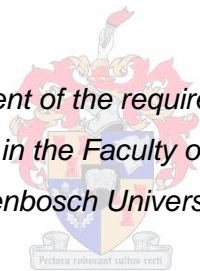


# **Black soldier fly (*Hermetia illucens*) pre-pupae as a protein source for broiler production**

by

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*Thesis presented in fulfilment of the requirements for the degree of  
Master of Science in the Faculty of AgriScience at  
Stellenbosch University*



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March 2015

## **Declaration**

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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## Summary

The aim of the study was to investigate the potential of black soldier fly (*Hermetia illucens*) pre-pupae meal (BSM) as a protein source in broiler chicken diets. The first part of the study determined the digestibility of black soldier fly pre-pupae meal in a trial where four treatment diets, i.e. 100% maize (control diet), BSM dried at 100 °C (BSM100), BSM dried at 65 °C (BSM65), and defatted BSM dried at 65 °C (DF-BSM) were fed to Cobb 500 broiler chicks, 43 days of age, for a period of three days. The chicks (n=64) allocated to the treatment groups were acclimatized to the treatment diets for a period of three days prior to the experimental feeding period. The chicks received a commercial diet, according to the guidelines of Cobb-Vantress, up to an age of 39 days. The DF-BSM diet was found to be more digestible than the BSM65 diet, and acceptable digestibility values (i.e. above 70%) were reported for all the treatment diets. The apparent metabolisable energy of the BSM65 diet was 16.52, with a crude protein coefficient of total intestinal tract digestibility (CTTD) of 86%, which is higher than that reported for soya bean meal. In the second part of the study, the inclusion of BSM65 in chicken diets and the effect on broiler production parameters; organ, gut and skeletal parameters, were investigated. Black soldier fly pre-pupae meal, dried at 65 °C (BSM), was included in broiler diets at levels of 0, 5, 10, and 15%, respectively and fed to 320 day-old Cobb 500 broiler chicks for a period of 35 days. For most of the production parameters studied, no significant differences were reported for average daily intake, live weight gain, feed conversion ratio and European protein efficacy factor. A significant effect was observed for average weekly feed intake and cumulative feed intake at day 18 of age. This part of the study indicated that BSM65 levels included at 15% sustained normal growth in birds used in the study, which indicated the viability of BSM to be used as a protein source in broiler diets. The findings also indicated that the inclusion of BSM in broiler diets did not influence organ weight, gizzard erosion score, tibia ash percentage, tibia breaking strength, tibia mineral content, small intestine pH, and histomorphology of the duodenal and jejunal regions. In the third part of the study, the inclusion of BSM65 in broiler diets on carcass characteristics and meat quality of broilers was investigated. Black soldier fly pre-pupae meal dried at 65 °C did not compromise the physical, sensory and chemical quality of the broiler meat. No significant effects were also found in terms of live slaughter weight, cold carcass weight and the commercial portions (i.e. breast, thigh, drumstick, wing and back). Overall, the study indicated that BSM, which is considered a non-traditional protein source, can be included in broiler diets at levels as high as 15%, without any adverse effect on normal broiler production, organ and skeletal parameters. The inclusion of BSM in broiler diets did not affect the quality of the meat nor compromised the eating quality of the meat produced, when compared to meat of broilers fed the control diet (commercial broiler diet).

## Opsomming

Die doel van die studie was om die potensiaal van die Venstervleig (*Hermetia illucens*) pre-papier meel (SSM) as 'n alternatiewe proteïenbron in braaikuiken diëte te ondersoek. In die eerste deel van die studie is die verteerbaarheid van SSM bepaal deur vier verskillende diëte, d.i. 100% mielies (kontrole diëet), SSM gedroog by 100 °C (SSM100), SSM gedroog by 65 °C (SSM65) en ontvette SSM gedroog by 65 °C (DF-SSM), aan 43 dae oue Cobb 500 braaikuikens te voer vir 'n tydperk van drie dae. Die kuikens (n=64) is ewekansig aan die onderskeie behandelingsgroepe toegeken en is drie dae voor aanvang van die eksperimentele voerperiode aangepas. Die kuikens het 'n kommersiële diëet, geformuleer volgens die riglyne van Cobb-Vantress, tot 'n ouderdom van 39 dae, ontvang. Die verteerbaarheid van die DF-SSM was hoër as die van die SSM65 diëet. Alle verteerbaarhede was hoogs aanvaarbaar met waardes bo 70%. Die skynbare metaboliseerbare energiewaarde van die SSM65 diëet was 16.52 MJ/kg, met 'n koëffisiënt verteerbaarheid (CTTD) van 86%, wat hoër is as die waardes vir sojaboonmeel. In die tweede deel van die studie is die insluiting van SSM65 in braaikuiken diëte en die effek op braaikuikenproduksie parameters, orgaan-, derm- en skeletale parameters, ondersoek. Venstervleig pre-papier meel, gedroog by 65 °C (SSM), is ingesluit in braaikuiken diëte teen vlakke van 0, 5, 10, en 15%, onderskeidelik en gevoer vir 'n tydperk van 35 dae aan 320 dag-oud Cobb 500 braaikuikens. Geen betekenisvolle verskille is gevind vir gemiddelde daaglikse inname, lewende massatoename, voeromsetverhouding of die Europese produksie effektiwiteitsfaktor nie. 'n Betekenisvolle verskil is gevin vir gemiddelde weeklikse voerinnamte en kumulatiewe voerinnamte waargeneem by dag 18 van ouderdom. Hierdie deel van die studie het aangedui dat SSM ingesluit teen vlakke so hoog as 15%, nie die normale groei van die kuikens in die studie beïnvloed het nie, wat dui op die potensiaal van SSM om as 'n proteïenbron in braaikuiken diëte gebruik te word. Die bevindinge het ook aangedui dat die insluiting van SSM in braaikuiken diëte nie orgaanmassas, spiermaag erosie telling, tibia mineraal inhoud, tibia breekkrag, dunderm pH en histomorfologie van die duodenum en jejunum beïnvloed het nie. In die derde deel van die studie, is die insluiting van SSM65 in braaikuiken diëte op die karkas eienskappe en vleiskwaliteit parameters van braaikuikens ondersoek. Venstervleig pre-papier meel, gedroog by 65 °C, het nie die fisiese-, sensoriese- en chemiese kwaliteitseienskappe van braaikuiken vleis beïnvloed nie. Geen betekenisvolle verskille is gevind vir slaggewig, koue karkasgewig of die massas van die onderskeie handelsnitte (d.i. bors, dy, boudjie, vlerk en rug) nie. Die algehele bevinding van die studie was dat SSM65, wat beskou word as 'n nie-tradisionele bron van proteïen, in braaikuiken diëte teen vlakke van so hoog as 15% ingesluit kan word, sonder enige nadelige invloed op die normale braaikuikenproduksie parameters, orgaan- en skeletparameters of vleiskwaliteit nie.

## **Dedication**

I would like to dedicate this document to my grandmother Monica Gideon (Gwakathepa), who has raised me to be the person I am today. I am grateful to her for granting me the opportunity to a better education. Despite our differences my experiences under your guidance has taught me how to be an independent and strong woman. Thank you for opening up your lovely home and setting a great foundation to life for me, which has groomed me till this point forth.

## Acknowledgements

I would like to extend my heartfelt gratitude towards the following people as the completion of this thesis would not have been possible without their support and contributions.

Firstly, I would like to give thanks to my Almighty heavenly Father, for giving me life and granting me this opportunity to do my MSc. studies at Stellenbosch University. In addition, thank You for being with me during every step of this journey, providing Your protection, guidance and lifting me up when I fell. Deuteronomy 31:8.

Secondly, a warm special thank you to my supervisors Prof L. C. Hoffman and Dr E. Pieterse for your guidance and continual grooming throughout my work.

Thirdly, I would like to extend a warm gratitude to my study fees sponsor and for making my dream become a reality.

Fourthly, to the technical team of Animal Sciences Department, Stellenbosch University, thank you for your support and immeasurable help that you have provided me. I also would like to give a special thank you to Ms G. Jordan for helping with my statistical analysis; your patience is much appreciated. To fellow postgraduate colleagues, Meat Science team, Mr G. Chingala, Dr. Dube and fellow Namibian students: thank you for your assistants and continual support.

Finally but not the least, I would like to thank my family and friends for their continual support, love and encouragement. To my parents Martha and Wilbard Uushona: thank you for the priceless gift of life, for your overwhelming love, continual support and always having faith in me and the choices I make.

## Notes

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

## Table of Contents

<b>Black soldier fly (<i>Hermetia illucens</i>) pre-pupae as a protein source for broiler production .....</b>	
Declaration.....	
Summary .....	ii
Opsomming .....	iii
Dedication.....	iv
Acknowledgements.....	v
Notes.....	vi
Table of Contents .....	vii
List of Tables .....	xi
List of Figures .....	xiii
List of Equations .....	xiv
Abbreviations .....	xv
<b>Chapter 1 .....</b>	<b>1</b>
<b>General introduction .....</b>	<b>1</b>
References.....	3
<b>Chapter 2 .....</b>	<b>5</b>
<b>Literature Review.....</b>	<b>5</b>
2.1 Introduction .....	5
2.2 Agricultural waste.....	6
2.1.2 Quantifying waste production .....	7
2.2 Recovery of protein/usable substances from agricultural waste .....	7
2.2.1 Manure .....	7
2.2.2 Abattoir waste.....	9
2.2.3 Waste water.....	10
2.3 Black Soldier Fly ( <i>Hermetia illucens</i> ) .....	11
2.3.1 Black soldier fly life cycle.....	12
2.4 Enzymatic activity of insects .....	13
2.4.1 Other beneficial characteristics of insect larvae .....	14
2.5 Poultry Nutrition.....	14
2.5.1 Protein .....	15
2.5.2 Use of alternative feed ingredients.....	17
2.6 Consumer perception.....	23
2.7 Conclusion .....	25
2.8 References.....	27



<b>Chapter 3 .....</b>	<b>38</b>
<b>Evaluation of the total intestinal track digestibility of black soldier fly (<i>Hermetia illucens</i>) pre-pupae meal in the diets of broiler chickens .....</b>	<b>38</b>
Abstract .....	38
3.1 Introduction .....	38
3.2 Materials and Methods.....	40
3.2.1 Experimental animals, layout and housing.....	40
3.2.2 Experimental diets, design and trail procedure .....	40
3.2.3 Data collection .....	41
3.2.4 Analytical methodologies.....	41
3.2.5 Coefficient of total tract digestibility .....	43
3.2.6 Statistical analysis .....	44
3.3 Results .....	44
3.4 Discussion .....	46
3.5 Conclusion .....	49
3.6 References.....	50
<b>Chapter 4 .....</b>	<b>54</b>
<b>Effect of black soldier fly (<i>Hermetia illucens</i>) pre-pupae meal on production parameters of broiler chickens .....</b>	<b>54</b>
Abstract .....	54
4.1 Introduction .....	54
4.2 Materials and Methods.....	56
4.2.1 Experimental treatments, layout and housing system .....	56
4.2.2 Management and handling of birds .....	56
4.2.3 Experimental diets formulations .....	57
4.2.4 Chemical composition analysis of the treatment diets .....	58
4.2.5 Production data collection .....	59
4.2.6 Statistical analysis .....	60
4.3 Results .....	60
4.4 Discussion .....	65
4.5 Conclusion .....	67
4.6 References.....	68
<b>Chapter 5 .....</b>	<b>72</b>
<b>The effects of black soldier fly (<i>Hermetia illucens</i>) pre-pupae meal on organ, gut and tibia bone parameters of broiler chickens .....</b>	<b>72</b>
Abstract .....	72
5.1 Introduction .....	72
5.2 Materials and methods.....	73

5.2.1 Organ sample .....	74
5.2.2 Intestinal samples.....	74
5.2.3 Tibia bone samples .....	76
5.2.4 Tibia bone strength and mineral content.....	76
5.2.5 Statistical analysis .....	77
5.3 Results .....	78
5.3.1 Organ weight and gizzard erosion .....	78
5.3.2 Intestinal pH and histomorphology.....	79
5.3.3 Tibia bone parameters .....	80
5.4 Discussion.....	81
5.4.1 Organ weight and gizzard erosion .....	81
5.4.2 Intestinal pH and histomorphology.....	82
5.4.3 Tibia bone parameters .....	83
5.5 Conclusion .....	84
5.6 References.....	85
<b>Chapter 6 .....</b>	<b>88</b>
<b>Effect of black soldier fly (<i>Hermetia illucens</i>) pre-pupae meal on the carcass characteristics, physical measurements, chemical quality and sensory attributes of broiler chicken meat.....</b>	<b>88</b>
Abstract .....	88
6.1 Introduction .....	88
6.2 Materials and methods.....	89
6.2.1 Experimental layout, handling and management.....	89
6.2.2 Slaughtering procedure .....	89
6.2.2 Carcass characteristics .....	90
6.2.3 Physical measurements .....	90
6.2.4 Chemical analysis .....	91
6.2.5 Sensory analysis .....	92
6.2.6 Statistical analysis.....	94
6.3 Results .....	94
6.3.1 Carcass characteristics .....	94
6.3.2 Physical measurements .....	95
6.3.3 Chemical analysis .....	96
6.3.4 Descriptive sensory analysis and correlations .....	101
6.4 Discussion.....	105
6.4.1 Carcass characteristics .....	105
6.4.2 Physical measurements .....	106
6.4.3 Chemical analysis .....	107
6.4.4 Descriptive sensory analysis and correlations .....	108

6.5 Conclusion .....	109
6.6 References.....	110
<b>Chapter 7 .....</b>	<b>113</b>
<b>General conclusion and recommendations.....</b>	<b>113</b>

## List of Tables

<b>Table 2.1</b> Animals produced during the 2012 financial year in South Africa (FAO, 2014) .....	7
<b>Table 2.2</b> Amino acid profile of slaughterhouse waste (adapted from Couillard & Zhu, 1993).....	10
<b>Table 2.3</b> Comparison of swine manure before and after vermi-composting using house fly larvae (adapted from Zhang <i>et al.</i> , 2012) .....	14
<b>Table 2.4</b> Dietary crude protein requirement (% dry matter) and ideal amino acid pattern (g/g lysine) of essential amino acids for growth of different animal species (adapted from Boland <i>et al.</i> , 2013) .....	16
<b>Table 2.5</b> Protein content and digestibility of protein in broiler chickens in percentage .....	16
<b>Table 2.6</b> Comparison of the nutritional value of insect meals with that of fish and soya bean meal.....	18
<b>Table 2.7</b> Chemical composition of black soldier fly larvae .....	20
<b>Table 2.8</b> Ideal amino acid (g/100g) requirement in poultry diets, adapted from NRC (1994) .....	21
<b>Table 3.1</b> Ingredient compositions of black soldier fly pre-pupae meal (BSM) digestibility treatment diets ...	41
<b>Table 3.2</b> Treatment diets' chemical composition .....	45
<b>Table 3.3</b> Mean ( $\pm$ standard error) for coefficient of total intestinal tract digestibility (CTTD) of black soldier fly pre-pupae meal (BSM)'s with apparent metabolisable energy (AME) in broiler chickens .....	46
<b>Table 4.1</b> Ingredients used for the Starter, Grower and Finisher diets with inclusion of black soldier fly pre-pupae meal (BSM).....	58
<b>Table 4.2</b> The number of birds lost during the production trial and weight at death (g), per dietary treatment as influenced by inclusion of.....	59
<b>Table 4.3</b> Analysed proximate and amino acid composition of trial Starter diets on dry matter basis, with inclusion of black soldier fly pre-pupae meal (BSM) .....	61
<b>Table 4.4</b> Analysed proximate and amino acid composition of trial Grower diets on dry matter basis, with inclusion of black soldier fly pre-pupae meal (BSM) .....	62
<b>Table 4.5</b> Analysed proximate and amino acid composition of trial Finisher diets on dry matter basis, with inclusion of black soldier fly pre-pupae meal (BSM) .....	63
<b>Table 4.6</b> The means ( $\pm$ standard error) of weekly feed intake (g), live weight (g) and cumulative feed intake (g) and the production ratios (ADG, FCR, EPEF and PER) of broilers as influenced by inclusion of black soldier fly pre-pupae meal (BSM) .....	64
<b>Table 5.1</b> Gizzard erosion scoring description (Johnson & Pinedo, 1971) .....	74
<b>Table 5.2</b> Mean ( $\pm$ standard error) of organ weight and organ weight relative to body weight as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets .....	79
<b>Table 5.3</b> Gizzard erosion scores as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken.....	78
<b>Table 5.4</b> Mean ( $\pm$ standard error) of small intestine pH as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets .....	79
<b>Table 5.5</b> Mean ( $\pm$ standard error) of duodenum and jejunum histomorphology sections ( $\mu$ m) as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets.....	80

<b>Table 5.6</b> Mean ( $\pm$ standard error) of tibia breaking force and strength of broiler chickens fed different levels of black soldier pre-pupae meal (BSM) in their diets.....	80
<b>Table 5.7</b> Mean ( $\pm$ standard error) of tibia bone ash percentage and mineral content of broiler chickens fed different levels of black soldier fly pre-pupae meal (BSM) in their diets.....	81
<b>Table 6.1</b> Definition and scale of each attribute used for the descriptive sensory analysis on breast portion	93
<b>Table 6.2</b> The means ( $\pm$ standard error) of live slaughter weight, cold carcass weight and dressing percentage of broilers as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets....	95
<b>Table 6.3</b> The means ( $\pm$ standard error) of broiler carcass portion yield (g) as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets .....	95
<b>Table 6.4</b> The means ( $\pm$ standard error) for skin, muscle and bone percentage of broiler carcasses breasts as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets.....	95
<b>Table 6.5</b> The means ( $\pm$ standard error) of physical measurements of broiler carcasses as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets .....	96
<b>Table 6.6</b> The means ( $\pm$ standard error) of the proximate analysis (g/100g; meat) of broiler cooked breast meat as influenced by the inclusion of black soldier fly pre-pupae meal (BSM) in their diets.....	97
<b>Table 6.7</b> The means ( $\pm$ standard error) of the amino acid composition (g/100g) of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets .....	97
<b>Table 6.8</b> The means of long chain fatty acid composition in Starter treatment diets percentage of fatty acids, as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets.....	98
<b>Table 6.9</b> The means of long chain fatty acid composition in Grower treatment diets percentage of fatty acids as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets.....	99
<b>Table 6.10</b> The means of long chain fatty acid composition in Finisher treatment diets percentage of fatty acids, as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets .....	100
<b>Table 6.11</b> The means ( $\pm$ standard error) of long chain fatty acid composition of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets (mg/g; meat)...	101
<b>Table 6.12</b> The means ( $\pm$ standard error) of mineral composition of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets.....	101
<b>Table 6.13</b> The means ( $\pm$ standard error) of sensory attributes as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets.....	102
<b>Table 6.14</b> Relevant positive correlation of sensory, physical and chemical attributes of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets .....	102
<b>Table 6.15</b> Correlation matrix showing the Pearson correlation coefficients ( $r$ ) and the $P$ -values for all the samples as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets on meat quality.....	103

## List of Figures

<b>Figure 2.1</b> Life cycle of the black soldier fly ( <i>Hermetia illucens</i> ) (Alvarez, 2012) .....	12
<b>Figure 4.1</b> Least square means with error bars for protein efficacy ratio at 5% significant level for treatment effect .....	65
<b>Figure 5.1</b> Photomicrograph of jejunum cross section indicating measurements taken for crypt depth, villi area, villi length and villi width from chicks that received the control treatment diet .....	75
<b>Figure 5.2</b> Photomicrograph of jejunum cross section indicating measurements taken for <i>muscularis mucosa</i> , inner circular fibres and outer longitudinal fibres from chicks that received the 15% treatment diet	76
<b>Figure 6.1</b> Descriptive analysis (DA) plot illustrating the classification of treatments based on the tested parameters .....	104
<b>Figure 6.2</b> Descriptive analysis (DA) plot illustrating prominent parameters as per treatment based on observation DA plot Figure 6.1 .....	104
<b>Figure 6.3</b> Principle component analysis (PCA) bi-plot indicating the means for each analysed parameter with the sensory attributes as per treatment replications .....	105

## List of Equations

<b>Equation 3.1</b> Acid insoluble ash .....	42
<b>Equation 3.2</b> Apparent metabolisable energy .....	43
<b>Equation 3.3</b> Nutrients consumed (g/trial) .....	43
<b>Equation 3.4</b> Nutrients excreted (g/trial) .....	43
<b>Equation 3.5</b> Digested nutrients (g/trial) .....	43
<b>Equation 3.6</b> Coefficient of total tract digestibility (g/kg) .....	44
<b>Equation 3.7</b> Coefficient of total tract digestibility of test ingredient (%) .....	44
<b>Equation 4.1</b> Average daily gain .....	59
<b>Equation 4.2</b> Feed conversion ratio .....	60
<b>Equation 4.3</b> Protein efficiency ratio .....	60
<b>Equation 4.4</b> European protein efficiency factor .....	60
<b>Equation 5.1</b> Breaking strength (N/mm <sup>2</sup> ) .....	77
<b>Equation 5.2</b> Breaking force per gram of weight (N/g) .....	77

## Abbreviations

a*	Red-green
AA	Amino Acids
ADG	Average daily gain
AgriLasa	Agriculture Laboratory Association of Southern Africa
AME	Apparent metabolisable energy
AMSA	American Meat Science Association
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists International
b*	Blue-yellow
B	Boron
BSE	Bovine Spongiform Encephalopathy
BSF	Black soldier fly
BSM	Black soldier fly pre-pupae meal
Ca	Calcium
CF	Crude fibre
CP	Crude protein
CTTD	Coefficient of total intestinal tract digestibility
cm	Centimetres
Cu	Copper
DA	Descriptive analysis
DAFF	Department of Agriculture, Forestry and Fisheries
DM	Dry matter
DSA	Descriptive sensory analysis
EPEF	European protein efficacy factor
FA	Fatty acids
FAME	Fatty acid methyl esters
FAO	Food & Agriculture Organization
FCR	Feed conversion ratio
Fe	Iron
g	Grams
GALT	Gut associated lymphoid tissue
GLM	General linear models
h	Hours
HF	House fly
IBD	Infectious <i>bursal</i> disease
K	Potassium
kg	Kilograms



L	Litres
L*	Lightness
Ma	Manganese
ME	Metabolisable energy
Mg	Magnesium
mg	Mili grams
MJ	Mega joules
MGM	Maggot meal
m	Meters
min	Minutes
ml	Mili liters
mm	Mili meters
MUFA	Mono-unsaturated fatty acids
N	Newton
n-3	Omega-3
n-6	Omega-6
Na	Sodium
NDF	Neutral detergent fibre
NPP	Non-phytin phosphorous
NRC	National Research Council
P	Phosphorous
PSE	Pale Soft Exudate
Pb	Lead
PCA	Principle component analysis
PER	Protein Efficiency Ratio
PMI	Post-mortem interval
PUFA	Poly-unsaturated fatty acids
r	Pearson's correlation coefficient
s	Seconds
SFA	Saturated fatty acids
µl	Micro litres
µm	Micro meters
vol	Volume
Zn	Zinc

## Chapter 1

### General introduction

The world population is increasing rapidly, creating a higher demand for food to feed this growing population (Cribb, 2010; Dar & Gowda, 2013). Due to the population increase and increase in disposable income, the global demand for animal protein is increasing with an expected continual rise in protein cost both for animal and human use (Food & Agriculture Organization [FAO], 2009). It is predicted that the world population will reach 9 billion by the year 2050 (DESA, 2009), while climate change is expected to create a warmer world over the next 50 years (Change, Intergovernmental Panel On Climate, 2007), which may lead to a reduction in crop yield (Dar & Gowda, 2013). Thus, a challenge to feed the world population is expected, which can only be addressed by an increase in global agricultural production of 70-100% by the year 2050 (Bruinsma, 2009). This increase in production requires improving the efficiency and cost effectiveness of food production systems, with food production needed to increase with minimal effect on the environment (Berg *et al.*, 2013). Enhanced agricultural investments are needed for improved land, water, and nutrient use to help counteract the negative effects of climate change on the global food security (Dar & Gowda, 2013). Protein is a major nutrient needed in the human diet and is abundant in livestock meat, thus human demand for livestock meat may also be expected to increase.

To feed the growing population, global agricultural food production output should increase production to approximately 200 million tons of livestock meat (Bruinsma, 2009). Furthermore, meat and meat products demand is set to increase despite the focus being on the continual price increase for meat proteins (Hoffman & Cawthorn, 2012). Broiler production represents one of the most economic and easiest means of bridging the supply-demand gap of animal protein, due to their rapid growth rate and superior feed conversion ratio (Khusro *et al.*, 2012). The main protein source for broiler production is legumes (Delgado *et al.*, 2001; Khusro *et al.*, 2012), which are also used by the biofuel industry that is expanding at a rapid rate (Biswas *et al.*, 2011). Thus, the biofuel industry has become the largest competitor for crop products, especially those high in fat (Biswas *et al.*, 2011). Therefore, there is an incentive to find an alternative feed source for broiler production that is not in competition with humans nor the biofuel industry.

Insects are rich in protein and fat; hence insects are consumed on a daily basis by free range animals and wild birds, making up a large portion of their diets (Miao *et al.*, 2005). In this regard, insect meals have been studied as potential feed ingredients in commercial animal diets, where they resulted in good growth performances, without compromising meat quality (Newton *et al.*, 2005a; Ijaiya & Eko, 2009; Hassan *et al.*, 2009; Barroso *et al.*, 2014; Pieterse *et al.*, 2014). Insects have high feed conversion efficiencies and act as bio-transformers converting organic waste to biomass and have the potential of reducing organic waste moisture content by over 50% (Diener *et al.*, 2009; Khusro *et al.*, 2012). Vermi-composting of organic waste produces larvae, pupae and pre-pupae which are high in protein (30-80%) and fat (14-35%); these ratios differing among insect species (Newton *et al.*, 2005b; Pieterse & Pretorius, 2014). The remaining vermi-composted biomass produced is usable as an animal feed ingredient or as a soil amender (DeFoliart, 1975).

