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THE POTENTIAL OF *MIMUSOPS ZEYHERI* SEED MEAL AS A DIETARY ENERGY SOURCE IN BROILER QUAIL DIETS

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

Johannesburg, 2019

Declaration

I declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine (Physiology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any University. I certify that all the experimental procedures used in this dissertation were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (AESC number: 2017/08/56B).

_____

Bayanda Mdoda

_____day of _____2019

Dedication

To the loving memory of my late mother

-Nongezile Rosy Mdoda

1958 - 2016

Abstract

The shortage of dietary energy sources for poultry feeds contributes to increased feed costs hence the need to search and develop alternative sources. *Mimusops zeyheri* seed meal (MZSM) has high energy content. This study evaluated the potential of MZSM to replace maize meal (MM) as an energy source in broiler quail finisher diets. The effects of dietary MZSM on the growth performance, feed utilisation efficiency, viscera morphometry, general health and meat quality of broiler quail were determined.

Thirty-two 5-week old male broiler quails were randomly allocated to 4 diets wherein MZSM replaced MM on gross energy basis at 0%, 12.5%, 25% and 37.5% (diet 1 to 4) and fed for 4 weeks. Body mass and feed intake (FI) was measured. Body mass gain (BMG), average daily gain (ADG) and feed conversion ratio (FCR) were computed. On slaughter, the carcasses were eviscerated and viscera macro-morphometry determined. pH and colour of the breast muscle were measured. Tissues were collected for physico-biochemical and histological assays. At 37.5% inclusion MZSM reduced (p<0.05) FI. Dietary MZSM did not affect ($p>0.05$) BMG, ADG and FCR but increased (p<0.0001) the mass of proventriculi, ventriculi and small intestine mass and the small intestine length of the quail. Visceral fat decreased (p<0.0001) with an increase in dietary MZSM. At 25% and 37.5% inclusion, MZSM increased ($p <0.05$) villus height and width, crypt depth and villus height:crypt depth ratio. MZSM had no effect ($p>0.05$) on fasting blood glucose concentration but at 25% inclusion level it increased ($p<0.05$) fasting blood triglyceride concentration. Dietary MZSM decreased ($p<0.05$) hepatic lipid content. At 25% inclusion MZSM increased ($p<0.05$) plasma urea concentration. Dietary MZSM did not affect ($p>0.05$) plasma uric acid concentration. At 25% inclusion, MZSM increased ($p<0.05$) plasma ALT activity and at 12.5% and 37.5% it increased ($p<0.05$) plasma ALP activity while at 25% and 37.5% inclusion it significantly increased ($p<0.05$) TBARs concentration. Dietary MZSM (25% and 37.5% inclusion) increased ($p<0.05$) plasma total bilirubin concentration. It had no effect on pHi and colour of the quail breast muscle (meat). MZSM had no effect ($p>0.05$) on pHu but at 37.5% inclusion it decreased ($p<0.05$) redness of the meat 24-hours post-slaughter. Dietary MZSM increased ($p<0.05$) water holding capacity of the meat. At 12.5% inclusion it reduced ($p<0.05$) cooking loss. MZSM (25% and 37.5% inclusion) decreased ($p<0.05$) shear force required to cut through fillets. Dietary MZSM had no effect ($p>0.05$) on the meat's moisture, dry matter and ash content. The meat's crude protein content increased ($p<0.05$) with

an increase in the meal and its fat decreased ($p<0.05$) with an increase in the meal. MZSM had no effect ($p>0.05$) on the meat's total saturated, monounsaturated and polyunsaturated fatty acid content. At 25% and 37.5% inclusion, MZSM decreased ($p<0.05$) the meat's trans and Cis fatty acid content but increased ($p<0.05$) the omega-3, -6 and -9 content.

Mimusops zeyheri seed meal can partially replace MM in quail finisher diets without compromising growth performance and feed utilisation efficiency, as well as viscera morphometry and meat quality. Caution has to be exercised in the use of *M. zeyheri* as a dietary energy source as it may compromise liver function and increase lipid peroxidation in the birds.

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List of symbols

ADF	Acid detergent fibre
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ANFs	Anti-nutritional factors
BUN	Blood urea nitrogen
CF	Crude fibre
CL	Cooking loss
CP	Crude protein
DAFF	Department of Forestry and Fisheries
DHA	Docosahexaenoic acid
DM	Dry matter
EAAs	Essential amino acids
EE	Ether extract
EFAs	Essential fatty acids
EOF	Erythrocyte osmotic fragility
EPA	Eicosapentaenoic acid
FA	Fatty acids
GE	Gross energy
GIT	Gastrointestinal tract
IFBTs	Indigenous fruit bearing trees
IMF	Intra-muscular fat
LDL	Low-density lipoprotein
MDA	Malonaldehyde
MM	Maize meal
MZSM	*Mimusops zeyheri* seed meal
n3PUFA	Omega-3 polyunsaturated fatty acids
n6PUFA	Omega-6 polyunsaturated fatty acids
NDF	Nutrient detergent fibre
ROS	Reactive oxygen species
SAPA	South Africa Poultry Association

SBM	Soyabean meal
SSA	Sub-Saharan Africa
TBARS	Thiobarbituric acid reactive substances
TFAs	Total fatty acids
TMUFA	Total monounsaturated fatty acids
TPUFA	Total polyunsaturated fatty acids
TSFA	Total saturated fatty acids
USDA	United State Department of Agriculture
WHC	Water holding capacity

Nomenclature

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

CHAPTER 1: INTRODUCTION AND JUSTIFICATION

1.1 Introduction

Globally the demand and consumption of poultry products, particularly poultry meat, has and continues to increase (Brenes and Roura, 2010). The growth in human population, urbanization and an increase in incomes are the major drivers of increased demand for foods of animal origin in the developing countries (Abdullah et al., 2011). It is estimated that the current global consumption of poultry meat stands at 117 608 thousand tonnes [Organisation for Economic Co-operation and Development of the Food and Agricultural Organisation (OECD and FAO, 2016)]. According to the OECD and FAO (2016) 2 795 thousand tonnes was consumed in sub-Saharan Africa (SSA), whereas 1 998 thousand tonnes was consumed in South Africa (SA). In 2014 the South African poultry industry produces 60% of the total broiler chicken meat consumed locally while 40% was covered by the imports from other countries (Esterhuizen, 2015). When compared to red meats poultry meat is a cheaper source of protein (Petracci et al., 2013) making it a major animal-derived protein source in human diets (Tan et al., 2018). Additionally, poultry meat has a health beneficial nutrient profile: low lipid content and moderately high carotenoid, vitamin and mineral contents (Nkukwana et al., 2014). It is also a source of essential amino acids (EAAs) and essential fatty acids (EFAs). The EAAs and EFAs are crucial for normal early development of the human brain (Mazza et al., 2007). While broiler chicken meat and eggs have been and continue to be the major sources of animal-derived protein-rich poultry products, a deficit in meat and eggs production exists, allowing for other potential poultry species besides chicken to be farmed. One such potential poultry species is the Japanese quail, *Cortunix cortunix japonicum*. Quails are small sized birds which require less initial investment as they demand less land area and less feed (Jatoi et al., 2013). Importantly, quail attain sexual maturity early and have a short generation interval compared to other poultry species (Jatoi et al., 2013). Quail farming would suit resource-limited farmers due to the comparatively lower establishment cost when compared to larger poultry species.

Japanese quail have higher average dressing percentage of 75% at the age of 5-6 weeks (Alkan et al., 2013) compared to 72% of broiler chicken at the same age (Kokosyznaki *et al.*, 2013). Importantly, quail meat has high amounts of iron and potassium, low lipid content (Raji et al., 2015), low calorific content, is tender and flavoursome thus making it healthier and wholesome alternative (Dauda et al., 2014). These positive attributes of quail and quail meat make quail not

only a feasible compliment to broiler chicken but a source of a more healthy product ideal for production by the rural resource-limited farming communities.

The poultry industry, particularly in developing countries is faced with the challenge of high feed costs (Abbas, 2013). Feed costs account for 70 - 80% of the total production costs (Begli et al., 2016; Baéza et al., 2015). The high cost of feed, scarcity of feed ingredients and the competition between human beings and animals for conventional dietary protein and energy sources (Godfray et al., 2010) has resulted in locally produced dietary protein [soyabean meal (SBM)] and energy sources (maize and other cereals) being unable to meet the requirements for monogastric (poultry: chicken, guinea fowl and quail) animal production (Uchegbu et al., 2010). Due to the competition-driven shortages, dietary protein and energy sources are generally channelled towards meeting human requirements first (Carlson and Frazao, 2012) resulting in less or poor quality ingredients being given to livestock (poultry) production (Sapkota et al., 2007). In SSA and SA, maize meal is the dominant dietary energy source in poultry feeds (Panda et al., 2014). Maize meal, the major dietary energy source, constitutes 60 to 65% of total metabolisable energy content and 20% of protein content in poultry (quail) feed (Cowieson, 2005). The high competition-driven cost of maize to the poultry industry (Biswas et al., 2015) is an impediment to the possible expansion of broiler chicken production as well the adoption of alternative poultry species such as quail and guinea fowl. There is thus a dire need to search and develop alternative dietary energy sources in order to ensure continued growth of the poultry industry (Onunkwo et al., 2015) in SSA and SA with a view to meet the increased demand of animal-derived (poultry: chicken, guinea fowl and quail) dietary protein sources for human consumption (Ncube et al., 2017; Donaldson et al., 2016; Geldenhuys et al., 2013).

The indigenous fruit-bearing trees (IFBTs), among them *Sclerocarya birrea caffra*, *Ximenia caffra* and *Mimusops zeyheri*, are widely distributed in SSA and produce fruit and nuts which are consumed by humans and animals (Maroyi, 2016; Nyau, 2013; Chivandi et al., 2011). Recent research has shown that dehulled *M. zeyheri* seeds contain 24.34 ± 0.56 MJ/kg gross energy, 9.3% crude protein, 48.3 ± 12.9 mg/100g calcium content, 33.2% neutral detergent fibre and 15.3% acid detergent fibre (Chivandi et al., 2011). *Mimusops zeyheri* seed meal's energy content is higher than that of maize thus making the seed meal a potential dietary energy source in feeds (Chivandi et al., 2011). Plant-derived non-conventional sources of nutrients, *M. zeyheri* seed meal

included, are known to contain anti-nutritional factors (ANFs) such as tannin, lectins, protease inhibitors and chelating agents (Chivandi, 2013). Preliminary studies in our laboratory have shown that *M. zeyheri* seed meal has a high energy content thus making it a potential dietary energy source in poultry feeds. Given the chemical nutrient composition, especially the energy content of the *M. zeyheri* seed meal against a background of shortages in conventional dietary energy sources for poultry feeds, this study, therefore, evaluated the potential of *M. zeyheri* seed meal to replace maize meal as a dietary energy source in finisher broiler quail diets.

1.2 Aim and objectives

The current study, executed in two experiments, had specific aims and objectives.

1.2.1 Aim(s) and objectives for experiment 1

This experiment determined the chemical nutrient composition of *M. zeyheri* seed meal for the purposes of getting the chemical nutrient composition data for use in the formulation of experimental *M. zeyheri* seed meal -based dietary treatments for the feeding trial. The specific objectives of this experiment were to determine the:

a) proximate composition [dry matter (DM), crude protein (CP), ether extract (EE and ash)] and gross energy (GE) content.
b) amino acid (non-essential and EAAs) and mineral (phosphorus, potassium magnesium and calcium) composition.
c) fibre (CF, NDF, and ADF) content and fatty acid profile.
d) anti-nutritional factors: tannin, tepernoid, flavonoid and saponin content.

1.2.2 Aim(s) and objectives for experiment 2

The aim of this experiment was to formulate broiler quail finisher diets wherein maize meal was replaced by MZSM on a gross energy basis at 0%, 12.5%, 25% and 37.5%. The specific objectives of this experiment were to determine the effects of dietary *M. zeyheri* seed meal on:

a) growth performance and feed conversion efficiency.
b) erythrocyte membrane fragility in serially diluted phosphate buffered saline.
c) serum metabolites (blood glucose and triglyceride) and stored (hepatic lipid) metabolic substrate content.

d) viscera macro-and micro-morphometry.

e) general health profile with a focus on markers of liver [alkanine transaminase (ALT), alkaline phosphate (ALP)] and kidney function (BUN, total bilirubin, uric acid), and markers of oxidative stress (TBARS).

f) physico-chemical properties of meat: colour, pH, tenderness, drip loss and water-holding capacity proximate, mineral, amino acid and fatty acid content.

1.3 Hypothesis

H_0: dietary MZSM as a substitute for maize meal in quail finisher diets does not affect growth performance (body weight indices, linear growth and viscera macro- and micro-morphometry), feed utilisation efficiency, the health profile and meat quality of male Japanese quail.

H_1: dietary MZSM as a substitute for maize meal in quail finisher diets affects growth performance (body weight indices, linear growth and viscera macro- and micro-morphometry), feed utilisation efficiency, the health profile and meat quality of male Japanese quail.

CHAPTER 2: LITERATURE REVIEW

2.0 Introduction

The world's population is anticipated to increase by 70 to 80 million every year (Alexandratos and Bruinsma, 2012). The human population in developing countries is estimated to increase to 9.7 billion by 2050 (United Nations Organisation, 2015). By 2050 the human population in Africa is estimated to be 2.4 billion (United Nations and World Population Prospects, 2015). The growth in the economies of developing countries [Sub-Saharan Africa (SSA), North Africa, and Asia] is driving fundamental changes in increasing global structure of food demand (Msangi et al., 2014). The combined increase in population and household incomes (Thornton, 2010) are key drivers of an increase in the demand of food including food of animal origin (Henchion et al., 2017). The demand and consumption of animal products worldwide, mainly poultry meat, has gradually increased and is expected to increase by 73% by 2050 (Alexandratos and Bruinsma, 2012; Brenes and Roura, 2010).

Poultry products (meat and eggs) are among the major forms of animal-derived dietary protein sources for human consumption (Magothe et al., 2012) and make a huge contribution to household food security in many developing countries (Alao et al., 2017; Chikwanha et al., 2017). Compared to red meats, poultry meat has as excellent flavour, is more tender, has a high protein and low lipid content and has moderately high carotenoid, vitamin and mineral content (Biswas et al., 2015; Nkukwana et al., 2014). Due to an increase in the dissemination of information in regard of the effects of food on human health, consumers have become more aware of the possible health-compromising effects of food and are thus demanding more healthful foods in the process of addressing malnutrition (Jeke et al., 2018; Goldberg et al., 2012). In the bid to safeguard health, consumers prefer natural foods rich in antioxidants (Kolarzyk et al., 2017) and leaner meat rich in EAAs and EFAs in order to mitigate possible diet-induced metabolic disease and cancers (Barbalho et al., 2016). Besides their physiological requirements to support normal physiology and health in adults, EAAs and EFAs are also crucial for early brain development in human (Nyaradi et al., 2013) and their deficiency at this critical stage of growth could lead to compromised mental health later in life (Cusick and Georgieff, 2016).

In the developing world, SSA included, the observed increase in the demand for poultry meat and eggs, which are relatively cheaper (Özturk and Kose, 2017), is premised on high population

growth and an increase in incomes. This increase in demand is occurring against a background of decreased animal (product) off-take from natural pastures due to a loss of the natural pasture to crop cultivation and urban settlements (Rust and Rust, 2013). This loss and or decrease in the contribution of farmed ruminants to food production for human consumption has and continues to necessitate the need to focus on alternatives such as poultry that do not depend on natural pasture. When compared to other farmed animal species, poultry has the advantages of a shorter generation intervals, less land requirement and importantly, relatively less product cost (meat and eggs) compared to other animal-derived protein sources. These relative merits contribute to the importance and centrality of the poultry industry in SSA and other developing regions in meeting the increased demand of animal products for human consumption.

2.1 Global and local poultry industry: an overview

The poultry industry has grown rapidly over the years (Brewer et al., 2012) and is the most dynamic agribusiness globally (Mehmood et al., 2013). World poultry production is estimated to increase by 24% over the next decade (OECD and FAO, 2017). Poultry products are estimated to reach 131.3 million metric tonnes by 2025 and poultry meat is deemed to be the most dominant (Heise et al., 2015). In 2017, the United States of America produced 20.6% of the global poultry production while Brazil, Europe and China contributed 14.7%, 13% and 12.9% respectively [United States Department of Agriculture (USDA, 2018)].

In SSA, poultry production plays a crucial role in the rural economies and food security of the populace as above 80% of rural households are actively involved in poultry production (Kryger et al., 2010). South Africa is one of the developing countries in SSA whose poultry industry is relatively well developed and produced 1.4% of the global poultry products while accounting for only 1.7% of worldwide consumption between 2013 and 2015 (OECD and FAO, 2016). The country (SA) was expected to increase its contribution to 4.76% of the global poultry production by 2018 (USDA, 2018). In 2015 and 2016, the South African poultry industry produced 1014.3 million day-old broiler chicks and 991.1 million day-old pullets, respectively [South African Poultry Association (SAPA, 2015)]. In 2015, 964.994 million broiler chickens were slaughtered while the number dropped to 935.572 million in 2016 (SAPA, 2016). Mnisi et al. (2017) contend that the poultry industry is one of the largest agricultural sectors and according to the SAPA

(2016) the industry contributes 64.4% of the total poultry protein consumed locally with the balance being imported from Brazil and the United States of America (OECD and FAO, 2016).

2.2 Chicken and the supply of animal protein in South Africa

Chicken is the dominant poultry species farmed in South Africa (Mkhabela and Nyhodo, 2011). Importantly, broiler chicken meat dominates the South African meat market. According to the Department of Agriculture, Forestry and Fisheries (DAFF, 2013), this dominance is premised on the relatively cheaper cost of chicken meat compared to other meats. In South Africa, the per capita consumption of chicken meat grew from 21.48kg in 2001 to 40.04kg in 2017 (DAFF, 2016), which growth is greater when compared to the per capita consumption of other meat products (beef, mutton and pork) which grew from 18.96kg in 2001 and 27.74kg in 2017 (DAFF, 2016). Rainbow and Astral are the major local poultry producers in South Africa (SAPA, 2015) whose combined production account for 55% of the total chicken meat consumed in the country (Hadebe, 2015). Production of the improved broiler and pullet chicken breeds is resource-intensive in terms of feed requirements, health demands and specialised housing requirements. These factors result in many potential smallholder farmers failing to adequately meet the requirements of the modern chicken production industry which (failure to meet the minimum requirements) compromises rural and smallholder household food and income security. Besides searching for and developing alternative feed resources, there is a need to also interrogate the potential of other poultry species that might be amenable to production by the smallholder farming community.

2.3 Alternative poultry species

Non-traditional poultry species such as Muscovy duck, Guinea fowl, and Japanese quail have been recommended as alternatives for meat and egg production in developing countries (Biswas et al., 2015). These non-traditional poultry species are characterised by many desirable attributes including hardiness with respect to feed, health and housing requirements (Gono et al., 2013; Kokoszyński et al., 2013), which make them relatively cheaper to produce by resource-limited smallholder farmers when compared to producing modern breeds of broiler and pullet chicken (Randall and Bolla, 2008). Currently, the meat and eggs from these non-conventional poultry species represent a very small portion of total output in the poultry industry and they (products) are found in specialist markets (Geldenhuys et al., 2013) thus making them unavailable to the

resource-limited consumer. There is a dire need to bring these non-conventional poultry species to be produced at household/ smallholder level as this will help alleviate the mismatch between the demand and supply of animal-derived products for human consumption (Biswas et al., 2015; Geldenhuys et al., 2013).

