Insect meal (*Hermetia illucens*) as an alternative protein source for Jumbo quails: Physiological and meat quality traits

F.G.T. Mbhele

orcid.org/0000-0003-3622-7920

Dissertation submitted in fulfilment of the requirements for the degree *Master of Science* in *Animal Science* at the North-West University

Supervisor: Dr C.M. Mnisi
Co-supervisor: Prof. V. Mlambo

Submission: November 2019
Student number: 23629096

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DECLARATION

I, Fezile Gcinile Thobeka Mbhele, declare that:

i. This thesis is my own original work

ii. It is hereby submitted by me, with the approval of my supervisors, for the degree of Masters of Science in Animal Science at the North-West University.

iii. It is not submitted in any institution other than the North West University.

iv. The use of reference material is duly acknowledged.

Student: Fezile Gcinile Thobeka Mbhele

Signature ..... Date .....

Main Supervisor: Dr C.M. Mnisi

Signature ..... Date .....

Co-Supervisor: Prof. V. Mlambo

Signature ..... Date .....

GENERAL ABSTRACT

Globally, soybean (*Glycine max*) meal is used as a standard vegetable protein source for poultry feeds. However, due to the negative ecological impact of soybean production, alternative natural and inexpensive protein sources such as insect meals ought to be identified and evaluated. Recently, there has been a substantial growth in the use of insect meal as a viable and sustainable alternative protein source for a number of meat animals but not in Jumbo quails (*Coturnix coturnix*). Insects are generally easy to rear, have a small carbon footprint and contain a high level of protein and essential amino acids. Therefore, the present study was conducted to determine the effect of partially replacing soya products with Black soldier fly (*Hermetia illucens*) larvae (BSFL) meal on feed intake, growth performance, haemato-biochemistry, internal organs, and carcass and meat quality traits of Jumbo quails. For a period of four weeks, 315, two-week-old, mixed gender Jumbo quails (53.79 ± 5.193 g live-weight) were fed five isonitrogenous and isoenergetic experimental diets, which were randomly allocated to 35 pens. The BSFL meal was included at a rate of 0, 25, 50, 75 and 100 g/kg (BSFL0, BSFL25, BSFL50, BSFL75 and BSFL100, respectively) as a replacement for soya products. The birds had access to the dietary treatments and water *ad libitum*. Infrared lights were placed in the pens to provide warmth as the trial was conducted during the winter season. Average weekly gain-to-feed ratio (G:F) was calculated using average weekly feed intake (AWFI) and average weekly body weight gain (ABWG) measured during the feeding trial. The birds were slaughtered at six weeks of age at a local abattoir. During slaughter, blood was collected from two birds that were randomly selected per pen. Repeated measures analysis showed a significant diet × week interaction on G:F. In weeks 3 and 5, there were linear trends for G:F in response to the inclusion level of BSFL meal. There were quadratic trends for overall feed intake [y = 605 (±17.56) + 2.1 (±0.82)x - 0.02 (±0.0077)x^2; R^2 = 0.20; P = 0.023] and overall weight gain [y= 155 (±4.70) + 0.57 (±0.22)x - 0.0049

(±0.002)x^2; R^2 = 0.22; P = 0.03]. Using the quadratic equation for overall weight gain, the optimal inclusion level for the BSFL meal was calculated to be 54 g/kg. There were no significant linear or quadratic trends for all haematological and serum biochemical parameters with the exception of albumin-to-globulin (ALB/GLOB) ratio in response to BSFL levels. Similarly, there were neither significant linear nor quadratic trends for size of internal organs except for gizzards, which linearly increased [y = 0.05 (±0.004) x + 2.3 (±0.085); R^2 = 0.18; P = 0.04] with BSFL levels. Quadratic trends were observed for size of large intestine [y = 1.4 (±0.077) + 0.0074 (±0.0036) x - 0.000073 (±0.0000337) x^2; R^2 = 0.16; P = 0.04] and breast weight [y = 21.9 (±0.637) + 0.054 (±0.0299)x - 0.00058 (±0.000278)x^2; R^2 = 0.168; P = 0.046]in response to BSFL levels. For all the meat quality parameters, there were neither linear nor quadratic (P>0.05) effects. There were no significant (P >0.05) differences on feed intake, physiological and meat quality responses across all dietary treatments. It was concluded that the inclusion of BSFL meal at the rate of 54 g/kg maximized weight gain in Jumbo quails without compromising any other traits of economic importance. These findings prove that BSFL meal at 54 g/kg is a viable, sustainable, low cost partial alternative to soybean protein for Jumbo quails.

Keywords: Growth; Insect meal; Jumbo quails; Meat quality; Soybean meal; Sustainable production.

ACKNOWLEDGEMENTS

Praise, Honour and Glory be unto God for granting me a healthy life and an opportunity to embark on this journey of doing a Masters degree.

Firstly, I would like to give thanks to the Almighty for crossing my path with my supervisors, Dr C.M. Mnisi and Prof. V. Mlambo, who tirelessly guided me towards the completion of this thesis. I greatly appreciate the support I received from them.

Secondly, I would like to extend my sincerest gratitude to AgriSeta Bursary for the financial support they provided me. I am indebted to Dr Molatelo Madibana (Department of Agriculture, forestry and fisheries) for supplying me with the insect meal used in this study.

Thirdly, I would like to thank my fellow postgraduate colleagues (Dr C. Kumanda, Berlinda Dlamini, Godfrey Mhlongo, Isaac Masenya, Didi Jonathan, Rudolf van Niekerk and Ashton Crafford) for their assistance during the feeding trial.

Lastly, I would like to thank my parents for always encouraging me even when I felt like I could not go on anymore, and for always supporting and having faith in me. To my brothers (Senzo, Mxolisi, Thapelo and Melusi), BoShangela, thank you for always being there when I needed financial support, and finally, to my one and only sister (Nozipho) thank you for the moral support. To Khwezi, my daughter, thank you for understanding that mommy has to study.

DEDICATION

This thesis is dedicated to the Mbhele and Manzini families. I would also like to dedicate it to my daughter, Khwezi.

TABLE OF CONTENTS

DECLARATION i
GENERAL ABSTRACT ii
ACKNOWLEDGEMENTS iv
DEDICATION v
TABLE OF CONTENTS vi
LIST OF TABLES ix
LIST OF PLATES x
LIST OF FIGURES xi
LIST OF ABBREVIATIONS xii
1 CHAPTER ONE – GENERAL INTRODUCTION 1
1.1 Background 1
1.2 Problem statement 2
1.3 Justification 3
1.4 Objective 4
1.5 Hypothesis 4
1.6 References 5
2 CHAPTER TWO – LITERATURE REVIEW 10
2.1 Introduction 10
2.2 Quail origin and development 11
2.2.1 Traits of quails 12
2.2.2 Potential constraints and challenges in quail farming 13
2.2.3 Nutritional requirements of quails 13
2.3 Soybean and insect meals as dietary protein sources 15
2.3.1 Soybean meal 15
2.3.2 Black soldier fly (Hermetia illucens) 16
2.3.3 Housefly (Musca domestica) 20
2.3.4 Yellow mealworm (Tenebrio molitor) 21
2.4 Mass production of insects 21
2.5 Effect of insect meals on feed utilisation and growth performance of poultry 22
2.6 Effect of insect meals on blood parameters of poultry 23
2.6.1 Haematology parameters 23

2.6.2 Serum biochemical parameters 24
2.7 Effect of insect meals on meat quality of birds 25
2.7.1 Meat pH 26
2.7.2 Meat colour 26
2.7.3 Water holding capacity 27
2.7.4 Drip loss 28
2.7.5 Meat tenderness 28
2.8 Consumer perception and acceptance 29
2.9 Summary 30
2.10 References 31
3 CHAPTER THREE – FEED INTAKE, GROWTH PERFORMANCE AND HAEMATO-BIOCHEMICAL PARAMETERS OF JUMBO QUAILS FED WITH BLACK SOLDIER FLY LARVAE MEAL-CONTAINING DIETS 47
Abstract 47
3.1 Introduction 48
3.2 Material and methods 49
3.2.1 Description of study site 49
3.2.2 Ingredient sources 50
3.2.3 Diet formulation 50
3.2.4 Chemical analyses 52
3.2.5 Experimental design and bird management 54
3.2.6 Feed intake and growth performance 54
3.2.7 Slaughter procedure and blood collection 55
3.2.8 Blood analysis 55
3.2.9 Statistical analysis 55
3.3 Results 57
3.3.1 Feed intake and growth performance 57
3.3.2 Haematological and serum biochemical parameters 58
3.4 Discussion 62
3.4.1 Feed intake and growth performance 62
3.4.2 Haematology and serum biochemical parameters 62
3.5 Conclusion 64
3.6 References 65

4 CHAPTER FOUR – SIZE OF INTERNAL ORGANS, CARCASS TRAITS AND MEAT QUALITY PARAMETERS OF JUMBO QUAILS FED WITH BLACK SOLDIER FLY LARVAE MEAL-CONTAINING DIETS 70
Abstract 70
4.1 Introduction 71
4.2 Material and methods 72
4.2.1 Study site, diet formulation and experimental design 72
4.2.2 Feeding trial and slaughter procedure 72
4.2.3 Measurements of carcass traits and internal organs 72
4.2.4 Meat quality attributes 73
4.2.5 Statistical analysis 75
4.3 Results 75
4.4 Discussion 81
4.4.1 Size of internal organs 81
4.4.2 Carcass characteristics 82
4.4.3 Meat quality parameters 83
4.5 Conclusion 84
4.6 References 85
5 CHAPTER FIVE – GENERAL DISCUSSION, CONCLUSIONS AN RECOMMENDATIONS 90
5.1 General Discussion 90
5.2 Conclusions 91
5.3 Recommendations and future prospects 92
5.4 References 93
6 LIST OF APPENDICES 96
Appendix 1: Article Accepted for Publication in Sustainability 96
Appendix 2: Ethics Certificate of the Study 97
Appendix 3: Language Editing Certificate 98

LIST OF TABLES

Table 2.1. Nutrient requirements of quails (g/kg unless otherwise stated)..... 15

Table 3.1. Ingredients composition (g/kg, *as fed* basis) of experimental diets..... 51

Table 3.2. Chemical composition of experimental diets on an as fed basis (g/kg) unless stated otherwise)..... 53

Table 3.3. Average weekly gain-to-feed ratio in Jumbo quails fed with diets containing the black soldier fly larvae meal as a partial replacement of soya products..... 57

Table 3.4. Overall feed intake (g/bird) and overall body weight gain (g/bird) of Jumbo quails fed the black soldier fly meal in place of soya products..... 58

Table 3.5. The effect of black soldier fly larvae meal-containing diet on haematological parameters of six-week old Jumbo quails..... 59

Table 3.6. The effect of black soldier fly larvae meal-containing diet on serum biochemical parameters of six-week old Jumbo quails..... 61

Table 4.1. Internal organs (g / 100 g HCWunless stated otherwise) of six weeks old Jumbo quails fed black soldier fly larvae meal-containing diets..... 76

Table 4.2. Carcass characteristics, slaughter weight and dressing out percentage of six-week old Jumbo quails fed black soldier fly larvae meal-containing diets..... 78

Table 4.3. The effect of black soldier fly larvae meal-containing diets on meat quality parameters of six-week old Jumbo quails..... 79

LIST OF PLATES

Plate 2.1. Jumbo quail (left) and Japanese quail (right)..... 12

Plate 2.2. Life cycle of the black soldier fly (Source: Diener, 2010). 19

LIST OF FIGURES

Figure 4.1. The effect of black soldier fly larvae meal-containing diets on cooking loss, water holding capacity and drip loss of six week old Jumbo quails..... 80

LIST OF ABBREVIATIONS

AA	:	Amino acids
ABWG	:	Average weekly body weight gain
ALB/GLOB	:	Albumin-to-globulin ratio
ALKP	:	Alkaline phosphate
ALT	:	Alanine transaminase
ANFs	:	Anti-nutritional factors
AWFI	:	Average weekly feed intake
BSFL	:	Black soldier fly larvae
CCW	:	Cold carcass weight
G:F	:	Gain-to-feed ratio
Hb	:	Haemoglobin
HCW	:	Hot carcass weight
LI	:	Large intestine
LSMEANS	:	Least square means
MCH	:	Mean corpuscular haemoglobin
MCHC	:	Mean corpuscular haemoglobin concentration
PCV	:	Packed cell volume
RBC	:	Red blood cells
SBM	:	Soybean meal
SI	:	Small intestine
WBC	:	White blood cells
WHC	:	Water holding capacity

1 CHAPTER ONE – GENERAL INTRODUCTION

1.1 Background

In the next 30 years, the world human population is anticipated to grow to 9.8 billion, with an increase of 83 million people expected every year (United Nations, 2019). Consequently, the agricultural sector is expected to increase food production owing to the growing population, climatic change, urbanization and economic development (Alexandratos & Brunsma, 2012). In order to meet the increasing demand for protein sources required to satisfy the nutritional requirements of both humans and livestock, there is a need to diversify and increase poultry production (FAO, 2013). The Jumbo quail is a new entrant into the poultry industry and is one type of domestic quail reared for commercial egg and meat production. It is a commercially more feasible option due to its several characteristics such as early maturity (six weeks of age), resistance to diseases and small rearing space that can accommodate several battery cages (Ali *et al.*, 2012; Puspamitra *et al.*, 2014). Intensive and semi-intensive quail production requires the use of commercial feeds that are formulated using expensive ingredients such as soybean and maize. These two feed ingredients have direct food value for humans and they are high in demand by the food and animal sector as well as the bio-fuel industry (Biswas *et al.*, 2011). This subsequently creates competition for these crop products (Khusro *et al.*, 2012) resulting in higher prices on the world market. This competition also has a negative impact on food and nutrition security in resource-poor communities (Semino *et al.*, 2009; von Witzke *et al.*, 2011). Therefore, there is a growing need to find alternative protein sources that are sustainable and inexpensive in order to maintain quail production. The potential protein alternatives to soybean meal (SBM) in quail production could be animal-based protein such as insect meals or plant protein sources such as rapeseed and sunflower meals (Ruviaro *et al.*, 2012; Gandhi & Zhou, 2014). For sustainable intensification

of quails, it is important to explore the alternative use of non-conventional animal protein sources such as the black soldier fly larvae (BSFL) meal. Research on insect meals as an alternative protein sources in animal diets has mainly focused on the housefly (*Musca domestica*) in both fish and poultry diets and black soldier fly larvae (BSFL) in fish diets (Bondari & Sheppard, 1981; Zuidhof *et al.*, 2003). This is despite the fact that the use of BSFL meal was proposed more than three decades ago as an efficient way to recover nutrients from organic waste (Diener *et al.*, 2011). Of late, the BSFL has gained more popularity for its ability to act as a bioreactor. These flies have the ability to access and extract energy from waste such as municipal waste, non-hazardous waste, non-industrial and agricultural waste to produce protein that can be included in animal feed (Van Huis *et al.*, 2013; Smetana *et al.*, 2016; Salomone *et al.*, 2017). In addition to their ability to recover protein from waste, the BSFL has high biological value and high feed conversion efficiencies (Makkar *et al.*, 2014).

1.2 Problem statement

The poultry industry has a dire need for sustainable protein sources owing to increasing demand for animal-protein food and the already high environmental costs associated with the production of commercial poultry feeds. Furthermore, large-scale production of soybean requires large spaces of land (Mungkung *et al.*, 2013), and has a high carbon footprint compared to the production of insect meals. Currently, the livestock sector alone accounts for approximately 14.5% of greenhouse gas emissions (Gerber *et al.*, 2013) mostly related to the production of feed (De Vries & De Boer, 2010). Expansion of arable land for feed production leads to deforestation resulting in more carbon emission (Foley *et al.*, 2005; Gibbs *et al.*, 2010) and depletion of carbon sinks. In order to address this problem, focus should be on feed

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resources that do not utilize large arable land resources and that cannot be directly used for human nutrition (Cassidy *et al.*, 2013). In literature, several studies are available on the effect of insect meal-based diets, mainly *Tenebrio molitor* and *Hermetia illucens* (De Marco *et al.*, 2015; Biasato *et al.*, 2016; Marono *et al.*, 2017) on broiler production and health. Animal-based protein sources tend to be richer in essential amino acids and thus have higher biological values (Newton *et al.*, 2005) than plant protein sources. Depending on their nutritional composition, some insects such as *Hermetia illucens* and *Tenebrio molitor*are considered more suitable than others when it comes to poultry nutrition. Insect meals have been nutritionally evaluated in chickens (Diener *et al.*, 2009), however, their potential as an alternative protein source in quail diets has not been assessed. This is despite the fact that adult larvae and pupae forms of insects are commonly consumed by wild birds and extensively reared chickens (Zuidhof *et al.*, 2003).

1.3 Justification

There are many advantageous motives for considering BSFL as a sustainable source of protein for Jumbo quails. The insect larvae of *Hermetia illucens* has the ability to feed on organic waste, converting low organic by-products to high value proteins (Van Huis & Oonincx, 2017). Inclusion of these larvae has been reported to decrease the incidence of metabolic skeletal disorders in broilers and improve the overall health of birds (Pieterse, 2014; Uushona, 2015). Cullere *et al.* (2016) reported that up to 15% of soya products can be replaced with BSFL meal without any negative impact on feed intake and growth performance of Japanese broiler quails (*Cotunix cotunix japonica*). The insect meal is particularly promising because it can convert and reach high growth rates on organic material that are unsuitable for human consumption and health (Diener *et al.*, 2011). It also has an

ideal amino acid composition, which is reported to be similar or even better than that of soybean (Veldkamp *et al.*, 2012). Józefiak *et al.* (2016) reported that poultry farmers could benefit immensely from the use of insects such as the *Hermetia illucens*, which contains active substances such as polyunsaturated fatty acids and antimicrobial peptides..No studies have attempted to establish the inclusion level of insect meal in Jumbo quail diets and there are also no recommended optimum inclusion levels of BSFL for these birds. The study, therefore, sought to investigate the inclusion level of Jumbo quails to BSFL meal in place of soya products and their effects on physiological and meat quality parameters of Jumbo quails.

1.4 Overall Objective

The broad objective of this study was to investigate the effect of different inclusion levels of black soldier fly larvae mealon feed intake, growth performance, blood metabolites, internal organs, and carcass and meat quality traits of Jumbo quails.

1.5 Overall Hypothesis

The alternative hypothesis of the study tested if the inclusion of incremental levels of black soldier fly larvae meal in place of soya products improved feed utilization, growth performance, haematology, serum biochemistry, internal organs, carcass characteristics and meat quality parameters of Jumbo quails.

1.6 References

Alexandratos, N. & Bruinsma, J., 2012. World agriculture towards 2030/2050: The 2012 revision. ESA Working Paper No. 12-03, FAO. Italy, Rome.

Ali, M.A., Hmar, L., Devi, L.I., Prava, M., Lallianchhunga, M.C. & Tokenkhomba, T.C., 2012. Effect of age on haematological and biochemical profile of Japanese quails (*Cotunix cotunix japonica*). Int. Multidiscip. Res. J. 2(8), 32-35.

Biasato, I., Gasco, L., De Marco, M., Renna, M., Rotolo, L., Dabbou, S., Capucchio, M.T., Biasibetti, E., Tarantola, M. & Sterpone., L., 2016. Yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for male broiler chickens: Effects on growth performance, gut morphology and histological findings. Poult. Sci. 97, 540-548.

Biswas, B., Scott, P.T. & Gresshoff, P.M., 2011. Tree legumes as feedstock for sustainable bio-fuel production: Opportunities and challenges. J. Plant Physiol. 168(16), 1877-1884.

Bondari, K. & Sheppard D.C., 1981. Black soldier fly larvae as feed in commercial fish production. Aquaculture, 24, 109-109.

Cassidy, E.S., West, P.C., Gerber, J.S. & Foley, J.A., 2013. Redefining agricultural yields: from tonnes to people nourished per hectare. Environ Res. 8, 034015.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S. & Dalle Zotte, A., 2016. Black soldier fly as dietary protein source for broiler quails: Apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal, 10(12), 1923-1930.