Furthermore, during aerobic composting of organic waste with insects and/or larvae, biogas is produced that is collectable and useable to generate electricity (Banks *et al.*, 2011; Gonzalez-Gonzalez *et al.*, 2013). Therefore, an efficient production of insect meals for use in animal diets can be combined with various production systems as mentioned, to increase their economies of scale.

Most research on insect meals as alternative protein sources in animal diets has been on the house fly (*Musca domestica*), both in fish and poultry diets (Sealey *et al.*, 2011; 2012; Pieterse *et al.*, 2014) and black soldier fly (*Hermetia illucens*) (BSF) in fish diets (Barroso *et al.*, 2014). There is paucity of information on the usage of BSF as a feed ingredient in poultry diets. Hale (1973) reported similar weight gains in chickens fed BSF larvae meal and those fed soya bean meal. Black soldier fly pre-pupae meal (BSM) is high in protein with an adequate amino acid profile and a high Ca and P content, which are essential for normal bird skeletal development and growth (Newton *et al.*, 2005b). Furthermore, apparent digestibility values of BSM need to be analysed, thereby enabling appropriate inclusion in diets during formulation. Since it is a non-traditional feed its effects on organ, gut and skeletal parameters also need to be documented as these are vital in determining the effective use of BSM in producing broiler chickens. The use of BSF larvae/pre-pupae as an alternative protein source in poultry feeds has however, not been fully investigated. This underscores the need to investigate the potential of using BSF meals (larvae/pre-pupae) in broiler production, with particular attention to its effect on growth performance and meat quality.

Therefore, the aim of this study is to investigate the potential of BSM as a protein source on broiler production. The specific objectives were to evaluate:

- i. The apparent metabolisable energy and apparent digestibility of nutrients in BSM;
- ii. The production performance of broiler chickens fed BSM;
- iii. The effect of BSM on organ, gut and skeletal parameters of broiler chickens;
- iv. The effect of BSM on carcass yield and characteristics, physical measurements, sensory attributes and chemical meat quality of broiler carcasses.

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## Chapter 2

### Literature Review

#### 2.1 Introduction

The world is facing a drastic increase in human population leading to more mouths to feed (Dar & Gowda, 2013). However, not all agricultural production systems are sufficiently sustainable to feed the growing population (Capper, 2013). Although “sustainable” has many definitions it can be defined as “meeting the needs of the present without compromising the ability of future generations to meet their own needs” (Burton, 1987). Capper (2013), in a review on sustainable livestock production, noted that sufficient food should be produced from a sustainable food system that must reach the consumers. Globally, the animal production industry is experiencing a shortage in supply of feed ingredients lowering animal production. These have led to minimal animal protein being produced and available for human consumption, especially in developing countries (Teguia & Beynen, 2005). Furthermore, due to increase in disposable income the demand for animal protein is also increasing (Food & Agriculture Organization [FAO], 2009).

The agricultural industry during production of food for humans has produced a huge tonnage of waste that is not recovered, but has potential for recovery and reuse in another sector of the industry (Cordell *et al.*, 2009). For example, organic waste biomass as composted by insects can be used as an animal feed ingredient (Diener *et al.*, 2009) or as a soil amender (Newton *et al.*, 2005b). Insects have proven to be feasible decomposers of organic waste and useful as a nutrient recovery tool from waste (Newton *et al.*, 2005a). During vermi-composting, insect larvae, pupae and/or pre-pupae are produced that are useable as animal feed ingredients. This has led to insect meals attracting research attention as animal feed sources and is proving to be feasible for animal production and reducing waste accumulation (Newton *et al.*, 2005b; Ogunji *et al.*, 2008a, b; Diener *et al.*, 2009; Pieterse *et al.*, 2014).

Defining ‘waste’ is a challenge; the term waste can be described as subjective and inaccurate because waste to one person is not waste to another. In 1981, the Food and Agricultural Organization defined waste as “wholesome edible material intended for human consumption, arising at any point in the food supply chain that is instead discarded, lost, degraded or consumed by pests” (Boland *et al.*, 2013). The term ‘discard’ is an elemental part of defining waste, to be approached effectively without posing a risk to the environment (Cheyne & Purdue, 1995).

On that point, waste can be classified from different origins such as solid municipal waste, non-hazardous waste, non-industrial and agricultural wastes and are mostly disposed of in landfills. However, with increase in livestock production, industrialisation and population, land is becoming a scarce resource and therefore, alternative disposal methods should be developed. Waste disposal as landfills are also detrimental to the environment and can cause technical and social issues, such as environmental pollution and the release of toxins into the air, soil, river and dams (Seng *et al.*, 2013). Amongst others: organic waste contains high energy and a nutrient content suitable as feed for other forms of life such as insect larvae (Lalander *et al.*,

2013). Therefore, organic waste can be composted using insect larvae leading to protein recovery (El Boushy, 1991; Li *et al.*, 2011), reduction of moisture and thus, providing a solution to waste disposal (Newton *et al.*, 2005a, b; Diener *et al.*, 2009; Kim *et al.*, 2011; Zhang *et al.*, 2012).

Black soldier fly (*Hermetia illucens*) larvae are known to consume decomposing organic matter reducing the dry matter of waste to about 60% (Newton *et al.*, 2005a; Kim *et al.*, 2011). Insect larvae, pre-pupae and pupae produced are high in energy and protein (Jeon *et al.*, 2011). Pre-pupae and larvae meal of black soldier fly (BSF) can be utilised as a feed ingredient in animal diets, and has been researched to a large extent in fish but minimally in monogastrics' and other animals (Bondari & Sheppard, 1981; Bondari & Sheppard, 1987; Newton *et al.*, 2005b; St-Hilaire *et al.*, 2007a; Sealey *et al.*, 2011).

This chapter therefore, aims to review the recovery of protein from various industry wastes, using insect larvae and hence their potential usage as animal protein sources. This study utilises BSF pre-pupae meal as a protein source in broiler production and thus in this review emphasis is placed on the BSF life cycle, organic waste decomposition and potential uses of their larvae and pre-pupae produced.

## 2.2 Agricultural waste

Smit & Nasr (1992) defined urban agriculture as "food and fuel grown within the daily rhythm of the city or town, produced directly for the market and frequently processed and marketed by the farmers or their close associates". With an increase in population growth, crop and livestock production, land is becoming a scarce resource making waste disposal more of a challenge (Seng *et al.*, 2013). As livestock production changes towards industrialization, it produces animal waste that exceeds available land for its storage (Mallin & Cahoon, 2003).

Waste from households, food industries, abattoirs, manure and sewage are known to be recyclable and can be used as soil amenders. However, the presence of pathogenic micro-organisms limits the use of some organic wastes because of potential health risks. Waste can also be detrimental to ground water and crops if not properly managed before being used. During composting, most pathogens are destroyed as the temperature rises (Georgacakis *et al.*, 1996). However during composting at temperatures between 15 and 45 °C, *Salmonella* spp. and *Escherichia coli* O157 can increase in numbers. Furthermore, *Salmonella tryphimurium* can grow and survive in household waste at 14, 24, and 37 °C (Elving *et al.*, 2010).

Kitchen waste also has the potential of spreading infectious diseases if not properly disposed of. The disposal of kitchen waste is a challenge; it contains high protein and fat content producing high concentrations of ammonia, causing accumulation of volatile fatty acids during anaerobic fermentation (Banks *et al.*, 2011; Dlabaja & Malat'ák, 2013). However, anaerobic digestion of organic waste produces biogas and recovered material is useable as a soil fertilizer/amender (Banks *et al.*, 2011).

### 2.1.2 Quantifying waste production

The amount of manure produced is increasing with livestock production, producing a vast quantity of this waste. According to Arkhipchenko *et al.* (2005) in the St Petersburg region, 11 million chickens produced 400 000-450 000 tons of manure and 150 000 pigs produced 1.5 million m<sup>3</sup> of liquid waste per annum. A dairy cow on average produces 57 litres (L) of excreta per day (Welsh Ministry of Agriculture, Fisheries & Food, 1991). In the 2012 financial year, South Africa had a livestock population of about 247.053 million (Table 2.1), mostly reared under intensive systems.

**Table 2.1** Animals produced during the 2012 financial year in South Africa (FAO, 2014)

Animal species	Total number (million)
Cattle	13. 888
Sheep	24. 391
Goats	6. 142
Pigs	1. 579
Poultry	201. 053
Total	247. 053

Another waste product that is causing concern due to its potential negative effect on the environment is the blood released during the slaughter of animals. One steer yields 10-12 L of blood and a sheep  $\pm$ 2.5 L of blood (Nollet & Toldrá, 2011). In the 2012 financial year, 2. 822 million cattle were slaughtered (excluding calves) in South Africa (Department of Agriculture, Forestry & Fisheries [DAFF], 2012) translating into approximately 28. 220 million L (27.17 tons) of blood produced as part of the abattoir waste. In the United Kingdom over 100 000 tons of blood is yielded every year, containing about 20 000 tons of protein (Arvanitoyannis & Ladas, 2008). Since the outbreak of bovine spongiform encephalopathy (BSE) many countries including the European Union have banned the use of animal origin protein as feed for livestock (Smith & Bradley, 2003; Hard, 2004). Therefore, alternative uses need to be found for this “new” waste product.

Briefly, a vast amount of waste is generated worldwide by agricultural production in its various sectors. The by-product wastes are mainly organic and can be composted either by aerobic or anaerobic processes. The composted material has potential use as an animal feed ingredient, soil enhancer, amender and/or fertilizer. There is currently a shortage of feed ingredients in the animal production sector especially those rich in protein, hence a need to find suitable feedstuff for use in animal diets. Protein recovery from waste is one of the possible routes to enhance environmental sustainability and prevent food shortage globally, with the ever growing human population. The following section reviews recovery of nutrients from waste.

## 2.2 Recovery of protein/usable substances from agricultural waste

### 2.2.1 Manure

Manure composition differs from species to species but on the whole is pre-determined by the diet of the animal (Kirchmann & Witter, 1992). Livestock manure contains large amounts of faecal bacteria. These microbes are however, not exposed to secondary treatment (disinfection) before disposal, as opposed to



human waste (Mallin & Cahoon, 2003). During composting of livestock manure, the temperature is raised to a high level that kills most microbes (Mawdsley *et al.*, 1995; Georgacakis *et al.*, 1996; Lalander *et al.*, 2014). Lalander *et al.* (2014) found viable viruses in pig manure and human faeces to have been reduced below detection limit after 14 days of vermi-composting with BSF larvae. Animal waste slurries do not reach lethal temperatures allowing microbes in animal waste slurries such as lagoon liquid to survive for extended periods (Mawdsley *et al.*, 1995). Mawdsley *et al.* (1995) reported that *E. coli* could survive up to 11 weeks in animal waste slurry. Alternative methods to decrease these microbiological loads need to be exploited. As example, BSF larvae feeding on cow manure reduced *E. coli* (Liu *et al.*, 2008) and on pig manure reduced *Salmonella* spp. (Lalander *et al.*, 2014) significantly as the temperature rose from 23 °C to 35 °C. Furthermore, horse manure inoculated with earthworms was found to reduce the pathogenic persistence of bacteria (Murry Jr & Hinckley, 1992).

Fly larvae, worms and beetles readily feed on fresh manure converting its residual protein and other nutrients into insectivorous biomass, which can be used as an animal feedstuff or soil fertilizer (DeFoliart, 1975). Biomass can be defined as the total amount of living material in a given sample. These can be plant material, animal or other organic waste that can be used for energy. Kim *et al.* (2011) noted that BSF were able to consume and digest raw organic waste materials (manure, kitchen waste, abattoir waste: blood and offal's etc.) more rapidly and resourcefully than the house fly (*Musca domestica*). This is due to the high digestive enzyme content secreted in the gut and salivary gland of the BSF. The larvae of BSF are known to be voracious consumers of organic matter. Li *et al.* (2011) observed that 1248.6 g fresh manure treated with 1200 BSF larvae would produce 15.8 g of biodiesel, 54.4 g of residual larvae and 96.2 g of sugar. The larvae of BSF have a protein content of 42% and 38% fat (Newton *et al.*, 1977), which varies depending on feed substrate fed to the larvae (Ramos-Elorduy *et al.*, 2002; St-Hilaire *et al.*, 2007). Once the larvae have metamorphosed into pre-pupae it contains about 40% crude protein and 30-35% fat (Newton *et al.*, 2005b; Diener *et al.*, 2009).

House fly (HF) larvae have been studied for its ability to compost animal manure (El Boushy, 1991; Zhang *et al.*, 2012; Wang *et al.*, 2013). Composting of manure using insect larvae can reduce the manure moisture content by 40-80% resulting in a total mass weight reduction of 55-65% depending on the insect species used (Newton *et al.*, 2005b; Diener *et al.*, 2009; Kim *et al.*, 2011; Zhang *et al.*, 2012; Wang *et al.*, 2013). In addition, insect larvae feeding on manure decrease the nutrient concentration and bulk of the manure residue leading to a 50-60% reduction of possible air pollution (Newton *et al.*, 2005b; Everest Canary & Gonzalez, 2012). Furthermore, adding bulk agents to manure during composting led to a reduction of nitrogen released into the atmosphere (Barrington *et al.*, 2002). Composting of manure reduces its moisture content, making it less attractive to insects and causing it to be easily handled and stored (Barrington *et al.*, 2002). In addition, the remaining decomposed manure can be used as a soil amender (Newton *et al.*, 2005b; Sheppard *et al.*, 2007) and/or as an animal feed ingredient (Diener *et al.*, 2009).

Broiler litter processed by deep stacking can replace 26% of cotton seed cake in ruminant rations as a protein source. Processed broiler litter as a feed ingredient in buffalo steers led to an increase in body weight

but a decrease when added over 26% (Chaudhry & Naseer, 2009). The price of cotton seed cake is increasing (Sarwatt *et al.*, 2004). Therefore, substituting processed broiler litter partially as a protein source in ruminants may reduce feed cost. Dehydrated poultry excreta used as feed in cattle had a nitrogen ruminal degradability of 78% and post-ruminal degradability escape of 27% nitrogen. The dehydrated poultry excreta are low in energy due to its high ash content (Zinn *et al.*, 1996), but can be formulated with other feed ingredients that are high in energy. However, most importantly poultry litter is banned for use as animal feed except in the United States but a withdrawal period of 21 days is required before slaughter due to pharmaceuticals used in poultry which may be deposited in the meat (Olson & Daniel, 2005).

Composting of organic matter emits ammonia, however ammonia produced in aerobic digestion can easily be eradicated by installing a proper ventilation system (Banks *et al.*, 2011). Anaerobic digestion of high energy food waste emits vast concentrations of ammonia which cannot be easily collected (Banks *et al.*, 2011). Furthermore, composting aerobically of animal excreta and other organic waste stabilize its carbon content, thereby transforming waste into a suitable soil amender. However, nitrogen in waste is better conserved in anaerobic digestion than with aerobic composting (Kirchmann & Bernal, 1997).

### **2.2.2 Abattoir waste**

Abattoirs produce vast amounts of organic waste. Abattoir waste, also called slaughter waste, is defined as the waste considered unfit for human consumption or not suitable for consumption for reasons associated with consumers' lifestyle (Weiers & Fischer, 1978). Abattoir waste consists of bones, hides, blood, gut and gut content, which are high in protein and fat (Adeyemi & Adeyemo, 2007). However, abattoir waste differs from country to country and place to place, in terms of what is regarded as inedible and edible. In most developing countries, blood and rumen content are considered as abattoir waste, hides are processed and the remaining (also known as offal) consumed by humans (Makinde & Sonaiya, 2010). In Nigeria it has been found that approximately 46% of a cow, 48% of a sheep, 38% of a pig and 28% of a chicken after processing is waste and is disposed of by dumping onto open fields or into municipal sewers (Adeyemi & Adeyemo, 2007).

Abattoir wastes can be a potential health hazard and may cause outbreak of food-borne diseases when not properly disposed of (Couillard & Zhu, 1993). *Echerichia coli* O157 which is pathogenic in humans, can survive in abattoir waste stored at 5 °C for 28 hours. Fresh blood has a lower bacterial load than aged blood creating a favourable environment for microbial pathogen growth (Hepburn *et al.*, 2002).

Abattoir waste can be treated by anaerobic digestion producing biogas whilst the remaining waste is usable as soil fertilizer (Adeyemi & Adeyemo, 2007). Abattoir wastes contain lipids and if treated through anaerobic digestion, it has a high potential for producing methane (Affes *et al.*, 2013), which can be used as fuel in electricity plants to provide energy (Gonzalez-Gonzalez *et al.*, 2013). Abattoir waste when treated by an aerobic thermophilic process recovered gross protein of 70% containing an acceptable amino acid profile (Table 2.2). An additional advantage is that digested slaughter waste effluents reclaim large volumes of water that can be used for field irrigation (Gonzalez-Gonzalez *et al.*, 2013). Thus, this enables recovering of

protein, recycling water and processing wastes before it's disposed of causing less negative impacts on the environment.

Feathers constitute about 5% of the total body weight of a bird. A broiler slaughterhouse with a 50 000 bird per day slaughter capacity produces about 2-3 tons of feathers per day (Dalev, 1994). If untreated these accumulate to 10-15 tons of waste feathers produced per week. Processing of bird feathers is a challenge on a large scale and there is need of a readily available non-toxic substance for the processing of feathers which can improve its nutritional value for possible use in animal diets (Dalev, 1994; Kumar *et al.*, 2012). Feathers that were pre-treated with sodium hydroxide, mechanically disintegrated and then hydrolysed by an enzyme led to total solubilisation of the feathers producing a powder high in protein (Karam & Nicell, 1997; Kumar *et al.*, 2012). Furthermore, Bertsch & Coello (2005) successfully processed feathers through fermentation using *K. rosea* LPB-3 a non-pathogenic bacterium. Feather powder contains large amounts of essential amino acids, usable as a feed component and as soil fertilizer, when corrected for sulphur (Dalev, 1994; Kumar *et al.*, 2012).

**Table 2.2** Amino acid (%) profile of slaughterhouse waste (adapted from Couillard & Zhu, 1993)

Amino acids	Recovered biomass (at 90% dry matter)
Arginine	3-4
Histidine	0.9
Isoleucine	1.8
Leucine	3-5
Lysine	2-4
Methionine + Cysteine	1.3
Phenylalanine + Tyrosine	3-8
Threonine	1-9
Tryptophan	0.2
Valine	2.1
Glycine	3.9
Proline	1.2

Since the outbreak of BSE disease, the use of animal origin feed has been banned in most countries and is strictly regulated by the European Union. This has contributed to the increase in competition between humans and animals for grains and legumes as food sources. Meat compost and powder were found to be good soil fertilizers increasing the yield of maize and mustard crops during drought seasons. In addition, meat products are banned as animal feed but not as soil fertilizer, providing a method of disposal and nutrient recovery (Ragályi & Kádár, 2012). The recovered protein from waste once treated, can be appropriately disposed of. Therefore, recovered nutrients may be recycled through soil and produce more food by enhancing soil quality.

### 2.2.3 Waste water

Waste water contains amongst others, toxic aromatic compounds which should be removed before disposing of the water. The use of enzymes for treatment of waste water seems promising. Enzymes can operate over

a broad aromatic concentration range and require low retention times; they are highly selective and can effectively treat and even dilute waste. Protease can solubilize proteins in waste streams producing dry solids or liquid concentrates that can be used in fish or livestock feed. Amylase can simultaneously scarify and ferment starch in waste waters, producing lactic acid that can be used in the production of photo- and bio- degradable plastics from waste such as cheese whey and potato by-products (Karam & Nicell, 1997). The use of enzymes has proven their ability to recover usable contents from waste solutions. The recovered material can be used to enhance production of another line within the industry, for example plastic production.

Cellulose containing agricultural waste materials has a potential in bio-sorption of metals. This is becoming an area of interest due to agricultural materials being cheap, abundant, renewable and usable as feed for animals. Depending on their chemical composition some materials bind to numerous heavy metals, of which some are specific. The capacity, affinity, specificity and physico-chemical nature of the bio-sorption material determines its efficiency in metal removal present in waste. Therefore, waste such as peanut shells, soya bean hulls, orange peels, maize cobs, hazelnut shells and jack fruit has been researched and found to be efficient in chromium removal from aqueous waste. Maize bran also has ability to optimally bio-sorb the metal lead (Pb) at 20-30 °C temperature and a pH of 3.0-6.5 in aqueous waste (Sud *et al.*, 2008). The recovered waste water is usable as field irrigation water, thus recovering nutrients and saving water.

Therefore, processing of agricultural waste in various ways reduces waste bulkiness and potential gas emissions into the atmosphere. The decomposed organic wastes can be used as a soil fertilizer or amender and as an animal feed ingredient. Therefore, this will minimize disposal problems of accumulating animal excreta caused by the increased production of animals. Alternatively, insect larvae have the potential of altering the remaining protein and several nutrients in organic waste into high quality organic matter which can be used as animal feedstuff or as a soil amender. This in turn will produce animal and plant protein, but research is warranted to evaluate its feasibility in animal diets and test whether produced products are fit for human consumption. In this study, the BSF species is utilized as a protein source in broiler chicken diets. Therefore, the origin, behaviour and life cycle of the BSF is of principle importance in understanding the fly species.

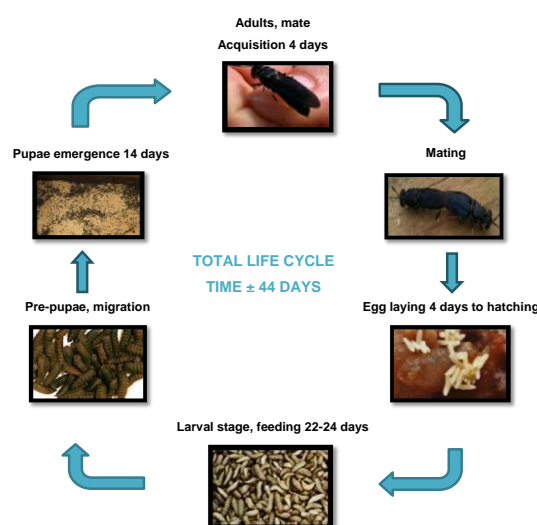
### **2.3 Black Soldier Fly (*Hermetia illucens*)**

Furman, Young and Catts were amongst the first researchers to carry out a contemporary study on *Hermetia illucens* in 1959 (Canary & Gonzalez, 2012). Black soldier fly (*H. illucens*) is a native insect common to the warm temperate region of south-eastern United States (Newton *et al.*, 2005b). According to Canary & Gonzalez (2012), BSF have been found all over South America and Asia but was native in Medellin, Colombia. The warming of climate is one factor that causes the spread of insects to other parts of the world (Turchetto & Vanin, 2004). Locomotion of humans and goods across the globe has also led to the wide spread of arthropods.

Black soldier fly larvae are known as ravenous consumers of decomposing organic matter, leading to adults being able to live without feeding using body fat storage for survival (Newton *et al.*, 2005b; Kim *et al.*, 2011). Their unique feeding enable them in their last developmental stage (pre-pupae) to migrate out of the waste to a dry place, making them simple to harvest. During the pre-pupae stage of BSF they no longer consume food, thus they have an empty gut and are high in stored energy (Sheppard *et al.*, 1994). Black soldier fly pre-pupae meal is a suitable potential feed ingredient for use in animal diets as it is high in protein and energy (Jeon *et al.*, 2011). Furthermore, BSF larvae when feeding on waste can eradicate the breeding of the HF thus reduce possible disease spreading by the HF (Bradley & Sheppard, 1984). Black soldier fly is a non-pest fly (Kim *et al.*, 2011) and the female flies lay only a single egg batch and die shortly thereafter (Tomberlin *et al.*, 2009). Fly fitness in the BSF species is more vital than longevity and is determined by energy reserves obtained while feeding. Black soldier flies can thrive at temperatures between 27-36 °C, and perform optimally at 30 °C with a declining survival rate and development at temperatures above 36 °C (Tomberlin *et al.*, 2009).

### 2.3.1 Black soldier fly life cycle

The life cycle of black soldier fly is 40-44 days as depicted in Figure 2.1 (Tomberlin *et al.*, 2009; Alvarez, 2012) but is pre-determined by the environment and the type of waste fed (Tomberlin *et al.*, 2002; Diener *et al.*, 2009; Jeon *et al.*, 2011). Food quality and the number of mating's determines the production of eggs in BSF. The flies fed an adequate diet and mated several times produce more eggs. The flies fed directly after hatching have a higher mating rate then their counterparts delayed in feeding. Black soldier flies can still reproduce without feeding after emerging into a fly, relying on fat reserves stored during larvae development. However, these flies die shortly after laying eggs which may be attributed to the depletion of fat reserves (Tomberlin *et al.*, 2002). With regards to the male BSF, no information was found on what happens to them after mating and forth.



**Figure 2.1** Life cycle of the black soldier fly (*Hermetia illucens*) (Alvarez, 2012)

## 2.4 Enzymatic activity of insects

Insects are known to protect themselves against microbial infections by producing antimicrobial substances from their digestive tract or surface exoskeleton. Furthermore, methanol extracts of BSF larvae have antibacterial activity that strongly inhibits the growth and proliferation of certain gram-negative bacteria (Choi *et al.*, 2012). These antimicrobial substances do not seem to be effective on gram-positive bacteria.

