Inclusion of mopane worm meal in Jumbo quail diets: Nutrient utilization, physiological and meat quality responses

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DECLARATION

I, Mveleli Marareni, hereby declare that this dissertation submitted to the North-West University is my own original work conducted under the supervision of Prof. C.M. Mnisi and has not been previously submitted to any other institution. Material and information adopted from other sources contained herein have been dully recognised and fully acknowledged.

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GENERAL ABSTRACT

There is an urgent need to mitigate the negative impact of soybean production on the environment while ensuring sustainable poultry production to meet the protein requirements for the growing human population. Insect meals such as mopane worm (*Imbrasia belina*) are considered as sustainable alternative protein sources because of their high biological value, small carbon footprint, and less water and space requirements compared to plant protein sources, particularly soybean. Replacing soybean products in poultry diets with insect protein sources could ensure sustainable intensification and profitable commercialisation of the poultry industry. This would also allow the diversification of the industry with poultry species such as quail (*Coturnix coturnix*) to complement the supply of dietary protein for human consumption. Therefore, this study was undertaken to determine the effect of partial substitution of soybean products with graded levels of mopane worm meal (MWM) on feed utilisation, growth performance, serum biochemistry, carcass characteristics, internal organ sizes, and meat quality parameters in Jumbo quails. A total of 384, two-week-old mixed-gender quails (71.2 ± 5.40 g live-weight), were evenly and randomly allocated to 32 replicate pens (experimental units) and reared using four isoproteic and isoenergetic experimental diets formulated by replacing soybean products in a commercial standard grower diet as follows: 1) MWM0 = a commercial standard grower diet without mopane worm meal; 2) MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal; 3) MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal; and 4) MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal. Average weekly gain-to-feed ratio (G:F) was calculated using average weekly body weight gain (ABWG) and average weekly feed intake (AWFI) measured during the four-week feeding trial. At six weeks of age, two quails per experimental unit were left behind for apparent nutrient

digestibility measurements and the rest of the birds were taken to a local abattoir for slaughter. During slaughter, blood was collected from two birds that were randomly selected per pen for analysis of serum biochemistry. Thereafter, the carcasses were eviscerated and used to measure carcass characteristics, internal organ sizes and meat quality parameters. Repeated measures analysis showed no significant week × diet interaction effect on AWFI, ABWG, and G:F. There were neither linear nor quadratic effects (P >0.05) on overall feed intake, overall body weight gain, final body weight, and overall G:F in response to increasing levels of MWM. Similarly, no significant dietary effects were observed on overall feed intake and overall growth performance traits of the quails. No significant linear and quadratic trends were observed for dry matter digestibility (DMD), organic matter digestibility (OMD), neutral detergent fibre digestibility (NDFD) and crude protein digestibility (CPD) with the exception of acid detergent fibre digestibility (ADFD), which linearly declined [y = 403.7 (±27.43) - 0.26 (±0.88) x; R^2 = 0.338, P = 0.003] in response to increasing levels of dietary MWM. Likewise, experimental diets affected (P >0.05) only ADFD. Regression analyses also showed no linear and quadratic responses for serum biochemical parameters as MWM levels increased. Similarly, no dietary influence (P >0.05) were observed on all serum biochemical indices. There were no significant linear or quadratic trends for all carcass traits and internal organs, except for weights and lengths of large intestines which linearly declined [R^2 = 0.138; P = 0.040] with MWM inclusion levels. At 24 h post-mortem, quadratic trends (P <0.05) were observed for breast meat redness (a*) [R^2 = 0.118; P = 0.046], yellowness (b*) [R^2 = 0.094; P = 0.001] and chroma [R^2 = 0.312; P = 0.0004] as MWM levels increased. There were significant dietary effects on b*, chroma and shear force. Diets MWM5 and MWM10 promoted higher (P <0.05) b* and chroma values than the control diet MWM0. Breast meat from quails on diet MWM5 had the highest (P <0.05) shear force (2.39 N) than breast meat from quails fed diets MWM0 and MWM10. It was concluded that MWM has a potential to replace soybean products because it promoted similar

growth performance, nutrient utilisation, serum biochemical indices, and carcass traits as the soybean-based control diet. An optimum inclusion level of MWM could not be determined using the measured parameters, suggesting a need to further investigate levels beyond 150 g/kg of MWM in diets of Jumbo quails.

Keywords: Blood parameters, Growth performance, Insect meal, Jumbo quails, Meat quality, Sustainable intensification

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DEDICATION

I dedicate this dissertation to my parents, Mr L.S. Marareni and Mrs P.M. Marareni, and to my entire family oMsimanga!

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PEER-REVIEW ARTICLE FROM THIS DISSERTATION

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LIST OF ABBREVIATIONS

AA	:	Amino acids
ADF	:	Acid detergent fibre
AWFI	:	Average weekly feed intake
AWG	:	Average weight gain
BSFL	:	Black soldier fly larvae
CCW	:	Cold carcass weight
CP	:	Crude protein
G:F	:	Gain-to-feed ratio
GHG	:	Greenhouse Gas emission
GLM	:	General linear model
HCW	:	Hot carcass weight
MW	:	Mopane worm
MWM	:	Mopane worm meal
NDF	:	Neutral detergent fibre
OM	:	Organic mater
SAS	:	Statistical analyses system
SBM	:	Soybean meal
WHC	:	Water holding capacity

1 CHAPTER ONE: INTRODUCTION

1.1 Background

The poultry industry is one of the largest agricultural sectors in South Africa that has sophisticatedly evolved from backyard household farming to large-scale commercial production systems over the past century. The industry has also seen the evolution of novel bird species known as quails (Mnisi *et al*., 2017). Quails (*Coturnix coturnix*) belong to the family of *Phasianidae* and the class *Galliformes* (Minvielle, 2004; Huss *et al*., 2008). The Food and Agriculture Organisation (FAO) has advocated for the recognition of alternate food sources as an initiative to ensure food security in the rising world population, which is expected to reach 9.6 billion by 2050 (Rosen *et al*., 2016). The addition of novel bird species of game-birds to the poultry industry such as the Jumbo quail presents a major opportunity for alleviating protein-energy malnutrition, predominantly in the most vulnerable areas of the world (Priti & Satish, 2014). Currently, quails are becoming popular throughout the world as a source of meat and eggs. The Jumbo quail is a diversified bird reared for egg and meat production (Priti & Satish, 2014). One favourable trait of a quail is its strong resistance against several poultry diseases, suggesting that no/little vaccination is required. These birds reach sexual maturity as early as six weeks and produce eggs in full potential in 50 days (Genchev *et al*., 2005; Huss *et al*., 2008; Vali, 2008). For optimal performance, quails require a well-balanced diet with high energy (2,600 - 3,000 ME kcal/kg) and protein levels (20 - 24%) as well as highly digestible amino acids (AA) (Altin *et al*., 2016). Currently, there is no commercial standard quail diet, but to attain optimal performance a game-bird diet is recommended (Randall & Bolla, 2008). However, this game-bird diet comes at a higher cost and, as a result, farmers resort to the use of commercial chicken diets, which results in suboptimal performance. Nonetheless, the constant rise in feed costs and the limited supply of commercial feed has resulted in an increased demand for alternative protein sources to use in place of soybean (Moreki *et al*., 2012).

In poultry feeds, soybean meal (SBM) is a vital ingredient that supplies high-quality dietary protein (43.8 - 49.9%) (Banaszkiewicz, 2011), and often times referred to as a gold standard. However, this protein source has a direct food value to human beings and is in high demand by the animal, food, and biofuel sectors. This has resulted in an increase in the market prices of soybean making it less, if not, accessible by resource-poor farmers (Beski *et al*., 2015). There is, therefore, a need to evaluate alternative protein sources that have a high biological value and a potential to supply essential amino acids (EAA) to enhance the performance of Jumbo quails without compromising their health status and meat quality attributes. Mopane worm (*Imbrasia belina*), is one such alternative with high-quality protein (55 - 57%) (Moreki *et al*., 2012). The mopane worm is a caterpillar of mopane moth (*I. belina*), which feed on leaves of mopane trees (*Colophospermum mopane*). When compared to beef and chicken meat, these worms have high protein, carbohydrates, fat, and valuable mineral contents (Chiripasi *et al*., 2013; Kwiri *et al*., 2014).

1.2 Problem statement

Feed costs account for approximately 70% of the total cost of production, which poses a major challenge for intensive production units (Delport *et al*., 2017). Quail diets are formulated from varieties of plant material such as maize, soybean meal, groundnut cake, sorghum, millet, and rice or wheat bran, which are generally low in EAA such as methionine and lysine (Altine *et al*., 2016). While commercial animal products such as fishmeal, bone meal, and blood meal are a good source of EAA, they are not sustainable to ensure profitable quail farming (Adeyemo & Longe, 2007). In addition, quails require high-quality dietary protein that is equivalent to that which is needed by humans due to the nature of their digestive system (Minvielle, 2004;

Altine *et al*., 2016). Sustainable intensification of quails may be prohibited by inaccessibility to protein sources such as SBM (Moreki *et al*., 2012), which is used as a standard protein source. The increasing demand of soybean beyond supply in feed production has necessitated the need to explore other potential protein sources for cost-effective quail production (Wickramasuriya *et al*., 2015). Furthermore, there are high variable costs (seed, fertilizer, fuel, chemicals, custom operations, repairs, purchased irrigation water, interest rates, and labour) associated with the production of soybean, which is currently experienced by the major soybean-producing countries (United States, Argentina and Brazil) (Sharma, 2016).

The demand for soybean as a protein source in the animal feed industry, food sector and other applications (biofuel, lubricant, and bioplastic) has increased tremendously in a way that its production has doubled (Castanheira & Freire, 2013). To this end, the expansion of soybean production areas has caused serious social and ecological problems (Brandão *et al*., 2010), since there is limited land available for other agricultural activities. Concerns have also arisen with regard to the high carbon footprint, which comes as a result of soybean production (Castanheira & Freire, 2013). Indeed, the cultivation of soybean is highly dependent on the use of fuel, machinery, pesticides and fertilisers, which in turn contribute to greenhouse gas emissions (GHG) (Arrieta *et al*., 2018). In addition, nitrogen oxide (N_2O) emitted by nitrogen fertilisers and mineralisation of organic matter are the major contributors of GHG in soybean production because N_2O has a high global warming affinity compared to carbon dioxide (Brandão *et al*., 2010).

1.3 Justification

The utilisation of insect protein sources such as MWM provides an opportunity to directly or indirectly increase the supply of dietary protein for human consumption. Thus, the use of insects as a protein source during feed formulations could lead to elevated conversion efficiency and smaller environmental footprint (Yen, 2009). Mopane worm meal is sustainable and can provide high-quality protein and minerals to supplement cereal-based diets (Ifie & Emeruwa, 2011). Manyeula *et al*. (2019) reported that MWM has a crude protein (CP) content of (55 - 57%) and high in lysine (3.95%) and methionine (0.9%) compared to SBM having 3.22% lysine and 0.69% methionine (Banaszkiewicz, 2011). The amino acid profile of MWM compares favourably well with that of SBM, but MWM is high in threonine, valine, phenylalanine and tryptophan concentrations (Madibela *et al*., 2009). Giddie *et al*. (2013) suggested that leafy material in the gut of the worms dilutes the CP content due to the presence of secondary plant metabolites, tannins, in the leaves. In addition, these leaves increases the fibre content of MWM. Therefore, degutting the worms prior to feeding improves the CP concentration of the worms by 10% (Madibela *et al*., 2009), and thereby increase protein bioavailability in the lower gastro-intestinal tract. Of interest is that MW contain unsaturated seed like oil acids rather than typically fat, which is a good source of fatty acids such as oleic linoleic and α-linolenic (Rapatsa & Moyo, 2019). In addition, meat from birds fed with rations containing 40% MWM had higher mineral content than those fed 20% MWM (Moreki *et al*., 2012).

Oppong (2013) noted that mopane worms are underutilized because people are not familiar with their potential benefits. The availability of mopane woodlands promotes a large occurrence of MW during the periods of October – November and again in February – March in Southern Africa (Oppong, 2013). Traditionally, rural households collect MW to supplement their daily diets with high-quality protein (Ndlovu *et al*., 2019). With regards to organoleptic properties of meat from Tswana hens, Manyeula *et al*. (2019) noted no differences in meat juiciness, tenderness, and odour in hens fed MWM from those fed with SBM-containing diets. Madibela *et al*. (2007) noted that MW that were degutted, intact, roosted or cooked all had an *in vitro* digestibility greater that 80%. Thus, the use of MWM in Jumbo quails provides a potential strategy to ensure sustainable intensification of these birds in a large-scale production system. Indeed, this study represents the first ever attempt to use MWM in place of soybean products in diets of Jumbo quails.

1.4 Aim and objective

The study was designed to investigate the feed value of mopane worm meal in place of soybean products in diets of Jumbo quails: The following specific objectives guided the study:

a. To determine the effect of graded levels of mopane worm meal in place of soybean products on feed utilisation, growth performance and serum biochemical parameters of Jumbo quails.

b. To investigate the effect of replacing soybean products with graded levels of mopane worm meal on carcass characteristics, internal organ sizes and meat quality traits of Jumbo quails.

1.5 Overall hypothesis

a. The study tested the hypothesis that inclusion of mopane worm meal in place of soybean products improves apparent nutrient digestibility, growth performance and serum biochemical indices in Jumbo quails

b. It further tested the hypothesis that replacing soybean products with mopane worm meal would not alter the internal organ sizes, carcass characteristics and meat quality parameters of Jumbo quails.

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2 CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

There is a dire need to increase agricultural production as a way to ensure food and nutrition security, and to sustain the ever-increasing global human population coupled with more demanding consumers. However, this would inevitably bring enormous pressure on food producers who are already struggling with overexploited scarce resources such as land, fertilizers, water and electricity. This consequently imposes an urgent need to identify alternate protein sources, whose production do not result in environmental pollution and degradation as well as deforestation (Van Huis *et al*., 2013). To attain optimal poultry production to satisfy the demands for animal protein, it is important to diversify both the bird species and the feed ingredients used. The use of alternative ingredients should seek to minimize the poultry industry's reliance on expensive ingredients such as maize and soybean grains, which have direct human food value and environmental repercussions (Mnisi & Mlambo, 2019). This predicament can be addressed by exploiting not only the already known unconventional protein sources (*Hermetia illucens*, *Tenibro molitor*, *Bombyx mori*, and *Musca domestica*) but also through the identification and introduction of new and lesser known alternative protein sources (*Imbrasia belina*). The production of these protein sources do not depend on the use of arable land, and thus do not compete with food crops for human nutrition (Ijaiya & Eko, 2009; Cassidy *et al*., 2013). A number of scholars believe that insect meals should be considered as a new alternative protein source to replace or complement the conventional protein sources used in animal feeds (Sánchez-muros *et al*., 2014; Bovera *et al*., 2016; Dabbou *et al*., 2018). The use of insect meals as alternative protein sources is attributed to the favourable features of insects, which includes high feed conversion efficiency, low GHGs emission, low risk of transmitting zoonotic infections, low water requirements, and have few animal welfares issues, although the ability of insect tolerance to discomfort is largely unknown (FAO, 2013).

2.2 Quails

Quails (*Coturnix coturnix*) are dual-purpose birds produced for meat and eggs. Quails develop rapidly and attain sexual maturity by the age of six weeks. Jumbo quail is a bigger and meatier version of the Japanese quail (*Coturnix coturnix japonica*) (Mbhele *et al*., 2019). The Japanese quail is regarded as a separate species from the common quail that migrated throughout Asia, Europe, and Africa. Japanese quail is not related to the native North American bobwhite (*Colinus virginianus*) or California quail (*Lophortyx californica*). The migratory quails are said to have been first domesticated in China in the 11th century and later brought to Japan in the 12th century, whereby they were bred for a decade and later introduced in China, Korea, and Taiwan (Huss *et al*., 2008). Extensive studies on Japanese quail revealed that it is a valuable bird for avian research and provides technical information that allowed for the modern production of quails (Minvielle, 2004). Moreover, raising quails for commercial purposes had unequal development around the word, for example in Asia quails were bred for egg production while in Europe they were bred for meat production. Nasr *et al*. (2017) stated that Jumbo quails have heavier body weight with the best carcass traits and meat quality attributes when compared to other quail breeds. Meat production from quails is reported to be a more lucrative farming enterprise when compared to chicken, duck, fowl, goose, ostrich, guinea fowl and turkey farming (Randall & Bolla, 2008). This is attributed to the fast growth rate which results in early maturity and small body size of quails that allows rearing of six to seven quails in the same space that caters for one matured chicken (Ali *et al*., 2012).