De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L. &Schiavone, A., 2015. Nutritional value of two insect meals (*Tenebrio molitor* and *Hermetia illucens*). Anim. Feed Sci. Technol. 209, 211-218.

De Vries, M. & De Boer I.J.M., 2010. Comparing environmental impacts for livestock products: A review of life cycle assessments. Livest. Sci. 128(1), 1-11.

Diener, S., Solano, N.M.S., Gutierrez, F.R., Zurbruegg, C. & Tockner, K., 2011. Biological Treatment of municipal organic waste using black soldier fly larvae. Waste Biomass Valori. 2, 357-363.

Diener, S., Zurbruegg, C. & Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. Waste Manage Res. 27(6), 603-610.

FAO, 2013. Organisation for Economic Cooperation and Development & Food and Agricultural Organisation of the United Nations (OECD-FAO). 2013. Agricultural Outlook 2013-2022. Paris, France.

Foley, J.A., De Fries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C. & Gibbs, H.K., 2005. Global consequences of land use. Science, 309, 570-574.

Gandhi, V.P. & Zhou, Z., 2014. Food demand and the food security challenge with rapid economic growth in the emerging economies of India and China. Food Res. Int. 63, 108-124.

Gerber, P.J., Hristov, A.N., Henderson, B., Makkar, H., Oh, J., Lee, C., Meinen, R., Montes, F., Ott, T., Firkins, J., Rotz, A., Dell, C., Adesogan, A.T., Yang, W.Z., Tricarico, J.M., Kebreab, E., Waghorn, G., Dijkstra, J. & Oosting, S., 2013. Technical options for the mitigation of direct methane and nitrous oxide emissions from livestock: A review. Animal, 7(2), 220-234.

Gibbs, H. K., Ruesch, A. S., Achard, F., Clayton, M. K., Holmgren, P., Ramankutty, N. & Foley J. A., 2010. Tropical forests were the primary source of new agricultural land in the 1980s and 1990s. Proc. Natl. Acad. Sci. 107(38), 16732-16737.

Józefiak, D., Józefiak, A., Kierończyk, B., Rawski, M., Świątkiewicz, S., Długosz, J. & Engberg R., 2016. Insects-a natural nutrient source for poultry: A review. Ann. Anim. Sci. 16(2), 297-313.

Khusro, M., Andrew, N. & Nicholas, A., 2012. Insects as poultry feed: A scoping study for poultry production systems in Australia. Worlds Poult. Sci. J. 68(03), 435-446.

Makkar, G., Tran, G., Heuze, V. & Ankers, P., 2014. State of the art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1-33.

Marono, S., Poccolo, G., Loponte, R., Parisi, G., Nizza, S., Lanonisi, V., Gasco, L. & Bovera, F., 2017. Effect of *Tenebrio molitor* larvae meal on growth performance, *in vivo* nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). Anim. Feed Sci. Technol. 226, 12-20.

Mungkung, R. & Aubin, J., 2013. Life cycle assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. Int. J. Life Cycle Assess. 11(1), 55-59.

Newton, L., Sheppard, C., Watson, D., Burtle, G. & Dove, R., 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. University of Georgia, College of Agriculture and Environmental Science.Annual Report, Tifton, Goergia.

Pieterse, E., 2014. Comparison of the production parameters, carcass and meat quality characteristics and gut parameters of broiler chicks grown on a diet containing either dried larvae and pre-pupae meal of the black soldier fly (*Hermetia illucens*) and larvae meal of the copper bottom blow fly (*Chrysomya chloropyga)* or soya as the main protein source. Internal report to Agriprotein. pp. 39.

Puspamitra, S., Mohanty, P.K. & Mallik, B.K., 2014. Haematological analyses of Japanese quails (*Coturnix coturnix japonica*) at different stages of growth. Int. Res. J. Biol. Sci. 3(11), 51-53.

Ruviaro, C.F., Gianezini, M., Brandão, F.S., Winck, C.A. & Dewes, H., 2012. Life cycle assessment in Brazilian agriculture facing worldwide trends. J. Clean. Prod. 28, 9-24.

Salomone, R., Saija, G., Mondello, G., Giannetto, A., Fasulo, S. & Savastano, D., 2017. Environmental impact of food waste bioconversion by insects: application of Life Cycle Assessment to process using *Hermetia illucens*. J. Clean. Prod. 140, 890-905.

Semino, S., Paul, H., Tomei, J., Joensen, L., Monti, M. & Jelsoe, E., 2009. Addressing the challenges of climate change-science meets industry, policy and public. IOP Conference Series-Earth and Environmental Science. Publishing Ltd, Bristol, UK.

Smetana, S., Palanisamy, M., Mathys, A. & Heinz, V., 2016. Sustainability of insect use for feed and food: Life cycle assessment perspective. J. Clean. Prod. 137, 741-751.

United Nations, 2019. The World Population Prospects Revision Department of Economics. New York, US. https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html

Uushona, T., 2015. Black soldier fly (*Hermetia illucens*) pre-pupae as a protein source for broiler production. MSc thesis, University of Stellenbosch, Western Cape, South Africa.

Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. & Vantomme, P., 2013. Edible insects-Future prospects for food and feed security. FAO Forestry Paper 171. Rome, Italy.

Van Huis, M. & Oonincx., D.G.A.B., 2017. The environmental sustainability of insects as food and feed. A review Agric. Sustain. Dev. pp. 43.

Veldkamp, T., Van Duinkerken, G., Van Huis, A., Lakemond, C.M.M., Ottevanger, E., Bosch, G. & Van Boekel, M.A.J.S., 2012. Insects as a sustainable feed ingredient in pig and poultry diets-a feasibility study. Report 638. Wageningen UR Livestock Research, Netherlands.

Von Witzke H. & Noleppa S., 2011. Why speculation is not a prime cause of high and volatile international agricultural commodity prices: An economic analysis of the 2007-08 price spike, The economics of Rumpelstiltskin, Humboldt Forum for Food and Agriculture, Berlin.

Zuidhof, M., Molnar, C., Morley, F., Wray, T., Robinson, F., Khan, B., Al-Ani, L. & Goonewardene, L., 2003. Nutritive value of house fly (*Musca domestica*) larvae as a feed supplement for turkey poults. Anim. Feed Sci. Technol. 105(1), 225-230.

2 CHAPTER TWO – LITERATURE REVIEW

2.1 Introduction

Worldwide, the animal production industry is experiencing a shortage in supply of feed ingredients resulting in poor animal production efficiency (FAO, 2009). Strategies for providing the industry with alternative feed ingredients include valorization of food and agro wastes or identifying feedstuff that cannot be used in the bio-fuel industry or as food for human (Delgade *et al.*, 2001). The deficiency of cost-effective feed ingredients has led to a shortage in animal protein for human consumption, especially in developing countries (Teguia & Beynen, 2005). To compound matters, an increase in disposable incomes has also seen an increase in the demand for animal protein (FAO, 2009).

Soybean is the preferred protein source in animal diets due to its high digestibility and high protein content (Makkar *et al.*, 2014). Despite its unique amino acid profile, soybean is costly and is associated with deforestation (Flachowsky & Meyer, 2015). Environmentally friendly protein sources that can provide the same advantages as soybean need to be identified and evaluated as alternatives (Bovera *et al.*, 2018). The agricultural industry produces large quantities of food waste that can be recovered and reused in other sectors of the industry (Cordell *et al.*, 2009). For instance, organic waste biomass is composted producing insects that can be used as an animal feed ingredient (Diener *et al.*, 2009) while the remaining compost can be used as a soil amender or to improve soil fertility (Newton *et al.*, 2005). Indeed, insects have been proven to be efficient decomposers of organic waste and useful as a nutrient recovery tool from waste (Newton *et al.*, 2005).

Waste can be classified according to its origins such as municipal waste, non-hazardous waste, non-industrial and agricultural waste, which is mostly disposed in landfills (Uushona, 2015). Alternative disposal methods should be developed because of the detrimental

environmental effects of burying waste, such as the release of toxins into the air, soil and dams (Seng *et al.*, 2013). Black soldier fly larvae are known to consume decomposing organic matter thus reducing the dry matter of waste to about 60% (Newton *et al.*, 2005). It is, therefore, ingenious to use this insect to reduce the amount of waste that require disposal while generating an insect meal that has potential for use as a protein source for birds of economic importance such as quails. Indeed, Bondari and Sheppard (1981) reported that pre-pupae and larvae meal of BSF can be utilized as a feed ingredient in animal diets and thus contribute to enhanced food and nutrition security. The use of BSFL as a protein source has mostly been investigated in fish and also in non-ruminants but never in the quail, an economically important, albeit new, bird with great potential as a source of animal protein for the ever-growing human population.

2.2 Quail origin and development

There are over 100 different breeds of quails and most of them are widely distributed in Asia, North America and Africa. These breeds are divided into two main groups, namely; the New-World quails and the Old-World quails (OMLET, 2004). In the Old-World quail group, the most common is the Japanese quail (*Coturnix coturnix japonica*) that belongs to the order *Galliformes* and the family *Phasianidae (*Minvielle, 2004). Quails are native wild birds of Japan, whose intensive production started in the 1920s in Japan followed by their successful introduction in China, India and Indonesia (Boni *et al.*, 2010). Minvielle (2004) highlighted that quails are very valuable in fowl research and have advanced the technical expertise required to execute modern quail production. The Jumbo quail was bred to create a larger and meatier version of the Japanese quail (*Coturnix coturnix japonica*).

Plate 2.1. Jumbo quail (left) and Japanese quail (right)

2.2.1 Traits of quails

The quail bird is the most recent entrant into the poultry industry. Over the years, they were known to be game birds that were later used for laboratory research as model animals (Egbeyale *et al.*, 2013). Of late, they are reared for commercial purposes (Onyewuchi *et al.*, 2013), dominating luxury markets such as restaurants, hotel and pubs. Quails are generally resistant to quite a number of avian diseases and are rarely vaccinated (Oluwatomi, 2010). A study conducted by Nain *et al.* (2011) revealed that quails are resistant to malathion, which is highly toxic to bees and other insects and moderately toxic to certain fish and birds.

Quails start laying at 8 weeks of age and produce 200 - 300 eggs per annum (Oluwatomi *et al.*, 2010). According to Tuleun (2011), quail meat is highly recommended for people who suffer from stress, digestive disturbance, gastric ulcers and hepatic diseases. The meat is known to be of high quality, with a high biological value and very low cholesterol content. Their eggs are high in vitamin D and antioxidants that promote the quality of food from animal origin in terms of colour, oxidative stability, tenderness and storage properties (Sahin

et al., 2008). Furthermore, quail production is relatively easy as it requires low investment while providing financial returns in a short period of time (Nasar *et al.*, 2016). Moreover, they reach sexual maturity at six weeks and therefore, reach market weight earlier (Tunsaringkorn *et al.*, 2013).

2.2.2 Potential constraints and challenges in quail farming

As a new entrant in the poultry industry, sufficient information and background knowledge on the production of quails is limiting, particularly the Jumbo quails. Feed cost remains a major hindrance for quail producers, especially for subsistence and emerging farmers. Some farmers are reluctant to venture into quail farming due to low markets availability and the high cost of feeding (Adeoti & Boruwa, 2019). Subsistence and emerging farmers are faced with challenges such as poor financial support from the department of agriculture in their quest to broaden animal production enterprises. Furthermore, female quails are characterized by poor brooding instincts hence the eggs need to be incubated artificially (Naibi *et al.*, 2009). However, since they lay eggs at high rates and have an average production of 200 – 300 eggs per bird annually, incubation is the only effective way to ensure maximum production (Adeoti & Boruwa, 2019). With that being said, farmers who cannot afford incubators are hindered from producing quails on a commercial scale.

2.2.3 Nutritional requirements of quails

During feed formulations, different ingredients are mixed to produce a nutritionally balanced diet that meets the nutritional requirements of the quails in order to sustain normal growth and production, as shown in Table 2.1. These nutrients can be divided into five classes, energy sources (carbohydrates and lipids), proteins, vitamins, minerals and water (Altine *et al.*, 2016). Feeding recommendations of quails differ according to the stages of growth and the type of production, i.e. whether they are reared for meat or egg production or breeding

purposes. Feed costs accounts about 65 - 75% of the total production costs for most types of poultry throughout the world (Bagopi *et al.*, 2014). Feed formulation requires detailed understanding of nutrient requirements of the class of poultry, feed ingredients (composition and constraint) in terms of nutrition, processing, cost and availability of the ingredients (Zaman *et al.*, 2008). A simple error in diet formulation such as deficiency of macro-minerals can cause reduced production, infertility problems and weakness of bones, which consequently affects profits for poultry producers (Hassan *et al.*, 2009). Since quails have a short digestive tract compared to other poultry species such as broilers and free-range chickens, they require a well-balanced diet to achieve high performance (Kidd *et al.*, 2001).

Quails require different feed ingredients (nutrient elements) in their diets to maintain various processes necessary for life, growth and reproduction (Altine *et al.*, 2016). These ingredients are mixed during feed formulations in optimum ratios to produce a balanced ration. Poultry feeds are referred to as 'complete' feeds because they contain all the required protein, energy, vitamins, minerals and other additives necessary for proper growth, egg production and health of the birds (Altine *et al.*, 2016). Jumbo quails have larger bodies and gain weight more rapidly than do the average quails (Boland *et al.*, 2013). Therefore, these birds ought to be fed diets with higher protein and energy levels to meet their nutrient requirements.

Table 2.1. Nutrient requirements of quails (g/kg unless otherwise stated)

	Broiler quails		Layer quails		
Nutrient	0-2weeks	3-5 weeks	0-2 weeks	3–5 weeks	>6 weeks
ME (Kcal/kg)	2800	2900	2750	2700	2650
Protein	270	240	240	200	190
Calcium	8.0	6.0	8.0	6.0	3.0
Phosphorus	3.0	3.0	3.0	3.0	4.5
Vitamin A (IU)	8000	8000	8000	8000	8000
Vitamin D3 (ICU)	1200	1200	1200	1200	1200
Riboflavin	0.006	0.006	0.006	0.006	0.006
Lysine	13.0	13.0	12.0	11.0	8.0
Methionine	4.8	4.5	4.5	4.0	3.3
Methionine + Cysteine	7.5	7.0	7.0	6.5	6.0

Adapted from Prabakan (2003).

2.3 Soybean and insect meals as dietary protein sources

2.3.1 Soybean meal

Soybean is customarily used as the gold standard protein source in poultry feed manufacturing. Its crude protein content ranges from 44 to 49% and this largely depends on the amount of hulls removed and the oil extraction method used during processing (NRC, 1994). However, other plant protein sources such as canola, sunflower and peas are extensively used as well (Baker *et al.*, 2011). In comparison to the protein meal of other oilseed grains, soybean is favoured due to its well-balanced amino acid (AA) profile, which

enables it to balance most cereal-based diets (Ravindran, 2013). However, inclusion of SBM in poultry diets is limited due to its high market prices and the presence of anti-nutritional compounds such as trypsin inhibitors that interfere with the digestion of protein and thus reduce their bioavailability and digestibility (Marsmon *et al.*, 1997; Mehri *et al.*, 2015).

On average, South African farmers produced about 1 550 880 tons of soybean in 2018 , which is not sufficient for human consumption, as an animal feed ingredient as well as for the bio-fuel industry (Khojely *et al.*, 2018). With the increase in the poultry sector and the world's population, farmers are failing to meet the increasing demand for soybeans. Due to shortages and cost considerations, it is inevitable that more consideration is given to alternative protein supplements to be utilised in poultry feeds in the near future (Khojely *et al.*, 2018). The high cost and shortage of commercial protein sources at times are known as some of the major limitations for efficient animal production. Alternative protein sources for poultry diets are necessary in order to reduce the farmer's dependence on standard or traditional sources of protein. Supply, availability and nutritional value are some of the necessary criteria in the search for viable alternatives (Berg *et al.*, 2013).

2.3.2 Black soldier fly (Hermetia illucens)

The black soldier fly belongs to the *Stratiomyidae* family and its native from the tropical, subtropical and warm temperate zones of America (Makkar *et al.*, 2014). The larva's phenotype is defined by a gloomy whitish colour on the body whereas the adult fly is characterized by a brownish colour (Diclaro & Kaufman, 2009). The BSFL feeds on decomposed organic matter and different kinds of waste that is unsuitable for human use, converting them into rich protein biomass (Makkar *et al.*, 2014). This process aids in reducing waste products resulting from the growing population (Makkar *et al.*, 2014). The use of the BSFL gained relevance due to their ability to efficiently produce at higher rates

(Makkar *et al.*, 2014; Bruni *et al.*, 2018). As emphasized by many authors, the protein content of the BSFL is of great value as it contains about 50% protein of higher biological value compared to soybean protein (Shumo *et al.*, 2019). Another desirable attribute of the BSFL is its similarities with fish meal in terms of the essential amino acid profile (Bruni *et al.*, 2018; Henry *et al.*, 2015). Table 2.2 shows the differences in total AA concentration of soybean and BSFL meal.

Table 2.2. Comparison of total amino acid concentration (g/kg, as fed) between soybean and BSFL meal

Amino acids	Soybean meal	BSFL meal
Arginine	35.3	18.5
Histidine	12.6	10.9
Isoleucine	22.6	16.0
Leucine	37.8	33.5
Lysine	31.3	17.7
Methionine	6.8	9
Phenylalanine	24	26.2
Threonine	18.2	18.1
Tryptophan	7	24.8
Valine	2.4	23.6
Cysteine	7.7	0.5
Glycine	20	30.5

Adapted from NRC (1994) and (Jansen, 2018)

Numerous studies have focused on the use of the insect larvae as a sustainable alternative to fish meal and soybean meal (Llagostera *et al.*, 2019). The sudden interest to use the BSFL is influenced by a number of advantages it possesses such as its low ecological impact and higher biological value. Under extensive rearing conditions, they require little land and water (Henry *et al.*, 2015; Van Huis & De Prins, 2013). Moreover, Makkar *et al.* (2014) reported that the adult flies do not feed and as such do not carry any diseases compared to other flies. The same authors reported that the adult flies are, in fact, able to reduce microbial load of substrate that may be present in the manure. However, the larvae become vulnerable to contamination if kept on contaminated raw material (Erickson *et al.*, 2004). Harvesting the larvae early and creating conducive environments in order to allow them to migrate from the waste before pupation occurs may aid in preventing contamination.

2.3.2.1 The life cycle of black soldier fly on waste

Organic waste conversion by insects is a potential valorization and recycling process that increases the value of the waste. Black soldier fly larvae feed on different decaying organic material such as rotting fruits and vegetables, animal manure and human excreta. The BSF has a relatively short life cycle of five to eight days. One female produces approximately 500 eggs in a dry environment near the edges of the decaying waste. After the eggs are laid, they hatch into larvae after four to five days (Makkar *et al.*, 2014). The larvae then feed on whatever waste is available until it reaches maturity at two weeks. The pupa stages follow after maturity and the larvae migrates from the decaying organic matter in search for dry environment before pupation takes place. The last larval stage (the pre-pupa), migrates from the feed source in search of dry and protected population site. The harvested pre-pupa is eaten by poultry; they can be live, chopped, dried or ground (Plate 2.1).

Plate 2.2. Life cycle of the black soldier fly (Source: Diener, 2010).

There have been attempts to make a defatted meal by cutting the larvae to let intracellular fat to pass out and then transfer the material to a tincture press (cylindrical pressured to extract liquids) (Kroeckel *et al.*, 2012). The BSF can be used commercially to resolve quite a number of environmental problems related with manure and other organic wastes. The adult black soldier fly is not attracted to human surroundings or foods thus they are not considered a problem in environments where people reside (Van Huis *et al.*, 2013). Large amounts of larvae can convert large quantities of organic waste into valuable biomass (van Huis *et al.*, 2013). Sheppard *et al.* (1994) and Barry (2004) as well as Newton *et al.* (2005) reported that larvae can lessen the accumulation of manure from laying hens and pigs by more than 50% without any added energy. Reduction values of 65 to 75% have been observed with household waste (Diener *et al.*, 2011). They rapidly utilise and process organic waste, stopping bacterial growth and consequently limit awful odours from decomposed waste.