Jeon *et al.* (2011) found that intestinal bacteria of BSF larvae led to the degradation and reduction of the organic compounds of the food fed to the larvae, producing probiotic compounds that can improve soil conditions. This was due to the unique gut microbiota, which was found in BSF larvae fed three different diets. However, it could not be determined whether the bacteria existed temporarily or as symbiotic organisms in the gut.

Larvae of *Hepialus gonggaensis* (ghost moth) were found to contain *Rahnella* and *Carnobacterium* strains amongst six others in the gut, which are known to reduce nitrate to nitrite, producing L-lactose from glucose and also producing acid from D-glucose. This plays a role in the metabolism and food utilization in insects (Yu *et al.*, 2008). Furthermore, Lalander *et al.* (2013) found BSF larvae to have significantly reduced *Salmonella* spp. in human faeces during composting. Black soldier fly larvae had no positive effect on the deactivation of *Ascaris suum* ova, recommending further treatment when used as crop fertilizer.

Insect larvae can compose and reduce organic waste content. Table 2.3 summarises the effects of composting swine manure with HF larvae. House fly significantly reduced the moisture, odour, faecal coliform, and fat content of the manure, while increasing its organic carbon and fibre content significantly (Zhang *et al.*, 2012). After one week of composting with HF larvae, there was an overall mass reduction of 55-65%. The organic carbon content in the waste as it decomposes might be a key indicator of the biochemical features and microbial functions of the larvae vermi-reactor. Since, results showed a correlation between microbial biomass and extracellular enzyme activities to the organic carbon content of waste before and after composting (Zhang *et al.*, 2012). Vermi-composting is the decomposition of organic waste by worms under aerobic and mesophilic conditions (Gómez-Brandón *et al.*, 2013).

**Table 2.3** Comparison of swine manure before and after vermi-composting using house fly larvae (adapted from Zhang *et al.*, 2012)

Manure content		Before vermi-composting		After vermi-composting		t-Test P-value
		Average	n	Average	n	
Moisture	%	78.3 ± 5.4	8	47.6 ± 1.6	8	<0.001
Organic carbon	%	32.5 ± 12.4	8	53.3 ± 4.7	8	<0.001
Crude fibre	%	17.2 ± 2.8	4	20.5 ± 2.7	4	0.008
Crude fat	%	4.61 ± 0.54	4	1.35 ± 0.43	4	0.002
TKN	% (N)	2.99 ± 0.65	8	2.20 ± 0.31	8	0.346
AN	% (N)	0.575 ± 0.079	8	0.441 ± 0.125	8	0.021
TP	% (P)	1.82 ± 0.54	8	2.86 ± 0.36	8	0.054
AP	% (P)	0.827 ± 0.43	8	1.15 ± 0.07	8	0.017
Odor (3-MI)	mg/kg	40.4 ± 7.5	2	2.24 ± 1.41	3	<0.001
Faecal coliforms	log/g	33.7 ± 16.9	2	3.01 ± 0.78	2	<0.001

n (Number of samplings)

TKN (Total Kjeldahl nitrogen)

AN (Available nitrogen)

TP (Total phosphorous)

AP (Available phosphorous)

#### 2.4.1 Other beneficial characteristics of insect larvae

Black soldier fly larvae and/or pre-pupae are used in forensic studies to estimate the post-mortem interval (PMI) on human corpses (Pujol-Luz *et al.*, 2008; Martínez-Sánchez *et al.*, 2011); with error implications that can occur as BSF development differs according to differences in temperature (Tomberlin *et al.*, 2009). The forensic PMI is defined as the time in which a dead body has been exposed to the environment (Turchetto & Vanin, 2004), which helps to determine the time of death.

Arrival time of insect species on corpses differ, with *Chrysomya megacephala* and *C. rufifacies* being usually the first flies to appear on the corpse within five minutes after death. Other species such as *H. illucens* and beetles arrive during the advanced decaying stage of the corpse. The larvae are collected, identified and human DNA is extracted from their gut. The extracted DNA is used in polymerase chain reaction for profiling (Chua & Chong, 2012). Proper identification of the specimen (fly species) is the key foundation in forensic entomology for PMI estimation (Chua & Chong, 2012; Turchetto & Vanin, 2004; Velásquez *et al.*, 2010; Martínez-Sánchez *et al.*, 2011).

Knowing the various benefits of insect larvae and their potential uses raised questions regarding food production utilising insect meals. This led into studying the nutritional composition of insect meals and their use as a feed ingredient in poultry diets.

## 2.5 Poultry Nutrition

When formulating feed rations different ingredients are mixed to produce a balanced diet with appropriate quantities of nutrients to sustain normal growth. Since poultry have a short digestive tract they require nutrient-dense diets to obtain high performances. Nutrients required by birds depend on the species, age



and type of production. Poultry cannot digest complex carbohydrates containing insoluble fibre but readily obtain energy from simple carbohydrates, fat and protein (Hetland *et al.*, 2004; Ravindran, 2013). However, when fed high concentrate diets containing insoluble fibre, the latter helps improve feed utilisation by the bird (Hetland *et al.*, 2004).

Anti-nutritional components within the feed also effect the efficient utilisation of the feed by the birds. They are usually found in small quantities but can alter the nutrient quality of the feed, as some nutrients will not be available for digestion by the bird (Aletor & Adeogun, 1995). Aflatoxins and ochratoxins included in broiler diets led to a reduction in the breaking strength of the chicken's bones and increased its flexibility (Huff *et al.*, 1980). Furthermore, the presence of anti-nutritional factors such as soluble non-starch polysaccharides (Rebolé *et al.*, 2010), protease and trypsin inhibitors (Clarke & Wiseman, 2000) and mycotoxins, aflatoxin and ochratoxins (Huff *et al.*, 1980; Awad *et al.*, 2006) can affect nutrient utilisation adversely and possibly decrease animal growth performance. It is however, not known whether insect larvae, pre-pupae and/or pupae contain any ant-nutritional factors that might inhibit its efficient utilisation in broiler chicken diets, which warrants research.

### **2.5.1 Protein**

Protein is an essential nutrient in animal diets required to ensure adequate growth and health. The performance of broilers (based on nutrient utilisation) is influenced by the metabolisable energy and crude protein (CP) content of the diet (Zaman *et al.*, 2008). However, adequate energy must be supplied so that the dietary protein is used for growth rather than metabolised for energy. A proper ratio of energy and protein should be maintained as excessive energy can cause reduced feed intake, resulting in decreased growth (Aletor *et al.*, 2000; Ravindran, 2013). However, a high CP diet in broilers is not economical as this increases the nutrient specification of the amino acids (AA). This will lead to an increase in the bird's metabolism in order to catabolize excess AA, thereby causing a loss of energy and hence decrease in body weight as compared to low protein diets (Kidd *et al.*, 2001). Alternatively, a high CP diet is to be supplemented with high energy source to ensure a balanced intake of AA, as birds will consume less feed with high energy diets (Ensminger, 1992).

Dietary proteins' function is to provide AA to the birds. Protein quality is determined by the available dietary AA that can be digested and absorbed by the animal to maintain its metabolic processes (Boland *et al.*, 2013). However, no single protein source contains the entire AA requirement in a balanced ratio to ensure maximum chicken performance and alternative strategies have to be applied to address this shortage. Production of synthetic AA for use in animal feed is one of the ways to bridge the protein supply chain and address this shortage. According to the FAO (2004), supplementing feed with synthetic AA can reduce protein levels in diets by 2% annually. This will save plant protein added to rations, reducing food source competition between humans, animals and emerging competitors (for example the biofuel industry).

The protein and AA requirement amongst different animal species that are needed for growth is indicated in Table 2.4. Optimum human and animal performance is dependent on the AA contained in the protein source,



amongst other nutrients needed for growth, health and reproduction. Amino acid composition of protein is important as not all AA can be synthesised by the body, hence supplementation is required (Boland *et al.*, 2013).

**Table 2.4** Dietary crude protein requirement (% dry matter) and ideal amino acid pattern (g/g lysine) of essential amino acids for growth of different animal species (adapted from Boland *et al.*, 2013)

Nutrients	Human	Pig	Poultry	Nile tilapia
Crude protein	10-15.00	15-29.00	18-23.00	30.00
Arginine	-	0.38	1.10	0.82
Histidine	0.33	0.32	0.32	0.34
Isoleucine	0.67	0.54	0.73	0.61
Leucine	1.30	1.00	1.09	0.66
Lysine	1.00	1.00	1.00	1.00
Methionine	0.33	0.27	0.38	0.52
Phenylalanine	0.83	0.60	0.65	0.73
Taurine	-	-	-	-
Threonine	0.50	0.64	0.74	0.73
Tryptophan	0.13	0.18	0.18	0.19
Valine	0.87	0.68	0.82	0.55

It is not only the amount of AA present that determines its nutritional value, but even more importantly, the digestibility of the AA. The digestibility of the protein and its AA in feed is predetermined by the protein source and is depended on the animal species for effective use (Boland *et al.*, 2013). The protein source should contain an appropriate AA profile (Table 2.4) and be highly soluble with limited or no ant-nutritional factors. Processing methods are another limiting factor to the bio-availability of protein in feed to the animals (Choct & Kocher, 2000). The use of heat and acid treatment may lead to protein denaturation during processing (Boland *et al.*, 2013). Lysine is the most affected AA by extreme heat processing as it is susceptible to Maillard reactions reducing its availability for use by the animal (Parsons, 1996). Therefore, protein ingredients for animal feed must be handled with care. It is evident that insect derived protein source meals are highly digestible in poultry as indicated in Table 2.5.

**Table 2.5** Protein content and digestibility of protein in broiler chickens in percentage

Protein source	Crude protein	Apparent digestibility	Reference
<b>Plant products</b>			
Soya bean meal	49.44	85-87.00	Boland <i>et al.</i> (2013)
<b>Animal by-products</b>			
Fish meal	60.20	91.30	Pieterse & Pretorius (2014)
<b>Insect products</b>			
HF larvae meal	60.38	69.00	Pieterse & Pretorius (2014)
HF pupae meal	73.26	79.00	Pieterse & Pretorius (2014)
Field cricket	58.30	92.90	Wang <i>et al.</i> (2005)
HF (House fly)			

Feed formulation of poultry has evolved over the years identifying that birds do not require protein '*per se*' but rather availability of AA within the protein source. Therefore, poultry diets are formulated based on

availability of AA in the feed ingredient used (Lemme *et al.*, 2004). The AA profile values for inclusion in a ration should be specific and not in excess or less than required, as this may lead to inhibition of growth especially with methionine, lysine and threonine in poultry diets (Lewis *et al.*, 1963). Furthermore, in poultry diets methionine or methionine plus cysteine is/are the first limiting amino acid(s) on bird growth followed by lysine and then threonine (Ravindran, 2013). Even though methionine is the first limiting AA, lysine is the preferred reference amino acid in diet formulation, since it is easier to analyse, least affected by metabolic functions and forms no metabolic conversions with other AA (Lemme *et al.*, 2004). Excess lysine inclusion in poultry diets may cause antagonism, while excess methionine may cause toxicity. Arginine and lysine have an antagonistic relationship which is prevented when supplied in a 1:1 ratio (Austic & Scott, 1975). Furthermore, in poultry diets if arginine is in excess lysine is added to correct for arginine to prevent losses on growth performance. However, the effect of lysine and arginine on body weight gain and feed conversion ratio is more evident when lysine is in excess than when arginine is in excess (Lewis *et al.*, 1963; Balnave & Barke, 2002). Chamruspollert *et al.* (2002) found increased methionine in broiler chicken diets to have improved growth and feed intake significantly. Furthermore, addition of methionine to a diet containing excess arginine was found to alleviate growth depression of broiler chickens (Chamruspollert *et al.*, 2002).

### **2.5.2 Use of alternative feed ingredients**

Broiler production cost is mainly dominated by the feed costs which amount to about 70% of the total production cost. Grains are the main feed ingredient in poultry diets but are also a staple food for humans in most developing countries (Teguia & Beynen, 2005). Moreover, with the increase in the human population, the demand for food has been increasing causing more of a challenge to feed the growing nation. Another factor influencing food availability rather than food competition and population increase is global warming, causing weather changes and thus reducing crop yields. These factors have contributed to an increase in grain commodity price attributing to a costly production of livestock and its products (Dar & Gowda, 2013). Therefore, there is a need for the search of alternative feed ingredients with an appropriate nutritional value for use in animal feeds, in this context focusing on poultry. These ingredients should be accessible, affordable and not in competition with humans. Alternative feed ingredients are not only needed in the poultry industry but also in other animal species used for farming. Insect meals are amongst the possible alternative feed sources under consideration owing to its performances when included in animal diets (Téguia *et al.*, 2002; Ogunji *et al.*, 2008a, b; Ijaiya & Eko., 2009; Sealey *et al.*, 2011).

There are about 10 million living species in the world, of which more than 60% are insects. Insect are the most diverse group of living organisms on earth. This may be due to their efficient defence systems providing them with an innate immunity enabling them to prosper amongst other living species (Masova *et al.*, 2010). DeFoliart (1989) estimated a total of about 500 insect species from 260 genera and 70 families of insect used as human food in central northern Africa, Asia, Australia and Latin America. However, DeFoliart (1989) further stated the estimated values to probably be more than the reported statistics. According to Jongema (2014) there are about 2 040 edible insect species recorded worldwide. This statistics substantiate statement by DeFoliart (1989) that there are more insect species than reported.

The diversity of insect species, availability and exceptional nutritional content makes them eligible sources for use as food. In animal diets, feed ingredients are analysed for their nutritional content to enable relatively accurate ration formulation that meet the nutrient requirements of the animals. Insect meals have an adequate amino acid content which is comparable or even better than the traditional feed ingredients: fish meal and soya bean meal (Table 2.6). This signifies insect meals to be potential protein sources for use in animal diets.

**Table 2.6** Comparison of the nutritional value of insect meals with that of fish and soya bean meal

Parameters	HFM <sup>1,a</sup>	M <sup>2,b</sup>	FC <sup>3,c</sup>	GH <sup>4,d</sup>	SWC <sup>5,e</sup>	FM <sup>6,f</sup>	SCM <sup>7,f</sup>	HFP <sup>8,g</sup>	BSM <sup>9,h</sup>
Proximate analysis (%)									
Crude protein	47.60	55.10	58.30	53.58	50.30	69.13	49.44	76.23	43.20
Crude fat	25.30	20.70	10.30	26.52	16.43	10.11	0.90	14.39	28.00
Crude fibre	7.50	6.30	8.70	9.21	10.90	0.54	7.87	15.71	-
Ash	6.25	10.40	2.96	4.31	12.03	-	5.90	7.73	16.60
Amino acids (%)									
Lysine	6.04	2.92	4.79	-	5.02	3.57	3.05	4.92	2.21
Methionine	2.28	-	1.93	-	3.02	1.09	0.70	1.37	0.83
Threonine	2.03	-	2.75	-	4.50	1.47	1.95	2.31	1.41
Mineral content (%)									
Ca	-	-	-	-	1.05	1.34	0.33	0.52	5.36
P	-	-	-	-	2.77	1.77	0.73	1.72	0.88

<sup>a</sup> Aniebo *et al.* (2009)

<sup>b</sup> Awoniyi *et al.* (2003)

<sup>c</sup> Wang *et al.* (2005)

<sup>d</sup> Hassan *et al.* (2009)

<sup>e</sup> Ijaiya & Eko (2009)

<sup>f</sup> National research council (2004)

<sup>g</sup> Pieterse & Pretorius (2014)

<sup>h</sup> Newton *et al.* (2005a)

<sup>1</sup> HFM (Housefly maggot, blood & wheat meal)

<sup>2</sup> M (Maggot meal)

<sup>3</sup> FC (Field cricket meal) meal

<sup>4</sup> GH (Grasshopper meal meal)

<sup>5</sup> SWC (Silkworm caterpillar

<sup>6</sup> FM (Fish meal dehydrated)

<sup>7</sup> SCM (Soya oil cake meal)

<sup>8</sup> HFP (Housefly pupae meal dried)

<sup>9</sup> BSM (Black soldier fly pre-pupae meal dried)

### 2.5.2.1 House fly meal

Calvert *et al.* (1969) evaluated the use of HF pupae meal (crude protein 63.1%) as a feed ingredient in poultry diets, partially substituting soya bean meal. The results obtained were favourable and warranted further research. The biological assay of HF larvae and pupae meal nutrients fed to broiler diets revealed HF to be highly digestible and their chemical analyses determined a complimentary nutrient composition of both larvae and pupae meals (Pieterse & Pretorius, 2014).

Pieterse *et al.* (2014), found significantly higher live and carcass weights in birds fed 10% HF meal but observed no treatment differences on sensory analysis of broiler chicken breast muscle for chicken aroma, initial juiciness, chicken flavour and tenderness. In another study by Zuidhof *et al.* (2003) on turkey poults fed HF larvae meal, a decrease in feed intake of poults receiving the larvae meal attaining a daily feed consumption of 39 g and an average weight gain of 34 g was observed during the digestibility study. However, the poults fed the commercial diet consumed about 60 g per day and gained 43 g. These results

look promising but no statistically analysed results were found to indicate liable differences in growth performance of the turkey poultts given it was a digestibility study and not a production study. Agunbiade *et al.* (2007) found maggots collected from poultry manure to have supported normal egg production without compromising its quality by analysing egg weight, shape, yolk colour and index, shell thickness and weight.

Ogunji *et al.* (2008a) evaluated HF maggot meal (MGM) as a protein source for *Oreochromis niloticus* (Nile tilapia). It was concluded that HF MGM has a good amino acid profile and can produce results similar to those from fish fed fishmeal. However, they observed improved metabolic functions of fish translating into optimal growth when the diet was enhanced with n-6 and n-3 fatty acids. In another study, Ogunji *et al.* (2008b) again evaluated HF MGM as a protein source for *O. niloticus*. It was noted that with an increase in HF MGM inclusion in Nile tilapia fish diets there was a decrease in feed conversion ratio (FCR) which is beneficial indicating increased efficiency in conversion of feed. Furthermore, the increase in MGM inclusion in the diet also led to an increased feed intake. On that note, acceptable performances were observed with up to 15% MGM inclusion in the tilapia diets. Aniebo *et al.* (2009), found no significant difference for growth parameters and nutrient utilisation of catfish fed HF MGM and those fed the standard meal at all inclusion levels (12.5 and 25%). Furthermore, catfish fed a 50% and 100% HF larvae meal compared with a standard commercial feed were evaluated for carcass quality and sensory characteristics; no significant differences were found (Aniebo *et al.*, 2011).

According to Tégua *et al.* (2002), HF MGM replacement of fish meal in broiler diets increased weight gain with no significant difference in carcass characteristics observed at inclusion levels of 5, 10 and 15%. They also found HF MGM had a better metabolisable energy (ME) than fishmeal. In another study, Okah & Onwujiariri (2012) reported 50% HF MGM replacement in finisher broiler chicken diets resulted in significantly higher weight gain and dressing percentage than the control, but a decrease in feed intake was observed in diets containing over 20% HF MGM, due to nutrient imbalances caused by higher maggot meal inclusion. Awoniyi *et al.* (2003) found HF MGM to be a suitable protein substitute for fishmeal in broiler diets. An optimal level of 25% was found to replace fish meal with no adverse effect on growth parameters and carcass characteristics. However, they further found an increase in HF MGM in broiler chicken diets over 25%, led to a reduction in feed intake. In a study by Pieterse *et al.* (2014), 10% HF meal substituted for fish meal in broiler diets resulted in significantly better live and carcass weight than those on the fishmeal and the control diet. Furthermore, the larvae meal diet yielded significantly higher breast and thigh muscle percentage of carcass weight than the control diet. The birds fed larvae meal and the control diet also had significantly higher leg muscle weights than those fed the fishmeal diet (Pieterse *et al.*, 2014). Thus, illustrating HF larvae meal to be a suitable feed ingredient in fish and poultry diets.

#### **2.5.2.2 Black Soldier fly larvae/pre-pupae meal**

Black soldier fly larvae and pre-pupae meal contains a number of essential amino acids and is high in minerals (Table 2.7). Bondari & Sheppard (1981) evaluated BSF larvae as a feed ingredient in tilapia and channel catfish diets. They observed refusal of fish to consume whole larvae, however when crushed both fish species resumed feeding with no refusal observed. They found no significant treatment difference on

growth. In addition, sensory analysis results indicated no significant treatment difference in aroma and texture of the fish. Thus, they concluded that BSF larvae to be a suitable feed ingredients for use in channel catfish and tilapia even at 100% inclusion or in combination with other ingredients. Similarly, in rainbow trout fed a 50% black soldier fly pre-pupae meal (BSM) for eight weeks, there was no significant effect on fish growth determined. Furthermore, the fish fillet was tested for quality through sensory techniques and no significant treatment differences were found compared to fish fed the control diet (Sealey *et al.*, 2011). Widjastuti *et al.* (2014) found quail (*Coturnix coturnix japonica*) fed 50% BSM to have led to a significantly higher feed consumption and an improved feed conversion ratio (reduced).

**Table 2.7** Chemical composition of black soldier fly larvae

Essential Amino Acids <sup>1</sup> (%)		Mineral and Proximate <sup>1</sup>		Fatty acid composition <sup>2</sup> (%)	
Methionine	0.83	P	0.88%	C10:0	0.9
Lysine	2.21	K	1.16%	C12:0	47.0
Leucine	2.61	Ca	5.36%	C14:0	6.5
Isoleucine	1.51	Mg	0.44%	C16:0	15.0
Histidine	0.96	Mn	348 ppm	C18:0	2.2
Phenylalanine	1.49	Fe	776 ppm	C16:1n=9	3.1
Valine	2.23	Zn	271 ppm	C18:1n=9	14.0
L-Arginine	1.77	Crude protein	43.2%	C18:1n-7	0.2
Threonine	1.41	Ether extract	28.0%	C20:1n=9	<0.1
Tryptophan	0.59	Ash	16.6	C22:1n=9	<0.1
				C22:1n=11	<0.1
				C18:2n=6	9.4
				C20:4n=6	<0.1
				C18:3n=3	0.8
				C18:4n=3	<0.1
				C20:5n=3	<0.1
				C22:5n=3	<0.1
				C22:6n=3	<0.1
				Σ SFA <sup>a</sup>	71.6
				Σ MUFA <sup>b</sup>	17.3
				Σ PUFA <sup>c</sup>	10.2

<sup>1</sup> Newton *et al.* (2005a)

<sup>2</sup> Kroeckel *et al.* (2012)

<sup>a</sup> Saturated fatty acid

<sup>b</sup> Mono-unsaturated fatty acid

<sup>c</sup> Poly-unsaturated fatty acid

### 2.5.2.3 Other insect meals

Field cricket and other insect meals have an adequate AA content that can sustain the set requirements of poultry AA inclusion in diets (Table 2.6 and 2.8, respectively). Field crickets were utilized as a protein source in broiler diets up to a 15% inclusion level with no treatment effect on growth noted. Furthermore, field crickets had a higher true amino acid digestibility coefficient of 92.9% than the 91.3% of fishmeal (Wang *et al.*, 2005). Grasshopper meal is another suitable feed ingredient in broiler starter diets. A 50% grasshopper meal diet partially substituting fishmeal had a feed conversion efficiency of 80%, while the 100% fishmeal diet had an efficiency of 92%. There was however, no significant treatment difference regarding weight gain.

The 100% grasshopper meal had a higher weight gain with a lower feed efficiency of 69% as compared to the 80% of the 50% diet (Hassan *et al.*, 2009). Therefore, grasshopper meal is a suitable feed ingredient in broiler starter diet but in combination with other protein sources. Ijaiya & Eko (2009) evaluated silkworm (*Anaphe infracta*) caterpillar meal as a protein substitute for fish meal in starter diets of broiler chickens. They found, no significant treatment difference regarding feed intake, weight gain, feed conversion ratio and protein efficiency ratio, with inclusion levels as high as 100%.

**Table 2.8** Ideal amino acid (g/100g) requirement in poultry diets, adapted from NRC (1994)

Nutrients	Poultry
Crude protein %	18-23.00
Arginine	1.10
Histidine	0.32
Isoleucine	0.73
Leucine	1.09
Lysine	1.00
Methionine	0.38
Phenylalanine	0.65
Threonine	0.74
Tryptophan	0.18
Valine	0.82

Generally, studies utilizing insect meals and other waste recovered protein sources as feed when fed to birds, fish and pigs resulted in acceptable growth rates. They also yielded carcasses with acceptable meat quality; most authors observed no difference in flavour as determined by consumers or trained taste panels. However, only few authors went further to investigate the effects of insect meals on the quality of the meat produced, thus minimal literature available. This review clearly outlined the potential of several alternative animal feed sources that can be utilised for animal based food production for safe consumption by humans. It was also found that insects can decompose organic material into biomass and reducing waste by over 50%, solving disposal problems by making it easy to store and properly dispose of composted waste. However, several studies have indicated insect meal to be costly, which was not discussed in this study as it's beyond the scope. Therefore, for sustainable mass production of insect meals that are cheaper, efficient and effective rearing techniques should be developed ensuring safety and a homogenous quality of insects meals produced. Moreover, this will increase the competitiveness of insect meals with other meals used.