2.3.1 Muscovy Duck

Muscovy ducks originated in central and south America (Suci et al., 2017; Yakubu, 2013) but they are also found in Africa (Téguia et al., 2008). Globally duck production for meat is a growing food industry (Mazurowski et al., 2016). In Africa, a variety of duck species including the Muscovy duck form the second most popular poultry species used for meat and egg production after chicken (Adzitey, 2012; Téguia et al., 2008). In 2012, Asia countries, Europe and Africa contributed 85%, 5.5% and 4.7%, of the duck meat, respectively while America only contributed 0.40% (Biswas et al., 2015). Duck breast and thigh meat contain significant amounts of aspartic acid and glutamic acid as well as the EAAs lysine and methionine (Aronal et al., 2012). The meat is also rich in vitamins, especially niacin and minerals such as selenium and iron (Adzitey, 2012). Selenium is a component of the antioxidant enzymes that help mop up excess ROS and thus helps protect the body against potential damage from ROS (Rotruck et al., 2013). On the other hand, iron is an essential component of every living organism that helps the body to synthesise its oxygen transport and storage proteins, haemoglobin and myoglobin as well as in the formation of heme enzymes (Hurrell, 1997). Despite a favourable fatty acid profile Muscovy duck meat has a higher overall fat content compared to chicken, thus making it less healthy and acceptable by health-conscious consumers (Zeng et al., 2015; Jiang et al., 2012).

2.3.2 Guinea fowl

Commercial production of Guinea fowl is similar to that of broiler chickens (Nahashon et al., 2011), with the species raised mainly for meat since the consumption of Guinea fowl eggs is not very popular (Nobo et al., 2012). The production of Guinea fowl is important in the developing countries as a relatively cost-effective approach to alleviating poverty and improving the sustainability of rural economies (Madzimure et al., 2011; Mwale et al., 2008). Despite their many desirable attributes (Bernacki and Kokoszynski, 2013; Gono et al., 2013), compared to chicken, Guinea fowl have a slower growth rate and would require up to 8-weeks to reach 1kg (Uaperendua Tjetjoo et al., 2013). While Guinea fowl meat is darker when compared to chicken

meat, the meat (Guinea fowl) is tastier, has a higher protein and lower fat content when compared to broiler chicken meat (Jiang et al., 2012; Madzimure et al., 2011) and thus is a more healthy product. Another alternative poultry species is the Japanese quail.

2.3.3 Japanese quail

The Japanese quail is native mainly to Asia (Saidu et al., 2014; Onyewuchi et al., 2013) although it is also found in the Middle East, America (Minvielle, 2004) and in Africa (Chazara et al., 2010). Commercial production started in Japan in the twenty century, spreading to China and Europe (Nuernberg et al., 2011; Minvielle, 2004). When compared to broiler chicken, quail grow faster and require less initial investment as they demand less land area and less feed (ElKatcha et al., 2015). Quail provide valuable niche products such as eggs and meat (Onyewuchi et al., 2013; Owen and Dike, 2013) and are also used in research (Huss et al., 2008; Ophir et al., 2005). Both quail meat and eggs are high nutritive value products that are low in fat and cholesterol (Daikwo and Momoh, 2013) hence more healthy (Mussah, 2017) compared to chicken meat and eggs.

2.3.3.1 Japanese quail meat: traits and nutritional value

Japanese quail have gained much popularity among consumers (Ikhlas et al., 2011). The quail is characterised by high meat yield which (meat) has less shrinkage during cooking and easy to cook (Awan et al., 2017). The meat is typically lean (Ikhlas et al., 2011), has a lower total lipid content when compared to broiler chicken meat (Ioniţă et al., 2010) and is thus a healthier alternative for consumers (Dauda et al., 2014). The consumption of two servings of quail meat a day provides the human body with 27 to 28g of protein, constituting 11g of essential amino acids that cover up to 40% of human protein requirement (Nasr et al., 2017). Eating quail meat two times a day fulfils the human daily requirement of lysine, leucine, phenylalanine, tyrosine and valine that are based on age, physiological condition and physical activity (Genchev et al., 2008). The meat is also credited with improving body and brain development in children and the quality of breast milk in nursing women (Onyewuchi et al., 2013).

Table 2.1: Chemical composition of breast muscle of quail and broiler chicken (value per 100g)

Parameter	Quail	Chicken
Water	69.7	66
Energy (Kcal)	192	215
Protein (g)	19.6	18.6
Total lipid (g)	12.1	15.1
Cholesterol (mg)	76	75

Source: Ioniţă et al., 2010

2.3.3.2 Japanese quail: egg production and quality

Japanese quail pullets are highly productive. On average each hen produced 300 to 350 eggs annually (Hrnčár et al., 2014). Compared to pullet chicken, pullet Japanese quail are more efficient utilisers of feed (Martin et al., 1998). Japanese quail eggs have a high potential to be developed as a cheaper source of protein which is affordable to many members of the community (Olawumi, 2015). Apart from being high sources of quality protein, Japanese quail eggs are good sources of the vitamin B series (folate, vitamin B_{12}, pantothenic acid and riboflavin) and the minerals iron, phosphorus and selenium (Oladipo and Ibukun, 2017). Despite their being approximately one third (10-13g) the size of the chicken egg; the Japanese quail eggs are reported to have a nutritional value that is three to four times greater than that of chicken eggs (Thomas et al., 2016).

Table 2.2: A comparison of the chemical composition of quail and chicken raw egg (value per 100g)

Parameters	Quail	Chicken
Calories (g)	158	147
Protein (g)	13.1	12.6
Carbohydrate (g)	0.4	0.8
Total lipid (g)	11.1	9.9
Cholesterol (mg)	844	423

Source: Tunsaringkarn et al., 2013

The positive nutritional attributes of quail meat and eggs put the Japanese quail on a platform where it can complement broiler and pullet chicken production, particularly in resource-limited communities. The capacity of resource-limited communities to tap into and benefit from poultry production is usually hindered by the unavailability and the high cost of feed ingredients.

2.4 Feed ingredients for broiler and pullet poultry

Commercial poultry production both at small- and large-scale is dependent on the availability and cost of key feed ingredients used for the formulation of broiler and pullet poultry diets. In terms of their quantity in the diets, dietary energy and protein sources constitute the major feed ingredients in poultry feeds.

2.4.1 Dietary energy sources for poultry feeds

Globally, cereal grains (maize, wheat, barley, and sorghum) are commonly used as dietary energy sources in the formulation of poultry diets (Ravindran, 2013). Majority of the carbohydrates in these cereal grains occur as starch (Lafiandra et al., 2014) which (starch) is readily digested by poultry (Ghazaghi et al., 2012). Due to their high digestibility in the gastrointestinal tracts of poultry species, meals from these cereal grains serve as sources of readily available energy for the birds (Bhuiyan et al., 2013). The high digestibility of these meals translates into increased feed utilisation efficiency by the birds that manifest in increased growth performance (Elangovan et al., 2004). Of the cereal grain-derived meals, maize meal is the most used dietary energy source in broiler diets (Panda et al., 2010). On average, in most poultry diets maize meal constitutes about 50 to 60% of the diet (Ibitoye et al., 2012). In 2017, the global maize usage in animal feed industry was estimated at 613 million tonnes, up by 2% from the previous year while in SSA maize usage in animal feed was estimated at 14 million tonnes, which is 2% higher than the previous year (Sihlobo, 2017).

In large-scale commercial production wheat, maize and barley are used as major energy sources in animal feeds (Silveira et al., 2017; Svihus et al., 2005) while small-scale farmers tend to use cheaper sorghum, millet, rice straw and cassava (Selle et al., 2010). Of these cheaper alternatives, sorghum grain is the most commonly used alternative by small-scale farmers. Sorghum meal is seen as an “excellent” replacer of maize meal in poultry feeds (Silveira et al., 2017; Moraes et al., 2016) largely due to its nutritional characteristics that closely match those of the maize (Carolino

et al., 2014). Compared to other cereal grains, sorghum grain has a higher energy density (Selle et al., 2010) and higher protein content but has a low vitamin A content (Etuk and Charles, 2012). Regardless of its similarities with maize in chemical nutrient composition, sorghum has been associated with sub-optimal and inconsistent poultry performance (Selle et al., 2018; Truong et al., 2017) resulting from its high tannin content which limits feed digestion and absorption by forming complexes with dietary proteins and digestive enzymes (Mabelebele et al., 2017; Naumann et al., 2017). Mansoori and Modirsanei (2012) reported that in broiler chicks, excessive dietary tannins increase excretion of salivary and intestinal mucoproteins, bile acids, and hypersecretion of enzymes and endogenous loss of minerals compromising the health and productive performance of the birds.

2.4.2 Dietary protein sources for poultry feeds

Dietary protein sources in poultry feeds are of twin origin: animal- and plant-derived sources (Wang et al., 2015). Animal-derived dietary protein sources are largely meat and bone meal, blood meal, feather meal and fish meal (Moutinho et al., 2017). Plant-derived dietary protein sources are largely by-products of oil expression from oilseed crops, especially rapeseed and soyabean (Wanasundara et al., 2016; Cornelis et al., 2014) with sunflower and cotton seed as other sources. Animal-derived dietary proteins are deemed to have a high value in poultry diets (Meeker and Meisinger, 2015; Oliva-Teles et al., 2015) due to their excellent digestibility, higher essential amino acid and phosphorus content (Beski et al., 2015). Fishmeal, although a good source of dietary protein, it price has increased with the increase in demand by aquaculture (Soliman et al., 2017; Ren et al., 2018) and importantly, it cannot be used in poultry finisher diets due to the odour tainting it imparts to products (Liang et al., 2017).

However, due to the high cost (Tacon and Metian, 2008) and problem of odour tainting of animal products (Liang et al., 2017) associated with dietary fishmeal as well as the risk of zoonoses when using animal-derived dietary protein sources (Brookes, 2001) producers tend to make use of plant-derived dietary protein sources in livestock and poultry production. Of the plant-derived dietary protein sources, soyabean meal (SBM) is the most widely used globally (Florou-Paneri et al., 2014). The widespread use of SBM in livestock and poultry feeds is premised on its high protein content typified with a high concentration and balance of essential amino acids (Taher et al., 2017) as well as its high palatability and digestibility (Chisoro et al., 2018).

A high competition between the human food and the livestock feed industry requirements, for plant-derived dietary protein sources, especially SBM, has been reported (Henchion et al., 2017; Schader et al., 2015). In the developing world, this competition is exacerbated by the inadequacy of local soyabean production to meet human and livestock requirements for SBM thus such countries depend on costly SBM imports (Ncube et al., 2016). This scenario is true of SSA and South Africa with the latter relying on imports from the Americas and Europe (Ncube et al., 2017). Between the year 2013 and 2016, SSA countries imported 3.3 million tonnes of SBM annually (Khojely et al., 2018) and SA was the leading importer with annual imports of over 1.5 million tonnes to meet the gap from local production (Rusike et al., 2012).

2.4.3 Feed cost and potential of non-conventional feed ingredients

Feed costs constitute about 60 to 70% of total poultry production costs (Swain, 2016; Thirumalaisamy et al., 2016). Maize production in SSA and rural SA does not meet the demands for human consumption which necessitates importation to meet the human and livestock (poultry) requirements. The inadequacy is not limited to the dietary energy source, maize, but to the dietary protein source, SBM. This scenario largely results in poultry being fed, where available, poor quality maize meal which compromises productive performance. There is, therefore, a great need to search and develop alternative dietary protein and energy sources which are locally available and pose little if any competition between humans and livestock. The development of such local alternatives with reduced and or no competition between animals and humans will result in the production of low-cost poultry feeds that would translate to increased production and more secure household food security.

2.4.3.1 Indigenous tree seeds as feed ingredients

Previous research in our laboratory has shown that indigenous tree seeds can be exploited as dietary protein (Chivandi et al., 2016) and energy (Chivandi et al., 2011) sources, respectively. In a study where mature and growing Sprague Dawley rats modelled monogastrics, the replacement of SBM as a dietary protein source with defatted *Ximenia caffra* kernel meal was observed not to affect total tract digestibility of nutrients, nitrogen balance and growth performance (as measured by body mass gain) of the rats (Chivandi et al., 2018) which is indicative of its potential (*X. caffra* kernel meal) as possible dietary protein source in monogastric animal feeds. *Mimusops*

zeyheri seed meal, although reported to have a low CP content (comparable to that of maize), thus cannot be used as a dietary protein source in poultry feeds, its energy content is reported to be 24.34 ± 0.56 MJ/kg DM which is 30 - 47% times greater than that of maize meal (Chivandi et al., 2011). Thus *M. zeyheri* seed meal can potentially be exploited as a dietary energy source in poultry feeds, hence the evaluation of its potential to replace maize meal as a dietary energy source in quail diets in the current study.

The use of these non-conventional feed ingredients, among other factors, influences the growth and productive performance, health and product quality of poultry, including quail.

2.5 Factors affecting poultry growth performance

While it is known that a multiplicity of factors singly and in combination influence the growth and productive performance, health and product quality of poultry, in regard of the current discourse, I intentionally focus my discussion on the following: animal genetic makeup, the environment as defined by the housing, ambient temperature, humidity, and diet.

2.5.1 Genetic make-up of the bird

Genetic selection along with the provision of adequate nutrients are some of the key factors that have enhanced the tremendous growth of modern broiler chicken industry (Kong et al., 2018; López-Andrés et al., 2018). The selection criteria in poultry have resulted in a transition from dual-purpose to specialised meat and egg producers (Muth et al., 2018). In comparison to their unselected indigenous broiler chickens (Zhao et al., 2018; Sokołowicz et al., 2016), modern broiler chickens are genetically selected for high growth rates, efficient feed conversion rates, high breast yield (Tallentire et al., 2018). In the mid-20th century (around 1953), it took broiler chicken producers more than 70-days to rear chickens to a final body weight of 1.5kg (Fouad and El-Senousey, 2014). In contrast, due to the effects of genetic selection for increased productivity, modern broiler only take 42 days for chicken producers to fatten broiler chicken to about 2.5kg body weight (Batkowska et al., 2017). The huge cut in the numbers of days it now requires to rear broiler chicken is ascribed to an improvement in the productive traits of the birds brought about by the genetic improvement. Zheng et al. (2014) stated that, despite the success of breeding programs in increasing meat production, the high selection intensity tend to result in negative impacts on meat quality characterised mostly by an increase in the total fat and cholesterol

content which (the lipids) are known to lead to obesity and an increase in the prevalence of obesity-associated metabolic diseases in consumers.

The selection for increased body weight in Japanese quail has been observed to improve in a few generations (Varkoohi and Kaviani, 2014). Importantly, the positive correlation between body weight and carcass traits has meant that by selecting for increased body weight, an added benefit of improved carcass traits (Daikwo and Momoh, 2013) has been realised and this correlation bodes well for meat and egg production which are fundamental for commercialisation (Grieser et al., 2018). While it is contended that the optimal age for slaughter for broiler quail is 4-5 weeks (Razee et al., 2016; Genchev et al., 2008) since it has been observed that their body weight gain increases until 4 weeks of age (Seker et al., 2007), it has to be noted that the age at slaughter is dependent on growth rate, feed consumption and feed conversion rate (Grieser et al., 2018; Sharif et al., 2018).

2.5.2 Housing

The provision of a conducive environment in poultry houses is fundamental to the maximisation of the genetic potential broiler chickens (Lima, 2011). Modern poultry housing is designed to minimise heat loss and improve energy efficiency (Costantino et al., 2018). Poor ventilation increases the environmental carbon dioxide and ammonia concentration that cause ocular abnormalities, eye lesion, structural damage to the lungs, skin and respiratory problems (Arruda et al., 2016; Wang et al., 2010) and sudden deaths (Gillespie et al., 2016; Olanrewaju et al., 2008). In addition, low ventilation levels in poultry houses allow for temperatures and humidity increases which (increases) compromises the thermoregulation abilities of the birds (Schwartzkopf-Genswein et al., 2012) which then (birds) respond with excessive thermal panting which is known to causes respiratory alkalosis, increases in blood pH and death of the chickens (Abidin and Khatoon, 2013).

One of the major determinants of conducive environmental housing conditions is the stocking density (Mesa et al., 2017). High stocking density strategies employed by some broiler chicken producers causes physiological stress and compromises carcass yield and quality (Arruda et al., 2016) and have been shown to reduce feed intake, weight gain and feed utilization efficiency (Mesa et al., 2017). In broiler chicken production, stocking density is a very important welfare

factor which impacts on the birds' walking space (Li et al., 2017) and developmental capability (Popoola et al., 2017). The National Chicken Council (2010) recommends 36.6kg/m^2 for a broiler chicken weighing 2.0 to 2.5kg and 62.8 to 76.2kg/m^2 for a chicken nearing market weight of 2.27kg. Jatoi et al. (2013) and Küçükönder et al. (2014) stated that for mature Japanese quail the stocking density is influenced by the housing type with a floor space of between 200 to 250cm^2 per bird recommended in a litter system and a floor space of 150 to 210cm^2 per bird desirable for the cage system.

2.5.3 Ambient temperature and humidity

Global warming phenomenon is a serious challenge that is faced by the poultry industry in the tropical and subtropical regions (Mehaisen et al., 2017) where season-induced changes in ambient temperature and humidity are common (El-Kholy et al., 2018). Hot and humid conditions that obtain during the rainy season (hot and wet) lead to heat stress (El-Kholy et al., 2018) which (stress) leads to behavioural and physiological adaptations that come at a cost to productive performance (Alagawany et al., 2017; Attia et al., 2017; Bölükbaşı et al., 2016). Heat stress activates the hypothalamus-pituitary-adrenal axis which manifests with increased corticosterone concentration and the production of inflammatory cytokines as well as lower thyroid hormone production; a cocktail which leads to poor bird productivity (Alhenaky et al., 2017; Soleimani et al., 2011). Additionally, heat stress causes oxidative stress and oxidative damage through the increment of mitochondria-generated reactive oxygen species (ROS) leading to a compromised antioxidant status (Akbarian et al., 2016; Huang et al., 2015). These behavioural and physiological adaptations that result from high ambient temperature induced stress negatively affect feed intake and feed utilization efficiency resulting in compromised growth performance (El-Kholy et al., 2018). The overall effect is an economic loss by producers (Bonfim et al., 2016) due to subnormal growth performance and an increase in mortality (Vale et al., 2016). The high ambient temperature-induced reduction in productive performance is well document in broiler chickens (El-Deep et al., 2014), pullet chickens (Oliveira et al., 2014), turkeys (Veldkamp et al., 2003) and quail (Ozbey and Ozcelik, 2004), hence the fundamental requirement that poultry housing provides for adequate ventilation in order to ensure the provision of an optimal production environment.