Following the quick processing of waste, the larvae species introduces air into the waste and quickly dries the manure thus reducing the odour (van Huis *et al.*, 2013).

The BSFL are competitive with housefly larvae as they make the manure wet and unfavourable for housefly, and their occurrence hinders the deposition of eggs by the housefly. In addition, several studies have shown that BSFL reduce housefly population in pig or poultry manure (Sheppard *et al.*, 1994; Newton *et al.*, 2005). Although this phenomenon is not well documented in the literature, Tingle *et al.* (1975) suggested that competition for food and space may be the cause. This makes them suitable to be used as a control measure in housefly dominated farms and places with poor sanitations, subsequently improving the overall health status of animals and people since the housefly are chief vectors of diseases such as cholera and tuberculosis through contamination (Sheppard *et al.*, 1994). Unlike the housefly, the BSF is not a vector of diseases since the adult fly only drinks water and does not eat but relies on its body reserves this can be attributed to the fact that the mouth parts do not function at this time (Newton *et al.*, 2005; Makkar *et al.*, 2014). Furthermore, the BSFL also have natural antibiotic properties (Newton *et al.*, 2008), which modify the microflora of manure and subsequently reduce harmful bacteria found in waste.

2.3.3 Housefly (Musca domestica)

This type of fly is found worldwide and is the most commonly studied *Diptera*, as it also contains essential amino acids similar to those of fishmeal (Makkar *et al.*, 2014). The *M. domestica* larvae has a crude protein content that ranges between 40 and 60% and a lipid content ranging between 9 and 26% (Llagostera *et al.*, 2019). They also have the ability to recover protein from waste but disadvantageous in that they are vectors of human and livestock diseases as they feed on manure and decaying organic wastes (Makkar *et al* 2014; Llagostera *et al.*, 2019). In a study conducted by Dankwa *et al.* (2002) on backyard chickens

fed live larvae of the housefly resulted in increased in body weight, egg weight and number of eggs hatched. Moreover, Khan *et al.* (2018) reported a decrease in feed intake of broilers fed the housefly larvae at an inclusion level of 60% and attributed these findings to the high lipid content contained in the larvae. However, Dordević *et al.* (2008) observed no significant effects of the housefly larvae meal on feed intake at inclusion level of 50% and 100% of broilers.

2.3.4 Yellow mealworm (Tenebrio molitor)

The *Tenebrio molitor* is an edible insect that is native to Europe and is currently distributed worldwide. They are regarded as pests that reduce the quality of grains. They are usually common in store rooms containing flour and wheat (Makkar *et al.*, 2014). Its protein content varies between 47 and 60%, whereas its lipid content ranges between 31 and 43% (Llagostera *et al.*, 2019). In some parts of the world, such as China and Africa, it is produced on a commercial scale and used as feed ingredient for both humans and livestock (Finke, 2002; Rumpold & Schluter, 2013). A feeding trial conducted by Bovera *et al.* (2015), where 30% of *T. molitor* larvae meal replaced soybean meal revealed no difference in live weight of broilers after 64 days. Another feeding trial conducted by Biasato *et al.* (2016) concluded that there was no difference in the growth rate of backyard chickens fed with yellow mealworm at 7.5% inclusion. However, Belforti *et al.* (2015) observed a significant decrease in feed intake of rainbow trout (*Oncorhynchus mykiss*) offered diets containing the yellow mealworm at 25 and 50% inclusion levels.

2.4 Mass production of insects

In order for insects or larvae meals to be utilized as protein sources during animal feed formulation, significant quantities need to be produced. Worldwide, the rearing of insects on an industrial mass scale is currently facing hindrances from the legislative and cultural

stereotype surrounding the use of insects (Van Huis, 2013). In general, consumers are sceptical about the use of insect meal in animal feed and find the act of eating insects unnatural and do not perceive them as a viable food source (Vantomme *et al.*, 2017). However, consumers who are conscious about their health, have changed their social attitude towards the use of insects as a sustainable food source (van Huis *et al.*, 2013).In South Africa, there are relatively few companies that are well facilitated and equipped to produce insect meals on an industrial or commercial scale. This is mostly likely due to lack of demand and knowledge about the utility of insect meal as a potential protein source and the associated ecological and economic benefits. Companies such as AgriProtein, which produce BSFL meal on a commercial scale, ensure that the fly eggs are grown on traceable waste in order to ensure quality of the larvae.

2.5 Effect of insect meals on feed utilisation and growth performance of poultry

Insect larvae mea lis a promising potential protein in poultry diets because their crude protein content is about 50% (Shumo *et al.*, 2019). This is dependent on the type of waste the larvae are produced on as well as the stage at which it is harvested (Tschirner & Simon, 2015). Digestibility forms part of the components that affect insect protein quality thus the digestibility of the BSFL can be restricted by the presence of chitin. Chitin has proven to be a difficult component to digest for ruminant. According to Diener *et al.* (2009), chitin is a linear polysaccharide that hinders nutrient degradation and absorption. The extent to which it affects the digestibility of feed is dependent upon the composition and development stages of the larvae (Makkar *et al.*, 2014). As the larvae move closer to the pre-pupae stage and eventually the pupation stage, chitin levels start to increase (Kramer & Koga, 1986). Low protein digestibility caused by the presence of chitin can be resolved by harvesting the insects at larval stages. Kumar *et* al. (2017) reported that exogenous enzymes such as chitinase can be used to degrade chitin and improve nutrient intake. According to Pearincott (1960), as the

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larvae approaches the pupae phase in metamorphosis they start to store more energy in the form of lipids and the larvae utilizes the protein in enzymatic reaction in the chitin layer (Kramer & Koga, 1986).

When a diet containing BSFL was used in place of soybean at a rate of 96%, it resulted in increased weight gain compared to chicks that were given the SBM control diet (Newton *et al.*, 2005). According to Onsongo *et al.* (2018) the inclusion of BSFL meal on broiler diets had no effect on aroma, taste and overall acceptability of cooked breast meat. An experiment by Schiavone *et al.* (2016) showed that partial and full replacement of soybean oil with insect oil does not affect broiler growth rate and does not affect bird health. Furthermore, Khatun *et al.* (2003) and Hwangbo *et al.* (2009) demonstrated that supplementation of silkworm pupae and housefly maggot (*Musca domestica*), respectively, in broiler diets improved breast and thigh muscle weights and dressing percentage.

2.6 Effect of insect meals on blood parameters of poultry

2.6.1 Haematology parameters

Haematological values are used to assess the health status and treatment of birds (Washington & Hoosier, 2012). These indices vary depending on age, sex, environmental and nutritional factors as well as health status of birds (Swenson, 1970). Ewoula *et al.* (2004) demonstrated that haematology profiles can reveal the type and amount of feed consumed. Blood parameters that are influenced by the diet include red blood cells (RBC), packed cell volume (PCV), glucose and plasma protein (Aletor & Egberongbe, 1992). Aro *et al.* (2013) reported that many conventional feedstuffs administered to rabbits do not compromise the well-being of the animals. Ratiu *et al.* (2013) reported that the comparison of haematological values is important in diet formulations as they may reflect the need to add certain nutrients at lower or higher inclusion level. According to Puspamitra *et al.* (2014), blood values of Japanese quails

increase with age. Onyinyechukwu *et al.* (2017) added that haematology analysis is undertaken to diagnose disorders relating to bone marrow. Although, quails are generally resistant to quite a number of diseases, it is important to analyse the haematological indices in order to diagnose and differentiate healthy birds from diseased ones (Esonu *et al.*, 2001). According to Isaac *et al.* (2013), parameters such as RBC, WBC, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) can be used to evaluate feed toxicity. Swenson *et al.* (1970) attributed allergic conditions, anaphylactic shock and certain parasitism to a low volume of WBC in the blood of organisms. According to Njidda *et al.* (2006), MCV, MCH and MCHC are used to diagnose anaemic conditions. Adamu *et al.* (2006) reported that dietary treatments have a significant influence on PCV, haemoglobin (Hb) and RBC. Moreover, Ahmed *et al.* (1994) observed that Hb concentration, MCV, MCH and MCHC are significantly influenced by the interaction between protein and energy levels, and that an increase in protein level decreases MCHC level in the diet of Japanese quails.

2.6.2 Serum biochemical parameters

Serum biochemical components are used as indicators of protein quality, amino acid requirements, liver damage and the degree of heart attack (Harper *et al.*, 1979). Evans (2009) reported that the condition of the liver is assessed by measuring total protein, albumin and globulins. Total serum protein includes both albumin and globulins and is influenced by age, nutritional status, physiological state, dehydration and sample handling (Washington & Hoosier, 2012). Severe malnutrition and failure of protein absorption results in low total protein or hypoproteinemia. Inclusion of BSFL meal in diets of Jumbo quails can be used as a preventative measure of such diseases. In contrast, high levels of total protein or hyperproteinaemia results from dehydration and chronic infection or shock (Washington & Hoosier, 2012). A decrease in the serum albumin concentration below the normal ranges is an

indication of liver dysfunction (Eugster *et al.*, 1966). The size of albumin-to-globulin ratio (ALB/GLOB) is measured to detect the cause of values below normal ranges of total serum protein values. A low ratio indicates a decreased level of albumin or an increased globulins resulting from certain autoimmune diseases (Washington & Hoosier, 2012).

Creatinine is also present in the serum and is used to determine renal function. An increase in creatinine levels results from poor glomerular filtration, necrosis as well as infections (Donsbough *et al.*, 2010). In order to diagnose a renal disorder, the ratio of blood urea nitrogen (BUN) to creatinine is often used (Adjarov *et al.*, 1976). Furthermore, an increase in BUN suggests kidney disorder whereas a decrease indicates muscle mass loss due to dental diseases (Melillo, 2007). Washington and Hoosier (2012), reported that enzymes such as alkaline phosphate (ALP),alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are found within the cells. Increased levels of these enzymes reflect cell damage. Another indicator of liver damage includes increased levels of bilirubin. According to Melillo (2007), increased levels of ALP are an indication lipid metabolism disorders whereas increased levels of ALT results from tissue damage and increased levels AST suggest diseases or environmental issues.

2.7 Effect of insect meals on meat quality of birds

Lawrie and Ledward (2006) described appearance, juiciness, tenderness and flavour as the most vital attributes of poultry meat quality. A consumer's preference of meat and meat products is greatly influenced by colour (Fletcher *et al.*, 2000). Muchenje *et al.* (2009) reported that sliced meat must have a uniform colour that is acceptable to consumers. Taking into consideration the rapid rise in human population, the demand for quality meat is remarkably increasing. This has led to the continued search for inexpensive and sustainable protein sources such as the BSFL to meet the increasing world population. Nowadays,

consumers are more concerned about their health and always opt for organic products as opposed to conventionally produced poultry meat reared using growth hormones and antibiotic growth promoters. Thus, quail meat is organically produced because there are no drugs or hormones usage during production.

2.7.1 Meat pH

Correa *et al.* (2010) reported that events taking place prior to slaughter have serious adverse effects on meat pH. Stress that may result from transporting animals from the farm to point of slaughter/ abattoirs affect the pH of the meat. In addition, fear and handling has been reported to cause a decline in glycogen reserves. Glycogen levels in a calm and laid-back live animal is generally high and easily converted to lactic acid that is partly responsible for the meats' tastefulness and tenderness post slaughter (Pethick *et al.*, 1995). However, under anaerobic conditions, lactic acid is produced causing a drop in muscle pH (Muchenje *et al.*, 2009). The normal pH is reported to be between 5.7 and 6.2 (Secci *et al.*, 2018). Meat pH levels below the range are regarded as pale, soft and exudative (PSE) whereas pH levels above the range are considered as dark firm and dry (DFD) (Faucitano *et al.*, 2010).Cullere *et al.* (2016) observed that the inclusion of BSFL meal at 10 and 15% in the diet of growing quails provided satisfactory results in terms of meat quality with pH values ranging from 5.67 and 5.78.

2.7.2 Meat colour

The consumer's choice of selection is influenced by appearance of the meat (Muchenje *et al.*, 2009). Indeed, Fletcher *et al.* (2000) reported that colour is one of the major contributors to appearance. Hutching (2003) added that the colour of the meat represents freshness and wholesomeness that is appealing to the consumer. Pigmentation of meat is due to the presence of myoglobin (Fletcher *et al.*, 2000). A fresh cut of meat contains three forms of

myoglobin, namely: deoxymyoglobin, oxymyoglobin and metmyoglobin (Mancini & Hunt, 2008). Under anaerobic conditions, myoglobin remains in its reduced state, whereas under aerobic conditions deoxymyoglobin is swiftly converted into oxymyoglobin giving meat the desired appearance appealing to consumers. Colour is generally measured according to the Commission International De I' Eclairage (CIE) colour system guidelines (Commission International De I' Eclairage, 1976). The guidelines stipulate three main colour coordinates, namely: L^*, which depicts lightness and measures the amount of light reflected (100 = white; 0 = black), a^* depicts positive red and/or negative green, and b^* depicts positive yellow and/or negative blue. Another form of determining colour objectively, is using values from L^*, a^*, and b^* to calculate hue angle $tan\,\theta = \frac{a^*}{b^*}$, which indicates colour from to yellow and Chroma ($\sqrt{a^{*2} + b^{*2}}$) which indicates the saturation of colour (Tapp III *et al.*, 2011). Dietary treatments may also affect colour as they are the main sources of pigments in animal life. Vegetable sources can be responsible for the high content of xanthophyllys and carotenoids (Kokoszyńskiet *al.*, 2013). Secci *et al.* (2018) concluded that the 1 kg of BSFL contains approximately 42 g of total tocopherols and 2 mg of total carotenoids. However, it is important to note that pigmentation results from all the ingredients contained in the dietary treatments.

2.7.3 Water holding capacity

According to Barbera (2019), water-holding capacity (WHC), is one of the most important parameters affecting meat quality and subsequently pricing. According to Gault (1985) and; Lawrie (1985), variation in weight from farm to point of slaughter, drip loss during thawing, juiciness and tenderness of meat is greatly affected by WHC. This parameter is measured using filter papers to measure water loss (Grau & Hamm, 1956). This method seeks the difference in meat before and after applying pressure by placing a specific weight for a few

minutes. It speculates that as pressure is exerted on the meat sample, the water retained by the filter paper forms an outer ring zone which is equivalent to the amount of loosely bound – water in meat. According to Genchev *et al.* (2008) the normal WHC range is between 18.05 and 21.7% for Japanese quails.

2.7.4 Drip loss

According to Logan *et al.* (2019), the freshness of meat is largely dependent on WHC, which is influenced by drip loss. Otto *et al.* (2004) demonstrated that drip loss is of high economic importance as it influences financial returns. It is also important because it represents the loss of fluid from meat cuts as a result of shrinkage of muscle proteins in the form of drip (Yu *et al.*, 2005). Drip loss occurs when proteins are exposed to low pH at high temperatures, which causes less water retention between actin and myosin filaments and thereby increases exudates. Genchev *et al.* (2008) observed that the drip loss of Japanese quails is between 0.6 and 1.07%.

2.7.5 Meat tenderness

Another important parameter of meat quality is tenderness. According to Hopkins and Geesink (2009), meat tenderness is influenced by collagen content and solubility, as well as the rate of muscle shortening and aging, which are all dependent on endogenous and exogenous factors (Sierra & Olivan, 2013). The degree of tenderness in meat can be evaluated in two ways. The commonly used method is the Warner-Bratzler shear force test (Van Wezemael *et al.*, 2014). According to Genchev *et al.* (2008) the normal shear force range is between 201 to 321 ^{0}P for Japanese quails. Higher shear force values indicate tougher meat quality (Genchev *et al.*, 2008). This test measures the amount of force (in Newtons) needed to cut through a piece of meat. Another method of testing meat tenderness is sensory evaluation using consumers. Consumers are used to determine preference using

attributes such as tenderness, flavour and aroma (Genchev *et al.*, 2008).This method is time consuming and quite costly (Hildrum *et al.*, 2009).

2.8 Consumer perception and acceptance

The future success of any product is dependent on the reaction and acceptance by the targeted consumers. These reactions are mostly likely to determine further continuation, expansion and future success of the product. In 2013, the FAO in the 'Edible Insect's Report' recognized the challenges accompanying mass rearing of insects used as food and as feed ingredient for livestock (Van Huis *et al.*, 2013). One of the major hindrances towards the utilization of insects as a feed ingredient include issues relating to food safety and conventional agricultural practices (Veldkamp *et al.*, 2012). In many countries, utilization and commercialization of insect-based meals is guided by rules and legislations. Smith and Pryor (2013) reported that, in Brazil, the Codex Alimmentarius, which is the international reference standard for food and feed developed by WHO and FAO points out that food and feed ingredients should be free of impurities and not pose danger towards human health. Till this day, the Codex has not been amended and still regards insects as impure. On the other hand, the European Union laws state that the rearing and utilization of insects as a feed ingredient should abide with the European Union animal by-product regulation 1069/2009 in order for the insects to become processed animal protein (Koeleman, 2014). In South Africa, a few companies are well equipped for mass rearing of insects. Little is known about the reactions of farmers, stakeholders and consumers towards the utilization of insects as a feed ingredient.

2.9 Summary

The use of soybean during feed formulations is one of the biggest challenges faced by the poultry industry. Soybean is widely used as the main vegetable protein source because it has a well-balanced amino acid profile in commercial feeds. The need to find alternative protein sources is driven by an increase in the market price for soybeans. In addition, the cultivation of soybean has a negative impact on the environment due to greenhouse gas emissions and the destruction of forests that are an important atmospheric carbon sink. As such, there is a need to explore alternative protein sources that are environmentally, financially and socially sustainable and can be produced at a large-scale.

2.10 References

Adamu, S., Thomas, A., Iseh, N. M., Fatihumi, M. Y. & Esieno, K. A. N., 2006. Normal values of haematology of Nigeria adopted albino rats (*Rattus norvegicus*) in Zaria. Proc. 31st Annual Conf. Nig. Soc. Anim. Prod. Zaria, Nigeria.

Adeoti, S.O. & Baruwa, O.I., 2019. Profitability and constraints of quail egg production in South western Nigeria. Obatemi Awolowo University, Osun State, Nigeria. J. Exp. Agri. Int. 33(3), 1-10.

Adjarov, D., Popov, S. & Ivanov, E., 1976. Studies on the mechanism of the changes in serum and liver gamma-glutamyl transpeptidase activity. I. Experimental extrahepatic cholestasis in rabbits. Enzyme Res. 21, 1-7.

Ahmed, M.K., Bargue, A.R., Nawaz, H. & Siddi, R.H., 1994. Effect of varying energy and protein levels of the haematology of Japanese quail. Pak. Vet. J. 14(4), 200-202.

Aletor, V.A. & Egberongbe O., 1992. Feeding differently processed soya bean. Part 2. An assessment of haematological responses in chickens. Die Nahrung. 36, 364-369.

Aro, S.O. & Akinmoegun, M. B., 2012. Haematology and red blood cell osmotic stability of pigs fed graded levels of fermented cassava peel based diets. Proc. 17th Annual Conf. Anim. Sci. Assoc. Nig. (ASAN). pp. 152-153.

Bagopi, E., Chokwe, E., Halse, P, Hausiku, J., Kalapula, W., Humavindu, M. & Roberts, S., 2014. Competition dynamics and regional trade flows in the poultry sector: the case of South Africa, Botswana, Namibia, and Zambia. Pre-ICN conference. Retrieved from http://www.africancompetitionforum.org/sites/default/files/docs/Poultry_ACF_ICNConfpaper.pdf

Baker, K.M., Utterback, P.L., Parsons, C.M. & Stein, H.H., 2011. Nutritional value of soybean meal produced from conventional, high-protein, or low-oligosaccharide varieties of soybeans and fed to broiler chicks. Poult. Sci. 90, 390-395.