#### **2.5.2.4 Effects of nutrition on organ, intestine and skeletal parameters in poultry**

Primarily, a diet is formulated to meet the nutrient requirements of the bird that sustains all its body functions. However, the overall development of an animal, its feed utilisation and digestive process is highly influenced by the intestinal microbiota and its metabolic activities (Rehman *et al.*, 2008). The diet can alter or affect the structural integrity of organs (Fasina *et al.*, 2006). When a chick is hatched its small intestinal mucosa is immature, hence it undergoes rapid intestinal development which is highly influenced by the presence of feed in the intestine. However, if chicks are fasted post hatch 24-48 hours it causes a reduction in the proliferation and migration rate of enterocytes producing fewer cells per villus and a smaller surface area is

produced reducing nutrient absorption (Geyra *et al.*, 2001). Therefore, the rapid and simultaneous development of the gut associated lymphoid tissue (GALT) after hatching is essential to ensure the survival of the chick. The GALT only become immunologically mature in all chicks ten days after hatching, which creates a window for pathogenic invasion if early immunization is not done. In addition, the GALT development and functionality rate of a chick is determined by the diet and time of feeding (Klipper *et al.*, 2000). Early fasting may cause damage to the hindgut for the first two weeks of the chick's life, due to a low intestinal antibody response leading to late colonization of the B and T lymphocytes in the hindgut and delayed lymphocyte populations in the cloacal *bursa* (Shira *et al.*, 2005). Avian lymphoid organs are vital as they assure that pathogens do not evade the host, resist infections and maintain productivity during infectious attacks (Fasina *et al.*, 2006).

The structure of the intestinal mucosa can be used as an indicator of the gut's condition and hence animal health, but it is affected by various factors (Xia *et al.*, 2004; Choct, 2009; Jönsson & Holm, 2010). According to Salim *et al.* (2013), modern broilers are hatched in a hygienic environment and raised in disinfected houses making the development of a balanced gut microflora difficult, thus chicks need microbial stimulants (probiotics) during the starter phase to enhance the development of the gut microflora. Naqi *et al.* (1970) found turkey poults to have had several bacterial species shortly after hatching. Apajalahti *et al.* (2004) noted on day one after hatching broiler chick's bacteria densities raised to  $10^8$  in the ileum and  $10^{10}$  in the caecum per gram of digesta. Furthermore, on day three bacteria densities rose to  $10^9$  and  $10^{11}$  in the ileum and caeca, respectively and remained stable until day 30 of age. The processing method of the feed and the rearing environment affects the intestinal microbial community load and its rate of development, as it derives its energy from the diet depending on the availability of sources (Apajalahti *et al.*, 2004).

A healthy gut has a high nutrient absorption and consequently an improved immune status (by increase in plasma immunoglobulin levels) of the host (Salim *et al.*, 2013). However, the gut requires more energy and protein compared to other organs due to its high maintenance costs (Xu *et al.*, 2003; Choct, 2009), utilizing 20% of all dietary energy to support absorptive and digestive processes (Weurding *et al.*, 2003; Apajalahti *et al.*, 2004). The digestion and absorption of nutrients occurs mostly in the small intestine. The absorptive capability of the intestinal villus area is determined by the villus size, the mutual proportion of enterocytes, goblet and entero-endocrine cells (Awad *et al.*, 2011). However, destruction of the gut wall (epithelium cells) directly affects intestinal barrier function, weakening absorption of nutrients and making the wall barrier permeable by luminal antigenic agents (Song *et al.*, 2014). The presence of toxins in the gut can be accessed by analysing the villi length and crypt depth (Choct, 2009). In addition, shorter villi's and deeper crypt are an indication that toxins where or are present in the gut of the animal. The length and width of the villi and the crypt depth gives an indication on the rate of tissue turnover of epithelial cells, energy requirement and the absorption capacity of nutrients in the gastro-intestinal tract (Pluske *et al.*, 1996; Xu *et al.*, 2003; Xia *et al.*, 2004; Choct, 2009; Awad *et al.*, 2009; Zhang *et al.*, 2013).

Modern poultry birds have been selected for fast growth. This has led to bone problems, as weight gain occurs at a faster rate than the bone development (Hocking *et al.*, 2009; Garcia *et al.*, 2013). The occurrence



of leg disorders is common in intensive poultry production systems. Osteoporosis is one of the common diseases causing leg disorders in the poultry industry and has been a problem since 1955, causing financial losses (Rubin *et al.*, 2007). Osteoporosis causes decline in bone mass and mineral content, and also alters the micro structure of the bone leading to bone fragility, and increased risk of fracturing (Gregory & Wilkins, 1989; Peck *et al.*, 1993; Bishop *et al.*, 2000; Rubin *et al.*, 2007). The fragility and porosity of the bones may lead to bones fragmenting during slaughter and deboning. Bone fragmenting can lead to discoloration of meat in close contact to the bone due to leaching of blood thereby producing a product that may not be appealing to the consumers (Gregory & Wilkins, 1989; Rath *et al.*, 2000; Garcia *et al.*, 2013). However, bone effects can be counter acted by supplying sufficient ratios of Ca to P amongst others, to support normal skeletal growth (Leeson & Summers, 2001). Therefore, during feed formulation, certain vitamins and minerals that cannot be synthesised by the birds are included in the diet as they are important to their health and attribute to birds attaining better production performances (Pandian *et al.*, 2012). In modern poultry diets, a commercial phytase enzyme can be used, aiding digestion and utilisation of phosphorus bound by phytic acid thereby reducing the use of inorganic phosphates in poultry diets (Huff *et al.*, 1998; Selle & Ravindran, 2007; Ravindran, 2013).

Furthermore, Rennie *et al.* (1997) and Fleming *et al.* (2003) reported Ca deficiency and retention, not to be the main cause of osteoporosis in laying hens. Feeding of Ca in particulate (as oyster shells) to laying hens led to a greater deposition of calcium in the skeleton (Rennie *et al.*, 1997). In other studies, feeding of calcium in particulate to laying hens led to a decrease in cancellous bone loss and increased growth of the medullary bone (Fleming *et al.*, 1998; Fleming *et al.*, 2003). According to Rama Rao *et al.* (2003), an increase in broiler dietary Ca level led to a decrease in growth and also Ca and P retention. These may be attributed to the high level of Ca to non-phytin phosphorous ratio of 2.85: 1. Furthermore, the level of Ca in the diet did not significantly influence Ca retention, feed intake, weight gain, leg score and serum P content in broiler chickens. Williams *et al.* (2000) found dietary Ca and available P content not to have affected bone reabsorption. Hence, they concluded that it may be due to genetic factors attributed to the different strains used. A 2Ca:1P ratio is the optimal set supplementation level in poultry (NRC, 1994), however not all feed ingredients are high in these minerals and are mostly bound by phytate reducing its availability. However, BSM utilised is known to contain high Ca and P levels (Newton *et al.*, 1977; Newton *et al.*, 2005b), which are essential to bone development in animals. Therefore, the use of nutrients and minerals that support proper bone growth during rearing can be a possible strategy to reduce leg problems (Gregory & Wilkins, 1989). To the authors knowledge no known study has been done on the effects of high Ca and P levels in BSM on bone strength and mineral composition.

## 2.6 Consumer perception

In the early 1980's consumers became increasingly concerned about excessive fat in red meat due to health reasons (Crouse *et al.*, 1984). However, in the 21<sup>st</sup> century, fat percentage is still of concern to consumers' preferring meat with less fat (Ngapo *et al.*, 2007; Troy & Kerry, 2010), especially saturated fatty acids in the diet (Wood & Enser, 1997). The United Kingdom Department of Health in 1994 recommended a diet



consisting of 35% of energy from fat of which 10% should be saturated fatty acids (Wood & Enser, 1997); this was done in attempts to reduce coronary heart disease and cancer.

Modern day consumers are more concerned about their health, making sure that what they consume is healthy. These consumers require meat portions that are considered good value for money with more muscle, less fat (just to maintain juiciness and flavour) and a consistent quality (Ward *et al.*, 1995). Consumers select meat products not only according to eating quality and price but also consider the ethical quality involving animal welfare issues (Kouba, 2003). These consumer concerns have led the food processing industry into producing products that meet their demands (McIlveen, 1994).

However, modern day consumers are opting to buy mimicked products that do not contain meat but meat alternatives due to health reasons, weight loss and convenience (McIlveen *et al.*, 1999). The purchasing power and behaviour of a consumer is controlled by social and psychological characteristics related to food risk of a particular product (Yeung & Morris, 2006). This leads to a consumer not willing to purchase a product if there are negative aspects related to food safety of the product. On that note, the outbreak of BSE since its first documentation in 1986, has led to a decrease in beef consumption leading to an increase in demand for chicken, pork and fish (Yeung & Morris, 2001; Ishida *et al.*, 2010). However, the outbreak of bird flu (avian influenza) led to a decrease in chicken meat consumption (Ishida *et al.*, 2010). Avian influenza H5N1 stain was documented in Asia during 2003, reaching Europe in 2005 and spread to Africa and Middle East around 2006. The H5N1 stain of the influenza virus is pathogenic and can cause death in humans ([http://en.wikipedia.org/wiki/Avian\\_influenza](http://en.wikipedia.org/wiki/Avian_influenza)). However, control measures and efforts from government to help control outbreaks of these diseases has led to stable situations and increase in consumption of these products overtime. It took longer for BSE outbreak situations to subside than compared to bird flu situations (Ishida *et al.*, 2010). According to Ravindran (2013), poultry meat consumption has shown steady increases over the years and it is predicted to keep rising. Poultry meat demand is increasing due to its acceptance in many societies (Bolan *et al.*, 2010).

When it comes to food safety of chicken meat, microbiological contamination is more of a concern to consumers, in which the consumers monitor it closely based on their choice of purchase outlet with most consumers' preferring to purchase from trusted supermarkets (Yeung & Morris, 2001). Food producing industries has recently (during the 21<sup>st</sup> century) shifted their production focus to meet the demands of modern day consumers (Grunert, 2002).

The use of alternative feed ingredients in broiler diets, especially the recent use of insects and their larvae, pupae or pre-pupae as a feed source has been of bacterial concern to consumers. Insects and their larvae are known to consume decaying organic matter such as manure which contains micro-organisms of which some are pathogenic; for example *E. coli*. Awoniyi *et al.* (2004) found that feeding maggot meal to broilers had no adverse effects on the erythrocyte numbers; this can be attributed to the defence mechanism of insects and larvae (they contain natural antibiotics which reduces transmission of pathogens from feed to insects) (Sheppard *et al.*, 2007), as well as the high body core temperature of the birds destroying some of

the organisms. Broiler chicken blood derived from birds fed maggot meal had a lower bacterial count than that from the control diet (not containing maggot meal) (Awoniyi *et al.*, 2004). This shows that maggot meal has no adverse bacterial threat to poultry meat and consumers can enjoy the flavourful meat produced. However, before birds were fed fish and soya bean meals they have been consuming insects and in fact, wild birds that still consume insects and their meat is consumed by humans with no reported health problems being experienced (Miao *et al.*, 2005).

According to studies in numerous countries in Europe and the United States, results show that consumers are willing to pay for meat products that result from animal friendly free-range production systems (Carlsson *et al.*, 2007). Country of origin has also been shown to be linked to animal welfare and the use of antibiotics as a quality cue for meat products in Sweden led to a shift from locally produced products to imported products (Hoffmann, 2000). Similarly, place of purchase as well as country of origin are main quality cues to consumers for fresh meat (Becker, 2000). Furthermore, consumers are shifting from beef, veal and lamb consumption to chicken and vegetables, as beef is not only expensive, but perceived to have a high calorie content and cholesterol. However, some consumers still find beef to be the best meat product as it is tastier, tender and juicy (Resurreccion, 2004). According to a study in Ethiopia, chicken, beef and chevon were preferred as opposed to fish and pork due to religious beliefs and sociocultural taboos (Teklebrhan, 2012), which are other factors that affects consumer product purchase choices.

Yeung & Yee (2002) reported chicken meat to have become a healthy and popular option in the United Kingdom. Similarly, Hoffman & Wiklund (2006) have found venison to be a healthier choice for the modern day consumer, as it is leaner. Interestingly, Yeung & Yee (2002) found retirees and non-degree holders, in need of food safety information to help reduce their perceived concerns about food risks. Moreover, Pirvutoiu & Popescu (2013) showed increased consumption of poultry meat with increase in education and income levels. It should therefore, be noted that not necessarily every potential hazard that occurs results in a food scare even though it has happened before. This is because food scares occurs in different ways of which some are detrimental and some not (Grunert, 2002).

It can, therefore, be said that these non-traditional feed ingredients derived from insects can be used as animal feed sources producing meat of similar quality as those fed traditional feeds or even better. Studies utilising insect meals as feed ingredients in animal diets, provided evidence that meat produced is safe for human consumption and of no inferior quality.

## **2.7 Conclusion**

Fish meal is currently the only meat origin protein used in poultry diets in most countries across the globe due to the ban of animal products as feed ingredients since the outbreak of BSE. However, in some parts of Africa, fish meal is a scarce commodity which has to be imported and is hence expensive. On the other hand, soya bean meal is the most used plant protein in poultry diets of which there has been an increase in prices due to increase in demand and reduction in quantity due to weather changes as well as the use thereof in

the biofuel industry. Therefore, animal scientists are searching for alternative feed ingredients to keep the animal production industry operating, especially broiler production. Since, broilers have a rapid growth rate and superior feed conversion ratio; their superior growth rate may be used as a production incentive to increase production of animal protein.

Insect meals have proven to be suitable protein sources in poultry diets. Therefore, a possible efficient production system of BSF larvae/pre-pupae can be one that would include composting of farm organic waste, produce biogas, biodiesel and pre-pupae that can be extracted for chitin, with the remaining larvae, pupae or pre-pupae after extraction usable as an animal feed ingredient. Therefore, recycling waste protein as feed for animals would help bridge the animal protein gap without shifting the human diet to plant protein. Black soldier fly larvae bred on poultry manure inoculated with bacteria led to faster development of the larvae, which can be a useful tool in waste management to fasten the process of vermi-composting.

Consumer perception regarding the use of non-traditional feed ingredients as animal feed might be a challenge, especially with the worldwide growing concern on animal welfare. The use of insects in animal diets is humane and reported to have no effects on the production efficiency of animals. However, waste protein recovery used as animal feed is nothing new to the world. Especially, the use of insects as they have been part of free range and wild birds' diets, which is considered by some people as organic due to their origin. Composting waste using insect and/or their larvae is environmental friendly, reducing gas emissions and waste disposal. Therefore, recovery and recycling of waste material with insects may solve waste management problems; produce animal feed and consequently increase production of food.

However, the scientific knowledge of the use of these various insects and their developmental stages as poultry feed is still sparse. Therefore, a study investigating the use of black soldier fly (*Hermetia illucens*) pre-pupae meal (BSM) as a protein source in chicken broiler diets will be initiated. Various attributes of importance in animal production will be evaluated to assess any possible effects arising from the use of BSM in chicken broiler diets. Furthermore, the quality of meat that's produced from the use of this non-traditional feed will also be documented. Black soldier fly pre-pupae meal has been studied extensively in fish but minimally in poultry. Therefore, it's vital to conduct a study enabling the scientific classification of BSM as a feed ingredient. If BSM proves to be a viable protein source in broiler chicken diets, this in turn will increase the availability of protein sources for broiler production that are sustainable and also increase meat protein for human consumption.

## 2.8 References

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## Chapter 3

### Evaluation of the total intestinal track digestibility of black soldier fly (*Hermetia illucens*) pre-pupae meal in the diets of broiler chickens

#### Abstract

This study investigated the nutrient (protein, fat, fibre, ash and amino acids) digestibility and apparent metabolisable energy (AME) of black soldier fly pre-pupae meal (BSM) in broiler chicken diets. Sixty four Cobb 500 broiler chickens at day 40 of age; two birds were randomly assigned to one of four dietary treatments. The treatment diets were each replicated eight times, yielding 32 experimental units. The treatment diets were 100% maize (control), BSM dried 100 °C (BSM100), BSM dried 65 °C (BSM65), and defatted BSM dried 65 °C (DF-BSM). The effect of drying temperature (100 °C and 65 °C) was also tested on the nutrient digestibility of BSM in a completely randomised design. The birds were acclimatised to the feed for three days where *ad libitum* feed intakes were determined. Thereafter, the digestibility trial commenced for three days and faecal samples were collection each morning. Treatment significantly affected the AME and coefficient of total intestinal tract digestibility (CTTD) of nutrients. Overall DF-BSM had high CTTD values for nutrients and AME. The nutrient CTTD values of BSM65 were significantly lower than DF-BSM. Results further revealed that the nutrients CTTD of BSM100 were not significantly different from those of DF-BSM for most nutrients and AME. Therefore, it can be concluded that nutrients in BSM dried at 65 °C and 100 °C are highly digestible in broiler chickens. These results will allow a more accurate inclusion of BSM into balance commercial broiler diets.

**Keywords:** CTTD, apparent digestibility, BSF, larvae meal, acid insoluble ash, amino acids

#### 3.1 Introduction

The extent to which nutrients are ingested, digested and absorbed by an animal determines the level of performance of an animal. Animals fed highly digestible feeds perform better than their counterparts fed feeds of low digestibility. Superior growth performance was observed in animals that consumed nutrients with higher digestibility coefficients than those fed low-digestibility nutrients (Thang *et al.*, 2010; Salem *et al.*, 2013). In broilers, high digestibility figures for dry matter, crude protein, ether extract and phosphorus were associated with superior growth performance (Emami *et al.*, 2013; Thiamhirunsopit *et al.*, 2014). In modern day intensive monogastric nutrition, use is made of feeding balanced diets of which the digestibility of the feedstuffs incorporated into the diet are known and included into the formulation process. This not only leads to better/best performance from the animals being fed, but also reduces the undigested waste. This implies that before any feed ingredient can be incorporated into an animal feed the digestibility of its nutrients should be ascertained using a digestibility study.

A digestibility study measures the digestion of certain nutrients in a feed component as consumed by animals (Scott & Boldaji, 1997). Digestibility methods differ between animal species, where the method used depends on the structure of the animal's digestive system. Thus, methods used in ruminants cannot be used in monogastrics or fish or *vice versa* (Scott *et al.*, 1998; Yahya *et al.*, 2011; Kroeckel *et al.*, 2012; Salem *et al.*, 2013). *In vivo* digestibility studies can be done through ileal digesta or excreta collection; however they are not well-suited for determining amino acid digestibility of individual feed ingredients (Parsons *et al.*,

2002). On the other hand, precision feeding (and excreta collection) is more advantageous as it is cheaper, faster and requires less feed samples, despite having been criticised for being less accurate (Parsons *et al.*, 2002). Markers (inert and external) are added in excreta assay diets to aid error minimization regarding inaccurate measurement of feed intake, excreta output and external contamination of excreta (Sibbald, 1987). Despite several studies stipulating that total excreta collection digestibility is less reliable than ileal digestibility, Ravindran *et al.* (1999) found no differences in digestibility of sorghum and maize utilizing both methods. They further found differences in wheat digestibility, while ileal amino acid (AA) digestibility was higher than in excreta assay; opposing literature also shows overestimated AA digestibility by excreta assay (Ravindran & Bryden, 1999; Parsons *et al.*, 2002).

Inert digestibility markers are routinely used to estimate the digestibility of dietary nutrients. In broilers, an indigestible inert marker is added to the birds' diet, where the digestibility of the nutrients is attained by the differences found between the proportion of the marker in the diet and that in the excreta. Oberleas *et al.* (1990) observed that the use of chromium as an inert digestibility marker may be negative for estimating rates of passage because it is unpalatable, reduces intake and is carried more readily by the fluid rather than the solid portion of the digesta, thus changing its digestibility. An ideal marker should accurately estimate total tract digestibility and provide results on the extent and direction of the effects induced by the diet, without changing the importance of the treatment effects to the animal (Huhtanen *et al.*, 1994). The faecal recovery rate of any marker is of significance in the reliability of the faecal marker to ensure accurate nutrient digestibility data analysis (Tamminga *et al.*, 1989; Sales & Janssens, 2003).

Feed naturally contains acid insoluble ash in low quantities; hence an exogenous source of acid insoluble ash can be added to help improve the accuracy of recovering the marker (Sales & Janssens, 2003). Scott & Boldaj (1997) observed that acid insoluble ash was more appropriate in conducting digestibility trials for broilers compared to chromium oxide. Digestibility trials have been conducted on diets of different compositions (Scott & Boldaji, 1997; Emami *et al.*, 2013; Thiamhirunsopit *et al.*, 2014). There are certain factors that can reduce the digestibility potential of feed by the animal. The use of heat and acid treatment may lead to protein denaturation during processing (Boland *et al.*, 2013). Lysine is the most affected AA by extreme heat processing as it is susceptible to Maillard reactions reducing its availability for use by the animal (Parsons, 1996). Thus these effects are to be tested to determine their effects on nutrient bioavailability to the animals. In monogastric animals fat content within the diet can affect the intake and growth of the animal. Crespo & Esteve-Garcia (2001) found a decrease in feed intake with an increase in dietary fat inclusion in broiler chickens. This may have been due to imbalances caused by metabolisable energy (ME) and protein ratio. The ME and protein content of the diet influences performance of birds (Zaman *et al.*, 2008). A higher ME to protein ratio in broiler chickens diets leads to excessive feed consumption in order to meet the required amino acids for maintenance and survival (Aletor *et al.*, 2000). An optimal and balanced ME and protein ratio ensures maximum nutrient and efficient dietary protein utilization under optimal rearing conditions (Zaman *et al.*, 2008; Ogunji *et al.*, 2008).



Despite the potential of black soldier fly larvae and pre-pupae meal as a potential diet for broilers (Hale, 1973), the extent of digestibility of its nutrients is not known in broiler chickens. Therefore, the objective of this study was to investigate the effect of heat and defatting on black soldier fly pre-pupae meal's (BSM) apparent metabolisable energy and apparent digestibility coefficients of nutrient composition and amino acids, utilising the following processed treatment diets; BSM dried at 100 °C, BSM dried at 65 °C and defatted BSM dried at 65 °C.

## **3.2 Materials and Methods**

### **3.2.1 Experimental animals, layout and housing**

Before the trial was commenced ethical clearance was obtained from Stellenbosch University; ethical clearance number SU-ACUM13-00026. For the trial, 64 day-old Cobb 500 broiler chicks were raised for the digestibility study at the Mariendahl experimental farm (33° 51' 0 S; 18° 49' 60 E) of Stellenbosch University.

On day 40 the birds were randomly selected and changed to different metabolic wire cages (0.45m X 0.3m) with two birds per cage, in the same grower pullet house. Each cage contained one nipple drinker and one tube feeder. The temperature in the grower pullet house was controlled with ventilation set at six air changes per hour. The artificial lighting was set at a pattern of 18 h of light and six hours of darkness.

### **3.2.2 Experimental diets, design and trial procedure**

For the first 39 days, the birds were fed commercial diets according to the nutrient specifications of Cobb-Vantress (2012). Feed and water was provided to the birds *ad libitum*. At day 40 the birds were randomly switched to one of the four experimental diets (Table 3.1) formulated according to Scott & Boldaji (1997), with 1% Celite (acid insoluble ash) inclusion. In a completely randomised design, the treatments were each replicated eight times yielding 32 experimental units (each unit representing two birds per cage (0.45m x 0.3m)). The black soldier fly (BSF) pre-pupae used in this study were harvested at different times of the year and were also fed different feed composition of organic kitchen waste, fruits and vegetables but the rearing methods and temperature were kept constant. The treatment diets were 100 % maize as a control, BSM dried at 100 °C (BSM100), BSM dried at 65 °C (BSM65) and defatted BSM dried at 65 °C (DF-BSM) shown in Table 3.1. The diets were mixed and administered to birds as mash diets.

The birds were acclimatised to the experimental diets on days 40-42. Feed intake and refusal were measured during the acclimatisation period for the determination of specific daily feed intake. The actual digestibility trial began on day 43 and ran for three days. The wire cages were fitted with faecal collection trays placed underneath each cage and outlined with a clean sheet of clear plastic. On this day the birds were provided with a specific amount of feed per day in grams based on the adjusted amounts of feed obtained during the acclimation period. The water was provided *ad libitum*. The digestibility assays of the various BSM were done according to the Acid Indigestible Assay as described by Scott & Boldaji (1997).

**Table 3.1** Ingredient compositions of black soldier fly pre-pupae meal (BSM) digestibility treatment diets

Ingredients	Treatment diets			
	100% maize	BSM100	BSM65	DF-BSM
Yellow Maize (fine)	100.00	50.00	50.00	60.00
BBSM		50.00	50.00	40.00
Acid insoluble ash (Celite™)**	1.00	1.00	1.00	1.00
Vit+min premix*	0.15	0.15	0.15	0.15

\*\* Celite™ included at a level as indicated by Scott & Boldaji (1997)

\* Vitamins and mineral are included according to the levels recommended by the National Research Council (1994)

### 3.2.3 Data collection

The broiler chickens were weighed at the beginning and end of the digestibility trail (day 40 and 46). Treatment feed samples were collected for further analyses. Excreta dropped were collected every morning at 08:00 (on day 44-46). The faeces were cleaned of any visible feathers, sealed in airtight zip lock bags and immediately frozen in a -18 °C freezer until further analysis. At the end of the trial period, the faeces collected were all pooled together per treatment replicate. In addition, feed intake and refusal were measured and samples of the refused feed were collected. However, the feed refused did not weigh more than 2 g per cage for the trial period and was thus not included in any further analyses.

### 3.2.4 Analytical methodologies

#### 3.2.4.1 Dry matter determination

The dry matter (DM) content of the samples was determined according to the Association of Official Analytical Chemists International (AOAC) (2002), official method 934.01. The samples were dried at 100 °C for 24 h.

#### 3.2.4.2 Crude protein determination

The crude protein content of each treatment feed and faecal samples was determined by measuring the total nitrogen content using a LECO FP528 machine, according to the Dumas combustion method 992.15 described by AOAC (2002). The nitrogen content was directly measured and used to calculate the crude protein content using a factor of 6.25.

#### 3.2.4.3 Crude fat determination

The crude fat content of each treatment feed and faeces sample were determined using the acid hydrolysis fat extraction method using diethyl ether, petroleum ether, ethanol and hydrochloric acid 38% reagent as described by the AOAC (2002), official method 920.39.