2.5.4 Diet

The formulation and provision of nutritionally adequate diets for animals, including birds, is a critical determinant of growth performance (Zancanela et al., 2015). Besides being critical determinants of poultry growth performance, poultry diets also strongly influence the flavour of the meat (Jayasena et al., 2013) and hence impacting the acceptability of the product. In birds, energy is the most important nutritional constituent from the standpoint of total cost and quantity of bird feed (Jahanian and Edriss, 2015). Thus the provision of adequate dietary energy is vital to broiler chicken/quail production (Baéza et al., 2015). One of the major dietary ingredients for broiler and pullet chicken and quail diets is lipid in the form of fat and or oil (Donaldson et al., 2016; Cherian, 2015). They (fat and or oil) are used to enhance the palatability of the diet, the absorption of fat-soluble vitamins, and to regulate the passage rate of the digesta in the gastrointestinal tract (Khatun et al., 2017). These dietary lipids also boost the energy content of the diets (Abdulla et al., 2017; Baéza et al., 2015). As is the case in other intensive animal production systems, energy is known to be the pacesetter of poultry production (Baéza et al., 2015) and dietary fatty acid profile has been shown to affect meat quality with diets rich in polyunsaturated fatty acid resulting in more tender and juicier meat (Abdulla et al., 2015) but whose shelf-life is compromised through increased susceptibility to oxidative damage that manifests in rancidity (Estévez, 2015).

In intensively farmed poultry species the supply of nutritionally balanced diets is crucial to meeting the birds' maintenance, temperature regulation and growth requirements (Beski et al., 2015; Mbajiorgu et al., 2011). Such diets usually derived 60 to 75% of their metabolisable energy content from cereal grains (Panda et al., 2014) and about 25% of their protein content largely from legumes and to some extent fishmeal (Andersson, 2014). In quail diets, protein quality is of great importance compared to the level in the diet for the maintenance of metabolic processes and growth performance (Dumont et al., 2017).

While the discourse above has focused more on the factors that affect the productive performance (growth and health) of the birds, it is essential that poultry producers have the requisite knowledge regarding the measurement of growth performance and the different types of indices used to determine growth performance.

2.6 Measures of growth performance

There are many ways of measuring the growth performance of poultry species, including chicken and quail. My discourse will focus more on the measures of body weight, empty carcass weight and the issues surrounding the use of weight-based measurements [gut fill, hydration status, body composition (lean versus fatty carcasses)].

Growth and development are important traits for all living things (Karadavut et al, 2017). Several methods such as body weight gain, average daily gain, lean weight gain and linear growth are used to estimate the animal growth performance (Lukuyu et al., 2016; Alves and Franzolin, 2015). In birds, growth of the GIT is stimulated when they have adequate access to nutrients and water. A fully developed GIT is instrumental to efficient nutrient digestion and absorption which manifest in two key elements: improved gut integrity and increased growth performance (Skinner-Noble and Teeter, 2004). Increasing and or decreasing feed intake causes differences in GIT fill and hydration status and has a bearing on body mass (Cameron, 2002). Thus making use full body mass as an indicator of growth performance is inaccurate as it falls foul to variances resulting from the amount of digesta and water in the GIT (Cameron, 2002). To account for the effect of the GIT fill, some authorities recommend the use of empty carcass mass as a measure of growth performance (Owens et al., 1995). Anti-gravity (long) bones, for example, the tibiae and femora, response to growth hormone in a dose-dependent manner (Rol De Lama et al., 2000), thus making them a better proxy for the estimation of growth performance (Sanchez and He, 2009). Hence the determination of linear growth based indices (length, weight and Seedor ratio) of such long bones gives a more accurate measure of animal growth performance (Eshet et al., 2004; Zeffer, 2003).

2.7 Factors affecting health

Over above the negative impact of environmental factors, the rearing system and dietary composition on the growth and health status of commercially reared chicken and quail, hygiene standards, overcrowding, air quality and anti-nutritional factors also affect bird health and product quality.

Poor control and management of environmental factors in poultry production result in poor bird welfare which results in increased mortalities and decline in meat quality (Moravej et al., 2012;

Sogunle et al., 2008). Birds reared for commercial purposes are raised under various stressors including heat stress due to high ambient temperature, nutritional stress due to dietary nutritional imbalance, overcrowding stress due to housing of a high number birds per unit space (Selvam et al., 2017; Fontana et al., 2015). Frequent occurrence of such stressors results in the excessive stimulation of the hypothalamic-pituitary-adrenal (Quinteiro-Filho et al., 2010) resulting in increased blood corticosterone concentration which induces oxidative derangements that negatively affect productive performance and health status of birds (Selvam et al., 2017).

While growth performance and the health status of birds (chicken and quail) are critical to successful poultry production, it is important to interrogate the effects/impact of management practices on product (meat and egg) quality. The discourse below briefly reviews the literature on meat quality.

2.8 Meat quality

Meat quality speaks to as the compositional quality and the palatability factors (Batkowska et al., 2017), that collectively coalesce to descriptions such as firmness, juiciness, tenderness, colour, odour and flavour (Mir et al., 2017). Carcass conformation, good sensory appraisal, aesthetics and nutritional composition are the key parameters assessed when evaluating for meat quality (Bogosavljevi et al., 2010). Furthermore, quantifiable properties including pH, colour, shear force, water-holding capacity and cooking loss, which largely determine acceptance of the meat by consumers, are also evaluated in the process of determining meat quality and acceptance by consumers (Narinc et al., 2013). A multiplicity of factors, among others, age at slaughter, slaughter process and breed impact meat quality (Radu and Popescu-Miclosanu, 2012).

Several studies have investigated the effect of intensive farming and free-range systems on meat quality, composition, and taste, but results are not consistent (da Silva et al., 2017). The skin and meat colour of slow-growing chickens reared in free-range was reported to be yellower and darker (Ponte et al., 2008) compared to that of fast-growing commercially reared broiler chickens (Sales, 2014). The yellow and dark colour are ascribed to the tainting effect of natural carotenoids that are found in grass and other plant parts consumed by the “grazing” chickens (Sales, 2014). The breast and thigh muscle of chicks kept on a free-range basis were reported to be darker when compared to those from intensively reared chicks (Mikulski et al., 2011) probably due to slower

growth rate which results in red (slow-twitch) muscle fibre that have large amounts of myoglobin in comparison to white muscle (Huff-Lonergan and Lonergan, 2005). The high myoglobin content allows greater oxygen storage and results in the red colour associated with dark poultry meat (Adzitey and Nurul, 2011). Carcasses from the slow-growing chickens were found to have a better ability to keep water in meat (muscle) after thermal processing but the meat was noted to be less tender compared to that of their fast-growing counterparts (Michalczuk et al., 2016). This indicates a loss of nutritional value through exudates due to exposure of birds to fluctuating temperatures and increased exercise in yards which affect the water content of the muscle (Wang et al., 2009).

2.8.1 Meat pH

The muscle pH is an important contributing factor to meat quality characteristics such as colour, tenderness, water-holding capacity and shelf-life (Kim et al., 2014). Biochemical and physical changes that take place at different times during post-mortem muscle metabolism influences quality characteristics (Tougan et al., 2013). Muscle metabolism is affected by pre-slaughter stress (Frizzell et al., 2017) and anaerobic glycolysis (Chauhan and England, 2018). The pre-slaughter stress is largely caused by poor transportation to slaughter, poor lairage conditions and slaughter protocol (Frizzell et al., 2017). Pre-slaughter stress causes depletion of muscle glycogen stores (Muchenje et al., 2009b) which translates into darker meat after slaughter (Ponnampalam et al., 2017). Anaerobic glycolysis converts the muscle glycogen to lactate (Chauhan and England, 2018) which then results in adenosine triphosphate hydrolysis (Warner et al., 2017). The breakdown of adenosine triphosphate and an increase of lactate causes a decline of muscle pH from 6.2 within the first 12 hours (pHi) to 5.6 (pHu) 24 hours post-slaughter (Ponnampalam et al., 2017; Glamoclija et al., 2015). This decrease in pH downfall results from the development of lactic acid derived from an anaerobic glycogenolytic pathway that uses glycogen to obtain glucose and energy (Ferguson and Gerrard, 2014). It impacts meat quality in the sense that it causes the development of rigour mortis (Warner, 2015).

Genchev et al. (2010) reported the pHu of Japanese quail meat to be between 6.00 and 6.17. It has been reported that meat with an pHu greater than 6.5 tends to be darker and firmer while meat with an pHu less than 5.8 is generally paler and softer meat (Garcia et al., 2010). Additionally, meat with higher pHu tends to have a higher water-holding capacity (WHC). The latter (higher

WHC) causes the meat to have a more compact structure (Bihan-Duval et al., 2018). This compact structure prevents oxygen diffusion in the muscle decreasing the amount of oxymyoglobin formed and reduces the amount of light reflected from the surface of the meat leading to meat of a darker colour (Harford et al., 2014). On the other hand, when meat is exposed to conditions which promote the oxidation of myoglobin to metmyoglobin, the colour of the meat becomes less dark (Barbut, 2015). A decrease in myoglobin concentration and increased light scattering at the surface of the meat contribute to meat being perceived as pale (Barbut, 2015). Both darker and paler meat defects reduce consumer acceptability, shelf life and yield of meat, thus consumers associate PSE and DFD meat with poor quality meat and that perception affects the profits tremendously (Adzitey and Nurul, 2011).

2.8.2 Meat colour

Poultry meat is unique because it is sold with and without its skin (Mir et al., 2017). The appearance and uniformity of both skin and meat are an important quality attribute (Ramirex-Hernandez et al., 2018). Meat colour is the primary quality characteristic that consumers usually become aware of before they purchase meat (Troy and Kerry, 2010; Karaoğlu et al., 2006). It (meat colour) is commonly quantified by the CIE-L^* (black and white), a^* (red-green) and b^* (blue-yellow) values (Girolami et al., 2013). The ideal value for quail breast meat lightness (L*) should be between 43 and 53 (Harford, Pavlidis and Anthony, 2014), whilst redness (a*) ranges from 0.96 and 4.50 and yellowness (b*) ranges from 6.7 to 13.5 (Narinc et al., 2013). Meat colour depends on the myoglobin concentration, haemoglobin chemical state and physical characteristics of the meat (Bak et al., 2017). These factors are influenced by the type of breed, age and diet (Mancini and Hunt, 2005). There are three chemical forms of myoglobin: deoxymyoglobin, oxymyoglobin and metmyoglobin, that are known to impact meat colour (Suman and Joseph, 2013). The relative proportion of these variants of myoglobin which invariably affects meat colour are influenced by storage conditions, availability of oxygen, active enzymes and reducing compounds in the muscle (Suman and Joseph, 2013).

Poultry meat has a lesser concentration of muscle myoglobin compared to red meat (Omana et al., 2011). The latter (muscle myoglobin) prolongs the red colour of meat which (red colour) consumers associate with freshness (Omana et al., 2011). The colour of raw chicken meat ranges from white to yellow and is dependent on the myoglobin concentration in chicken portions: with

breast muscle tending to be light (white) coloured while the thigh muscle tends to be of a red colour (Bak et al., 2017; Kruk et al., 2011). There are several factors that influence the chicken skin colour, such as rearing system, handling and slaughter conditions, processing, and packaging of meat (Radu and Popescu-Miclosanu, 2012; Petracci et al., 2005). Additionally, fat content, active acidic pH and the structure of muscle tissue also affect meat colour (Kong et al., 2018; Bak et al., 2017). Muscle fibre types are important for meat quality because they characterise the white and dark meat (Genchev et al., 2010). A high percentage of white myofibrils (fast twitch, glycolytic) is associated with light-coloured meat with low fat and myoglobin, whereas darker meat has a higher content of oxidative or oxido-glycolytic muscle fibres, which contain more myoglobin and fat because of their higher oxidative metabolism (Zerehdaran et al., 2012).

2.8.3 Meat tenderness

Meat tenderness is the most important eating quality trait because it strongly influences consumer's perception of acceptability (Lee et al., 2017) which is determined by the contractile state of the muscle, endomysium thickness and fibre diameter (Ismail and Joo, 2017). Many external factors contribute to the extensive differences in meat tenderness such as the bird strain, gender, age at slaughter, antemortem and post-mortem handling and cooking method (Ismail and Joo, 2017; Maiorano et al., 2012). Tenderness is mainly affected by the amount and solubility of connective tissue, composition and contractile state of muscle fibres and the extent of proteolysis during muscle rigour (Joo et al., 2013a). The network of collagen and elastic fibres in the matrix of proteoglycan is made up of endomysium, perimysium, and epimysium (An et al., 2010; Listrat et al., 2015). Although the perimysium represents approximately 90% of total connective tissues in muscles (Mccormick, 1999), its amount fluctuates from one muscle to another compared to the amount of endomysium (An et al., 2010). Liu et al. (2016) found that the shear force value of poultry meat increases linearly with the increasing thickness of perimysium in bird muscle.

The big diameter of the muscle fibres and a low score of fat tissue produce tough meat, while the small diameter of muscle fibres and a high score of fat tissue produce tender meat (Ismail and Joo, 2017; Chartrin et al., 2006). Muscles having a high activity level usually have a big muscle fibres diameter. It is due to the high activity that increases muscle contractions which cause muscle hypertrophy and causes the enlargement of the muscle fibres diameter (Kartikayudha et al., 2013). High activity in muscle also causes a low score of fat tissue because fat is depleted as

it is oxidised in support of the exercise activity (Kartikayudha et al., 2013). Intramuscular fat (IMF) content is reported to indirectly affect meat tenderness with greater marbling associated with increased tenderness (Lee et al., 2017) which is linked to increased consumer acceptability of the meat.

2.8.4 Water-holding capacity

Water-holding capacity (WHC) is defined as the ability of fresh meat to retain its own water (Pearce et al., 2011). Water represents approximately 75% of muscle tissue thus it is a major component of meat (Pearce et al., 2011). The water in meat is organised in layers located around polar molecules and among stratums of cellular materials (Pearce et al., 2011). It is (WHC) branded as one of the major quality characteristics of fresh meat and influences product yields for processors and sensory quality for consumers (Bowker et al., 2014). Pearce et al. (2011) reported that factors such as pH, ionic strength, osmotic pressure and sarcomere length affect WHC as they all influence the distance between myosin and actin/tropomyosin. Meat parameters including water-holding capacity, cooking loss (CL) and pH are strongly correlated. An increase in meat pH is positively correlated to WHC but negatively correlated to CL (Jiang et al., 2011). Fluid loss during meat processing and packaging result in fresh meat being criticised by the consumers because of the changes in palatability; a sensory property of meat (Wright et al., 2005).

The following chapter details the materials used in the trial, the design of the feeding trial and data collection during the trial as well as the methods used in carrying out various assays on collected tissue samples. Additionally, the procedures on data analysis and presentation are given.

CHAPTER 3: MATERIALS AND METHODS

3.1 Experiment 1: Chemical Nutrient and Anti-Nutrient Composition of *Mimusops Zeyheri* Seed Meal

3.1.1 Mimusops zeyheri seed source and processing

The *Mimusops zeyheri* seeds used were extracted from *M. zeyheri* fruits harvested from trees in Matopos National Park, Zimbabwe. Matopos National Park on GPS coordinates: 20° 25' S, 28° 29' E and altitude: 1320m. The area has a mean annual rainfall of 609mm, mean minimum temperature of 10.8°C and mean maximum temperature of 25.9 °C (Mushove et al., 1995). Prior to chemical characterisation, the *M. zeyheri* seeds were mechanically dehulled using a manual crusher. The dehulled seed was separated from the seed hulls by blowing them (hulls) off using a Kenwood electric fan (IF450, China). The resultant dehulled seeds (kernels) were then ground into a seed meal using a blender (Household Grain Mill, Bioexcel, Jiangsu, China).

Picture: A – *M. zeyheri* seed

Picture: B – dehulled *M. zeyheri* seed

Figure 3.1: *Mimusops zeyheri* seed and dehulled *Mimusops zeyheri* seed

3.1.2 Determination of the chemical nutrient composition of the seed meal

The chemical nutrient composition (proximate, energy, fibre, mineral and amino acid content and fatty acid profile) of *M. zeyheri* seed meal was assayed at the Agricultural Research Council`s Irene Analytical Service Laboratories (Pretoria, South Africa) and energy content was assayed at the University of Pretoria's Nutrilab (Pretoria, South Africa). All samples were assayed in triplicate.

3.1.3 Determination of proximate composition

The proximate composition of *Mimusops zeyheri* seed meal [dry matter (DM), crude protein (CP), ether extract (EE) and ash] content was determined as described by the Association of Official Analytical Chemists (AOAC) (2005; method numbers 930.15, 984.01, 920.39 and 942.15, respectively). Organic matter was estimated as the difference between dry DM and ash content. The gross energy (GE) of the meal was determined using an MC-1000 Modular Calorimeter attached to a personal computer with MC1000 software (Energy Instrumentation, Centurion, South Africa).

3.1.4 Determination of the amino acid content

The amino acid concentration content of the seed meal 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 hours 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). Identification of the amino acids was done at an excitation wavelength of 264nm and an emission wavelength of 340nm. A PC equipped with TSP software was used for quantification.

3.1.5 Determination of the mineral composition of the seed meal

The mineral content of the seed meal was determined as described by Zasoski and Burau (1977). Briefly, 0.5g of seed meal sample was digested into 25ml of 65% nitric acid and 5ml of perchloric acid at 200°C. The digest solution was then used to spectrophotometrically determine the mineral (calcium, magnesium and phosphorus) 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).

3.1.6 Determination of the fibre content

The neutral detergent fibre (NDF) and acid detergent fibre (ADF) components of the *M. zeyheri* seed meal were determined as described by Van Soest et al. (1991). Briefly, NDF was determined by refluxing 0.5g of the seed meal in 100ml of the neutral detergent solution of sodium lauryl sulphate and ethylenediamine-tetraacetic acid to which a heat-stable alpha-amylase (20 350 IU/ml) (dietary fibre kit, Sigma-Aldrich) was added. After refluxing for 1 hour, the mixture was filtered; the residue was dried and then weighed. The ADF was determined by refluxing 0.5g of seed meal in 20g cetyl-trimethyl ammonium bromide (acid detergent solution) dissolved in 1L NH_2SO_4 for 1 hour. After an hour of refluxing, the mixture was filtered and the remainder of the mixture was desiccated and weighed.

3.1.7 Determination of the fatty acid profile

The Soxhlet method was used to extract the oil from the seed meal as described by AOAC (2005; method number 920.39) and fatty acid profiles were determined as described by Christopherson and Glass (1968). Briefly, the oil extracts were trans-methylated with 2mol/L methanol sodium hydroxide. The resulting fatty acid methyl esters were extracted in heptane, filtered and dried under nitrogen after which they are separated by a temperature gradient over 45 minutes on a gas chromatograph 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 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.

3.1.8 Determination of the phytochemicals content

Preliminary qualitative phytochemical screening tests were carried out to determine the major classes of phytochemicals present in the *M. zeyheri* seed meal.

3.1.8.1 Test for saponins

The presence of saponins was determined as described by Ejikeme et al. (2014). Briefly, 30ml of distilled water was added into 0.30g of *M. zeyheri* seed meal and boiled for 10 minutes in a water bath and filtered using a filter paper. A mixture of distilled water (5ml) and filtrate (10ml) was

agitated vigorously for a stable persistent froth. The formation of emulsion on the addition of three drops of olive oil showed a positive result.