2.3 Quail production systems

Quail farming can be categorized into extensive, semi-intensive and extensive production systems (Permin & Pedersen, 2002). These systems are built upon a diverse collection of structures, styles and technologies for management. They vary significantly in terms of necessary expenditure, choice of quail breed, farming practices as well as inputs i.e. feed, labour, power and transport. The requirements of feed ingredients, feeding methods and type of ration differ greatly depending on the system used. The choice of a method of production is generally a decision of the farmer. Initially, farmers consider their financial capabilities, which influences the method of production to embark on (Mulaudzi *et al*., 2019). Time as well as space are also some of the considerations to be considered by farmers when selecting a production system, for instance the intensive production system requires daily or frequent supervision, whereas the extensive production system needs large amount land for farming (Sonaiya, 2003).

2.3.1 Extensive production system

The most common method of quail production in low-income and less modernised countries is the extensive rearing system. In this system, quails are allowed to roam around the homestead throughout the day in search for home scraps, kitchen waste products as well as environmental resources i.e. seeds, worms, insects, scattered grains as well as green forages (Goromela *et al.*, 2006). Water and other inputs such as supplementary feeds, shelter, vaccination, and medicine are not routinely given to birds. Drinking water is supplied irregularly in tins or broken bits of clay (King'ori, 2004). Extensive poultry systems are most useful for meat production in rural households, as most farmers require strong birds that can feed on home-made rations or scavenge for themselves, remain alive and produce enough meat before slaughter (Bosch, 2011). Nevertheless, in regions with limited natural resources, low rainfall, predation, as well diseases, quail rearing can be a challenge resulting in low yield (Ravindran, 2013). Extensive quail rearing plays a major role in ensuring food security in most non-industrialized nations, and is widely practiced by communal farmers. This system is suggested for resource-poor farmers, since quails can scavenge for their own feed without proper shelter. The system is characterized by minimal inputs, with birds hunting for feedstuff, no investment beyond the base stock, a handful of grain every day and simple night sheds (Saina, 2005).

. No shelter is given in some situations, if available it is improper for accommodating a large flock, but houses the birds at night. Farmers may be deprived of the birds 'highest benefit by using feed services that do not meet the birds' nutritional requirements, thus reducing their productivity. This production system simulates organic farming and remains the public's first choice since no antibiotics are used, and are inexpensive to operate and labour-free meaning that every member of the community can practice it (Mulaudzi *et al*., 2019).

2.3.2 Semi-intensive production system

The semi-intensive system is often found in both urban and peri-urban and rural settlements (FAO, 2010). It shares similar characteristics as the intensive production system because they both include the rearing of conventional breeds (Kitalyi, 1998; Musundire, 2016). Ahlers *et al*. (2009) stated that intermediate farming systems involve small to medium flock sizes ranging from 50-500 quails and are constrained overnight and permitted to scavenge during the day. Farmers make available fresh green forage as well as water in troughs to the birds, and supplementary feed is also available. Chicks intended for breeding are pinioned to prevent them from flying (Musundire, 2016). There's remarkable low expenditure on feed ingredients coupled with high returns in terms of better product quality of lean and low-fat meats compared to birds reared in intensive production systems (Koster & Coetzee, 1996).

Mapiye *et al*. (2008) stated that the semi-intensive production system consists of moderate levels of management, advanced breeds as well as intensive labour. To minimize feed costs, the majority of farmers have resorted to feeding homemade rations under the semi-intensive units (Tadelle *et al*., 2003). Control of diseases is practiced, mainly vaccinations against economically significant diseases such as Newcastle disease and infectious bronchitis

(Muchadeyi, 2007). The output level in the system is, nevertheless, lower than in the intensive production system. Quails mate only inside the enclosure, and this system needs considerable effort. The provided shelter is made of different materials including woody and leafy structures from local trees or shrubs. The housing as well as the fencing offer some security against theft and predators (Musundire, 2016).

2.3.3 Intensive production system

This system is prominent in both industrialized as well as non-industrialized nations, distinguished by the integration of highly advanced processing units with high-performing birds and electronic machinery (Karousa *et al.,* 2015). In this system, quails are reared without access to an outdoor enclosure. Musundire (2016) claimed that the purpose of raising poultry in confinement is to establish optimum temperature and lighting conditions, and to manipulate the daytime length as a way to maximize yield. This is in response to the great demand for animal protein in most non-industrialized nations owing to the growing urban populations (Marangon & Busani, 2006). When farming with quails or any other poultry bird, feed must be available *ad libitum* with the required nutrients that can satisfy their daily requirements. Labour is necessary to make sure that the birds are fed, given water and the environment is clean. The success of large-scale quail farming relies heavily on the use of protein as well as energy content of the feed, which is extracted mainly from soybean as well as cereals. High productivity and performance in quails depends on feeding a nutritionally balanced ration designed to suit the quail's nutritional requirements. Chowdhury *et al*. (2006) reported that with nutritionally balanced diets and proper management conditions, good health and high productivity increases. Economically non-industrialized nations invest in intensive systems of quail production to produce meat as well as eggs for the rising human population (Mulaudzi *et al*., 2019).

2.4 Introduction and importance of quails into the South African poultry industry

According to Mogesse (2007) and Nkukwana (2018), the term 'poultry' refers to all domesticated birds which are raised for the purpose of producing meat and eggs for human consumption and for wealth creation. It includes chickens, ducks, turkeys, guinea fowls, geese, quails, and other farmed birds (Singh, 2000). The poultry industry is a major division of the agricultural sector in South Africa, adding more than 16% to the domestic gross product (Lombard & Bahta, 2018). This industry provides direct and indirect employment for more than hundred thousand people throughout its value chain and industry related activities (Lombard & Bahta, 2018). In the past decades, it has grown from just backyard extensive production to a highly advanced commercial enterprise, which had also seen the addition on new species known as quails (Mnisi & Mlambo, 2018). Ncobela (2014) stated that the South African poultry sector is dominated by exotic and indigenous chickens. However, the recent diversification of the industry through the addition of quails may be attributed to their desirable characteristics. Quails attain early sexual maturity, short generation interval, lower feed and space requirement, high rate of egg production, and are resistant to most poultry diseases (Nasr *et al*., 2017). Hossain *et al*. (2015) stated that quails reach market weigh of 140 to 220 g and optimum egg production within 5 to 8 weeks of age (Altine *et al*., 2016). Guèye (2009) stated that malnutrition in resource poor homesteads can be forestalled by consumption of quail meat and eggs which are a rich source of high-quality protein, and also contributes to wealth creation through rapid income return with low production investment. Scavenging birds are, however, faced with a lot of nutritional and environmental challenges that hinder them to reach optimal growth and egg production (Ncobela, 2014). Despite the challenges, these birds contribute significantly to the livelihood of rural communities in South Africa. However, there is scarcity of information on the status of quail development in most of South Africa's communal areas.

This data is critical for the proper preparation of conservation efforts and the encouragement of the use of quail genetic resources in commercial establishment (Mtileni *et al*., 2008).

The rapid growth of the global human population has imposed the need of additional source of animal protein to close the gap of protein deficiency or malnutrition (Mohammed & Ejiofor, 2015). Thus, in most developing countries, quails are gaining popularity as a new venture of diversification in fulfilling the needs of human protein and as a way of earning rapid economic return from commercial farming (Hossain *et al*., 2015). Gecgel *et al*. (2015) noted that quails have high meat to bone ratio, with their carcass composed of 79% meat, 14% bones, and 10% skin. The benefits of consuming quail meat are linked to its high levels of essential fatty acids, protein, and minerals such as iron, sodium and potassium. The high rate of metabolism in these birds is reported to be responsible for the increasing glycogen levels thus resulting into high quality meat (Hossain *et al*., 2015). Furthermore, due to the thin skin and low-fat accumulation under the skin, quail meat is recommended for low fat diets and conscious consumers. In addition, quail eggs are high in crude protein, crude fat and minerals when compared to the eggs from other poultry species. With regards to the nutrition of quail eggs, Jeke *et al*. (2018) reported that a crude protein content of 13.09%, and is characterised by adequate amounts of essential amino acids such as lysine, valine, and leucine.

2.5 Nutritional requirements of quails

Poultry birds require a well-balanced diet with a precise amount of highly digestible essential amino acids and nitrogen rather than crude protein (CP) per se, so as to be able to synthesize the non-essential amino acids. To achieve this, poultry diets should be formulated to meet the requirements of amino acid digestibility instead of CP (Awad *et al*., 2016). Furthermore, the National Research Council (NRC, 1994) emphasised that birds utilise the amino acids (AA) attained from dietary protein to accomplish a variety of structural and protective tissue functions such as feathers, skin, ligaments and bone matrix, along with soft tissues including organs and muscles. Protein requirements in quails are influenced by the stage of production and the metabolisable energy (ME) content as well as the ingredients used to formulate the rations. Therefore, the type of protein ingredients to be used in quail diets must come from a high-quality protein source that is high in AA content (Altine *et al*., 2016). Thus, it is worth evaluating mopane worm meal (MWM) as a potential protein ingredient in quails because of the high CP content (54 – 59%) and an AA profile comparable to that of fish meal and SBM (Rapatsa & Moyo 2019). According to Madibela *et al*. (2007) MWM has notably higher amounts of lysine, methionine, valine, threonine, tryptophan, and phenylalanine than soybeans.

NRC (1994) proposed that quails should be reared on a high protein diets with 24% CP during the growing phase and 20% CP in the production phase, as shown in Table 2.1. However, it is worth noting that these recommended protein levels are suitable for quails that are reared in temperate regions (NRC, 1994). Currently, there is no standard ration for broiler or layer quails in South Africa (Mnisi & Mlambo, 2019), suggesting that the performance of the birds may be compromised with low nutrient intake. For optimum performance, a commercial game-bird diet that containing 250 g/kg CP and 12.6 MJ/kg ME and 10 g/kg calcium should be fed to growing quails (Randall & Bolla, 2008). As aforementioned, this game-bird diet is expensive and inaccessible, resulting in farmers feeding a standard chicken ration with a CP content ranging around 180 – 220 g/kg, which is associated with low growth rates in quails (Randall & Bolla, 2008). Therefore, when formulating quail diets, it is crucial to use a high-quality protein ingredient with a well-balanced AA content (Soares *et al*., 2004).

Table 2.1. Nutrient requirements of Jumbo quails in different phases versus the nutrient content of mopane worm meal (g/kg DM)

Nutrient	Starting and Growing	Breeding	Mopane worm nutrient content
Protein	240	200	568.3
Arginine	12.5	12.6	32.0
Glycine + serine	11.5	11.7	-
Histidine	3.6	4.2	16.5
Isoleucine	9.8	9.0	22.2
Leucine	16.9	14.2	35.0
Lysine	13.0	10.0	35.8
Methionine	5.0	4.5	8.9
Methionine + cysteine	7.5	7.0	-
Phenylalanine	9.6	7.8	25.1
Phenylalanine + tyrosine	18.0	14.0	-
Threonine	10.2	7.4	27.3
Tryptophan	2.2	1.9	6.8
Valine	9.5	9.2	31.4

Sources: NRC (1994); Rapatsa & Moyo (2019).

For efficient utilisation of dietary protein, the quail diet must have sufficient energy (10.89 to 12.56 MJ/kg ME) because quail performance is dependent on an optimum energy-to-protein ratio and not only on the protein content (Tarasewicz *et al*., 2006). For example, Reda *et al*. (2015) postulated that 22% CP and 12.14 MJ/kg ME are sufficient in the first few weeks of the growth phase. While the findings of a trial conducted by Jahanian & Edriss (2015) suggested that CP and energy levels of 26% and 12.56 MJ/kg ME, respectively, are adequate for growth phase, meaning that it is important to balance the protein-to-energy ratio in quail diets to increase their performance. An increase in protein levels from very low to high levels has a positive effect on body weight gain of growing quails (Gheisari *et al*., 2011). Hence, it is expected that standard rations that constitute of a high-quality protein ingredient would ensure superior productivity. For this reason, poultry scientists and feed manufacturers are in a quest to find inexpensive and sustainable feed ingredients or supplements to manufacture poultry diets of high quality (Hossain *et al*., 2017).

2.6 Soybean (*Glycine max*) meal

According to Heuzé *et al*. (2016), soybean meal (SBM) is identified as the most essential type of protein that forms two-thirds of the total protein used in animal feeds worldwide. Its nutritional value is unbeatable by any other type of plant protein, hence it is often regarded as the "gold standard" to which the protein values of all other protein sources are generally compared to (Beski *et al*., 2015). The superiority of this legume seed is due to its valuable protein composition of 44 - 49% (Table 2.2), and its excellent profile of highly digestible amino acids and 20% fat content (Banaszkiewicz, 2011). The protein structure of soybean is composed of two main globular proteins 11s glycinin and 7s β-conglycinin. Glycinin constitute of an acid/base peptide unit that contains higher level of sulphur-containing amino acids (methionine and cysteine), thus it determines the AA status of protein (O'keefe *et al*., 2015). Soybean meal is a standard protein ingredient used to formulate diets of farm animals (Cámara *et al*., 2016). The protein content of SBM in the feed market is standardized at a range of 44 to 49% with considerable amount of lysine (6.2 g / 16 g N) but limited in sulphur amino acids (2.6 g / 16.2 g N) (Banaszkiewicz, 2011). Nevertheless, SBM is an exceptional source of high-quality lysine, which is comparable to that of pork muscle protein ranging from 6.5 to 7.0% lysine. Furthermore, Stein *et al*. (2008) stated that lysine is highly digestible such that when compared to the daily requirements of chicks (per unit of protein), it exceeds the lysine requirements. Furthermore, the excellent digestibility of SBM amino acids in poultry diets and the high lysine content make it possible to develop diets that provide less total protein than other forms of protein and less surplus nitrogen in the feed, minimizing the excretion of nitrogen into the biosphere (Heuzé *et al*., 2016). A variety of soybean with high quality protein content and a low content of oligosaccharides has been developed (baker *et al*., 2011).

Despite SBM being an exceptional source of protein for animals, the presence of antinutritional factors (ANFs) in raw soybeans may require pre-treatment before incorporation into animal diets. The main ANFs in raw soybean that are responsible for the poor nutritional value are trypsin inhibitors and lectins (Vasconcelos *et al*., 2001). These ANFs could affect protein digestibility by binding to protein and increasing the gut viscosity, thereby increases the excretion of metabolic nitrogen (Erdaw *et al*., 2017; Erdaw & Beyene, 2018). Soybean meal also constitute appreciable amounts phytates and oligosaccharides that interfere with nutrient utilisation and may result in a negative post-ingestive feedback (Shi *et al*., 2017), thereby compromising the well-being of the quails. Efforts have been directed towards ameliorating the amounts of these ANFs, with special attention being directed to purifying the protease inhibitors and lectins because of their biochemical usefulness (Franco-Fraguas *et al*., 2003). Antinutritional factors such as trypsin inhibitors and lectins are inactivated by proper heat treatment such as (boiling, roosting, cooking and autoclaving) or by irradiation and treatment with exogenous enzymes (Kocher *et al*., 2003; Yasothai, 2016). Thermal processing of soybean at optimum temperatures such as autoclaving at 121 °C for 15 minutes has been reported to reduce more than 90% of antitrypsin activity in processed soybean (Yasothai, 2016; Avilés-Gaxiola *et al*., 2018). Thus, processing soybean increase the bioavailability of amino acids by destroying ANFs (such as trypsin inhibitors), which promote high nutrient utilisation and growth performance in quails (Yu *et al*., 2007).