#### 3.2.4.4 Ash determination

The duplicate samples used in the dry matter determination (3.2.4.1) were retained and used to analyse the ash content of the feed and faeces (AOAC, 2002; official method 942.05). The samples were combusted in a furnace oven at 500 °C for 6 h.

**3.2.4.5 Crude fibre determination**

The crude fibre in the feed and faeces samples was analysed according to the official method 962.09 (AOAC, 2002) on a Fibertec/Dosifiber extrusion apparatus. The samples were dried in a 100 °C oven for 48 h and then combusted at 500 °C for 6 h.

**3.2.4.6 Amino acid hydrolysis and analysis**

Amino acid hydrolysis was according to the official method 994.12 of the AOAC (2002). The feed and faecal samples with addition of 6 mm of 6N hydrochloric acid containing 15% phenol solution was sealed under nitrogen and were left to hydrolyse for 24 h in a 110 °C oven. The samples were removed and allowed to cool then poured into eppendorf tubes and were frozen at -18 °C until amino acid analysis.

The amino acid analysis was done using a Water API Quattro Micro instrument with the samples being subjected to the Water AccQ Tag Ultra derivitization kit for cleaning. The amino acid standard used was purchased from Waters (P/N: WAT088122) and prepared by adding 40 µl of standard plus 760 µl Water and 200 µl Internal standard. The samples were diluted according to their protein content where 8-20% protein was diluted 10x, 20-60% protein was diluted 20x and 60-100% protein was diluted 50x. The samples were then prepared by adding 10 µl of sample, 70 µl Borate buffer and 20 µl of reconstituted AccQ Tag reagent, vortexed and allowed to stand at room temperature (28 °C) for 1 min and placed in a heating block at a temperature of 55 °C, for 10 min. Thereafter, 1 µl was injected into the apparatus for the actual test and the different amino acids were determined in g/100g sample.

**3.2.4.7 Acid insoluble ash**

In duplicates, 5 g of samples were weighed and placed into combustible crucibles and were combusted in a furnace for 12 h at 550 °C. After combustion the samples were transferred to 500 cm<sup>3</sup> Erlenmeyer flasks and 100 ml of 2 mol hydrochloric acid solution was added. Thereafter, the mixed substances were boiled on a hot plate for five minutes. Subsequently, the hot solution was filtered through a Whatman® No 41 filter paper. Hot distilled water (85-100 °C) was used to rinse the flask and wash the samples free of acid. The filter papers with the ash residue were placed in combustible crucibles and re-combusted for 12 h at 550 °C. After 12 h of combustion the samples were removed, placed in a desiccator for 30 min and then weighed. The acid insoluble ash content of the sample was calculated using Equation 3.1 as outlined with the procedure by Van Keulen & Young (1977).

**Equation 3.1,**

$$\text{Acid insoluble ash (\%)} = \frac{\text{Weight of crucible (g)} + \text{Weight of ash (g)} - \text{Weight of empty crucible (g)}}{\text{DM weight of sample (g)}} \times \frac{100}{1}$$

**3.2.4.8 Gross energy**

The gross energy of the feed and faecal samples was determined using the CP 500 isothermal bomb calorimeter. The CP 500 isothermal bomb calorimeter apparatus was calibrated before commencing of

analysis. At the start of the analysis two benzoic acid tablets were analysed separately, the values obtained were used to standardize the samples gross energy obtained as a correction factor. The bomb was sealed with pure oxygen and the gross energy was directly measured in MJ/kg. The values were obtained and used to calculate for the apparent metabolisable energy (AME) of each treatment diet and faeces using Equation 3.2, as described by Scott & Boldaji (1997).

**Equation 3.2,**

$$\text{Apparent metabolisable energy (AME)} = \text{Gross energy}_{\text{diet}} - \left[ \text{Gross energy}_{\text{excreta}} \times \left( \frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{excreta}}} \right) \right]$$

**3.2.4.9 Mineral Analysis**

At the institute of Animal Production, Western Cape Department of Agriculture at Elsenburg, the mineral composition of the sample (feed and faeces) was analysed according to the Combustion Method No. 6.1.1 as described by Agriculture Laboratory Association of Southern Africa (AgriLASA) (2007). Briefly, 5 ml of 6 M hydrochloric acid was added to 0.5 g sample. The samples were then placed in a 50 °C oven for 30 minutes. After removal of samples, 35 ml of distilled water was added and filtered into a 50 ml bottle topped with distilled water to fill to the 50 ml mark. The minerals were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. The iTEVA Analyst software was used to calculate for the mineral concentrations. The wavelengths at which Ca and P were eluted are 317.933 and 177.495 nm, respectively.

**3.2.5 Coefficient of total tract digestibility**

The coefficient of total tract digestibility of each nutrient was calculated using the following Equations 3.3-3.6:

**Equation 3.3,**

$$\text{Nutrients consumed (g/trial)} = \text{Nutrient}_{\text{analysed in feed}} \times \text{DM}_{\text{intake}} \text{ (g/trial)},$$

**Equation 3.4,**

$$\text{Nutrients excreted (g/trial)} = \text{Nutrient}_{\text{analysed in excreta}} \times \text{DM}_{\text{excreta}} \text{ (g/trial)},$$

**Equation 3.5,**

$$\text{Digested nutrient (g/trial)} = \text{Nutrient}_{\text{consumed}} - \left[ \text{Nutrient}_{\text{excreta}} \times \left( \frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{excreta}}} \right) \right], \text{ and}$$

**Equation 3.6,**

$$\text{Coefficient of total tract digestibility (g/kg)} = \frac{\text{Digested nutrient}}{\text{Nutrient consumed}} \cdot$$

The 100% maize meal diet coefficient of total intestinal tract digestibility (CTTD) obtained was used for digestibility correction of the pre-pupae-maize meal diets according to Aksnes *et al.* (1996), using Equation 3.7:

**Equation 3.7,**

$$\text{CTTD}_{\text{test ingredient}} = \text{CTTD}_{\text{maize}} + \left( \frac{\text{CTTD}_{\text{test ingredient}} - \text{CTTD}_{\text{maize}}}{\% \text{ Test ingredient in test diet}} \right)$$

**3.2.6 Statistical analysis**

The data from the trial were analysed using the general linear models (GLM) procedure of SAS (2009). The analysis of variance (ANOVA) assumptions for normality and homoscedasticity were investigated before further analyses were done. The tests were considered significant at  $P > 0.05$ . The data adhered to the normality and homoscedasticity test and thus a one-way analysis of variance (ANOVA) with Bonferroni's *post hoc* (least square means) test was used for statistical analysis. In cases where the homoscedasticity assumption for the data was not satisfied, a Welch's ANOVA for unequal variances was used. The 5% significance level was used for the statistical tests and treatment differences were declared at  $P < 0.05$ .

The model for the one-way ANOVA is indicated by,  $Y_{ij} = \mu_i + \alpha_j + \varepsilon_{ij}$  where the terms in the model are defined as; the treatment effect response ( $Y_{ij}$ ), the overall mean ( $\mu_i$ ), treatment effect ( $\alpha_j$ ) and the unexplained error ( $\varepsilon_{ij}$ ).

**3.3 Results**

The chemical composition of the treatment diets is shown in Table 3.2. All nutrients analysed for coefficient of total intestinal tract digestibility (CTTD) were influenced ( $P < 0.05$ ) by the treatment diets except CTTD for crude fat, leucine and methionine (Table 3.3). Apparent metabolisable energy of BSM100 was higher ( $P < 0.05$ ) than BSM65 but similar to DF-BSM (Table 3.3). Treatment differences were observed for Ca CTTD with the BSM100 diet being ( $P < 0.05$ ) higher than BSM65, but both did not differ from the DF-BSM diet. Treatment also ( $P < 0.05$ ) influenced phosphorous CTTD, with the BSM100 and DF-BSM diet attaining higher CTTD's than the BSM65 diet. Treatment differences ( $P < 0.05$ ) were found regarding organic matter with the BSM65 being lower, while the BSM100 and DF-BSM were similar. Furthermore, unequal variances were found regarding the data of organic matter and hence the Welch's ANOVA value was reported. The ash CTTD differed ( $P < 0.05$ ), with the DF-DSM being higher.

Similar CTTD results were observed regarding crude protein, crude fibre, valine and isoleucine were the DF-BSM diet attained higher CTTD ( $P<0.05$ ) than BSM65 but was similar to that of BSM100. However, the BSM100 CTTD did not differ from that of BSM65 ( $P<0.05$ ). Furthermore, the CTTD of histidine, tyrosine, arginine, aspartic acid, glutamic acid, proline, threonine, cysteine, lysine, phenylalanine were observed to be similar. Pertaining to the above, the BSM65 attained CTTD lower ( $P<0.05$ ) than DF-BSM and BSM100 which were similar. The CTTD of serine was influenced ( $P<0.05$ ) by treatment with DF-BSM being higher, while BSM65 was lower and BSM100 being intermediate.

**Table 3.2** Chemical composition of the digestibility treatment diets fed to broiler chickens

Parameters	Units	Treatment diets			
		100% maize	BSM100 <sup>#</sup>	BSM65 <sup>#</sup>	DF-BSM <sup>#</sup>
AME <sup>1</sup>	MJ/kg	14.82	17.40	16.52	16.85
Crude protein	%	8.95	26.19	25.52	24.66
Ether extract	%	3.70	19.46	15.64	10.21
Ash	%	4.74	7.17	7.97	6.69
Crude fibre	%	2.70	6.51	7.68	5.72
Histidine*	g/100g	3.39	3.77	3.39	3.78
Serine	g/100g	6.04	5.43	5.56	6.17
Arginine*	g/100g	5.87	6.47	5.78	6.47
Glycine	g/100g	5.64	7.01	7.47	7.54
Aspartic acid	g/100g	7.73	9.44	9.65	8.78
Glutamic acid	g/100g	22.17	14.34	14.61	15.37
Threonine*	g/100g	4.73	4.73	4.93	4.95
Alanine	g/100g	8.23	7.47	7.73	7.53
Proline	g/100g	11.49	7.80	7.83	8.53
Cysteine	g/100g	0.55	0.20	0.21	0.26
Lysine*	g/100g	3.29	5.55	5.26	5.13
Tyrosine	g/100g	4.87	8.00	8.20	8.28
Methionine*	g/100g	0.79	1.87	2.08	1.67
Valine*	g/100g	5.65	7.24	7.32	7.04
Isoleucine*	g/100g	3.94	5.07	5.08	5.06
Leucine*	g/100g	14.00	9.61	10.03	10.18
Phenylalanine*	g/100g	6.19	5.54	6.26	5.97
Calcium	%	1.88	1.62	1.08	1.21
Phosphorus	%	0.50	0.59	0.45	0.36

\* Essential amino acids

<sup>1</sup> Apparent metabolisable energy

<sup>#</sup> BSM (Black soldier fly pre-pupae meal)

**Table 3.3** Mean ( $\pm$  standard error) for coefficient of total intestinal tract digestibility (CTTD) of black soldier fly pre-pupae meal (BSM)'s with apparent metabolisable energy (AME) in broiler chickens

Parameters	Units	Treatment diets			P-value
		BSM100	BSM65	DF-BSM	
AME	%	17.40 <sup>a</sup> $\pm$ 0.200	16.52 <sup>b</sup> $\pm$ 0.220	16.85 <sup>ab</sup> $\pm$ 0.104	0.013
Organic matter	%	0.94 <sup>a</sup> $\pm$ 0.004	0.91 <sup>b</sup> $\pm$ 0.009	0.95 <sup>a</sup> $\pm$ 0.004	0.006
Crude protein	%	0.91 <sup>ab</sup> $\pm$ 0.015	0.86 <sup>b</sup> $\pm$ 0.014	0.97 <sup>a</sup> $\pm$ 0.010	0.003
AH <sup>1</sup> fat extract	%	1.02 $\pm$ 0.001	1.00 $\pm$ 0.004	1.01 $\pm$ 0.001	0.101
Ash	%	0.85 <sup>b</sup> $\pm$ 0.024	0.83 <sup>b</sup> $\pm$ 0.017	0.92 <sup>a</sup> $\pm$ 0.016	0.002
Crude fibre	%	0.74 <sup>ab</sup> $\pm$ 0.015	0.70 <sup>b</sup> $\pm$ 0.022	0.81 <sup>a</sup> $\pm$ 0.017	0.005
Histidine*	%	0.95 <sup>a</sup> $\pm$ 0.006	0.90 <sup>b</sup> $\pm$ 0.009	0.96 <sup>a</sup> $\pm$ 0.005	<0.001
Serine	%	0.91 <sup>b</sup> $\pm$ 0.006	0.87 <sup>c</sup> $\pm$ 0.011	0.95 <sup>a</sup> $\pm$ 0.004	<0.001
Arginine*	%	0.98 <sup>a</sup> $\pm$ 0.002	0.95 <sup>b</sup> $\pm$ 0.005	0.99 <sup>a</sup> $\pm$ 0.004	<0.001
Glycine	%	0.88 <sup>b</sup> $\pm$ 0.011	0.83 <sup>b</sup> $\pm$ 0.019	0.94 <sup>a</sup> $\pm$ 0.011	0.000
Aspartic acid	%	0.95 <sup>a</sup> $\pm$ 0.005	0.93 <sup>b</sup> $\pm$ 0.008	0.96 <sup>a</sup> $\pm$ 0.003	0.001
Glutamic acid	%	0.94 <sup>a</sup> $\pm$ 0.005	0.91 <sup>b</sup> $\pm$ 0.009	0.95 <sup>a</sup> $\pm$ 0.004	0.002
Threonine*	%	0.94 <sup>a</sup> $\pm$ 0.004	0.91 <sup>b</sup> $\pm$ 0.013	0.96 <sup>a</sup> $\pm$ 0.005	0.001
Alanine	%	0.92 <sup>b</sup> $\pm$ 0.008	0.89 <sup>b</sup> $\pm$ 0.013	0.96 <sup>a</sup> $\pm$ 0.005	0.000
Proline	%	0.92 <sup>a</sup> $\pm$ 0.004	0.89 <sup>b</sup> $\pm$ 0.011	0.94 <sup>a</sup> $\pm$ 0.004	0.001
Cysteine	%	0.86 <sup>a</sup> $\pm$ 0.024	0.77 <sup>b</sup> $\pm$ 0.028	0.88 <sup>a</sup> $\pm$ 0.011	0.005
Lysine*	%	0.97 <sup>a</sup> $\pm$ 0.004	0.94 <sup>b</sup> $\pm$ 0.009	0.98 <sup>a</sup> $\pm$ 0.004	0.002
Tyrosine	%	0.95 <sup>a</sup> $\pm$ 0.005	0.93 <sup>b</sup> $\pm$ 0.006	0.98 <sup>a</sup> $\pm$ 0.008	0.000
Methionine*	%	0.97 $\pm$ 0.008	0.95 $\pm$ 0.009	0.98 $\pm$ 0.009	0.070
Valine*	%	0.92 <sup>ab</sup> $\pm$ 0.005	0.90 <sup>b</sup> $\pm$ 0.011	0.95 <sup>a</sup> $\pm$ 0.006	0.001
Isoleucine*	%	0.94 <sup>ab</sup> $\pm$ 0.004	0.92 <sup>b</sup> $\pm$ 0.012	0.96 <sup>a</sup> $\pm$ 0.005	0.003
Leucine*	%	0.92 $\pm$ 0.010	0.94 $\pm$ 0.005	0.94 $\pm$ 0.007	0.444
Phenylalanine*	%	0.96 <sup>a</sup> $\pm$ 0.003	0.94 <sup>b</sup> $\pm$ 0.005	0.97 <sup>a</sup> $\pm$ 0.003	0.001
Calcium	%	0.90 <sup>a</sup> $\pm$ 0.004	0.80 <sup>b</sup> $\pm$ 0.032	0.83 <sup>ab</sup> $\pm$ 0.013	0.021
Phosphorous	%	0.85 <sup>a</sup> $\pm$ 0.021	0.63 <sup>b</sup> $\pm$ 0.023	0.85 <sup>a</sup> $\pm$ 0.006	<0.001

<sup>(a,b,c)</sup> Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

\* Essential amino acids

<sup>1</sup> Acid hydrolysis

### 3.4 Discussion

The chemical analyses revealed that BSM contains an adequate composition of nutrients yielding favourable CTTD for all nutrients analysed and AME. Black soldier fly pre-pupae meal is known to be high in Ca and P content (Newton *et al.*, 2005b), but its bioavailability and digestibility rate are not known. Modern broilers are selected for fast growth which has led to leg problems as weight gain occurs at a faster rate than bone development causing bones to become porous and fragile (Hocking *et al.*, 2009; Garcia *et al.*, 2013). Thus, the Ca and P content in poultry diets should be available for bio-absorption by the bird and used to sustained bone development and growth, making the quantification of Ca and P digestibility values of various feed sources vital. This study revealed BSM CTTD for Ca was high with the lowest CTTD obtained by BSM65 diet being 80%. The phosphorous CTTD of BSM65 diet was significantly lower (63%) than BSM100 and DF-BSM diet both, attaining 85% CTTD. Further research is warranted on the bio-availability of Ca and P from BSM in poultry given their CTTD values obtained in broiler chickens and their importance in bone development and growth. The CTTD of crude protein for BSM100, BSM65 and DF-BSM were 91%, 86% and 97%,



respectively. Treatment ( $P<0.05$ ) lowered CTTD of organic matter in the BSM65 diet, while the ash content was ( $P<0.05$ ) higher in the DF-BSM diet. The crude protein CTTD of BSM is higher than the 79% of house fly pupae meal (Pieterse & Pretorius, 2014) and the 80.7% soya oil cake meal (Sebastian *et al.*, 1997). Hwangbo *et al.* (2009) found soya bean meal protein digestibility to be 98%, which is similar to the 97% digestibility of DF-BSM in this study. The adequate AA profile of BSM (Newton *et al.*, 2005a) might have led to the high protein digestibility in this study given that various meals contain different AA profiles which determine the protein quality of a feed (Boland *et al.*, 2013).

Arginine, an essential AA for broiler growth, was found in BSM with a CTTD of 95-99% across treatment diets. Arginine deficiency or excess supply can cause decreased bird performance influenced by the antagonistic relationship of arginine and lysine which may arise if not supplied in an equal ratio (Chamrupollert *et al.*, 2002). Furthermore, Kwak *et al.* (1999) observed that arginine-deficient diets caused poor development of lymphoid organs, affecting thymus growth to a greater extent. Therefore, the combination of BSM with other raw ingredients may easily balance the arginine requirement in broiler chicken diets and thus prevent lysine-arginine antagonism. Glutamic acid has been classified as an AA of interest regarding broiler growth improving live weight and carcass weight (Moran & Stilborn, 1996) and it is therefore important to quantify its digestibility value in BSM. Results showed high CTTD for glutamic acid in BSM diets, with BSM65 attaining a CTTD of 91% which is lower ( $P<0.05$ ) than DF-BSM (95%) and BSM100 (94%).

Methionine being the first limiting AA in poultry diets, lysine second and threonine third, their CTTD are considered important. The study results indicated BSM has high CTTD for methionine ranging between 95-98% and no treatment differences were observed ( $P>0.05$ ). The methionine digestibility values obtained in this study are higher than the 91.6% of soya bean meal reported by Sebastian *et al.* (1997) and the 93% reported by Hwangbo *et al.* (2009). Lysine CTTD of DF-BSM was 98%, BSM100 (97%) and BSM65 (94%) being different ( $P<0.05$ ). Lysine is vital in poultry diets being the second limiting AA and used as a reference AA in feed formulation and important for muscle growth (Lemme *et al.*, 2004). L-Threonine in poultry diets optimises the use of body protein deposition and weight gain, in complement with lysine, and is also vital in the immune responses of the birds (Taghinejad-Roudbaneh *et al.*, 2013). In this study the threonine CTTD in BSM was high at 96% for the DF-BSM being different ( $P<0.05$ ) from BSM65 which attained a CTTD of 91%. It can therefore be concluded that BSM contain the first three limiting AA in poultry diets and their CTTD digestibility values are above 91%.

Fat was the highest digested nutrient attaining a CTTD of 100% with no treatment differences observed ( $P>0.05$ ). However, the homoscedasticity test for the fat was not met indicating unequal variances and thus a Welch's ANOVA was done attaining  $P=0.101$ . Newton *et al.* (1977) found an 83.6% apparent fat digestibility of BSF larvae meal in pigs; lower than that attained in this study in broiler chickens. Even though the treatment diets contained different levels of fat, it is possible to attain a similar CTTD for fat. According to Zollitsch *et al.* (1997) the fatty acid profile of the dietary fats determines its digestibility and not total fat *per se*. The amount of fat in poultry diets is important, Crespo & Esteve-Garcia (2001) found a decrease in feed



intake with an increase in dietary fat inclusion. This may help explain the significantly higher CTTD of nutrients obtained in the DF-BSM diet, given that their fat level was lower than its counterpart treatment diets. This seems to indicate that removal of fat from BSM has led to higher digestibility coefficients of nutrients ( $P>0.05$ ) in broiler chickens. Therefore, a further study is warranted on dietary fat percentage correlation to dietary fatty acid profile in BSM and its effects on fat CTTD, and their corresponding effects on broiler growth.

Processing methods, especially over-heating of feed ingredients high in protein, is considered to be the main cause in reducing the bioavailability of amino acids to animals (Parsons, 1996). One of the objectives of this study was to test the effect of heat on digestibility coefficients of BSM. The results revealed that heat processing did not affect CTTD of protein and AA in BSM diets, as the highest heat treatment at 100 °C performed comparably to those dried at 65 °C (Table 3.3). Insect pupae and pre-pupae meals covered in chitin are expected to yield lower digestibility values (Diener *et al.*, 2009). Furthermore, Razdan & Pettersson (1994) and Kroeckel *et al.* (2012) found an increase of chitin inclusion in chicken and fish diets to have caused a decrease in nutrient digestibility. Although, chitin levels in this study's treatment diets were not investigated, BSM are known to be high in chitin (Newton *et al.*, 2005b). In this study the CTTD of the nutrients analysed across the treatment diets were above 70%. Coefficients of total tract digestibility of nutrients above 70% are acceptable (Emami *et al.*, 2013; Thiamhirunsopit *et al.*, 2014). Therefore; the chitin layer might have protected the nutrients in the pre-pupae meal from the heat effects on nutrients and consequently, the nutrients were made available for digestion in the gastrointestinal tract. Furthermore, Newton *et al.* (2005b) observed that fractioning of pre-pupae cuticle layer containing chitin might improve the availability of nutrients for digestion. Therefore, further studies are warranted on the effect of chitin in insect pre-pupae/pupae meals on the digestibility of nutrients by determining their chitin levels, fractioning and removal of chitin.

Furthermore, according to McDonald (2002) milling of feed ingredients increases the surface area of feeds thereby availing more nutrients for absorption and enhancing digestion. The BSM used in this study were minced and this process might have partitioned the chitinous cuticles thereby improved digestibility of BSM. Wenk (2001) noted that increased animal dietary fibre content in the diet on one hand reduces digestion rate of nutrients in the upper digestive track and on other hand increases digestion in the lower digestive tract. In this regard it was noted that the DF-BSM diet had a low crude fibre (5.72%) content compared to BSM65 (7.68%). It was observed that DF-BSM diet had better CTTD of nutrients analysed compared to BSM65. The CTTD obtained in this study could also be related to the fibre content as illustrated by Wenk (2001), since the diets with low fibre content had better CTTD than those with high fibre content. Furthermore, Wenk (2001) observed an increase in dietary fibre to reduce the diet's metabolisable energy. Similarly, Pieterse & Pretorius (2014) noted AME values and acid detergent fibre content in broiler chicken diets to be related; a low acid detergent fibre value in the diet led to an increase in diet AME value. It was observed that BSM65 had a low AME value of 16.52 while DF-BSM had a value of 16.85 which, did not differ from each other ( $P>0.05$ ).

Overall, the digestibility efficiency of feed sources by animals is dependent on the quantity and composition of the feed sources in the diet (Wenk, 2001). Briefly, the differences found in dietary fibre content and fat as mentioned amongst treatments may explain why DF-BSM had significantly higher CTTD of nutrients analysed. Another possible explanation on treatment differences and overall high digestibility values obtained in this study could be due to the feed substrate used and/or age at harvest of the pre-pupae (Ramos-Elorduy *et al.*, 2002; St-Hilaire *et al.*, 2007) or simply superiority of diets. The feed substrate fed to the larvae during its growth stage can alter its fat and fatty acid composition. Sheppard *et al.* (1994) found differences in weight and crawl off patterns of larvae between seasons. St-Hilaire *et al.* (2007) found differences in fat composition of BSM fed cow manure and that of cow manure enriched with fish offal, reporting an increased fat percentage and omega-3 content with the enriched diet. The BSF pre-pupae used for the BSM in this study were harvested at different times of the year and were thus fed different feed composition of organic kitchen waste, fruits and vegetables, although the larvae rearing methods and temperature were kept constant.

The CTTD of AME and nutrients analysed in this study utilising BSM in various processed forms are similar and comparable to that of soya bean as reported by Newton *et al.* (1977); Sebastian *et al.* (1997) and Hwangbo *et al.* (2009). However, the bioavailability of nutrients in soya bean meal may be lower due to the presence of phytate binding to nutrients and minimizing their availability for digestion (Omogbenigun *et al.*, 2004). Anti-nutritional factors in BSM were not analysed and if any; they are not known. The CTTD obtained for BSM indicated it to be a viable protein source for use in broiler chicken diets, warranting further research on BSM effects on broiler production. Furthermore, Zuidhof *et al.* (2003); Hwangbo *et al.* (2009) and Pieterse & Pretorius (2014) also found insect meals to be highly digestible, noting results similar to the current study on BSM. Further digestibility studies of insect meals are needed as very little is currently being researched with no study having been found on BSM digestibility in broiler chickens.

