CP basis as follows: - diet 1 - 0% MNM CP + 100% SBM CP, diet 2 - 25% MNM CP + 75% SBM CP, diet 3 - 50% MNM CP + 50% SBM CP, diet 4 - 75% MNM CP + 25% SBM CP and diet 5 - 100% MNM CP + 0% SBM CP. The hens were fed their respective diets for 8 weeks.

5.2.6 Measurements during the feeding trial

The hen's body mass (BM) and feed intake (FI) were measured as described in chapter 4; subheading 4.2.6. Eggs were collected manually and counted daily and egg mass was determined daily using an electronic balance (Snowrex, model: EQ: 1200, Taiwan, China). Egg length, width as well as eggshell thickness (following removal of albumen and yolk) were measured daily using a digital Vernier calliper (SDP-S-ETP-1001, Major Tech, Johannesburg, South Africa). Yolk and associated albumen diameter and height were also measured using the same digital Vernier calliper. Egg yolk mass and albumen mass were measured daily using an electronic balance (Snowrex, model: EQ: 1200, Taiwan, China).

5.2.7 Pre-termination computations

The BMG, ADG and FCR computations were done using equations as described in chapter 4; under subheading 4.2.7 above.

5.2.7.1 Laying performance indices

The percentage of laying birds was determined using the following equation:

$$\text{Laying birds (\%)} = \frac{\text{total number of laying birds}}{\text{total number of birds}} \times 100$$

Percentage lay on a daily basis was determined using the formula previously reported by Ali *et al.* (2003).

$$\text{Percentage lay on a daily basis} = \frac{\text{number of egg produced on a daily basis}}{\text{number of birds available in the flock on that day}} \times 100$$

The number of eggs per diet per week was determined using the formula reported by North, (1984).

$$\text{Number of eggs per diet per week} = \frac{\text{total number of eggs produced per week}}{\text{total number of hens per diet}}$$

However to determine how many eggs each laying bird contributed to the total number of eggs, the following equation was developed:

$$\text{Number of eggs per laying hen per diet per week} = \frac{\text{total number of eggs produced per week}}{\text{total number of laying hens per diet}}$$

Egg shape index (SI) was computed as described by Romanoff and Romanoff (1949) using the equation:

$$\text{SI} = \frac{\text{egg width}}{\text{egg length}} \times 100$$

5.2.8 Terminal procedures and measurements

The terminal procedures and measurements were done as described in chapter 4; under subheading 4.2.8 above, except that samples for meat quality tests were not harvested.

5.2.9 Determination of long bone indices

The tibia length was used to determine linear growth of the hens. Briefly, the tibia from the right leg was dissected out of the carcasses following which the bones were dried in an oven (Salvis®, SalvisLab, Schweiz, Switzerland) at 40 °C until they reached constant mass (±7 days). Bone length was measured using vernier digital calipers (SDP-S-ETP-1001, Major Tech, Johannesburg, South Africa). The bones were then weighed on a Precisa 310 M electronic balance (Precisa Instruments AG, Switzerland) to determine their dry mass. The tibia Seedor ratio (bone density) was then determined as described by Seedor *et al.* (2009) using the equation:

$$\text{Seedor ratio} = \frac{\text{bone mass (mg)}}{\text{bone length (mm)}}$$

5.2.10 Determination of health status

The plasma preparation and storage and determination of surrogate markers of liver and kidney function and liver lipid content were also done as described in chapter 4; under subheading 4.2.9 and 4.2.10, respectively.

5.2.10.1 Determination of plasma markers of health

The plasma concentrations of uric acid, phosphorus and calcium concentration were determined using a colorimetric-based clinical chemistry analyzer (IDEXX VetTest® Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) as per the manufacturer's instructions. The assay was done as described in chapter 4; under subheading 4.2.9 above.

5.2.11 Determination of liver lipid content

The liver lipid content was determined using a Soxtec system HT 1043 Extraction Unit (Foss Analytic, HillerØd, Denmark) as described in chapter 4; under subheading 4.2.10.

5.2.12 Determination of egg quality

The yolk indexes (YI), yolk ratio (YR) and albumen index (AI) were computed as described by Romanoff and Romanoff (1949) using the equations:

a. Yolk index (%) $= \frac{\text{yolk height (cm)}}{\text{yolk diameter (cm)}} \times 100$;

b. Yolk ratio (%) $= \frac{\text{yolk weight (g)}}{\text{egg weight (g)}} \times 100$ and,

c. Albumen index (%) $= \frac{\text{albumen height (mm)}}{\text{average of albumen length and width (mm)}} \times 100$

The egg yolk/albumen (Y/A) ratio was computed by dividing the yolk weight by the albumen weight as described by Elnagar and Abd-Elhady (2009). The Haugh units were calculated according to the equation by Wells (1968):

HU = 100 log (h – 1.7 W0.37+ 7.6);

where: HU – Haugh units, h – albumen height (mm), W – egg weight (g).

5.2.13 Determination of the egg yolk cholesterol content

The yolk cholesterol content was determined using an automated Soxhlet apparatus (Soctec Avanti 2055, Foss, Sweden) at the Agricultural Research Council's Animal Production Institute laboratory, Irene, Pretoria, South Africa. Briefly, egg yolks were first freeze dried and milled. Two grams sample of yolk was transferred into an extraction thimble. A fat-free cotton wool plug was place in the top of the thimble. Thereafter, the thimble was placed in the extraction tube before 60 ml of petroleum ether was added into the extraction cup. The extraction cup containing ether was placed on the heating pad and boiled for 30 min. After the boiling time, the condensed ether was used to rinse the extraction cup for about 15 min. At the end of the rinsing period, the extraction cup was removed and allowed to cool. Thereafter, the ether was evaporated under nitrogen in a waterbath (R-300, BÜCHI, Switzerland) at 40 °C. Twenty milligrams of extracted fat was put into a glass tube and 1 ml of Stigmasterol was added. The mixture was dried under nitrogen gas in a waterbath (R-300, BÜCHI, Switzerland) at 40 °C. After drying, 2 ml ethanol / KOH mixture [Ethanol / 30% potassium hydroxide (4:1 ratio)] was added and mixed before being incubated in a waterbath (R-300, BÜCHI, Switzerland) at 70 °C for 2 h. After cooling, 2 ml hexane was added followed by 1 ml distilled water and vortexed. The resultant hexane layer was transferred into another glass tube and dried under nitrogen gas in a waterbath (R-300, BÜCHI, Switzerland) at 40 °C. The sample was cooled and dissolved into 0.5 ml carbon disulfide before being filtered through 0.45 µ millipore filter and injected in gas chromatograph.

The working standard (1 ml internal standard dried under N_2 gas at 40 °C and dissolved in 0.5 ml standard) was injected. A control sample was analysed with the sample and the result plotted on a control chart. The cholesterol concentration was determined using the following equation:

mg cholesterol/100 g egg yolk: $\frac{A\,(c)}{A\,(is)} \times \frac{R_f\,(c)}{R_f\,(is)} \times \frac{Mass\,(is)\,(mg)}{1} \times \frac{\%Fat}{m}$

Where Rf is the response factor for cholesterol and stigmasterol, and is given by

A (c): Peak area (or height) of cholesterol

A (is): Peak area (or height) of internal standard

Rf (c): Response factor of cholesterol

Rf (is): Response factor of internal standard

m: Mass of fat in g

% Fat: Total fat in sample

5.2.14 Determination of egg yolk fatty acid profile

The egg yolks were freeze dried prior the experiment and an automated Soxhlet apparatus (Soctec Avanti 2055, Foss, Sweden) was used to extract the oil from the egg yolks as described by the Association of Official Analytical Chemists (AOAC, 2006; method number 925.32) in a way similar to that described in chapter 4; under subheading 4.4.2 above.

5.3 Statistical analysis

Results are presented as mean ± SD. GraphPad Prism, version 5 (GraphPad Software, San Diego, California, USA) was used to analyse data. Data on weekly feed intake, weight gain, feed conversion efficiency and egg production data were analysed using the repeated-measures ANOVA. Treatment means were compared using Tukey's *post hoc* test. Significance was set at $P < 0.05$. The following statistical linear model was employed:

$Y_{ij} = \mu + T_i + W_j + E_{ij}$

where: Y_{ij} = dependent variable (weekly feed intake, weight gain, feed conversion efficiency and egg production data)

μ = population mean

T_i = effect of diets (1,2….,5)

W_j = effect of week (1,2…..5)

E_{ij} = random error

Data on overall feed intake, overall weight gain, overall feed conversion efficiency, and surrogate markers of health, carcass characteristics, external and internal egg quality were analysed using a one-way ANOVA. Treatment means were compared using Tukey's *post hoc* test. Significance was set at $P < 0.05$. The linear statistical model employed was as follows:

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

where: Y_{ij} = dependent variable (overall feed intake, overall weight gain, overall feed conversion efficiency, surrogate markers of health, carcass characteristics, external and internal egg quality)

μ = population mean

T_i = effect of diets (1,2….,5)

E_{ij} = random error

5.4 Results

5.4.1 Effect of dietary Marula nut meal on growth and feed economy

The effects of graded dietary substitution of SBM with MNM on the growth performance, feed intake and feed utilisation efficiency of layer Japanese quail are presented in Table 5.2. There was no interaction between diet and week of feeding observed. The induction body masses and terminal body masses of the hens were similar ($P > 0.05$) across dietary treatments. However, the hens grew significantly ($P < 0.05$) during the course of the trial. The BWG, ADG, FI and FCR of the quail hens were similar across dietary treatments ($P > 0.05$)

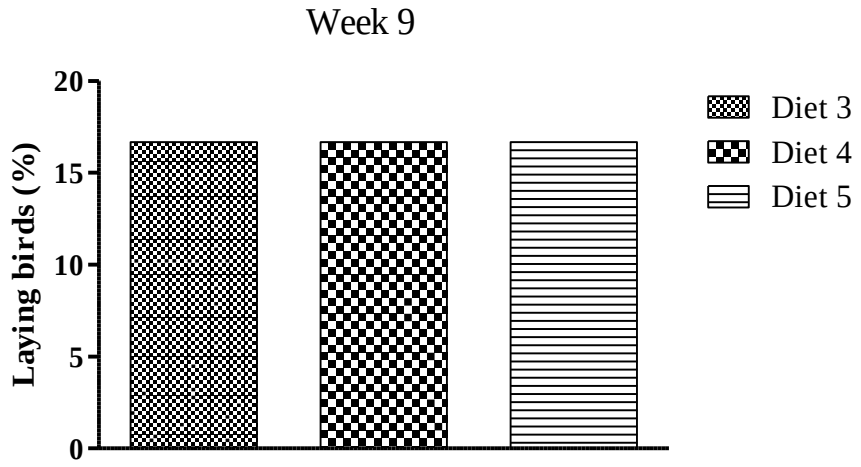
Table 5. 2: Effect of graded dietary substitution of soybean meal with Marula nut meal on the growth performance, feed intake and feed conversion ratio of Japanese quail hens

Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
Induction body mass (g)	144.20 ± 39.20[a]	140.70 ± 37.09[a]	160.80 ± 27.54[a]	150.00 ± 49.98[a]	158.80 ± 35.85[a]	ns
Terminal body mass (g)	225.50 ± 23.07[a]	218.00 ± 15.73[a]	236.50 ± 32.02[a]	222.50 ± 29.51[a]	215.80 ± 31.14[a]	ns
Body weight gain (g)	81.33 ± 9.06[a]	77.27 ± 9.84[a]	75.64 ± 14.42[a]	72.50 ± 9.35[a]	62.40 ± 13.85[a]	ns
Average daily gain (g)	1.45 ± 0.16[a]	1.38 ± 0.18[a]	1.35 ± 0.26[a]	1.30 ± 0.17[a]	1.11 ± 0.25[a]	ns
Feed intake (g)	358.70 ± 19.43[a]	336.30 ± 37.01[a]	333.80 ± 30.28[a]	347.70 ± 26.25[a]	347.20 ± 13.13[a]	ns
Feed conversion ratio	2.34 ± 1.75[a]	2.51 ± 1.12[a]	1.67 ± 1.14[a]	4.02 ± 5.08[a]	3.01 ± 2.28[a]	ns

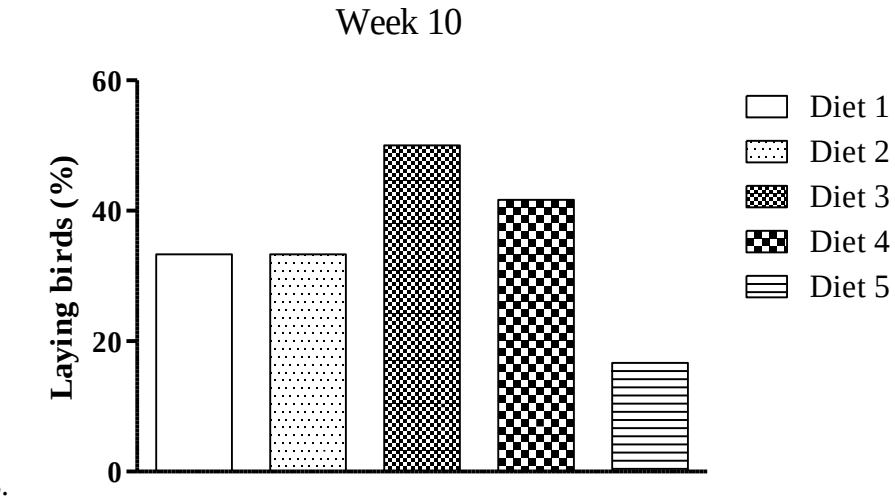
ns = not significant, P >0.05; No significant differences were found in the induction body mass, terminal body mass, body weight gain, average daily gain, feed intake and feed conversion ratio of quail birds across dietary treatments. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 12 birds.

5.4.2 Effect of dietary Marula nut meal on percent hens in lay

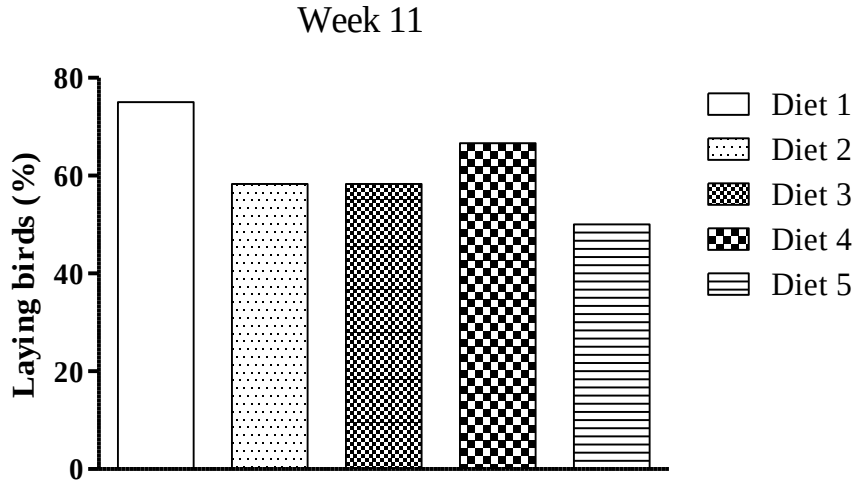
The effect of graded dietary substitution of SBM with MNM on the percent of laying Japanese quail hens by dietary treatment is presented in figure 5.1 A to E. At week 9, birds fed diet 1 and 2 were not laying but 16.67% of the birds fed diets 3, 4 and 5 were laying. At week 10, birds fed diet 3 had the highest number (50%) of laying birds while those fed diet 5 had the lowest percentage (16.67%). At week 11, seventy five percent of birds fed diet 1 had the highest laying birds while birds fed diet 5 had the lowest at 50%. At week 12, birds fed diets 1, 2, 3 and 4 had similar percentage of laying (83.33%) but birds fed diet 5 had lower percentage of laying birds (75%). Higher percentage (91.66%) of laying birds from birds fed diets 2 and 4 was observed at week 13.