Table 2.2. Chemical composition (g/kg DM) of mopane worm meal versus soybean meal

Nutrient	Mopane worm meal	Soybean meal
Protein content	550 – 570	490
Ash	82.6	77.0
Neutral detergent fibre	278	668
Acid detergent fibre	160	391
Minerals (g/kg)		
Potassium	1.07	2.56
Calcium	5.72	0.43
Phosphorus	2.40	0.78
Sodium	26.70	0.079

Sources: Chiripasi *et al*. (2012); Kwiri *et al*. (2014); Rapatsa & Moyo, (2019)

2.6.1 Lectins

Lectins are glycoproteins capable of agglutinating erythrocytes and binding the constituents of sugar. Lectins are not broken down in the intestine, but attach to mucosal cells that harm the intestinal wall and reduce nutrient absorption (Yasothai, 2016). Growth can be decreased by raw soybean and cause animal mortality rate to rise (Banaszkiewicz, 2011). Lectins are heat sensitive and therefore are only existing in soybean products at residual levels. Heat treatment in soy products for the inactivation of anti-nutritional factors is less effective for antigen than for trypsin inhibitors or lectins (Van Eys *et al*., 2004). One can estimate the amount of soy lectins by measuring the hemagglutination activity. Lectins or agglutinins are carbohydrate-binding proteins found in significant proportions in soybean and can potentially interfere with animal metabolism (Fasina *et al*., 2003). Heavy lectin effects virtually disappear during or after autoclaving.

2.6.2 Trypsin inhibitors

Trypsin inhibitors are the Kunitz and Bowman-Birk factors that are found in raw soybeans (Winiarska-Mieczan, 2007), which inhibit proteolytic enzyme activities in the digestive tract. According to Yasothai (2016), they minimize the activity of trypsin (a protease secreted by the pancreas) and, to a lesser extent, chymotrypsin and eventually hinder the digestion of proteins in monogastric animals. The Bowman-Birk inhibitor is steady to heat, acid, and proteolytic digestion since it has a rigid tertiary structure comprising of seven cross-linkages with disulfide (Castro, 2014). Nevertheless, both trypsin inhibitors can be inactivated by means of steaming as well as extrusion during the oil extraction process (Refstie *et al*., 2001). Twenty percent (20%) of Bowman-Birk and Kunitz inhibitors stay active during heat treatment (Friedman & Brandon, 2001). Care must be given when heat is utilized in processing soybean, because while undesirable substances can be removed, other proteins' functional and nutritional properties may be destroyed (Castro, 2014). According to Castro (2014), the degree of protein damage is usually due to the temperature, moisture content, velocity of the screw, shear forces as well as length of heating throughout processing.

2.6.3 Phytates

Phytate (phytic acid salt) is a polyphosphorylated carbohydrate that serves as phosphorous as well as mineral storage. It is the largest source of soy phosphorus (Wu & Kang, 2011), accounting for 7% of total soy phosphorus (Castro, 2014). It may lead to mineral deficiencies in humans since it acts as a powerful chelator of calcium, magnesium, iron and zinc (Wu & Kang, 2011). For the same cause, these necessary cations appear to be inaccessible to other monogastric animals (Castro, 2014) including fish (Yang *et al*., 2011). In addition, phytate interacts with proteins that form phytate-mineral-protein complexes, reducing protein bioavailability in monogastric animals (Phumee *et al*., 2011; Morales *et al*., 2012). Water extraction at pH 5.0 extracts about 75% of the phytate content (Castro, 2014). Treatment of SBM with an exogenous enzyme, phytase, can release phosphorus and chelated cations from the phytate-mineral-protein complexes, enhancing both protein digestibility (Morales *et al*., 2012) and phosphorus bioavailability (Imanpoor & Bagheri, 2012). Rainbow trout studies have shown that phosphorus absorption and retention increase when SBM is supplemented with phytase (Phumee *et al*., 2011).

2.6.4 Saponins

According to Knudsen *et al*. (2008) and Güçlü-Üstündağ & Mazza (2007), saponins are triterpenoid or steroid aglycones connected to one or more units of naturally occurring soybean sugars. The concentration of saponins in soybeans and soybean products is relatively high (Hu *et al*., 2002). Soybean meal contains alcohol-soluble factors that interfere with digestive processes and eventually affect feed intake and development, for instance saponins (Refstie *et al.*, 2005). The soybean saponins, content is roughly 4.3 – 6.7 g/kg (Chen, 2011). The occurrence of saponins in soybean is of particular significance due to their growing utilization as a medicine or nutraceutical sources (Tarade *et al*., 2006). However, in animal diets, saponins are believed to have many negative effects, e.g. dietary saponins derived from different plants have been held responsible for feed intake depression, weight gain reduction, inhibition of active nutrient intake including vitamins and minerals in the intestine, reduction of protein digestibility (Francis *et al*., 2001). Saponins are heat-stable ANFs that can be ameliorated by either aqueous, solvent extraction or exogenous enzyme treatment (Jacobsen *et al*., 2018).

2.6.5 Oligosaccharides

Oligosaccharides are a class of carbohydrates produced in small amounts when three to ten simple sugars are linked together by glycosidic bonds (Laurentin & Edwards, 2012). These substances reduce nutrient digestibility and cause intestinal hypertrophy (Salgado *et al*., 2002).

Oligosaccharides could induce fluid retention and increase the rate of digestive flow, resulting in lower nutrient utilization and absorption (Baker *et al*., 2014). Indeed, raffinose a family of oligosaccharides has been reported to increase the digesta passage rate which in turn reduces fibre fermentation of soybean in the gut (Choct *et al*., 2010). In addition, Li *et al*. (2017) reported a negative correlation between raffinose content and ileal digestibility of amino acids in growing pigs. According to Rubio *et al*. (1998), oligosaccharides affect the number of micro-organisms in the intestines, which destabilize the gut microflora and nutrient utilisation. Animals' reactions to soybean anti-nutritional substances rely on animal species as well as age. Adult ruminants are not responsive to these substances, while chickens, pigs, calves, and rats were observed to have reduced growth rates when raw soybean was given. Reaction of hens as well as adult pigs fed on diets containing 10% raw soybean seeds is minimal, therefore simple non-ruminants can utilize a little amount raw soybean product with no adverse effect on performance (Banaszkiewicz, 2011). Oligosaccharides are not inactivated by heat treatment during processing of soybeans, however, processing of soybean meal with ethanol extraction would remove oligosaccharides (Ghazi *et al*., 2003). Two studies by Leske *et al*. (1991, 1993) revealed that treating soybean by ethanol extraction increases true metabolisable energy.

2.7 Mopane worm

Mopane woodlands are found in the tropical parts of Southern Africa and hosts one of the valuable larvae known as mopane worm (Timberlake & Chidumayo, 2010). Mopane trees occurs on nutrient rich clay soil at an altitude of 200 - 1200 m, but normally from 300 to 900 m receiving an annual rainfall of 400 - 700 mm. The species can be also found in drier areas of north-western Namibia, where rainfall can be 150 to 250 mm per annum (Makhado *et al*., 2014). The mopane worm is eaten in significant quantities as part of family diets and as a food source in rural areas (Potgieter *et al*., 2012). However, its consumption has religious restrictions to a large part of the population in South Africa. The life cycle of MW begins in summer whereby the female month mate and lay an egg cluster of about 30 to 335 eggs, which would later hatch to MW-larva. The larva feeds mostly on mopane tree leaves and to lesser extent feeds on other leaves within the mopane woodland (Kumirai, 2017). On average, MW life cycle takes about 4 to 6 weeks, and is divided into 5 growing stages called instar (instar I being the earliest growth stage and instar V being the last) (Gondo *et al*., 2010), and grows to approximately 80 mm long (Kumirai, 2017).

2.7.1 Importance of mopane worm in rural communities

Mopane worms occur in two periods: October-November and again in February-March (Makhado *et al*., 2014). As a result, harvesting periods of MW creates part-time employment opportunity to rural communities. The revenue generated from MW harvest can be used for various purposes such as purchasing foodstuff, kitchen utensils, paying school fees, medical bills, and for traveling (Lucas, 2011). Ghazoul (2006) suggested that the ideal time to collect MW is when they descend the trees for pupation because at this stage, they voluntary empty their guts before going underground and degutting would not be necessary thus speeding harvest. Nonetheless, most MW are harvested from trees while still feeding and the undigested material in their gut needs to be thoroughly processed (Lucas, 2011). When the worms are collected while still feeding on trees, traditional methods such as tree shaking and branch shear looping to speed up harvest are used, yet these methods results in unsustainable MW production (Stack *et al*., 2003).

Mopane worm are consumed as food by people residing mostly in rural communities and also partially gaining attention from people in urban areas (Lucas, 2011). Makhado *et al*. (2012) noted that in Giyani (Limpopo, South Africa), 78% of the worms are harvested and used to supplement household diets, while only 28% is sold as dry MW. In Zimbabwe, the primary goal of MW harvest is to earn income such that 59% of harvested MW is commercialised and the remaining used as food.

2.7.2 The life cycle of mopane worm

The availability of mopane worm is predetermined by the factors that include prevailing weather conditions, consumption by birds and harvesting. High amounts of rainfall in summer promote high egg-laying by the emperor moth (Makhado *et al*., 2012). Mopane worms are bivoltine producing two generations in a given year, with their first outbreak occurring in October-November and again in February-March. When mating, males would follow chemical pheromones secreted by the females in which the mated female would lay a cluster of 50 - 200 eggs around twigs and the leaves of host plants. The eggs hatch after about 10 days to produce tiny black larvae (caterpillars) (Gondo *et al*., 2010). During the growing phase, the larvae passes through 5 (Plate 2.4) instar stages with each phase lasting for about 5 - 7 days. During instar stages I-III, the caterpillars cluster together in groups of 20 - 200 whereby they feed on the leaves of mopane trees and of those trees that grow in close proximity to the mopane woodland (Dube & Dube, 2010). When the larvae pass stage IV, they begin to molt and displace from the unit, it is at this phase when they are being referred to as mopane worm. During instar V, mopane worms cease feeding and begin to descend the tree trunk to barrow through the soil and form pupae. The whole life cycle occurs in a period of 4 - 6 weeks, during which time their body mass increases 4000 times (Moyo *et al*., 2019). The last phase (Instar V) before pupations is regarded as a favourable harvesting period because the gut does not contain the indigested material (Gondo *et al*., 2010)

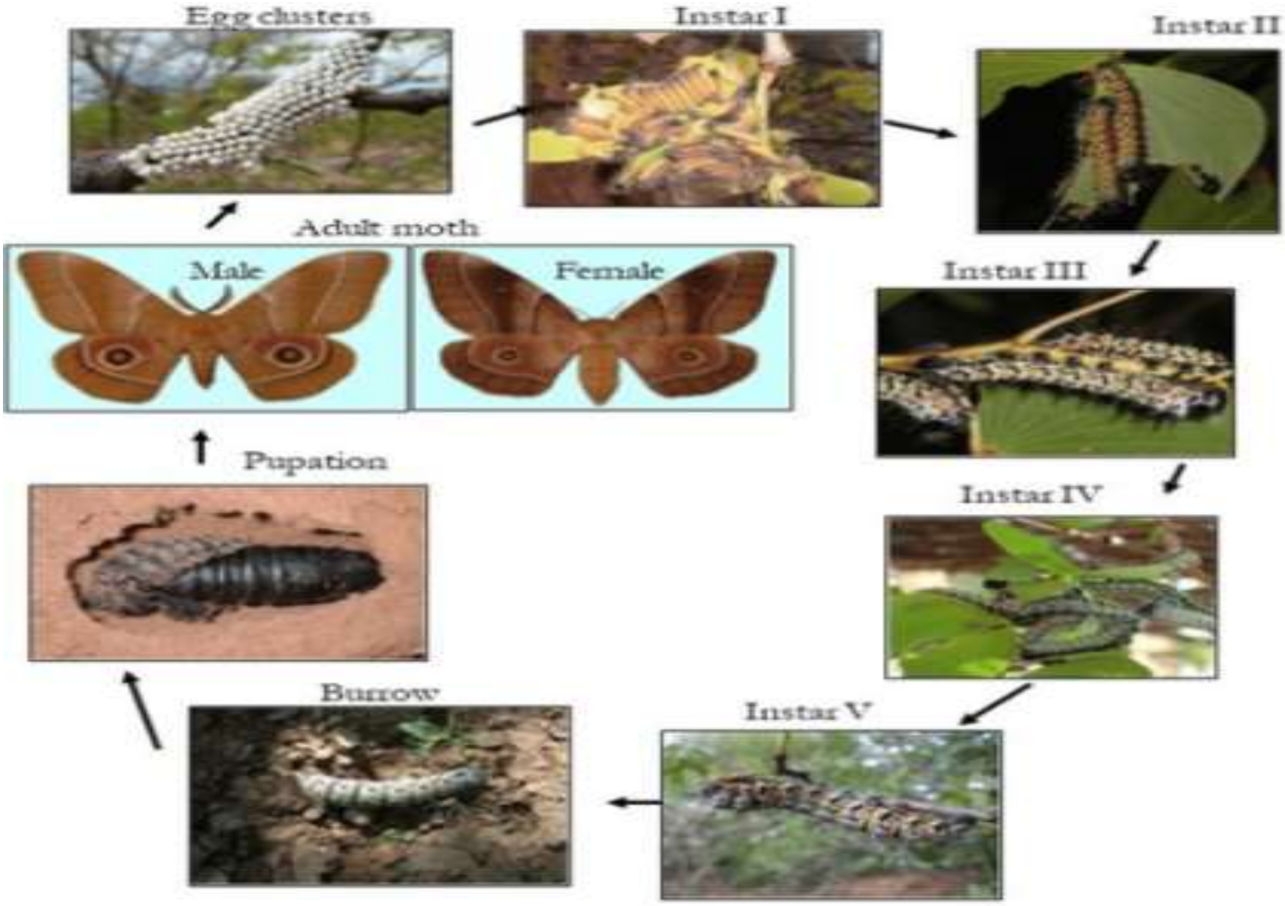

Plate 2.1. Different instar phases of mopane worm life's cycle

2.7.3 Mopane worm harvest and processing

Mopane worms that are harvested while feeding undergo a series of separate processing stages. The first phase of processing is to degut the worms while collecting by pushing from the head towards the anus in-between two fingers to remove the undigested material in the gut (Thomas, 2013). The second phase involves boiling or roosting worms in brine for 20 to 60 minutes followed by sun-drying for a period of 2 - 4 days. This step is important in removing spines (roosting) and also prolongs the shelf life of worms up to a year (Sekonya *et al.*, 2020).

2.7.4 Chemical composition of mopane worm

Mopane worm is a rich source of protein (55 - 57%) and lipids (14-17%) (Nobo *et al.*, 2012) (Table 2.3). The protein content of MW is three times that of beef and also exceeds other protein sources such as chicken, such that a 100 g of dried MW will provide approximately 76% of protein to humans and many of the essential vitamins and minerals as they contain significant quantities of phosphorus, iron and calcium (Potgieter *et al*., 2012). In addition, MW is rich in threonine, valine, phenylalanine and tryptophan more than soybean or fishmeal. In addition, lysine and methionine content of MWM is greater than that of SBM (Nobo *et al*., 2012). The keratinized heads of MW increase the ash content when supplemented to diets (Ohiokpehai, 2006). The amount of protein content of MW is said to be diluted by the leafy material in the gut since mopane tree leaves are low in protein and have condensed tannins (Madibela *et al*., 2007). Furthermore, Madibela *et al*. (2007) observed that MW that had a protein content of 521.4 g/kg DM when intact where improved by almost 10% upon degutting the worms. Amino acid concentration of the worms was determined by Ohiokpehai *et al*. (2006) and found that it is comparable to that of soybean, however MW have a high concentration of methionine, lysine, valine, tryptophan, phenylalanine, and threonine. In addition, the methionine and lysine levels, which are important in poultry rations, were also reported to be equivalent to that observed in fishmeal diets. Inclusion of MW would reduce the feeding cost in quail production since they are naturally occurring in abundance in mopane woodlands, this is true for quail producers who harvest the worms for themselves without purchasing it. Additionally, the inclusion of MWM in diets of quails would improve the growth traits of the birds due to its high protein and amino acids levels compared to SBM.

Table 2.3. Composition and true amino acids digestibility (g/kg DM) of mopane worm meal versus soybean meal

	Composition		True amino acid digestibility	
Amino acid	MWM	SBM	MWM	SBM
Methionine	8.90	6.7	-	920
Lysine	35.80	30.2	-	910
Tryptophan	6.8	6.5	-	900
Arginine	32.0	34.8	-	940
Tyrosine	35.7	18.2	-	900
Histidine	16.5	12.8	-	900
Threonine	27.3	1.85	-	880
Isoleucine	22.1	21.6	-	890
Leucine	35.0	36.6	-	890
Phenylalanine	25.1	23.9	-	880
Valine	31.4	22.7	-	910

Sources: Camara *et al*., (2016); Stein *et al*., (2008); (-) means not reported.