### **3.5 Conclusion**

The DF-BSM diet's CTTD values showed the highest digestible potential. The study results revealed BSM processed in different forms to be generally more digestible than soya bean meal. However, further research is warranted on the effects of BSM on broiler production, given its digestibility and waste reduction potential. Since BSF larvae are known to be voracious consumers of organic waste, their use as animal protein sources may increase their use as organic waste decomposers, which will help reduce waste accumulation and thus simplify waste disposal. Future research warranted on BSM, on broiler production may have the potential to indicate whether the BSM CTTD values for nutrients obtained have the potential to be efficiently utilised by broiler chickens, converting it to growth and ultimately into meat.

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## Chapter 4

### Effect of black soldier fly (*Hermetia illucens*) pre-pupae meal on production parameters of broiler chickens

#### Abstract

The study was conducted to investigate the effect of black soldier fly (*Hermetia illucens*) pre-pupae meal (BSM) as a protein source in broiler diets on chicken production parameters. Four treatment diets with BSM included at 0%, 5%, 10% and 15% replacing soya-bean meal, were used. Three hundred and twenty day-old Cobb 500 broiler chickens were randomly allocated to the treatments, where each treatment was replicated eight times. These broiler chickens were raised to slaughter at day 35 of age. Addition of BSM as a protein source did not significantly affect average live weight, average daily gain (ADG), feed conversion ratio (FCR), and European protein efficacy factor (EPEF). Weekly feed intake and cumulative feed intake differed significantly between treatments only at day 18. Treatment had an effect on protein efficacy ratio (PER), with chicks receiving the 5% diet being significantly lower than their counterparts. It is therefore, concluded that BSM can be used in broiler diets up to 15% as a protein source, with no effect on the growth performance of broilers.

**Keywords:** Poultry, BSF, magmeal, larvae meal, FCR, ADG, EPEF

#### 4.1 Introduction

About 70% of the total costs in broiler production are attributed to feed costs (Teguia & Beynen, 2005) where soya bean meal is a major protein source. There is a fierce competition for soya beans and grains among humans, livestock (Teguia & Beynen, 2005; Khusro *et al.*, 2012) and the biofuel industry (Biswas *et al.*, 2011). In Asia, soya beans and their products are used mainly for human consumption and minimally for animal production (Ravindran & Blair, 1992). Nonetheless, crop yields have decreased due to climate change influenced by global warming (Dar & Gowda, 2013). It is argued that the competing needs for plant products between humans, livestock and the biofuel industry will increase due to rapid human population growth and climate changes (Delgado *et al.*, 2001; Cribb, 2010). These effects have led to price increase of protein and other feed ingredients; thus an urgent need to find alternative protein sources that are acceptable and sustainable (Téguia *et al.*, 2002).

In South Africa, the poultry industry is the largest utilizer of soya bean meal for animal production. On average South Africa produces about 0.5 million tons of soya beans per annum which is not sufficient (Department of Agriculture, Forestry & Fisheries [DAFF], 2010). The production of soya bean requires an enormous amount of land and capital for the infrastructure necessary for input and harvesting. In Brazil soya bean cultivation is increasing and shifting into the Amazonia causing losses of natural ecosystems, driven by the expanding market (Fearnside, 2001). Soya beans are being considered for biodiesel production due to their extractable fat content and availability (Biswas *et al.*, 2011). The use of soya beans for biodiesel extraction in South Africa will not be feasible due to the high soya bean price coupled with low cultivation and yield (Sparks *et al.*, 2011). Soya bean meal is the preferred protein source widely used in the poultry industry, attributed to its high protein content with an adequate amino acid (AA) profile supporting normal



animal growth (Kocher *et al.*, 2002). The protein quality of a feed ingredient is determined by its AA content in monogastric animals, particularly in poultry diets being formulated on the basis of digestible AA as requirement by the animal (Corzo *et al.*, 2005; Boland *et al.*, 2013). A balanced amino acid profile (synthetic or natural) in a feed ration is essential as it regulates broiler growth and maintenance regardless of the protein percentage level in the diet (Deschepper & DeGroot, 1995; Aletor *et al.*, 2000; Rezaei *et al.*, 2004; Corzo *et al.*, 2005). In poultry, methionine is the first limiting amino acid followed by lysine and threonine. However, if lysine is added in excess it may cause antagonism with arginine, while excess methionine leads to toxicity (Lemme *et al.*, 2004). The deficiency of limiting amino acids in poultry diets causes depressed bird growth (Ravindran, 2013). Hence, it is vital to supply animal diets with various protein sources that contain adequate amounts of amino acids balancing the ratio's leading to sustained normal growth.

Insect meal as an alternative protein source in poultry and fish production is attracting research interest globally. Insect meal is prepared either from larvae, pupae or pre-pupae. Insect meals have been identified to contain high protein levels, as evidenced by their usage in human (Ramos-Elorduy, 1997; van Huis, 2013), poultry (Okah & Onwujariri, 2012; Pieterse *et al.*, 2014) and fish diets (Ogunji *et al.*, 2008a, b; Aniebo *et al.*, 2009). Several authors have reported improved performance of poultry and fish supplemented with insect meal (Sealey *et al.*, 2011; Okah & Onwujariri, 2012; Pieterse *et al.*, 2014). This can be attributed to their high protein content (36.2-76.23%) with sufficient quantities of essential AA required by animals (Newton *et al.*, 2005a; Hassan *et al.*, 2009; Ijaiya & Eko, 2009; Barroso *et al.*, 2014; Pieterse *et al.*, 2014). Furthermore, the protein content of larvae, pupae and pre-pupae do not differ (Newton *et al.*, 1977; Newton *et al.*, 2005b; Diener *et al.*, 2009) within distinct insect species. Insect meals also contain adequate amounts of essential minerals (Newton *et al.*, 2005a) and a fatty acid composition (Raksakantong *et al.*, 2010; Pieterse & Pretorius, 2014) necessary for animal growth. Insect meals' crude protein is digestible attaining apparent digestibility ranging between 69 and 98.8% in poultry (Zuidhof *et al.*, 2003; Hwangbo *et al.*, 2009; Pieterse *et al.*, 2014), comparable to that of soya bean meal's 80.7-87% (Sebastian *et al.*, 1997; Boland *et al.*, 2013). The feed consumed by insects may affect its nutritional value, pertaining to fat and fatty acid content (Ramos-Elorduy *et al.*, 2002), which may account for some differences in nutritional values observed by various authors investigating insect meals.

Organic wastes are suitable growth media for rearing insect larvae, yielding an insect meal high in protein and suitable as a feed ingredient in fish and poultry diets (Ogunji *et al.*, 2008a, b; Pieterse & Pretorius, 2014). This also solves waste disposal and storage problems, thereby reducing waste accumulation and possible pollution (El Boushy, 1991; Li *et al.*, 2011). The black soldier fly (BSF) is a non-pest species and its larvae is known as a voracious consumer of decomposing organic matter reducing its moisture to about 60% (Newton *et al.*, 2005a; Kim *et al.*, 2011). The life cycle of BSF is about 40-44 days and it migrates out of waste in its last developmental stage (pre-pupae), making it easily harvestable (Bradley & Sheppard, 1984). This last immature stage of BSF no longer feed and thus it has an empty gut and is high in stored energy (Sheppard *et al.*, 1994), making it a suitable potential feed ingredient for use in high protein and energy diets for animals (Jeon *et al.*, 2011). Studies on BSF larvae or pre-pupae meal as a feed ingredient indicated that



they are good sources of nutrients for chickens (Hale, 1973), pigs (Newton *et al.*, 1977) and fish (Bondari & Sheppard, 1981), which the pre-pupae is digestible in broiler chickens (Chapter 3).

Despite the availability of black soldier fly in South Africa and the need for alternative protein sources in the poultry industry, black soldier fly pre-pupae (BSM) has not been used as a feed ingredient in poultry diets. The objective of the study was to investigate the effects of BSM as a protein source, at different inclusion levels on broiler chicken production parameters.

## **4.2 Materials and Methods**

### **4.2.1 Experimental treatments, layout and housing system**

The experiment consisted of four treatment diets: three diets based on black soldier fly (*H. illucens*) pre-pupae meal (BSM) at inclusion levels of 0%, 5%, 10% and 15% replacing soya in the diet. The four treatments were each replicated eight times yielding 32 experimental units (cages). A completely randomised design was used for treatment allocation across the poultry house. Ethical clearance was obtained from Stellenbosch University; ethical clearance number SU-ACUM13-00026.

Three hundred and twenty Cobb 500 day-old broiler chicks were received at Mariendahl Experimental Farm (33° 51' 0 S; 18° 49' 60 E) of Stellenbosch University, Western Cape, South Africa. The chicks were vaccinated against infectious *bursal* disease (IBD) and Newcastle disease at the hatchery. The chicks obtained were from the same parental group. Upon arrival the chicks were randomly selected and weighed in groups of ten and then randomly allocated to the treatment cages. From day one to day three the chicks received a commercial starter diet before switching to the respective treatment diets. The heating component in the poultry house intended for the trial malfunctioned and emergency repairs were done, thus the actual trial started on day four. At day four, the chicks were moved to a chicken house equipped with wire cages (0.9 x 0.6 m; 10 birds/m<sup>2</sup>), each containing a hanging tube feeder and two nipple drinkers. Artificial lighting was provided at a pattern of 18 h of light alternating with six hours of darkness. The ventilation of the house was set to six air changes per hour. The chicks were fed a mash diet per bird: 900 g for starter consumed within 15 days, 1200 g of grower consumed within 9 days and 1200 g of finisher consumed within 8 days. The birds were provided with feed and water *ad libitum*.

### **4.2.2 Management and handling of birds**

The broilers were cared and managed based on the Cobb 500 management guide (Cobb-Vantress, 2012), from day one to slaughter age at day 35. The chicks were vaccinated against IBD and Newcastle disease at the hatchery; repeated at day 17 for IBD and Newcastle at day 20 and 31 of age. The vaccinations were administered using purified water and given to the birds through chick water fountains. Prior to vaccination, the drinkers were lifted for an hour to ensure all the birds drink the vaccination. The birds were further checked for blue beaks and/or blue surroundings around their eyes to ensure that each bird had been vaccinated.

The birds were reared in a temperature controlled room and the ventilation in the house was set to provide a minimum of six air changes per hour. Temperature of the house was checked and recorded every morning at eight o'clock. The birds were monitored every two hours for the first week of age and thereafter every four hours, except in the darkness hours. Check-ups on the birds were done, for any abnormal behaviour with regards to sickness, cannibalism, activeness, eating and water drinking patterns. All mortalities were accounted for and underwent post-mortem inspection to determine the cause of death.

#### ***4.2.3 Experimental diets formulations***

The four treatment diets were formulated according to Cobb 500 nutrient specifications (Cobb-Vantress, 2012), using table nutrient values. Treatment diets were mixed at the Mariendahl experimental farm (Stellenbosch University). The diets were mixed at room temperature (28 °C) and administered to birds as mash diets. The black soldier fly (BSF) pre-pupae used in this study were harvested at different times of the year and were also fed different feed composition of organic kitchen waste, fruits and vegetables, but the rearing methods and temperature were kept constant. Furthermore, the BSM used in this study was dried at 65 °C. The ingredients used to formulate the treatment diets are shown in Table 4.1 for the starter, grower and finisher diets.

**Table 4.1** Ingredients used for the Starter, Grower and Finisher diets with inclusion of black soldier fly pre-pupae meal (BSM)

Ingredients	Unit	Treatment diets			
		Control	5% BSM	10% BSM	15% BSM
<b>Starter diets</b>					
Yellow Maize Fine	%	50.091	48.424	46.757	52.813
Soybean full fat	%	15.392	16.606	17.821	13.995
Soybean	%	30.138	25.618	21.099	13.553
BSM <sup>1</sup>	%		5.000	10.000	15.000
L-Lysine	%	0.136	0.129	0.121	0.337
DL Methionine	%	0.125	0.119	0.113	0.143
Vit+Min Premix*	%	0.150	0.150	0.150	0.150
Limestone	%	1.660	1.650	1.640	1.677
Salt	%	0.129	0.131	0.133	0.090
Monocalcium Phosphate	%	1.639	1.637	1.635	1.644
Sodium Bicarbonate	%	0.540	0.536	0.532	0.598
<b>Grower diets</b>					
Yellow Maize Fine	%	45.682	47.342	51.707	49.037
Soybean full fat	%	44.172	34.676	16.459	
Soybean	%	6.147	8.974	17.784	26.418
BSM <sup>1</sup>	%		5.000	10.000	15.000
L-Lysine	%	0.045	0.060	0.093	1.094
DL Methionine	%	0.145	0.124	0.094	0.764
Vit+Min Premix*	%	0.150	0.150	0.150	0.150
Limestone	%	1.632	1.642	1.667	1.679
Salt	%	0.275	0.262	0.238	
Monocalcium Phosphate	%	1.632	1.634	1.640	1.661
Sodium Bicarbonate	%	0.118	0.135	0.168	0.725
Oil - Sunflower	%				3.471
<b>Finisher diets</b>					
Yellow Maize Fine	%	47.164	47.292	47.420	47.798
Soybean full fat	%	48.887	43.776	38.665	33.294
Soybean	%				
BSM <sup>1</sup>	%		5.000	10.000	15.000
L-Lysine	%				0.006
DL Methionine	%	0.134	0.115	0.097	0.080
Vit+Min Premix*	%	0.150	0.150	0.150	0.150
Limestone	%	1.640	1.641	1.642	1.644
Salt	%	0.290	0.284	0.279	0.271
Monocalcium Phosphate	%	1.634	1.634	1.634	1.635
Sodium Bicarbonate	%	0.100	0.107	0.113	0.122

\*Vitamins + Minerals premix included according to levels set by the National Research Council (1994)

#### 4.2.4 Chemical composition analysis of the treatment diets

The proximate analysis of the feed samples was analysed according to acceptable standard methods as provided by the Association of Official Analytical Chemists International (2002). Refer to Chapter 3 regarding methodological analysis of dry matter, crude protein, ash content, crude fibre and amino acid analysis under sections 3.2.4.1, 3.2.4.2, and 3.2.4.4-3.2.4.6, respectively.

#### 4.2.4.1 Crude fat determination

The crude fat content of each treatment feed sample was determined by the ether extract official method 920.39 using diethyl ether reagent on a Tecator Soxtec System HT 1043 Extraction Unit (AOAC, 2002).

#### 4.2.5 Production data collection

The birds were weighed on day four (before they were changed to their experimental diets) and weekly thereafter. All the birds in each cage were weighed together and the average weight for an individual chicken in each cage was calculated. Left-over feed was also weighed during the weighing of the birds. The measurements of live weight and left-over feed were used for calculating the average live weight, weekly feed intake, cumulative feed intake, feed conversion ratio (FCR), average daily gain (ADG) and the European production efficiency factor (EPEF) and protein efficiency ratio (PER). The PER (Equation 4.3) and EPEF (Equation 4.4) were calculated according to Boling-Frankenbach *et al.* (2001) and Awad *et al.* (2009), respectively. The EPEF takes liveability into account. The number of birds that died during the trial is shown in Table 4.2. The death of the birds were found to be caused by excess weight which was revealed by the post mortem diagnosis, while those that were culled were due to observed cannibalism and leg problems. Only one bird was culled due to neck cannibalism from the group of chicks receiving the 10% treatment diet on day 23. Liveability of the birds was calculated as a percentage of birds that survived till the end of the trial over the total number of birds placed at the start of the trial.

**Table 4.2** The number of birds lost during the production trial and weight at death (g)

Pen number	Date of death	Cause of death	n	Treatment diets			
				Control	5% BSM <sup>#</sup>	10% BSM <sup>#</sup>	15% BSM <sup>#</sup>
2	28	Mortality	1		985.70		
4	29	Mortality	1			1897.00	
7	9	Culled <sup>3</sup>	1	165.78			
12	7	Culled <sup>3</sup>	1				160.95
14	18	Morbidity <sup>1</sup>	1			330.00	
18	23	Culled <sup>2</sup>	1			918.00	
22	24	Mortality	1		1207.90		
31	13	Culled <sup>3</sup>	1	294.40			
32	15	Mortality	1			564.50	
<b>Total</b>			<b>9</b>				

<sup>n</sup> Number of birds that died per treatment

<sup>1</sup> Removal of bird from its replicate group (but not killed) due to large differences in bird average weight

<sup>2</sup> Culled due to cannibalism

<sup>3</sup> Culled due to leg problems

<sup>#</sup> BSM: Black soldier fly pre-pupae meal

The following formulae were used for calculating the ratios and efficient coefficients (Equation 4.1-4.4):

#### Equation 4.1,

$$\text{Average daily gain} = \frac{\text{Average live weight per chick (g)}}{\text{Age (days)}}$$

**Equation 4.2,**

$$\text{Feed conversion ratio} = \frac{\text{Cumulative feed intake (g)}}{\text{Average live weight per chick (g)}}$$

**Equation 4.3**

$$\text{Protein efficiency ratio} = \frac{\text{Body weight gain (g)}}{\text{Crude protein intake (g)}}$$

**Equation 4.4**

$$\text{European production efficacy factor} = \frac{\text{Liveability \%} \times \text{Live weight (g)}}{\text{Age (days)} \times \text{Feed conversion ratio}} \times \frac{100}{1}$$

**4.2.6 Statistical analysis**

The statistical analysis was done using the general linear models (GLM) procedure of SAS (2009). The analysis of variance (ANOVA) assumptions for normality and homoscedasticity were investigated before further analyses were done. The tests were considered significant at  $P > 0.05$ . A one-way analysis of variance (ANOVA) with Bonferroni's *post hoc* (least square means) test was used for statistical analysis. The ADG slope was calculated by means of a regression. The ADG slope comparison of the treatments was analysed using a one-way analysis of variance (ANOVA) with Bonferroni's *post hoc* test. The 5% significance level was used for all the statistical tests and treatment differences were declared at  $P < 0.05$ .

The statistical model for the ANOVA test is indicated by;  $Y_{ij} = \mu_i + \alpha_j + \varepsilon_{ij}$  where the terms in the model are defined as: the treatment effect response ( $Y_{ij}$ ), the overall mean ( $\mu_i$ ), treatment effect ( $\alpha_j$ ) and the unexplained error ( $\varepsilon_{ij}$ ).

The statistical model for the regression test is indicated by;  $Y_i = \beta_0 + \beta_1 X_i + \varepsilon_i$  where the terms in the model are defined as: the treatment value of the dependent variable ( $Y_i$ ), the intercept of the best-fitting line ( $\beta_0$ ), the slope of the best-fitting line ( $\beta_1$ ), the treatment value of the independent variable ( $X_i$ ), the unexplained error associated with the treatment effect not explained by the regression line ( $\varepsilon_i$ ).

**4.3 Results**

The chemical composition of the treatment diets are summarized in Tables 4.3, 4.4 and 4.5 for the starter, grower and finisher, respectively.

**Table 4.3** Analysed proximate and amino acid composition of trial Starter diets on dry matter basis, with inclusion of black soldier fly pre-pupae meal (BSM)

Parameters	Units	Treatment diets			
		Control	5% BSM	10% BSM	15% BSM
Dry matter	%	89.28	90.02	89.71	88.78
Crude protein	%	27.23	29.58	29.54	26.50
Crude fat	%	5.08	7.67	9.62	9.11
Ash	%	7.75	8.16	7.82	8.62
Crude fibre	%	4.35	4.81	5.27	4.25
Histidine*	g/100g	0.54	0.53	0.56	0.47
Serine	g/100g	1.06	1.16	1.01	0.86
Arginine*	g/100g	1.33	1.38	1.38	1.06
Glycine	g/100g	0.91	0.96	1.01	0.81
Aspartic acid	g/100g	1.93	1.80	1.93	1.48
Glutamic acid	g/100g	3.46	3.27	3.33	2.65
Threonine*	g/100g	0.78	0.79	0.79	0.66
Alanine	g/100g	0.88	0.92	0.97	0.83
Proline	g/100g	1.16	1.11	1.15	1.00
Cysteine	g/100g	0.09	0.08	0.07	0.07
Lysine*	g/100g	1.08	1.06	1.15	1.09
Tyrosine	g/100g	0.74	0.92	0.88	0.75
Methionine*	g/100g	0.24	0.27	0.25	0.23
Valine*	g/100g	0.86	0.93	1.01	0.79
Isoleucine*	g/100g	0.74	0.78	0.85	0.62
Leucine*	g/100g	1.60	1.56	1.64	1.33
Phenylalanine*	g/100g	1.10	1.16	1.09	0.87

\* Essential amino acids

**Table 4.4** Analysed proximate and amino acid composition of trial Grower diets on dry matter basis, with inclusion of black soldier fly pre-pupae meal (BSM)

Parameters	Units	Treatment diets			
		Control	5% BSM	10% BSM	15% BSM
Dry matter	%	90.03	89.55	89.03	88.28
Crude protein	%	26.97	27.78	25.73	29.74
Crude fat	%	9.60	9.98	9.48	10.78
Ash	%	6.75	7.66	7.84	8.23
Crude fibre	%	4.60	6.57	5.26	4.19
Histidine*	g/100g	0.54	0.61	0.51	0.52
Serine	g/100g	0.99	1.12	0.86	1.00
Arginine*	g/100g	1.40	1.40	1.22	1.29
Glycine	g/100g	0.90	1.00	0.88	1.00
Aspartic acid	g/100g	1.78	1.60	1.51	1.79
Glutamic acid	g/100g	3.24	2.91	2.82	3.06
Threonine*	g/100g	0.74	0.74	0.71	0.79
Alanine	g/100g	0.83	0.83	0.81	0.99
Proline	g/100g	1.11	1.04	1.03	1.19
Cysteine	g/100g	0.07	0.07	0.07	0.08
Lysine*	g/100g	0.93	0.87	0.89	1.63
Tyrosine	g/100g	0.74	0.89	0.90	0.95
Methionine*	g/100g	0.22	0.28	0.25	0.74
Valine*	g/100g	0.87	0.94	0.86	0.95
Isoleucine*	g/100g	0.76	0.79	0.72	0.79
Leucine*	g/100g	1.51	1.49	1.48	1.59
Phenylalanine*	g/100g	1.11	1.17	1.06	1.08

\* Essential amino acids

**Table 4.5** Analysed proximate and amino acid composition of trial Finisher diets on dry matter basis, with inclusion of black soldier fly pre-pupae meal (BSM)

Parameters	Units	Treatment diets			
		Control	5% BSM	10% BSM	15% BSM
Dry matter	%	89.61	89.84	90.63	90.02
Crude protein	%	22.21	24.73	21.55	21.45
Crude fat	%	7.98	11.21	14.95	12.79
Ash	%	8.76	4.20	9.82	9.45
Crude fibre	%	5.89	7.98	5.77	6.08
Histidine*	g/100g	0.51	0.53	0.49	0.41
Serine	g/100g	0.81	0.89	0.78	0.70
Arginine*	g/100g	1.17	1.34	0.92	0.93
Glycine	g/100g	0.77	0.87	0.87	0.76
Aspartic acid	g/100g	1.39	1.67	1.31	1.13
Glutamic acid	g/100g	2.67	3.04	2.30	2.17
Threonine*	g/100g	0.61	0.69	0.63	0.56
Alanine	g/100g	0.73	0.78	0.88	0.76
Proline	g/100g	1.00	1.08	1.04	0.97
Cysteine	g/100g	0.07	0.07	0.06	0.04
Lysine*	g/100g	0.76	0.85	0.72	0.62
Tyrosine	g/100g	0.70	0.76	0.94	0.79
Methionine*	g/100g	0.32	0.18	0.30	0.24
Valine*	g/100g	0.76	0.85	0.85	0.74
Isoleucine*	g/100g	0.66	0.73	0.66	0.60
Leucine*	g/100g	1.36	1.44	1.38	1.24
Phenylalanine*	g/100g	1.00	1.04	0.85	0.84

\* Essential amino acids

The production parameters for the chickens fed different levels of black soldier fly pre-pupae meal (BSM) included at 0, 5, 10 and 15% in broiler chicken diets, under a three phase feeding system (starter, grower and finisher) are summarised in Table 4.6.

During the experimental period birds were in good health, experiencing low bird mortalities of 2.81% (Table 4.2). Liveability results indicated that mortality of birds was not related to treatment, attaining  $P=0.433$ . Inclusion of BSM (treatment) did not ( $P>0.05$ ) affect the average live weight of the birds at day 11, 18, 25, 32 and 35 of age. Also, at day 11, 25, 32 and 35 of age, no treatment differences ( $P>0.05$ ) were found regarding weekly feed intake and cumulative feed intake. However, at day 18 of age treatment did ( $P<0.05$ ) affect weekly feed intake and cumulative feed intake of broiler chicks. The chicks receiving the 5% inclusion of BSM had a higher weekly feed intake and cumulative feed intake, while the 10% pre-pupae meal had significantly lower values. Weekly feed intake and cumulative feed intake did not differ significantly between chicks that received the 0% and 15% treatment diets, however being similar to the 5 and 10% treatment diets chicks.

The inclusion of BSM in the diets of broiler chickens did not ( $P>0.05$ ) influence average daily gain (ADG), feed conversion ratio (FCR), liveability and European protein efficacy factor (EPEF). Treatment did ( $P<0.05$ )



influence protein efficiency ratio (PER). The chicks receiving the 5% BSM diet differed significantly from the 0, 10 and 15% diets, attaining a ( $P<0.05$ ) lower PER value (Figure 4.1).