3.1.8.2 Test for terpenoids

The presence of terpenoids was determined as described by Ejikeme et al. (2014). Briefly, 0.30g of *M. zeyheri* seed meal was weighed into a beaker and then carefully mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid to form a layer at the bottom. The formation of a reddish-brown colouration of the interface indicated the presence of terpenoids.

3.1.8.3 Test for tannins

The presence of tannins was determined as described by Ejikeme et al. (2014). Briefly, 0.30g of *M. zeyheri* seed meal was weighed into a beaker containing 30ml of distilled water. The mixture was then boiled in a water bath for 10 minutes and then filtered using a filter paper (Whatmann®, No 1, size 185mm, pore size 7-11µm, England). Three (3) drops of 0.1% ferric chloride were added to the 5ml of the filtrate. The development of a brownish-green colouration showed confirmed the presence of tannins.

3.1.8.4 Test for flavonoid

The presence of flavonoids was determined as described by Ejikeme et al. (2014). Briefly, 0.30g of *M. zeyheri* seed meal was weighed into a beaker and mixed with 30ml of distilled water and then the mixture was allowed to stand for 2 hours following which it was filtered using a filter paper (Whatmann®, No 1, size 185mm, pore size 7-11µm, England). Five (5) ml of 1.0M dilute ammonia solution was added to 10ml of the aqueous filtrate followed by the addition of 5ml of concentrated sulphuric acid. The development of a yellow colouration which disappeared on standing indicated the presence of flavonoids.

3.2 Experiment 2: *In vivo* evaluation of *M. zeyheri* seed meal on growth, health and meat quality of broiler quail

3.2.1 Study site

The study was conducted at the Central Animal Service facility and the School of Physiology laboratories, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

3.2.2 Animal ethical clearance

Ethical clearance for the study was obtained from the University of the Witwatersrand Animal Ethics Screening Committee (AESC number: 2017/08/56/B). The experimental procedures carried out during the study complied with the internationally accepted principles and guidelines on the care and use of laboratory animals (National Research Council, 2010).

3.2.3 Feed ingredients and diet preparation

The *M. zeyheri* seeds were obtained from Matopos National Park, Zimbabwe while whole yellow maize, canola oil and salt were purchased from Makro Wholesalers, Woodmead, Johannesburg, South Africa. Methionine, lysine, and vitamin and mineral premix were supplied by Trouw Nutrition Group, Isando, Johannesburg, South Africa. Meadow Feed Company (Johannesburg, South Africa) supplied wheat bran and limestone while the Agricultural Research Council's Irene Animal Production Institute (Pretoria, South Africa) supplied SBM. The whole yellow maize grain was ground into a meal using a hammer mill prior to use in the diet formulation. Four iso-caloric and iso-nitrogenous finisher dietary treatments were formulated to meet the nutrient requirements for finisher broiler Japanese quail as prescribed by the National Research Council (NRC, 1994). The *M. zeyheri* seed meal (MZSM) replaced maize meal (MM) on a gross energy basis. The upper MZSM concentration in the test diets was determined in a pilot study where it was discovered that replacement of the MM with MZSM, on a gross energy basis, beyond 37.5% compromised feed intake. The dietary ingredients of the dietary treatments are shown in Table 3.1.

Table 3.1: Ingredient of the dietary treatments

	Diets			
Ingredients (g/kg)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Soyabean meal	403.00	407.00	415.00	395.00
Yellow maize meal	410.00	364.00	316.00	263.00
**Mimusops zeyheri* seed meal (%)	0.00 (0)	36.00 (12.5)	74.00 (25)	110.00 (37.5)
Wheat bran	136.00	145.00	147.00	201.00
Soyabean oil	21.00	19.00	18.00	4.00
Limestone	19.00	16.00	17.00	14.00
DL-Methionine, 99%	2.00	2.00	2.00	2.00
L-Lysine HCL 98.5%	1.00	1.00	1.00	1.00
Salt	4.00	5.00	5.00	5.00
Vitamin and mineral premix	4.00	5.00	5.00	5.00
Total	**1000**	**1000**	**1000**	**1000**
Chemical composition				
Dry matter (g/kg)	924.60	914.30	902.30	907.50
Crude protein (g/kg)	240.50	240.40	240.60	240.60
Ether extract (g/kg)	46.10	50.60	57.00	51.90
Calcium	8.20	8.30	8.60	8.40
Phosphorus	4.60	4.40	4.20	4.20
Gross energy (MJ/kg)	17.03	17.02	17.02	17.07

The figures in parenthesis show the percent replacement of MM with MZSM on a gross energy basis.
**M. zeyheri* seed meal inclusion is on a percentage (%) basis.

3.2.4 Study animals, housing and feeding

Thirty-two (32) male broiler quail used in the study were sourced from Rockliff Farm, East London, South Africa. Following veterinary approval [Application under section 20 of the animal disease ACT. 1984 (Act No 35 of 1984)] the birds were transported from the Eastern Cape Province to the University of the Witwatersrand's Central Animal Services unit at the Faculty of Health Sciences in Johannesburg. On arrival, the birds were each housed individually in a cage (0.60m length x 0.60m width x 0.80m height) and allowed a 2-day habituation period and de-

wormed using piperazine (Kyron Laboratories Pty Ltd, Johannesburg, South Africa) at a dose of 90mg/L in drinking water. Each cage had a cardboard box for shelter and perches for environmental enrichment. Clean straw was used for bedding. The bedding was changed twice weekly. Each bird had *ad libitum* access to feed and clean drinking water. Room temperature was maintained at 25±2°C throughout the course of the experiment. A 12-hour lighting cycle was provided: lights on from 19:00h to 07:00h.

3.2.5 Experimental design

Thirty-two (32) five-week old male broiler quail with the mean induction body weight of 156 ± 16.97g were randomly allocated to the four dietary treatments (n = 8) wherein MZSM replaced MM on a gross energy basis as follows diet 1: 0% MZSM + 100% MM, diet 2: 12.5% MZSM + 87.5% MM, diet 3: 25% MZSM + 75% MM and diet 4: 37.5% MZSM + 62.5% MM and fed for 4 weeks.

3.3 Measurements

3.3.1 Body mass

The birds' body masses were determined immediately on arrival, following the two-day habituation period and thereafter twice weekly as part of the routine health monitoring. An electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan) was used to measure the body masses.

3.3.2 Feed intake

The birds` feed intake was determined by subtracting refusal from the total feed given to each bird on a daily basis. Feed offered and refusals were weighed on an electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan). The feed refusals were measured and recorded prior topping up the feed craft again each morning.

3.3.3 Computations

Body weight gain (BWG) over the experimental period was computed using the equation: BWG = final body weight – induction body weight. Average daily gain (ADG) was computed using the equation: ADG (g) = body weight gain / duration (days) of feeding trial. Feed intake (FI) was computed using the equation: FI (g) = feed offered – feed refusals; with feed offered weighed in

the morning and refusal weighed the following day in the morning. Feed conversion ratio (FCR) was computed using the equation: FCR = feed intake (g) / weight gain (g).

3.4 Terminal procedures and measurements

At the end of the 4-week feeding trial, the birds were subjected to a 4-hour fasting period prior to termination but had access to clean drinking water. Each bird was humanely killed using a guillotine to decapitate. Following decapitation, blood was collected into 10ml heparinised blood collection tubes (Becton Dickinson Vacutainer Systems Europe, Meylan Cedex, France). The heparinised blood samples were centrifuged at 5500×*g* for 15-min in a centrifuge (Hermle Centrifuge Z230 A, Berthold Hermle AG, German) to yield plasma. The plasma was then harvested and stored in microtubes (Eppendorf, Hamburg, Germany) at -20°C till assay for plasma surrogate markers of liver and kidney function and general health profile.

3.4.1 Determination of viscera macro morphometry

After collection of blood, feathers were plucked off. The bird`s abdomen was dissected through a midline incision using a pair of scissors. Viscera (proventriculus, stomach, ventriculus, small and large intestines, liver, caecum, visceral fat and pancreas, heart and testis) were carefully dissected out. Prior to the weighing of GIT viscera, digesta was gently removed from the respective GIT viscera. The small and large intestines had their lengths measured using a ruler attached on cooled dissection board. The weights of each visceral organ were measured using an electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan). Samples of the liver and small intestine were preserved in 10% phosphate-buffered formalin for histology. The rest of the liver was stored in a freezer (Haier Biomedical, China) at -20°C pending the determination of hepatic lipid content.

3.4.2 Determination of erythrocyte osmotic fragility

Fifty (50) microlitres of freshly collected heparinised blood from each bird was pipetted into a set of 13 test tubes containing 5ml of serially diluted concentrations (0% to 0.85%) of phosphate-buffered saline (pH 7.4). The test tubes were carefully inverted to allow the appropriate mixing of blood and the saline solutions. After 30-min of incubation at room temperature, the contents of the test tubes were centrifuged at 5500×*g* for 15-mins using a centrifuge (Hermle Centrifuge Z 230 A, Berthold Hermle AG, German). The supernatant was carefully transferred into respective

cuvettes (Cuvette Macro PS, Lasec) and the absorbance of each of the supernatants was determined using a Spectronic-20 Spectrophotometer (Beckman Coulter Du 720, UV Spectrophotometer, USA) at a wavelength of 540nm.

3.4.3 Determination of Thiobarbituric acid reactive substances (TBARS)

The plasma antioxidant activity, an indirect measure of the antioxidant status of the live birds, was determined as described by Chirico (1994), using a TBARS parameter assay kit (KGE013, Minneapolis, MN, USA) according to the manufacturer's instructions. The assay procedure is based on the formation of malonaldehyde (MDA) from lipid peroxidation. In the presence of heat, the MDA reacts with thiobarbituric acid to yield a coloured product that absorbs light which can then be measured as TBARS. The plasma samples were thawed and acid treated with TBARS acid reagent to precipitate interfering substances. The samples were then incubated at room temperature for 15min and then centrifuged in a micro-centrifuge (Eppendorf, Hamburg, Germany) at 12 000×g for 4-min. The supernatant was then removed and used for the analysis. The optical density of each sample was then pre-read at 540nm using a microplate reader (Bio-Tek Instruments, Winooski, VT, USA) prior to incubation. The samples were then incubated at 48°C in an oven (Labcon, South Africa) for two and a half hours and then the optical density of each of the samples was read again following incubation. The initial optical densities were then subtracted from the final readings. A standard curve was generated from the standards and used to determine the TBARS concentration of the test samples.

3.4.4 Determination of the general health profile

The plasma activities of alanine transaminase (ALT) and alkaline phosphatase (ALP) and plasma glucose, triglyceride, uric acid, total bilirubin and blood urea nitrogen concentration of the broiler quail were determined using a colorimetric-based clinical chemistry analyser (IDEXX VetTest® Clinical Chemistry Analyser, IDEXX Laboratories Inc., USA) as per the manufacturer's instructions. Briefly, the stored plasma samples were allowed to thaw at room temperature and gently inverted to mix the contents. Three hundred microlitres (300µL) of plasma from each sample were drawn using a pipette and transferred into a catalyst sample cup which was then inserted in a colorimetric-based clinical chemistry analyser along with pre-loaded disks for analyses of the various health profile components. The machine automatically analysed the samples and a print out of results was provided.

3.4.5 Determination of hepatic lipid content

The hepatic storage of lipids was determined using a solvent extraction method as described by Bligh and Dyer (1959). Briefly, approximately 5g of the liver sample was steeped in 150ml of 2:1 chloroform: methanol solution in a flat-bottomed 250ml flask overnight in a refrigerator at 4°C. The mixture from each beaker was then filtered through a Whatmann filter paper (Whatmann®, No 1, size 185mm, pore size 7-11μm, England) into a 250ml 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 20ml with chloroform. An aliquot of 2ml of the extract was then placed into a dried, pre-weighed vial, and dried at 50°C for 30-min in an oven (Salvis ®, Salvis Lab, Switzerland) and then cooled in a desiccator and then reweighed to determine the lipid content. The liver lipid content was computed on the basis of the dry liver weight.

3.4.6 Liver and small intestines histology

The preserved liver and small intestine were embedded in paraffin wax, sectioned at 5μm and then stained with haematoxylin and eosin (H and E) on a glass slide and covered with a glass coverslip (Reyes-Gordillo et al., 2007). The changes in hepatocellular, specifically hepatocyte size and total hepatocytes (single and double nuclei) numbers and Kupffer cells were counted within the slide area ($3.7x10^{-2}$μm) were viewed under a light microscope at a magnification of 400 X using an eyepiece micrometer (Reichert®, Austria). Photographs of the slides sections were captured using a camera mounted onto the microscope. Liver sections were examined for steatosis (the accumulation of fat droplets within the liver tissue) and assigned a score relative to the level of lipid deposits in the sample as per the classification by Kleiner et al. (2005) where a score of 0 indicates no steatosis, 1 indicated mild steatosis, 2 indicated moderate steatosis and 3 indicated severe steatosis. Changes in size of the small intestines villus height and width and crypt depth were also measured within a slide area ($3.7x10^{-2}$ μm) and viewed under a light microscope at a magnification of 400 X using an eyepiece micrometer.

3.5 Determination of the physical traits of meat

3.5.1 Determination of pH

The pH of the *Pectoralis major* (breast) muscles was determined using a digital pH meter with a piercing electrode (Crison pH25, Crison instruments, SA, Allena, Spain), which was inserted in three different positions around each section of the breast. The digital pH meter was calibrated using three standard buffer solutions pH 4.01, pH 7.00 and pH 9.21**.** The pHi of meat was determined 30-min post slaughter and the pH_u was determined 24-hours of post-slaughter as following the storage of the carcasses in the cold room at 4°C.

3.5.2 Determination of meat colour

Meat colour [Lightness (L*), Redness (a*) and Yellowness (b*)] of the breast muscles was determined 30-min and 24-hours post-slaughter using Lovibond Colour meter (LC 100 Spectrophotometer, LASEC, South Africa) following the manufacturer's instructions. Three points from each of the breast muscle were sampled in the determination of colour. Following overnight storage of the carcasses at 4°C, the ultimate meat colour of the breast muscle was then determined as previously described.

3.5.3 Determination of water holding capacity

The water holding capacity (WHC) of the breast muscle was determined as described by Bertram et al. (2001). About 0.5g breast samples 2.0cm long and 0.4 x 0.4cm in cross-sectional area cut parallel to the fibre direction (after 24-hours of carcass storage at 4°C) were centrifuged (Hermle Centrifuge Z 230 A, Berthold Hermle AG, German) at 4000×*g* for 1-hour at 4°C in centrifuge tubes. Each centrifuge tube had beads to separate the meat from the expelled liquid. After the centrifugation each sample was then reweighed on an electronic balance (Snowrex EQ-1200, Snowrex International Company, Taipei, Taiwan) and the water holding capacity computed using the equation: $WHC = [1 \ (W_3 - W_2) / W_1 * 100]$; where W_1 is the weight of the sample after centrifuging (g) W_2 is the weight of the tube, including the beads, after centrifuging (g) and W_3 is the weight of the tube after the sample was removed and after centrifuging (g).

3.5.4 Determination of tenderness

The breast samples were kept frozen until the assay date. Tenderness of the breast muscle samples was determined at the Meat Quality Analysis Laboratory at ARC-API, Irene, Pretoria.

The samples were thawed at 4ºC for 24-hours and cooked according to a standardised dry heat cooking method in a conventional calibrated oven at 160°C to an endpoint temperature of 68-70°C. The samples were cooled to room temperature (centrally controlled at 22°C). The fillet cuts obtained from the cooked breast muscles were sheared once in the centre in order to avoid the hardened parts of the fillet during broiling. Shearing was done by using a Warner-Bratzler shear (WBS) machine mounted on a Universal Instron Machine (Model 4301) (Instron Corporation, 1990). The shear force was determined at a cross speed of 200mm/min with a 1kN load cell as recommended by the American Meat Science Association,(1995). The results of shear force were recorded on the computer screen mounted to the Universal Instron machine.

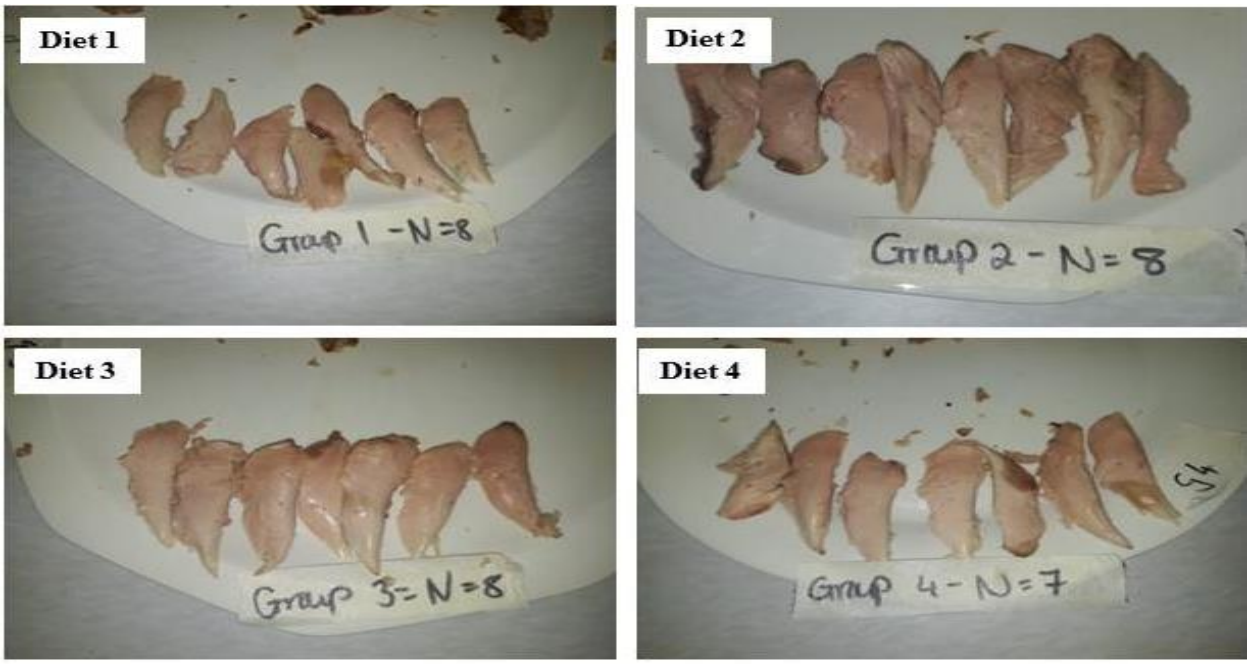

Figure 3.2: Photographs of fillets from the breast muscle used in the determination of tenderness by dietary treatment

3.6 Determination of the chemical composition of the meat

3.6.1 Determination of the proximate composition

The proximate analysis (DM, CP, EE and Ash content) of the breast muscles was determined as described by the AOAC procedures (AOAC, 2005; method numbers: 930.15, 984.01, 920.39 and 942.15, respectively). Organic matter was estimated as the difference between dry matter and ash.