2.8 Insect meals

There are many considerations to remember when using insects as nutrient sources for animal feeds, which comprises the normal eating habits of many animals such as chickens, pigs and fish as well as the nutrient requirement (Sánchez-Muros *et al*., 2014). Accordingly, Van Huis *et al*. (2013) reported that insects are a natural food source for many fish and free-range chickens/birds. Most birds voluntarily consume worms and larvae from the topsoil and litter, whereas in recreational fishing they use maggots as fish baits. However, the feeding value and biochemical properties of insect meals are not well known or documented due to the mere recent curiosity in using insects as an alternative source of protein. Chemical composition of many insects especially mopane worm has been previously studied for human nutrition, and demonstrated a good composition for use as human food. Nonetheless, despite the great potential exhibited by insects such as mopane worm as a potential ingredient in animal feeding, its utilisation in animal feeds is largely unknown (Banjo *et al*., 2006; Sánchez-Muros *et al*., 2014), specifically in Jumbo quail diets.

2.9 The value of insects as animal feed

Plant and animal protein are two major quality protein sources that are used in poultry production. Plant protein sources that are currently used in poultry diets includes SBM, canola meal, and sunflower meal, although SBM is regarded as an excellent protein source in poultry feeds (Ravindran, 2013). The use of animal protein has added limitations in the poultry production due to its ability to transmit diseases (Womeni *et al*., 2012), whereas cultivated feed ingredients have been identified as the major contributors to land occupation, using enormous amount of water and consequently contributes to climate change, acidification, and power use (Mungkung *et al*., 2013). To reduce the environmental impact caused by feed production, it is critical to explore other sustainable high-quality feed ingredient that can be used in aquaculture and livestock production. Therefore, there is a need to transform the current insect production systems into sustainable and profitable production systems in order to address the current nutritional challenges as well as to achieve the sustainable development goal set by the United Nations (Sayed *et al*., 2019).

Edible insects have been noted be a potential candidate that can be used as sustainable alternative in animal feeds because of their high-value protein and comparable low environmental impact (Bovera *et al*., 2016). Additionally, there has been a developing interest by animal nutritionists on the use of novel protein ingredients such as insects with an objective of addressing availability, high cost, and better efficiency of conventional feed ingredients (Van der Spiegel *et al*., 2013). Edible insects are proposed to play a crucial role as a renewable source of feed because of their potential to convert and valorise low-nutrition materials into high-quality feed with reduced demand on global land, energy, and water supplies. Insects form part of natural food sources for poultry birds, such that birds that have access to outdoor areas can be seen catching worms and larvae from topsoil and debris. Given insects natural role as food for most domesticated livestock, it is worth noting their role as a feed ingredient for particular poultry birds (Van Huis *et al*., 2013). Insects have a similar fat (30 - 40% DM) with higher protein content (40 - 60% DM basis) to SBM, suggesting that insect meal has a potential to serve as an alternative protein source to poultry diets (Makkar *et al.*, 2014). Adopting the use of insect meal instead of traditional protein sources could lead to a more effective use of environmental resources and a reduced carbon footprint as well as mitigating eutrophication in water streams (Van Zenten *et al.*, 2014).

2.10 Mass production of insects for feed production

Utilisation of insect meals as potential protein sources in livestock diets requires large-scale production of insects that will maintain a significant production of insects required for the production of animal feed (Sánchez-Muros *et al*., 2014). Most of the insects that are utilised by mankind as food or feed occur naturally in the wilderness, where they are being collected. But to a lesser extent, insects with economical value have been domesticated for commercial production. The concept of insect farming is a relatively new venture of diversification, which encompasses rearing of insects in a confined area (i.e. a farm) and the rearing conditions, diet and food quality of insects are controlled. Farmed insects are farmed in captivity and are therefore isolated from their natural communities (Van Huis *et al*., 2013). The indoor and semi-outdoor insect farming in a monitored or controlled ecosystem ensure a successful mass production of insects (Gahukar *et al.,* 2016). Commercialising insects of *Lepidoptera* genus such as MW and mulberry silkworms is an economically viable business, because these worms are prolific in nature and have many generations per year with MW being bivoltine (Gondo *et al*., 2012), while mulberry silkworm is polyvoltine (Gahukar *et al*., 2016).

Production of insects on a large-scale has been challenged by various factors such as reliability, quality, and cost-effectiveness (FAO, 2013). According to Gahukar *et al*. (2016), successful insect farming has been achieved by some countries such as India, China, and Korea whereby mulberry silkworm are produced by utilising readily available indigenous material that are sustainable and low-cost. Plate 2.2 shows built rearing sheds on mud wall fields, and used nylon nets as well as bamboo poles to build mulberry silkworm rearing beds. This procedure has served to preserve moisture and temperature and is economical reasonable and viable (Sathe *et al*., 2008).

Intensive production of mopane worms has been investigated in Zimbabwe, South Africa and Botswana to ensure continuous availability (Rapatsa & Moyo, 2017). Industrial scale worm production coupled with sustainable worm breeding and processing technology can ease the challenges of worm availability and reduce the selling price of MW (Raheem *et al*., 2018). Gondo *et al*. (2001) investigated various breeding methods for MW, whereby shade houses were built around mopane tress and larvae were collected and placed in wooden boxes, drums, and shelves filled with soil at the end of the final (5^{th}) instar pre-pupation stage (Plate 2.3 and 2.4) so that they can be protected from virus and parasitoid infection until the next outbreak. Mopane worm production can also be increased by collecting eggs in high-outbreak areas and moving them to areas with low outbreak levels. Twigs or leaves that carries eggs should be covered with a white sleeve as a way of protection from parasitoid infections or alternatively eggs can be stored in a white container in a house until they hatch. After hatching the larvae should be distributed to mopane trees with low outbreaks (Potgieter *et al*., 2012). Gardiner (2003), demonstrated that a captive breeding population of MW can be established and sustained for over three years. The success of all-year-round mass production would, indeed, increase the availability of the worms and allow sustainable intensification.

Plate 2.2. Mulberry silkworm breeding beds

Plate 2.3. Shelving for mopane pupal hatching within the shade-house.

Plate 2.4. Egg-house used for mating mopane moths and hatching of eggs

2.11 The effect of insect meals on performance traits of poultry

Several studies have reported a decrease in feed intake of broilers that had been fed insect meal as a replacement of SBM. The decrease in feed intake has been attributed to high amounts of proteins and lipids in most insect-based diets, which result into broilers satisfying their nutritional requirements earlier than SBM (Makkar *et al*., 2014; Khan *et al*., 2018). Indeed, Moreki *et al*. (2012) stated that the high nutritive value of MWM leads to low feed intake and enhanced feed conversion efficiency thus resulting in higher body weight gain. In some cases, the presence of chitin, a fibrous substance that forms a major component in insects' exoskeleton, may reduce the utilisation of MWM in simple non-ruminants because is said to be a non-digestible amino polysaccharide (Moreki *et al*., 2012). Indeed, high levels of chitin in shrimp meal has been reported to negatively influence feed intake, body weight gain, feed efficiency and dry matter digestibility of growing broiler chickens (Khempaka *et al*., 2006).

2.11.1 Silk worm pupa

The silkworm (*Bombyx mori*) is a common moth belonging to phylum Arthropoda and order *Lepidoptera* (Mishra *et al*., 2003). Domestication of silkworm began around 2650 BC in China where it was mainly produced for silk used in cloths production (Omotoso, 2015). Currently, silk worm pupa (SWP) is used as a protein-rich ingredient with high nutritive value (Makkar *et al*., 2014). It is a waste product of the silk industry that can be used in poultry feeds as a protein and energy substitute of conventional protein sources due to its high nutritional status following proper processing (Khan, 2018). Silkworm pupa contains high-quality protein with a balanced amino acid ratio as well as other essential nutrients and unsaturated fatty acids (Mlcek *et al*., 2014). Hertrampf & Piedad-Pasual (2000) found that SWP contains 88.9% dry matter, 89.6% organic matter, 55.1% CP, 23.2% crude fat, 3.8% ash, and 5.5% crude fibre. Additionally, Zhou & Han (2006) reported that the dried SWP powder contained 71.9% CP, 20.1% EE and 4.0% ash on a DM basis. The high CP content in silk worm meal makes an ideal contender when formulating concentrate isolates with improved protein quality that can be used in quail diets (Longvah *et al*., 2011). Furthermore, the protein content of SWP is made out of 18 known AAs with all the EAAs including sulphur-containing AAs (Zhou & Han, 2006).

The amount of minerals and micro-nutrients of SWP and silk worm prepupae that were either grown in castor or tapioca leaves were assessed by Longvah *et al*. (2011), and found that there was an average mineral content of 1.25% in prepupae, which was similar to most of other insect's larvae such as MW. In addition, the minerals analysis of SWP shows that it is high in potassium (34.0 mg/g) (Zhou & Han, 2006), but has a low sodium-to-potassium ratio and heavy metal content (Khan, 2018). According to Omotoso (2015), phosphorus (37.66 mg/100g) is the highest macro-element found on silk worm larvae. The SWP is also a viable source of fatty acids particularly α-linolenic acid (36.3%), which is a major component (Tomotake *et al*., 2010). Kwon *et al*. (2012) stated that SWP is an excellent source of essential fatty acids (α-linoleic acid + linoleic acids; 49.0%) and non-essential fatty acids (19.9% oleic acid, 2.5% palmitoleic acid, 8.6% steric acid, 19.7% palmitic acid, and 0.3% eicosapentaenoic acid).

A study conducted by Ullah *et al*. (2017), in which SBM was replaced with SWP at (25, 50, 75 and 100), showed that 75% inclusion of SWP promoted the highest feed intake and body weight gain as compared to the other treatments. These findings were in line with those observed by Khatun *et al*. (2003), who stated that the high feed intake in broilers fed high amounts of SWP was due to the palatability and acceptability by both broilers and layers. Although, when SWP was included at 100% it was noted to decrease feed intake subsequently lowering production performances (Ijaiya & Eko, 2009; Ullah *et al*., 2017). The decrease in feed intake and poor growth performance in broilers when SWP is included at 100% could be attributed to effects of high fat content and the incapacity of young chicks to digest the crude fibre integral in the exoskeleton of the silkworm caterpillar (Makkar *et al*., 2014, Khan *et al*., 2018). In addition, Fadiyimu *et al*. (2003) stated that the protein requirements of broilers depend mostly on amino acid profile rather than to the total nitrogen content during the finisher phase.

2.11.2 **Cirina forda** ***(Westwood)***

The *Cirina forda* (*Lepidoptera*: *Saturniidae*) is an edible caterpillar of *Saturniidae* that is commonly found in Nigeria, Zambia, Zimbabwe, South Africa, the Central African Republic and the Democratic Republic of Congo (Badanaro *et al*., 2014). *Cirina forda* is one of the larvae that are mostly consumed as a delicacy, and usually served as snacks or taken with carbohydrate food in the Southern parts of Nigeria (Omotoso, 2005). A number of studies have reported a varying protein content (DM basis) of *C. forda*, which was 64.49% (Ande, 2002), 52.6% (Adepoju & Daboh 2013), and the lowest protein content being 20% (Osasona & Olaofe, 2010). The variation in the protein content has been attributed to the different processing methods prior to analysis. Osasona & Olaofe (2010) stated that boiling and sun drying of the larvae may have desaturated the protein. Nonetheless, *C. forda* has considerable amounts of EE (12.5%), ash (8.7%) and carbohydrates (54.3%) (Khan, 2018). Moreover, Ande (2002) reported a rather high amount of EE (21.45%) in *C. forda* larvae. *Cirina forda* is also an outstanding source of essential fatty acids that encompasses 41.6% unsaturated fatty acids. The notable essential fatty acid is 33.84% linoleic acid, which is vital in the formation of nerve and functioning of the retina (Ande, 2002). Broiler chicks have the ability to efficiently utilise *C. forda* meal as the conventional fishmeal, and this has been confirmed by a feeding trial conducted by Oyegoke *et al*. (2006), who found that replacing fishmeal with *C. forda* at 50 and 100% did not affect growth performance. This suggests that the compounded larval diets constituted a protein source that is similar in quality to that present in the commercial fish meal.

2.11.3 Black soldier fly

The black soldier fly (*Hermetia illucens*) is a *Diptera* belonging to the *Stratiomyidae* family, which is native to American tropical, subtropical and hot temperate areas, but currently found worldwide (Martínez-Sánchez *et al*., 2011). This fly is naturally found in large numbers and typically occurs among large chickens, pigs, and cattle manure piles. Additionally, the larvae also appear on wide range of decomposing kitchen waste such as vegetables, coffee bean pulp, distillery waste and fish offal, in very dense habitats (FAO, 2013; Nguyen *et al*., 2015). The black soldier fly larvae (BSFL) can be used commercially to address a range of environmental problems related to manure and other organic material, such as reduction of manure density, moisture content and aggressive odours. The BSFL consist of an admirable amount of protein (40 - 44%) with an excellent amino acid profile comparable to that of SBM (Makkar *et al*., 2014; Tran *et al*., 2015). The fat content of BSFL is said to be influenced by the type of substrate with which the insect was reared upon. For example, the fat content of BSFL was reported to be 42–49% on oil-rich food waste (Barry, 2004), 35% on cattle droppings (Newton *et al*., 1977), 28% on pig manure (Newton *et al*., 2005), and 15 – 25% for larvae fed on poultry manure (Arango Gutierrez *et al*., 2004),

A number of studies have evaluated the potential of BSFL to partially or completely replace SBM and Fish meal. The high biological value of BSFL as well as its low ecological impact are the main factors that have led to intensive utilisation of this novel protein source (Henry *et al*., 2015; Llagostera *et al*., 2019). Using BSFL as a component of a complete diet have shown positive results by promoting healthy growth in chicks. Chicks that were raised on BSFL attained similar growth rate of 96% with those fed with soy meal. However, there was significant difference in feed intake whereby only 93% of feed was consumed by chicks reared on BSFL as compared to reared soy product which consumed 100% (Newton *et al*., 2005), suggesting higher feed conversion efficiency of diet containing the larvae meal. Another study evaluated the inclusion of BSFL in starter broiler diets, and yielded comparable results as the broilers fed traditional fishmeal (Elwert *et al*., 2010).

2.12 Dietary influences of insect meals on serum biochemical indices

Poultry management requires a thorough understanding of how diseases affect the biochemical function of the body in order to detect any possible abnormalities (Gylstorff & Grimm, 1987). When assessing the health status of livestock, conducting serum profiling assists in the diagnosis and clinical monitoring of diseases (Ali *et al*., 2012). Serum biochemical analysis assists in evaluating the degree of damage to multiple essential organs such as liver, renal and pancreatic functions, and also useful in assessing the state of illness (Ali *et al*., 2012). With reference to biochemical changes of blood, Washington & van Hoosier (2012) reported that an accumulation of enzymes beyond normal in serum chemistry is an indication of cell damage because enzymes are normally stored and secreted by the cells that synthesise them. The damage to liver cells may be noticed by elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Blood urea nitrogen and creatinine would increase with poor glomerular filtration, thereby depicting a renal dysfunction (Donsbough *et al*., 2010). Moreover, blood urea nitrogen increases when animals are offered a high-protein diet or subjected to vigorous exercises (McLaughlin & Fish, 1994). Thus, creatine is a more reliable biomarker of renal function as it is not influenced by protein catabolism or gastrointestinal bleeding, rather it is an end-product of muscle metabolism; thus, relatively higher and lower concentrations may be attributable to well-muscled and muscle-wasted animals, respectively (Siegel & Walton, 2020).