**Table 4.6** The means ( $\pm$  standard error) of weekly feed intake (g), live weight (g) and cumulative feed intake (g) and the production ratios (ADG, FCR, EPEF and PER) of broilers as influenced by inclusion of black soldier fly pre-pupae meal (BSM)

Production days	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
<b>Day 11</b>					
Average live weight	267.6 $\pm$ 4.22	270.4 $\pm$ 3.48	265.2 $\pm$ 4.33	262.1 $\pm$ 5.28	0.587
Weekly feed intake	221.6 $\pm$ 2.77	222.3 $\pm$ 1.99	216.7 $\pm$ 3.78	210.7 $\pm$ 5.05	0.100
Cumulative feed intake	221.6 $\pm$ 2.77	222.4 $\pm$ 1.99	216.7 $\pm$ 3.78	210.7 $\pm$ 5.05	0.810
<b>Day 18</b>					
Average live weight	604.3 $\pm$ 7.59	626.8 $\pm$ 6.38	610.8 $\pm$ 8.19	625.0 $\pm$ 8.46	0.133
Weekly feed intake	491.1 <sup>ab</sup> $\pm$ 6.21	499.0 <sup>a</sup> $\pm$ 3.95	475.0 <sup>b</sup> $\pm$ 3.44	489.6 <sup>ab</sup> $\pm$ 7.28	0.033
Cumulative feed intake	715.6 <sup>ab</sup> $\pm$ 6.28	721.3 <sup>a</sup> $\pm$ 4.70	691.7 <sup>b</sup> $\pm$ 5.39	710.3 <sup>ab</sup> $\pm$ 9.28	0.022
<b>Day 25</b>					
Average live weight	1122.4 $\pm$ 9.22	1135.0 $\pm$ 17.88	1131.8 $\pm$ 27.30	1147.4 $\pm$ 18.46	0.835
Weekly feed intake	764.4 $\pm$ 10.00	789.7 $\pm$ 5.98	788.8 $\pm$ 12.82	769.6 $\pm$ 6.49	0.140
Cumulative feed intake	1489.8 $\pm$ 17.68	1511.0 $\pm$ 8.92	1499.9 $\pm$ 27.99	1479.9 $\pm$ 11.12	0.652
<b>Day 32</b>					
Average live weight	1758.3 $\pm$ 16.40	1800.9 $\pm$ 17.00	1783.0 $\pm$ 24.19	1794.8 $\pm$ 23.66	0.488
Weekly feed intake	1033.7 $\pm$ 17.39	1064.4 $\pm$ 12.80	1068.2 $\pm$ 15.35	1033.6 $\pm$ 14.23	0.216
Cumulative feed intake	2523.5 $\pm$ 27.10	2617.3 $\pm$ 37.23	2608.5 $\pm$ 43.09	2513.4 $\pm$ 20.62	0.063
<b>Day 35</b>					
Average live weight	2033.4 $\pm$ 23.80	2082.4 $\pm$ 17.13	2077.5 $\pm$ 25.90	2076.6 $\pm$ 26.97	0.441
Weekly feed intake	480.3 $\pm$ 10.06	490.7 $\pm$ 10.86	492.0 $\pm$ 5.98	478.1 $\pm$ 12.24	0.690
Cumulative feed intake	3003.8 $\pm$ 35.27	3108.0 $\pm$ 42.05	3100.5 $\pm$ 41.96	2991.6 $\pm$ 30.63	0.067
<b>ADG<sup>1</sup> (g)</b>	64.3 $\pm$ 0.68	65.9 $\pm$ 0.59	65.6 $\pm$ 0.95	65.8 $\pm$ 0.84	0.666
<b>FCR<sup>2</sup></b>	1.6 $\pm$ 0.01	1.6 $\pm$ 0.01	1.6 $\pm$ 0.01	1.5 $\pm$ 0.01	0.121
<b>EPEF<sup>3</sup></b>	400.7 $\pm$ 12.22	411.6 $\pm$ 14.81	390.9 $\pm$ 17.80	431.0 $\pm$ 14.57	0.288
<b>PER<sup>4</sup></b>	2.5 <sup>b</sup> $\pm$ 0.02	2.4 <sup>a</sup> $\pm$ 0.02	2.5 <sup>b</sup> $\pm$ 0.02	2.5 <sup>b</sup> $\pm$ 0.02	<0.001
<b>Liveability</b>	1.0 $\pm$ 0.02	1.0 $\pm$ 0.02	1.0 $\pm$ 0.02	1.0 $\pm$ 0.01	0.433

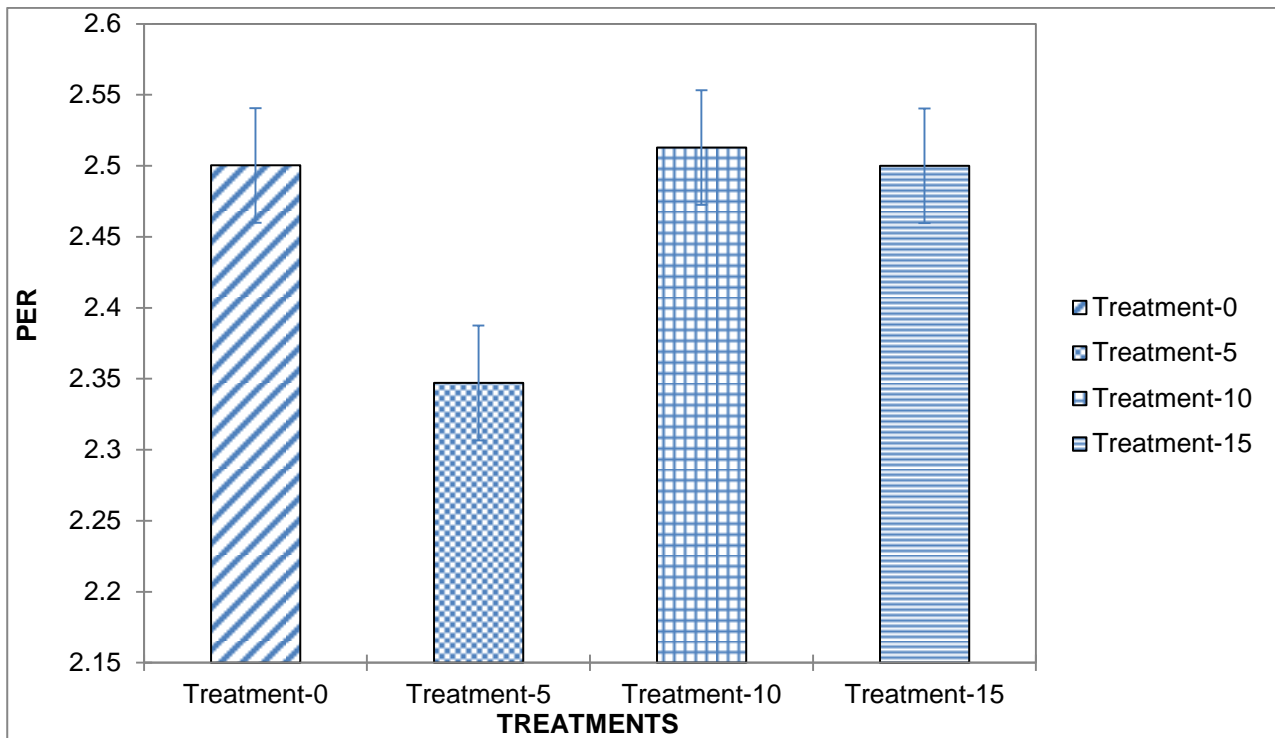
(<sup>a,b</sup>) Means with different superscripts within the same row differ significantly ( $P<0.05$ )

<sup>1</sup> Average daily gain

<sup>2</sup> Feed conversion ratio

<sup>3</sup> European protein efficacy factor

<sup>4</sup> Protein efficiency ratio



**Figure 4.1** Least square means with error bars for protein efficacy ratio at 5% significant level for treatment effect

#### 4.4 Discussion

The effect of BSM on growth results reported in this study on day 11, 18, 25, 32 and 35 (except at day 18 for weekly feed intake and cumulative feed intake), were similar with those reported in chickens by Wang *et al.* (2005) and in fish (Newton *et al.*, 2005b; Ogunji *et al.*, 2008a, b; Aniebo *et al.*, 2009) who used house fly maggot meal (HFMM) in their experiments. No treatment differences ( $P > 0.05$ ) were observed regarding average live weight, weekly feed intake, cumulative feed intake, ADG, FCR, EPEF and liveability, but treatment differences ( $P < 0.05$ ) were observed at day 18 for weekly feed intake and cumulative feed intake. The production parameters results indicated that BSM inclusion in broiler chicken diets did not positively or negatively affect growth but sustained normal bird growth, yielding results similar to the chicks receiving the control treatment diet. Contrastingly, Téguia *et al.* (2002), Okah & Onwujiariri (2012) and Pieterse *et al.* (2014) found improved performance in broiler chickens fed house fly meal as a protein source. Furthermore, Cole (2007) found treatment differences with growth parameters measured in alligators, fed diets containing different inclusion of BSM leading to a decrease in growth. However, treatment difference found by Cole (2007) may have been due to a shorter trial period (3 months) observing a substantial increase in growth of male alligators then females, even though mixed sex was used per treatment, differences were noted. In the study by Newton *et al.* (2005b), the addition of BSM over 7.5% had no positive effect on growth, in fact Awoniyi *et al.* (2003) found a decrease in weight gain with increased inclusion of HFMM at levels  $> 25\%$  in broiler chicken diets. This might be due to an oversupply of proteins that may arise with high inclusion levels of insect meals which was also observed by Pretorius (2011) in broiler chickens fed house fly larvae meals.

However, in this study BSM was included up to 15% obtaining no declining effect on growth parameters investigated but rather comparable performance results to the control were observed.

To obtain production efficiency in broiler birds' production the following should be achieved: acceptable EPEF value  $\geq 260$  units, with ADG of  $\geq 50$  g, a FCR  $\leq 1.85$  and slaughter live weight of 1.5-2 kg at 35 days, reared under optimal management and adequate nutrition (Butcher & Nilipour, 2002). The results obtained in the current study for EPEF, ADG, FCR and live weight (Table 4.6) were above the stated standards, indicating BSM to be a viable protein source in broiler diets.

In this study a 2.81% (Table 4.2) bird mortality was obtained. The accepted mortality percentage of a bird flock is 2%. However, 1.56% (4 birds) of the death experienced in the study was due to heart attacks and 1.25% (4 birds) were culled and 0.31% (1 bird) was morbid. Only one bird was culled due to neck cannibalism from chicks receiving the 10% treatment diets. Three birds were culled due to leg disorders with two birds from the 0% (control) and one from the 15% treatment groups. Based on the post-mortem inspection done on the mortality birds, it was revealed that they died due to excess weight with increased heart size to body weight percentage. Furthermore, there was no ( $P > 0.05$ ) treatment difference observed regarding liveability (Table 4.6), indicating that the inclusion of BSM in broiler chickens did not cause death of the birds.

The chicks receiving the 5% treatment diet differed ( $P < 0.05$ ) from those receiving the 10% diet, but both did not differ from those fed the control and 15% diets regarding weekly feed intake and cumulative feed intake at day 18 of age (Table 4.6). However, the treatment differences ( $P < 0.05$ ) found in this study at day 18 are difficult to explain, since a general trend of no significant treatment differences was observed in the study. The treatment difference observed was thought to have been attributed to a slightly higher crude fibre (CF) percentage found in the 5% treatment diet. However, slightly higher CF content was found in all three feeding phases of the 5% diet but only affected feed intake significantly at day 18 of age. Furthermore, a slightly higher CF content was also observed with the remaining treatment diets in starter, grower and finisher which however, did not lead to treatment differences in feed intake at any given day. Nevertheless, it is reported that birds consume slightly more feed when CF is higher in their diets (Ranjhan, 2001). Furthermore, the significant increase in weekly feed intake and cumulative feed intake found at day 18 did not affect ( $P > 0.05$ ) the overall treatments' live weight, ADG, FCR and EPEF ( $P > 0.05$ ), but may have affected PER where ( $P < 0.05$ ) treatment differences were observed. The chicks receiving the 5% treatment diet had a ( $P < 0.05$ ) lower PER and a higher weekly feed intake and cumulative feed intake. The differences observed might be attributed to a measuring error that possibly occurred during measurement of the birds and feed but no outliers were found, thus there seems to be no biological explanation to the differences observed.

The PER explains the protein utilization by the animal: a low PER value ( $< 1.5$ ) is an indicator of low protein quality in the diet and its utilization by the animals (Johnson & Parsons, 1997). However, in this study even though treatment ( $P < 0.05$ ) affected PER, the values obtained indicate that dietary protein was efficiently utilized with a minimum of 2.35 PER (Table 4.6). This indicates that dietary protein quality was not the cause

for PER treatment differences observed as it would have affected the 10% and 15% treatment diet more than the 5% diet, as they have a higher inclusion of BSM. However, a balanced amino acid profile in an animal diet is more essential than the protein percentage, as the amino acids regulates growth and determines the protein quality of the feed source (Boland *et al.*, 2013). The BSM protein and AA digestibility values (Table 3.3) may help explain the lack of treatment differences attained given that it attained a higher protein digestibility than soya bean meal, as indicated by Sebastian *et al.* (1997), thus proving that nutrients were available for use by the animal for conversion into growth.

Furthermore, the amino acid content of BSM closely fits that of the ideal amino acid requirements for poultry diets, shown in Table 2.7 and 2.8, respectively. This may further explain why no treatment differences were obtained amongst the production parameters investigated as the quality of the BSM enabled formulation of balanced diets according to the nutritional requirements for this chicken line (Cobb 500). A balanced amino acid profile (synthetic or natural) in a feed ration is essential as it regulates broiler growth and maintenance regardless of the protein percentage level in the diet (Deschepper & DeGroot, 1995; Aletor *et al.*, 2000; Rezaei *et al.*, 2004; Corzo *et al.*, 2005). The amino acid content of BSM summarized in Table 2.7 has proven to be adequate, producing broiler chickens with production parameters results similar to those fed a soya bean meal based diet. Therefore, the results obtained in this study proved BSM to be a possible protein source in broiler bird's diets as it has supported normal growth of broiler chickens.

#### **4.5 Conclusion**

The use of back soldier fly (*H. illucens*) pre-pupae meal in broiler chicken diets resulted in a similar production efficiency when compared to soya bean meal (the preferred protein source in the poultry industry). The lack of treatment differences noted for the growth parameters indicate that BSM can successfully be used in broiler chicken diets with no detrimental effects on broiler production up to the evaluated inclusion level of 15%. The use of insect meals in animal diets will however, lead to an increase in organic waste vermi-composting thereby minimizing waste effects, and in the process yield larvae and/or pupae which will increase protein availability for animal use and minimize usage of crop products used for human consumption in broiler diets. Since, BSM proved to have supported broiler chickens growth with no adverse effects, further research is warranted on BSM's effect on organs, gut and bone parameters of broiler chickens.

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## Chapter 5

### The effects of black soldier fly (*Hermetia illucens*) pre-pupae meal on organ, gut and tibia bone parameters of broiler chickens

#### Abstract

This study investigated the effect of black soldier fly (*Hermetia illucens*) pre-pupae meal (BSM) as a protein source included at 0, 5, 10 and 15% in starter, grower and finisher diets of broiler chickens on organ weight, gastrointestinal pH, histomorphological measurements of the small intestine (duodenum and jejunum) and tibia bone parameters. Three hundred and twenty Cobb 500 broiler chicks were randomly assigned to the four dietary treatments each replicated eight times and were fed for 35 days. At day 35 of age, eight birds were selected from each treatment and slaughtered. The excised organs from the carcasses were weighed and the gizzards were scored for erosion. The pH of the duodenum, jejunum and ileum were taken and histomorphology evaluations were done on the duodenum and jejunum. The tibia bone was measured for breaking strength, ash percentage and mineral content. Inclusion of BSM did not significantly affect organ weight, gizzard erosion score, intestinal pH, tibia bone ash percentage, tibia breaking force (N), tibia breaking force per gram of tibia weight (N/g) and tibia breaking strength (N/mm<sup>2</sup>). Treatment did not significantly affect tibia bone minerals except for calcium (Ca) where a significantly increased Ca content was observed for chicks fed BSM but this did not differ from the control. It could therefore, be concluded that the use of black soldier fly pre-pupae meal in broiler chickens did not affect organs, small intestine and tibia bone development.

**Keywords:** Tibia, bone breaking strength, minerals, Instron, gastrointestinal tract, histomorphology, BSF

#### 5.1 Introduction

The major protein and energy sources in broiler chicken diets are soya bean and maize meal, respectively. There has been a decline in production yield of these important crops, attributed to climate change due to global warming, consequently increasing their prices (Dar & Gowda, 2013). The low supply and raise in price of maize and soya bean meal forced the livestock industry to search for alternative feed ingredients (Téguia *et al.*, 2002). Primarily a diet is formulated to meet the nutrient requirements of the animal and maintain its body functions, as nutrient imbalances affect animal growth and development (Awad *et al.*, 2009).

In monogastric nutrition the major gut functions being digestion, absorption and intestinal barrier, are vital for efficient production and should be optimised at minimal nutrient use (Van der Klis & Jansman, 2002). Briefly, apart from hygiene and vaccines, an adequate nutritional supply to the bird is vital for optimal health and immune responsiveness (Goddeeris *et al.*, 2002). Furthermore, the feed may alter or affect the structural integrity of organs depending upon its nutritional composition (Fasina *et al.*, 2006) and feed granule size (Engberg *et al.*, 2002). The deficiency of essential nutrients, such as low arginine amongst others in avian diets, causes poor development of organs, including lymphoid organs which are essential for immune responses of birds. Avian lymphoid organs assure that pathogens do not evade the host, resist infections and maintain productivity during infectious attacks (Kwak *et al.*, 1999). Diets must therefore, be balanced for essential nutrients for proper development and growth of birds, which is dictated by the feed ingredient's nutritional quality (Ensminger, 1992).

The skeletal structure and strength of avian bones is determined by physical, nutritional and physiological factors (Rath *et al.*, 2000). Avian bone is composed of the organic and inorganic matrix responsible for bone stiffness, tensile and compositional strength. Calcium (Ca), phosphorous (P) and dietary vitamin D available to the bird aids the development of its bone's inorganic matrix (Rath *et al.*, 1999). The fast growth of modern broiler chickens has led to bone problems, as weight gain occurs at a faster rate than bone development, causing bones to become porous and fragile and thus being unable to support the excessive weight of the birds. In addition, the porosity of the bones cause them to become fragile, due to insufficient P and Ca mobilization from the bones to support mineral metabolism and support the rapid growth, influenced by mineral deficiency in the diet (Hocking *et al.*, 2009; Garcia *et al.*, 2013). Porous and fragile bones also easily fragment during slaughter and processing of the birds, leading to discoloration of meat which is in close contact with the bone. This is due to leaching of blood and is met with consumer resistance (Rath *et al.*, 2000; Brenes *et al.*, 2003; Garcia *et al.*, 2013).

Most ingredients used in poultry diets are deficient in P and Ca increasing occurrence of bone breakage and leg defects (Brenes *et al.*, 2003). Black soldier fly larvae and pre-pupae meal are potential alternative feed ingredients gaining popularity in monogastric nutrition as a feed ingredient and at the same time as a waste reduction tool. Black soldier fly pre-pupae meal (BSM) is known to contain high levels of Ca and P (Newton *et al.*, 2005). However, studies utilizing BSM have not evaluated its effects on bone strength despite it being high in Ca and P which are essential for bone development. There is also very limited literature available on evaluation of BSM's toxic effects, if any in broiler chickens. It is however, vital to study the effects of any new feed ingredient in animal diets for toxicity on organs (Téguia *et al.*, 2002). Therefore, research is merited to find out whether BSM would affect the skeletal, gut and organ development of animals.

The objectives of this study were two-fold. Firstly, investigate the effect of BSM on the organ and gut parameters of broiler chickens. The study focused on charactering the possible changes in the gastrointestinal tract and organ size of broilers in response to BSM in diets. Secondly, investigate the effect of BSM on the tibia bone of broiler chickens by evaluating its breaking strength, ash percentage and mineral content.

## 5.2 Materials and methods

The materials and methods on the experimental treatments, layout, housing, experimental diets procedures, management and handling of birds are outlined in Chapter 4 under sections 4.2.1, 4.2.3, and 4.2.2, respectively. Briefly, 320 Cobb 500 day-old chicks were feed four diets (0, 5, 10 and 15%) based on inclusion of black soldier fly pre-pupae meal (BSM) till day 35. The treatment diets where each replicated eight times. One bird per cage was randomly selected from the middle weight group for slaughter at day 35 of age. The birds were slaughtered according to acceptable slaughtering standard methods used for commercial chickens (Department of Agriculture, Forestry & Fisheries [DAFF], 2006).

### 5.2.1 Organ sample

At slaughter the birds were rendered unconscious by electrical stunning (50-70 volts; 3-5 s) then exsanguinated and allowed to bleed out for about 2 min. Thereafter, the organs were immediately excised from the carcass using scalpels and scissors from dissection kits. Organs excised were the heart, spleen, liver, gizzard and *bursa of Fabricius*. The gizzard was cut open then rinsed with clean water and scored for gizzard erosion, using an ordinal scale according to Johnson & Pinedo (1971) shown in Table 1. Then organs were immediately weighed using a PC 400 Mettler laboratory scale (Mettler-Toledo, Switzerland). These organs were removed with care to avoid any damage to the organs. The organ weight relative to body weight was calculated as a percentage of organ weight to live weight of the bird.

**Table 5.1** Gizzard erosion scoring description (Johnson & Pinedo, 1971)

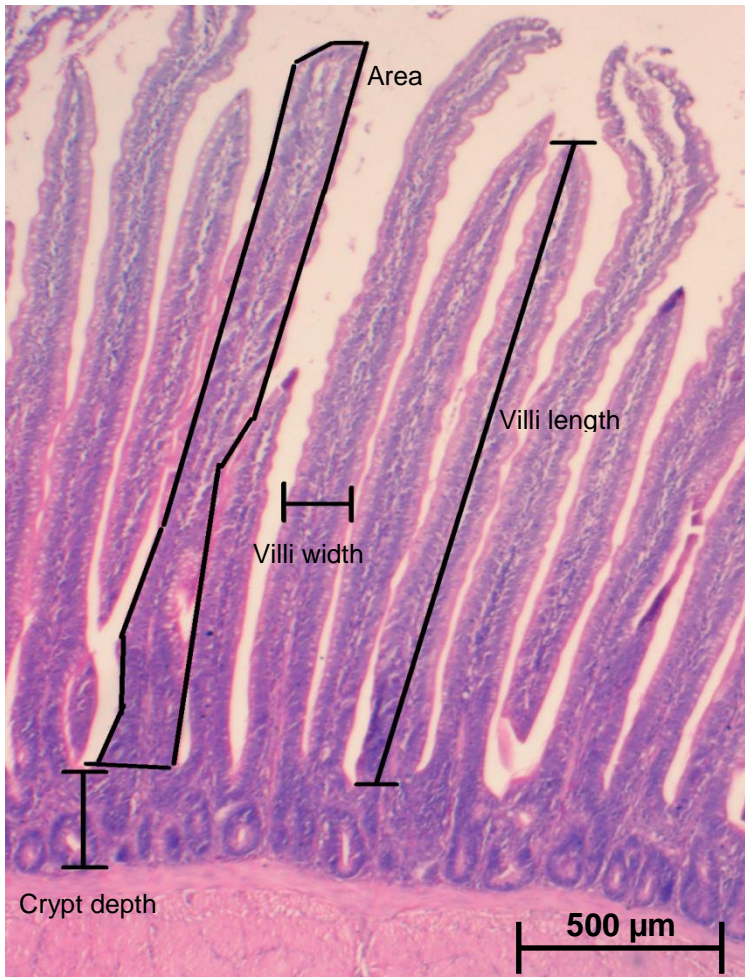
Score	Description
0	No erosion
1	Light erosion (minimal roughness of the epithelia)
2	Modest erosion (roughness and minimal gaps of the epithelia)
3	Severe erosion (roughness, gaps and ulcers on wall showing slight haemorrhaging)
4	Extreme erosion (roughness, gaps and haemorrhagic ulcers on stomach wall and visible separation of epithelia from stomach wall)

### 5.2.2 Intestinal samples

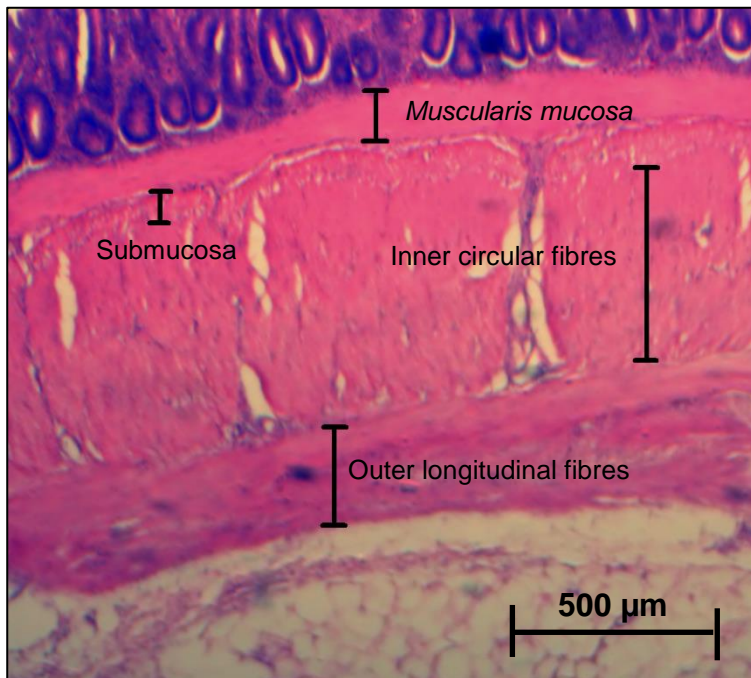
Within 15 min after slaughter the small intestine was removed to obtain the gut samples: duodenum, jejunum and ileum. The duodenum section for histology samples was cut on the gizzard side of the duodenum, while the jejunum section was taken from the centre of the jejunum and the ileum was taken 5 mm from Meckel's diverticulum to the ileocecal junction. After sectioning, the pH of the duodenum, jejunum and ileum contents were measured using a calibrated (standard buffers pH 4.0 and 7.0 at 25 °C) portable Crison pH 25 meter (Alella, Barcelona), by inserting the pH electrode into the centre of the intestinal section to be measured. Distilled water was used to thoroughly rinse the probe between each reading. Approximately a 2 cm long segment of each sample (duodenum, jejunum and ileum) was then dissected and cut open longitudinally and rinsed with 0.9% saline solution and then fixed in 10% buffered formalin solution until further analysis.