Week 9
Laying birds (%)
20
15
10
5
0
Diet 3
Diet 4
Diet 5

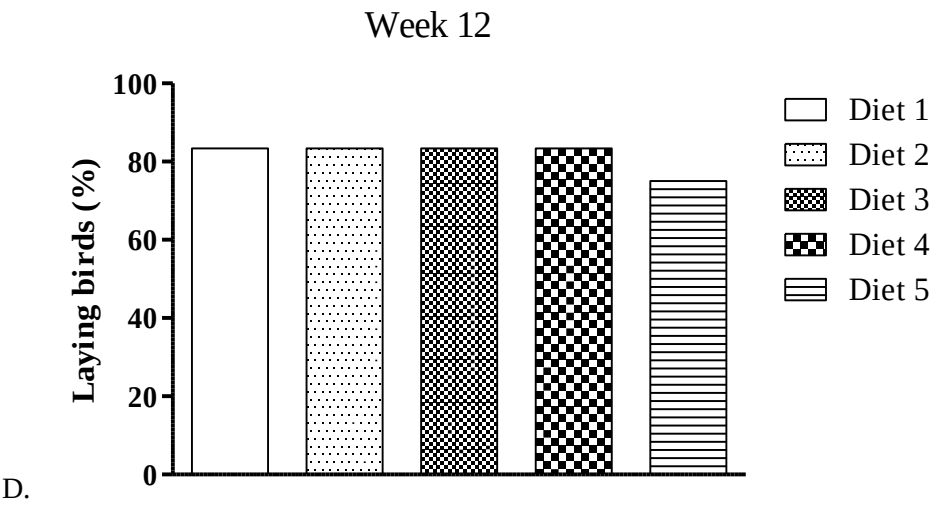
A.


Week 10
Laying birds (%)
60
40
20
0
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

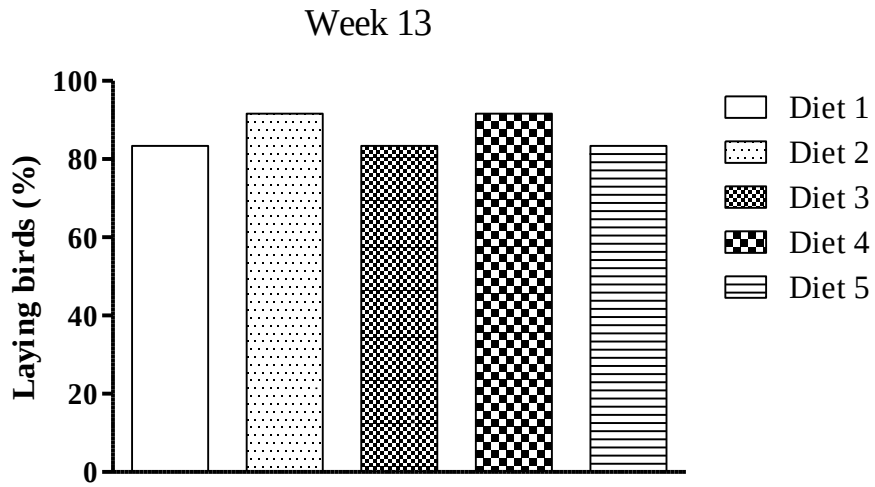
B.


Week 11
Laying birds (%)
80
60
40
20
0
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

C.



D.



E.

Figure 5.1: A, B, C, D and E: Effect of graded dietary substitution of soybean meal with Marula nut meal on the percentage of laying birds per dietary treatment per week

Quail hens fed diets 3, 4 and 5 started laying at 9 weeks of age but there was no significant difference in the percent laying hens (Figure 5.1 A). At week 10, the percentage of laying birds that were fed on diets 1 and 2 were similar (33.33%). Fifty percent of the birds that were fed diet 3 were laying. The percentage of laying birds from birds fed diet 4 and 5 was 41.66 and 16.66%, respectively (Figure 5.1 B). At age 11 weeks, 75% of the birds given diet 1 were laying while the percentage of laying birds was similar from for birds fed diets 2 and 3 (58.33%). The percentage of laying birds was 66.67 and 50% for birds fed diets 4 and 5 (Figure 5.1 C). The percentage of laying birds was similar for birds fed diets 1, 2, 3 and 4 (83.33%) but the percentage of laying birds was lower (75%) for birds fed diet 5 (Figure 5.1 D). At age 12 weeks, the laying percentage of birds fed diets 1, 3 and 5 was similar at 83.33% while those fed 2 and 4 was also similar at 91.66% (Figure 5.1 E). Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis.

5.4.3 Effect of dietary Marula nut meal on egg production

Effect of dietary MNM on percentage lay on a daily basis is presented in figure 5.2. There were no significant differences (P >0.05) in the percentage lay on a daily basis across dietary treatment groups.

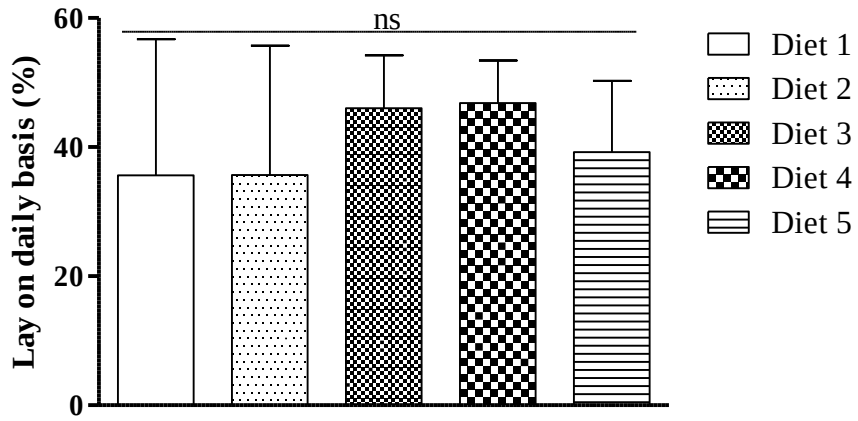
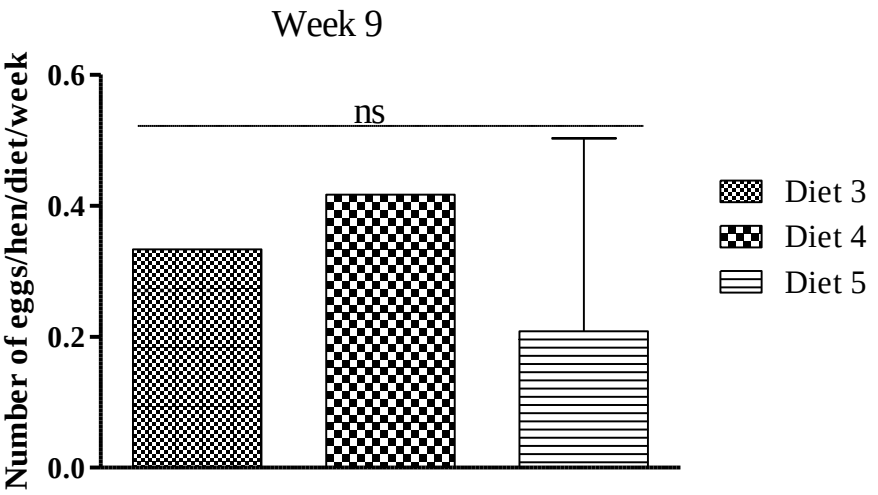


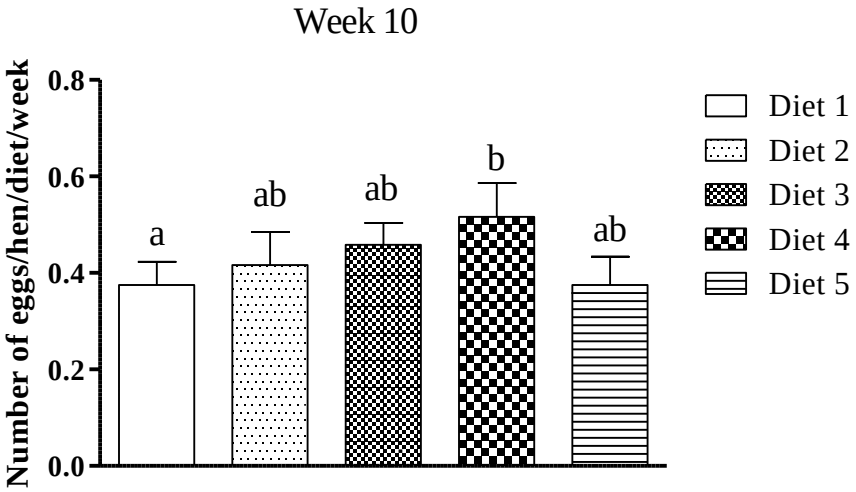
Figure 5.2: Effect of graded dietary substitution of soybean meal with Marula nut meal on the percentage lay on a daily basis by quail hens.

There were no significant difference (P >0.05) in the percentage lay by quail hens. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD. n = 12 birds.

The effect of graded dietary substitution of soybean meal with Marula nut meal on the number of eggs produced by quail hens per dietary treatment per week is presented in figure 5.3. Hens fed diet 4 had a higher ($P < 0.05$) number of eggs per hen as compared to those fed on diet 1 at week 10. While at week 11 quail hens fed diet 3 had a higher ($P < 0.05$) number of eggs compared to those fed diet 1, during week 9 only birds fed diet 3, 4 and 5 were laying and the number of eggs per hen and per week was similar ($P > 0.05$) amongst them. Also at weeks 12 and 13, the number of eggs per hen and per week was similar ($P > 0.05$).

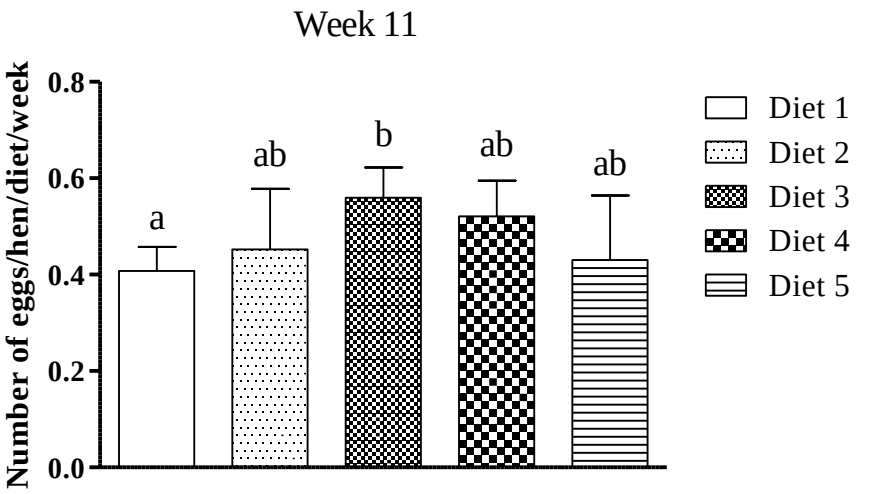

Week 9
Number of eggs/hen/diet/week
0.6
0.4
0.2
0.0
ns
Diet 3
Diet 4
Diet 5

A.

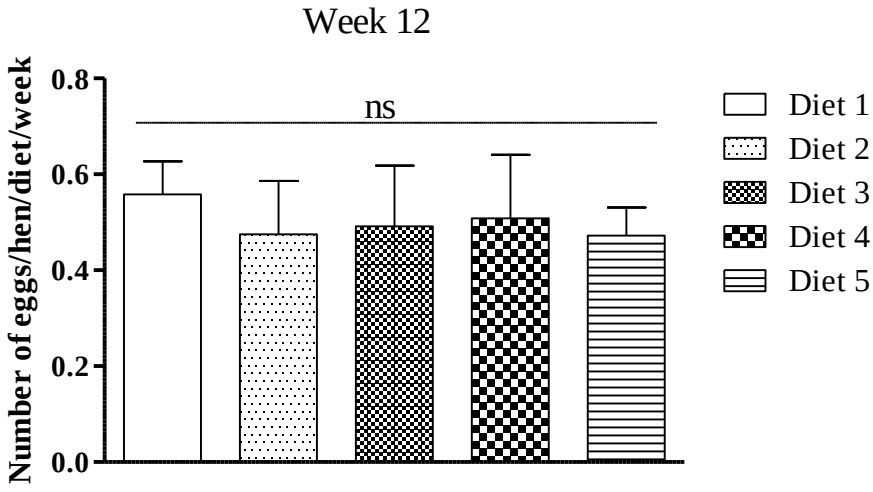

Week 10
Number of eggs/hen/diet/week
0.8
0.6
0.4
0.2
0.0
a
ab
ab
b
ab
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

B.

C.


Week 11
Number of eggs/hen/diet/week
0.8
0.6
0.4
0.2
0.0
a
ab
b
ab
ab
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

D.


Week 12
Number of eggs/hen/diet/week
0.8
0.6
0.4
0.2
0.0
ns
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

E.