Barbera, S., 2019. WHC trend, an up-to-date method to measure water holding capacity in meat. Department of Science Agriculture, Forestry and food, University of Torino, Largo Paolo Braccini 2, 10095, Grugliasco, Italy.

Barry, T., 2004. Evaluation of the economic, social, and biological feasibility of bio converting food wastes with the black soldier fly (*Hermetia illucens*). PhD Dissertation, University of Texas, Texas. pp. 176.

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., De Marco, M., Dabbou, S., Schiavone, A., Zoccarato, I. & Gasco, L., 2015. *Tenebrio molitor* meal in rainbow trout (*oncorhynchusmy*) diets: effects on animal performance, nutrient digestibility and chemical composition of fillets. Ital. J. Anim. Sci. 14, 670-675.

Berg, A., De Noblet-Ducoudré, N., Sultan, B., Lengaigne, M. & Guimberteau, M., 2013. Projections of climate change impacts on potential C4 crop productivity over tropical regions. Agric. Meterol. 170, 89-102.

Biasato, I., De Marco, M., Rotolo, L., Renna, M., Dabbou, S., Capucchio, M.T. & Schiavone, A., 2016. Effects of dietary *Tenebrio molitor* meal inclusion in free-range chickens. J. Anim. Physiol. Anim. Nutr.100, 1104-1112.

Boland, M.J., Rae, A.N., Vereijken, J.M., Meuwissen, M.P., Fischer, A.R., van Boekel, M.A., Rutherfurd, S.M., Gruppen, H., Moughan, P.J. & Hendriks, W.H., 2013. The future supply of animal derived protein for human consumption. Trends Food Sci. Technol. 29(1), 62-73.

Bondari, K. & Sheppard D.C., 1981. Black soldier fly larvae as feed in commercial fish production. Aquaculture, 24, 109-109.

Boni, I., Nurul, H. & Noryati, I., 2010. Comparison of meat quality characteristics between young and spent quails Int. Food Res. J. 17, pp. 661-666.

Bovera, F., Loponte, R., Pero, M.E., Cutrignelli, M.I., Calabro, S., Musco, N., Vassalotti, G., Panettieri, V., Lombardi, P., Piccolo, G., Di Meo, C.C., Siddi, G., Fliegerova, K. & Moniello, G., 2018. Laying performance, blood profiles, nutrient digestibility and inner organs traits of hens fed an insect meal from *Hermetia illucens* larvae. Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, Via Federico Delpino 1, 80137, Napoli, Italy.

Bovera, F., Piccolo, G. Gasco, L., Marono, S., Loponte, R., Vassalotti, G. & Nizza, A., 2015. Yellow mealworm larvae (*Tenebrio molitor*) as a possible alternative to soybean meal in broiler diets. Brit. Poultry Sci. 56(5), 569-575.

Bruni, L., Pastorelli, R., Viti, C., Gasco, L. & Parisi, G., 2018. Characterisation of the intestinal microbial communities of rainbow trout (*Oncorhynchus mykiss*) fed with *Hermetia illucens* (black soldier fly) partially defatted larva meal as partial dietary protein source. Aquaculture, 487, 56-63.

Commission International De I' Eclairage, 1976. Calorimetry (2nded.). Vienna, Switzerland.

Cordell, D., Drangert, J. & White, S., 2009. The story of phosphorus: Global food security and food for thought. Global Environ. Change. 19(2), 292-305.

Correa, J.A., Torrey, S., Devillers, N., Laforest, J.P., Gonyou, H.W. & Faucitano, L., 2010. Effects of different moving devices at loading on stress response and meat quality in pigs. J. Anim. Sci. 88(12), 4086-4093.

.

Dankwa, D., Nelson, F.S., Oddove, E.O.K. & Duncan, J.L., 2002. Housefly larvae as a feed supplement for rural poultry. Ghana J. Agric. Sci. 35, 185-187.

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. & Courbois, C., 2001. Livestock to 2020: The next food revolution. Outlook Agric. 30(1), 27-29.

Diclaro, I.I., Joseph, W. & Kaufman P.E., 2009." Black Soldier Fly" – *Hermetia illucens.* University of Florida, USA.

Diener S., 2010. Valorisation of organic solid waste using the black soldier fly, *Hermetia illucens*, in low and middle-income countries. PhD thesis. ETH Zürich, Zürich.

Diener, S., Solano, N.M.S., Gutierrez, F.R., Zurbruegg, C. & Tockner, K., 2011. Biological Treatment of municipal organic waste using black soldier fly larvae. Waste Biomass Valori.2, 357-363.

Diener, S., Zurbruegg, C. & Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. Waste Manage. Res. 27(6), 603-610.

Diener, S., Zurbrugg, C., Roa Gutiérrez, F., Nguyen, H.D., Morel, A., Koottatep, T. & Tockner, K., 2011. Black soldier fly larvae for organic waste treatment: prospects and constraints. Paper presented at the Waste Safe 2011, 2nd International

Conference on Solid Waste Management in Developing Countries, Khulna, Bangladesh.

Donsbough, A.L., Powell, S., Waguespack, A., Bidner, T.D. & Southern, L.L., 2010. Uric acid, urea and ammonia concentrations in serum and uric acid concentration in excreta as indicators of amino acid utilization in diets for broilers. Poult. Sci. 89, 287-294.

Dordević, M., Radenković-Damnjanović, B., Vučinić, M., Baltić, M., Teodorović, R., Janković, L., Vukašinović, M. & Rajković, M., 2008. Effects of substitution of fishmeal with fresh and dehydrated larvae of the house fly (*Musca domestica* L) on productive performance and health of broilers. Acta. Vet. 58, 357-368.

Egbeyale, L.T., Fatoki, H.O. & Adeyemi, O.A., 2013. Effect of egg weight and Oviposition time on hatchability and post hatch performance of Japanese quail (*Coturnix coturnix japonica*). Niger. J. Anim. Prod. 40, 102-110.

Erickson, M., Islam, M., Sheppard, C., Liao, J. & Doyle, M., 2004. Reduction of *Escherichia coli* O157:H7 and *Salmonella enterica serovar Enteritidis* in chicken manure by larvae of the black soldier fly. J. Food Prot. 67, 685-690.

Esonu, B.O., Emenalom, O. O., Udedibie, A. B. I., Berbert, U., Ekpor, C. F., Okoli, E. C. & Iheukwumere, F. C., 2001. Performance and chemistry of weaner pigs fed raw *Mucuna bean* (Velvet bean) meal. Trop. Anim. Prod. Invest. 4, 49-54.

Eugster, A.K., Albert, P.J. & Kalter, S.S., 1996. Multiple enzyme determinations in sera and livers of tumor-bearing hamsters. Proc. Soc. Exp. Biol. Med. 123, 327-331.

Evans, G.O., 2009. Animal clinical chemistry. CRC Press, Boca Raton FL.

Ewuola, E.O., Folayan, O.A., Gbore, F.A, Adebunmi, A.I., Akanji, R.A., Ogunlade, J.T. & Adeneye, J.A., 2004. Physiological response of growing West African dwarf goats fed ground nut shell-based diet as the concentrate supplement. Bowen J. Agric. 1, 61-69.

FAO, 2009. State of food and agriculture: livestock in the balance. Food and Agriculture Organization. Rome, Italy.

Faucitano, L., Chevillon, P. & Ellis, M., 2010. Effects of feed withdrawal prior to slaughter and nutrition on stomach weight, and carcass and meat quality in pigs. Livest. Sci. 127(2–3), 110-114.

Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21, 269-285.

Flachowsky, G.& Meyer, U., 2015. Sustainable production of protein of animal origin the state of knowledge. Part 1. Resources and emissions as factors affecting sustainability. J. Anim. Feed Sci. 24, 273-282.

Fletcher, D.L., Qiao, M. & Smith, D.P., 2000. The raw broiler breast meat colour and pH to cooked pH. Poult. Sci. 79, 784-788.

Gault, N.F.S., 1985. The relationship between water-holding capacity and cooked meat tenderness in some beef muscles as influenced by acidic conditions below the ultimate pH. Meat Sci. 15, 15-50.

Genchev, A., Mihaylova, G., Ribarski, S., Pavlov, A. & Kabakchiev, M., 2008. Meat quality and composition in Japanese quails. Trak. J. Sci. 6, 72-82.

Harper, A.E., Rodwell, V.W., Mayes, P.A., 1979. Review of Physiological Chemistry. 17th ed. Lang Medical, Los Altos, California 9442. 60(81), 188-216.

Hassan, A., Sani I., Maiangwa, M. & Rahman, S., 2009. The effect of replacing graded levels of fishmeal with grasshopper meal in broiler starter diet. Polym. Adv. Technol. 5(1), 30-38.

Henry, M., Gasco, L., Piccolo, G. & Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: past and future. Anim. Feed Sci. Technol. 203, 1-22.

Henry, M.A., Gasco, L., Chatzifotis, S. & Piccolo, G., 2018. Does dietary insect meal affect the fish immune system? The case of mealworm, *Tenebrio molitor* on European sea bass, *Dicentrarchus labrax*. Dev. Comp. Immunol. 81, 204-209.

Hildrum, K.I., Rodbotten, R., Hoy, M., Berg, J., Narum, B. & Wold, J.P. 2009. Classification of different bovine muscles according to sensory characteristics and Warner Bratzler shear force. Meat Sci. 83(2), 302-30.

Hopkins, D. L. & Geesink, G. H., 2009. Protein degradation post-mortem and tenderization. In M. Du & R. J. McCormick (Eds.), Applied muscle biology and meat science. Boca Raton: CRC Press, Taylor & Francis Group. 149-166.

Hutchings, J.B., 2003. Expectations and the food industry. The impact of colour and appearance. Kluwer Academic/Plenum Publishers, New York, USA.

Hwango, J., Hong, E.C., Jang, A., Kang, H.K., Oh, J.S., Kim, B.W. & Park, B.S., 2009. Utilization of housefly-maggot as feed supplement in the production of broiler chickens. 30(4), 609-614.

Jansen, Z., 2018. The nutritional potential of black soldier fly (*Hermetia illucens*) for layer hens. MSc thesis, University of Stellenbosch, Western Cape, South Africa.

Khan, M., Chand, N., Khan, S., Khan, R.U. &Sultan, A., 2018. Utilizing the housefly (*Musca domestica*) larva as an alternative to soybean meal in broiler ration during the starter phase. Braz. J. Poult. Sci. 20, 9-14.

Khatun, R., Howlider, M.A.R., Rahman, M.M. & Hasanuzzaman, M., 2003. Replacement of fishmeal by silkworm pupae in broiler diets. Pak. J. Biol. Sci. 6(11), 955-958.

Khojely, D.M., Ibrahim, S.E., Sopey, E. & Han, T., 2018. History, current status and prospects of soybean production and research in Sub Saharan Africa. J. Crop. Prod. 6(3), 226-235.

Kidd, M., Gerard P., Heger J., Kerr B., Rowe D., Sistani K. & Burnham D., 2001. Threonine and crude protein responses in broiler chicks. Anim. Feed Sci. Technol. 94(1), 57-64.

Kokoszyński, D., Bernacki, Z., Koeytkowska, H., Wilkanowska, A. & Frieske, A., 2013. Carcass composition and meat quality of Grey partridge (*Perdix perdix L.*). J. Cent. Eur. Agric. 14(1), 378-387.

Kramer, K.J. & Koga, D., 1986. Insect chitin. Insect Biochem. 16(6), 851-877.

Kroeckel, S., Harjes, A. G. E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. & Schulz, C., 2012. When a turbot catches a fly: evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fishmeal substitute - growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture, 364/365, 345-352.

Kumar, K., Borsra, M.Z.S., Ganesan, D., Kuppusamy, G., Herriman, M., Salter, A. & Ali, S.A., 2017. Efficacy of insect larvae meal to replacefishmeal in juvenile barramundi (*Late calcarifer*) reared in fresh water. Int. Aquat. Res. 9(4), 303-312.

Lawrie R.A. & Ledward, D.A., 2006. Lawrie's meat science (7thed.). Woodhead Publishing. Cambridge, England.

Lawrie, R.A., 1985. Meat Science (4th ed.), Pergamon Press, Oxford, UK.

Llagostera, F., Kalla, Z., Reig, L. & de Gea, D., 2019. The use of insect meal as a sustainable feeding alternative in aquaculture: Current situation, Spanish consumers' perceptions and willingness to pay. J. Clean. Prod. 229, 10-21.

Logan, B.G., Bush, R.D., Biffin, T.E., Hopkins, D.L. & Smith, M.A., 2019. Measurement of drip loss in alpaca (*Vicugna pacos*) meat using different techniques and sample weights. University of Sydney, Sydney, Australia.

Makkar, G., Tran, G., Heuze, V. & Ankers, P., 2014. State of the art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1-33.

Mancini, R.A., Hunt, M.C., 2005 Current research in meat colour. Meat Sci.71(1), 100-121.

Marono, S., Poccolo, G., Loponte, R., Parisi, G., Nizza, S., Lanonisi, V., Gasco, L. & Bovera, F., 2017. Effect of *Tenebrio molitor* larvae meal on growth performance, *in vivo*nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). Anim. Feed Sci. Technol. 226, 12-20.

Marsman, G.J., Gruppen, H., van der Poel, A.F., Kwakkel, R.P., Verstegen, M.W. & Voragen, A.G., 1997. The effect of thermal processing and enzyme treatments of

soybean meal on growth performance, ileal nutrient digestibility, and chyme characteristics in broiler chicks. Poult. Sci. 76, 864-872.

Mehri, M., Sabaghi, V. & Bagherzadeh-Kasmani, F., 2015. *Mentha piperita* (peppermint) in growing Japanese quails' diet: Serum biochemistry, meat quality, humoral immunity. Anim. Feed Sci. Technol. 206, 57-66.

Melillo, A., 2007. Rabbit clinical pathology. J. Exot. Pet. Med. 16, 135-145.

Minvielle, F., 2004. The future of Japanese quails for research and production. World Poult. Sci. J. 60, 500-506.

Muchenje, V., Dzama, K., Chimoyo, M., Strydom, P.E., Hugo, A & Raats, J.G., 2009. Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chem. 112, 279-289.

Naibi, S.A., Zahraddeen, D., Kalla, D.J.U. & Nathaniel C., 2009. Effects of storage length and positioning on hatchability of Japanese quail eggs (*Coturnix coturnix japonica*) in a sub-tropical environment. Proc. 34th Ann. Conf. Nig. Soc. for Anim. Prod. 15th-18thMarch, Uyo. 457-460.

Nain, S., Bour, A., Chalmers, C.& Smits, J.E., 2011. Immunotoxicity and disease resistance in Japanese quail (*Coturnix coturnix japonica*) exposed to malathion. Ecotoxicology, 20(4), 892-900.

Nasar, A., Rahman, A., Hoque, N., Talukder, K.A. & Das, Z.C., 2016. A survey of Japanese quail (*Coturnix coturnix* japonica) farming in selected areas of Bangladesh. Vet. World.9(9), 940-947.

Newton, G.L., Sheppard, D.C. & Burtle, G., 2008. Black soldier fly pre-pupae: a compelling alternative to fishmeal and fish oil. Public comment on alternative feeds for aquaculture, NOAA 15/11//2007 - 29/2/2008.

Newton, L., Sheppard, C., Watson, D., Burtle, G. & Dove, R., 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Annual Report. University of Georgia, College of Agriculture and Environmental Science, Georgia.

Njidda, A.A., Igwebuike, J.U., Ngoshe, A.A. & Tijjani, A.O., 2006. Effect of substituting maize with graded levels of cane molasses on the performance of broiler finisher birds in the semi-arid region of Nigeria. J. Sustain. Agric. Environ. 8(1), 1-13.

NRC, 1994. Nutrient Requirements of Poultry (9th Ed.). National Academy Press, Washington, DC, USA.

Okah, U., Onwujiariri, E.B., 2012. Performance of finisher broiler chickens fed maggot meal as a replacement for fishmeal. J. Agric. Technol. 8, 471-477.

Oluwatomi, O., 2010. Raising Quails: A Beginners Introduction. Tribune; 2010. Retrieved from https://bestfarmanimals.com/the-complete-guide-to-raising-quail/. Accessed 17 July 2019.

OMLET, 2004. History of Quails. Retrieved and accessed on 17 July 2019.https://www.omlet.ie/guide/quails/about_quail/history.

Onsongo, V.O., Osuga, I.M., Gachuiri, C.K., Wachira, A.M., Miano, D.M., Tanga, C.M., Ekesi, S., Nakimbugwe, D. & Fiaboe K.K.M., 2018. Insects for income generation through animal feed: Effect of dietary replacement of soybean and fish meal with

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black soldier fly meal on broiler growth and economic performance. J. Econ. Entomol. 111, 1966-1973.

Onyewuchi, U.U., Offor, I.R. & Okoli, C.F., 2013. Profitability of quail bird and egg production in IMO state. Nig. J. Agric. Food Environ. 9(1), 40-44.

Onyinyechukwu, A.A. Wilfred, S.E. & Ezinwa, M.I., 2017. The haematology and serum biochemistry profile of adult Japanese quail (*Coturnix coturnix japanica*). Not. Sci. Biol. 9(1), 67-72.

Otto, G., Roehe, R., Looft, H., Thoelking, L. & Kalm, E., 2004.Comparison of different methods for determination of drip loss and their relationships to meat quality and carcass characteristics in pigs. Meat Sci. 68(3), 401-409.

Pearincott, J. V., 1960. Changes in the lipid content during growth and metamorphosis of the house fly, Musca domestica *Linnaeus*. J. Cell. Physiol. 55(2), 167-174.

Pethick, D.W., Rowe, J.B. & Tudor, G., 1995. Glycogen metabolism and meat quality. Recent Advances in Animal Nutrition. University of New England, Armidale. 97-102.

Piccolo, G., Laconisi, V., Marono, S., Gasco, L., Loponte, R., Nizza, S., Bovera, F. & Parisi, G., 2017. Effect of *Tenebrio molitor* larvae meal on growth performance, *in vivo* nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). Anim. Feed Sci. Technol. 226, 12-20.

Puspamitra, S., Mohanty, P.K. & Mallik, B.K., 2014. Haematological analyses of Japanese quail (*Coturnix coturnix*) at different stages of growth. Int. Res. J. Biol. Sci. 3(11), 51-53.

Ratiu, T.A., Aderinola, O.A., Akinwumi, A.O., Alab, T.A. & Shittu, M.D., 2013. Performance and blood chemistry of broiler chickens fed *Moringa oleifera* leaf meal. Proc. 18th. Annual Conf. Anim. Sci. Assoc. Nig. 294.

Ravindran V., Hew L. I., Ravindran G.& Bryden W. L., 2005. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. Anim. Sci. 81, 85-97.

Rumpold, B.A. & Schluter, O.K., 2013. Potential and challenges of insects as an innovative source for food and feed production. Inno. Food Sci. Emerg. Technol. 17, 1-11.

Sahin, N., Akdemir, F., Orhan, C., Kucuk, O., Hayirli, A. & Sahin, K., 2008. Lycopene-enriched Quail Egg as Functional Food for Humans. Food Res. Int. 41, 295-300.

Schiavone, M., Cullere, M., DeMarco, M., Meneguz, I., Biasato, S., Bergagna, S., Dezzutto, F., Gai, S., Dabbou, L. & Gasco, L., 2016. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens*) fat in broiler diets: effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality Ital. J. Anim. Sci. 16, 93-100.

Seng, B., Hirayama, K., Katayama-Hirayama, K., Ochiai, S. & Kaneko, H., 2013. Scenario analysis of the benefit of municipal organic-waste composting over landfill, Cambodia. J. Environ. Manage. 114, 216-224.

Sharma, S., Pradhan, K., Satya, S. & Vasudevan, P., 2005. Potentiality of earthworms for waste management and in other uses: A review. J. Anim. Sci. 1, 4-16.