Serum biochemical indices are a preferred method of analysis over haematology when assessing the health status of farm animals. This is because, during sample preparation for haematology, an anticoagulant such as ethylene diamine tetra acetic acid or citrate is added to prevent clotting by chelating calcium ions that are cofactors in enzyme assays, thus has a potential to influence the results (Washington & van Hoosier, 2012). However, the application of serum biochemical analysis in commercially raised poultry flocks can only be reliable if reference values of serum indices are defined for that particular species and preferable using the same method (Verstappen *et al*., 2002). Scholtz *et al*. (2009), stipulated that serum biochemical indices reference values can offer helpful information about individuals' physical condition and assist in differentiating between normal and healthy animals from abnormal or sick ones. But, the implementation of serum biochemical analysis has been constrained by insufficient acceptable reference ranges. Besides, much of the available data is based on limited parameters, inadequate sample numbers, and often old analytical techniques (Ali *et al.*, 2012). Another major setback with this technique is the fact that serum values are apparently influenced by a number of different factors such as age, breed, diet, stage of production, and presence of haemolysis (Verheyen *et al*., 2007). Scholtz *et al*. (2009) revealed that laying hens undergo several sex-related physiological changes in metabolism, which result in absurdly high circulating of triglyceride and cholesterol concentrations than in males. In addition, the secretion of oestrogen just before egg-laying has been shown to elevate the total protein concertation due to induced hyperproteinaemia as a response to an increase in the yolk precursors viz., vitellogenin and lipoproteins (Scholtz *et al*., 2009; Agina *et al*., 2017). Several studies have shown that partial substitution of conventional protein source with insect meal does not compromise the health status of quails (Schiavone *et al*., 2017; Dabbou *et al*., 2018; Mbhele *et al*., 2019). Bovera *et al*. (2015) observed that inclusion of yellow mealworm did not affect total protein, albumin and globulin, except for albumin/globulin ratio which decreased with increasing levels of the yellow mealworm. This observation is in agreement with Marono *et al*. (2017) and Loponte *et al*. (2017) who reported higher globulin levels with lower albumin-to-globulin ratio and lower creatinine levels when soybean products were substituted with either *Hermetia illucens* or a combination of *H. illucens* and *Tenibro molitor*. Lowered albumin-to-globulin ratio when insect meal is incorporated in poultry diets has been associated with better immune response and disease resistance (Griminger & Scanes, 1986). This observation is ascribed to the prebiotic effect of chitin, which improve colonic function by restoring compositional balance microbial population (Bovera *et al*., 2016).

2.13 Meat quality attributes

Meat quality is a very diverse trait that encompasses visual, compositional, and sensory characteristics of a carcass (Lawrie & Ledward 2006). Muchenje *et al*. (2009) stated that meat quality is a function of physical, chemical, sensory, technological, nutritional, and culinary properties perceived as desirable by meat consumers. Meat quality attributes are greatly influenced by the pH and colour, whereby meat pH is used as a predictor of tenderness, shelf life, water holding capacity, and drip loss (Ponsuksili *et al*., 2008; Traore *et al*., 2012; England *et al*., 2014). The extent and rate at which muscle pH drop is a factor of reserved muscle glycogen which would be converted into lactic acid during anaerobic respiration after slaughter (King, 2006). Appearance is an important attribute when selecting food commodities such as poultry meat. The physical attributes greatly impact the product's external appearance thus having an immense impact on a buyer’s or consumer’s choice (Oguz *et al*., 2004). Meat colour on the market is thus seen as a characteristic that defines the freshness of meat and other meat items as it influences consumer buying behaviour (Fanatico *et al*., 2007).

2.14 Dietary influences of insect containing diets on meat quality traits

A number of researchers have noted that insect-based meals influence meat colour through the deposition of insect pigment. Schiavone *et al*. (2019) noted an elevated redness index value when broiler chickens were reread on a diet containing 150 g/kg of BSFL, whereas diets containing *Musca domestica* larvae meal in broiler diets were found to significantly decrease lightness in the pectoral muscle (Pieterse *et al*., 2014). These observations are different to those of Pieterse *et al*. (2019), who did not find any significant effect of dietary BSFL meal on broiler meat colour, also Bovera *et al*. (2016) did not find any dietary effects on the colour of raw and cooked meat, or the skin of broiler chickens fed with *Tenebrio molitor* meal. The variation in meat colour of broiler chickens and quails fed with insect meal may be attributed to different nutritional composition and insect pigment which is a result of species difference, life stage, and rearing condition of the insect (Zadeh *et al*., 2019)

2.15 Summary

The poultry industry is the largest agricultural farming enterprise in South Africa, providing employment throughout its value chain. Poultry products are the cheapest source of high-quality animal protein, and are more affordable compared to red meats. Recently, the poultry industry has seen the addition of a new bird species known as quail. For optimum quail production, high-quality diets are offered to the birds on a daily basis for the entire production cycle. These high-quality diets involve the use of maize and soybeans, which are in high demand thereby increasing the cost of the feed. In addition, soybean is not produced in appreciable quantities in South Africa, thus it is imported at exorbitant prices since soybean producing countries (United States of America, Brazil and Argentina) incurs high variable costs. Soybean production also contributes to the emissions of GHGs through the use of fuel, machinery, pesticides and chemical fertilizers, which has a detrimental effect on the environment. Thus, the continued use of soybean products in quail diets is unsustainable for large-scale production. Alternatively, insect meals can be a potential substitute for soybean in quail diets. Insects can be produced in large quantities with minimal inputs, low carbon foot print, and low water and land requirements. Insect meals such as mopane worm are rich sources of high-quality protein, fats and lipids. Thus, the use of mopane worm in Jumbo quail diet can ensure sustainable intensification, high performance and acceptable meat quality attributes, without compromising the health status of the birds. However, MW is seasonal and only occurs twice a year. Thus, this necessitate an urgent need to fully domesticate mopane worms, so as to ensure all year-round availability of this worm. This can be achieved by intensive breeding of MW and moving eggs from high outbreak areas to the trees that have low outbreak. In addition, harvesters should be monitored so as to ensure that there is a large number of MW that undergoes pupa stage.

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3 CHAPTER THREE: EFFECT OF GRADED LEVELS OF MOPANE WORM MEAL ON GROWTH PERFORMANCE, SERUM BIOCHEMICAL INDICES AND APPARENT NUTRIENT DIGESTIBILITY OF JUMBO QUAILS

Abstract

The study was undertaken to evaluate the effect of partial replacement of soybean products with mopane worm meal (MWM) on feed intake, growth performance, serum biochemical indices and apparent nutrient digestibility of Jumbo quails. A total of 384, two-week-old, unsexed Jumbo quails (71.2 ± 5.40 g live-weight) were randomly and equally distributed to four isoproteic and isocaloric diets formulated as follows: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal. Experimental diets and freshwater were offered *ad libitum* throughout the four-week feeding trial. Average weekly feed intake (AWFI) and average weekly body weight gain (ABWG) were recorded and used to calculate weekly gain-to-feed ratio (G:F). At the end of the growth trial (six weeks of age), two quails were left in each replicate pen (experimental unit) to allow for a five-day digestibility trial. Feed intake was measured daily, and fresh faecal samples were collected and frozen at -15ºC pending analyses. The rest of the quails were slaughtered after stunning in a local abattoir and while bleeding, about 2 ml of blood samples were collected from two birds randomly selected per pen for serum biochemistry analyses. Repeated measures analysis showed no significant week and diet interaction effect on AWFI, ABWG and G:F. Neither linear nor quadratic trends

(P >0.05) were observed for overall feed intake, ABWG, final body weight, G:F and serum biochemical parameters in response to incremental levels of MWM. Similarly, there were no significant dietary influences on overall feed intake, overall growth performance and serum biochemical indices of Jumbo quails. Acid detergent fibre digestibility (ADFD) linearly declined [y = 403.7 (±27.43) - 0.26 (±0.88) x; R^2 = 0.338, P = 0.003] as MWM levels increased. Dietary treatments had no effect (P >0.05) on apparent nutrient digestibility values, except on ADFD. The inclusion of MWM in the diets of Jumbo quails promoted similar results in terms of performance and serum biochemistry as those fed the soybean-based control diet. Thus, it can be concluded that mopane worm meal can partially substitute soybean products in Jumbo quail diets. An optimum level could not be determined using feed utilisation measurements and physiological responses, meaning that inclusion levels beyond 150 g/kg requires further research.

Keywords: *Coturnix coturnix,* Feed utilisation, Health status, Physiological response, Protein source, Sustainability

3.1 Introduction

The global demand for poultry meat and eggs is estimated to grow to about 121% and 65%, respectively (Mottet & Tempio, 2017). This increasing demand of poultry products is linked to the predicted 9.6 billion human population size by 2050 (FAO, 2013). The growing human population has surged the demand for animal protein, which continues to add pressure on food producers (Agina *et al*., 2017). As a consequence, efforts must be directed at increasing livestock production without negatively affecting the environment (Cullere *et al*., 2016). To address this expected upsurge in animal protein demand, the use of fast-growing poultry species, such as quails, is inevitable (Hagan *et al*., 2016). The success of quail production across different countries is attributable to the birds' fast growth rates, adaptability to harsh environmental conditions, resistance to several poultry diseases, as well as fast returns on investments because of their short generation intervals, early sexual maturity and low maintenance cost (Khosravi *et al*., 2016).

Quail diets ought to be nutritionally balanced in order to meet their daily nutrient requirements for high productivity and efficiency. These diets should constitute at least 38 dietary nutrients in appropriately balanced rations (Altine *et al*., 2016). This can be accomplished through diet formulation, which emphasizes on using ingredients that would maximize nutrient availability, rather than simply meeting energy or amino acid levels (Ravindran *et al*., 2005). Priti & Satish (2014) reported that quails require about 30 to 35 g of feed per day but the feed should be available *ad libitum* to promote rapid growth. The key components of poultry feed are dietary energy and protein, which are commonly provided through maize and soybean. Two major ingredients that continue to be in high demand, and consequently increasing feed costs. Concerns have risen with regards to soybean production (Da Silva *et al*., 2010), which is associated with ecological problems such as high-water dependency and loss of biodiversity as a result of deforestation and overuse of agricultural chemicals. In addition, soybean supply chain requires a much longer transportation distance to meet global demand (Jia *et al*., 2020). Thus, for sustainable intensification, the use of insect meal such as mopane worm presents a potential strategy. Mopane worms (*Imbrasia belina*) are an excellent source of high-quality protein, with a protein content of (55 – 57%) which contains amino acids that compares favourably well with soybean. The MWM has significant high proportion of lysine, tryptophan and methionine, which are limiting in soybean (Kwiri *et al*., 2020). The protein value of MWM suggests that it can be a suitable protein ingredient for Jumbo quails because of its balanced composition of essential amino acid (Moyo *et al*., 2019). However, no studies have investigated the feed value of MWM in diets of Jumbo quails as a substitute of soybean products. This study was, therefore, designed to investigate the effect of graded levels of MWM in place of soybean products on feed intake, growth performance, serum biochemical indices, and apparent nutrient digestibility of Jumbo quails. It was hypothesised that partial replacement of soybean products with MWM would improve feed intake, growth performance, serum biochemical indices and apparent nutrient digestibility in Jumbo quails.

3.2 Materials and methods

3.2.1 Description of the study site and ingredient source

The study was carried out at Molelwane Research Farm (25°40.459′S, 26°10.563′E) of the North-West University (North West, South Africa). The feeding trial was carried out during summer whereby ambient temperatures around the area ranges from 17ºC to 37ºC. Mopane worms were purchased from a street vendor in Gaborone, Botswana. During collection, the worms were degutted by hand and thereafter roasted in a brine for 20 minutes to remove the spines and prevent spoilage. The degutted and roasted worms were then sun-dried for four days and thereafter packed in paper bags. Soybean products (soya oilcake and oil crude soya) and all the other feed ingredients were purchased from Nutroteq (Gauteng, South Africa).

3.2.2 Chemical analyses of mopane worm meal

The worms were milled to pass through a 2 mm-sieve (Polymix PX-MFC 90 D) to produce the meal (MWM) prior blending to the other ingredients. The MWM was subjected to preliminary analysis for proximate composition using the Official Analytical Chemists International Methods (AOAC, 2005) prior to diet formulations. For laboratory dry matter (DM) (AOAC, 2005: method no. 930.15) determination, 1 g of MWM triplicate samples were added into pre-weighed crucibles and oven-dried at 105°C for 12 h. After 12 h, samples were removed from the oven, placed in desiccators to cool, and then weighed. The DM was calculated as the difference between initial sample weight and moisture content weight. Organic matter (OM) content (AOAC, 2005: method no. 924.05) was determined by ashing the dried samples in a muffle furnace set at 600°C for 12 h. The loss in weight was measured as OM content and the residue as ash content. Crude protein (CP) content was determined following the standard macro-Kjeldahl method (AOAC, 2005: method no. 984.13). An ANKOM^{2000} Fibre Analyser (ANKOM Technology, New York) was used to determine neutral detergent fibre (NDF) and acid detergent fibre (ADF) for 60 and 70 min, respectively, as described by van Soest *et al*. (1991). The MWM contained 905.3 g/kg of DM, 826.7 g/kg DM of OM, 78.5 g/kg DM of ash, 577.7 g/kg DM of CP, 229.3 g/kg DM of NDF, and 313.4 g/kg DM of ADF.

3.2.3 Diet formulations and analyses

Four isonitrogenous and isoenergetic dietary treatments diets were formulated through partial inclusion of MWM in place of soybean products in a commercial standard grower diet as follows: 1) MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, 2) MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, 3) MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, 4) MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal, as shown in Table 3.1.

Table 3.1. Ingredient composition (g/kg *as fed* basis) of dietary treatments

	[1]Diets			
	MWM0	MWM5	MWM10	MWM15
Mopane worm meal	0	50.0	100.0	150.0
Yellow maize	605.5	635.0	664.6	659.0
Soya oil cake 47%	322.8	260.9	199.1	143.3
Oil crude soya (degummed)	29.03	15.04	1.05	0
Di-calcium phosphate	24.51	14.92	5.33	0
Limestone	3.20	9.98	16.77	20.35
Salt-fine	4.39	3.76	3.13	2.51
Kaoline	5.00	5.00	5.00	5.00
Methionine (Dl 98%)	2.18	1.82	1.46	1.14
Lysine (Sint 78%)	0.95	1.18	1.41	1.52
Threonine (98%)	0.25	0.12	0	0
Br Starter	1.00	1.00	1.00	1.00
Choline Cl (60%)	0.70	0.70	0.70	0.70
Coxistac 12	0.50	0.50	0.50	0.50
Sand	0	0	0	14.99

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

The formulated experimental diets (MWM0, MWM5, MWM10, and MWM15) were thereafter milled (Polymix PX-MFC 90 D) to pass through a 1 mm sieve for chemical analyses (Table

3.2). Crude fibre was determined using the ANKOM^{2000} Fibre analyser with 0.255 N crude fibre acid solution and then with 0.313 N crude fibre base solution. Metabolisable energy (ME) contents and amino acids (lysine, methionine, cysteine, threonine, and tryptophan) were predicted using the Near-Infrared Reflectance Spectroscopy (SpectaStar XL, Unity Scientific, Emu Heights, Australia). Mineral content levels (Calcium (Ca), Phosphorus (P), Sodium (Na), Chlorine (Cl) and Potassium (K)) were determined using the atomic ICP spectrophotometer (AAS – Buck) as guided by the Agri Laboratory Association of Southern Africa guidelines (AgriLASA, 1998).

Table 3.2. Chemical composition of experimental diets (g/kg, as fed basis) unless stated otherwise.

	[1]Diets			
	MWM0	MWM5	MWM10	MWM15
Dry matter	879.8	880.4	881.1	885.2
Metabolisable energy (MJ/kg)	12.27	12.25	12.23	12.21
Crude protein	200.0	200.0	200.0	200.0
Crude fat	58.04	50.53	43.01	47.15
Crude fibre	26.76	25.27	23.78	21.63
Ash	57.34	56.49	55.65	55.71
Calcium	8.0	8.0	8.0	8.0
Phosphorus	8.42	7.38	6.34	6.10
Sodium	1.80	1.80	1.80	1.80
Chlorine	3.32	3.14	2.96	2.74
Potassium	9.26	8.57	7.87	7.19
Lysine	11.39	11.19	10.99	10.80
Methionine	5.24	4.87	4.50	4.14
Cysteine	3.30	3.56	3.82	4.07
Threonine	7.81	7.66	7.52	7.51
Tryptophan	2.28	2.17	2.07	1.98

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

3.2.4 Growth trial

A total of 384, one-week-old mixed-gender, Jumbo quails were purchased from T/A R&G Poultry in Walkerville (Gauteng, South Africa). The quails were randomly and evenly allocated to 32 replicate pens (experimental unit) with each pen carrying 12 birds that were replicated eight (8) times per dietary treatment. In their replicate pens (100 cm long 142 × 60 cm wide × 30 cm high), the quails were offered stress pack for three consecutive days upon arrival, while being adapted to the dietary treatments for a week under continuous fluorescence of infrared lamps to provide warmth until two weeks of age. The pens were made of wire mesh floor that requires no bedding material; however, a polythene plastic was used to cover the floor and were regularly changed. On average the temperature and humidity inside the quail house during the experimental period were 30°C and 40%, respectively. Fresh, clean water and dietary treatments were offered *ad libitum* during the experimental period, and rearing was conducted under natural lighting (12 h of daylight). Ventilation was enforced by opening windows in the mornings and closing them in the evenings.