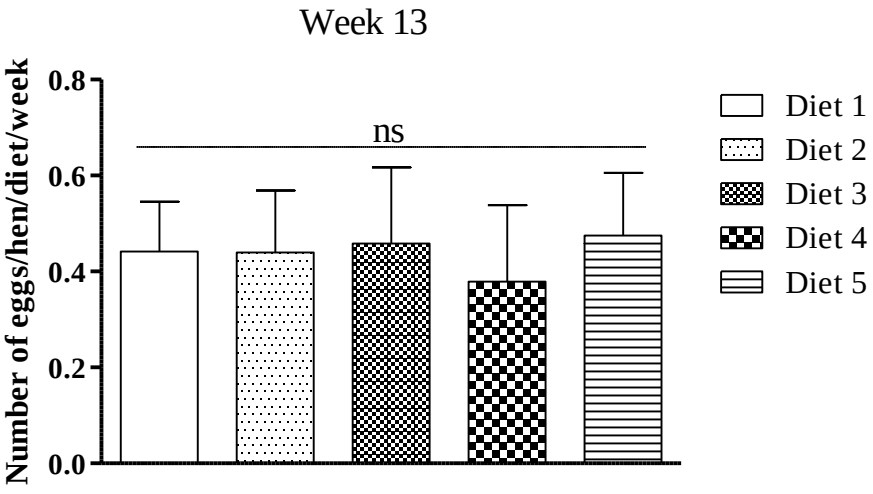
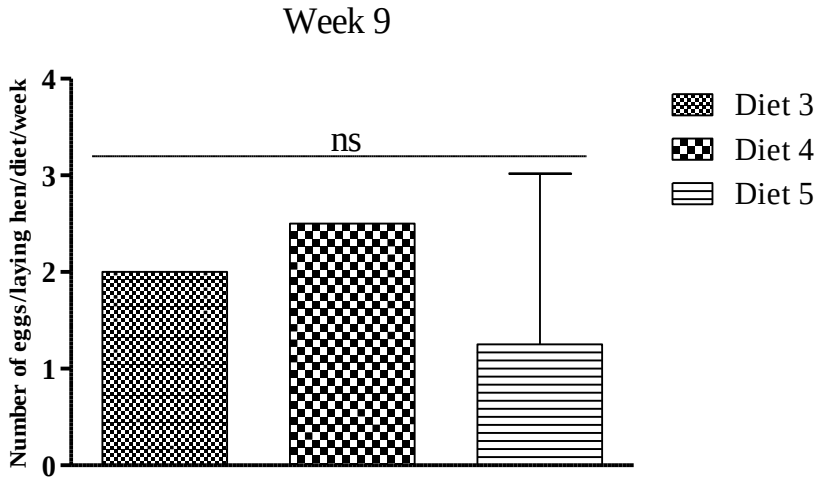

Week 13
Number of eggs/hen/diet/week
0.8
0.6
0.4
0.2
0.0
ns
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

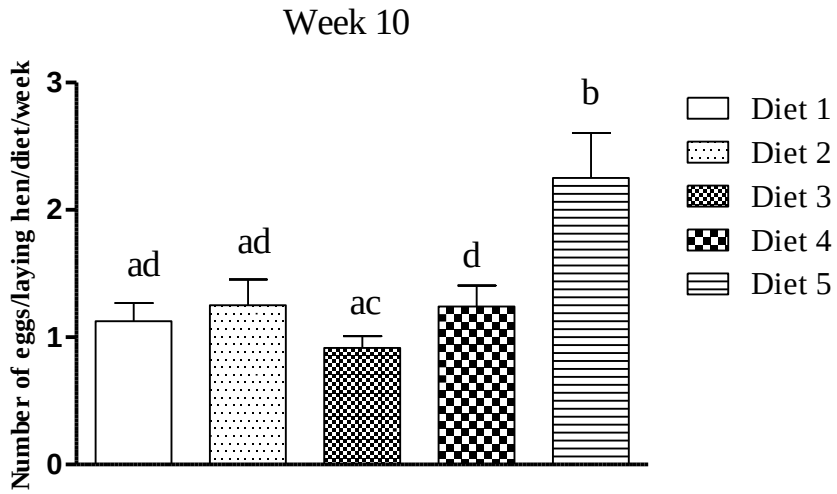
Figure 5.3: Effect of graded dietary substitution of soybean meal with Marula nut meal on the number of eggs produced by quail hens per dietary treatment per week per diet per week (A, B, C, D and E).

There was no significant difference (P >0.05) in the number of eggs per diet per week at week 9 (Figure 5.2 A). The number of eggs from birds fed diet 4 was significantly higher (P <0.05) from those fed diet 1 at week 10 (Figure 5.2 B). Birds fed diet 3 had a significantly higher (P <0.05) number of eggs per diet per week compared to those fed diet 1 at the age of 11 weeks (Figure 5.2 C). There were no significant differences (P >0.05) in the number of eggs per diet per week across dietary treatment at weeks 12 and 13. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD. n = 12 birds.

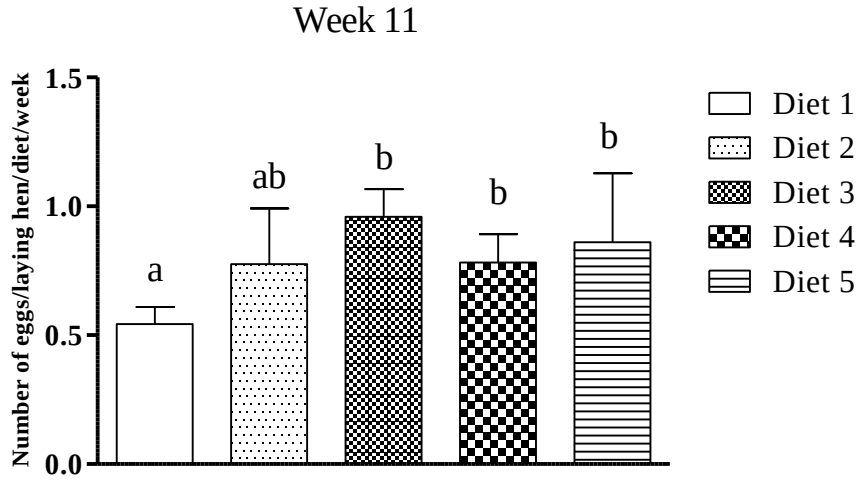
The effect of graded dietary substitution of SBM with MNM on the number of eggs produced by the total number (by the end of the study) of laying quail hens per dietary treatment per week is presented in figure 5.4. At week 10 the number of eggs laid by quail hens fed diet 5 was higher ($P < 0.0001$) than from hens fed diets 1 through to 4. During the same week hens fed diet 4 had a higher ($P < 0.05$) number of eggs by total number of laying hens per diet compared to that from counterparts fed diet 3. Birds fed diets 3 and 4 had lower ($P < 0.0001$) number of eggs by total number of laying hens per diet compared to those fed diet 5. At week 11, birds fed diet 1 had a lower ($P < 0.0001$) number of eggs by total number of laying hen per diet compared to those fed diets 3, 4 and 5.


Week 9
Number of eggs/laying hen/diet/week
0
1
2
3
4
ns
Diet 3
Diet 4
Diet 5

A.

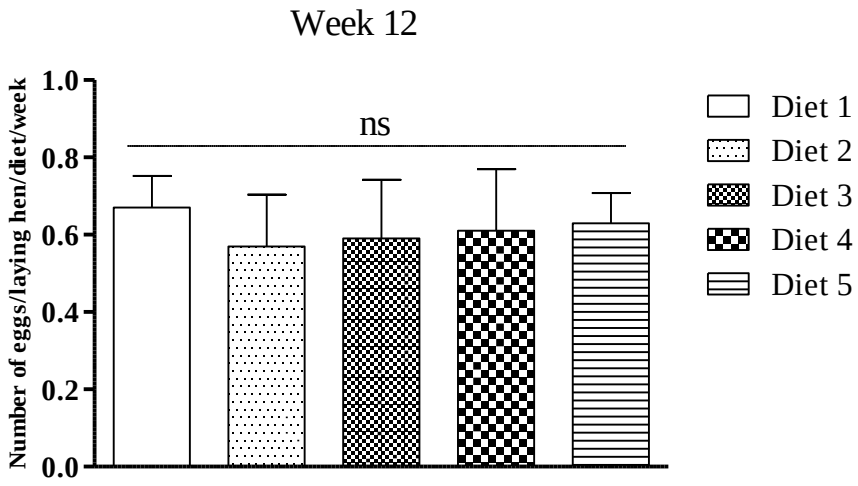

Week 10
Number of eggs/laying hen/diet/week
0
1
2
3
ad
ad
ac
d
b
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

B.


Week 11
Number of eggs/laying hen/diet/week
0.0
0.5
1.0
1.5
a
ab
b
b
b
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

C.

D.



E.

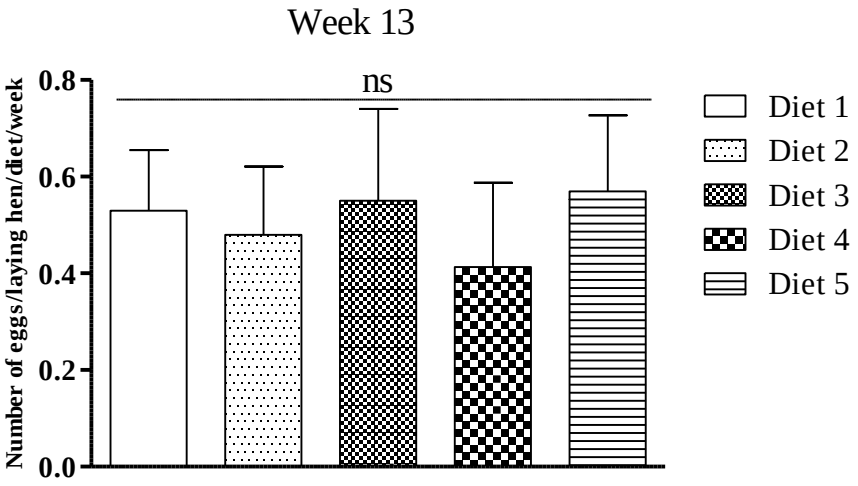
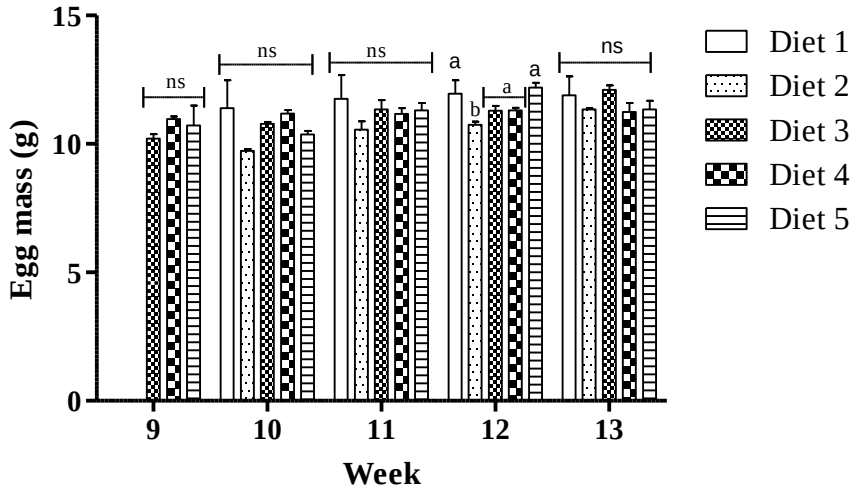


Figure 5.4: A, B, C, D and E: Effect of graded dietary substitution of soybean meal with Marula nut meal on the number of eggs per laying hen per dietary treatment per week.

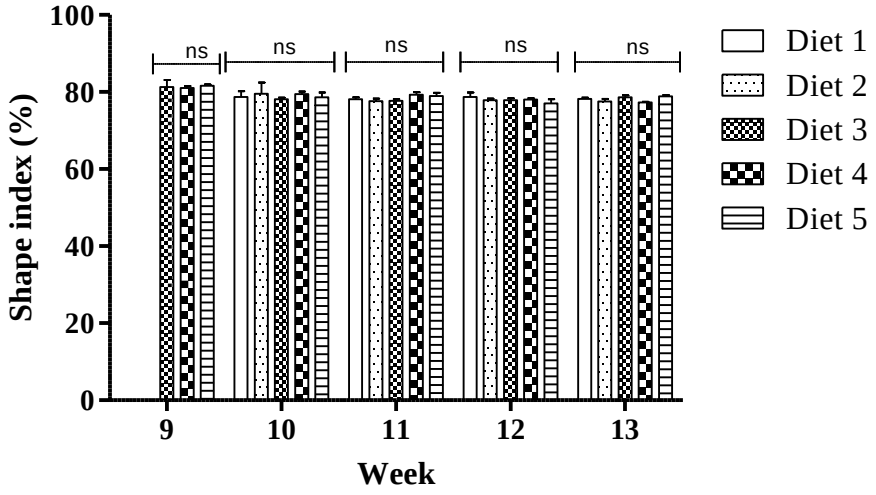
At weeks 9, 12 and 13, the number of eggs per laying hen per week per diet was similar (P >0.05). In week 10, birds fed diets 1 and 2 had significantly lower (P <0.0001) number of eggs per laying hen per diet compared to those fed diet 5. Birds fed diet 2, 4 and 5 had a significantly lower (P <0.0001) number of eggs per laying hen per diet (Figure 5.3 B). Birds fed diet 1 had a significantly lower (P <0.0001) number of eggs per laying hen compared to those fed diet 3, 4 and 5 (Figure 5.3 C). Hens fed diet 4 had a significantly higher (P <0.05) number of eggs per laying hen compared to that of counterparts fed diet 3 (Figure 5.3 B). There were no significant differences number of eggs per laying hen per diet at both week 12 and 13. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis ; Data presented as mean ± SD, week 9: n = 2 birds; week 10 (diet 1:n = 4, diet 2:n = 4, diet 3:n = 6, diet 4:n = 5 and diet 5:n = 2);week 11 (diet 1:n = 9, diet 2:n = 7, diet 3:n = 7, diet 4:n = 8 and diet 5:n = 6); week 12 (diet 1:n = 10, diet 2:n = 10, diet 3:n = 10, diet 4:n = 10 and diet 5:n = 9); week 13 (diet 1:n = 10, diet 2:n = 11, diet 3:n = 10, diet 4:n = 11 and diet 5:n = 10).

5.4.3 Effect of dietary marula nut meal on egg quality

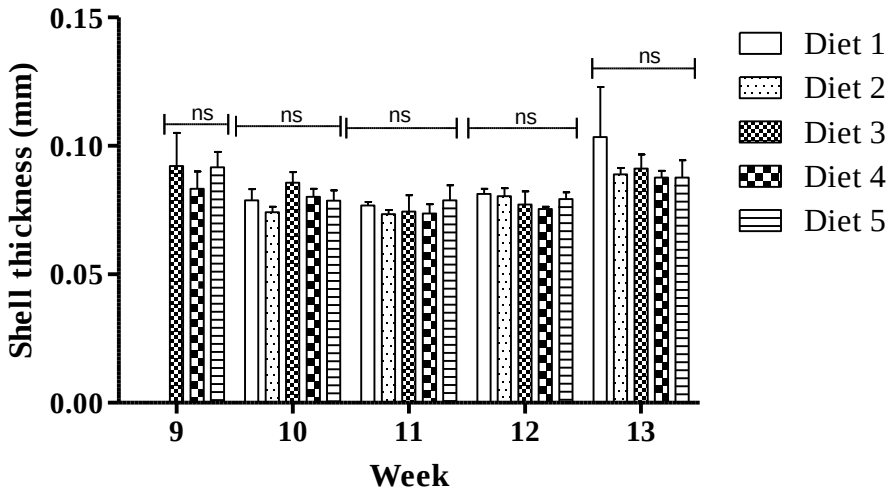
Figure 5.5 A, B and C show mean weekly egg mass, shape index and eggshell thickness, respectively from pullet quail fed graded levels of dietary MNM. The egg mass of the laying birds was similar ($P < 0.05$) at week 9, 10 and 11 birds. At week 12, hens fed diet 2 produced lighter ($P > 0.05$) eggs than eggs from hens fed diets 1, 3, 4 and 5. The eggs' shape index was similar ($P < 0.05$) across dietary treatments throughout the trial period. The eggshell thickness from the hens was similar throughout the experimental period.