Sheppard, D.C., Newton, G.L., Thompson, S.A. & Savage, S., 1994. A value technology. Bioresour. Technol. 50, 275-279.

Sheppard, D.C., Tomberlin, J.K., Joyce, J.A., Kiser, B.C.& Sumner, S.M., 2002. Rearing methods for the black soldier fly (Diptera: *Stratiomyidae*). J. Med. Entomol. 39(4), 695-698.

Shumo, M., Osuga, I.M., Khamis, F.M., Tanga, C.M., Fiaboe, K.K.M., Subramanian, S., Ekesi, S., van Huis, A. & Borgemeister, C., 2019. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Sci. Rep. 9, 10110.

Sierra, V. &Olivan, M., 2013. Role of mitochondria on muscle cell death and meat tenderization. Recent Pat. Endocr. Metab. Immune Drug Discov. 7, 120-129.

Smith, R. & Pryor, R., 2013. Enabling the exploitation of insects as sustainable source of protein for animal feed and human nutrition. Work package 5: Pro – insect Plata form in Europe, Minerva. pp. 1-24.

Swenson, M.J., 1970. Physiological properties, cellular and chemical constituents of blood. Dukes' physiology of domestic animals (ed.). Cornstock Publishing Associates, Cornell University Press, Ithaca, London. pp. 75-83.

Tapp III, W.N., Yancey, W.S. & Apple, J.K., 2011. How is the instrumental colour of meat measured? Department of Animal Science, University of Arkansas Division of Agriculture, Fayetteville, USA.

Teguia, A. & Beynen, A., 2005. Alternative feedstuffs for broilers in Cameroon. Livest. Res. Rural Dev. 17, 3.

Tingle, F.C., Mitchell, E.R. & Copeland. W.W., 1975. The Black soldier fly (*Hermetia illucens*) in poultry houses in North Central Florida. J. Ga. Entomol. Soc. 10, 179-183.

Tschirner, M.& Simon, A., 2015. Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed J. Insect Food Feed. 1(4), 249-259.

Tuleun, C.D., Adenkola, A.Y. & Afele, T., 2011. Effect of dietary ascorbic acid supplementation on the performance of Japanese (*Coturnix coturnix japonica*) quails in a tropicalenvironment. J. Anim. PlantSci. 10(2), 1268-1275.

Tunsaringkarn, T., Tungjaroenchai, W. & Siriwong, W., 2013. Nutrient benefits of quail (*Coturnix Coturnix japonica*) eggs. Int. J. Sci. Res. 3, 1-8.

Uushona, T., 2015. Black soldier fly (*Hermetia illucens*) pre-pupae as a protein source for broiler production. MSc thesis, University of Stellenbosch, Western Cape, South Africa.

VanWezemael,L., DeSmet, S., Uelandand, Ø.&Verbeke, W., 2014. Relationships between sensory evaluations of beef tenderness, shear force measurements and consumer characteristics. Meat Sci. 97, 310-315.

Vantomme, P., 2017. Creating an enabling environment. Insects as food and feed: From production to consumption, Wageningen Academic Publishers, Wageningen, The Netherlands. pp. 381-397.

Vantomme, P., Mertens, E., Van Huis, A. & Klunder, H., 2012. Assessing potential of insect as food and feed in assuring food security. FAO, Rome, Italy.

Veldkamp, T., Van Duinkerken, G., Van Huis, A., Lakemond, C.M.M., Ottevanger, E., Bosch, G. & Van Boekel, M.A.J.S., 2012. Insects as a sustainable feed ingredient in

pig and poultry diets – a feasibility study. Wageningen UR Livestock Research Report 638.

Washington, I.M. & Van Hoosier, G., 2012. Clinical biochemistry and haematology. University of Washington, Seattle, WA, USA. pp. 57-116.

Yu, L.H., Lee, E.S., Jeong, J.Y., Paik, H.D., Choi, J.H. & Kim, C.J., 2005. Effects of thawing temperature on the physicochemical properties of pre-rigor frozen chicken breast and leg muscles. Meat Sci. 71(2), 375-382.

Zaman, Q., Mushtaq, T., Nawaz, H., Mirza, M., Mahmood, S. Ahmad, T., Babar, M. & Mushtaq, M., 2008. Effect of varying dietary energy and protein on broiler performance in hot climate. Anim. Feed Sci. Technol. 146(3), 302-312.

3 CHAPTER THREE – FEED INTAKE, GROWTH PERFORMANCE AND HAEMATO-BIOCHEMICAL PARAMETERS OF JUMBO QUAILS FED WITH BLACK SOLDIER FLY LARVAE MEAL-CONTAINING DIETS

Abstract

The study was conducted to investigate the effect of substituting soya products with black soldier fly larvae (BSFL) meal on feed intake, growth performance, haematology and serum biochemistry of Jumbo quails for a period of four weeks. Three hundred and fifteen, two-week-old, mixed gender, Jumbo quails (53.79 ± 5.193 g live-weight) were randomly allocated to five isonitrogenous and isoenergetic experimental diets formulated by replacing soy components with BSFL meal as follows: 1) commercial chicken grower diet with no BSFL meal (BSFL0), 2) = commercial chicken grower diet in which soya products components were replaced with BSFL meal at a rate of 2.5% (BSFL25), 5% (BSFL50), 7.5% (BSFL75) and 10% (BSFL100). The experimental diets and fresh water were provided *ad libitum*. Average weekly feed intake (AWFI) and average weekly body weight gain (ABWG) were determined and used to calculate average weekly gain-to-feed ratio (G:F). At 42 days of age, all the quails were slaughtered and blood collected for haemato-biochemical analyses. Repeated measures analysis showed a significant diet × week interaction on average weekly G:F. There were significant linear trends for G:F in week 3 and 5 in response to BSFL levels. Quadratic trends were observed for overall feed intake [y = 605 (±17.56) + 2.1 (±0.82)x - 0.02 (±0.0077)x^2; R^2 = 0.20; P = 0.023]and overall bodyweight gain [y = 155 (±4.70) + 0.57 (±0.22)x - 0.0049 (±0.002)x^2; R^2 = 0.22; P = 0.03] from which an optimum BSFL inclusion level was calculated to be 5.4%. There were neither linear nor quadratic (P> 0.05) trends for blood parameters, except for albumin-to-globulin ratio. It was concluded that BSFL meal has

the potential to replace soya products in Jumbo quail diets at an optimal inclusion level of 5.4% without compromising their productive performance, health status and quality of the meat.

Keywords: Blood parameters; Production; Insect meal; Soybean meal.

3.1 Introduction

Sustainable intensification of poultry production requires the diversification of bird species as well as the use of feed ingredients with lower carbon footprints and higher returns. The Jumbo quail is a recently developed meat type breed that grows very fast and reaches sexual maturity as early as six weeks of age. It was bred to create a larger and meatier version of the Japanese quail (*Coturnix coturnix japonica*). For sustainable intensification of Jumbo quail production, alternative dietary protein sources with lower environmental costs are required (Mungkung *et al.*, 2013). Recently, quails form part of the poultry industry that contributes high quality dietary protein for human consumption (Mulaudzi *et al.*, 2019). In many countries, they are mostly reared for household consumption (Siddique & Mandal, 1996; Ali *et al.*, 2012). Quails are popular among consumers who are conscious about their health and mostly sold at niche markets (Adeoti & Boruwa, 2019). In South Africa, only a few farmers grow quails on a commercial scale, and this may be due to lack of information on quail farming and management. As such, quail farming should be encouraged to increase employment rate and provide income (Nasar *et al.*, 2016). The need to find sustainable and ecologically friendly protein sources other than soybean remains a challenge for researchers and farmers. The immense growth of the human population indicates the need to find alternative protein sources that do not occupy a large space of land as much as plant protein. Soybean meal has been widely used as the major source of dietary protein for poultry diets. This has resulted in a higher quantity demand for soybean, which has subsequently increased

its market price. The inclusion of insect meal larvae such as that of *Hermetia illucens* and *Tenebrio molitor* have proven without doubt its capabilities to be used in place of soybean. Generally, insects are efficient in converting waste products into high nutritive valuable protein (Makkar *et al.*, 2014). According to van Huis *et al.* (2013), the protein content is most abundant in the BSFL larvae than in any other insect species. Insect larvae inclusion has been reported to decrease the incidence of metabolic skeletal disorders in broilers and improve the overall health of birds (Pieterse, 2014). The effect of *Tenebrio molitor* and *Hermetia illucens* meals on broiler production and health has been reported by Oyegoke *et al.* (2016) and Biasato *et al.* (2017). A feeding trial by De Marco *et al.* (2015) of broiler chickens fed BSFL meal at an inclusion level of 15 and 25% showed no dietary effect on overall feed intake and meat quality traits suggesting that the inclusion of the BSFL meal can be a potential animal protein source to replace SBM. Also, Moula *et al.* (2017) demonstrated that the inclusion of BSFL meal at a rate of 8% improved growth performance of free range chickens. This study was, therefore, designed to determine the optimum inclusion level of black soldier fly larvae protein as a partial replacement for soya products using growth performance, haematology and serum biochemistry, of Jumbo quails as response indicators.

3.2 Material and methods

The rearing and slaughter procedures of the quails were approved by the Animal Research Ethics Committee of the North-West University (approval no. NWU-00483-18-A5) according to established guidelines for use and care of research animals.

3.2.1 Description of study site

The feeding trial was conducted at the North-West University Molelwane farm (25°40.459′S, 26°10.563′E), in the North West province of South Africa. The study was carried out during winter season when ambient temperatures ranged between -3°C and 25°C. Infrared lamps

were used to provide warmth to the quails. Rearing was done under natural lighting (12 hours of daylight).

3.2.2 Ingredient sources

Dried and defatted black soldier fly larvae meal was acquired from AgriProtein (PTY) LTD, a feed manufacturing company located in Cape Town (Western Cape, South Africa). According to the company, the BSFL meal is produced on organic waste before undergoing quality control checks. The chemical composition of BSFL meal was quoted by the supplier as 900 g/kg dry matter, 500 g/kg crude protein, 130 g/kg crude fibre and 100 g/kg crude fat. Soya products (soya oilcake and full-fat soya) and all other ingredients were purchased from Optifeeds (PTY) LTD situated in Lichtenburg (North-West, South Africa).

3.2.3 Diet formulation

The BSFL meal was milled (2 mm; Polymix PX-MFC 90 D) prior to blending with other dietary ingredients. The experimental diets were formulated to be isonitrogenous and isoenergetic by replacing the soya products with BSFL meal as follows: 1) BSFL0 = commercial grower diet with no BSFL meal inclusion, 2) BSFL25 = commercial grower diet in which 25 g/kg of soya products was replaced with BSFL meal, 3) BSFL50 = commercial grower diet in which 50 g/kg of soya products was replaced with BSFL meal, 4) BSFL75 = commercial grower diet in which 75 g/kg of soya products was replaced with BSFL meal, and 5) BSFL100 = commercial grower diet in which 100 g/kg of soya products was replaced with BSFL meal (Table 3.1).

Table 3.1. Ingredients composition (g/kg, *as fed* basis) of experimental diets

	[1]Diets				
Ingredients	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100
BSFL meal	0.0	25.0	50.0	75.5	100.0
Soya Oilcake	193.0	201.0	149.0	142.0	92.0
Full-Fat Soya	74.0	15.0	15.0	15.0	15.0
Sunflower Oilcake	25.0	31.0	71.0	64.0	100.0
Lysine	2.65	2.44	2.42	1.73	1.41
Methionine	2.44	2.34	2.11	1.92	1.68
Threonine	0.52	0.55	0.53	0.39	0.30
Yellow maize	661.0	681.0	669.0	660.0	631.0
Wheaten bran	10.0	10.0	10.0	10.0	30.0
Feed lime	10.4	10.4	10.5	10.8	10.6
[2]MDCP	12.7	12.2	11.4	10.4	10.0
Salt-Fine	3.40	3.27	3.06	3.7	3.01
Sodium bicarbonate	1.51	1.57	1.74	0.40	1.56
Axtra Phy 10000 P	1.0	1.0	1.0	1.0	1.0
Hemicell® (β-mannanase)	3.0	3.0	3.0	3.0	3.0
Salinomycin (12%)	0.50	0.50	0.50	0.50	0.50
Zinc bacitracin (15%)	0.50	0.50	0.50	0.50	0.50
Premix no spec	2.5	2.5	2.5	2.5	2.5

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae.
[2]MDCP = Mono-dicalcium phosphate.

3.2.4 Chemical analyses

Subsamples of the formulated diets (BSFL0, BSFL25, BSFL50, BSFL75 and BSFL100) were analysed using the AOAC international methods (AOAC, 2005) for laboratory dry matter (method no. 930.15), ash (method no. 924.05), crude fibre (method no. 978.10), crude fat (method no. 920.39), and crude protein (method no. 984.13). Metabolisable energy (ME) contents were predicted using the near infrared reflectance spectroscopy (NIRs) SpectaStar XL (Unity Scientific, Australia). Mineral content levels (Calcium (Ca), Phosphorus (P), Sodium (Na), Chlorine (Cl) and Potassium (K) were determined using the atomic ICP spectrophotometer (AAS – Buck 205) as guided by Agri Laboratory Association of Southern Africa guidelines (AgriLASA, 1998).

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

	[1]Diets				
	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100
Dry matter	900.00	899.8	901.0	895.4	904.0
Ash	51.1	50.1	49.2	49.5	48.9
[2]ME (MJ/Kg)	120.4	120.4	120.4	120.4	120.4
Crude protein	190	190	190	190	190
Crude fat	44.2	40.1	45.4	50.6	56.5
Crude fibre	40.9	42.1	49.2	48.1	56.5
Calcium	7.9	7.9	7.9	7.9	7.9
Phosphorus	5.5	5.6	5.8	5.9	6.0
Sodium	1.8	1.8	1.8	1.8	1.8
Chlorine	3.0	3.0	3.0	3.0	3.0
Potassium	7.2	7.1	7.1	6.7	7.5
Lysine	11.3	11.5	11.6	11.8	12.0
[3]M + C	8.4	8.5	8.5	8.6	8.7
Threonine	7.4	7.4	7.4	7.5	7.6
Tryptophan	2.0	2.0	2.0	2.0	2.0

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae.

[2]ME: Metabolisable energy.

[3]M + C = Methionine + Cysteine

3.2.5 Experimental design and bird management

A total of 315, day-old mixed gender, Jumbo quails were purchased from a Quail Breeder in Valhala (Gauteng, South Africa). The quails were reared using a commercial chicken starter mash purchased from Nutrifeeds (PTY) LTD, which is located in Mafikeng, (North West, South Africa) until one week of age. The five dietary treatments were randomly allocated to 35 pens (experimental units) and were replicated seven times per dietary treatment with each replicate pen (100 cm long × 60 cm wide and 30 cm high) carrying nine quails. The pens had wire mesh floor but without bedding. On average the temperature and humidity inside the poultry house during the experimental period was 30°C and 40%, respectively. The birds were then adapted to the dietary treatments for one week before the commencement of measurements at two weeks of age. For the first two weeks, the quails were reared under continuous lighting via infrared lamps to provide warmth because at this stage they are sensitive to cold.

3.2.6 Feed intake and growth performance

Average weekly feed intake (AWFI) per bird was measured by subtracting the weight of the feed refused from that offered and dividing the difference by the total number of quails in the pen. At two weeks of age, initial live-weights of the birds were measured (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA) and subsequently weighed weekly per pen until they were six weeks of age (final weight). The live weights obtained weekly were used to calculate the average weekly body weight gain (ABWG) per bird as follows:

$$ABWG\ (t_{\circ}, T) = \frac{W(T) - W(t_{\circ})}{T - t_{\circ}}$$

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

3.2.7 Slaughter procedure and blood collection

At 42 days of age, all the quails were transported to Rooigrond poultry abattoir (North West, South Africa) for slaughter. Upon arrival, the quails were electrically stunned and slaughtered by cutting the jugular vein using a sharp knife and left hanging until bleeding ceased. While bleeding, four mL of blood was collected from two randomly selected birds per pen using syringes and immediately transferred into two sets of sterilized tubes.

3.2.8 Blood analysis

For haematological analysis, tubes containing ethylenediaminetetraacetic acid were used during blood collection. Haematological parameters: haemoglobin, white blood cells (WBC), heterophils, lymphocytes, monocytes and eosinophils were determined using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc., Maine, US). Clotted blood was centrifuged at 1000 *g* for 15 minutes to generate serum for biochemical analysis (Washington & van Hoosier, 2012). Albumin-to-globulin ratio (ALB/GLOB), albumin, alkaline phosphate (ALKP), alanine transaminase (ALT), amylase, calcium, creatinine, globulin, glucose, lipase, phosphorus, total bilirubin, total protein and urea were analysed using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc. Maine, US).

3.2.9 Statistical analysis

Feed intake, growth performance and haemato-biochemistry data were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis (Proc

RSREG; SAS, 2010) was used to determine the optimum inclusion level of BSFL for Jumbo quails using the following quadratic model:

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

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

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 employed:

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

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.

Overall feed intake, overall body weight gain, blood parameters data were analysed using the general linear model procedure of SAS (2010). The linear statistical model employed was as follows:

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

Where Y_{ij}= dependent variable, μ = population mean, D_i = effect of diets, and E_{ij} = random error associated with observation ij, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P \leq 0.05$. Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

3.3 Results

3.3.1 Feed intake and growth performance

Repeated measures analysis showed a significant diet × week interaction for G:F but not for AWFI and ABWG. Table 3.3 shows that there were significant linear trends for G:F in week 3[y = 0.40 (±0.016) - 0.0009 (±0.00077)x; R^2 = 0.159; P = 0.047] and week 5[y = 0.189 (±0.0217) + 0.0023 (±0.00101)x; R^2 = 0.290; P = 0.011] in response to BSFL levels. However, there were neither linear nor quadratic effects (P>0.05) for G:F in weeks 4 and 6. For the entire duration of the study, there were no significant dietary influences on G:F of Jumbo quails.

Table 3.3. Average weekly gain-to-feed ratio in Jumbo quails fed with diets containing the black soldier fly larvae meal as a partial replacement of soya products

	[1]Diets						Significance	
	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100	[2]SEM	Linear	Quadratic
Week 3	0.396	0.386	0.357	0.347	0.352	0.015	0.047	0.572
Week 4	0.345	0.359	0.340	0.340	0.356	0.012	0.533	0.888
Week 5	0.235	0.261	0.274	0.277	0.281	0.029	0.011	0.117
Week 6	0.186	0.125	0.129	0.150	0.131	0.025	0.312	0.322

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae meal inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae meal, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae meal.
[2]SEM: Standard error of the mean.

Table 3.4. Overall feed intake (g/bird) and overall body weight gain (g/bird) of Jumbo quails fed with the black soldier fly meal in place of soya products

	[1]Diets						Significance	
	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100	[2]SEM	Linear	Quadratic
Overall FI	598.9	651.3	650.7	656.0	627.7	15.54	0.543	0.023
Overall BWG	151.2	170.0	166.7	168.1	164.3	5.094	0.263	0.026

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae meal inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae meal, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae meal.

[2]SEM: Standard error of the mean.

There were no significant linear effects for overall feed intake and overall weight gain (Table 3.4.). However, there were significant quadratic trends for overall feed intake [y = 605 (±17.56) + 2.1 (±0.82)x - 0.02 (±0.0077)x^2; R^2 = 0.20; P = 0.023] and overall BWG [y = 155 (±4.70) + 0.57 (±0.22)x - 0.0049 (±0.002)x^2; R^2 = 0.22; P = 0.03], from which an optimum BSFL inclusion level was calculated to be 5.4%. There were no dietary effects (P>0.05) on overall feed intake and overall weight gain of Jumbo quails (Table 3.4).