3.6.2 Determination of the amino acid content

Amino acid concentration of the meat was determined as described by Einarsson et al. (1983). Briefly, the meat (matured breast muscle) was hydrolysed in an acid 6M HCl at a temperature of 110°C for 24-hours and pre-column fluorescence derivatisation of amino acids with 9-flourenylmethyl chloroformate. The amino acids were extracted with pentane and separated by gradient elution on a chromatograph. The chromatograph consisted of a SpectraSystem P4000 Quaternary high-performance liquid chromatograph 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). Identification of the amino acids was done at an excitation wavelength of 264nm and an emission wavelength of 340nm. A PC equipped with TSP software was used for quantification.

3.6.3 Determination of the mineral content

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

3.6.4 Determination of the fatty acid profile

The Soxhlet method was used to extract the oil from the breast muscle sample as described by AOAC (2005; method number 920.39) and fatty acid profiles were determined as described by Christopherson and Glass (1968). Briefly, the oil extracts were trans-methylated with 2mol/L methanol sodium hydroxide. The resulting fatty acid methyl esters were extracted in heptane, filtered and dried under nitrogen after which they are 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.

3.7 Data analysis

Parametric data are expressed as mean±SD and non-parametric data are expressed as median and range (mean, max). The data were analysed using GraphPad Prism 5 software (Graph-Pad Software Inc., San Diego, CA, USA). Weekly within group data were analysed using repeated measures ANOVA. Multiple-group data (growth performance and feed utilisation efficiency, viscera macro-and micro-morphometry, health profile and the meat's physical and chemical traits) were analysed using the one-way analysis of variance. The differences between the treatment means were determined using Tukey's *post hoc* test. Multiple group steatosis data were analysed using the Kruskal-Wallis test followed by a multiple-comparisons Dunn's *post hoc* test. Statistical significance was set at $P < 0.05$.

The statistical model used for the one-way ANOVA was as $Yijk = \mu + Ti + Bj + Ck + eijk$ where:
Yijk = dependent variable of interest (growth performance indices, blood and stored metabolic substrates, viscera macro-and-micro morphometry, general health profile, meat physical attributes and chemical composition).
μ = overall mean effect
T_i = is the fixed effect of the i^{th} dietary treatments (i = 1, 2...4)
Bj = the fixed effect of individual bird (j = 1, 2, 3….32)
Ck = is the effect of the j^{th} batch (j = 1, 2...4)
eijk = random residual error

CHAPTER 4: RESULTS

4.1 Experiment 1: Chemical composition of the seed meal

The proximate, gross energy, fibre content and mineral content of *M. zeyheri* seed meal are shown in Table 4.1.

Table 4.1: Proximate, fibre, mineral and energy content of *M. zeyheri* seed meal

Constituent	Mean ± SD
Proximate composition (% DM)	
Dry matter	95.72 ± 0.11
Organic matter	92.38 ± 0.09
Crude protein	9.20 ± 0.15
Ash	3.34 ± 0.03
Ether extract	25.28 ± 0.68
Fibre components (% DM)	
Neutral detergent fibre	24.34 ± 0.25
Acid detergent fibre	7.38 ± 0.14
Macro-mineral content (% DM)	
Calcium	0.85 ± 0.04
Phosphorus	0.14 ± 0.00
Energy content (MJ/kg DM)	
Gross energy	24.18 ± 0.76

Data presented as mean±SD.

The amino acid profile of *M. zeyheri* seed meal is shown in Table 4.2 below.

Table 4.2: Amino acid content of *M. zeyheri* seed meal

Amino acid	Mean ± SD
Essential amino acids (g/100g DM)	
Arginine	0.65 ± 0.00
Histidine	0.29 ± 0.03
Isoleucine	0.31 ± 0.01
Leucine	0.54 ± 0.03
Lysine	0.54 ± 0.06
Methionine	0.05 ± 0.01
Phenylalanine	0.24 ± 0.01
Threonine	0.32 ± 0.01
Tyrosine	0.37 ± 0.13
Valine	0.41 ± 0.01
Non-essential amino acid (g/100g DM)	
Alanine	0.46 ± 0.03
Aspartic acid	0.60 ± 0.01
Glumatic acid	1.01 ± 0.00
Glycine	0.32 ± 0.00
Hydroxyproline	0.03 ± 0.00
Proline	0.35 ± 0.01
Serine	0.29 ± 0.00
Total	**6.78**

Data presented as mean±SD.

From the 17 assayed amino acids, glumatic acid was the highest concentrated amino acid in the seed meal constituting 1.01% of the crude protein content of the seed meal. The least concentrated amino acid in the MZSM was methionine at 0.05% of the *M. zeyheri* seed meal CP content.

The fatty acid profile of *M. zeyheri* seed meal is shown in Table 4.3 below.

Table 4.3: Fatty acid profile of *M. zeyheri* seed meal

Fatty acids	**Percent (%)**
Saturated fatty acids	
C 12:0 (lauric acid)	0.01 ± 0.00
C 14:0 (myristic acid)	0.07 ± 0.00
C 15:0 (pentadecanoic acid)	0.04 ± 0.00
C 16:0 (palmitic acid)	13.46 ± 0.29
C 17:0 (margaric acid)	0.09 ± 0.00
C 18:0 (stearic acid)	9.44 ± 0.01
C 20:0 (arachidic acid)	0.83 ± 0.02
C 21:0 (heneicosanoic acid)	0.03 ± 0.02
C 22:0 (behenic acid)	0.59 ± 0.35
C 24:0 (lignoceric acid)	0.19 ± 0.01
TSFAs	**24.56 ± 0.12**
Mono-unsaturated fatty acids	
C 16:1 (palmitoleic acid)	0.14 ± 0.00
C 17:1 (heptadecenoic acid)	0.02 ± 0.00
C 18:1n9c (oleic acid)	54.40 ± 0.05
C 20:1 (11-eicosenoic acid)	0.53 ± 0.01
C 22:1n9 (erucuc acid)	0.03 ± 0.00
C 24:1 (nervonic acid)	0.06 ± 0.01
TMUFAs	**55.18 ± 0.06**
Poly-unsaturated fatty acids	
C 18:2n6c (linoleic acid)	20.05 ± 0.01
C 18:3n3 (α-linolenic acid)	0.02 ± 0.01
C 18:3n6 (γ-linolenic acid)	0.12 ± 0.00
C 20:2 (eicosadienoic acid)	0.02 ± 0.00
C 22:2 (docosadienoic acid)	0.01 ± 0.01
TPUFAs	**20.23 ± 0.00**
Cis fats	74.45 ± 0.04
Omega-3 fats	0.07 ± 0.08
Omega-6 fats	20.08 ± 0.00

Omega-9 fats	54.42 ± 0.05
TPUTA: TSFA	0.82:1
n3PUFA: n6PUTA	0.003:1

TSFA - Total saturated fatty acids, TMUFAs - Total mono-unsaturated fatty acids, TPUFAs - Total poly-unsaturated fatty acids, n3PUFAs - Omega-3 poly-unsaturated fatty acids, n6PUFAs - Omega-6 poly-unsaturated fatty acids and n9PUFAs – Omega-9 poly-unsaturated fatty acids; Data presented as mean±SD.

The fatty acid profile of *M. zeyheri* seed meal showed that TMUFAs contributed more than 50% of the fatty acid content of the *M. zeyheri* seed oil. In terms of polyunsaturated fatty acids, the n9PUFAs were most concentrated in the seed meal.

The anti-nutritional factors composition (qualitative) of *M. zeyheri* seed meal is depicted in Table 4.4 below.

Table 4.4: Qualitative anti-nutritional factors content of *M. zeyheri* seed meal

Parameter	Present/Absent
Terpernoids	+++
Tannins	++
Flavonoids	+
Saponins	+

Key: +++ = (very high), ++ = (high), + = (low).

4.2 Experiment 2: *In vivo* evaluation dietary *M. zeyheri* seed meal on growth, health and meat quality of broiler quail

4.2.1 Growth performance

Table 4.5 below shows effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on the growth performance and feed utilisation economy of broiler quail.

Table 4.5: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on growth performance, weekly feed intake (FI), and feed conversion ratio (FCR) of broiler quail

Parameter	Week	Dietary treatments				Significance level
		Diet 1	Diet 2	Diet 3	Diet 4	
Induction mass (g)		147.50 ± 19.68[a]	151.50 ± 15.33[a]	149.75 ± 22.40[a]	148.14 ± 14.61[a]	ns
Terminal body mass (g)		193.50 ± 16.87[a]	192.75 ± 7.01[a]	189.50 ± 12.46[a]	185.64 ± 9.85[a]	ns
Weekly BWG (g)	1	14.00 ± 3.21[a]	12.25 ± 5.06[a]	11.25 ± 4.86[a]	8.91 ± 2.59[a]	ns
	2	11.50 ± 7.54[a]	10.50 ± 4.50[a]	10.25 ± 5.18[a]	10.88 ± 2.58[a]	ns
	3	10.75 ± 4.40[a]	9.25 ± 2.61[a]	9.26 ± 3.20[a]	9.43 ± 1.40[a]	ns
	4	9.75 ± 4.33[a]	9.24 ± 2.61[a]	9.25 ± 3.19[a]	8.88 ± 1.81[a]	ns
Total BWG (g)		**46.00 ± 8.82[a]**	**41.25 ± 11.06[a]**	**39.75 ± 10.39[a]**	**37.50 ± 6.21[a]**	**ns**
Weekly ADG (g)	1	2.00 ± 0.46[a]	1.75 ± 0.72[a]	1.61 ± 0.69[a]	1.26 ± 0.37[a]	ns
	2	1.64 ± 1.08[a]	1.50 ± 0.64[a]	1.47 ± 0.74[a]	1.55 ± 0.37[a]	ns
	3	1.54 ± 0.63[a]	1.32 ± 0.43[a]	1.32 ± 0.46[a]	1.35 ± 020[a]	ns
	4	1.39 ± 0.62[a]	1.32 ± 0.37[a]	1.32 ± 0.46[a]	1.26 ± 0.26[a]	ns
Overall ADG		**1.64 ± 0.31[a]**	**1.47 ± 0.40[a]**	**1.42 ± 0.37[a]**	**1.34 ± 0.21[a]**	**ns**
Weekly FI (g)	1	124.07 ± 13.89[b]	120.27 ± 11.95[b]	113.66 ± 8.59[b]	85.40 ± 4.10[a]	***
	2	138.79 ± 16.83[b]	132.94 ± 9.01[b]	123.66 ± 11.04[ab]	115.57 ± 4.36[a]	**
	3	170.88 ± 11.26[a]	162.06 ± 12.25[a]	160.71± 7.79[a]	157.42 ± 5.87[a]	ns
	4	171.93 ± 15.19[a]	166.66 ± 8.26[a]	165.96 ± 8.54[a]	164.88 ± 8.39[a]	ns

Total FI (g)		**605.56 ± 34.98^{b}**	**581.93 ± 26.77^{ab}**	**563.99 ± 24.12^{a}**	**557.56 ± 12.19^{a}**	******
Weekly FCR	1	8.86 ± 2.09^{a}	9.82 ± 4.41^{a}	10.10 ± 2.73^{a}	9.58 ± 5.97^{a}	ns
	2	12.07 ± 7.69^{a}	12.66 ± 5.43^{a}	12.06 ± 4.05^{a}	10.62 ± 4.79^{a}	ns
	3	15.89 ± 4.96^{a}	17.51 ± 2.53^{a}	17.37 ± 2.49^{a}	16.69 ± 2.91^{a}	ns
	4	18.10 ± 3.61^{a}	18.01 ± 3.80^{a}	17.92 ± 5.66^{a}	18.56 ± 3.87^{a}	ns
Overall FCR		**65.58 ± 17.14^{a}**	**63.16 ± 12.92^{a}**	**64.25 ± 19.79^{a}**	**57.79 ± 8.10^{a}**	**ns**
Empty carcass mass		134.63 ± 20.07^{a}	131.38 ± 9.98^{a}	128.63 ± 12.65^{a}	124.71 ± 12.08^{a}	ns
Dressing percentage		70.05 ± 4.64^{a}	68.11 ± 3.48^{a}	67.81 ± 3.94^{a}	66.95 ± 3.46^{a}	ns

ns = not significant, $p > 0.05$, ** $p < 0.01$, *** $p < 0.0001$, ^{ab} Within row means with different superscripts are significantly different at $p \leq 0.05$. Birds fed diet 4 had the lowest ($p < 0.0001$) feed intake in week 1 of the experiment. Birds fed diet 4 had significantly lower ($p < 0.0001$) weekly feed intake compared to that of counterparts fed diet 1 and 2 in week 2 of the experiment. Birds fed diets 3 and 4 had significantly lower ($p < 0.01$) total feed intake compared to that of counterparts feed diet 1. Dietary *M. zeyheri* has no significant effect ($p > 0.05$) on the birds' total body weight gain, weekly and overall ADG, weekly and overall FCR, empty carcass mass and dressing percentage. ADG – average daily gain, FCR – feed conversion ratio; Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4

Birds fed diet 4 (37.5% partial replacement of MM with MZSM on a gross energy basis) had the lowest feed intake during weeks 1 and 2 and lower overall total feed intake compared to that of birds fed diet 1 (Table 4.5). However, dietary *M. zeyheri* seed meal had no effect on the growth (total BWG, ADG and FCR) performance of the birds. Importantly it did not affect the meat yield as measured by empty carcass mass and dressing percentage.

4.2.2 Viscera macro-morphometry

The effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on viscera macro-morphometry of broiler quail is shown in Table 4.6.

Table 4.6: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on viscera macro-morphometry of broiler quail

Parameter	Dietary treatments				Significance
	Diet 1	Diet 2	Diet 3	Diet 4	Level
Heart (g)	1.69 ± 0.25[a]	1.69 ± 0.33[a]	1.61 ± 0.21[a]	1.56 ± 0.31[a]	ns
(% body mass)	0.91 ± 0.11[a]	0.90 ± 0.18[a]	0.91 ± 0.13[a]	0.88 ± 0.16[a]	ns
Liver (g)	2.99 ± 0.33[a]	3.67 ± 0.34[ab]	3.42 ± 0.65[ab]	4.08 ± 0.80[b]	**
(% body mass)	1.62 ± 0.11[a]	1.95 ± 0.16[ab]	1.91 ± 0.27[ab]	2.30 ± 0.52[b]	**
Pancreas (g)	0.46 ± 0.12[a]	0.54 ± 0.12[a]	0.52 ± 0.11[a]	0.73 ± 0.44[a]	ns
(% body mass)	0.25 ± 0.07[a]	0.29 ± 0.06[a]	0.29 ± 0.06[a]	0.41 ± 0.26[a]	ns
Proventriculus (g)	0.65 ± 0.11[a]	0.83 ± 0.07[b]	0.71 ± 0.09[ab]	0.79 ± 0.09[b]	**
(% body mass)	0.35 ± 0.04[a]	0.44 ± 0.04[b]	0.40 ± 0.03[ab]	0.45 ± 0.06[b]	***
Ventriculus (g)	2.99 ± 0.60[a]	3.84 ± 0.42[b]	3.71 ± 0.54[ab]	3.79 ± 0.58[b]	*
(% body mass)	1.60 ± 0.19[a]	2.05 ± 0.27[b]	2.08 ± 0.24[b]	2.13 ± 0.31[b]	**
Small intestines (g)	3.99 ± 0.50[a]	5.12 ± 0.88[b]	4.64 ± 0.70[a]	5.69 ± 0.76[b]	***
(% body mass)	2.16 ± 0.26[a]	2.73 ± 0.52[b]	2.59 ± 0.25[ab]	3.20 ± 0.51[b]	***
Small intestines length (mm)	536 ± 59.52[a]	620 ± 22.55[ab]	618 ± 54.97[ab]	700 ± 56.35[b]	***
Large intestines (g)	0.45 ± 0.13[a]	0.56 ± 0.29[a]	0.49 ± 0.05[a]	0.58 ± 0.05[a]	ns
(% body mass)	0.27 ± 0.10[a]	0.30 ± 0.17[a]	0.25 ± 0.06[a]	0.30 ± 0.04[a]	ns
Large intestines length (mm)	59.38 ± 8.63[a]	59.13 ± 5.89[a]	58.75 ± 8.35[a]	61.43 ± 6.27[a]	ns
Caecum (g)	0.85 ± 0.14[a]	1.08 ± 0.14[ab]	1.19 ± 0.21[b]	1.29 ± 0.27[b]	**
(% body mass)	0.46 ± 0.07[a]	0.58 ± 0.10[ab]	0.66 ± 0.08[b]	0.72 ± 0.17[b]	***
Visceral fat (g)	2.75 ± 1.72[b]	1.29± 0.74[a]	1.43 ± 0.59[ab]	0.12 ± 0.19[a]	***
(% body mass)	1.40 ± 0.83[b]	0.64 ± 0.42[a]	0.80 ± 0.34[ab]	0.08 ± 0.11[a]	***

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. Birds fed diet 4 had significantly heavier (p < 0.01) absolute and relative (% body mass) liver masses compared to birds fed diet 1. Birds fed diet 2 had significantly heavier (p < 0.01) proventriculi absolute and relative (% body mass) masses, as well as ventriculi absolute and relative (% body mass) masses compared to birds fed diet 1. Birds fed diet 2 and 4 had significantly heaviest (p < 0.0001) absolute and relative (% body mass) small intestine masses compared to birds fed Diet 1 and 3. The lengths of small intestines significantly (p < 0.0001) increased across the dietary treatments. Birds fed diet 4 had significantly heavier (p < 0.0001) absolute and relative (% body mass) caecum masses compared to birds fed diet 1. On the other hand, birds fed diet 4 MZSM had significantly lowest (p < 0.0001) absolute and relative (% body mass) visceral fat masses compared to birds fed diet 1. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Dietary *M. zeyheri* seed meal, at all levels of inclusion, did not affect the mass of the hearts, pancreata and the mass and length of the large intestines of the broiler quail (Table 4.6). However, dietary *M. zeyheri* seed meal at 37.5% substitution of the MM gross energy supply in the diet resulted in significantly heavier livers, proventriculi, ventriculi, small intestines and caeca and significantly longer small intestines compared to those from quail feed the control diet. Importantly, dietary *M. zeyheri* seed meal at 12.5% (Diet 2) and 37.5% (Diet 4) replacement of MZSM (on a gross energy basis) in the diets, resulted in a significant lowering of the visceral fat mass (VFM) of the birds compared to the VFM from birds fed the control diet (Table 4.6).

4.2.3 Erythrocyte osmotic fragility

Figure 4.1 below shows the effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on erythrocyte osmotic fragility of broiler quail while Table 4.7 shows the range of concentrations of phosphate-buffered saline solutions (%) at which IH, MCF and MH of erythrocytes from broiler Japanese quail occurred.