15
10
5
0
Egg mass (g)
ns
ns
ns
a
b
a
a
ns
9
10
11
12
13
Week
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

A.


100
80
60
40
20
0
Shape index (%)
ns
ns
ns
ns
ns
9
10
11
12
13
Week
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

B.

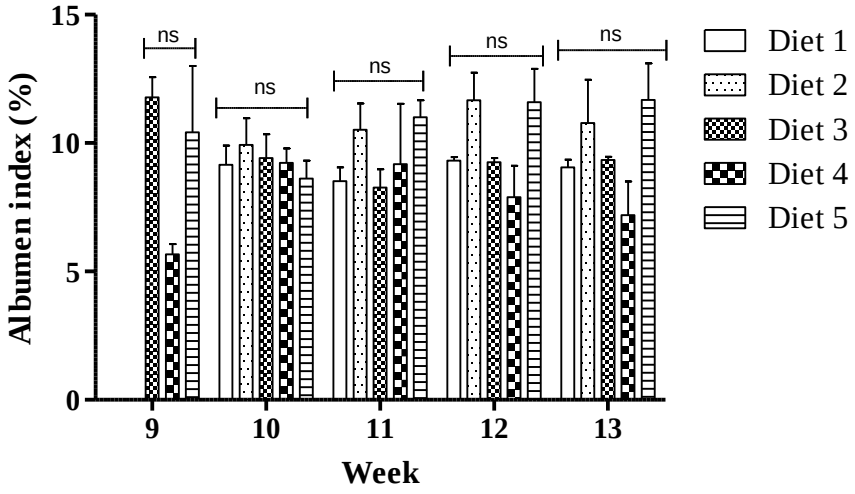


C.

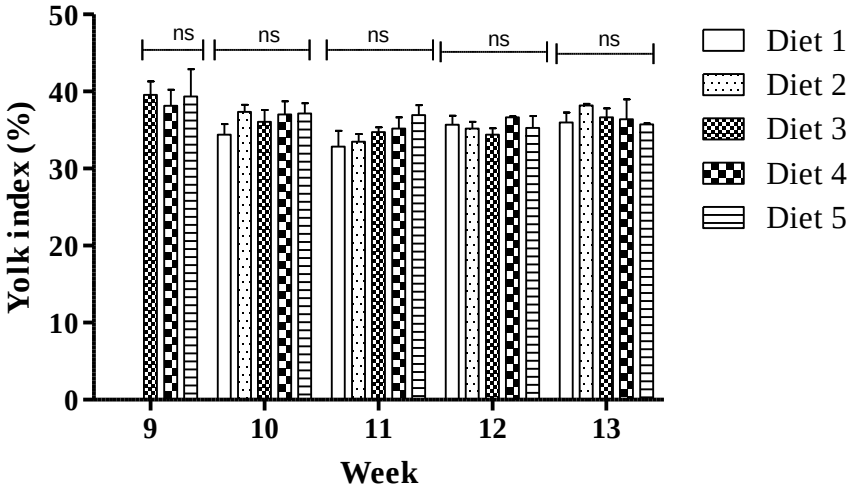
Figure 5. 5: Effect of graded dietary substitution of soybean meal with Marula nut meal on mean weekly egg mass (A), shape index (B) and eggshell thickness (C) of layer Japanese quail.

Figure A: Eggs produced by quail fed diets 1, 3, 4 and 5 were significantly heavier ($P < 0.05$) compared those from counterparts fed diet 2 (Figure 5.4 A).The egg shape index and eggshell thickness were similar ($P > 0.05$) across dietary treatments (Figure 5.4 B and C, respectively). Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD. n = 12 eggs.

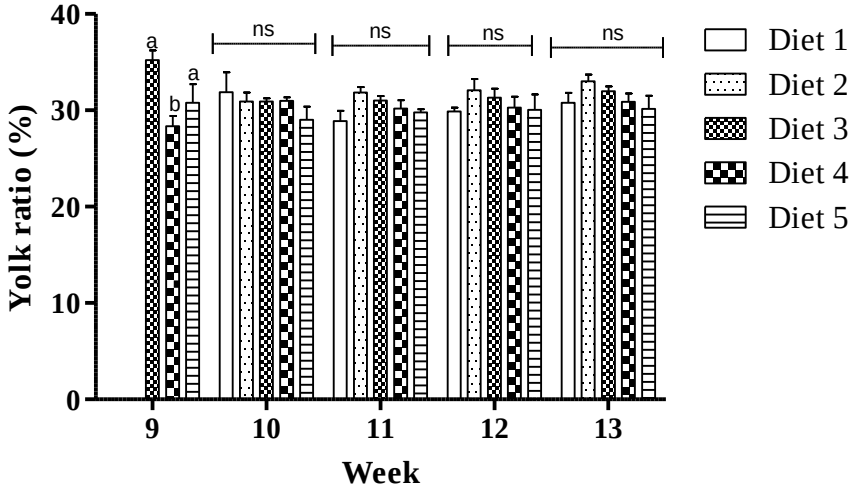
Figure 5.6 shows mean weekly albumen index, yolk index (%), yolk ratio, Haugh unit (%) and yolk/albumen ratio of from pullet quail fed graded levels of dietary MNM. The albumen index, yolk index, yolk ratio, Haugh unit and yolk/albumen ratio were similar ($P > 0.05$) across dietary treatments throughout the experimental period.


Albumen index (%)
15
10
5
0
ns
ns
ns
ns
ns
9
10
11
12
13
Week
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

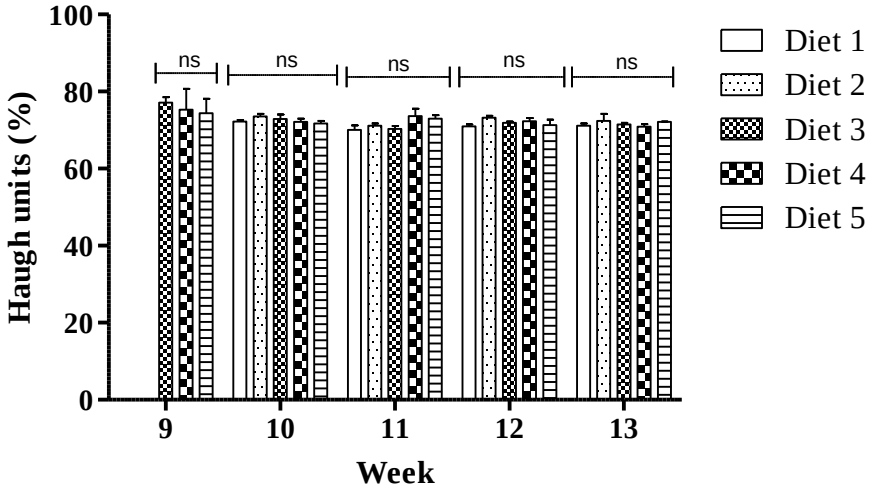
A.


Yolk index (%)
50
40
30
20
10
0
ns
ns
ns
ns
ns
9
10
11
12
13
Week
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

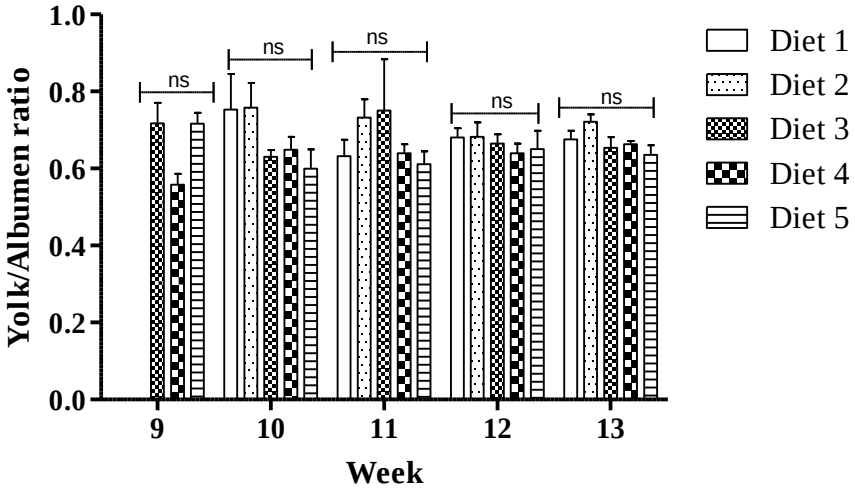
B.


Yolk ratio (%)
40
30
20
10
0
a
b
a
ns
ns
ns
ns
9
10
11
12
13
Week
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

C.


Haugh units (%)
100
80
60
40
20
0
ns
ns
ns
ns
ns
9
10
11
12
13
Week
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

D.


Yolk/Albumen ratio
1.0
0.8
0.6
0.4
0.2
0.0
ns
ns
ns
ns
ns
9
10
11
12
13
Week
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5

E.

Figure 5. 6: Effect of graded dietary substitution of soybean meal with Marula nut meal on mean weekly albumen index (A), yolk index (B), yolk ratio (C), Haugh unit (D) and yolk/albumen ratio (E) of layer Japanese quail.

There were no significant differences (P >0.05) in the albumen index, yolk index, yolk ratio, Haugh unit and yolk/albumen ratio of the eggs across dietary treatments throughout the experimental period (Figure A, B, C, D and E, respectively). Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD. n = 12 eggs.

The effect of graded dietary substitution of SBM with MNM on the overall egg quality parameters of layer Japanese quail is presented in Table 5.3. The overall egg albumen index from quail hens fed diets 2 and 5 were higher (P >0.05) compared to that of hens fed diet 4 while the overall yolk ratio of eggs from hens fed diet 1 was lower (P >0.05) compared to that of hens fed diet 3.

Table 5. 3: Effect of graded dietary substitution of soybean meal with Marula nut meal on the overall egg quality parameters of layer Japanese quail

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Exterior egg quality						
Egg mass (g)	11.29 ± 0.34^{a}	10.85 ± 0.19^{a}	11.45 ± 0.29^{a}	10.93 ± 0.29^{a}	11.32±0.64^{a}	ns
Shape index (%)	78.01 ± 0.65^{a}	78.47 ± 0.67^{a}	78.50 ± 0.58^{a}	79.13 ± 1.04^{a}	77.08±1.70^{a}	ns
Shell thickness (mm)	0.09 ± 0.00^{a}	0.08 ± 0.00^{a}	0.08 ± 0.00^{a}	0.08 ± 0.00^{a}	0.08±0.00^{a}	ns
Interior egg quality						
Albumen index (%)	9.31 ± 0.27^{ab}	12.24 ± 1.39^{a}	9.39 ± 0.39^{ab}	7.49 ± 0.70^{b}	11.72 ± 0.62^{a}	*
Yolk index (%)	35.97 ± 0.81^{a}	37.59 ± 0.87^{a}	35.13 ± 0.83^{a}	35.56 ± 1.25^{a}	35.12 ± 0.98^{a}	ns
Yolk ratio (%)	29.80 ± 0.40^{a}	32.34 ± 0.70^{ab}	32.91 ± 0.95^{b}	30.38 ± 0.72^{ab}	29.58 ± 0.83^{a}	*
Yolk/albumen ratio	0.66 ± 0.01^{a}	0.71 ± 0.02^{a}	0.69 ± 0.02^{a}	0.66 ± 0.02^{a}	0.63 ± 0.02^{a}	ns
Haugh unit (%)	71.56 ± 0.31^{a}	72.87 ± 0.73^{a}	71.92 ± 0.40^{a}	72.00 ± 0.59^{a}	72.34 ± 0.69^{a}	ns

ns = not significant, P >0.05, * P <0.05; ^{ab} Within row means with different superscripts are significantly different at P <0.05.The albumen index of eggs from quail hens fed diet 4 was significantly lower (P <0.05) than that of eggs from quail hens fed diets 2 and 5. The yolk ratio from hens fed diets diet 3 was significantly higher (P <0.05) than that from counterparts fed diets 1 and 5. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD. n = 12 eggs.

The effect of graded dietary substitution of SBM with MNM on Japanese quail egg yolk fat and cholesterol is presented in Table 5.4. The egg yolk from quail hens fed diets 2 and 3, respectively had a higher ($P < 0.0001$) fat content compared to that of egg yolks from hens fed diets 1 and 5. Quail hens fed diets 2 and 3 produced egg yolks with a higher ($P < 0.01$) cholesterol content compared to that of eggs from their counterparts fed diets 1, 4 and 5.

Table 5. 4:Effect of graded dietary substitution of soybean meal with Marula nut meal on the Japanese quail egg yolk fat and cholesterol content

Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
Fat (%)	54.75 ± 0.19^{a}	55.70 ± 0.25^{b}	55.45 ± 0.27^{b}	55.25 ± 0.02^{ab}	54.49 ± 0.33^{ac}	***
Cholesterol (mg/100g)	2044 ± 177.60^{a}	3077 ± 493.70^{b}	2402 ± 161.90^{ab}	2316 ± 188.90^{a}	2104 ± 174.50^{a}	**

** P <0.01, *** P <0.0001; [abc] Within row means with different superscripts are significantly different at P <0.05. The egg yolk from birds fed diets 2 and 3 had significantly different fat content compared to those fed diets 1 and 5 (P <0.0001). The cholesterol content of the egg yolks from birds fed diets 2 and 3 was significantly higher compared to that of eggs from hens fed diets 1, 4 and 5 (P <0.01). Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD; n = 12 eggs.

The effect of graded dietary substitution of soyabean meal with Marula nut meal on the fatty acid profile of Japanese quail egg yolks is presented in table 5.5. The TSFA of the egg yolks from eggs laid by Japanese quail were similar ($P > 0.05$) across dietary treatments. The TMUFA percentage of the egg yolks of eggs laid by quail fed diets 1, 2, 3, 4 and 5 were significantly different ($P < 0.0001$) across dietary treatments with egg yolks from eggs laid by quail hens fed diet 1 having the lowest and those from quail hens fed diet 5 having the highest percentage. The TPUFA percentage of the egg yolks of eggs laid by quail hens fed diet 4 was similar ($P > 0.05$) compared to those fed diet 5 but lower ($P < 0.0001$) in comparison to those fed diets 1, 2 and 3.