3.2.5 Feed intake and growth performance

Average weekly feed intake (AWFI) per bird was determined by subtracting the weight of the feed refused from feed offered and dividing the difference by the total number of quails in the pen. After two weeks of age, all quails per experimental unit were measured (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA) to determine the initial body weights, and thereafter body weight gain was measured weekly by subtracting the weight of the previous week with the weight of the current week until six weeks of age. The live weights obtained weekly were used to calculate the average weekly body weight gain (ABWG) per bird as follows:

$$ABWG\ (t_0, T) = \frac{W(T) - W(t0)}{T - t0}$$

Where, t_0 = initial time (days); T = final time; W (T) = final body weight/bird (g), and W (t0) = initial body weight (g). The ABWG and AWFI were then used to calculate average weekly gain-to-feed ratio (G:F).

3.2.6 Slaughter procedure, and blood collection and analysis

Six-week-old Jumbo quails were taken to Rooigrond poultry abattoir where they were electrically stunned and humanely slaughtered by cutting the jugular vein with a very sharp knife and left hanging until bleeding stopped. While bleeding, sterile tubes were used to collect blood from two randomly selected quails from each pen. Serum was generated by following guidelines stipulated by Washington & van Hoosier (2012), whereby clotted blood was centrifuged at 1000 *g* for 15 minutes. The acquired serum was analysed for albumin-to-globulin ratio (ALB/GLOB), albumin, alkaline phosphate (ALKP), alanine transaminase (ALT), symmetric dimethylarginine (SDMA), calcium, creatinine, globulin, glucose, phosphorus, bilirubin, total protein, and urea using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories S.A. PTY, Gauteng, South Africa).

3.2.7 Digestibility trial

At the end of the growth trial, two quails per replicate pen were left behind for digestibility measurements. The 64 birds were fed the same diets (MWM0, MWM5, MWM10, and MWM15) as in the growth trial. The birds were allowed a three days adaptation period to the diets and afterward samples of feed offered, feed refusal, and faeces were collected, pooled, weighed, and processed every day for the five days digestibility trial duration as described by (Manyeula *et al*., 2019). Apparent digestibility values of dry matter (DMD), organic matter

(OMD), crude protein (CPD), neutral detergent fibre (NDFD), and acid detergent fibre (ADFD) were determined using the following formula:

$$\text{Apparent nutrient digestibility} = \frac{\text{Nutrient intake} - \text{Faecal nutrient}}{\text{Ntrient intake}} \times 100$$

3.2.8 Statistical analysis

Average weekly feed intake, average weekly body weight gain and average weekly gain-to feed ratio data were analysed using the repeated measures analysis (SAS, 2010). The following statistical linear model was utilised:

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

Where Y_{ijk} = dependent variable, μ = population mean, D_i = effect of diets, W_j = effect of week, $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed.

Data on feed intake, growth performance, serum biochemistry, and apparent nutrient digestibility were evaluated for linear and quadratic effects using polynomial contrast. A response surface regression analysis (SAS, 2010) was employed to estimate the optimum dietary inclusion level of MWM using the following quadratic equation.

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

Where: y = dependent variable, a and b are the coefficients of the quadratic equation, and c is dietary MWM levels. The x value for optimal response was determined as: $\frac{-b}{2a}$

Overall feed intake, body weight gain, gain-to-feed ratio, serum biochemical parameters and apparent nutrient digestibility data were analysed using the general linear model (GLM) procedure of SAS (2010) in a completely randomized design, with diet as the only main factor. The linear statistical model employed was as follows:

$$Yij = \mu + Di + Eij$$

Where Yij = dependent variable, μ = population mean, Di = effect of diets, and Eij = random error associated with observation ij, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P < 0.05$ and least squares means were compared using the probability of difference.

3.3 Results

3.3.1 Feed intake and growth performance

Repeated measures analysis showed no significant week and diet interaction effect on average weekly feed intake, average weekly body weight gain, and average gain-to-feed ratio. Table 3.3 indicates that there were neither significant linear nor quadratic effects for overall feed intake, overall BWG, final body weight, and overall G:F of Jumbo quails in response to MWM levels. Similarly, there were no significant dietary effects on overall feed intake and overall growth performance of Jumbo quails.

Table 3.3. Effect of partially replacing soybean products with mopane worm meal on overall feed intake (g/bird), overall weekly body weight gain (g/bird), final body weight (g/bird), and overall weekly gain-to-feed ratio in of Jumbo quails

	[1]Diets					Significance	
	MWM0	MWM5	MWM10	MWM15	[2]SEM	Linear	Quadratic
Overall feed intake	654.9	625.2	645.8	628.0	14.01	0.353	0.680
[3]Overall BWG	171.9	169.0	167.3	161.7	4.510	0.115	0.755
Final body weight	247.6	237.6	235.3	234.2	5.009	0.064	0.374
[4]Overall G:F	0.262	0.270	0.260	0.258	0.007	0.447	0.455

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

[2]SEM = standard error of the mean.

[3]Overall BWG = overall body weight gained in the four-week feeding period.

[4]Overall G:F = gain-to-feed ratio for the entire duration of the feeding period.

3.3.2 Serum biochemical indices

Table 3.4 shows that there were no significant linear or quadratic effects for serum biochemical parameters in response to incremental levels of MWM. Likewise, no dietary influences (*P* >0.05) were observed on serum biochemical parameters of Jumbo quails.

Table 3.4. The effect of replacing soybean products with mopane worm meal-containing diets on serum biochemical parameters of six-week-old Jumbo quails.

	[1]Diets					Significance	
[2]Parameters	MWM0	MWM5	MWM10	MWM15	[3]SEM	Linear	Quadratic
Glucose (mmol/L)	5.34	4.68	5.37	4.98	1.538	0.959	0.712
Urea (mmol/L)	1.19	1.01	0.98	1.46	0.300	0.612	0.954
Calcium (mmol/L)	3.53	2.99	6.11	3.21	1.473	0.903	0.834
Total protein (g/L)	52.26	49.38	53.31	55.31	3.481	0.417	0.367
Albumin (g/L)	21.31	15.56	19.75	16.69	3.187	0.544	0.702
Globulin (g/L)	37.13	34.31	33.53	38.75	2.082	0.389	0.284
Cholesterol (mmol/L)	4.29	5.23	4.62	5.05	0.462	0.850	0.387
SDMA (µg/dL)	21.13	17.69	17.21	21.56	2.918	0.753	0.071
Creatinine (µmol/L)	12.56	10.81	11.44	9.14	1.549	0.740	0.215
Phosphorus (mmol/L)	6.11	5.16	4.94	5.08	0.559	0.837	0.706
ALB/GLOB	0.436	0.450	0.444	0,438	0.015	1.000	0.461
ALT (U/L)	35.83	50.57	43.00	44.33	12.54	0.967	0.639
ALKP (U/L)	211.1	215.1	219.7	172.9	18.40	0.840	0.072
Bilirubin (µmol/L)	9.14	11.25	10.79	13.29	3.135	0.506	0.982

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

[2]Parameters: ALB/GLOB = albumin/globin ratio, ALKP = alkaline phosphatase, ALT = alanine aminotransferase, SDMA = serum symmetric dimethylarginine.

[3]SEM = standard error of the mean.

3.3.3 Apparent nutrient digestibility

Table 3.5 shows that there were neither significant linear nor quadratic trends for dry matter digestibility (DMD), organic matter digestibility (OMD), neutral detergent fibre digestibility (NDFD) and crude protein digestibility (CPD) in response to increasing levels of dietary MWM. However, increasing levels of MWM resulted in a linear decrease for acid detergent fibre digestibility (ADFD) [y = 403.66 (±27.43) - 0.26 (±0.88) x; R^2 = 0.338, P = 0.003]. Experimental diets had no effect (P >0.05) on DMD, OMD, NDFD and CPD, but significantly influenced ADFD. Quails fed diet MWM15 had higher (P <0.05) ADFD (523.6 g/kg) compared to those fed the other diets, which did not differ (P >0.05).

Table 3.5. Apparent nutrient digestibility (g/kg DM, unless stated otherwise) of Jumbo quails fed mopane worm meal-containing diets

	Diets					Significance	
[2]Parameters	MWM0	MWM5	MWM10	MWM15	[3]SEM	Linear	Quadratic
DMD (g/kg)	681.2	711.0	685.9	679.6	15.68	0.675	0.262
OMD	705.9	739.0	704.6	704.8	15.70	0.604	0.315
NDFD	370.2	378.2	420.1	444.1	28.81	0.050	0.781
ADFD	409.3[a]	392.0[a]	467.9[a]	523.6[b]	28.25	0.003	0.207
CPD	175.0	183.6	125.6	139.2	19.28	0.074	0.899

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.
[2]Parameters: DMD = dry matter digestibility, OMD = organic matter digestibility, NDFD = neutral detergent fibre digestibility, ADFD = acid detergent fibre digestibility, and CPD = crude protein digestibility.
[3]SEM = standard error of the mean.

3.4 Discussion

There is a need to identify potential protein sources that can be used in poultry feeds. Insect meals have been proposed as alternative sources of protein with high biological value (Veldkamp *et al*., 2012; Van Huis *et al*., 2013). The use of insects as feed ingredients is associated to their high quality and quantity of protein Makkar *et al*. (2014) and reduction of environmental carbon footprint (Sánchez-Muros *et al*., 2014). Currently, a variety of insects such as black soldier fly (Culler *et al*., 2016; Schiavone *et al*., 2019; Mbhele *et al*., 2019), yellow mealworm (Bovera *et al*., 2015; Biasato *et al*., 2016) and *Musca domestica* (Pieterse *et al*., 2014) have been shown to give similar or better performance when used in place of SBM in poultry diets. However, with regards to mopane worm, there is a lack of information on its effect on nutrient utilisation, growth performance and pathophysiological status in poultry birds, especially the Jumbo quail. This study represents, therefore, the first ever attempt to evaluate the inclusion of MW in diets of Jumbo quails. Repeated measures analyses indicated a significant week and diet interaction effect on AWFI, AWG and G:F, which implies that the inclusion of MWM did not influence the performance of the birds as they grew older. Results showed that substituting soybean products with MWM up to 150 g/kg does not alter overall feed intake and growth performance of Jumbo quails. According to Dewi & Setiohadi (2010), feed consumption in broilers is determined by several factors such as age, size of the birds, and feed quality and palatability. This means that feeding the quails with MWM-containing diets did not render the diets unpalatable and indigestible (De Marko *et al*., 2015). This is consistent with the findings of other researchers who reported that partial replacement of commercial protein source with insect meals does not alter the palatability and quality of the diet (Maurer *et al*., 2016; Dabbou *et al*., 2018; Mbhele *et al*., 2019). However, in a study conducted by Mareko *et al*. (2010), higher MWM inclusion levels of 20 and 40% reduced BWG of broilers, which was not the case in this study presumably because the levels of MWM were capped at

15%. Thus, higher inclusion levels of MWM should be investigated in Jumbo quail diets so as to establish the optimum inclusion level. The reduction in performance at higher inclusion levels could be due to the presence of chitin, which forms about 27% dry weight of the exoskeleton of the insects. Chitin has been linked with reduced nutrient utilisation since this outermost layer physically blocks digestive enzymes from hydrolysing lipids and protein (Mahata *et al*., 2008).

Nutrient digestibility refers to the availability of nutrients that are absorbed and assimilated as the ingested feed passes through the gastrointestinal tract of birds (Nhlane *et al*., 2020). Morono *et al*. (2015) stated that the fibre content of insects' exoskeleton, mostly measured as acid detergent fibre, consists mainly of chitin, which is associated with a significant amount of cuticular proteins. These cuticle-bound proteins interfere with protein utilisation, since chitin is not degraded and absorbed in the small intestine (Vidanarachchi *et al*., 2010). In this study, the dietary inclusion of MWM in Jumbo quail diets did not affect DMD, OMD, NDFD and CPD values of the birds. These current findings concur with the results of De Marco *et al*. (2015) who reported that partial replacement of soybean products at 250 g/kg with either *Tenibro molitor* or *Hermetia illucens* had no influence on DMD, OMD, and CPD of broiler chickens. Culler *et al*. (2016) also reported that dietary substitution of soy products with *Hermetia illucens* at the rate of 100 and 150 g/kg did not alter overall apparent nutrient digestibility of broiler quails. Acid detergent fibre digestibility was affected by the increasing levels of MWM, where quails on diet MWM15 had the highest ADFD value. This observation was surprising because the presence of chitin in MW was expected to reduce ADFD in quails fed MWM15, because chitin is composed of a hard-indigestible fibrous substance found in the exoskeleton (Schiavone *et al*., 2017). This could have been an error during measurement.

According to Scholtz *et al*. (2009), reference serum biochemistry values provide useful information on the physical condition of animals, and are useful in distinguishing normal and healthy animals from abnormal or sick ones. Furthermore, Ali *et al*. (2012) stated that serum biochemical indices are used to monitor the health status and to detect subclinical diseases because biochemical changes are a result of abnormal conditions in the body. Thus, serum biochemical analysis is a preferred method of detecting the extent of damage in vital organs and the status of the disease (Ali *et al*., 2012). Indeed, serum analysis is the most reliable clinical test because, during sample preparation for serum profiling, no anticoagulants are used, thereby eliminating possible interference by the actions of an anticoagulant (Washington & van Hoosier 2012). In the present study, all serum blood parameters were not affected by the inclusion levels of MWM. This indicates that the inclusion of MWM up to 150 g/kg does not compromise the health status of Jumbo quails. In addition, the serum blood indices obtained in the current study are consistent with the reference values reported by Scholtz *et al*. (2009) for Japanese quails fed with soybean products. The lack of differences among the dietary treatments on serum biochemical indices could be attributed to the almost similar chemical composition of both MWM and SBM (Ohiokpehai *et al*., 1996).

According to Washington & van Hoosier (2012), biochemical enzymes are usually stored by the cells that synthesised them and high levels of serum enzymes indicate cell damage. This confirms that in the current trial there were no liver damages as a result of including MWM, given that all the liver enzymes (AST, ALP, and ALT) used as markers of liver function (Washington & van Hoosier 2012) were not altered. Bovera *et al*. (2015) noted an elevated amount of AST in broilers fed yellow mealworm as a complete replacer of soy products, indicating a deficiency in methionine, selenium, or vitamin E, which leads to liver damage (Gylstorff & Grimm, 1987). Evans (2009) reported that the body's defence mechanism is measured by the amount of total serum protein and its components albumin and globulin, because they are all synthesized in the liver. Jumbo quails had similar blood protein concentration in all dietary treatments, which was not surprising since experimental diets were isonitrogenous and also blood protein is not affected by partial changes of protein in the diet (Bovera *et al*., 2007). This could also explain why there were no changes in blood urea levels because it is directly influenced by the amount of protein in the diet (Bovera *et al*., 2015).

Biochemical indices such as creatinine, uric acid, and blood urea nitrogen that are biomarkers of renal function (Donsbough *et al*., 2010), were also not influenced by the inclusion levels of MWM. Moreover, creatinine is the preferred indicator of measuring renal function because it is not influenced by factors such as diet and hydration (Mitruka & Rawnsley, 1981), compared to blood urea nitrogen, which increases as a result of high protein diet or vigorous activity and also decreases when a low-protein diet is offered or when an animal is experiencing liver failure (McLaughlin & Fish, 1994). Van Huis (2013) highlighted that using insect meal reduces the need of using synthetic antibiotics in the poultry industry because the presence of chitin in the exoskeleton of insects acts as a prebiotic by exhibiting a bacteriostatic effect on gram-negative bacteria such as *Escherichia coli*, *Vibrio cholerae*, *Shigella dysenteriae* and *Bacteriodes fragile* (Vidanarachchi *et al*., 2010). Thus, the antimicrobial activities of MWM in Jumbo quail diets require further research.

3.5 Conclusion

Results showed that mopane worm meal can replace soybean products in Jumbo quail diets without compromising growth performance, nutrient utilisation and the health status of the birds. An optimum inclusion level was not determined using the performance data, suggesting a need to investigate higher inclusion levels beyond 150 g/kg of MWM.