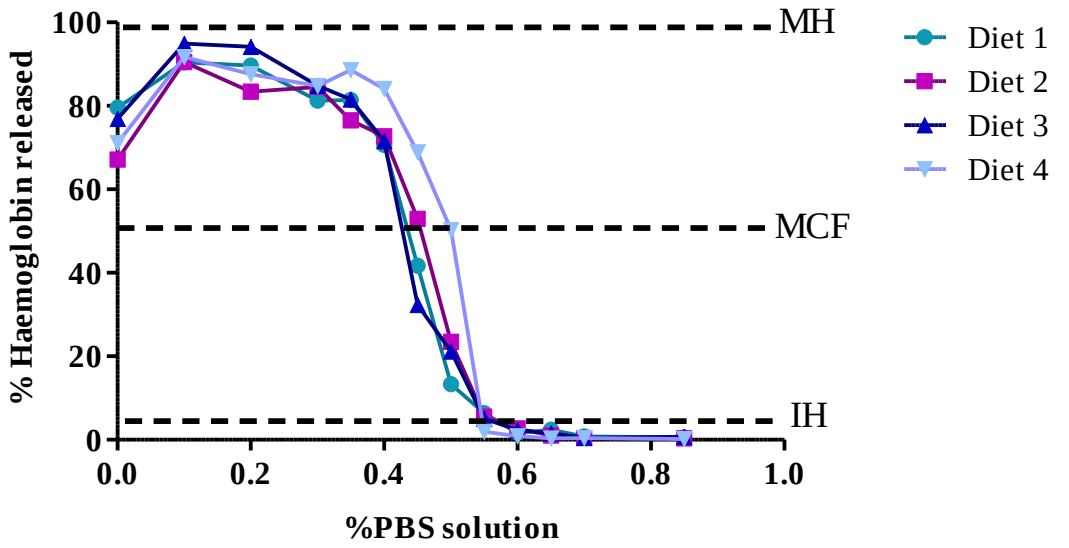

Figure 4.1: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on erythrocyte osmotic fragility broiler quail

Birds fed diet 4 had higher MCF (0.50-0.60) compared that of birds (0.40-0.50) fed diet 1, 2 and 3. IH – initial haemolysis, MCF - mean corpuscular fragility, MH – maximum haemolysis, PBS – phosphate buffered saline. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Table 4.7: Range of concentrations of phosphate-buffered saline solutions (%) at which initial haemolysis, mean corpuscular fragility and maximal haemolysis of erythrocytes from broiler Japanese quail occurred

	% PBS solution			
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
Initial haemolysis	0.60-0.70	0.60-0.70	0.60-0.70	0.60-0.70
MCF	0.40-0.50	0.40-0.50	0.40-0.50	0.50-0.60
Maximal haemolysis	0.10-0.20	0.10-0.20	0.10-0.20	0.10-0.20

PBS = phosphate-buffered saline solution; MCF = mean corpuscular fragility or 50% haemolysis. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

The initial haemolysis (IH) of erythrocytes of quail fed Diet 4 (37.5%) occurred at the PBS concentration range between 0.70 – 0.80% while that of birds on other dietary treatments occurred at a PBS compared concentration range of 0.60 – 0.70% PBS (Table 4.7). The mean corpuscular fragility (MCF) of birds fed diet 4 occurred at the PBS range 0.50 – 0.60% while that of birds fed diet 1 and 3 occurred at PBS concentration range 0.40 – 0.50%. The maximal haemolysis (MH) of birds across the dietary treatments occurred within 0.10 – 0.20% at PBS concentration (Table 4.7).

4.2.4 General health profile

The effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on plasma markers of general health of broiler quail is shown in Table 4.8.

Table 4.8: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on plasma markers of general health of broiler quail

Parameter	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Glucose (mmol/L)	11.01 ± 4.89[a]	13.78 ± 1.22[a]	8.82± 4.77[a]	12.02 ± 3.55[a]	ns
Triglyceride (mmol/L)	1.3 ± 0.34[a]	1.1 ± 0.22[a]	1.7 ± 0.66[b]	1.1 ± 0.24[a]	*
Urea (mmol/L)	0.89 ± 0.27[ab]	0.71 ± 0.01[a]	1.10 ± 0.41[b]	0.71 ± 0.01[a]	*
Uric acid (mmol/L)	398.52 ± 54.42[a]	537.18 ± 181.02[a]	492.20 ± 168.08[a]	440.15 ± 204.35[a]	ns
Total bilirubin (mmol/L)	1.90 ± 0.62[a]	1.94 ± 0.24[a]	4.95 ± 2.88[b]	4.79 ± 2.74[b]	**
ALP (U/L)	190.38 ± 129.06[a]	279.88 ± 128.56[bc]	133.88 ± 81.83[a]	213.43 ± 42.26[c]	**
ALT (U/L)	3.09 ± 2.63[a]	1.39 ± 0.49[a]	6.88 ± 5.16[b]	1.06 ± 0.15[a]	**
TBARS (nM/mL)	1.03 ± 0.02[a]	1.04 ± 0.02[a]	2.07 ± 0.02[b]	2.06 ± 0.01[b]	**

ns = not significant, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, [ab] Within row means with different superscripts are significantly different at $p \leq 0.05$. Plasma glucose and uric acid values were observed similar ($p > 0.05$) across the dietary treatments. Birds fed diet 3 had the highest ($p < 0.05$) plasma triglyceride, urea and ALT concentration levels compared birds fed diet 2 and 4. Total bilirubin of birds fed diet 3 and 4 was significantly higher ($p < 0.01$) compared to birds fed diet 1 and 2. The plasma ALP of birds fed diet 3 was significantly lower ($p < 0.05$) compared to the ALP of birds fed diet 2. The TBARS concentration of birds fed Diet 3 and 4 were higher ($p < 0.01$) compared to birds fed diet 1 and 2. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Across treatments, dietary *M. zeyheri* seed meal had no effect on plasma glucose and uric acid concentrations of the quail (Table 4.8). Plasma urea and bilirubin concentrations, as well as plasma ALP, increased with increasing dietary *M. zeyheri* seed meal concentration. The replacement of MM at 25% gross energy supply with *M. zeyheri* seed meal (Diet 3) caused a significant increase in plasma ALT activity of the birds (Table 4.8). Partial replacement of MM at 25% and 37.5% of its gross energy value with *M. zeyheri* seed meal resulted in a significant increase in the birds' thiobarbituric acid reactive substance (TBARS) concentration (Table 4.8).

4.2.5 Liver lipid content

Figure 4.2 below shows the effect of partial replacement of MM with MZSM as a dietary energy source on liver lipid content of broiler quail.

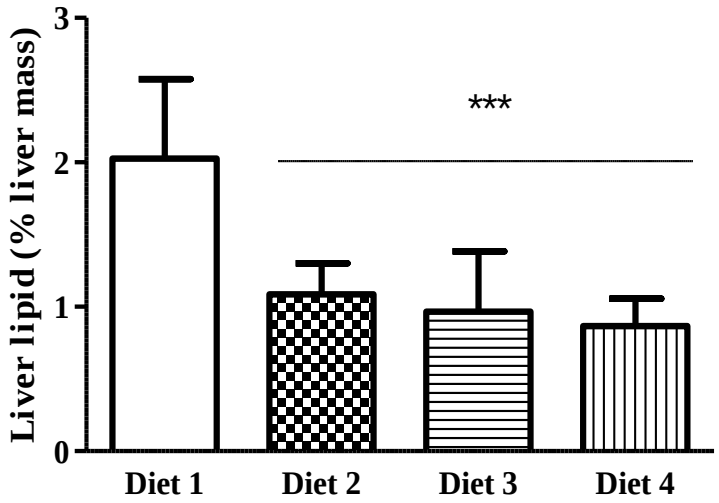

Figure 4.2: Effect of partial replacement of maize meal with *Mimusops zeyheri* seed meal as a dietary energy source on liver lipid content of broiler quail

*** $p < 0.0001$. Birds fed diet 1 had significantly higher ($p < 0.0001$) liver lipid content compared to the liver lipid content of counterparts fed diet 2, 3 and 4. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

After the feeding period of 4-weeks, broiler quail fed diet 1 had significantly higher liver lipid content compared to that from birds fed diets 2, 3 and 4 (Figure 4.2).

4.2.6 Liver histology

The representative liver histology sections (haematoxylin and eosin staining, 400 X magnifications) are shown in Figure 4.3. Hepatocyte cell sizes and numbers of hepatocytes, and Kupffer cells and the observed hepatic macro-vesicular steatosis in slide area ($3.7x10^{-2}$ μm) are shown in Table 4.9.

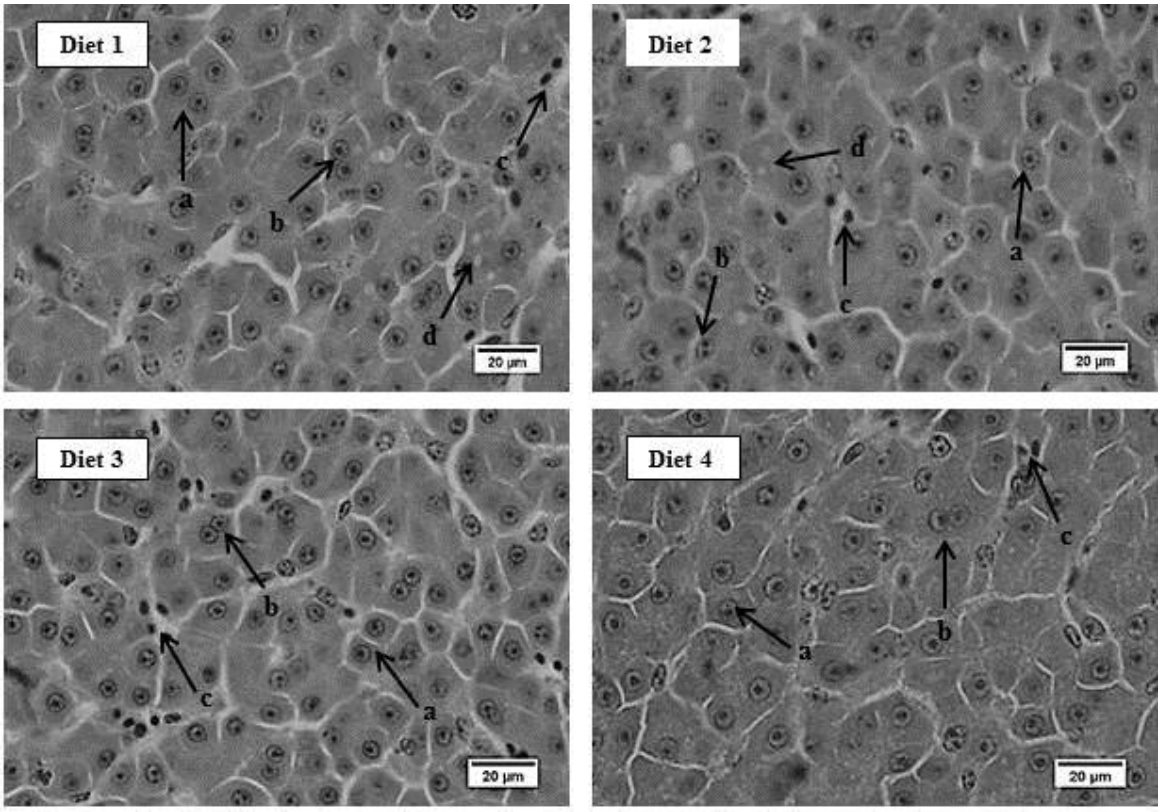

Figure 4.3: Liver histology sections (haematoxylin and eosin staining, 400 X magnifications)

Arrows a: single nuclei hepatocytes, arrows b: double nuclei hepatocytes, arrows c: Kupffer cells and arrows d: steatosis. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Table 4.9: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on the histology of liver sections from broiler quail

Item	n	Dietary treatments				Significant level
		Diet 1	Diet 2	Diet 3	Diet 4	
Hepatocytes size (μm)	4	131.54 ± 19.87^{a}	152.86 ± 21.79^{ab}	161.74 ± 10.34^{b}	170.06 ± 5.67^{b}	**
Hepatocytes density ($N/\mu m^2 x10^{-2}$)	4	8.58 ± 5.52^{c}	8.14 ± 5.12^{c}	7.37 ± 5.79^{b}	5.96 ± 4.82^{a}	***
Kupffer cell density ($N/\mu m^2 x10^{-2}$)	4	1.83 ± 0.35^{c}	1.58 ± 0.38^{bc}	1.49 ± 0.32^{b}	1.18 ± 0.02^{a}	**
Steatosis score	4	$2(1, 2)^{b}$	$1(1, 2)^{ab}$	$0(0, 1)^{ab}$	$0(0, 0)^{a}$	*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, ^{abc} Within row means with different superscripts are significantly different at $p \leq 0.05$. The sizes of hepatocytes in a slide area significantly increased ($p < 0.01$) across the dietary treatments, while the numbers of hepatocytes ($p < 0.0001$) and Kupffer cells ($p < 0.01$) significantly decreased. Dietary *M. zeyheri* seed meal significantly decreased ($p < 0.05$) the amount of hepatic macro-vesicular steatosis in the liver in all the diets. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; Data presented as mean ± SD with the exception on the steatosis score presented as median and range (min, max); n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Dietary *M. zeyheri* seed meal significantly increased the size of hepatocytes, while it significantly decreased the density of hepatocytes and Kuppfer cells in a slide area ($3.7x10^{-2}$ μm) as dietary MZSM inclusion increased (Table 4.9). The birds fed *M. zeyheri* seed meal based diets 3 and 4 had the lowest numbers of hepatic macro-vesicular steatosis compared to birds in diet 1 and 2 (Table 4.9).

4.2.7 Small intestine histology

The representative small intestine histology sections (haematoxylin and eosin staining, 400 X magnifications) are shown in Figure 4.4. Table 4.10 shows micro-morphometry of the small intestine of broiler quail fed graded levels of *M. zeyheri* seed meal.

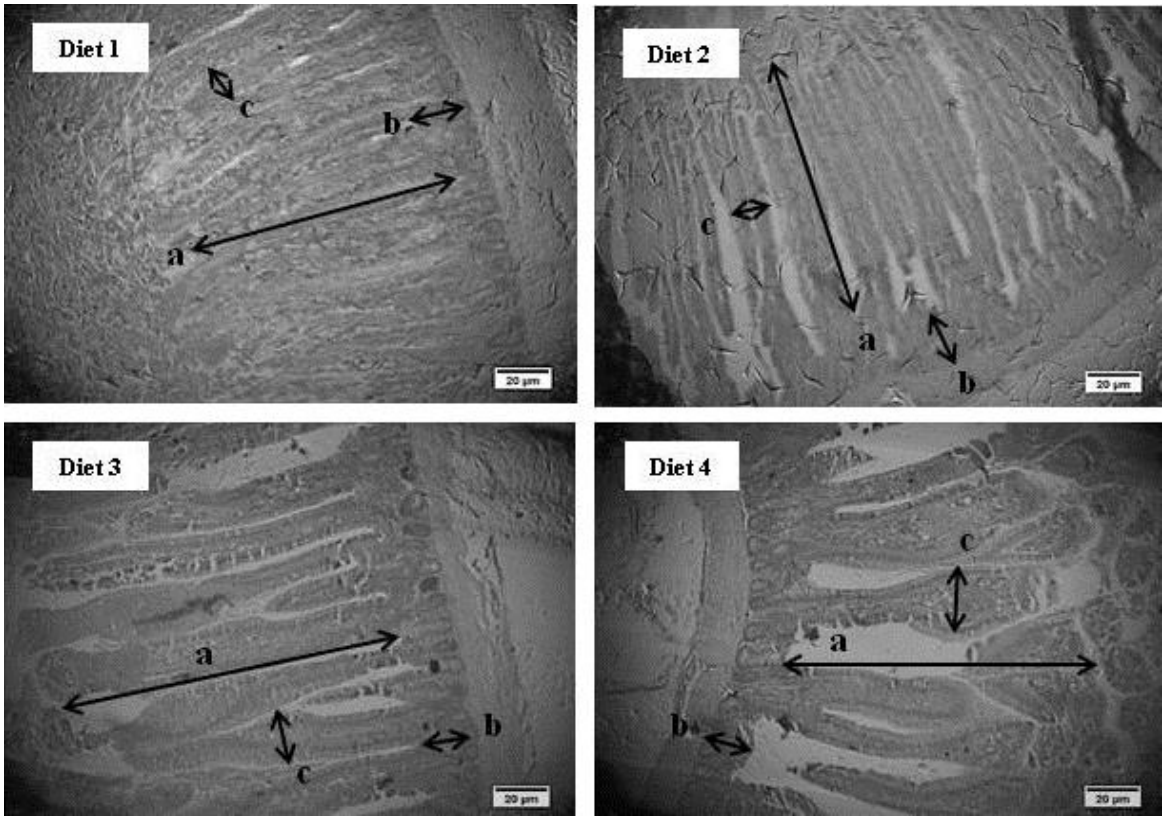

Figure 4.4: Small intestines histology sections (haematoxylin and eosin staining, 400 X magnifications)

Double headed arrows a: villus height, double-headed arrows b: crypt depth and double-headed arrows c: villus width. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Table 4.10: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on small intestines micro-morphometry of broiler quail

Item	n	Dietary treatments				Significance level
		Diet 1	Diet 2	Diet 3	Diet 4	
Crypt depth (µm)	4	23.67 ± 3.02^{c}	19.46 ± 2.75^{b}	14.09 ± 1.49^{a}	11.13 ± 2.87^{a}	***
Villus height (µm)	4	87.04 ± 11.01^{a}	92.33 ± 10.81^{a}	117.82 ± 10.88^{b}	127.93 ± 15.54^{c}	**
Villus width (µm)	4	10.56 ± 1.44^{a}	54.13 ± 2.87^{a}	56.78 ± 1.46^{a}	65.51 ± 1.85^{b}	***
Villus height:crypt depth	4	4.43 ± 2.32^{a}	6.09 ± 1.67^{a}	10.08 ± 2.87^{b}	13.08 ± 1.03^{b}	**

** $p < 0.001$, *** $p < 0.0001$, ^{abcd} Within row means with different superscripts are significantly different at $p \leq 0.05$. The depth of the *Crypts of Lieberkühn* significantly ($p < 0.0001$) decreased across the dietary treatments, while villus height and villus width significantly increased ($p < 0.0001$). The ratio of villus height/crypt depth significantly increased with an increase of *M. zeyheri* seed meal inclusion in diets. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Dietary *M. zeyheri* seed meal significantly decreased small intestine crypt depth, despite the increase of villus height and villus width across the dietary treatments (Table 4.10). Villus height/crypt depth ratio significantly increased with increase *M. zeyheri* seed meal inclusion (Table 4.10).

4.2.8 Meat quality

4.2.8.1 Physical attributes of broiler quail breast meat

Tables 4.11 shows the effect of partial replacement of maize meal with *Mimusops zeyheri* seed meal as a dietary energy source on the physical attributes of broiler quail breast muscle.