Table 5. 5: Effect of graded dietary substitution of soyabean meal with Marula nut meal on the fatty acid profile of Japanese quail egg yolks

Fatty acid (%)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
Saturated						
C14:0 (myristic acid)	0.44 ± 0.01^{a}	0.47 ± 0.01^{bc}	0.46 ± 0.01^{b}	0.46 ± 0.00^{b}	0.49 ± 0.01^{c}	***
C16:0 (palmitic acid)	27.84 ± 0.02^{a}	27.62 ± 0.12^{ab}	27.47 ± 0.06^{b}	27.39 ± 0.08^{b}	26.17 ± 0.22^{c}	***
C17:0 (margaric acid)	0.11 ± 0.00^{a}	0.11 ± 0.00^{a}	0.10 ± 0.01^{a}	0.11 ± 0.00^{a}	0.11 ± 0.00^{a}	ns
C18:0 (stearic acid)	9.20 ± 0.17^{a}	8.55 ± 0.10^{b}	8.55 ± 0.08^{b}	8.39 ± 0.09^{b}	8.62 ± 0.16^{b}	***
C20:0 (arachidic acid)	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.02 ± 0.01^{a}	0.03 ± 0.00^{a}	ns
C21:0 (henicosanoic acid)	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	ns
C22:0 (docosanoic acid)	0.12 ± 0.01^{a}	0.16 ± 0.01^{a}	0.17 ± 0.00^{a}	0.13 ± 0.03^{a}	0.17 ± 0.01^{a}	ns
C23:0 (tritricosanoin acid)	1.81 ± 0.04^{a}	1.79 ± 0.03^{ab}	1.86 ± 0.02^{b}	1.69 ± 0.01^{a}	1.88 ± 0.02^{b}	*
TSFA	**39.57 ± 0.26^{a}**	**38.75 ± 0.27^{a}**	**38.66 ± 0.18^{a}**	**38.21 ± 0.22^{a}**	**37.49 ± 0.42^{a}**	**ns**

Monounsaturated						
C14:1 (myristoleic acid)	0.08 ± 0.00^{a}	0.01 ± 0.00^{b}	0.09 ± 0.00^{c}	0.09 ± 0.01^{bc}	0.08^{a} ± 0.00	***
C16:1 (palmitoleic acid)	4.71 ± 0.08^{a}	5.55 ± 0.11^{b}	5.27 ± 0.04^{c}	5.15 ± 0.04^{c}	4.65 ± 0.04^{a}	***
C18:1n9c (oleic acid)	43.67 ± 0.22^{a}	44.79 ± 0.05^{b}	45.57 ± 0.08^{c}	45.58 ± 0.15^{c}	47.81 ± 0.47^{d}	*
C18:1n9t (elaidic acid)	0.19 ± 0.00^{a}	0.18 ± 0.00^{b}	0.19 ± 0.00^{a}	0.18 ± 0.01^{ab}	0.17 ± 0.01^{c}	**
C20:1 (eicosenoic acid)	0.11 ± 0.00^{a}	0.12 ± 0.01^{a}	0.13 ± 0.00^{b}	0.11 ± 0.00^{a}	0.12 ± 0.00^{a}	***
C24:1 (sphingomyelin acid)	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.03 ± 0.00^{a}	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	ns
TMUFA	**48.79 ± 0.31^{a}**	**50.67 ± 0.17^{b}**	**51.28 ± 0.12^{c}**	**51.14 ± 0.22^{c}**	**52.86 ± 0.52^{d}**	*******
Polyunsaturated						
C20:2 (eicosadienoic acid)	0.09 ± 0.00^{a}	0.08 ± 0.00^{bc}	0.08 ± 0.01^{ac}	0.08 ± 0.01^{bc}	0.08 ± 0.00^{bc}	**
C18:2n6c (linoleic acid)	8.46 ± 0.08^{a}	7.31 ± 0.15^{b}	7.06 ± 0.04^{b}	6.11 ± 0.05^{c}	6.14 ± 0.15^{c}	***
C18:3n3 (α-linolenic acid)	0.22 ± 0.01^{a}	0.15 ± 0.00^{b}	0.13 ± 0.01^{c}	0.08 ± 0.00^{d}	0.05 ± 0.00^{e}	***
C18:3n6 (γ-linolenic acid)	0.13 ± 0.01^{a}	0.14 ± 0.01^{a}	0.13 ± 0.00^{a}	0.11 ± 0.01^{b}	0.12 ± 0.00^{b}	**

C20:3n6 (eicosatrienoic acid)	0.12 ± 0.01[a]	0.12 ± 0.01[a]	0.12 ± 0.01[a]	0.07 ± 0.04[a]	0.27 ± 0.02[b]	***
C22:6n3 (docosahexaenoic acid)	0.56 ± 0.02[a]	0.47 ± 0.01[b]	0.43 ± 0.01[b]	0.37 ± 0.01[c]	0.27 ± 0.02[d]	ns
TPUFA	**9.58 ± 0.13[a]**	**8.27 ± 0.18[b]**	**7.95 ± 0.08[c]**	**6.82 ± 0.12[d]**	**6.93 ± 0.19[d]**	*******

ns = not significant, P >0.05, *P <0.05, ** P <0.01, *** P <0.0001; [ab] Within row means with different superscripts are significantly different at P <0.05. TSFA = total saturated fatty acids, TMUFA = total monounsaturated fatty acids, TPUFA = total poly-unsaturated fatty acids. The TSFA of the egg yolks from eggs laid by Japanese quail were not significantly different across dietary treatments. The myristic acid percentage of the egg yolks of eggs laid by quail fed diet 1 were significantly different (P <0.0001) from those fed diets 2, 3, 4 and 5. The palmitoleic acid percentage of the egg yolks of eggs laid by quail fed diet 1 were significantly different (P <0.0001) from those fed diets 2, 3, 4 and 5. The stearic acid percentage of the egg yolks of eggs laid by quail fed diet 1 were significantly different (P <0.0001) from those fed diets 2, 3, 4 and 5. The tritricosanoin acid percentage of the egg yolks of eggs laid by quail fed diet 1 and 4 were significantly different (P <0.05) from those fed diets 2, 3 and 5. The margaric acid, arachidic acid, henicosanoic acid and docosanoic acid percentage of the egg yolks from eggs laid by Japanese quail were not significantly different across dietary treatments. The TMUFA percentage of the egg yolks of eggs laid by quail fed diets 1, 2, 3, 4 and 5 were significantly different (P <0.0001) across dietary treatments. The myristoleic acid percentage of the egg yolks of eggs laid by quail fed diet 1 and 5 were significantly different (P <0.05) from those fed diets 2, 3 and 4. The palmitoleic acid percentage of the egg yolks of eggs laid by quail fed diet 1 and 5 were significantly different (P <0.05) from those fed diets 2, 3 and 4. The oleic acid percentage of the egg yolks of eggs laid by quail fed diets 1, 2, 3, 4 and 5 were significantly different (P <0.05) across dietary treatments. The elaidic acid percentage of the egg yolks of eggs laid by quail fed diets 1 and 3 were significantly different (P <0.05) from those fed diets 2, 4 and 5. The eicosenoic acid percentage of the egg yolks of eggs laid by quail fed diet 3 was significantly different (P <0.01) from those fed diets 1, 2, 4 and 5. The eicosadienoic acid percentage of the egg yolks of eggs laid by quail fed diet 1 was similar (P >0.05) to those fed diet 3 but significantly different (P <0.0001) from those fed diets 2, 4 and 5. The α-linolenic acid percentage of the egg yolks of eggs laid by quail fed diets 1, 2, 3, 4 and 5 were significantly different (P <0.0001) across dietary treatments. The γ-linolenic acid percentage of the egg yolks of eggs laid by quail fed diets 4 and 5 were significantly different (P <0.01) from those fed diets 1, 2 and 3. The eicosatrienoic acid percentage of the egg yolks of eggs laid by quail fed diet 5 was significantly different (P <0.0001) from those fed diets 1, 2, 3 and 4. The TPUFA percentage of the egg yolks of eggs laid by quail fed diet 4 was similar (P >0.05) to those fed diet 5 but

significantly different (P <0.0001) from those fed diets 1, 2 and 3. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 12 egg yolks.

5.4.4 Effect of dietary Marula nut meal on hepatic lipid content

The effect of graded dietary substitution of soybean meal with MNM on the liver lipid content of layer Japanese quail is presented in figure 5.7. The liver lipid content of the laying Japanese quail hens was similar (P >0.05) across dietary treatments.

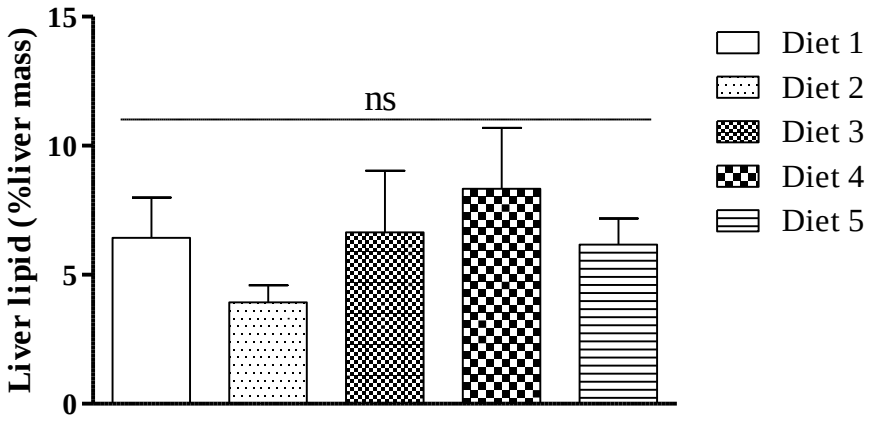


Figure 5.7: Effect of dietary Marula nut meal on the liver lipid content of Japanese quail hens.

There were no significant differences (P >0.05) in the liver lipid content of the layer Japanese quail hens across the dietary treatments. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD, n = 12 birds.

5.4.5 Effect of dietary Marula nut meal on plasma metabolites

The effect of dietary MNM on the plasma calcium, phosphate and uric acid of laying Japanese quail hens is presented in Table 5.6. Hens fed diet 4 had higher ($P < 0.05$) plasma phosphorus concentration compared to that of hens fed diet 1.

Table 5. 6: Effect graded dietary substitution of soybean meal with Marula nut meal on plasma calcium, phosphorus and uric acid concentration of the layer Japanese quail

Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
Calcium (mmol/L)	3.76 ± 0.24^{a}	4.00 ± 0.00^{a}	3.74 ± 0.24^{a}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}	ns
Phosphate (mmol/L)	2.23 ± 0.71^{a}	4.29 ± 0.94^{ab}	3.68 ± 1.80^{ab}	4.66 ± 1.07^{b}	3.14 ± 1.65^{ab}	*
Uric acid (µmol/L)	440.20 ± 46.15^{a}	502.60 ± 80.72^{a}	418.30 ± 59.69^{a}	405.50 ± 52.92^{a}	355.70 ± 27.41^{a}	ns

ns = not significant, P >0.05, * P <0.05; ^{ab} Within row means with different superscripts are significantly different at P <0.05. The plasma phosphate concentration of quail hens fed diet 4 was significantly higher (P <0.05) compared to that of hens fed diet 1. The plasma calcium and uric acid concentration were similar across dietary treatments. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein (CP) basis, Diet 3 – 50% MNM inclusion on CP basis, Diet 4 – 75% MNM inclusion on CP basis, Diet 5-100% MNM inclusion on CP basis; Data presented as mean ± SD; n = 12 birds.

Effect of graded dietary substitution of soybean meal with Marula nut meal on the mass, length and mass to length ratio of tibiae from layer Japanese quail is presented in Table 5.7. The tibia mass, length and mass to length ratio of tibiae from the quail hens were similar across dietary treatments ($P > 0.05$).

Table 5. 7: Effect of graded dietary substitution of soybean meal with MNM on the mass, length and density of the tibiae from layer Japanese quail

Parameter	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Significance level**
Mass (g)	5.19 ± 0.20[a]	5.04 ± 0.18[a]	5.57 ± 0.25[a]	5.18 ± 0.22[a]	5.08 ± 0.23[a]	ns
Length (mm)	25.55 ± 0.37[a]	25.24 ± 0.23[a]	25.68 ± 0.57[a]	25.75 ± 0.31[a]	25.47 ± 0.32[a]	ns
Density (g/mm)	0.20 ± 0.01[a]	0.20 ± 0.01[a]	0.22 ± 0.01[a]	0.20 ± 0.01[a]	0.20 ± 0.01[a]	ns

ns = not significant, P >0.05; There were no significant differences in the tibia mass, length and density (seedor index) of quail birds across dietary treatments. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 12 birds.

5.4.6 Effect of dietary Marula nut meal on viscera

The effect of graded dietary substitution of soybean meal with MNM on viscera macro-morphometry of the layer Japanese quail is presented in Table 5.8. The mass and length (where relevant) of the hens' GIT viscera (proventriculus, ventriculus, small intestine, caecum, large intestines) and that of their visceral fat, pancreata, ovaries and fallopian tubes, were similar ($P < 0.05$) across dietary treatments. However, the livers from hens fed diet 5 were heavier ($P < 0.0001$) than those from hens fed diets 1 through to 4.

Table 5. 8 Effect of graded dietary substitution of soybean meal with MNM on viscera macro-morphometry of the layer Japanese quail

Organ	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Significance level
Proventriculus						
Absolute (g)	0.94 ± 0.05[a]	0.99 ± 0.03[a]	1.04 ± 0.08[a]	0.93 ± 0.04[a]	0.85 ± 0.04[a]	ns
Relative to body mass (%)	0.42 ± 0.02[a]	0.46 ± 0.02[a]	0.44 ± 0.03[a]	0.42 ± 0.01[a]	0.40 ± 0.03[a]	ns
Relative to tibia length (g/mm)	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.04 ± 0.00[a]	ns
Ventriculus						
Absolute (g)	3.87 ± 0.16[a]	3.62 ± 0.11[a]	3.82 ± 0.19[a]	3.52 ± 0.15[a]	3.96 ± 0.16[a]	ns
Relative to body mass (%)	1.73 ± 0.08[a]	1.67 ± 0.05[a]	1.62 ± 0.07[a]	1.59 ± 0.07[a]	1.86 ± 0.08[a]	ns
Relative to tibia length (g/mm)	0.15 ± 0.01[a]	0.14 ± 0.00[a]	0.15 ± 0.01[a]	0.14 ± 0.01[a]	0.15 ± 0.01[a]	ns
Small intestines						
Absolute (g)	5.61 ± 0.30[a]	5.91 ± 0.19[a]	5.75 ± 0.25[a]	5.39 ± 0.23[a]	5.46 ± 0.27[a]	ns
Relative to body mass (%)	2.49 ± 0.13[a]	2.72 ± 0.09[a]	2.43 ± 0.07[a]	2.42 ± 0.05[a]	2.57 ± 0.14[a]	ns
Relative to tibia length (g/mm)	0.22 ± 0.01[a]	0.23 ± 0.01[a]	0.22 ± 0.01[a]	0.21 ± 0.01[a]	0.21 ± 0.02[a]	ns
length (mm)	609.20 ± 21.79[a]	613.60 ± 17.76[a]	615.90 ± 8.230[a]	587.10 ± 29.26[a]	614.80 ± 21.75[a]	ns
Caecum						
Absolute (g)	0.86 ± 0.07[a]	0.86 ± 0.05[a]	1.02 ± 0.09[a]	0.72 ± 0.05[a]	0.87 ± 0.09[a]	ns
Relative to body mass (%)	0.38 ± 0.03[a]	0.39 ± 0.02[a]	0.43 ± 0.04[a]	0.32 ± 0.02[a]	0.40 ± 0.03[a]	ns
Relative to tibia length (g/mm)	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.00[a]	ns
Large intestines						
Absolute (g)	0.72 ± 0.05[a]	0.72 ± 0.06[a]	0.74 ± 0.04[a]	0.59 ± 0.03[a]	0.66 ± 0.06[a]	ns
Relative to body mass (%)	0.32 ± 0.02[a]	0.33 ± 0.03[a]	0.32 ± 0.01[a]	0.27 ± 0.01[a]	0.30 ± 0.02[a]	ns
Relative to tibia length (g/mm)	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.00[a]	ns