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4 CHAPTER FOUR: EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN PRODUCTS WITH MOPANE WORM MEAL ON CARCASS TRAITS, INTERNAL ORGANS AND MEAT QUALITY PARAMETERS OF JUMBO QUAILS

Abstract

This study was conducted to determine the effect of feeding Jumbo quails with mopane worm meal (MWM)-containing diets on carcass characteristics, internal organ sizes, and meat quality traits. Three-hundred and eighty-four quails were reared in 32 pens (experimental units), each carrying 12 birds replicated 8 times per dietary treatment. The experimental diets were formulated to be isonitrogenous and isoenergetic as follows: a commercial standard grower diet with no mopane worm meal inclusion (MWM0), and a commercial standard grower diet in which 50 (MWM5), 100 (MWM10) and 150 g/kg (MWM15) of soybean products were replaced with MWM. At six weeks of age, all the quails with the exception of those in the digestibility trial were slaughtered for the determination of carcass characteristics, internal organ sizes, and meat quality parameters. Regression results showed that there were no linear or quadratic trends (*P* >0.05) for all carcass traits and internal organs, except for weights [R^2 = 0.138; *P* = 0.040] and lengths [R^2 = 0.247; *P* = 0.004] of large intestines which linearly declined as MWM levels increased. Similarly, no significant dietary effects were observed on carcass traits and internal organ sizes of Jumbo quails. Neither linear nor quadratic effects (*P* >0.05) were observed for meat quality parameters except for redness (*a**) [R^2 = 0.118; *P* = 0.046], *b** [R^2 = 0.094; *P* = 0.001] and chroma [R^2 = 0.312; *P* = 0.0004], which quadratically responded (*P* <0.05) to increasing levels of MWM at 24 h post-slaughter. Dietary treatments had influenced (*P* <0.05) shear force, *b** and chroma values measured 24 h post-mortem. Quails on diets MWM5 and MWM10 had higher (*P* <0.05) *b** and chroma values than those fed the control diet MWM0. Quails on diets MWM5 and MWM15 had the highest shear force values. Diet MWM0 promoted similar (P >0.05) shear force values as diets MWM10 and MWM15. It can be concluded that inclusion levels of mopane worm meal up to 150 g/kg in Jumbo quail diets did not compromise carcass characteristics, internal organ sizes and meat quality attributes of the birds.

Keywords: Carcass, Insect meal, Poultry meat, Soy protein, Visceral organs

4.1 Introduction

In South Africa, poultry meat forms the largest source of high-quality protein with a per capita consumption of 41.2 kg for the year 2016/2017 compared to its counterpart beef, pork and mutton (DAFF, 2017). This shift in meat consumption is attributed to the fact that poultry meat products are widely available, comparatively cheap, rich in polyunsaturated fatty acids, leaner, and low in both cholesterol and sodium content (Ribarski & Genchev, 2013). Meat quality of poultry species is determined by two separated quality attributes, namely visual quality and eating quality (Pieterse *et al*., 2019). In addition, the quality of meat is a function of flavour, pH, nutritive value, juiciness, tenderness, and colour (Lawrie & Ledward, 2006). The appearance of meat is the most important quality attribute in the market because it is the main factor that influences or attracts consumers to buy a poultry product, while texture and sensory features determine the eating satisfaction and intent to repurchase (Fletcher, 2002). The above-stated meat quality attributes together with water holding capacity (WHC) and cooking loss can be predicted by measuring the pH and colour of meat. For example, when meat pH is low such that it is approaching the Iso-electric point of muscle protein, the meat tends to be lighter in colour and subsequently lower WHC as well as a high incidence of cooking loss (Barbut 1997; Qiao *et al*., 2001). Meat pH from quails is generally higher than that of chickens, partridges, and turkeys (Ribarski & Genchev, 2013).

To produce high quality meat and meat products acceptable by conscious consumers, there is a need to use natural protein sources with antimicrobial and meat-enhancing properties. The use of insect-based meal in poultry feeds has been identified as a potential alternative to conventional protein sources around the world (Dabbou *et al*., 2019). A recent study conducted by Cullere *et al*. (2016) revealed that feeding quails with an insect-based diet did not alter meat quality parameters. In addition, Bovera *et al*. (2016) reported that feeding yellow mealworms to broilers does not affect the physical properties of meat such as colour and tenderness. The effective utilisation of insect meal is dependent on the integrity of the gastrointestinal tract and gut microbial population, which together play a pivotal role in the development of immunity, disease resistance, and nutrient absorption (Shang *et al*., 2018). This study was, therefore, undertaken to investigate meat quality parameters, carcass traits, and internal organ sizes of Jumbo quails fed graded levels of mopane worm meal in place of soybean products. It was hypothesized that the inclusion of mopane worm meal in place of soybean products would not compromise the internal organs, and carcass and meat quality parameters of Jumbo quails

4.2 Materials and methods

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

4.2.1 Carcass traits and internal organs

At six weeks of age, the birds were slaughtered as described in Chapter 3, Section 3.2.6. After slaughter, the carcasses were manually eviscerated and individually measured to determine the hot carcass weights (HCW). The carcasses were then chilled in a cold room for 24 hours and reweighed to determine cold carcass weight (CCW). Carcass yield was calculated as the proportion of HCW on slaughter weight (final body weight). Weights of internal organs (gizzard, liver, proventriculus, and small and large intestine including their lengths) and carcass cuts (breast, wing, thigh, and drumstick) were measured using a digital balance (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA).

4.2.2 Meat pH and temperature measurements

Meat pH and temperature were recorded immediately and 24 h post-slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) fitted with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland). After every 10 measurements, the pH meter was calibrated with pH 4, pH 7, and pH 10 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland) meant for this purpose.

4.2.3 Meat colour attributes

Meat colour coordinates: $L*$ (lightness), $a*$ (redness) and $b*$ (yellowness) were measured from the breast meat samples immediately and 24 hours post-slaughter using a Minolta colour-guide 45/0 BYK- Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-daylight, 10º observation angle after 30 minutes blooming time. Hue angle was calculated as arc Tan (θ) $\frac{a*}{b*}$ and chroma was calculated as $\sqrt{a^{*2} + b *^2}$ as described by Priolo *et al*. (2002).

4.2.4 Cooking loss and meat tenderness

Raw breast meat samples were individually weighed to obtain initial weight, and followed by cooking the samples in an oven set at 140ºC for 20 min (Honikel, 1998). The samples were allowed to cool before measuring final weight. The following formula was employed to measure the cooking loss:

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

After determination of cooking losses, breast samples were sheared using a Meullenet-Owens razor shear blade mounted on a Universal Instron apparatus (crosshead speed = 200 mm / minute, one shear in the centre of each core). The reported value represented the average shear force value of each sample in Newtons.

4.2.5 Water holding capacity (WHC)

Water holding capacity was determined by pre-weighing (~ 10 g) of freshly cut breast samples which were placed in-between a pre-weighed 18 Whatman filter-paper and pressed under a pressure of 60 kg for 5 min using dumbbell weights (Grau & Hamm, 1957). The water expressed from fresh meat was absorbed by filter bags and thereby used to calculate water holding capacity expressed in proportion of the initial weight as shown in the formula below:

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

4.2.6 Drip loss

Drip loss was determined using a method adapted from Zhang *et al*. (2009), whereby duplicate samples from each pen weighing approximately 2 g (w1) each were left hanging inside an airtight container. The suspended samples were stored in a cold room that was pre-set at 4°C for 72 hours. The meat samples were then reweighed to obtain weight after drip (w2). The weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

$$Drip\ loss\ (\%) = \frac{w1 - w2}{w1} \times 100$$

4.2.7 Statistical analysis

Data on internal organs, carcass traits, and meat quality were evaluated for linear and quadratic effects using polynomial contrast in a response surface regression analysis (SAS, 2010) as described in Chapter 3, Section 3.2.8. The one-way ANOVA (Proc GLM; SAS, 2010) model employed in Chapter 3 was also used to determine the effects of MWM inclusion on internal organs, carcass characteristics, and meat quality parameters. Significance was declared at $P < 0.05$, and least squares means were compared using the probability of difference option in SAS.

4.3 Results

4.3.1 Internal organs and carcass traits

Table 4.1 shows the effect of replacing soybean products with MWM on sizes of internal organs in Jumbo quails. Regression results showed that there were no linear or quadratic trends ($P > 0.05$) observed for the liver, gizzard, proventriculus and weight and length of small intestine. A significant linear trend was observed for the weight and length of large intestine [$y = 1.09 (\pm 0.082) - 0.023 (\pm 0.026) x$; $R^2 = 0.138$; $P = 0.040$] and [$y = 11.16 (\pm 0.201) - 0.051 (\pm 0.065) x$; $R^2 = 0.247$; $P = 0.004$], respectively, in response to increasing MWM inclusion levels. There were no significant dietary effects on all internal organ sizes of the Jumbo quails.

Table 4.1. Sizes of internal organs (g/100 g HCW, unless stated otherwise) of six-week-old Jumbo quails reared on diets containing incremental levels of mopane worm meal

	[1]Diets					Significance	
	MWM0	MWM5	MWM10	MWM15	[2]SEM	Linear	Quadratic
Liver	3.23	3.01	3.68	3.36	0.198	0.267	0.831
Gizzard	2.30	2.13	2.38	2.25	0.089	0.824	0.854
Proventriculus	2.30	2.38	2.25	2.13	0.045	0.270	0.496
Small intestine	0.56	0.46	0.52	0.56	0.276	0.728	0.596
Large intestine	4.290	4.503	4.216	4.295	0.084	0.040	0.808
Small intestine (cm)	67.20	63.35	66.74	65.56	2.066	0.871	0.528
Large intestine (cm)	11.12	10.98	10.51	10.33	0.208	0.004	0.925

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

[2]SEM = standard error of the mean.

Table 4.2 shows the effect of partial replacement of soybean products with MWM on carcass characteristics of Jumbo quails. There were no linear and quadratic trends (P >0.05) observed for all carcass characteristics as well as carcass yields. Likewise, there were no significant effect on carcass characteristics of Jumbo quails amongst the dietary treatments.

Table 4.2. Effect of feeding diets containing inclusion levels of mopane worm meal on carcass characteristics (g/100 g HCW, unless stated otherwise) of Jumbo quails

	[1]Diets					Significance	
	MWM0	MWM5	MWM10	MWM15	[4]SEM	Linear	Quadratic
Carcass yield (%)	65.30	68.05	65.25	67.24	1.259	0.609	0.777
[2]HCW (g)	161.2	161.7	153.2	157.7	3.670	0.265	0.5934
[3]CCW (g)	159.8	161.4	153.2	154.3	3.344	0.113	0.955
Thigh	6.26	6.20	6.20	5.86	0.186	0.157	0.453
Wing	4.38	4.38	4.21	4.24	0.138	0.343	0.931
Drumstick	4.01	4.10	3.90	3.98	0.097	0.491	0.947
Breast	21.78	21.765	20.95	20.96	1.095	0.503	0.990
Back (cm)	10.51	10.33	10.49	10.23	0.187	0.432	0.841
Thigh (cm)	4.68	4.75	4.60	4.64	0.178	0.723	0.922
Drumstick (cm)	5.68	5.70	5.32	5.48	0.167	0.202	0.665
Wing (cm)	10.59	12.55	10.58	30.22	9.835	0.201	0.371

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

[2]HCW = hot carcass weight.

[3]CCW = cold carcass weight.

[4]SEM = standard error of the mean.

4.3.2 Meat quality parameters

Table 4.3 shows the effect of MWM in place of soybean products on meat pH, temperature and colour measured immediately after slaughter of Jumbo quails. Neither linear nor quadratic effects (P >0.05) were observed for pH, temperature and colour measured immediately after slaughter in response to incremental levels of MWM. Similarly, dietary treatments had no significant effects on breast meat pH (5.97 - 6.07), temperature (22.07 – 25.75 ºC), *a** (3.22 - 3.63), *b** (7.21 - 8.20), *L** (50.15 - 53.42), chroma (8.01 - 8.91) and hue angle (1.13 - 1.18).

Table 4.3. Effect of replacing soybean products with mopane worm meal on meat pH, temperature and colour measured immediately after slaughter in Jumbo quails

	[1]Diets					Significance	
	MWM0	MWM5	MWM10	MWM15	[2]SEM	Linear	Quadratic
pH	6.05	5.97	6.01	6.07	0.071	0.774	0.277
Temp (ºC)	24.90	22.07	24.70	25.75	1.230	0.359	0.129
*L** (lightness)	51.43	50.24	50.15	53.42	1.143	0.253	0.058
*a** (redness)	3.22	3.63	3.42	3.45	0.245	0.656	0.445
*b** (yellowness)	7.90	7.77	7.21	8.20	0.441	0.869	0.221
Chroma	8.55	8.60	8.01	8.91	0.449	0.806	0.353
Hue angle	1.18	1.13	1.13	1.17	0.027	0.826	0.103

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

[2]SEM = standard error of the mean.

Table 4.4 shows the effect of MWM in place of soybean products on breast meat pH, temperature and colour measured 24 h post-slaughter of Jumbo quails. No linear or quadratic effects (P >0.05) were observed for pH, temperature, lightness and hue angle with MWM levels. However, quadratic trends (P <0.05) were observed for a^* [$y = 4.66\ (\pm 0.185) + 0.151\ (\pm 0.059)\ x - 0.008\ (\pm 0.004)\ x^2$; $R^2 = 0.118$; $P = 0.046$], b^* [$y = 8.83\ (\pm 0.236) + 0.306\ (\pm 0.0758)\ x - 0.017\ (\pm 0.005)\ x^2$; $R^2 = 0.094$; $P = 0.001$] and chroma [$y = 0.343\ (\pm 0.075)\ x + 10.0\ (\pm 0.234) - 0.019\ (\pm 0.005)\ x^2$; $R^2 = 0.312$; $P = 0.0004$] as MWM levels increased. Dietary treatments had no significant effect on pH, temperature, L^*, a^* and hue angle, but had effect (P <0.05) on b^* and chroma. Breast meat from quails on diets MWM5 and MWM10 had higher (P <0.05) b^* and chroma values than breast meat from those fed the control treatment MWM0. There were no significant differences between breast meat from quails on diet MWM0 and breast meat from those on diet MWM15. Quails fed the MWM-containing diets had similar (P >0.05) b^* and chroma values.

Table 4.4. Meat pH, temperature and colour measured 24 h after slaughter of six weeks Jumbo quails fed with diets containing the mopane worm meal

	[1]Diets				[2]SEM	Significance	
	MWM0	MWM5	MWM10	MWM15		Linear	Quadratic
pH	5.32	5.49	5.42	5.60	0.084	0.055	0.913
Temp (°C)	15.18	16.80	14.65	14.77	0.911	0.425	0.428
*L** (lightness)	51.32	51.87	50.77	50.86	0.743	0.462	0.762
*a** (redness)	4.70	5.20	5.50	5.10	0.190	0.070	0.046
*b** (yellowness)	8.74^{a}	10.20^{b}	9.89^{b}	9.60^{ab}	0.235	0.046	0.001
Chroma	9.94^{a}	11.41^{b}	11.33^{b}	10.87^{ab}	0.239	0.018	0.000
Hue angle	1.08	1.11	1.06	1.08	0.017	0.695	0.781

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.

[2]SEM = standard error of the mean.

Table 4.5 shows the effect of replacing soybean products with MWM on meat quality parameters of Jumbo quail. No linear or quadratic effects ($P > 0.05$) were observed for cooking loss, shear force, drip loss, and WHC. There were no significant dietary influences ($P > 0.05$) on cooking loss, drip loss and WHC, with the exception of shear force ($P < 0.05$). Breast meat from quails on diet MWM5 had the highest ($P < 0.05$) shear force value (2.39 N) than the breast meat from those on diets MWM0 and MW10, which were similar ($P > 0.05$). The control diet

MWM0 also promoted the same shear force value as diet MWM15. Breast meat from quails on diet MWM5 had similar (P >0.05) shear force value as those in diet MWM15.

Table 4.5. Meat quality attributes (%, unless stated otherwise) of Jumbo quails fed with mopane worm meal-containing diets

	[1]Diets					Significance	
	MWM0	MWM5	MWM10	MWM15	[3]SEM	Linear	Quadratic
Cooking loss	16.60	17.84	16.02	16.34	1.131	0.614	0.684
Shear force (N)	2.18[a]	2.39[b]	2.14[a]	2.23[ab]	0.048	0.732	0.318
Drip loss	3.42	3.38	3.29	3.30	0.170	0.547	0.879
[2]WHC	6.30	5.63	5.72	5.50	0.307	0.103	0.470

[1]Diets: MWM0 = a commercial standard grower diet with no mopane worm meal inclusion, MWM5 = a commercial standard grower diet in which 50 g/kg of soybean products were replaced with mopane worm meal, MWM10 = a commercial standard grower diet in which 100 g/kg of soybean products were replaced with mopane worm meal, and MWM15 = a commercial standard grower diet in which 150 g/kg of soybean products were replaced with mopane worm meal.
[2]WHC = water holding capacity
[3]SEM = standard error of the mean.