Table 4.11: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on broiler quail breast muscle quality attributes

Parameters	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
30 mins post-slaughter					
Muscle pH_i	6.14 ± 0.17[a]	6.01 ± 0.15[a]	6.16 ± 0.12[a]	6.09 ± 0.28[a]	ns
Lightness (L*)	54.34 ± 7.70[a]	48.05 ± 3.45[a]	56.34 ± 15.41[a]	51.20 ± 3.56[a]	ns
Redness (a*)	3.75 ± 1.65[a]	3.93 ± 1.59[a]	2.74 ± 1.14[a]	2.46 ± 1.85[a]	ns
Yellowness (b*)	9.95 ± 2.06[a]	7.58 ± 2.50[a]	9.76 ± 3.50[a]	8.39 ± 2.12[a]	ns
Chroma (C*)	11.08 ± 1.45[a]	8.66 ± 2.09[a]	10.14 ± 3.63[a]	9.10 ± 1.32[a]	ns
Hue (H*)	70.78 ± 10.07[a]	68.91 ± 11.85[a]	84.53 ± 17.30[a]	82.19 ± 8.93[a]	ns
24 hrs post-slaughter					
Muscle pH_u	5.92 ± 0.52[a]	5.94 ± 0.46[a]	5.93 ± 0.55[a]	5.79 ± 0.55[a]	ns
Lightness (L*)	43.14 ± 2.44[a]	40.99 ± 2.26[a]	50.24 ± 18.88[a]	42.33 ± 2.09[a]	ns
Redness (a*)	4.33 ± 3.44[b]	4.16 ± 2.50[b]	2.64 ± 1.29[b]	1.54 ± 1.20[a]	*
Yellowness (b*)	11.20 ± 2.70[a]	12.08 ± 1.27[a]	10.61 ± 3.74[a]	9.87 ± 1.80[a]	ns
Chroma (C*)	12.56 ± 2.67[a]	11.99 ± 2.61[a]	10.61 ± 3.46[a]	9.90 ± 1.77[a]	ns
Hue (H*)	75.91 ± 9.07[a]	79.00 ± 13.91[a]	84.53± 13.12[a]	89.77 ± 12.70[a]	ns
Moisture characteristics					
Water holding capacity	17.02 ± 1.31[a]	21.34 ± 0.94[b]	20.94 ± 1.71[b]	21.45 ± 1.35[b]	**
Drip loss %	0.66 ± 0.04[b]	0.13 ± 0.05[a]	0.13 ± 0.05 [a]	0.10 ± 0.01[a]	***
Cooking loss %	19.21 ± 0.50[b]	14.77 ± 0.54[a]	17.93 ± 1.64[b]	17.86 ± 0.90[b]	***

Tenderness					
Shear force (N/cm^2)	1.60 ± 0.02^{b}	1.57 ± 0.02^{b}	1.40 ± 0.02^{a}	1.34 ± 0.09^{a}	***

ns = not significant, $p > 0.05$, * $p < 0.05$, ^{ab} Within row means with different superscripts are significantly different at $p \leq 0.05$. There were no differences ($p > 0.05$) in breast meat colour coordinates at initial L*, a*, b*, C*, H* and pH across the dietary treatments. Twenty four (24) hours post-slaughter redness (a*) of breast muscle from birds fed diet 4 was lower ($p < 0.05$) compared to redness of birds in diet 1, 2 and 3. Dietary MZSM significantly increased the percentages of WHC across the dietary treatments, while cooking loss and drip loss were decreasing ($p < 0.001$). Shear force values of birds fed significantly decreased across the dietary treatments. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

At the 30-mins post-slaughter, the inclusion of *M. zeyheri* seed meal as an energy source in the quail finisher diets had no effect on the muscle (meat) initial pH and colour (Table 4.11). However, at 24-hours post-slaughter, dietary inclusion of *M. zeyheri* seed meal at 37.5% of the contribution of MM to the gross energy of the diet resulted in a significant reduction in the redness of the meat (Table 4.11). Substitution MM at 12.5% of its gross energy supply to the diet with *M. zeyheri* seed meal resulted in meat with the lowest cooking loss. Substituting MM with *M. zeyheri* seed meal at the highest level (Diet 4) resulted in tenderer breast fillet (Table 4.11).

4.2.8.2 Chemical composition of broiler quail breast meat

Table 4.12 and below shows the effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on the proximate content of the quail breast muscle while Table 4.12 shows the effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on the fatty acid composition of the quail breast muscle.

Table 4.12: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on the proximate content of broiler quail breast meat

Proximate components	Dietary treatments				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Dry matter (% DM)	31.35 ± 0.37[a]	31.13 ± 3.65[a]	31.94 ± 3.05[a]	28.94 ± 0.75[a]	ns
Moisture (% DM)	68.65 ± 0.37[a]	68.87 ± 3.65[a]	68.06 ± 3.05[a]	71.06 ± 0.75[a]	ns
Ash (% DM)	4.43 ± 0.18[a]	4.41 ± 0.06[a]	4.55 ± 0.10[a]	4.61 ± 0.10[a]	ns
Protein (% DM)	78.22 ± 1.14[a]	82.67 ± 0.25[b]	82.93 ± 0.72[b]	86.27 ± 0.54[c]	***
Fat (% DM)	10.92 ± 0.24[c]	7.55 ± 0.07[b]	7.49 ± 0.10[b]	4.23 ± 0.12[a]	***

ns = not significant, $p > 0.05$, *** $p < 0.0001$. [abc] Within row means with different superscripts are significantly different at $p \leq 0.05$. Dry matter, moisture and ash components of the breast muscles were similar ($p > 0.05$) across the dietary treatments. Protein content of breast muscles significantly increased with the increase of MZSM inclusion in quail diets, while on the other hand fat content significantly decreased ($p < 0.0001$) across the dietary treatments. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri seed* meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Table 4.13: Effect of partial replacement of maize meal with *M. zeyheri* seed meal as a dietary energy source on the fatty acid profile of broiler quail breast meat

Fatty acids (%)	Dietary treatment				Significance level
	Diet 1	Diet 2	Diet 3	Diet 4	
Saturated fatty acids					
C10:0 (capric acid)	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	
C11:0 (undercanoic)	1.41 ± 0.27	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
C12:0 (lauric acid)	0.03 ± 0.00	0.38 ± 0.03	0.04 ± 0.00	0.02 ± 0.00	
C14:0 (myristic acid)	0.45 ± 0.04	0.70 ± 0.00	0.60 ± 0.00	0.51 ± 0.00	
C15:0 (pentadecanoic acid)	0.11 ± 0.00	0.14 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	
C16:0 (palmitic acid)	20.14 ± 0.05	20.12 ± 0.02	21.21 ± 0.02	19.28 ± 0.02	
C17:0 (heptadeconoic acid)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
C18:0 (stearic acid)	5.87 ± 0.01	6.83 ± 0.02	7.57 ± 0.01	10.19 ± 0.01	
C20:0 (arachidic acid)	0.12 ± 0.01	0.12 ± 0.00	0.16 ± 0.00	0.21 ± 0.00	
C21:0 (heneicosanoic acid)	0.02 ± 0.01	0.03± 0.00	0.05 ± 0.00	0.08 ± 0.00	
C22:0 (behenic acid)	0.00 ± 0.00	0.02 ± 0.00	0.15 ± 0.01	0.13 ± 0.00	
TSFA	**28.14 ± 2.10[a]**	**28.36 ± 0.40[a]**	**29.92 ± 0.61[a]**	**30.55 ± 0.65[a]**	**ns**
Mono-unsaturated FA					
C14:1 (myristoleic acid)	0.13 ± 0.01	0.11 ± 0.01	0.16 ± 0.00	0.14 ± 0.00	
C16:1 (palmitoleic acid)	7.61 ± 0.01	5.55 ± 0.09	7.45 ± 0.01	6.12 ± 0.01	
C17:1 (heptadecenoic acid)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
C18:1n9t (elaidic acid)	0.09 ± 0.01	0.12 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	

C18:1n9c (oleic acid)	41.13 ± 0.08	42.34 ± 0.11	40.15 ± 0.08	41.90 ± 0.07	
C20:1 (11-eicosenoic acid)	0.27 ± 0.00	0.34 ± 0.01	0.27 ± 0.00	0.37 ± 0.00	
C22:1n9 (erucuc acid)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	
	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
C24:1 (nervonic acid)					
TMUFA	**49.28 ± 1.64[a]**	**48.49 ± 0.34[a]**	**48.18 ± 0.38[a]**	**48.73 ± 0.92[a]**	**ns**
Poly-unsaturated FA					
C18:2n6t (linolelaidic acid)	0.05 ± 0.01	0.09 ± 0.00	0.11 ± 0.00	0.08 ± 0.00	
C18:2n6c (linoleic acid)	19.60 ± 0.05	19.54 ± 0.03	17.54 ± 0.02	17.89 ± 0.03	
C18:3n6 (γ-linolenic acid)	0.19 ± 0.01	0.16 ± 0.01	0.12 ± 0.01	0.06 ± 0.00	
C18:3n3 (α-linolenic acid)	1.14 ± 0.00	1.19 ± 0.00	0.86 ± 0.01	1.06 ± 0.00	
C20:2 (eicosadienoic acid)	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.00	1.10 ± 0.00	
C20:3n6 (dihomo-y-linolenic acid)	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
C20:3n3 (eicosatrienoic acid)	0.12 ± 0.00	0.23 ± 0.00	0.16 ± 0.00	0.21 ± 0.00	
C20:4n6 (arachidonic acid)	0.07 ± 0.01	0.15 ± 0.00	0.10 ± 0.00	0.14 ± 0.00	
C20:5n3 (eicosapentaenoic acid)	1.13 ± 0.01	1.47 ± 0.00	3.25 ± 0.01	3.29 ± 0.01	
C22:2 (docosadienoic acid)	0.06 ± 0.01	0.09 ± 0.01	0.26 ± 0.00	0.28 ± 0.00	
C22:6n3 (docosahexaenoic acid)	0.05 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	
TPUFA	**22.52 ± 0.59[a]**	**23.06 ± 0.09[a]**	**22.53± 0.70[a]**	**23.11 ± 0.14[a]**	**ns**
Trans fatty acids	28.29 ± 0.30[b]	28.60 ± 0.05[b]	30.08 ± 0.02[b]	20.71 ± 0.74[a]	***
CIS	49.23 ± 0.09[b]	48.55 ± 0.01[b]	48.30 ± 0.07[b]	45.01 ± 0.07[a]	***

Omega-3	22.31 ± 0.04^{ab}	22.69 ± 0.01^{ab}	21.39 ± 0.05^{a}	23.96 ± 0.04^{b}	**
Omega-6	0.15 ± 0.01^{a}	0.20 ± 0.01^{a}	0.27 ± 0.01^{b}	0.23 ± 0.01^{ab}	**
Omega-9	20.96 ± 004^{a}	21.35 ± 0.03^{ab}	20.19 ± 0.03^{a}	22.59 ± 0.03^{b}	*
EPA	60.73 ± 0.12^{b}	61.89 ± 0.14^{b}	57.71 ± 0.10^{ab}	56.79 ± 0.09^{a}	**
DHA	1.26 ± 0.01^{bc}	1.31 ± 0.01^{c}	1.18 ± 0.02^{b}	0.06 ± 0.01^{a}	***
TPUFA: TSFA	0.80:1	0.81:1	0.72:1	0.79:1	
n6PUFA: n3PUFA	0.007:1	0.009:1	0.013:1	0.010:1	

ns = not significant, $p > 0.05$, * $p < 0.05$, **$p<0.01$, *** $p < 0.0001$. ^{abc} Within row means with different superscripts are significantly different at $p \leq 0.05$. Total SFAs, MUFAs and PUFAs were observed similar $p > 0.05$ across the dietary treatments. Birds fed diet 4 had lowest *trans* fatty acids and CIS, while Omega 3, 6 and 9 fatty acids were significantly higher compared to birds in other dietary treatments. Dietary *M. zeyheri* seed meal significantly decreased EPA and DHA in birds fed diet 4 compared to diet 1 and 2. Diet 1 – 0% inclusion of *M. zeyheri* seed meal (control), Diet 2 – 12.5% *M. zeyheri* seed meal inclusion on energy basis, Diet 3 – 25% *M. zeyheri* seed meal inclusion on energy basis, Diet 4 – 37.5% *M. zeyheri* seed meal inclusion on energy basis; TSFA = total saturated fatty acid; TMUFA = total monounsaturated fatty acid; TPUFA = total polyunsaturated fatty acid; Data presented as mean ± SD; n: 8 for dietary treatments 1 to 3 and 7 for dietary treatment 4.

Substituting MM with MZSM on energy gross basis caused no differences in breast muscle DM, moisture and ash content across the dietary treatments. Muscle protein content significantly increased across the dietary treatment as fat content was decreasing (Table 4.12). Oleic acid was significantly higher compared to other fatty acids in breast meat, while there were no differences observed in total SFAs, MUFAs and PUFAs across the dietary treatments. TMUFAs contributed more than 45% of the fatty acid content found in the breast muscle. In terms of polyunsaturated fatty acids, the n3PUFAs were most concentrated in the muscle, followed by the n9PUFAs and the n6PUFAs are the least concentrated.

CHAPTER 5: DISCUSSION

This study determined the chemical nutrient and phytochemical (antinutrient) composition of dehulled *M. zeyheri* seed meal and the formulated test diets whereby the *M. zeyheri* seed meal was used in the graded substitution of maize meal as an energy source and evaluated the effects of the substitution on the growth performance, health and meat quality of broiler quail.

5.1 Nutrient and phytochemical composition

In the current study, the proximate content of the *M. zeyheri* seed meal revealed that the meal had 95.72%, 9.20%, 92.38%, 3.37% and 24.18±0.76 MJ/kg dry matter (DM), crude protein (CP), organic matter (OM), ash, and gross energy (GE), respectively. The current results are comparable to the 91.10% DM, 9.30% CP, 88.34% OM, 2.8% ash content and 24.34±0.56 GE MJ/kg in *M. zeyheri* seed meal as reported by Chivandi et al. (2011). Since the *M. zeyheri* seed used in the current study was collected from Matopos National Park in Zimbabwe, the slight differences could be ascribed to seasonal effects and soil condition. What is interesting is that in the current study the *M. zeyheri* seed meal with a GE content of 24.18±0.76 MJ/kg confirmed its potential as a possible dietary energy source in feeds. Similar to the findings by Chivandi et al. (2011), the fatty acid profile of the *M. zeyheri* seed meal had more of oleic acid.

In the tropics browse (twigs, leaves, fruits and seeds) are frequently used to supplement livestock nutrition especially during the long dry season (Szczerbińska et al., 2015). While this browse is of a higher nutrient value compared to the moribund dry season veld grass, its utilisation is limited to some extent due to the presence of phytochemicals such as tannins, alkaloids, saponins and lectins (Cowan, 1999; Lu and Jorgensen, 1987). These phytochemicals have anti-nutritive effects in animals (Acimovic et al., 2016). In the current study saponins, terpenoids, tannins and flavonoids were detected to be present in the *M. zeyheri* seed meal hence its use in diets has to be with caution as higher dietary concentrations may lead to poor feed utilisation efficiency, poor growth performance and may elicit damage to vital organs such as the liver and kidneys. Indeed in the pilot study prior to the current study dietary concentration of *M. zeyheri* seed meal beyond 37.5% of the GE contribution of MM resulted in reduced feed intake most likely due to the bitterness of tannins (Nyamambi et al., 2007) and foaming effect of saponins (Azzaz et al., 2011) in the meal which would reduce palatability and cause decreased feed intake.

5.2 Growth performance

In the current study, dietary *M. zeyheri* seed meal had no effect on the quails' BWG [both weekly and overall], ADG [by week and overall], empty carcass mass and dressing percentage (Table 4.5). However, at 25% and 37.5% inclusion level of MZSM's gross energy contribution to the diet, dietary *M. zeyheri* seed meal resulted in significant decrease in feed intake (FI) albeit there were no significant differences in FCR of the birds across dietary treatments. These observations suggest that *M. zeyheri* seed meal did not compromise growth performance as measured by BWG, ADG and FCR neither did it compromise carcass yield as measured by empty carcass weight and dressing percentage (Table 4.5). Results from the current study suggest that despite the significant decrease in FI at 25% (Diet 3) and 37.5% (Diet 4) substitution level, the birds fed these diets managed to grow at the same rate as their counterparts suggesting higher feed utilisation efficiency at higher dietary *M. zeyheri* seed meal levels. Górka et al. (2016) reported that the presence of tannins in the animal feed lowers the dry matter and protein digestibility which reduces overall feed intake thus it can be speculated that the reduction in FI with an increase in dietary *M. zeyheri* seed meal could have been due to the effects of tannins that were qualitatively confirmed to be present in the meal. In this study, the ADG and FCR ranged from 1.32g to 2.00g and 8.86 to 18.56, respectively. The slaughter weight and dressing percentage that ranged from 185.64g to 191.50g and 66.95% to 70.05%, respectively. Several authors have reported ADG ranges of 1.2g to 23g, FRC ranging from 7.49 to 65.63, slaughter weights ranging from 8g to 300g and dressing percentages ranging from 65% to 70% in Japanese quail (Mnisi et al., 2017; Karthika and Chandirasekaran, 2016; Randall and Bolla, 2008; Vali, 2008; Almeida et al., 2002). In the current study, ADG, FCR, slaughter weights, and dressing percentage of the quail on the control and test diets fall within the range reported by various researchers. These findings suggest that *M. zeyheri* seed meal can be used to replace MM as a dietary energy source (up to 37.5% of MZ's GE contribution to the diet) without compromising growth performance, the economy of feed utilisation and carcass yield in male broiler quail.

5.3 Viscera morphometry

Apart from being the major site for nutrient digestion and absorption the GIT also plays a protective role against exogenous pathogens and is the body's largest immunological organ (Choct, 2009). The growth and development of the different organs of the GIT in birds and indeed other animals is influenced by the nutrient composition of the diet (Ranjitkar et al., 2016;

Svihus, 2014). In young birds diets with 3-4% fibre content (moderate fibre content) have been shown to stimulate GIT development and digestive enzyme production (Sacranie et al., 2012; Jiménez-Moreno et al., 2009) which translate into a healthy digestive physiology and growth performance in broiler chicken (Gonzalez-Alvarado et al., 2010). The small intestine is the major site for nutrient absorption and an increase of villi height/crypt depth ratio is associated with an increased nutrient absorptive capacity (Ranjitkar et al., 2016). In the present study, dietary *M. zeyheri* seed meal did not affect the mass of the large intestines of the quail, but caused a significant increase in the masses of the proventriculi, ventriculi, small intestines and caeca as well as the length of the small intestines of the quail (Table 4.6). The increase in the mass and length (small intestine only) of these GIT organs increased with an increase in the concentration of *M. zeyheri* seed meal in the diet. This increment of small intestine sizes was also confirmed by the results of micro-morphometry (Table 4.10). These findings suggest that dietary *M. zeyheri* seed meal positively impacted the growth and development of the proventriculi, ventriculi, small intestines and caeca of the quail in a dose-dependent manner. The ventriculi and caeca are major sites of digestion in birds and the small intestine is the major site of nutrient absorption. Results of the current study suggest that despite the decrease in FI in quail fed the diet with the highest *M. zeyheri* seed meal inclusion, by virtue of them having longer small intestines with longer villi these birds had a more efficient feed digestion and absorption as well as better nutrient utilisation as measured by their FCR (Table 4.5). The more efficient feed utilisation observed in this study can be speculated to have emanated from positive effects of the dietary *M. zeyheri* seed meal on the proventriculi, ventriculi, small intestines and caeca of the quail.