length (mm)	73.75 ± 3.59[a]	76.82 ± 3.65[a]	80.45 ± 2.57[a]	74.17 ± 2.44[a]	81.50 ± 4.17[a]	ns
Visceral fat						
Absolute (g)	1.59 ± 0.68[a]	1.05 ± 0.25[a]	1.79 ± 0.32[a]	1.11 ± 0.20[a]	1.49 ± 0.52[a]	ns
Relative to body mass (%)	0.69 ± 0.29[a]	0.48 ± 0.12[a]	0.74 ± 0.12[a]	0.48 ± 0.08[a]	0.67 ± 0.23[a]	ns
Relative to tibia length (g/mm)	0.06 ± 0.03[a]	0.04 ± 0.01[a]	0.07 ± 0.01[a]	0.04 ± 0.01[a]	0.06 ± 0.02[a]	ns
Liver						
Absolute (g)	6.01 ± 0.29[a]	5.41 ± 0.22[a]	5.94 ± 0.22[a]	6.50 ± 0.29[ab]	7.63 ± 0.38[b]	***
Relative to body mass (%)	2.66 ± 0.11[a]	2.48 ± 0.09[a]	2.53 ± 0.07[a]	2.94 ± 0.13[ab]	3.56 ± 0.16[b]	***
Relative to tibia length (g/mm)	0.23 ± 0.01[a]	0.21 ± 0.01[a]	0.23 ± 0.01[a]	0.25 ± 0.01[ab]	0.29 ± 0.02[b]	***
Pancreas						
Absolute (g)	0.54 ± 0.03[a]	0.59 ± 0.32[a]	0.58 ± 0.04[a]	0.54 ± 0.03[a]	0.51 ± 0.02[a]	ns
Relative to body mass (%)	0.24 ± 0.01[a]	0.02 ± 0.09[a]	0.25 ± 0.02[a]	0.24 ± 0.01[a]	0.24 ± 0.01[a]	ns
Relative to tibia length (g/mm)	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	ns
Ovaries						
Absolute (g)	5.88 ± 0.77[a]	6.16 ± 0.41[a]	7.11 ± 0.77[a]	6.56 ± 0.42[a]	6.14 ± 0.60[a]	ns
Relative to body mass (%)	2.63 ± 0.33[a]	2.82 ± 0.18[a]	2.96±0.28[a]	2.95±0.18[a]	2.84±0.26[a]	ns
Relative to tibia length (g/mm)	0.23 ± 0.02[a]	0.24 ± 0.02[a]	0.27±0.03[a]	0.25±0.01[a]	0.24±0.03[a]	ns
Fallopian tube						
Absolute (g)	6.99 ± 0.54[a]	6.72 ± 0.29[a]	8.14 ± 1.17[a]	6.32 ± 0.62[a]	7.43 ± 0.71[a]	ns
Relative to body mass (%)	3.11 ± 0.23[a]	3.09 ± 0.14[a]	3.32 ± 0.34[a]	2.83 ± 0.28[a]	3.39 ± 0.22[a]	ns
Relative to tibia length (g/mm)	0.27 ± 0.02[a]	0.27 ± 0.01[a]	0.31 ± 0.05[a]	0.24 ± 0.02[a]	0.27 ± 0.03[a]	ns
Length (mm)	375.00 ± 21.55[a]	379.10 ± 13.95[a]	415.90 ± 34.74[a]	456.70 ± 30.41[a]	416.30 ± 23.38[a]	ns

ns = not significant, P >0.05, *** P <0.0001; [ab] Within row means with different superscripts are significantly different at P <0.05. No significant differences (P >0.05) in the masses of the proventriculus, ventriculus, small intestine, caecum, large intestines, visceral fat, pancreas, ovaries and fallopian tube of the quail across dietary treatments.

Livers from quail hens fed diet 5 were heavier (P <0.0001) than those from counterparts fed diets 1 through to 4. Diet 1 – 0% inclusion of MNM (control), Diet 2 – 25% MNM inclusion on crude protein basis, Diet 3 – 50% MNM inclusion on crude protein basis, Diet 4 – 75% MNM inclusion on crude protein basis, Diet 5-100% MNM inclusion on crude protein basis; Data presented as mean ± SD; n = 12 birds.

5.5 Discussion

The discourse under this subheading discuss the findings of the effects of dietary Marula nut meal on percent lay, egg production, egg quality, quail hen health, growth performance and feed economy of pullet quail.

5.5.1 Effect on growth and feed economy

In the current study, MNM did not affect terminal body mass (TBM) BMG, ADG, FI and FCR and the hens grew significantly during the course of the trial (Table 5.2). Additionally, it did not affect the mass, length and bone mass to length ratio of the tibiae of the Japanese quail hens (Table 5.6). These findings suggest that dietary hexane-extracted MNM at all levels of inclusion did not compromise growth performance, FI and feed utilisation efficiency. Hexane has been previously reported to remove toxic compounds and ANFs such as Gossypol and aflatoxin (Saetae and Suntornsuk, 2011; Saxena *et al.*, 2011; Panhwar, 2005). Anti-nutritional factors reduce nutrient digestion and absorption (Collett, 2012), which translates to compromised growth thus findings from the current study suggest that either the MNM had a low ANFs concentration or that the hexane extraction reduced the ANFs concentration to low levels that did not elicit toxicity. Negative effects on growth performance may negatively affect egg production as there is a direct correlation between bird size and egg mass (Woyengo *et al.*, 2017). Laying quail hens weigh about 200 to 250g (Rahman *et al.*, 2016). The terminal body masses of the quail hence ranged from 215.80 ± 31.14 to 236.50 ± 32.20g (Table 5. 2) thus the terminal body weights of the hens were within the body weight range of laying quail as reported by Rahman *et al.* (2016) indicating that the MNM did not compromise their growth. It can also be speculated that hexane-mediated extraction of the MNM reduce its residual fat content to concentrations that did not elicit the development of rancidity when used in diet formulation thus the resultant diets did not develop off-flavours which are known to negatively impact feed intake. Additionally the similarities in the feed intake and feed utilisation efficiency in the quail across dietary treatments suggests that the MNM supplied nutrients to the same degree as SBM and the MNM-derived nutrients were equally and efficiently utilised as those supplied by SBM. These findings suggest that MNM could be used in pullet quail feeds without compromising the growth performance, feed intake and feed conversion efficiency.

5.5.2 Effect on egg production

In the current study, the quail, procured at 5-weeks of age, only started laying at 9-weeks of age with only hens fed diets 3, 4 and 5 coming into lay. Quail hens fed diets 1 and 2 only came into lay at 10-weeks of age (Figure 5.2). Japanese quail normally start laying at 5 to 6 weeks of age (Randon, 2013). The observed delay in laying in the current study could have been due to a number of factors including environmental temperature, photoperiod and stress.

The recommended temperature for laying quails is about 25 to 27 °C (Raharjo *et al.*, 2018), while temperatures exceeding 29 °C cause stress to the laying birds (Raharjo *et al.*, 2018), minimum temperatures above 23.8 °C may also lead to stress in Japanese quail (El-Tarabany, 2016). In the current study the room temperature in the animal unit is set at 23 ± 2 °C as per the South African national Standards recommendations for animal housing. Thus whilst the temperatures did not exceed 27 °C, the low ambient temperature may have impacted on the delayed laying of the birds.

Optimum temperatures have to be coupled to good lighting conditions as they have a huge impact on egg production. Adequate lighting programs are crucial for egg production, as the light stimulus directly influences the physiological responses of the bird (Nunes *et al.*, 2016). According to Freitas *et al.* (2005), light stimulates the release of reproductive hormones, may accelerate or delay sexual maturation, and stimulate egg laying. Guibert *et al.* (2010), Memon (2018) and Planck and Johnson (1975) reported range of 14 to16 h photo period during laying. The photoperiod used in the study was within that recommended (14 h) by Guibert *et al.* (2010); Memon (2018) and Planck and Johnson (1975). Thus the photoperiod was not likely to have had an impact on the start of lay. However, light intensity can also impact the start of lay (Etches, 1994). In the current study, due to technical limitations, we were unable to measure light intensity and therefore control for its possible impact on the study.

Stressors such as noise and transportation can affect laying performance (Campo *et al.*, 2005; Hamm, 1967). Whilst every effort was made to minimise the former during housing, the hens were transported from the Eastern Cape to Gauteng (1 h 25 m flight) and although given a 2-day habituation period before the commencement of the trial, it may have not been sufficient to counter the effect of travel stress. Duval *et al.* (2013) and Guibert *et al.* (2010) habituated laying quail for 14 and 25 days respectively, compared to that from the current study, the habituation period was shorter as there was need to urgently commence the feeding trial due to the age of the birds. Considering the possible stressors, if any were applicable to the current study, the impact

would have been on all the birds and hence changes noted would be attributable to the dietary interventions.

Importantly, laying performance has been reported to be affected by feed composition (Kaye *et al.*, 2017). Murakani *et al.* (1993) recommended 18% CP for laying Japanese quails while Pinto *et al.* (1998) recommended 22.42% CP. The NRC (1994) recommended levels 20% CP in laying Japanese quail, thus the observed staggered commencement in laying could also be ascribed to differences in the inclusion level of MNM in the diet. Findings from the current study suggest that at higher dietary inclusion levels, the hexane-extracted MNM stimulated laying at a younger age. It is important to note that the delay in commencement to laying, under practical farming conditions, would cost the producer, as it results in reduced egg production. However considering that diet 1 had no MNM inclusion, results of the current study suggest that dietary MNM inclusion can potentially be utilised, in place of SBM, to mitigate the effects of inadequate habituation of point of quail hens.

Birds fed diet 4 had a higher number of laying quail hen when compared to those fed diet 1 at week 10 and in week 11, birds fed diet 3 had a higher number of laying birds as compared to those fed diet 1. These findings suggest that the increasing dietary inclusion of MNM reduced the age of the onset of laying (in poorly habituated hens) as well as increased the number of hens in lay. It could therefore be speculated that dietary MNM can potentially mitigate stress induced-delay in laying and hence egg productivity. While laying percentage is important, it does not tell the full story regarding the number of eggs laid per diet thus there was a need to determine the total number of eggs laid per diet (Figure: 5.3). Hens fed diets 3 and 4 had a higher number of eggs compared to that from hens fed diet 1. These results suggest that higher dietary inclusion of the hexane-extracted MNM improved egg production by causing an increase in laying percentage and number eggs per diet up to 11 weeks of age.

In the current study, the quail hens reached peak laying at 11 weeks of age. Piao *et al.* (2004) reported that quails reach peak laying at 10 to 12 weeks of age thus peak lay age reported in the current study findings falls within the range reported in literature (Piao *et al.*, 2004) thus it can be inferred that hexane extracted MNM can be utilised as a dietary protein source without the risk of compromising Japanese quail peak lay age. Despite peak lay age in the current study being within the age range reported by Piao *et al.* (2004), it has to be emphasised that the observed delay to come into lay, which cannot be ascribed to dietary MNM, compromises overall egg production.

5.5.3 Effects on egg quality

5.5.3.1 External egg quality

External egg quality, typified by factors such as texture, colour, shape, size (mass), and cleanliness, is important as it is the main attraction of consumers at the point of purchase (Đukić Stojčić *et al.*, 2012). Importantly, the shell of each egg should be smooth, clean and free of cracks. Also, the eggs should be uniform in colour, size and shape (Moula *et al.*, 2013). Key egg quality parameters used in the poultry industry include egg mass (EM), shape index (SI) and shell thickness (ST). Eggshell quality, largely dependent on dietary calcium levels, is of utmost importance in egg production (Abdelqader, 2017). Kul and Seker (2004), Vali (2008) and Vali *et al.*(2009) reported that Japanese quail egg mass ranged from 7 to 11 g. In the current study, the mean egg mass ranged from 10.85 ± 0.19 to 11.45 ± 0.29g (Table 5.2) thus while their upper mass was within that reported in the literature, their lower mass was higher. These findings suggest dietary MNM improves the egg mass which is linked with high nutrient content. On average, an avian egg has 2.3g calcium in the shell, and approximately 25mg in the yolk (Etches, 1987). Throughout the experimental period and overall findings from the current study showed that dietary MNM had no effect (P >0.05) on egg weight, shape index and shell thickness. It can, therefore be inferred that MNM can be used to substitute SBM as a dietary protein source in pullet quail feeds without compromising the external egg quality of layer Japanese quail hens.

In this study, the shape index and shell thickness range from 78.01 ± 0.65 to 79.13 ± 1.04% and 0.08 to 0.09 mm respectively. These findings are generally within the 77.64 to 78.8% shape index range reported by Sari *et al.* (2012) and Zita *et al.* (2013). The shell thickness reported in this study is thinner compared to that reported in the literature. Yannakopoulos and Tserveni-Gousi, (1986), Orhan and Erensayın, (2001), Kul and Seker, (2004), Đukić Stojčić *et al.* (2012) and Zita *et al.* (2013) reported a shell thickness range of 0.186 to 0.223 mm in Japanese quail eggs. The lower eggshell thickness in the current study may have been affected by the several factors such as Ca deposition (Shaker *et al.*, 2019) and the oviposition time (Ketta and Tůamová, 2016). Furthermore, altitude may have been responsible for thinner eggshells, Hempleman *et al.*, (1993) reported thicker eggshells from eggs laid at lower altitudes (1200 m) compared to eggs laid at higher altitude (3800m). A change in altitude from 133 m above sea level in East London to 1753 m in Johannesburg may have affected the eggshell thickness. High altitudes result in hypoxia (low environmental oxygen) which results in increased breathing rate and minute volume but decreased tidal volume which may decrease plasma carbon dioxide. The large decrease in arterial carbon dioxide is associated decreases in bicarbonate (Brackenbury *et al.*,

1982). Eggshell quality is affected by the reduction of bicarbonate concentration in the lumen of the shell gland (Frank and Burger, 1965; Balnave *et al.*, 1989). The reduced bicarbonate concentration results in a reduction in ionized blood calcium thus reducing the amount of calcium available for eggshell formation (Poultryworld, 2015).

Regarding the external egg quality, compared to what has been reported in other studies, dietary MNM has no effect on the egg mass and shape index but may result in reduced shell thickness of Japanese quail eggs. The thinner shell thickness may result in easy breakage of the egg shell thus resulting in egg losses to the producer. However, there were no egg shell breaking or damage observed during the feeding trial but supplementation of Ca may be needed to improve the shell thickness.