4.4 Discussion

Gastrointestinal tract (GIT) and the gut microbial population play crucial roles in nutrient utilisation, immune production, and disease resistance (Shang *et al*., 2018). The function of the gut is not only limited to nutrient digestion and absorption, but it also acts as the first defensive system against pathogens that may colonize and penetrate the host cells and tissues. Hence, the gut is referred to as the largest immunological organ in the body, meaning that healthier animals possess a more robust gut, and in turn, digest and utilizes nutrients more effectively (Yang *et al*., 2009). In this study, the inclusion of MWM in place of soybean products had no adverse effect on visceral organs of the Jumbo quails, as there were no linear or quadratic trends observed on most of the visceral organs in response to the incorporation of MWM. The lack of dietary differences across dietary treatments especially for gizzard weights indicates that the chitin amount in MWM did not challenge the digestion of the quails when the inclusion level is capped at 150 g/kg. It is worth noting that it is not always the case that simple non-ruminants would be challenged by fibre containing diets because their gut development requires physical stimulation by hard, solid particles of feed (Hetland *et al*., 2004). However, the results of the current study differ with those of Mbhele *et al*. (2019), who reported that incorporating BSFL meal at 100 g/kg in Jumbo quail diets caused a significant linear increase in gizzard weights, which was explained to be an anatomical adaption to cope with the fibrous nature of chitin. Moreover, the inclusion levels of MWM in the diets showed a linear decrease in both length and weight of large intestine. This finding was not consistent with the observations of Ballitoc & Sun (2013), who found that the intestinal weights increased when broilers were fed ground yellow mealworms at 10 g/kg. It was not surprising that the small intestine sizes did not change with increased MWM levels because chitin is not digested and assimilated in the small intestine and thereby passes unchanged to the large intestine (Vidanarachchi *et al*., 2010; Bovera *et al*., 2015). However, the linear decrease in large intestine weight and length was surprising because it was expected that prolonged hindgut fermentation of chitin would result in a highly developed large intestine. Large intestines are known to have a more diverse, rich, and stable microbial community including anaerobes inhabiting the cecum (Salanitro *et al*., 1974; Videnska *et al*., 2013).

Muscular development and carcass conformation are greatly influenced by nutrition, sex, age and genetic make-up of the birds (López-Pedrouso *et al*., 2019). In this study, all these factors except for the diet were nullified, which could explain the similar carcass traits such as carcass weights and yield as well as the commercial cuts (breast, thigh, wing and drumstick). There were neither linear nor quadratic effects on all commercial cuts in response to increasing level of MWM. These results are in agreement with the findings of Cullere *et al*. (2016) who observed no dietary effect on commercial cuts of broiler quails fed diets containing Black soldier fly larvae a rate of 100 g/kg and 150 g/kg. However, Hwangbo *et al*. (2009) reported an improvement in dressing yield, breast muscle, and thigh muscle of broiler chicken fed with increasing levels (5, 10, 15, and 20%) of maggot meal. This finding is consistent with the observations of Khatun *et al*. (2003) and Ballitoc & Sun (2013) who noted positive results in carcass traits of broiler chickens when the inclusion levels of silkworm pupa and yellow meal worm, respectively, were included at a rate of 100 g/kg. Overall, the lack of dietary effects on the carcass characteristics of the Jumbo quails shows that partial substitution of soybean products with MWM does not compromise these traits.

The major attributes of meat quality entails the appearance, texture, juiciness, taste, and functionality (Fletcher *et al*., 2002). These meat quality attributes are predicted by measuring pH and colour of the muscle. Thus, normal quail meat is described to have an ultimate pH (5.62 – 6.22), meat colour coordinates $L*$ (46.21 – 58.84), $a*$ (7.10 – 14.61), and $b*$ (4,86-15,31) when the pectoral muscles have attained rigor mortis 24-hour post-slaughter (Genchev *et al*., 2008; Ribarski & Genchev, 2013). In the current study, meat quality traits measured immediately after slaughter fell within the reference ranges reported for normal quail meat by (Cullere *et al*., 2016; Mnisi *et al*., 2017; Mulaudzi *et al*., 2019) and showed no linear or quadratic responses in response to MWM levels. In addition, the inclusion of MWM did not affect most of the meat quality traits measured 24 h post-slaughter, except for $b*$ and chroma values. Additionally, quadratic responses were observed for $a*$, $b*$ and chroma values with increasing MWM levels. These findings may have been influenced by the potential deposition of MWM carotene pigments on intramuscular fat (Schiavone *et al*., 2019). Various studies have reported different findings on meat colour of various poultry birds fed with dietary insect meal.

For example, Leiber *et al*. (2017), Altmann *et al*. (2018), Mbhele *et al*. (2019) and Pieterse *et al*. (2019) did not find any significant effect of dietary BSFL meal on broiler meat colour. Whereas, Cullere *et al*. (2016) and Schiavone *et al*. (2019) noted that 150 g/kg of BSFL increased the redness value. Bovera *et al*. (2016) also did not find any dietary effect on meat colour parameters of broiler chickens fed with yellow mealworm. Pieterse *et al*. (2014) found that meat lightness was decreased in broiler chicken fed with diets containing *Musca domestica* meal. This various reports or inconsistent results could be due to the different insect meal types and the species type used in the respective studies.

Breast meat pH did not differ amongst the treatment groups. These results differed from those of Bovera *et al*. (2016), who found that chickens fed with yellow mealworm containing diets had higher ultimate pH. Nonetheless, similar pH readings across the treatment groups may explain the lack of dietary difference on cooking loss, drip loss, and WHC as there is a strong correlation between muscle pH and these quality traits (Fletcher, 2002). It is well known that when pH value reaches a protein isoelectric point, the ability of meat to hold water is lowered thus reduces its moisture content (Barbut, 1997). Meat shear force was significantly affected by the inclusion of MWM, this observation was not expected given that the control diet promoted the same shear force value as the 150 g/kg MWM diet, and this could have been a systematic error during measurement.

4.5 Conclusion

Meat colour attributes such as redness and yellowness were affected by graded levels of mopane worm meal suggesting that carotene deposition to muscles influenced the pigmentation of meat. Nonetheless, this study has demonstrated that partial substitution of soybean products with MWM does not compromise the carcass characteristics, sizes of internal organs and meat quality. More studies should be conducted in order to fully understand the effect of insect meals, particularly mopane worm meal, on visceral organs, carcass and meat quality of Jumbo quails.

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5 CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The United Nations predicted that the human population of sub-Saharan Africa would have grown by 50% in the year 2050 (United Nations, 2019) and the recent statistics of the year 2017 estimates that nearly a quarter of people living in this region are undernourished (Fraval *et al*., 2019). This prevailing situation can be addressed by farming with fast-growing birds such as Jumbo quails and utilisation of lesser-known unconventional feed ingredients such as mopane worm, yellow mealworm, black soldier fly, and other insects could ensure sustainable intensive poultry production in sub-Saharan Africa. Previous studies that have attempted to partially replace soybean products with insect meal have demonstrated that, indeed, this meal has potential properties that can promote similar or even improve poultry performance and meat quality, without compromising the well-being of the birds. This has been shown by comparable results in terms of nutrient utilisation, growth performance, health status, and carcass and meat quality traits (Van Huis, 2013; Biasato *et al*., 2013; Bovera *et al*., 2015; Schiavone *et al*., 2019). Likewise, similar findings were reported for the aforementioned parameters in this study. The inclusion levels of MWM were expected to improve nutrient utilisation, growth performance and serum biochemical indices. However, Chapter three revealed that inclusion levels of MWM on Jumbo quail diets does not alter the palatability of the ration, this has been shown by lack of dietary differences in performance traits. Furthermore, the inclusion levels of MWM up to 150 g/kg did not affect any of the serum biochemical indices, suggesting that partial substitution of soybean products did not alter the health status of the quails. Also, the optimum inclusion level of MWM was not established using performance parameters, thereby suggesting that future research should focus on investigating the inclusion of MWM beyond 150g/kg. Chapter four showed that dietary treatments did not influence the internal organ sizes and carcass characteristics. These results were consistent with those reported by Makkar *et al*. (2014), Moreki *et al*. (2012) and Khan (2018). However, large intestine weights linearly decreased in response to the inclusion levels of MWM. This response of larger intestine was surprising since it was expected that the presence of chitin would challenge the utilisation of MWM-containing diets, and thereafter cause an enlargement of the large intestines as an adaptive mechanism to utilise fibre (chitin) (Vidanarachchi *et al*., 2010; Bovera *et al*., 2015). The replacement of soybean products with MWM had no influence on carcass traits and visceral organs but affected the values of meat yellowness, chroma, and shear force. Changes in meat yellowness may be attributed to possible deposition of mopane worm carotene pigment. In addition, it not clear as to why quails reared on MWM5 diet had a higher shear force value than those on the control and MW10 diets, provided that the MWM5 diet promoted the same shear force value as the MWM15 diet, this could have been a result of an error during measurement. Thus, this warranty further investigation in understanding the true effects of MWM on meat quality traits.

5.2 Conclusions

This study showed that partial replacement of soybean products with graded levels of mopane worm meal did not compromise nutrient utilisation, performance, health status, internal organs, and carcass and meat quality traits of Jumbo quails. The use of MWM in quail diets represents an environmentally friendly and sustainable way of providing high quality and organically produced meat products.

5.3 Recommendations and future prospects

An optimum inclusion level of mopane worm meal could not be established using the physiological and meat quality data. Thus, more research is required on the potential to replace soybean products with mopane worm in the diet of Jumbo quail at higher inclusion levels beyond 150 g/kg. Future studies can be designed to investigate the use of exogenous fibrolytic enzymes on chitin, a fibrous substance, when mopane worm meal is included at higher levels in quail diets. In addition, future research should focus on determining the least cost-effective means of domesticating and mass production of mopane worms to ensure all-year-round availability. The antimicrobial activities of mopane worm meal in Jumbo quail diets also require further research.

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6 LIST OF APPENDINCES

6.1 Ethics Certificate of the study

Private Bag X1290, Potchefstroom
South Africa 2520
Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: http://www.nwu.ac.za
Research Ethics Regulatory Committee
Tel: 018 299-4849
Email: nkosinathi.machine@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 01/11/2019, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Partial replacement of soybean meal with mopane worm meal in Japanese quail diets: Nutrient utilization, physiological, and meat quality parameter.

Study Leader/Supervisor (Principal Investigator)/Researcher: Dr K Mnisi

Student: Mveleli M

Ethics number: N W U - 0 1 8 8 5 - 1 9 - S 5
Institution / Study Number / Year / Status
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 2019/11/06 **Risk Category:** 2
Expiry date: 2020/10/05
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:*

1

- *request access to any information or data at any time during the course or after completion of the study;*
- *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
- *withdraw or postpone approval if:*
  - *any unethical principles or practices of the study are revealed or suspected;*
  - *it becomes apparent that any relevant information was withheld from the NWU-AnimProdREC or that information has been false or misrepresented;*
  - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
  - *new institutional rules, national legislation or international conventions deem it necessary.*
- *NWU-AnimProdREC can be contacted for further information or any report templates via upenyu.marume@nwu.ac.za or 018 389 2725.*

The NWU-AnimProdREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the NWU-AnimProdREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely

Prof Upenyu Marume
Chairperson NWU Animal Production Sciences Research Ethics Committee

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5 December 2018

File reference: 9.1.5.4.2

6.2 Language Editing Certificate

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Date: 16th November, 2020

To Whom It May Concern,

REF: Language Editing and Proof-reading of Dissertations/Theses

Dear Sir or Madam,

This serves to confirm that I have proof-read and edited the MSc dissertation of **M Marareni** (31971873; **orcid.org/0000-0001-5452-8933**) entitled: **Inclusion of Mopane worm meal in Jumbo quail diets: Nutrient utilization, physiological and meat quality responses.** The candidate then later corrected all the identified language and technical errors to my and the supervisor's utmost satisfaction. Thus the document presented here is of sufficient and acceptable academic standards.

Editor

Prof. O Ruzvidzo

Supervisor

Dr C.M. Mnisi

6.3 Publication in Veterinary and Animal Science (Elsevier)

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Growth performance, serum biochemistry and meat quality traits of Jumbo quails fed with mopane worm (*Imbrasia belina*) meal-containing diets

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ARTICLE INFO

Keywords:
Coturnix coturnix
Insect meal
Meat quality
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Sustainable production

ABSTRACT

Alternative protein sources such as mopane worm (*Imbrasia belina*) meal (MWM) are essential for sustainable poultry production. To date, no studies have attempted to investigate the effect of replacing soybean products with MWM in Jumbo quail diets. Thus, this study was designed to investigate the optimum inclusion level of MWM in place of soybean products on feed intake, physiological and meat quality responses of Jumbo quails. A total of 384 two-week-old mixed-gender quails (71.2 ± 5.40 g live-weight) were allotted to four isoproteic and isocaloric dietary treatments formulated by replacing soybean products with MWM at 0 (MWM0), 50 (MWM5), 100 (MWM10), and 150 (MWM15) g/kg. Neither linear nor quadratic effects ($P > 0.05$) were observed for feed intake, physiological responses, carcass traits and internal organs except for large intestines, which linearly decreased ($P < 0.05$) with MWM levels. There were significant quadratic trends for meat redness (a^*), yellowness (b^*) and chroma values in response to MWM levels. No dietary influences ($P > 0.05$) were observed on feed intake, physiological responses, internal organ weights, and carcass and meat quality parameters, except on b^*, chroma and shear force. Diets MWM5 and MWM10 promoted higher ($P < 0.05$) b^* and chroma values than MWM0. Whereas diet MWM5 promoted the highest ($P < 0.05$) shear force (2.39 N) than diets MWM0 and MWM10. We concluded that MWM has the potential to replace soybean products in quail diets without compromising their performance, health and meat quality. An optimum MWM inclusion level could not be determined suggesting that higher levels of MWM should be further investigated.

Introduction

Novel bird species such as the Jumbo quail (*Coturnix coturnix*) has great potential for use in the alleviation of food and nutrition insecurity, particularly in the most disadvantaged parts of the world. Currently, quails are gaining worldwide popularity as a source of high-quality protein in the form of meat and eggs (Genchev, Ribarski, Afanasjev, & Blohin, 2005; Priti & Satish, 2014), and are steadily cementing their place in the poultry industry (Mnisi & Mlambo, 2018a). This could be attributed to their fast growth rates, early sexual maturity, short generation intervals, lower feed and space requirements, high egg production rates, and resistance to several poultry diseases (Huss, Poynter, & Lansford, 2008; Mnisi & Mlambo, 2018b). For optimal performance, quails require a high energy and protein diet with highly digestible essential amino acids (Altine, Sabo, Muhammad, Abubakar, & Saulawa, 2016). Indeed, as with any intensive bird production system, the contribution towards sustainable food and nutrition security depends on innovative and low-cost feeding strategies. For many decades, soybean has been used as a source of high-quality protein in animal diets (Beski, Swick, & Iji, 2015). However, due to its high market prices, the continued use of soybean in animal feeds may reduce the contribution of Jumbo quails to food and nutrition security. The demand for soybean by the animal, food and biofuel industries has increased tremendously in such a way that its production has doubled (Castanheira & Freire, 2013). From an environmental and economic viewpoint, soybean production incurs high variable costs and is highly dependent on the use of fuel, machinery, pesticides and chemical fertilizers (Sharma, 2016; Arrieta, Cuchietti, Cabrol, & González, 2018; Ishiwata & Furuya, 2020). According to Brandão, Clift, Milà, and Basson (2010), the emission of nitrogen oxide from nitrogen fertilizers and mineralization of organic matter is the major contributor of greenhouse gases in soybean production. Indeed, the production of soybean has a larger carbon footprint due to deforestation and other land preparation activities as well as transportation (Castanheira & Freire, 2013). Thus, the

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6.4 Turnitin report

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Submitted to University of Fort Hare