3.3.2 Haematological and serum biochemical parameters

Table 3.5 shows that there were no significant linear and quadratic effects for haemoglobin (10.82 - 12.18 g/dL), white blood cells (22.23 - 41.81 $\times 10^9$/L), heterophils (3.60 - 9.90$\times 10^9$/L), lymphocytes (16.37 - 22.63 $\times 10^9$/L), monocytes (2.12 - 9.54 $\times 10^9$/L) and eosinophils (0.28 - 0.61 $\times 10^9$/L) in response to black soldier fly meal inclusion level. No

dietary effects (P>0.05) were observed on all the haematological parameters of Jumbo quails (Table 3.5).

Table 3.5. The effect of black soldier fly larvae meal-containing diet on haematological parameters of six-week old Jumbo quails

	[1]Diets						Significance	
Parameters	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100	[3]SEM	Linear	Quadratic
Haemoglobin (g/dL)	10.82	12.31	11.31	12.14	12.18	0.942	0.442	0.918
[2]WBC ($\times 10^9$/L)	31.33	30.98	30.22	41.81	22.23	10.14	0.475	0.074
Heterophils ($\times 10^9$/L)	4.22	5.93	4.38	9.90	3.60	1.975	0.474	0.165
Lymphocytes ($\times 10^9$/L)	17.96	19.88	22.63	21.66	16.37	5.294	0.562	0.077
Monocytes ($\times 10^9$/L)	8.67	4.58	2.84	9.54	2.12	4.370	0.562	0.290
Eosinophils ($\times 10^9$/L)	0.41	0.46	0.28	0.61	0.41	0.181	0.633	0.955

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae meal inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae meal, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae meal.

[2]WBC = white blood cells.

[3]SEM: Standard error of the mean.

Table 3.6 shows that there were no significant linear and quadratic effects of serum biochemical parameters, except for albumin to globulin ratio[y = 0.39 (±0.065) + 0.008 (±0.0031)x- 0.00007 (±0.000027)x^2; R^2 = 0.337; P = 0.030], which showed a quadratic trend

in response to BSFL meal inclusion levels. There were no dietary effects (P >0.05) observed on all the serum biochemical parameters of Jumbo quails (Table 3.6).

Table 3.6. The effect of black soldier fly larvae meal-containing diet on serum biochemical parameters of six-week old Jumbo quails

	[1]Diets						[4]Significance	
[2]Parameters	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100	[3]SEM	Linear	Quadratic
ALB/GLOB	0.37	0.60	0.55	0.45	0.45	0.05	0.878	0.030
Albumin (g/L)	29.00	23.25	26.00	20.83	19.64	5.75	0.398	0.337
ALKP (U/L)	602.5	414.2	500.0	40.0	511.7	240.6	0.428	0.121
ALT (U/L)	46.50	60.08	14.83	245.00	19.64	67.21	0.903	0.709
Amylase (U/L)	410.5	316.9	357.7	131.8	294.3	81.53	0.515	0.781
Calcium (mmol/L)	3.83	3.04	3.22	3.38	3.45	0.40	0.821	0.114
Creatinine (µmol/L)	33.25	19.92	40.90	30.17	12.36	15.66	0.644	0.881
Globulin (g/L)	59.00	37.00	50.10	41.50	47.00	14.62	0.291	0.129
Glucose (mmol/L)	23.84	12.30	17.23	26.22	20.02	4.81	0.703	0.535
Lipase (U/L)	181.1	263.4	172.4	190.0	199.8	59.65	0.757	0.965
Phosphorus (mmol/L)	4.50	4.82	4.59	4.20	4.34	0.58	0.595	0.560
Bilirubin (µmol/L)	22.75	11.70	14.50	12.43	10.07	6.79	0.153	0.392
Total protein (g/L)	95.00	68.42	76.00	100.00	66.79	14.74	0.221	0.235
Urea (mmol/L)	1.240	0.975	1.210	0.833	0.629	0.40	0.976	0.884

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae meal inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae meal, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae meal.

[2]Parameters: ALBGLOB = albumin/globin, ALKP = alkaline phosphate, ALT = alanine aminotransferase.

[3]SEM: Standard error of the mean.

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3.4 Discussion

3.4.1 Feed intake and growth performance

Limited data is available in literature on the use of insect meal specifically BSFL on physiological parameters of Jumbo quails. The inclusion of the BSFL meal in diets has been extensively investigated on fish and broiler chickens (Bovera *et al.*, 2015; Iaconisi *et al.*, 2017; Cummins *et al.*, 2017; Marono *et al.*, 2017). In this study, the inclusion of BSFL had no effect on overall feed intake and overall weight gain on Jumbo quails. However, repeated measures analyses showed a significant diet and week interaction effect on G:F, indicating that the efficiency of the birds in converting the dietary treatments into body mass depended on their age. In this study, the optimum inclusion level for the BSFL meal was calculated to be 54 g/kg, revealing that substituting soya products with BSFL meal at higher rates depresses overall feed intake and body weight gain. Indeed, at three weeks of age, G:F linearly decreased with BSFL meal levels, confirming that higher inclusion levels reduced the feed conversion efficiency. This could be due to the presence of chitin in the larvae, a fibrous substance, which is reported to reduce nutrient utilization and digestibility (Bovera *et al.*, 2015). Indeed, Marono *et al.* (2017) reported a decrease in feed intake in laying hens fed BSFL meal. However, Cullere *et al.* (2016) reported that overall performance of Japanese quails reared on BSFL meal was comparable to the ones fed de-fatted soybean meal and soybean oil. Similarly, a feeding trial conducted by Maurer *et al.* (2016) with Leghorn laying hens where SBM was partly substituted with de-fatted BSFL meal at inclusion levels of 50 and 100% showed no significant dietary effects.

3.4.2 Haematology and serum biochemical parameters

Haematology and blood chemistry values reflect the health condition of an organism (Haynes & Skidmore, 1991). Moreover, Sparling *et al.* (1999) reported that haematological parameters

values could vary depending on age, season, gender, nutrition, bacterial and viral infections. There is a dearth of information on blood indices of Jumbo quails fed BSFL meal. In this current study, the inclusion of the BSFL meal did not have any negative effect on the blood parameters. There were also no dietary influences observed on all blood parameters, suggesting that adding BSFL meal promoted similar health status as the soya products containing control diet in Jumbo quails. According to Piotrowska *et al.* (2011), total serum protein and albumin are the two most important parameters used to assess the nutritional status and body condition of birds. Total protein plays a vital role in platelet adhesion and coagulation and is also responsible for haemostasis maintenance, whereas albumin plays a key role in the maintenance of intravascular colloid osmotic pressure in the liver (Filipovic *et al.*, 2007). In this study, both these parameters were similar across all the dietary treatments indicating that the health status of the quails was not compromised. A quadratic response was observed for ALB/GLOB ratio, indicating that at higher inclusion levels the BSFL may compromise autoimmunity of the quails and cause plasma cell disorders. Indeed, Griminger and Scanes (1986) reported that low ALB/GLOB ratio is an indicator of low immune response for the birds. The fact that the ALB/GLOB initially increased and then declined at even higher inclusion levels of BSFL meal confirms that its inclusion must be capped at 54 g/kg to avoid overproduction of globulins, which may cause autoimmune diseases. Nonetheless, no dietary influences were observed for all blood parameters including the liver enzymes (ALKP and ALP), suggesting that partial replacement of soya products with BSFL meal did not compromise the health status of the birds. Furthermore, the haemato-biochemical values reported in this study fell within the normal ranges reported for quails (Mnisi & Mlambo, 2017; Mulaudzi *et al.*, 2019). In addition, several studies also revealed no significant dietary effects on blood parameters of broilers (Bovera *et al.*, 2015), laying hens

(Marono *et al.*, 2017) and Barbary partridge (Loponte *et al.*, 2017) when fed with a variety of insect meals.

3.5 Conclusion

The optimal inclusion level was calculated to be 54 g/kg. The partial replacement of soybean meal with BSFL meal did not compromise the production performance and the health status of the Jumbo quails proving that it can be used as an alternative protein source. What may limit the contribution of BSFL to sustainable intensification of the Jumbo quail is the possible reduction in bird performance at inclusion rates beyond 54 g/kg. The anti-nutritional effects of chitin most likely impose this upper limit in the tolerance level of BSFL. The use of the enzyme chitinase may allow for the inclusion of higher levels of BSFL meal in Jumbo quail diets.

3.6 References

Adeoti, S.O. & Baruwa, O.I., 2019. Profitability and constraints of quail egg production in Southwestern Nigeria. Obatemi Awolowo University, Osun State, Nigeria. J. Exp. Agric. Int. 33(3), 1-10.

AgriLASA, 1998. Feed and Plant Analysis Methods. Agri Laboratory Association of Southern Africa, Pretoria, South Africa.

Ali, M.A., Hmar, L., Devi, L.I., Prava, M., Lallianchhunga, M.C. & Tolenkhomba, T.C., 2012. Effect of age on the haematological and biochemical profile of Japanese quails. Int. Multidiscip. Res. J. 2(8), 32-35.

AOAC, 2005. Official Methods of Analysis of AOAC International (16th Ed). Association of Official Analytical Chemists, Arlington, VA, USA.

Biasato, I., Gasco, L., Marco De M., Rennab, M., Rotolob, L., Dabboub, S., Capucchioa, M.T., Biasibettia, I.E., Tarantolaa M., Bianchia, C., Cavallarinc, L., Gai, F., Pozzo, L., Dezzutto, D., Bergagna S. & Schiavone, A., 2017. Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. Anim. Feed. Sci. Technol. 234, 253-263.

Bovera, F., Piccolo, G., Gasco, L., Marono, S., Loponte, R., Vassalotti, G., Mastellone, V., Lombardi, P., Attia, Y.A. & Nizza, A., 2015. Yellow mealworm larvae (*Tenebrio molitor)* as a possible alternative to soybean meal in broiler diets. Brit. Poult. Sci. 56, 569-575.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S. & Dalle Zotte, A., 2016. Black soldier fly as dietary protein source for broiler quails: Apparent

digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal, 10(12), 1923-1930.

Cummins, V.C., Jr, Rawles, S.D., Thompson, K.R., Velasquez, A., Kobayashi, Y., Hager, J. & Webster, C.D., 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture, 473, 337-344.

De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L. & Schiavone, A., 2015. Nutritional value of two insect meals (*Tenebrio molitor* and *Hermetia illucens*). Anim. Feed Sci. Technol. 209, 211-218.

Filipovic, N., Stojeviae, Z., Milinkoviae-Tur, S., Ljubiae, B.B. & Zdelar-Tuk, M., 2007. Changes in concentration and fractions of blood serum proteins of chickens during fattening. Vet. Archive. 77, 319-326.

Griminger, P. & Scanes, C.J., 1986. Protein metabolism in Avian Physiology.4th Ed, New York, US.

Haynes, J.I. & Skidmore, G.W., 1991. Haematology of the dasyurid marsupials*Sminthopsis crassicaudata* and *Sminthopsis macroura*.Aust. J. Zool. 39, 157-169.

Iaconisi, V., Marono. S., Parisi, G., Gasco, L. & Genovese, L., 2017. Dietary inclusion of *Tenebrio molitor* larvae meal: Effects on growth performance and final quality treats of blackspot sea bream (*Pagellus bogaraveo*). Aquaculture, 476, 49-58.

Loponte, R., Nizza, S., Bovera, F., Riu De, N., Fliegerova, K., Lombardi, P. & Moniello, G., 2017. Growth performance, blood profiles and carcass traits of Barbary partridge

(*Alectoris barbara*) fed two different insect larvae meals (*Tenebrio molitor* and *Hermetia illucens*). Res. Vet. Sci. 115, 183-188.

Makkar, G., Tran, G., Heuze, V. & Ankers, P., 2014. State of the art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1-33.

Marono, S., Loponte, R., Lombardi, P., Vassalotti, G., Pero, M.E., Russo, F., Gasco, L., Parisi, G., Piccolo, G., Nizza, S., Di Meo, C., Attia, Y.A. & Bovera, F., 2017. Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 week of age. Poult. Sci. 96, 1783-1790.

Maurer, V., Holinger, M., Amsler, Z., Früh, B., Wohlfahrt, J., Stamer, A. & Leiber, F., 2016. Replacement of soybean cake by *Hermetia illucens* meal in diets for layers. J. Insects Food Feed.2, 89-90.

Mnisi, C.M. & Mlambo, V., 2017. Growth performance, haematology, serum biochemistry and meat quality characteristics of Japanese quails (*Coturnix coturnix japonica*) fed canola meal-based diets. Anim. Nutr. 4(1), 34-43.

Moula, N., Scippo, M.L., Douny, C., Degand, G., Dawans, E., Cabaraux, J.F., Hornick J.L., Medigo, R.C., Leroy, P., Francis, F. & Detilleux, J., 2017. Performances of local poultry breed fed black soldier fly larvae reared on horse manure. Anim. Nutri. 4(1), 73-78.

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2019. Dietary *Moringa oleifera* leaf meal improves growth performance but not haemo-biochemical and meat quality parameters in female Japanese quails. Pak. J. Nutr. 18(10), 953-960.

Mungkung, R. & Aubin, J., 2013. Life cycle assessment for environmentally sustainable aquaculture management: a case study of combined aquaculture systems for carp and tilapia. Int. J. Life Cycle Assess. 11(1), 55-59.

Nasar, A., Rahman, A., Hoque, N., Takukder, A.K. & Das, Z.C., 2016. A survey of Japanese quail (*Coturnix coturnix japonica*) farming in selected areas. Banglesh. Vet. World. 9(9), 940-947.

Oyegoke, O.O., Akintola, A.J. & Fasoranti, J.O., 2006. Dietary potentials of the edible larvae of *Cirinaforda* (westwood) as a poultry feed. Afr. J.Biotechnol. 5, 1799-1802.

Pieterse, E., 2014. Comparison of the production parameters, carcass and meat quality characteristics and gut parameters of broiler chicks grown on a diet containing either dried larvae and pre-pupae meal of the black soldier fly (*Hermetia illucens*) and larvae meal of the copper bottom blow fly (*Chrysomya chloropyga)* or soya as the main protein source. Internal report to Agriprotein. pp. 39.

Piotrowska, A., Burlikowska, K. & Szymeczko, R., 2011. Changes in blood chemistry in broiler chickens during the fattening period. Folia Biol. Krakow. 59(3-4), 183-187.

Prabakaran, R., 2003. Good practices in planning and management of integrated commercial poultry production in South Asia. Food and Agricultural Organization of the United Nations. Rome, Italy. pp. 97.

Siddique, S.A. & Mandal, M.A., 1996. Economics of Japanese quail farming in Dhaka metropolitan city. Banglesh. J. Agric. Econs. 19, 71-84.

Sparling, D.W., Dag, D. & Klein, P., 1999. Acute toxicity and sub lethal effects of white phosphorous in mute swans, *Cygnusolor*. Arch. Environ. Con. Tox. 36, 316-322.

Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. & Vantomme, P., 2013. Edible insects-Future prospects for food and feed security. FAO Forestry Paper 171.Rome, Italy.

Washington, I.M. & Van Hoosier, G., 2012. Clinical biochemistry and haematology. University of Washington, Seattle, WA, USA. pp. 57-116.

4 CHAPTER FOUR – SIZE OF INTERNAL ORGANS, CARCASS TRAITS AND MEAT QUALITY PARAMETERS OF JUMBO QUAILS FED WITH BLACK SOLDIER FLY LARVAE MEAL-CONTAINING DIETS

Abstract

The objective of this study was to evaluate the size of internal organs, carcass characteristics and meat quality parameters of Jumbo quails fed on diets containing incremental levels of black soldier fly larvae meal over a four-week feeding trial. The experimental diets were formulated to be isonitrogenous and isoenergetic by replacing the soya products with BSFL meal as follows: 1) BSFL0 = commercial grower diet with no BSFL meal inclusion, 2) BSFL25 = commercial grower diet in which 25 g/kg of soya products was replaced with BSFL meal, 3) BSFL50 = commercial grower diet in which 50 g/kg of soya products was replaced with BSFL meal, 4) BSFL75 = commercial grower diet in which 75 g/kg of soya products was replaced with BSFL meal, and 5) BSFL100 = commercial grower diet in which 100 g/kg of soya products was replaced with BSFL meal. The quails were fed on the experimental diets until slaughter at 42 days of age. The slaughtering procedure was as described in the Chapter Three. After slaughter, the carcasses were taken to the Animal Science laboratory of the North-West University for determination of size of internal organs, carcass characteristics and meat quality parameters. No linear effects on size of internal organs were observed, except for empty gizzard weight, which linearly increased [y = 0.054(±0.004) x + 2.28 (±0.085); R^2 = 0.179; P = 0.037] with BSFL levels. There were significant quadratic effects for large intestine [y = 1.38 (±0.077) + 0.0074 (±0.0036) x - 0.000073 (± 0.0000337) x^2; R^2 = 0.159; P = 0.040], and breast weights [y = 21.9 (±0.637) + 0.0539 (±0.0299) x - 0.00058 (±0.000278) x^2; R^2 = 0.168; P = 0.046] in response to BSFL levels. There were neither linear nor quadratic trends for meat quality parameters in response

to dietary BSFL levels. The dietary treatments had no influence on internal organs, carcass characteristics and meat quality traits of Jumbo quails. It can be concluded that partial replacement of soybean component with BSFL meal does not negatively affect meat quality traits.

Keywords: *Hermetia illucens*, Protein source, Meat quality, Internal organs, Carcass traits

4.1 Introduction

The global demand for meat has increased from 1961 to 2013 from 70 million tons to 320 million tons (Ritchie & Roser, 2019). According to Ryna *et al.* (2011), meat is a valuable source of proteins, amino acids, vitamins, minerals and fats required for human growth. The inevitable changes in the meat industry brought by the increased demand for meat and its by-products has put pressure on food producers and researchers to find alternative means to increase the supply of quality meat products that are free from prophylactic antibiotic growth promoters (Tao & Peng, 2014). Quail farming shows a great potential to supply high quality dietary protein for human consumption. Moreover, it has been recognized as part of the solution to eradicate problems related to food insecurity and malnutrition (Nchinda *et al.*, 2011). Quail meat is of high quality (Tunsaringkarn *et al.*, 2013) while the birds themselves are resistant to several avian diseases thus leading to a reduction in required antibiotics use. Moreover, Ayyub *et al.* (2014) demonstrated that the quality of quail meat is similar to that of chicken and even more nutritious. Attributes of meat such as pH, water-holding capacity, texture, oxidative stability and sensory evaluation are considered the most important characteristics of meat quality that influence the meat industry and consequently consumer acceptance (Lawrie *et al.*, 2006). For quails to reach their optimal performance a game-bird diet is recommended (Mnisi *et al.*, 2017) but such diets are very expensive and not affordable for resource-poor farmers. For this reason, quail farmers tend to resort to conventional

chicken diets to reduce production costs. One potential source of protein is the black soldier fly larvae, which has been shown to improve meat quality and meet the protein requirements for quails (Cullere *et al.*, 2019), Barbary partridges (Secci *et al.*, 2018), and broiler chickens (Schiavone *et al.*, 2019). This study was, therefore, designed to evaluate the effect of replacing soya products components with incremental levels of BSFL on sizes of internal organs, carcass characteristics and meat quality parameters of Jumbo quails. It was hypothesized that that size of internal organs, carcass characteristics and meat quality parameters in Jumbo quails vary in response to incremental levels of BSFL meal.

4.2 Material and methods

4.2.1 Study site, diet formulation and experimental design

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

4.2.2 Feeding trial and slaughter procedure

The feeding trial as well as the slaughter procedure were also as described in Chapter 3.

4.2.3 Measurements of carcass traits and internal organs

Immediately after slaughter and for each replicate treatment group, the carcasses were labelled and placed in tagged transparent plastic bags. The carcasses were immediately weighed (Explorer EX224, 0.01 g readability (2 decimal places), supplied by OHAUS Corp, Parsippany, NJ, USA) to determine hot carcass weight (HCW). After chilling for 24 hours, the carcasses were reweighed to determine cold carcass weights (CCW). Thereafter, dressing percentage was calculated as the proportion of HCW on slaughter weights. The weights of the livers, hearts, proventriculi, full and empty gizzards, wings, drumsticks, thighs, breasts small and large intestine were measured using the aforementioned digital weighing scale and

expressed as the proportion of HCW. The lengths of the small and large intestines were determined using a tape measure.