5.5.3.2 Internal egg quality

The internal quality of an egg encompasses albumen and yolk quality (Duman *et al.*, 2016). The albumen consists of about 88% water, 11% protein, 1% carbohydrates, lipids and minerals (Abeyrathne *et al.*, 2013). The albumen proteins impart the functional and antimicrobial characteristics of the albumen (Kovacs-Nolan *et al.*, 2005). The egg yolk is a central part that lies within the albumen and generally yellow in colour but its pigmentation may vary depending on the feed type and composition (Mendes de Souza *et al.*, 2019). The yolk harbours most of the nutrients found in the egg, including lipids (34%), and proteins such as globular proteins (Juneja and Science, 1996). Antinutritional factors in some oilseed meals compromise nutrient digestion and absorption negatively impacting albumen and yolk quality (Khajali and Slominski, 2012). The findings from the study show that at week 9 eggs from birds fed diet 4 had a lower albumen index as compared to that of eggs from hens fed diets 3 and 5, respectively (Table 5.2). The overall albumen index of birds fed diet 2 and 5 was higher than those fed diet 4. These findings suggest the differences that occurred in week 9 may have an effect on the overall albumen index outcome. The egg albumen index is the measure of the concentration of nutrients available in the albumen of the egg (Heiman and Wilhelm, 1937). These however would need to be measured in the eggs, which were did not do due to technical limitations.

While the overall yolk ratio of eggs from birds fed diet 1 was lower as compared to those fed diet 3, the higher yolk ratio of eggs yolks from the birds fed diet 3 shows that they had a higher nutrient content compared to that of egg yolks from hens fed diet 1. All the other internal egg parameters were the same throughout the laying period. Therefore dietary MNM did not have adverse effects on both weekly and overall internal egg quality traits, hence the meal can be

utilised as a dietary protein source in Japanese quail pullet diets without adversely affecting the aforementioned parameters.

Findings from the current study showed overall albumen index, yolk index, yolk ratio, yolk: albumen ratio and Haugh unit ranges of 7.4 ± 0.70 to 12.24 ± 1.39%, 35.13 ± 0.83 to 35.97 ± 0.81%, 29.80 ± 0.40 to 32.91 ± 0.95%, 0.66 ± 0.01 to 0.71 ± 0.02% and 71.56 ± 0.31 to 72.89 ± 0.73%, respectively in Japanese quail eggs. Kul and Seker (2004) reported albumen index, yolk index, and yolk ratio at 9.37 ± 0.10%, 36.70 ± 0.28% and 32.1 ± 0.12%, respectively while Genchev (2012) and Zita *et al.* (2013) reported quail egg albumen index ranges of 10.5 to 12.33%). Thus, the albumen index, yolk index and yolk ratio values reported in the current study are within range of those reported by Kul and Seker (2004), Genchev (2012), Zita *et al.* (2013). It has been reported that the normal albumen index and yolk index range from 10 to 15% and 48 to 54%, respectively (Gonzalez, 1995; Waheda *et al.*, 1999). Therefore, the albumen index from the current study falls within the previously reported range but the yolk index falls below that range. Zita *et al.* (2013) reported a higher yolk index (47.66 ± 0.22%) and Haugh unit (90.88 ± 0.46%) while Alkan *et al.* (2010) reported higher yolk index and albumen index percentages at 43.28 ± 0.42 to 47.33 ± 0.34% and 9.33 ± 0.18 to 10.67 ± 0.25%, respectively. However, Haugh unit may not be a direct measure of egg quality as it decreases with age of the bird or rather not change at all (Nazligül *et al.*, 2001; Orhan and Erensayın, 2001). According to Adeogun and Adeoye (2004) a higher yolk index is more desirable because it means that the egg has high interior quality.

Despite some differences in the yolk index, albumen index and Haugh unit values reported in the current study compared with those reported by Zita *et al.* (2013) and Alkan *et al.* (2010) when compared to other values reported elsewhere in literature (Gonzalez, 1995; Waheda *et al.*, 1999, Kul and Seker, 2004, Genchev, 2012) they are similar. Therefore findings from the current study fall within the previously reported internal egg quality values suggesting that dietary hexane-extracted MNM did not compromise internal egg quality.

5.5.4 Effects on egg yolk fat and cholesterol content

Eggs form a very important part of the human diet: they are the most consumed source of protein in developing countries and they possess numerous nutritive and functional properties (Bragagnolo and Rodriguez-Amaya, 2003). However, the excessive consumption of eggs has raised the concern of excessive fat and cholesterol intake which is associated with cardiovascular diseases (Bragagnolo and Rodriguez-Amaya, 2003; Mahabadi *et al.*, 2008). However, recent reports on the association of egg consumption and cardiovascular diseases, Melough *et al.* (2019)

have reported that egg consumption actually lowers the risk of cardiovascular diseases while Qin *et al.* (2018) reported no effect.

Although there are statistical differences in the egg yolk fat content in the current study. However, the differences may not be biologically significant as there was less than 1% difference across the dietary treatment groups. Genchev (2012), Polat *et al.* (2013) and Sinanoglou et al. (2011) reported quail egg yolk fat content ranging from 27.45 to 33.47%. Polat *et al.* (2013) reported a 30.60 to 33.22% range in egg yolk fat of goose, duck, turkey, peacock, guinea fowl, pheasant, quail, and partridge.

Findings from the current study show egg yolk fat ranging from 54.49 ± 0.33 to 55.70 ± 0.25% which is higher compared to that reported by Sinanoglou *et al.* (2011) and Genchev (2012). Additionally, findings from the current study report egg yolk cholesterol content ranges of 2104 ± 174.04 to 3077 ± 493.70 mg/100g. Genchev (2012) reported the cholesterol content from quail egg yolk to range from 4120 to 4760 mg/100g while Bragagnolo and Rodriguez-Amaya (2003) reported it at 1210 mg/100g.

Chicken eggs are the most consumed poultry eggs (Sinanoglou *et al.*, 2011) and this makes them important comparison and their egg yolk fat and cholesterol range from 8.48 to 33.42% and 152.87 to 333.33 mg/100, respectively (Farrell, 2013; Naviglio *et al.*, 2012; Abdul Rehman *et al.*, 2016; Chepkemoi *et al.*, 2017; Polat *et al.*, 2013) and this compares unfavourably with that of quail egg yolk reported in this study. Despite the reported the egg cholesterol findings from the current study being higher than those reported by Bragagnolo and Rodriguez-Amaya (2003), it is important to note that they are lower than those reported by Genchev (2012). Based on the variances in literature values of quail egg yolk cholesterol content, it cannot be concluded that dietary hexane-extracted MNM negatively impacted quail egg yolk cholesterol content. The MNM had a higher residual TSFA and OA content (Appendix 1). It has been reported that supplementing poultry diets with fats and or oils results in increasing palatability and feed utilisation efficiency (Poorghasemi *et al.*, 2013) and also that the fat profile in the ration is mirrored in the products (Shahryar *et al.*, 2011). The high egg yolk fat content, compared to that reported in literature, of eggs from quail fed MNM-based diets could have resulted from the deposition of the residual TSFA and OA in the egg yolks. However, in order to fully determine the potential health impact of consuming these egg yolks, there may be need to identify the fatty acid profile of the quail egg yolk from the current study as saturated and trans-fats are known to be detrimental to human health (Mayurasakorn *et al.*, 2008; Chekaniazar, 2011). High amounts of saturated (predominantly low density lipoprotein) and trans fats increase high density

lipoprotein which may lead to higher risk cardio vascular diseases (German and Dillard, 2004; Mozaffarian *et al.,* 2007) as opposed to monounsaturated and polyunsaturated fat.

5.5.5 Effects on egg yolk fatty acid profile

To fully establish the health benefits/nutrition value of eggs, it is important to profile their fatty acid composition (Khan *et al.,* 2017). The egg yolk contains triacylglycerols and phospholipids which are the source of energy and structural lipids for the developing embryo (Sinanoglou *et al.*, 2011). The egg yolk fatty acid content changes depending on dietary manipulation (Oliveira *et al.*, 2010). There are variations in the fatty acid content of egg yolk reported in literature. Genchev (2012) reported that quail egg yolks contain 36.89 to 45.44% TSFA and 9.94 to 23.84 % TPUFA. Lower TSFA and higher TPUFA percentage compared to Genchev (2012) were reported in other studies. Adachi *et al.* (1978) and Polat *et al.* (2013) reported that quail egg yolks contain 29.90 to 40.70% TSFA, 45.02 to 48.70% TMUFA and 25.09 % TPUFA.

In the current study, quail egg yolks contained 37.49 ± 0.42 to 39.57 ± 0.26% TSFA, 48.79 ± 0.31to 51.14 ± 0.22% TMUFA and 6.82 ± 0.12 to 9.58 ± 0.13% TPUFA. Palmitic acid, Oleic acid and linoleic acid were the dominant SFA, MUFA and PUFA ranging from 26.17 ± 0.22 to 27.39 ± 0.08%, 43.67 ± 0.22 to 47.81 ± 0.47% and 6.11 ± 0.05 to 8.46 ± 0.08%, respectively. The TSFA and TMUFA reported in the current study are within the range but had lower TPUFA compared to findings by Adachi *et al.* (1978), Genchev (2012) and Polat *et al.* (2013). There was increase in the TMUFA content of the egg yolks TMUFA with increasing dietary MNM. The MNM had a higher concentration of oleic acid, a MUFA, compared to the oleic acid content of SBM. Livestock and poultry products (meat and eggs) have been shown to mirror the dietary ingredient and or diet lipid profile (Tuunainen *et al.,* 2016, Lopez-Ferrer *et al.,* 2001), thus it can be speculated that the higher TMUFA in the egg yolks from quail fed MNM-based diets could have come from the oleic acid. The egg yolk TPUFA content shows a decreasing trend from the eggs of hens fed diet 1 through to 5; this might be as a result of the linoleic acid as SBM contains high amounts as compared to MNM.

Thus oleic acid comprised of almost half of the fat content in the egg yolk. Oleic acid in the diets has been associated with health benefits such as reduction in the risk of cardiovascular diseases (Lopez *et al.,* 2010). Also, oleic acid is highly stable to oxidation (Hernandez, 2015) thus can extend the shelflife of egg and egg products.

5.5.6 Effect of dietary Marula nut meal on general health of the quail

5.5.6.1 Effect on liver lipid content

Diet quality plays a critical role in the aetiology of fatty livers in poultry (Hu *et al.*, 2017; Sánchez-Polo *et al.*, 2015). In addition to diet-mediated lipid accumulation in the liver, the physiological status of the bird is also critical, for example the onset of egg-laying causes an increase in the hepatic synthesis of triglycerides, phospholipids and cholesterol (Agina *et al.*, 2017; Kaur *et al.*, 2014; Scholtz *et al.*, 2009) which result in higher liver lipid content. Findings from the current study showed similarity in the liver lipid content of the hens across dietary treatments. This finding suggests that dietary MNM did not alter the metabolism of lipids in the quail's livers. Importantly, it can be speculated that MNM can be utilised in pullet quail diets without the risk of eliciting abnormal deposition of lipids in the liver which can cause fatty liver disease. Maurice and Jensen (1979) reported mean liver lipid content ranging from 4.4 to 7.4% in adult female Japanese quail. In the current study, despite being in lay (which increases liver lipid accumulation), the liver lipid content of the quail hens ranged from 3.94 ± 2.18 to 8.33 ± 8.19 (figure 5.7), which is within the range reported in adult quail fed a cereal-based diet reported by Maurice and Jensen (1978). The lower lipid content observed in the current study suggests that MNM could be used in pullet quail feeds without the risk of eliciting derangement of liver lipid metabolism that could trigger fatty liver disease.

5.5.6.2 Effect on plasma surrogate markers of health

Calcium and phosphate are critical constituents of the eggshell and thus are critical during the egg-laying period (Vries *et al.*, 2010; Rodrigues *et al.*, 2013; Bölükbasi *et al.*, 2005). Their plasma concentration increases when birds start laying (Kashap *et al.*, 2017; Pavlík *et al.*, 2009; Albokhadaim *et al.*, 2012). According to Summers *et al.* (1992), anti-nutritional factors such as phytic acid oxalates reduce calcium availability resulting in thinner eggshells which makes the eggs prone to breaking. The onset of eggshell formation decreases plasma Ca concentration which then stimulates parathyroid hormone-mediated bone resorption in order to maintain plasma calcium homeostasis (Berto *et al.*, 2009).

Findings from the current study showed similarities in the plasma Ca concentration of the quail hens across dietary treatments (Table 5.3) but the plasma phosphate concentration of hens fed diet 4 was higher than that of hens fed diet 1. The high plasma phosphate concentration of the birds fed diet 4 compared those fed diet 1 may be due to a possible malfunction in the maintenance of plasma Ca and phosphate balance. According to Li *et al.* (2018), Ca and

phosphorus (phosphate) balance is maintained by the absorption in small intestine, reabsorption and excretion of kidney, and deposition and mobilization of bone. Any fluctuation in the maintenance of Ca and phosphorus ratio may lead to an increase or decrease in either mineral in the blood thus compromising egg quality parameters such as shell thickness. Poultry diet composition impact the plasma metabolite content (Mushtaq *et al.*, 2013).

In the current study, the plasma Ca and phosphate concentration of the quail hens ranged from 3.74 ± 0.24 to 4.00 ± 0.00 and 2.23 ± 0.29 to 4.66 ± 0.44 mmol/L, respectively. The Ca concentrations reported in the study were higher than the 0.67 mmol/L reported by Awal and Das (2016). Compared to findings from the current study, Berto *et al.* (2009) reported lower plasma Ca and phosphate concentration of 1.05 mmol/L and 0.66 mmol/L respectively from layers fed diets containing 4.5% Ca and phosphate.

Uric acid is a major end product of nitrogen metabolism in poultry species (Simoyi *et al.*, 2015). Plasma uric acid is often used in clinical practice to determine avian renal function (Kolmstetter and Ramsay, 2000). Diets with a high CP content result in increased metabolic cost to eliminate the excess nitrogen (Millet *et al.*, 2018). Poultry feeds rich in CP have been shown to result in increased uric acid excretion (Araújo *et al.*, 2004; Azzam *et al.*, 2019). Findings from the current study showed similarities in plasma uric acid concentration in quail hens across dietary treatments (Table 5.4). The similarities in plasma uric acid concentration suggests that the hexane-extracted MNM did not alter protein metabolism of the quail suggesting that the MNM can potentially be utilised without negative effects on nitrogen metabolism in quail hens.

Therefore, MNM can be used as a source of dietary protein in poultry feeds without compromising the kidney function. Also comparing to plasma Ca and phosphate concentrations of layer quails reported in literature, MNM increases the plasma Ca and phosphate concentrations in laying Japanese quails.