The observed increase in the masses of pancreata with an increase in dietary *M. zeyheri* seed meal (Table 4.6) might have resulted in increased production and release of pancreatic digestive enzymes resulting in more efficient nutrient extraction (digestion and absorption). This might explain the similarity in FCR in the birds across dietary treatments despite the observed decreased FI at higher *M. zeyheri* seed meal inclusion levels (Table 4.5).

Fouad and El-Senousey (2014) contend that in avian species dietary lipid supply influences body fat accretion in the birds. In another study, Hashiguchi and Esumi (2013) observed that feeding a diet with alpha-lipoic acid in male quail caused an increase in liver mass by some 1.47% to 2.65% of body mass, it, however, caused a linear decrease in body mass. Importantly

phytochemicals present in the dietary ingredients are known to impact visceral adiposity in birds (Acimovic et al., 2016). In broiler chicken, Haščík et al. (2015) reported that flavonoids extracted from propolis decreased abdominal fat from 2.05% to 1.87%. In the current study, the phytochemical screening of the *M. zeyheri* seed meal revealed the presence of flavonoids (Table 4.4). In the current study, the consumption of *M.zeyheri* seed meal based diets resulted in a decrease in visceral fat mass (Table 4.6). This reported decrease in visceral fat mass could be attributed to the presence of flavonoids in the *M. zeyheri* seed meal.

In the current study, while the mean liver mass increased with an increase in dietary *M. zeyheri* seed meal, liver lipid content decreased with an increase in the concentration of the meal in the diet (Table 4.6, Figure 4.2). The higher liver mass of quails fed diet 4 could possibly be explained by the fact that hepatocytes from these quail were larger compared to those from quails fed diets 1 to 3 (Table 4.6). Despite the observed differences in the liver masses of the quail, on average the observed liver masses across treatment diets are in agreement with those reported by Imam et al. (2016). Although the mean liver mass fall within the range reported by other researchers, caution must be taken in the use of *M. zeyheri* seed meal as it caused morphometric changes in hepatocytes (Table 4.6, Figure 4.3).

5.4 Erythrocyte osmotic fragility

Erythrocyte osmotic fragility (EOF), a measure of erythrocyte membrane stability in hypotonic solution (Salka Minka, 2013), is also an important biomarker of oxidative stress (Asala et al., 2011). Serum biomarkers of health as wells as EOF are influenced extrinsic and intrinsic factors (Mafuvadze and Erlwanger, 2007). Tuleun and Adenkola (2013) also point out diet composition as one of the major factors that impact EOF. The consumption of diets rich in polyunsaturated fatty acids has been shown to effect a reduction in EOF while the consumption of diets rich in saturated fatty acid increases erythrocyte membrane rigidity which (membrane rigidity) results in increased fragility and susceptibility to lysis (Tuleun and Adenkola, 2013). In the current study, IH and MH of birds were similar across treatment diets but the MCF of quail fed diet 4 was higher (Table 4.7, Figure 4.1). This suggests that the *M. zeyheri* seed meal caused the erythrocyte membranes to be fragile.

5.5 Plasma markers of general health of broiler quail

5.5.1 TBARS and circulating metabolic substrate content

Plasma malondialdehyde (MDA) concentration is a vital indicator of stress (Tsikas, 2017) that results from lipid peroxidation (Ferdinand et al., 2017). Lipid peroxidation generates free radicals that oxidise dietary polyunsaturated fatty acids (Gasparovic et al., 2017). Feeding Japanese quail Hempseed meal which had a high ratio of n-3 and n-6 fatty acids was reported to decrease MDA concentration (Konca et al., 2014). Results of the current study indicated an increase in MDA concentration with an increase in dietary *M. zeyheri* seed meal (1.03 to 2.07nM/mL). An increase in lipid peroxidation has been reported to spawn increased MDA concentration (Seven and Seven, 2009). Results of the current findings, therefore, suggest that dietary *M. zeyheri* seed meal increased lipid peroxidation as denoted by increased MDA concentration with increasing dietary *M. zeyheri* seed meal. The increased MDA concentration with increasing dietary *M. zeyheri* seed meal could be speculated to have resulted from the low n-3 to n-6 fatty acid ratio in the *M. zeyheri* seed meal since a high n-3 to n-6 fatty acid ratio has been reported to decrease MDA concentration (Konca et al., 2014).

Changes from the effect of pollutants in the biochemical blood profile mirror changes in metabolism and biochemical processes of the organism (Bamidele et al., 2015). Blood glucose level plays a very important role in the regulation of glucose in the tissues and production of energy for use in the metabolic processes (Kian et al., 2011). In the current study, no differences were observed in plasma glucose concentration of the birds across the dietary treatments but triglycerides (TGs) were significantly increased in birds fed diet 3 (Table 4.8). The current findings on blood glucose concentration suggest that dietary *M. zeyheri* seed meal did not compromise blood glucose regulation. Exogenous TGs originate from food while endogenous TGs are formed in the liver. In the current study, the plasma triglyceride concentration of quail fed diet 3 (25% inclusion of *M. zeyheri* seed meal) was the highest when compared to that from birds fed other diets. It is difficult to ascribe the increased plasma TGs to be either of dietary origin or to be from increased export of TGs from the liver into the blood. However, since liver lipid decreased with the increase of dietary *M. zeyheri* seed meal inclusion, we speculate that the observed increase blood TGs concentration in quail fed diet 3 likely originated from increased export of TGs from the liver to the blood.

5.5.2 Surrogate markers of kidney and liver function

The kidneys play several vital roles in animals and birds: they eliminate metabolic wastes (Lierz and Vet, 2002), detoxify metabolites (Mobini and Abdollahi, 2016) and help regulate blood pressure (Dhyaa, 2014) and volume (Batah, 2012). In birds, uric acid is an end product of amino acid and purine catabolism (Nazifi et al., 2011) and is excreted through the kidneys. Plasma urea and uric acid concentrations are used to assess avian renal function (Maiuolo et al., 2016). Kidney malfunction manifests with increased plasma urea and uric acid concentration (Akbarian et al., 2016). However other non-renal factors, such as hydration status, diet and gastrointestinal haemorrhage (Dossetor, 1966) can cause elevated plasma urea concentration (Cockcroft and Gault, 1976). Results of the current study indicated that the concentration of plasma uric acid was similar in the quail across the dietary groups, while plasma urea concentration was increased in birds fed diet 3. These findings suggest that dietary inclusion of *M. zeyheri* seed meal at 12.5% and 37.5% did not elicit kidney malfunction while its inclusion at 25% might be associated with compromised kidney function as reflected by the increased plasma urea concentration. The increased plasma urea concentration might be indicative of a failure by the kidneys to efficiently and effectively excrete the urea. However, the lack of trend in this particular observation and it not easy to explain.

Plasma bilirubin is formed from the haem component of haemoglobin (Gilmore and Garvey, 2013). Bilirubin is conjugated in the liver by enzymes in hepatocytes making it soluble such that it can be excreted either by the kidneys (Kalakonda and John, 2018) and or through faeces. An elevated plasma total bilirubin concentration indicates compromised liver function (Takeda et al., 2015). Results of the current study showed an increase in plasma bilirubin concentration with an increase in dietary *M. zeyheri* seed meal. Importantly, the current study reports a decrease in hepatocyte density per field and an increase in hepatocyte size with an increase in dietary *M. zeyheri* seed meal. These observations suggest that the *M. zeyheri* seed meal might have compromised hepatic micro-histomorphometry, bilirubin metabolism and excretion. It could also be speculated that the increased erythrocyte fragility with increased dietary *M. zeyheri* seed meal increased the supply of haem for bilirubin formation. Therefore the use of *M. zeyheri* seed meal as a feed ingredient in quail at inclusion levels of 25% and possibly above in finisher diets should be done with caution as it may contain some toxic compounds that are harmful to the liver.

Indeed the qualitative phytochemical characterisation of the meal revealed the presence of saponins that are known to be potentially toxic to the liver (Bone and Mills, 2013). Increased plasma ALT and ALP are surrogate biomarkers for intra-hepatic liver damage (Rafter et al., 2012; Robles-díaz et al., 2016) and of post-hepatic liver disease, respectively (Giboney, 2005). Hepatocytes, which contain transaminases including ALT an AST, are the major metabolic sites (Hall and Cash, 2012). Damage to hepatocytes results in a leakage of transaminases into the plasma (Gowda et al., 2009). In the current study, dietary *M. zeyheri* seed meal inclusion at 25% increased plasma ALT activity. Additionally, dietary inclusion of *M. zeyheri* seed meal at 12.5% and 37.5% increased plasma ALP activity (Table 4.8). These results suggest that dietary *M. zeyheri* seed meal might have damaged hepatocytes and bile duct cells as manifested by increase plasma ALT and ALP activity, respectively. Despite the observed normal growth and feed efficiency shown by the birds across dietary treatments, any probable use of *M. zeyheri* seed meal as a feed ingredient must be done with caution as evidence from the current study suggest it might compromise liver function in broiler quail.

5.6 Liver lipid content and morphometry

The liver is a metabolic centre. Compromising liver function and micromorphometry manifests with altered physiological function (Jafargolipour et al., 2017). In the current study, the graded dietary inclusion of *M. zeyheri* seed meal reduced total liver lipid content and steatosis (Table 4.9, Figure 4.2). This finding suggests that the *M. zeyheri* seed meal may have exerted lipolytic and or anti-lipogenic effects in the liver of quail. Qualitative screening for phytochemicals revealed the presence of saponins, terpenoids, flavonoids and tannins that are well known for their lipolytic and anti-lipogenic effects. Therefore, it can be speculated that the decrease in total liver lipid can be ascribed to the phytochemicals that were present in the *M. zeyheri* seed meal that was used in the current study.

Additionally, findings from the current study showed that dietary *M. zeyheri* seed meal while it increased hepatocyte size it reduced the hepatocyte and Kupffer cell density (Table 4.9). Kupffer cells are part of the reticuloendothelial system which phagocytoses xenobiotics (Yona and Gordon, 2015). While the *M. zeyheri* seed meal could potentially reduce the risk of developing fatty-liver disease in quail, it might expose the birds to decreased hepatic xenobiotic detoxification. However, a decrease in Kupffer cell density is associated with decreased

inflammation and reduced steatosis (Huang et al., 2010; Sakai et al., 2010). Based on the current study findings it can also be speculated that dietary *M. zeyheri* seed meal might have reduced inflammation which translates into the observed Kupffer cell density, liver lipid content and steatosis. It can also be inferred that dietary *M. zeyheri* seed meal inclusion was beneficial to the health of the liver of the birds when compared to their counterparts fed the control diet.

The observed increase in hepatocyte size and decrease hepatocyte density with increasing dietary *M. zeyheri* seed meal inclusion though difficult to explain, it might be speculated that the increase in plasma bilirubin concentration might be indicative of reduced bile excretion. Reduced bile excretion results from biliary system obstruction that is characterised by increased plasma ALP activity. Therefore it can be speculated that dietary *M. zeyheri* seed meal might cause biliary system obstruction that translates into increased hepatocyte size and reduced density (Table 4.9).

5.7 Meat quality attribute

5.7.1 Physical attributes of broiler quail breast meat

Consumers consider the meat's appearance, juiceless, texture, tenderness and flavour among the critical determinants that influence the acceptability of the product (Mir et al., 2017). Some of these quality-determining factors are influenced by the immediate pre-slaughter as well as the poster slaughter handling.

5.7.1.1 Colour

After slaughter, biochemical changes, converting muscle to meat, determine final meat quality (Mir et al., 2017). The quality of poultry meat can be predicted by the initial pH (Ristic and Damme, 2010). Dark firm and dry (DFD) meat usually has a pH of 6.58 (Genchev et al, 2010) while pale, soft and exudative (PSE) meat is generally characterised by a pH of 5.8 (Ristic and Damme, 2010). Japanese quail meat generally has pHu of 5.61 to 6.17 which is lower (6.02 to 6.41) compared to that of meat from other poultry species (Riegel et al., 2004). In the current study, pHi of broiler quail breast muscle across the dietary treatments ranged from 6.29 to 6.43 while pHu ranged from 6.01 to 6.16 (Table 4.11). However, the lack of differences in the pHi and pHu of the quail breast muscle across dietary treatments suggests that *M. zeyheri* seed meal did not affect the meat`s pH. Findings from the current study are at variance with those by Riegel et al. (2004) since instead of having a pHu which is lower, the pHu of the quail breast muscle was

within the range of other poultry species. The observed decrease in pH of the muscle from pHi to pHu values is most likely due to post mortem anaerobic conversion of glycogen stored in the muscle to lactic acid.

The colour of meat, which is species dependent, is an organoleptic trait which is indicative of the freshness and tenderness of the meat (Ribarski and Genchev, 2013). The significant differences in colour range among meat from different species are due to the differences in the content of myoglobin (Mb) in muscle (Joo et al., 2013). The myoglobin content, in turn, is influenced by factors such as exercise, genetic and environmental factors such as diet (Joo et al., 2013). Importantly myoglobin concentration is positively associated with the redness and preferred acceptability of meat (Khliji et al., 2010). In the current study, there were no differences in the colour of meat 30-min post-slaughter across dietary treatments. However, 24-hours post-slaughter the breast muscle from quail fed the diet with the highest inclusion of *M. zeyheri* seed meal was lighter with respect to redness. This suggests that at a higher dietary concentration the *M. zeyheri* seed meal might have decreased the myoglobin concentration in the breast muscle. Consumers prefer meat which is redder (Khliji et al., 2010) and therefore it can be speculated that higher dietary *M. zeyheri* seed meal inclusion might compromise consumer acceptability of the meat.

Besides myoglobin concentration the colour of meat is also influenced by the pH: with lighter muscles (L*>50) having higher pH and darker muscles (L*<45) having a lower pH (Allen et al., 1998). In the current study the observed decrease in the breast (meat) muscle lightness (L*) 24-hours post-slaughter cannot be explained on the basis of the pHu since it was similar in the breast muscle across the dietary treatments.

5.7.1.2 Moisture characteristics

In raw meat poor water holding capacity (WHC) results in diminished organoleptic appeal leading to poor acceptability of the meat (Huff-Lonergan and Lonergan, 2005). A decrease in the pH of meat is associated with a reduction in WHC (Qiao et al., 2001) which results in compromised tenderness and juiciness on cooking (Hughes et al., 2014). In the current study, dietary *M. zeyheri* seed meal increased WHC which resulted in decreased in-drip and cooking

loss (Table 4.11). Therefore, it can be inferred that dietary *M. zeyheri* seed meal improved tenderness and juiciness on the meat.

5.7.1.3 Shear force

Tenderness is the single most important sensory property affecting the acceptability of meat (Muchenje et al., 2009a). The texture of meat is affected by several factors inclusive of WHC, intramuscular fat, actomyosin complex and collagen type (Coro et al., 2003). In birds, perimysium and endomysium thickness and muscle fibre diameter determine tenderness and toughness of meat (An et al., 2010). In the current study, the shear force required to cut through breast fillets decreased with an increase in dietary *M. zeyheri* seed meal inclusion. It can be speculated that the consumption of *M. zeyheri* seed meal results in tenderer meat.

5.7.2 Chemical composition of the meat

5.7.2.1 Proximate content

Japanese quail produce lean meat (Boni et al., 2010) that is characterised by high degree of unsaturated FAs, high protein content and low fat and cholesterol levels (Gecgel et al., 2015). Meat from quail breast muscle on average contains 19.6% protein, 12.1% fat and 0.076% cholesterol (Ioniţă et al., 2010). In the current study, the major proximate findings were an increase in the quail breast meat protein content with an increase in dietary *M. zeyheri* seed meal inclusion and a concomitant decrease in fat. Results of the current study suggest that dietary *M. zeyheri* caused greater accretion of lean meat which translates to a better product viz consumer health thus it can be inferred that dietary *M. zeyheri* seed meal could be exploited to produce leaner meat cuts.

5.7.2.2 Fatty acid profile

Dietary lipid composition affects the quantity and quality of the fat of meat (Costa et al., 2016). The presence of high concentrations of SFAs and trans FAs in poultry meat is one of the major contributors to diet-induced causes of cardiovascular (Mayneris-Perxachs et al., 2010) and other metabolic diseases (Kwon, 2016). Unlike SFAs and trans FAs, MUFAs and PUFAs in the diet have been shown to reduce the risk of cardiovascular diseases (Gecgel et al., 2015). In quail breast meat the TSFAs, TMUFA and TPUFA range between 28.17%-30.53%, 44.43%-55.71% and 22.51%-24.11%, respectively (Gecgel et al., 2015). Results of the current study showed that

the quail breast meat TSFAs, TMUFA and TPUFA ranged from 28.17% to 30.53%, 44.73% to 49.28% and 22.51% to 24.11%, respectively and are in tandem with those reported by Gecgel et al. (2015). Findings of the current study suggest that the inclusion of dietary *M. zeyheri* seed meal neither improves nor compromises the fatty acid profile of the meat. Another important finding of the current study is that the TMUFA and TPUFA were the major lipid constituents in the quail breast meat. This finding further reiterates the health beneficial fatty acids profile of the meat.

The next chapter gives a summary of the key conclusions drawn from the study, limitations of the current study and some recommendations for future studies.

CHAPTER 6: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusions

The aim of this study was to investigate whether the graded substitution of MM in broiler quail finisher diets with *M. zeyheri* seed meal, on a gross energy basis, would have any effect on the growth performance, feed utilisation efficiency, macro-and-micro viscera morphology, health profile and meat quality of broiler quail.

Mimusops zeyheri seed meal can partially replace MM in quail finisher diets without compromising growth performance and feed utilisation efficiency. The meal increased villi height and width and villi height:crypt ratio suggesting that it increased absorption surface area. Dietary *M. zeyheri* seed meal exerted anti-lipogenic properties. Caution has to be exercised in the use of *M. zeyheri* as a dietary energy source as it may compromise liver function and increase lipid peroxidation in the birds. The seed meal produced leaner meat with higher protein content. Moreover, finding on meat quality suggest that the dietary *M. zeyheri* seed meal improved the WHC, tenderness and juiciness.

Limitations

A limitation of the current study was the failure to determine all factors that impact meat colour. Additionally, the study evaluated the antioxidant status of the birds using indirect markers.

Recommendations for future studies

Based on the findings from the current study *M. zeyheri* seed meal can be utilised as a dietary energy source in quail finisher diets. However, caution such as processing the meal to reduce possible ANFs effect should be taken as it may have adverse effects on liver function.

CHAPTER 7: REFERENCES

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APPENDICES

Appendix 1

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2017/08/56/B

APPLICANT: Ms B Mdoda

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: The potential of Mimusops zeyheri seed meal as a dietary energy in broiler quail diet

Number and Species

75X 5 weeks old male Jumbo Japanese quail (coturnix coturnix)

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 2019/10/25.
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: 27/10/2017

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: 27 October 2017

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

Works 2000/lain0015/AESCCert.wps

Appendix 2

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

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

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

Appendix 3

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 4073.

28 August 2017.

To: Department of Agriculture Forestry and Fisheries

Attention; Mr. Mdoda

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

Dear Mr. Mdoda

The following researcher, Mr. Bayanda Mdoda, 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. Mdoda requires 75 (5 week-old) broiler 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
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