4.2.4 Meat quality attributes

4.2.4.1 Meat pH and temperature

To determine post-mortem pH and temperature, the measurements were done on the pectoralis major muscle using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) as guided by Stanford *et al.* (2003). The pH meter was calibrated after every 10 measurements with standard solutions (Ingold Messtechnik AG, Udorf, Switzerland).

4.2.4.2 Meat colour

The colour (L^* = Lightness, a^* = Redness and b^* = Yellowness) of the meat was determined according to the Commission International De I' Eclairage (CIE, 1976) using a Minolta colour-guide (BYK – Gardener GmbH, Geretsried, Germany), on a 20 mm diameter measurement area and illuminant D65-day light, and at a 10°C observation angle. The colour meter was calibrated using the Zero and White standard before measurements. Colour recording was done on the thigh area. According to Priolo *et al.* (2002), hue angle was calculated as $tan\ \theta = \frac{a*}{b*}$and Chroma values were calculated as $\sqrt{a^{*2} + b^{*2}}$.

4.2.4.3 Cooking losses and shear force

Cooking loss was determined using triplicate breast samples from each replicate treatment group. The breast samples were weighed (initial weight) and then cooked in an oven set at 130 °C for 20 minutes to attain cooked weight (cooked weight). The percentage cooking loss was calculated as follows:

$$Cooking\ losses\ (\%) = \frac{initial\ weight - cooked\ weight}{initial\ weight} \times 100$$

The cooked breast samples were then sheared using a Warner-Bratzler shear device mounted on a Universal Instron apparatus (cross head speed = 200 mm/minute, one shear in the centre of each core) of a Texture Analyser (TA XT plus, Stable Micro Systems, Surrey, UK) to determine the average shear force of each sample, expressed in Newtons.

4.2.4.4 Water holding capacity and drip loss

Water holding capacity (WHC) was determined using the filter paper press method developed by Grau and Hamm, (1957) where triplicate samples per replicate treatment group were cut from the pectoral major muscle (~10 g; w1) and were held under pressure (60 kg of pressure; w2). The WHC was calculated using the following formula:

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

For drip loss measurements, triplicate breast meat samples from each replicate treatment group were sliced with a knife into strips (~3 g; *w1*) and thereafter sewed on a flat cardboard using wool and hung on top of the cold room (4°C) sealing so that the samples did not touch the cardboard. After 72 hours, the samples were gently removed and re weighed (*w2*). The percentage drip loss was calculated according to the following formula:

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

4.2.5 Statistical analysis

Results were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis (Proc RSREG; SAS, 2010) was used to determine the optimum inclusion level of BSFL for Jumbo quails based on the following quadratic model:

$$y = ax^2 + b + c,$$

Where y = dependent variable, *a* and*b* are the coefficients of the quadratic equation, c is dietary BSFL levels and *–b/2a* is the *x* value for optimal response.

Data on internal organs, carcass characteristics and meat quality data were analysed using the GLM procedure of SAS (2010). The linear statistical model employed was as follows:

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

Where, Y_{ij}= dependent variable, μ = population mean, D_i = effect of diets, and E_{ij} = random error associated with observation *ij,* assumed to be normally and independentlydistributed. For all statistical tests, significance was declared at $P \leq 0.05$. Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

4.3 Results

Table 4.1 shows that there were no significant linear effects for liver, large intestine (LI), small intestine (SI), proventriculi, heart, full gizzard, except for empty gizzard in response to dietary levels of BSFL. The size of empty gizzard linearly increased[y = 0.05 (±0.004) x + 2.3 (±0.085); R^2 = 0.18; P = 0.04] with BSFL levels. Similarly, there were no significant

quadratic effects for the aforementioned internal organs, with the exception of large intestine [$y = 1.4\ (\pm 0.077) + 0.0074\ (\pm 0.0036)\ x - 0.000073\ (\pm\ 0.0000337)\ x^2$; $R^2 = 0.16$; $P = 0.04$].

Table 4.1. Internal organs (g / 100 g HCW, unless stated otherwise) of six weeks old Jumbo quails (n = 9±1) fed with black soldier fly larvae meal-containing diets

	[1]Diets						Significance	
Parameters	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100	[2]SEM	Linear	Quadratic
Liver	2.90	3.03	2.98	3.20	3.12	0.162	0.252	0.645
Large intestine	1.37	1.52	1.57	1.43	1.42	0.072	0.881	0.040
Small intestine	3.69	3.84	4.00	4.11	3.87	0.159	0.242	0.155
Proventriculi	0.58	0.52	0.64	5.59	0.55	2.470	0.550	0.463
Heart	1.15	1.17	1.22	1.06	1.14	0.055	0.507	0.703
Full gizzard	3.00	3.11	3.22	2.87	3.23	0.161	0.627	0.877
Empty gizzard	2.32	2.38	2.45	2.52	2.52	0.082	0.037	0.445
Large intestine (cm)	10.46	11.62	10.43	10.45	9.97	0.723	0.351	0.474
Small intestine (cm)	52.64	55.57	53.67	48.40	55.45	3.867	0.896	0.609

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae meal inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae meal, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae meal.

[2]SEM: Standard error of the mean.

Table 4.2 shows that there were no significant linear and quadratic trends for all carcass characteristics, slaughter weight and dressing percentage except for breast weight, which showed a quadratic response [$y = 21.9\ (\pm 0.637) + 0.054\ (\pm 0.0299)x - 0.00058\ (\pm 0.000278)x^2$; $R^2 = 0.168$; $P = 0.046$] to BSFL levels.

Table 4.2. Carcass characteristics, slaughter weight and dressing out percentage of six-week old Jumbo quails (n = 9±1) fed black soldier fly larvae meal-containing diets

	[1]Diets						Significance	
[2]Parameters	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100	[3]SEM	Linear	Quadratic
HCW (g)	140.4	154.0	147.5	148.7	148.7	4.26	0.460	0.511
CCW (g)	139.5	152.7	145.6	147.3	147.8	4.23	0.355	0.322
SW (g)	207.3	223.6	213.4	218.2	217.2	6.21	0.428	0.557
Dressing out (%)	67.74	68.84	69.15	68.17	68.51	0.462	0.652	0.493
Back (cm)	8.51	9.60	9.83	8.97	10.69	0.68	0.114	0.860
Wing (cm)	10.75	10.47	9.83	10.71	10.16	0.37	0.551	0.689
Thigh (cm)	4.31	4.53	4.53	4.49	4.52	0.12	0.343	0.343
Drumstick (cm)	5.45	5.63	5.60	5.47	5.68	0.09	0.120	0.667
Wing (g /100 g HCW)	4.29	4.29	4.29	4.49	4.26	0.12	0.815	0.322
Thigh (g/100 g HCW)	6.04	6.27	6.14	6.25	6.27	0.21	0.649	0.448
Drumstick (g/ 100 g HCW)	4.25	4.22	4.26	4.26	4.25	0.13	0.973	0.510
Breasts (g/ 100 g HCW)	22.39	21.92	22.25	22.34	21.25	0.75	0.425	0.044

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae meal inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae meal, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae meal.

[2]Parameters: HCW = hot carcass weight, CCW = cold carcass weight, SW = slaughter weight.

[3]SEM: Standard error of the mean.

Table 4.3 shows that there were neither linear nor quadratic trends (P>0.05) for meat quality parameters whose ranges were as follows: meat pH (6.1 – 6.2), meat temperature (11.5 – 11.9), L^* (44.8 – 44.2), a^* (5.7 – 6.7), b^* (9.9 – 10.5), hue angle (1.0), Chroma (11.5 – 12.2) and shear force (6.2 – 7.6). Similarly, the experimental diets had no effects on meat quality parameters of Jumbo quails.

Table 4.3. The effect of black soldier fly larvae meal-containing diets on meat quality parameters of six-week old Jumbo quails (n = 9±1)

	[1]Diets						Significance	
Parameters	BSFL0	BSFL25	BSFL50	BSFL75	BSFL100	[2]SEM	Linear	Quadratic
pH	6.1	6.1	6.1	6.1	6.2	0.03	0.777	0.875
Temperature (°C)	11.8	11.9	11.9	11.6	11.5	0.27	0.287	0.272
L^* (lightness)	43.8	44.5	44.3	44.2	44.6	0.44	0.157	0.641
a^* (redness)	5.8	6.2	6.7	5.9	5.7	0.38	0.310	0.188
b^* (yellowness)	9.9	10.5	10.2	9.9	10.3	0.37	0.807	0.940
Hue angle	1.0	1.0	1.0	1.0	1.0	0.02	0.134	0.129
Chroma	11.5	12.2	12.2	11.6	11.8	0.46	0.791	0.608
Shear force (N)	6.2	6.9	7.6	6.4	6.6	0.76	0.523	0.291

[1]Diets: BSFL0 = a commercial grower diet with no black soldier fly larvae meal inclusion, BSFL25 = a commercial grower diet in which 25 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL50 = a commercial grower diet in which 50 g/kg of soya products was replaced with black soldier fly larvae meal, BSFL75 = a commercial grower diet in which 75 g/kg of soya products was replaced with black soldier fly larvae meal, and BSFL100 = a commercial grower diet in which 100 g/kg of soya products was replaced with black soldier fly larvae meal.

[2]SEM: Standard error of the mean.

There were neither linear nor quadratic (P>0.05) trends observed for cooking loss, WHC and drip loss in response to the BSFL levels (Figure 4.1). Experimental diets had no significant effect (P >0.05) on cooking loss (17.29 – 22.07), water holding capacity (6.09 – 7.40) and drip loss (40.21 – 43.36).

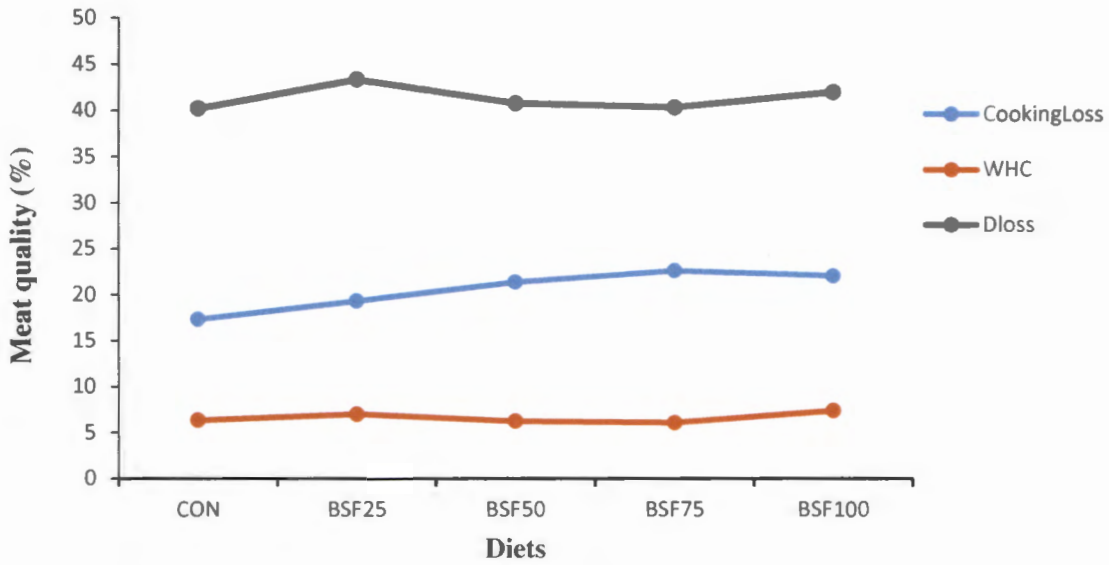

Figure 4.1. The effect of black soldier fly larvae meal-containing diets on cooking loss, water holding capacity and drip loss of six-week old Jumbo quails

4.4 Discussion

4.4.1 Size of internal organs

Internal organ sizes are indicative of an animal's digestive health and function (Kuzmuk *et al.*, 2005). Several factors such as feed, genetics and housing systems affect the function and size of internal organs (Young *et al.*, 2001; Havenstein *et al.*, 2003; Brickett *et al.*, 2007). Providing a balanced diet and a conducive environment promotes good health and productivity. In the present study, gizzard weights linearly increased with the inclusion of BSFL meal, indicating that the fibrous nature of chitin present in the larvae meal challenged the birds. As a result, they responded by developing enlarged gizzards as an anatomical adaptation mechanism to physically degrade this structural polysaccharide. A quadratic response was observed for large intestines, which was expected since there was a linear effect for gizzard weights, implying that by the time the digesta reached the intestinal tract it was well digested hence the size of the large intestines declined with dietary BSFL meal. For all the other internal organs, there were no significant dietary effects, which is in agreement with several studies that reported a lack of dietary effects on size of internal organs of broiler quails fed BSFL meal (Cullere *et al.*, 2016) and broiler chickens fed mealworm (Biasato *et al.*, 2017). However, a feeding trial conducted by Bovera *et al.* (2016) on broilers fed mealworm-containing diets showed significant dietary effects on both weight and length of the ileum and ceca. The authors ascribed these findings to the amount of chitin contained in the mealworm larvae. Moreover, Ballito and Sun (2013) together with Schiavore *et al.* (2014) observed an increase in the weights of small intestines of broilers when they were fed mealworm-containing diets at a rate of 10% and 25%. In addition, Smits and Annison (1996) demonstrated that when broilers are fed with diets that are high in fibre, the size of the intestinal tract increased. There is limited scientific evidence on the effect of insect meals on

the intestinal tract and gut physiology of quails, as many studies focus on the growth aspects of these birds. In addition, it is worth taking into consideration that different bird species do not have the same digestive capacity when challenged with diets containing high levels of chitin.

4.4.2 Carcass characteristics

Breast weights showed a quadratic response to dietary levels of BSFL meal. This trend was not surprising because a quadratic trend was also observed for overall feed intake and overall weight gain, verifying that including BSFL meal beyond 54 g/kg depressed voluntary feed intake and subsequently reduced the development of the pectoralis major muscle. Replacing soy components with BSFL meal had no effect on carcass characteristics, slaughter weight and dressing percentage of Jumbo quails. The results from this study are in accordance with the findings of Cullere *et al.* (2016), who reported no significant differences on carcass weights, dressing percentage, breast meat and breast meat yield of quails fed BSFL-containing diets at a rate of 10% and 15%. In a similar study by Secci *et al.* (2018) where Barbary partridge birds were fed BSFL meal and *T. molitor* larvae meal at a rate of 25% and 50%, respectively, as a protein substitutes, showed a lack of statistical significance for legs, chest and wings. Furthermore, Biasato *et al.* (2017) reported a lack of statistical effects on live and carcass weights, breast and thighs of broilers fed with *T. molitor* larvae meal. However, Loponte *et al.* (2017) observed significant dietary effects on carcass weights and distal legs for Barbary partridge fed both *T. molitor* larvae meal and BSFL meal at 25% and 50% as alternatives to soybean. For the regression analyses, only breast weights showed a quadratic trend in response to dietary levels of BSFL. This trend was expected because a quadratic trend was observed for overall feed intake and overall body weight gain, which resulted in a reduction of the development of the pectoralis major muscles

4.4.3 Meat quality parameters

The assessment of meat quality parameters is performed to evaluate physical, biological and chemical characteristics of meat such as pH, tenderness, texture, colour and storage qualities (Van Laack *et al.*, 2000). According to Zhang and Barbut (2005), the ultimate pH range for poultry species is regarded as normal if it is between 5.7 and 6.1. In this present study, the dietary treatments had no effect on meat pH, which ranged from 6.1 to 6.2. These findings are in line with those of Secci *et al.* (2018), who observed a lack of dietary effects on meat pH of Barbary partridge fed *T. molitor* larvae meal and BSFL meal. In literature, meat pH observed from different avian species is contradictory, for example, Cullere *et al.* (2016) reported significant dietary effects on meat pH (5.67 - 5.76) of quails fed BSFL meal at an inclusion level of up to 15%. Moreover, Genchev *et al.* (2008) reported meat pH values of up to 6.17 in Japanese quails, and attributed their findings to the morphology of quail muscle that is mostly composed of aerobic red muscle fibres. According to Berri *et al.* (2005), the variation in meat pH is a result of the changes in the glycogen levels before slaughter, which subsequently affect the post-mortem metabolism of the muscle. Terlouw *et al.* (2005) and Tavaneillo *et al.* (2014) also ascribed the variation in meat pH to ante-mortem stress, which uses up glycogen, and also to the type of breed and variation within breeds. Nonetheless, the pH values observed from these studies were within the normal meat pH reported for quails Genchev *et al.* (2008).

Generally, external features of the carcass influence the purchasing decision making process of consumers (ElMasry *et al.*, 2012). Taheri-Garavand *et al.* (2019) demonstrated that freshness and tenderness are the two most important factors affecting meat quality. Tenderness of the meat is determined using slice shear force. In this study, no dietary effects were observed for shear force, which is consistent with the findings of Mnisi *et al.* (2017) and

Mulaudzi *et al.* (2019), who reported no dietary effects on meat tenderness of Japanese quails fed canola meal and *Moringa* leaf meal, respectively. Pigmentation of carcasses is partly affected by the colour of the feed. According to Secci *et al.* (2018), the BSFL meal contains approximately 2.15 mg/kg of carotenoids, which is responsible for the yellowness of the bird's carcasses. In this present study, colour was not influenced by the dietary treatments. This could be explained by the fact that the BSFL meal was included at lower inclusion levels (10%). Nonetheless, these findings are consistent with Dalle Zotte *et al.* (2018) who observed no significant differences across all dietary treatments of rabbits when fed BSFL containing diets. Aaslyng *et al.* (2003) described high cooking loss as a mechanism that gives meat a less preferential option from consumers. An increase in cooking loss reduces carcasses juiciness (Schönfeldt& Strydom, 2011). In this study, no significant effect related to BSFL meal inclusion in experimental diets was observed for cooking loss, drip loss and water holding capacity. Similarly, Secci *et al.* (2018) and Schiavone *et al.* (2019), observed no significant influence on the cooking loss and drip loss of Barbary partridge and broilers fed BSFL meal.

4.5 Conclusion

It was concluded that feeding Jumbo quails with diets containing BSFL meal promoted similar performance in terms of health status, and carcass and meat quality parameters with those fed the commercial soybean-containing diet.

4.6 References

Aaslyng, M.D., Bejerholm, C., Ertbjerg, P., Bertram, H.C. & Andersen, H.J., 2003. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. Food Qual. Prefer. 14, 277-288.

Ayyub, R.M., Mushtaq, M.H., Bilal, M. & Akram, M.R., 2014. A study of consumer behaviour regarding quail meat product development and marketing. Sci. Int. (Lahore). 26(2), 871-874.

Ballitoc, D.A. & Sungo, S., 2013. Ground yellow mealworms (*Tenebrio molitor* L.) feed supplementation improves growth performance and carcass yield characteristics in broilers. Open Sci. Reposit. Agric.23050425. doi:10.7392/openaccess.23050425.

Berri, C., Debut, M., Sante-Lhoutellier, V., Arnould, C., Boutten, B., Sellier, N., Baeza, E., Jehl, N., Jego, Y., Duclos, M.J. & LeBihan-Duval, E., 2005. Variations in chicken breast meat quality: Implications of struggle and muscle glycogen content at death. Brit. Poult. Sci. 46, 572-579.

Biasato, I., Gasco, L., Marco De M., Rennab, M., Rotolob, L., Dabboub, S., Capucchioa, M.T., Biasibettia, I.E., Tarantolaa M., Bianchia, C., Cavallarinc, L., Gai, F., Pozzo, L., Dezzutto, D., Bergagna S. & Schiavone, A., 2017. Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. Anim. Feed. Sci. Technol. 234, 253-263.