5.5.7 Effect on viscera macro-morphometry

Bird growth can be estimated by the sum of organ weights and several factors may influence organ growth and development including among others genetics, gender, environment, management and nutrition (Grieser *et al.*, 2015). Seed meals used as feed ingredients are known to contain several ANFs that hinder digestion and absorption of nutrients (Saetae and Suntornsuk, 2011). The effect of the ANFs on GIT organ function may result in compromised

organ macro- and micro-morphometry that manifest with reduced digestive and absorptive. The findings from this study show that dietary MNM had no effect (P >0.05) on the GIT visceral organs morphometry. This may be as a result of reduced ANFs courtesy of defatting with hexane. The GIT accessory organs such as the liver and pancreas are normally affected by the nutritional quality of the feed (Zaefarian *et al.*, 2019; Emiola *et al.*, 2007). Velu *et al.* (1971) reported that the liver mass can be affected by deficiencies in protein and amino acids. The hens fed diet 5 had a heavier liver (absolute and relative to body mass and tibia length) compared liver masses from hens fed diets 1, 2 and 3. These findings could be a result of general organ growth because there were no differences in the liver lipid content (Figure 5. 6) of the hens which have been reported as major causes of increased liver size. Histological examination of the liver samples could have provided some insights into the cause of the increased liver mass in hens fed diet 5. It is thus recommended that further studies do histological evaluations on the possible effects of such non-conventional dietary protein sources on liver architecture. Based on the findings from the current study, it can be inferred that the hexane extracted MNM did not compromise the macro-morphometry of GIT viscera but its use at 100% substitution of SBM be done with caution as it might lead to hepatomegaly.

5.6 Conclusion

Dietary MNM can be used in pullet Japanese quail diets without compromising the egg production, egg nutrient composition, internal egg quality parameters, egg mass, egg shape index as well as bird health, growth performance, feed intake and feed utilisation efficiency, liver lipid content and macro-morphometry of GIT viscera. However, its used resulted in thinner egg shells which might increase the eggs' susceptibility to breakages thus use of MNM in pullet diets might require supplementation with Ca and phosphorus.

CHAPTER SIX - CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

6.0 Conclusions

Previous studies using mechanically defatted MNM have shown limited capacity for use of MNM in chicken feed. The hexane defatted MNM and SBM were characterised for the purpose of assessing the meal's potential to be used as a source of dietary protein in poultry feeds particularly Japanese quail. The current study sought to further defat mechanically defatted MNM in order to reduce its residual oil content and evaluate its suitability as a dietary protein source in broiler and pullet quail feeds. Solvent (hexane) extraction of the mechanically defatted MNM produced a meal with a higher CP, amino acid, GE and Ca content that SBM but with lower phosphorus content. Additionally, although the hexane-extraction significantly reduced the fat content of the meal, compared to SBM, the MNM had higher residual TSFA and OA content. Solvent extraction of mechanically produced meal can potentially be used as a dietary protein source in feeds. The commercialisation of MNM based feeds would therefore make it possible for resource limited farmers that want to expand their businesses in the poultry and livestock industry. Furthermore, this will not only help improve food security in SSA, it will also create job opportunities up and downstream in the production chain thus strengthening the economy.

Based on the outcomes of the *in vivo* evaluation it can be concluded that hexane-extract MNM can used as dietary protein source in quail broiler and pullet diets without compromising growth performance, feed intake, feed conversion ratio, visceral macromorphology, health status and meat (pH, thawing loss and cooking loss) and egg quality. Importantly, the meal increased the redness and OA content of the meat. Thus the hexane-extracted MNM can be exploited to improve acceptability and nutritional value of the quail and probably poultry meat. Despite it demonstrated potential to support egg production and not to compromise the egg nutrient composition, internal egg quality parameters in quail, use of the hexane-extracted MNM in pullets needs a cautious approach as it caused thin egg shells which can lead to losses through increased breakages.

Overall my study has shown that MNM can be used as a source of dietary protein in broiler and layer diets without compromising productivity (growth and egg production), bird health as well as meat and egg quality. These findings suggest that hexane-extract MNM can be used as an alternative to SBM in quail broiler and pullet feeds. This can potentially lead to cheaper feeds which would translate to increased poultry meat and egg production for human consumption.

The use of MNM as a source of dietary protein will also add to the value chain of Marula in SSA and reduced the importation of SBM thus cutting costs.

6.1 Limitations and recommendations

Determination of ileal and total tract digestibility of the MNM-based diets would have shed more light on the possible mechanisms and basis of the observed efficacy of MNM as a replacement of SBM in quail diets.

While some attempt was done to determine effects of dietary MNM nut meal on the health of the quail by evaluating its effects on plasma metabolites, an evaluation of its effects on the architecture of key organs such as the liver and kidneys would have provided more insight on its health effects, especially in view of its effect on liver lipid content.

In the current study, sensory evaluations of the meat to determine the meat's eating quality were not done. Such tests would have confirmed MNM's effects on aroma, flavour and taste which are some of the qualities buyers also consider when consuming the meat.

Future studies should consider determining digestibility (ileal, essential amino acid and total tract) of MNM-based diets in quail diets and other poultry. It is recommended that liver and kidney histology be examined in future studies as plasma surrogate markers of health may not be specific in evaluating effects on key organs in order to determine possible key organ damage.

Future studies need to investigate the concentrations of the types of cholesterol (LDL and HDL) in eggs yolks from quail fed MNM in order to give a clear recommendation on the health effects consuming the eggs might impose.

Future studies should include measurements of bone turnover in order to fully understand growth performance and get clear explanations of thinner eggshells.

To study the long term impact, the feeding of layers should have started at an earlier age from day one; however the difficulty is sexing males and females at a younger age and this had to be factored in the study design.

It is also recommended that future studies should explore the fecundity of the eggs, as this would have a bearing on the breeding success of quail fed on MNM based diets.

The MNM based feed's keeping quality should also be determined in future studies so farmers may be able to use the meal while it is still palatable and nutritious to animals.

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8.0 Appendices
Supplementary data

Appendix 1: Fatty acid content of Marula nut meal and soyabean meal lipid

Fatty Acids (g/100g DM)	MNM	SBM	Significance level
Saturated			
C20:0 (arachidic acid)	nd	3.00 ± 0.71	-
C22:0 (behenic acid)	5.64 ± 0.61	2.05 ± 2.08	***
C12:0 (lauric acid)	1.13 ± 0.05	0.15 ± 0.06	***
C24:0 (lignoceric acid)	1.44 ± 0.16	0.31 ± 0.12	***
C17:0 (margaric acid)	1.30 ± 0.11	0.28 ± 0.14	***
C14:0 (myristic acid)	1.20 ± 0.12	0.19 ± 0.10	***
C16:0 (palmitic acid)	104.00 ± 0.85	17.84 ± 2.59	***
C15:0 (pentadecanoic acid)	nd	1.20 ± 0.15	-
C18:0 (stearic acid)	56.40 ± 12.06	6.71 ± 0.81	**
C23:0(tricosanoic acid)	nd	1.08±0.73	-
TSFA	**171.11 ± 19.96**	**32.81 ± 7.49**	***
Monounsaturated			
C18:1n9 (oleic acid)	484.40 ± 18.70	30.12 ± 14.95	***
C22:1n9 (erucic acid)	2.34 ± 1.16	0.22 ± 0.20	*
TMUFA	**486.74 ± 19.86**	**30.34 ± 15.15**	***
Polyunsaturated			
C18:3n3 (α-linolenic acid)	1.20 ± 0.11	7.11 ± 5.52	ns
C20:5n3 (Eicosapentaenoic acid)	2.37 ± 0.76	6.47 ± 3.80	ns
C18:2n6t (linolelaidic acid)	1.12 ± 0.11	0.20 ± 0.10	***
C18:2n6c (linoleic acid)	49.09 ± 4.59	68.96 ± 18.86	ns
C18:3n6 (γ-linolenic acid)	1.20 ± 0.11	0.30 ± 0.26	**

TPUFA	**54.98 ± 5.68**	**83.04 ± 28.54**	*******
Trans fatty acids	1.07 ± 0.36	0.20 ± 0.10	*
Cis fatty acids	551.10 ± 17.68	34.93 ± 15.09	***
Omega-3	5.22 ± 0.66	0.18 ± 0.03	***
Omega-6	53.49 ± 9.07	61.17 ± 6.19	ns
Omega-9	493.80 ± 2.01	33.89 ± 15.25	***
EPA (Eicosapentaenoic acid)	1.76 ± 0.14	0.15 ± 0.04	***
DHA (Docosahexaenoic acid)	0.40 ± 0.11	nd	-

ns = not significant, P >0.05, *P <0.05 ** P <0.01, *** P <0.0001, [ab] Within row means with different superscripts are significantly different at P <0.05, nd = not detected. Marula nut meal had a high content of TSFA (P <0.0001) where C22:0, C12:0, C24:0, C17:0, C14:0, C16:0 and C18:0 were in high (P <0.0001) amounts compared to SBM. The TMUFA content of MNM was higher (P <0.0001) compared to that of SBM, where C18:1n9 and C22:1n9 were both in high amounts. However, SBM contained larger amounts of TPUFA compared to MNM where C18:3n3, C20:5n3 and C18:2n6c were the highest amino acids. Marula nut meal had high amounts of Trans fatty acids, Cis fatty acids, Omega-3, Omega-9 and EPA compared to SBM. MNM = solvent extracted Marula nut meal; SBM = soyabean meal; data presented in mean±SD; n = 3.

Appendix 2: Amino acid content of the Marula nut meal and soyabean meal

Amino acid (g/100g DM)	MNM	SBM	Significance level
Alanine	158.20 ± 0.92	101.20 ± 0.61	***
Arginine	969.40 ± 30.18	184.60 ± 4.24	***
Aspartic acid	159.90 ± 12.46	218.60 ± 21.15	*
Glumatic acid	702.80 ± 2.62	393.50 ± 9.68	***
Glycine	255.00 ± 7.94	92.22 ± 1.56	***
Histidine	224.10 ± 1.92	88.44 ± 4.25	***
Hydroxy-proline	6.27 ± 0.99	4.08 ± 0.43	*
Isoleucine	233.80 ± 20.56	100.20 ± 2.07	***
Leucine	322.10 ± 2.92	164.60 ± 7.82	***
Lysine	148.90 ± 3.76	239.80 ± 79.71	ns
Methionine	93.12 ± 1.61	27.41 ± 3.49	***
Phenylalanine	248.20 ± 4.65	112.20 ± 1.10	***
Proline	181.50 ± 9.13	112.70 ± 1.29	***
Serine	254.50 ± 22.43	120.60 ± 1.39	***
Threonine	124.70 ± 3.03	90.55 ± 1.96	***
Tyrosine	137.40 ± 2.91	133.40 ± 4.76	ns
Valine	245.50 ± 1.38	95.57 ± 1.68	***

ns = not significant, P >0.05, *P <0.05, *** P <0.0001, ab Within row means with different superscripts are significantly different at p <0.05, nd = not detected. Alanine, Arginine, Glumatic acid, Glycine, Histidine, Hydroxy-proline, Isoleucine, Leucine, Methionine Phenylalanine, Proline, Serine, Threonine and Valine were all high (P < 0.0001) in MNM as compared to SBM. MNM = solvent extracted Marula nut meal; SBM = soyabean meal; data presented in mean ± SD; n = 3.

Appendix 3: proximate content of Japanese quail breast and thigh meat

CP %	EE %	Ash %	REFERENCES
21.40	3.70	0.97	Fakolade, 2015
17.90	1.55	1.28	Raji et al., 2015
22.20 to 22.44	0.83 to 1.57	1.25 to 1.45	Lukanov et al., 2018
-	1.4 to 1.64	1.28 to 1.44	Gecgel et al., 2015
22.23 to 27.51	2.21 to 2.75	1.51 to 1.61	Genchev et al., 2008

CP = crude protein, EE = ether extract.

Appendix 4: ethical clearance certification

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2017/08/54/B

APPLICANT: Mr B Mazizi

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: The potential of Sclerrocarya birrea caffra nut meal as a dietary protein source in broiler and layer Jumbo Japanese quail (Cortunix cortunix japonicum)

Number and Species

100X 7-day old male Jumbo Broiler Japanese quail (Cortunix cortunix japonicum), 100X 7-day old female Jumbo Broiler Japanese quail (Cortunix cortunix japonicum) and 60X 5-week old female Jumbo Layer Japanese quail (Cortunix cortunix japonicum)

Approval was given for the use of animals for the project described above at an AESC meeting held on 2017/08/29. This approval remains valid until 2019/10/11.
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:

Signed: _____ (Chairperson, AESC) Date: 17 October 2017

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: 16 October 2017

cc: Supervisor: Professor E Chivandi, Professor K Erlwanger and Professor V Mukhenje

Appendix 5: Animal transportation permits

Province of the
EASTERN CAPE
RURAL DEVELOPMENT AND
AGRARIAN REFORM

VETERINARY SERVICES – AMATOLE
ANIMAL HEALTH, STATE VETERINARY OFFICE,
EAST LONDON

20 Church Street, Private Bag X9022 , East London, 5201, Eastern Cape, REPUBLIC OF SOUTH AFRICA.
Website:www.drdar.gov.za
Tel: +27(0)43 722 3081. Fax: +27(0)43 743 4075.

28 August 2017.

To: Department of Agriculture Forestry and Fisheries

Attention; Mr. Mazizi

RE: APPLICATION UNDER SECTION 20 OF THE ANIMAL DISEASES ACT, 1984. (Act No 35 of 1984).

Dear Mr. Mazizi

The following researcher, Mr. Bulelani Elvis Mazizi, School of Physiology, Medical School. University of the Witwatersrand, has submitted a request to my office in terms of the above Section of the Act. He has requested that I issue a letter as required in section 6.3 of the application. This has reference to a declaration as to disease restrictions relating to the sourcing area.

Although Mr. Mazizi requires 200 (7 day-old) broiler Quail and 60 (5 week-old) layer Quail for his research project (Section 6.1 of the application) he cannot, at this time, confirm either the dates on which the birds are to be moved or the numbers of birds to be moved on each occasion.

I am able, at this time, to declare the following:

- There are, as of the above date, no restrictions relating to notifiable or controlled diseases affecting Quail in place in the State Veterinary Area;
- SA Quail Breeders (Section 6.2 of the application) is a business that falls within my State Veterinary area;
- With special reference to the dynamic situation concerning Highly Pathogenic Avian influenza that exists in South Africa at this time it is impossible to be able to confirm that when Mr. Mazizi is ready to move these birds I would be able to make a similar declaration as to disease restrictions;
- Therefore every application to move birds from my area to Gauteng would need to be individually assessed on its merits at the time and a specific movement permit issued.

Should any further information be required you are welcome to contact me.

Yours sincerely
Dr MJ Ndzengu
State Veterinarian
Animal Health
East London.
Cell: 083 303 6982.
e-mail: mzikazi.ndzengu@gmail.com.

DEPT. OF RURAL DEVELOPMENT
AND AGRARIAN REFORM
VETERINARY SERVICES, EAST LONDON
28 AUG 2017
PRIVATE BAG X9022, EAST LONDON, 5200
TEL : 043 722 3081 FAX : 043 743 4073

" Vibrant, Equitable, Sustainable Rural Communities and Food Security for All"

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

20 September 2017

Dear Mr Mazizi and Mdoda

RE: Support for undertaking of feeding research in Quails at the University of the Witwatersrand

Your enquiry about the State veterinarian letters refers. The completed Section 20 application was received the 13th of September 2017 (copies attached)

I have no objection to the movement of the quails into Gauteng should the disease situation in the EC warrant the movement of quails from origin in the EC to the University at the time of movement.

The letter regarding absence of disease restrictions at origin should be sourced from EC at the time of movement.

Please forward your application and EC SV letter to DAFF.

Kind Regards

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