Bovera, F., Loponte, R., Marono, S., Piccolo, G., Parisi, G., Iaconisi, V., Gasco, L. & Nizza, A., 2016. Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. J. Anim. Sci. 94, 639-647.

Brickett, K.E., Dahiya, J.P., Classen, H.L. & Gomis, S., 2007. Influence of dietary nutrient density, feed form, and lighting on growth and meat yield of broiler chickens. J. Poult. Sci. 86, 2172-2181.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S. & Dalle Zotte, A., 2016. Black soldier fly as dietary protein source for broiler quails: Apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal, 10(12), 1923-1930.

Cullere, M., Woods, M.J., van Emmenes, L., Pieterse, E., Hoffman, L.C. &Dalle Zotte, A.D., 2019. *Hermetia illucens* larvae reared on the different substrates in broiler quail diets: effect on physiochemical and sensory quality of quail meat. Animals, 9, 595.

Dalle Zotte, A., Cullere, M., Martins, C., Alves, S.P., Freire, J.P.B., Falcao-e-Cunha, L., Bessa, R.J.B., 2018. Incorporation of Black Soldier Fly (*Hermetia illucens* L.) larvae fat or extruded linseed in diets of growing rabbits and their effects on meat quality traits including detailed fatty acid composition. Meat Sci. 146, 50-58.

ElMasry, G., Barbin, D.F., Sun, D.W. & Allen P., 2012. Meat quality evaluation by hyperspectral imaging technique: An overview. Crit. Rev. Food Sci. Nutr. 52(8), 689-711.

Genchev, A., Mihaylova, G., Ribarski, S., Pavlov, A. & Kabakchiev, M., 2008. Meat quality and composition in Japanese quails. Trak. J. Sci. 6, 72-82.

Havenstein, G.B., Ferket, P.R. & Qureshi., M.A., 2003. Carcass Composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. J. Poult. Sci. 82, 1509-1518.

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Kuzmuk, K.N., Swanson, K.S., Tappenden, K.A., Schook, L.B. & Fahey Jr, G.C., 2005. Diet and age affect intestinal morphology and large bowel fermentative end-product concentrations in senior and young adult dogs. J. Nutr. 135, 1940-1945.

Lawrie R.A. & Ledward, D.A., 2006. Lawrie's meat science (7thed.). Woodhead Publishing. Cambridge, England.

Loponte, R., Nizza, S., Bovera, F., Riu De, N., Fliegerova, K., Lombardi, P. & Moniello, G., 2017. Growth performance, blood profiles and carcass traits of Barbary partridge (*Alectoris Barbara*) fed two different insect larvae meals (*Tenebrio molitor* and *Hermetia illucens*). Res. Vet. Sci. 115, 183-188.

Mnisi, C.M. & Mlambo, V., 2017. Growth performance, haematology, serum biochemistry and meat quality characteristics of Japanese quails (*Cotunix cotunix japanica*) fed Canola meal-based diets. Anim. Nutr. 4(1), 34-43.

Mulaudzi, A., Mnisi, C.M. & Mlambo, V., 2019. Dietary *Moringa oleifera* leaf meal improves growth performance but not haemo-biochemical and meat quality parameters in female Japanese quails. Pak. J. Nutr. 18(10), 953-960.

Nchinda, V.P., Thieme, O., Ankers, P., Crespi, V. & Ariste, S., 2011. Food security and economic importance of family poultry (chicken) husbandry program in Artibonite and South departments of Haiti. Livest. Res. Rural Dev. 23(201). http://www.lrrd.org/lrrd23/9/nchi23201.htm.

Ritchie, H. & Roser, M., 2019. Meat and Dairy Production. Oxford University, Oxford, UK. Retrieved from: https://ourworldindata.org/meat-production.

Ryan, J.T., Ross, R.P., Bolton, D., Fitzgerald, G.F. & Stanton C., 2011. Bioactive peptides from muscle sources: Meat and fish. Nutrients, 3(9), 765-791.

Schiavone, A., Dabbou, S., Petracci, M., Zampiga, M., Sirri, F., Biasato, I., Gai, F. & Gasco, L., 2019. Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on carcass traits, breast meat quality and safety. Animal, 13(10), 2397-2405.

Schiavone, A., Marco De, M., Rotolo, L., Belforti, M., Martinez Mirò, S., Madrid Sanchez, J., Hernandez Ruiperez, F., Bianchi, C., Sterpone, L., Malfatto, V., Katz, H., Zoccarato, I., Gai, F. & Gasco. L., 2014. Nutrient digestibility of *Hermetia illucens* and *Tenebrio molitor* meal in broiler chickens. In: Proc. 1st Int. Conf. Insects to Feed the World, Wageningen, The Netherlands. pp. 73.

Schönfeldt, H.C. &Strydom, P.E.,2011. Effect of age and cut on cooking loss, juiciness and flavour of South African beef. Meat Sci. 87, 180-190.

Secci, G., Moniello, G., Gasco, L., Bovera, F. & Parisi, G., 2018. Barbary partridge meat quality as affected by *Hermetia illucens* and *Tenebrio molitor* larva meals in feeds. Food Res. Int. 112, 291-298.

Smits, C. & Annison, G., 1996. Non-starch polysaccharides in broiler nutrition towards a physiologically valid approach to their determination. World Poult. Sci. J. 52, 203-221.

Stanford, K., Aalhus, J.L., Dugan, M.E.R., Wallins, G.L., Sharma, R. & McAllister, T.A., 2003. Effects of feeding transgenic canola on apparent digestibility, growth performance and carcass characteristics of lambs. J. Anim. Sci. 83(2), 299-305.

Tao, F. & Peng, Y., 2014. A method for non-destructive prediction of pork meat quality and safety attributes by hyper spectral imaging technique. J. Food Eng. 126, 98-106.

Tarehi-Garavand, A., Fatahi, S., Omid, M. & Makino, Y., 2019. Meat quality evaluation based on computer vision technology. A review. Meat Sci. 156, 183-195.

Tavaniello, S., Maiorano, G., Siwek, M., Knaga, S., Witkowski, A., Di Memmo, D. & Bednarczyk, M., 2014. Growth performance, meat quality traits, and genetic mapping of quantitative trait loci in 3 generations of Japanese quail populations (*Coturnixjaponica*). Poult. Sci. 93, 2129-2140.

Terlouw, C., 2005. Stress reactions at slaughter and meat quality in pigs: Genetic background and prior experience: A brief review of recent findings. Livest. Prod. Sci. 94, 125-135.

Tunsaringkarn, T., Tungjaroenchai, W. & Siriwong, W., 2013. Nutrient benefits of quail (*Coturnix coturnix japonica*) eggs. Int. J. Sci. Res. 5(3), 1-8.

Van Laack, R.L., Liu, C.H., Smith, M.O. & Loveday, H.D., 2000. Characteristics of pale, soft, exudative broiler breast meat. Poult. Sci. 79, 1057-1061.

Young, L.L., Northcutt, J.K., Buhr, R.J., Lyon, C.E. & Ware, G.O., 2001. Effects of age, sex, and duration of post-mortem aging on percentage yield of parts from broiler chicken carcasses. Poult. Sci. 80, 376-379.

Zhang, L. & Barbut, S., 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. Br. Poult. Sci. 46, 687-693.

5 CHAPTER FIVE – GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General Discussion

The United Nations estimates that by 2050 the population in Sub-Saharan Africa would have increased by 50% (United Nations, 2019). In light of these demographic patterns, the demands of consumers are expected to change and increase to accommodate their standard of living. A shift in economic growth and environmental conservation is also expected to increase to match the increasing human population. According to Biasato *et al.* (2017), the increase in human population will increase the demand for food and other resources, primarily animal protein. Poultry production is one of the easiest means to meet the demand for dietary protein and for this reason, the production of other poultry species such as quails must be increased to complement and meet the nutritional requirements of humans. Quails are mid-sized, ground dwelling birds previously used as game birds and conserved to improve restocking and habitat maintenance (Scandura *et al.*, 2010). Of late, they are considered as a source of nutritious eggs and meat, especially for the niche market. Tuleun *et al.* (2011) reported that consumption of quail meat could help treat people suffering from digestive problems, gastric ulcers and liver problems. Moreover, the demand for dietary protein other than plant protein i.e. soybean will increase to meet the nutritional requirements of both humans and livestock. Insects form part of the natural diet of birds suggesting that the inclusion of insect larvae in their diet is not a new phenomenon (FAO, 2013). Józefiak *et al.* (2016) reported that the BSFL and yellow mealworm have a major potential to be used as a standard protein ingredient for commercial feeds. Satisfactory results in terms of growth performance, blood metabolites, carcass characteristics and meat quality parameters have been achieved when the aforementioned insects were included in the diets of free range

chickens, broilers, barbary partridges and Japanese quails (Bovera *et al.*, 2017; Biasato *et al.*, 2017; Secci *et al.*, 2018; Cullere *et al.*, 2019). Likewise, the results from the present research revealed similar findings in terms of blood parameters, internal organs, and carcass and meat quality parameters. Chapter Three of this study showed that the inclusion of BSFL meal had no significant dietary influence on growth performance and blood parameters of Jumbo quails, indicating that the dietary inclusion of BSFL meal does not compromise growth performance and has no negative effect on blood parameters. The optimum BSFL inclusion level in Chapter Three was calculated to be 5.4%, revealing that higher inclusion levels would depress feed intake and body weight gain. Chapter Four also yielded comparable results to those in literature (Barroso *et al.*, 2014; Dabbou *et al.*, 2018; Onsongo *et al.*, 2018; Spranghers *et al.*, 2018). The dietary treatments had no influence on the internal organs, carcass characteristics and meat quality parameters. However, a quadratic effect for empty gizzards and a linear effect for the large intestine and breasts were observed. The linear trend observed in this study suggests that the inclusion level of BSFL meal was not sufficient for these parameters, which made it difficult to acquire the optimum inclusion level for this chapter. Nonetheless, all the results for internal organs, carcass characteristics and meat quality traits in this present study fell within the normal ranges reported for quails, suggesting that the inclusion of BSFL meal can be a suitable protein source for Jumbo quails.

5.2 Conclusions

The inclusion of black soldier fly larvae meal in the diet of Jumbo quails provided comparable results to those fed the standard control diet and, therefore, BSFL meal can be considered as a possible protein alternative to soybean in quail diets. Overall, the use of BSFL meal in diets of Jumbo quails forms part of a sustainable solution to alleviate production costs associated with the feeding of animals.

5.3 Recommendations and future prospects

It is recommended that farmers include BSFL meal at 54g/kg in quail diets because the presence of chitin has a negative effect on growth performance. Thus, pre-treatment of BSFL meal with the enzyme chitinase can be used if higher inclusion levels of the larvae meal are to be included in quail diets.The presence of peptides and chitin in the BSFL meal are also known to have antimicrobial properties, but their activity has not been demonstrated in Jumbo quails. Thus a follow up study can be conducted to test the antimicrobial activities of these compounds in place of prophylactic antibiotics in quail diets.

5.4 References

Barroso, F.G., de Haro, C., Sánchez-Muros, M.J., Venegas, E., Martínez-Sánchez, A. & Pérez-Bañón C., 2014. The potential of various insect species for use as food for fish. Aquaculture, 422, 193-201.

Biasato, I., Gasco, L., Marco De M., Rennab, M., Rotolob, L., Dabboub, S., Capucchioa, M.T., Biasibettia, I.E., Tarantolaa M., Bianchia, C., Cavallarinc, L., Gai, F., Pozzo, L., Dezzutto, D., Bergagna S. & Schiavone, A., 2017. Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. Anim. Feed. Sci. Technol. 234, 253-263.

Bovera, F., Loponte, R., Marono, S., Piccolo, G., Parisi, G., Iaconisi, V., Gasco, L. & Nizza, A., 2016. Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: effect on growth performance, nutrient digestibility, and carcass and meat traits. J. Anim. Sci. 94, 639-647.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S. & Dalle Zotte, A., 2016. Black soldier fly as dietary protein source for broiler quails: Apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal, 10(12), 1923-1930.

Cullere, M., Woods, M.J., van Emmenes, L., Pieterse, E., Hoffman, L.C. & Dalle Zotte, A.D., 2019. *Hermetia illucens* larvae reared on the different substrates in broiler quail diets: effect on physiochemical and sensory quality of quail meat. Animals, 9, 595.

Dabbou, S., Gai, F., Biasato, I., Capucchio, M.T., Biasibetti, E., Dezzutto, D., Meneguz, M., Plachà, I., Gasco, L. & Schiavone, A., 2018. Black soldier fly defatted meal as a

dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. J. Anim. Sci. Biotechnol. 9, 49.

FAO, 2013. Edible insects -Future prospects for food and feed security. FAO Forestry Paper. Rome, Italy.

Józefiak, D., Józefiak, A., Kierończyk, B., Rawski, M., Świątkiewicz, S., Długosz, J. & Engberg, R.M., 2016. Insects – A natural nutrient source for poultry – A review. Ann. Anim. Sci. 6, 297-313.

Onsongo, V.O., Osuga, I.M., Gachuiri, C.K., Wachira, A.M., Miano, D.M., Tanga, C.M., Ekesi, S., Nakimbugwe, D. & Fiaboe K.K.M., 2018. Insects for income generation through animal feed: Effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. J. Econ. Entomol. 111, 1966-1973.

Scandura, M., Iacolina, L., Apollonio, M., Dessì-Fulgheri, F.& Baratti, M., 2010. Current status of the Sardinian partridge (*Alectoris barbara*) assessed by molecular markers. Eur. J. Wildl. Res. 56, 33-42.

Secci, G., Moniello, G., Gasco, L., Bovera, F. & Parisi, G., 2018. Barbary partridge meat quality as affected by *Hermetia illucens* and *Tenebrio molitor* larva meals in feeds. Food Res. Int. 112, 291-298.

Spranghers, T., Michiels, J., Vrancx, J., Ovyn, A., Eeckhout, M., De Clercq, P. & De Smet S., 2018. Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. Anim. Feed Sci. Technol. 235, 33-42.

Tuleun C.D., Adenkola A.Y. & Afele T., 2011. Effect of dietary ascorbic acid supplementation on the performance of Japanese (*Coturnix coturnix japonica*) quails in a tropical environment. J. Anim. Plant Sci. 10(2), 1268-1275.

United Nations, 2019. The World Population Prospects Revision Department of Economics. New York, US. https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html.

6 LIST OF APPENDICES

Appendix 1: Article Accepted for Publication in Sustainability

sustainability

MDPI

Article

A Nutritional Evaluation of Insect Meal as a Sustainable Protein Source for Jumbo Quails: Physiological and Meat Quality Responses

Fezile Gcinile Thobeka Mbhele [1,2], Caven Mguvane Mnisi [1,2,*] and Victor Mlambo [3]

[1] Department of Animal Science, Faculty of Natural and Agricultural Science, North-West University, Mafikeng, South Africa; mbhelefezile@gmail.com

[2] Food Security and Safety Niche area, Faculty of Natural and Agricultural Science, North-West University, Mafikeng, South Africa

[3] School of Agricultural Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Nelspruit, South Africa; victor.mlambo@ump.ac.za

* Correspondence: mnisiecm@gmail.com or 23257539@nwu.ac.za; Tel.: +27-18-389-2738

Received: 04/11/2019; Accepted: 15/11/2019; Published: date

Abstract: The use of insect meals in place of soybean meal in poultry diets is critical for sustainable intensification but the utility of this alternative dietary protein source is unknown for the Jumbo quail. Therefore, this study investigated the effect of partial substitution of soybean with black soldier fly larvae (BSFL) meal on feed intake, physiological and meat quality parameters of Jumbo quails. Three hundred and fifteen mixed gender quails (53.8 ± 5.19 g live-weight) were reared on five isonitrogenous and isoenergetic experimental diets formulated by replacing soybean products with BSFL meal at 0 (BSFL0), 25 (BSFL25), 50 (BSFL50), 75 (BSFL75) and 100 g/kg (BSFL100). Feed intake, body weight gain, gain-to-feed ratio (G:F), blood parameters, and carcass and meat quality traits were determined. Quadratic trends were observed for overall feed intake [$(y = 605 (\pm 17.56) + 2.1 (\pm 0.82)x - 0.02 (\pm 0.008)x^2$; $R^2 = 0.20$; $P = 0.023$] and overall body weight gain [$(y = 155 (\pm 4.70) + 0.57 (\pm 0.22)x - 0.005 (\pm 0.002)x^2$; $R^2 = 0.22$; $P = 0.026$]. There were neither linear nor quadratic trends for meat quality and blood parameters, except for albumin-to-globulin ratio. Only gizzard size linearly increased [$y = 0.05 (\pm 0.004)x + 2.3 (\pm 0.09)$; $R^2 = 0.18$; $P = 0.04$] with BSFL levels. We concluded that BSFL meal could replace soybean products in Jumbo quail diets at 54 g/kg without compromising weight gain and meat quality.

Keywords: feed production; growth performance; insect meal; jumbo quails; meat quality; soybean; sustainable intensification

1. Introduction

The production and use of feed in the livestock sector accounts for approximately 14.5% of total greenhouse gas emissions [1]. An anticipated increase in the demand of poultry products means more arable land will be required for feed grain production. This will inevitably lead to higher levels of atmospheric carbon [2] due to deforestation and land preparation activities. The demand for soybean, the main protein source in poultry diets, has also steadily increased due to competition between animal and human needs [3]. This has led to higher market prices for this vegetable protein thus compromising its contribution to food and nutrition security. Sustainable intensification of poultry production requires the use of feed ingredients whose production requires less water, land, and results in lower greenhouse gas emissions [4]. This approach will ensure environmental, financial and social sustainability of the poultry industry. The sustainability of the poultry industry also requires diversification of bird species used to supply animal protein.

Appendix 2: Ethics Certificate of the Study

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Research Ethics Regulatory Committee
Tel: +27 18 299 4849
Email: Ethics@nwu.ac.za

ETHICAL CLEARANCE LETTER OF STUDY

Based on approval by **Animal Research Ethics Committee, Mafikeng Campus** on **03/08/2018** after being reviewed at the meeting held on **03/08/2018**, the North-West University Research Ethics Regulatory Committee (NWU-RERC) hereby **approves** your study as indicated below. This implies that the NWU-RERC 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: Insect meal as an alternative protein source for Jumbo quails: physiological and meat quality traits

Study Leader/Supervisor: Dr C Mnisi & Prof Mlambo
Student: Mbhele

Ethics number: NWU-00483-18-A5

Application Type: N/A
Commencement date 2018-08-03 **Expiry date** 2019-08-03 **Category:** 1

Special conditions of the approval (if applicable):

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 (principle investigator) must report in the prescribed format to the AnimCare/REC:*
  - *annually (or as otherwise requested) on the monitoring of the study, and upon completion 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.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the AnimCare, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-RERC and AnimCare reserves the right to:*
  - *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 AnimCare or that information has been false or misrepresented,
    the required amendments, annual (or otherwise stipulated) report and 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.
- *AnimCare can be contacted for further information or any report templates via Ethics-AnimCare@nwu.ac.za or 018 299 2197.*

The RERC 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 RERC or AnimCare for any further enquiries or requests for assistance.

Yours sincerely

Prof Upenyu Marume
Chair NWU Animal Research Ethics Committee

Appendix 3: Language Editing Certificate

NWU®
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NOORDWES-UNIVERSITEIT
YUNIBESITHI YA BOKONE-BOPHIRIMA

DEPARTMENT OF BOTANY

Tel: 27 18 299 2289 Fax: 018 386 2154
Cell: +27 72 626 8446 E-mails: Ozaniel.Ruzvidzo@nwu.ac.za

Date: 18th November, 2019

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 **FGT Mbhele** (23629096; **orcid.org/0000-0003-3622-7920**) entitled: **Insect meal *(Hermetia illucens)* as an alternative protein source for Jumbo quails: Physiological and meat quality traits.** 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 CM Mnisi