The duodenum and jejunum histology samples were processed according to Presnell & Schreiber (1997). The samples were cut to size, placed into embedding cassettes, processed and impregnated with paraffin wax (Histosec, Merck) followed by tissue processing using an automated tissue processor (TISSUE TEK II, model 4640B, Lab-Tek division, Miles Laboratories Inc, Naperville, IL). Thereafter, 5 µm cross-sections were cut using a rotary microtome (Reichert Jung, Heidelberg, Austria) and stained with haematoxylin and eosin to enable subjective visualisation of the immune cell infiltration. The slides were examined with an Olympus IX70 microscope equipped with a digital camera (color view II) and analysed with Analysis Imaging Software (build 5.1.0.2640) supplied by the Olympus company. The 2X magnification objective lens was used for villi height, width and area, and crypt depth, while a 4X magnification objective lens was used for analysing the outer longitudinal fibres, inner circular fibres and *muscularis mucosa*. The villi area and length were measured from the tip of the villi to the villous-crypt junction (in areas with intact villi's), while crypt depth was

measuring as the vertical distance from the villous-crypt junction to the lower limit of the crypt as indicated in Figure 5.1. Furthermore, the outer longitudinal fibres, inner circular fibres and *muscularis mucosa* were measured as indicated in Figure 5.2. Each parameter was estimated by measuring 10 consecutive measurements and the average was used.



**Figure 5.1** Photomicrograph of jejunum cross section indicating measurements taken for crypt depth, villi area, villi length and villi width from chicks that received the control treatment diet



**Figure 5.2** Photomicrograph of jejunum cross section indicating measurements taken for *muscularis mucosa*, submucosa, inner circular fibres and outer longitudinal fibres from chicks that received the 15% treatment diet

### 5.2.3 Tibia bone samples

After slaughter of the birds, the internal organs and intestines were immediately removed as discussed. Thereafter, the tibia bones were detached from the carcass by carefully cutting between the periosteum of the tibia and femur bone, using a knife without damaging the periosteum. These samples were frozen and stored at  $-18\text{ }^{\circ}\text{C}$  with their muscle and skin still attached to the bone until further analysis. Prior to analysis, the samples were thawed for  $\pm 12\text{ h}$  at  $4\text{ }^{\circ}\text{C}$  in a refrigerator. The right tibia bone from each sample bird were deboned and cleaned of all visible adherent tissues and fat. Thereafter, the fibula and periosteum were detached. These were done with outmost care to avoid any damage to the bone. The length of the bone was measured using a Vernier calliper, weighed and finally bone breaking strength was determined using an Instron (Fleming *et al.*, 1998).

### 5.2.4 Tibia bone strength and mineral content

A three-point bending test was used to determine the breaking strength of each bone using an Instron tensile/compression machine fitted with a 50 kg load cell. The machine was set at a total distance of 30 mm span between the two supporting ends. Each bone was placed onto the machine in a stable position, with the mid-diaphyseal diameter at the centre of the breaking probe. The mid-diaphyseal diameter of the bone at the site of impact was measured using a Vernier calliper. The bending force (strain: which is the total force the object can endure before it goes into failure) of the bone was determined using a 10 mm diameter probe crosshead which approached the bone at a constant speed of 30 mm/s. The Instron machine is computer



controlled using an HBM MVD25010 signal conditioning and data acquisition system. This system records the force (Newton) readings with its displacement value every 0.02 seconds while the bending test is conducted. The values and methodology used for the set up were according to a method described by Fleming *et al.* (1998). The peak of each loading curve as generated by the computer was used to obtain the failure point in Newton's which was used to determine the breaking strength of each tibia bone. Bone strength is measured by the load in N and the cross-sectional area ( $\text{mm}^2$ ), defined as a force in  $\text{N}/\text{mm}^2$  (kilograms force per square millimetre) which indicates the modulus measures of stiffness or rigidity, as related to stress and strain (Rath *et al.*, 1999; Baird *et al.*, 2008).

The formulas for bone breaking strength ( $\text{N}/\text{mm}^2$ ) and breaking force per gram of bone were derived from breaking strength units according to Hibbeler (2005) and thus calculated using Equation 5.1 and Equation 5.2, respectively:

**Equation 5.1,**

$$\text{Breaking strength (N/mm}^2\text{)} = \frac{\text{Force (N)} \times \text{Span between supports (m)}}{\pi \times \text{radius}^2}$$

**Equation 5.2,**

$$\text{Breaking force (N/g)} = \frac{\text{Force (N)}}{\text{Weight of bone (g)}}$$

After the bones were broken, all the pieces were collected, weighed and then dried in a 100 °C oven for 48 hours to obtain a constant dry weight for each sample. The samples were weighed again after drying to determine moisture lost during drying. Thereafter, the bone samples were incinerated in a furnace at 600 °C for 24 hours continuously, following weighing of the samples. This was done to obtain the total ash percentage for each bone sample as described in Chapter 3 section 3.2.4.4. Weight measurements taken of the bone were determined using a Mettler AE 200 scale with an accuracy of 0.0001 g (Mettler-Toledo, Switzerland). Thereafter, combusted bones were ground to powder using a mortar and pistol and sent to the Institute of Animal Production, Western Cape Department of Agriculture at Elsenburg for mineral analysis. The tibia bone mineral composition was analysed as described in Chapter 3 section 3.3.4.9. It should be noted that the minerals were eluted at a wavelength of 2497 for B, Ca (317.933), Cu (324.754), Fe (259.94), K (766.49), Mn (257.61), Na (589.592), P (177.495) and Zn (213.856).

### **5.2.5 Statistical analysis**

Statistical analysis were analysed using the general linear models (GLM) procedure of SAS (2009). The analysis of variance (ANOVA) assumptions for normality and homoscedasticity were investigated before further analyses were done. The tests were considered significant at  $P > 0.05$ . Treatment effects of all parameters except for gizzard erosion score were analysed using one-way ANOVA with Bonferroni's *post hoc* (least square means) test. In cases where the homoscedasticity assumption for the data was not satisfied, a Welch's ANOVA for unequal variances was used. The gizzard erosion scores were analysed

using the Chi-squared test of SAS (2009). The significance level of 5% of all tests was used and significant treatment differences were declared at  $P < 0.05$ .

The statistical model for ANOVA is indicated by;  $Y_{ij} = \mu_i + \alpha_j + \varepsilon_{ij}$  where the terms in the model are defined as: the treatment effect response ( $Y_{ij}$ ), the overall mean ( $\mu_i$ ), treatment effect ( $\alpha_j$ ) and the unexplained error ( $\varepsilon_{ij}$ ).

The Chi-square (goodness of fit) test statistic is indicated by;  $\chi^2 = \sum \frac{(O - E)^2}{E}$  where the terms are defined as: treatment effect response ( $\chi^2$ ), summation ( $\Sigma$ ), the observed frequencies ( $O$ ) and expected frequencies ( $E$ ).

## 5.3 Results

### 5.3.1 Organ weight and gizzard erosion

Inclusion of black soldier fly pre-pupae meal (BSM) at 0, 5, 10 and 15% in broiler diets did not ( $P > 0.05$ ) affect gizzard, liver, heart, *bursa*, spleen and the spleen:*bursa* weights and their weights relative to body weight (Table 5.2). In addition, treatment did not ( $P > 0.05$ ) affect gizzard erosion score (Table 5.3).

**Table 5.2** Mean ( $\pm$  standard error) of organ weight and organ weight relative to body weight as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets

Parameters	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
<b>Organ weight (g)</b>					
Gizzard	32.7 $\pm$ 1.29	34.0 $\pm$ 1.14	33.1 $\pm$ 0.70	31.9 $\pm$ 0.75	0.514
Liver	36.4 $\pm$ 1.52	36.4 $\pm$ 1.18	37.6 $\pm$ 1.20	36.4 $\pm$ 0.71	0.855
Heart	10.6 $\pm$ 0.44	11.3 $\pm$ 0.40	10.8 $\pm$ 0.49	10.5 $\pm$ 0.45	0.612
<i>Bursa</i>	3.5 $\pm$ 0.58	3.4 $\pm$ 0.28	4.1 $\pm$ 0.64	3.9 $\pm$ 0.59	0.746
Spleen	2.4 $\pm$ 0.11	2.3 $\pm$ 0.16	2.7 $\pm$ 0.25	2.3 $\pm$ 0.09	0.338
Spleen: <i>Bursa</i>	0.7 $\pm$ 0.09	0.7 $\pm$ 0.5	0.6 $\pm$ 0.09	0.7 $\pm$ 0.14	0.916
<b>Organ weight relative to body weight (%)</b>					
Gizzard	1.6 $\pm$ 0.04	1.6 $\pm$ 0.06	1.6 $\pm$ 0.05	1.5 $\pm$ 0.04	0.527
Liver	1.8 $\pm$ 0.08	1.8 $\pm$ 0.05	1.8 $\pm$ 0.05	1.7 $\pm$ 0.03	0.800
Heart	0.5 $\pm$ 0.02	0.5 $\pm$ 0.02	0.5 $\pm$ 0.02	0.5 $\pm$ 0.02	0.635
Spleen	0.1 $\pm$ 0.01	0.1 $\pm$ 0.01	0.1 $\pm$ 0.01	0.1 $\pm$ 0.00	0.234
<i>Bursa</i>	0.2 $\pm$ 0.03	0.2 $\pm$ 0.01	0.2 $\pm$ 0.03	0.2 $\pm$ 0.03	0.449
<i>Bursa</i> : spleen	0.04 $\pm$ 0.010	0.03 $\pm$ 0.000	0.03 $\pm$ 0.000	0.03 $\pm$ 0.010	0.894

**Table 5.3** Gizzard erosion scores as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets

Treatment diets	Gizzard erosion score			
	0	1	2	3
0% (Control)	0	2	5	1
5% BSM	2	4	2	0
10% BSM	0	5	3	0
15% BSM	3	3	2	0
<b>Chi-Square P-value</b>	0.1891			

### 5.3.2 Intestinal pH and histomorphology

Black soldier fly pre-pupae meal as a protein source in broiler diets did not ( $P>0.05$ ) influence pH of the duodenum, jejunum and ileum sections of the small intestine (Table 5.4). Treatment did not ( $P>0.05$ ) influence most histomorphology measurements of the duodenum and jejunum, except for the duodenum crypt depth and outer longitudinal fibres, and jejunum area (Table 5.5). Regarding inner circular fibres no treatment differences were found even though they biologically different. The 15% treatment diets chicks had significantly lower crypt depths compared to the control and the 5 and 10% treatment chicks were intermediate. The 0, 10 and 15% treatment diets outer layer did not differ from one another while the 5% treatment diet had a higher ( $P<0.05$ ) outer longitudinal fibres. The 10% treatment diet had a higher ( $P<0.05$ ) jejunal area than the 5 and 15% treatment diet while the control was intermediate. Due to unequal variances in the duodenum outer longitudinal fibres data the Welch's ANOVA was used.

**Table 5.2** Mean ( $\pm$  standard error) of small intestine pH as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets

Parameters	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
Duodenum	6.3 $\pm$ 0.08	6.2 $\pm$ 0.07	6.2 $\pm$ 0.08	6.0 $\pm$ 0.08	0.222
Jejunum	6.3 $\pm$ 0.05	6.4 $\pm$ 0.03	6.4 $\pm$ 0.04	6.4 $\pm$ 0.04	0.132
Ileum	6.8 $\pm$ 0.09	6.8 $\pm$ 0.11	6.8 $\pm$ 0.08	6.9 $\pm$ 0.07	0.924



**Table 5.3** Mean ( $\pm$  standard error) of duodenum and jejunum histomorphology sections ( $\mu\text{m}$ ) as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets

Parameters	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
<b>Duodenum</b>					
Villi area	119094.0 $\pm$ 2838.05	139245.9 $\pm$ 8630.96	122791.3 $\pm$ 4182.13	124279.8 $\pm$ 5345.61	0.085
Villi length	1203.9 $\pm$ 48.64	1229.1 $\pm$ 77.33	1203.2 $\pm$ 53.62	1214.3 $\pm$ 47.48	0.988
Villi width	127.1 $\pm$ 6.80	119.0 $\pm$ 8.02	123.4 $\pm$ 7.05	122.8 $\pm$ 8.23	0.900
Crypt depth	187.4 <sup>a</sup> $\pm$ 6.89	170.2 <sup>ab</sup> $\pm$ 5.99	169.9 <sup>ab</sup> $\pm$ 7.31	153.4 <sup>b</sup> $\pm$ 8.05	0.021
<i>Muscularis mucosa</i>	37.3 $\pm$ 1.00	37.6 $\pm$ 1.43	36.9 $\pm$ 2.74	38.7 $\pm$ 1.84	0.911
Submucosa	32.8 $\pm$ 0.51	33.5 $\pm$ 0.58	32.7 $\pm$ 0.41	32.5 $\pm$ 0.50	0.534
Inner circular fibres	188.0 $\pm$ 15.71	231.1 $\pm$ 11.93	189.7 $\pm$ 14.58	214.3 $\pm$ 13.72	0.113
Outer longitudinal fibres	80.8 <sup>b</sup> $\pm$ 2.12	107.2 <sup>a</sup> $\pm$ 7.55	79.8 <sup>b</sup> $\pm$ 2.67	87.7 <sup>b</sup> $\pm$ 4.76	0.001
<b>Jejunum</b>					
Villi area	108397.5 <sup>ab</sup> $\pm$ 3705.8	100766.8 <sup>b</sup> $\pm$ 1811.98	116803.3 <sup>a</sup> $\pm$ 2511.62	106607.7 <sup>b</sup> $\pm$ 2832.08	0.000
Villi length	1122.6 $\pm$ 26.25	1117.0 $\pm$ 37.16	1090.1 $\pm$ 50.30	1135.9 $\pm$ 37.65	0.864
Villi width	92.4 $\pm$ 3.36	99.1 $\pm$ 7.18	90.2 $\pm$ 4.57	89.0 $\pm$ 4.79	0.523
Crypt depth	165.5 $\pm$ 8.06	155.5 $\pm$ 6.08	169.2 $\pm$ 4.09	152.9 $\pm$ 5.04	0.191
<i>Muscularis mucosa</i>	47.8 $\pm$ 1.59	45.0 $\pm$ 3.52	48.5 $\pm$ 3.38	49.5 $\pm$ 3.10	0.747
Submucosa	52.4 $\pm$ 1.44	47.8 $\pm$ 0.81	49.6 $\pm$ 1.59	49.6 $\pm$ 1.04	0.110
Inner circular fibres	277.9 $\pm$ 11.22	256.8 $\pm$ 18.35	252.2 $\pm$ 23.56	243.9 $\pm$ 17.46	0.600
Outer longitudinal fibres	92.7 $\pm$ 4.73	86.0 $\pm$ 5.10	93.7 $\pm$ 4.75	84.6 $\pm$ 4.21	0.420

<sup>a,b</sup> Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

### 5.3.3 Tibia bone parameters

Results on tibia bone ash percentage and mineral content are shown in Table 5.7. Treatment had no ( $P > 0.05$ ) effect on bone ash percentage and mineral content except for calcium (Ca) content ( $P = 0.03$ ; Welch's ANOVA) and Ca:P ( $P = 0.048$ ). The chicks receiving the 5% diet had a Ca content significantly different (lower) than that of the 15% but did not differ significantly from chicks receiving the 0 and 10% diets. However, the 15% was not statistically different from the 0 and 10% diet chicks. Treatment affected ( $P = 0.048$ ) the Ca:P ratio, however based on the Bonferroni *post hoc* test none of the treatments were significantly different from each other. Tibia bone breaking force (N), breaking force per gram of bone weight (N/g) and breaking strength (N/mm<sup>2</sup>) were not ( $P > 0.05$ ) influenced by treatment (Table 5.6).

**Table 5.6** Mean ( $\pm$  standard error) of tibia breaking force and strength of broiler chickens fed different levels of black soldier pre-pupae meal (BSM) in their diets

Parameters	Units	Treatments				P-value
		Control	5% BSM	10% BSM	15% BSM	
Breaking force	N	335.7 $\pm$ 26.68	384.1 $\pm$ 26.68	378.0 $\pm$ 26.68	429.2 $\pm$ 26.68	0.140
Breaking strength	N/mm <sup>2</sup>	75.4 $\pm$ 7.20	80.0 $\pm$ 7.20	78.1 $\pm$ 7.20	68.5 $\pm$ 7.20	0.694
Breaking force per gram of weight	N/g	32.6 $\pm$ 2.52	33.2 $\pm$ 2.52	36.1 $\pm$ 2.52	37.4 $\pm$ 2.52	0.495

**Table 5.7** Mean ( $\pm$  standard error) of tibia bone ash percentage and mineral content of broiler chickens fed different levels of black soldier fly pre-pupae meal (BSM) in their diets

Parameters	Units	Treatment diets				P-value
		Control	5% BSM	10% BSM	15% BSM	
Ash	%	22.2 $\pm$ 0.48	21.6 $\pm$ 0.48	22.9 $\pm$ 0.48	22.6 $\pm$ 0.48	0.272
Ca	%	41.0 <sup>ab</sup> $\pm$ 0.65	40.3 <sup>a</sup> $\pm$ 0.38	41.8 <sup>ab</sup> $\pm$ 0.56	42.7 <sup>b</sup> $\pm$ 0.56	0.034
P	%	19.6 $\pm$ 0.24	19.6 $\pm$ 0.12	19.8 $\pm$ 0.26	20.3 $\pm$ 0.18	0.116
Ca:P	%	2.1 $\pm$ 0.02	2.1 $\pm$ 0.01	2.1 $\pm$ 0.01	2.1 $\pm$ 0.02	0.048
Potassium	%	1.0 $\pm$ 0.03	0.9 $\pm$ 0.02	1.0 $\pm$ 0.09	1.0 $\pm$ 0.07	0.566
Magnesium	%	0.7 $\pm$ 0.01	0.7 $\pm$ 0.01	0.7 $\pm$ 0.01	0.7 $\pm$ 0.01	0.427
Sodium	mg/kg	12.8 $\pm$ 0.26	13.2 $\pm$ 0.81	12.8 $\pm$ 0.20	13.3 $\pm$ 0.57	0.763
Copper	mg/kg	2.0 $\pm$ 0.23	1.7 $\pm$ 0.05	1.8 $\pm$ 0.15	1.7 $\pm$ 0.07	0.592
Zinc	mg/kg	303.1 $\pm$ 14.33	294.1 $\pm$ 9.78	309.5 $\pm$ 5.18	307.6 $\pm$ 4.78	0.661
Iron	mg/kg	234.0 $\pm$ 12.00	200.1 $\pm$ 8.02	247.6 $\pm$ 19.56	200.6 $\pm$ 15.67	0.069

<sup>a,b</sup> Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

## 5.4 Discussion

### 5.4.1 Organ weight and gizzard erosion

In this study, internal organ weights and their weight relative to live weights of birds investigated were not influenced ( $P > 0.05$ ) by BSM inclusion in the diets. Similarly, Tégua *et al.* (2002) reported lack of treatment differences regarding gizzard, heart and liver weights in broiler chicken fed house fly (HF) maggot meal. Although, Okah & Onwujiariri (2012) reported broiler chickens fed HF larvae meal had higher gizzard weights and lower heart weights, but no treatment differences were found with liver weights. Okah & Onwujiariri (2012) evaluated HF maggot meal on older broilers beyond day 35 of age, which might account for the significant differences. In a review by Bedford (2000) it's stated that as birds increase in age their digestive capacity also increases, which may be attributed to the subsequent increase in microfloral population. Hence, this may have attributed to the higher gizzard weights observed by Okah & Onwujiariri (2012).

In this study no significant treatment differences regarding organ percentage relative to live weight were obtained. According to Pope (1991) the decrease of *bursa* weight relative to body weight is a possible indicator of potential increase in immunosuppression of the immune system. The *bursa* is the only lymphoid organ that acts as both a primary and secondary lymphoid organ in avian species. Furthermore, in the *bursa* B-cells are produced that are responsive to antigens for immune protection of the bird (Glick, 1991). As *bursa* plays a vital part in maintaining the immune health of the bird, it is essential to evaluate the effect of BSM inclusion in broiler diets on the *bursa*. In this investigation BSM inclusion in the birds' diets did not affect *bursa* weight.

Gizzard erosion score is a visual score analysis of any possible lesion occurrence or change within the gizzard lining as influenced by treatment diets (Table 5.1). Gizzard erosion occurs mostly in broiler chickens characterized by rough inner lining of the gizzard and in severe cases manifestations as erosions and ulceration of the inner muscle layer occurs (Wessels & Post, 1989). In this study there were no severe erosions observed in the gizzard, which was scored mostly between zero and two, with two and below being

acceptable while four and above is not acceptable (Johnson & Pinedo, 1971). Therefore, BSM included up to 15% in broiler chickens diets did not have any adverse effects on the gizzard lining.

#### **5.4.2 Intestinal pH and histomorphology**

In this study the minimum and maximum values of the intestinal pH values obtained were within the normal pH range of the duodenum (5.5-6.2), jejunum (5.8-6.9) and ileum (6.3-8.0) in healthy poultry (Van der Klis & Jansman, 2002). Furthermore, the intestinal pH conditions were not ( $P>0.05$ ) affected by the inclusion of BSM in broiler chickens diets. Intestinal pH is considered important for the growth and maintenance of the gastro intestinal tract microbial community of birds. However, intestinal pH is not the only determinants for a bird's health status but organ size and the morphology of the intestines should also be considered (Van der Klis & Jansman, 2002). The different inclusion levels of BSM had no vital influence ( $P>0.05$ ) on any of these.

The structure of the intestinal mucosa can be used as an indicator of the gut's condition and hence animal health (Xia *et al.*, 2004; Choct, 2009; Jönsson & Holm, 2010). A healthy gut has a high nutrient absorption and consequently an improved immune status (indicated by increase in plasma immunoglobulin levels) of the host (Salim *et al.*, 2013). The digestion and absorption of nutrients occurs mostly in the small intestine, with the jejunum being the major site of absorption (Nourmohammadi & Afzali, 2013). The absorptive capability of the intestinal villus area is determined by the villus size and its mutual proportion of enterocytes, goblet and entero-endocrine cells (Awad *et al.*, 2011). No major treatment differences were observed with the histomorphology data on the duodenum and jejunum sections hence, the ileum was not analysed. In this study treatment significantly influenced the jejunum area with the 10% treatment diet being higher ( $P<0.05$ ) and the control intermediate. Indicating that inclusion of BSM at 10% in the diet might have increase the absorptive capacity of the jejunum even though it was not ( $P<0.05$ ) different from the control. Area of the duodenum was not influenced by treatment ( $P>0.05$ ). Furthermore, shortening of the villi subsequently reduce its surface area and decreases the absorptive capacity of the intestine. The villi length and width was not influenced ( $P>0.05$ ) by treatment for both duodenum and jejunum. In addition, destruction within the gut wall (epithelium cells) can directly affect intestinal barrier function, weakening absorption of nutrients and also making the barrier permeable by luminal antigenic agents (Song *et al.*, 2014). Hence, it is essential to analyse the small intestinal histomorphology given that it's the major cite of absorption for any affects that may arise by feeding broiler chickens BSM.

The presence of toxins in the gut is associated with shorter villi and deeper crypts (Choct, 2009). The results revealed there was minimal to none toxins present, as no adverse treatment differences were observed ( $P>0.05$ ) regarding the villi length and crypt depth of the duodenum and jejunum. However, treatment differences were observed regarding duodenum crypt depth with the 15% diet chicks attaining ( $P<0.05$ ) lower crypt depth than the control diet chicks but this was not of concern as the 5 and 10% treatment diets were intermediate, thus minimising the impact of the differences. Furthermore, the length and width of the villi and the crypt depth gives an indication on the rate of tissue turnover of epithelial cells, energy requirement and the absorption capacity of nutrients in the gastro-intestinal tract (Awad *et al.*, 2009; Choct, 2009; Zhang *et al.*, 2013). Treatment did not ( $P>0.05$ ) affect the *muscularis mucosa*, submucosa, inner

circular fibres and outer longitudinal fibres of the jejunum. Treatment neither affected ( $P>0.05$ ) the *muscularis mucosa*, submucosa and inner circular layer of the duodenum but affected ( $P<0.05$ ) the outer layer. The *muscularis mucosa*, submucosa, inner circular fibres and outer longitudinal fibres ensure the luminal contents remain mixed, enabling contractions of the muscles for efficient transfer of materials into the small intestinal sections for digestion and absorption (Rogers, 1983). Therefore, treatment ( $P<0.05$ ) increased the outer longitudinal fibres of the duodenum but this did not affect the inner circular layer, submucosa and *muscularis mucosa*. Since modern broiler chickens have been selected for fast growth, any alternative feed source must support normal bird growth and must not cause any adverse effects on the chicken's health. The inclusion of BSM in broiler chickens diets did not affect the histomorphology of the small intestine sections (duodenum and jejunum), indicating to have sustained normal development, growth and optimal functioning of the tested gut morphology.

### **5.4.3 Tibia bone parameters**

Diets with BSM included ( $P>0.05$ ) affected tibia bone Ca content and Ca:P. However, tibia bone breaking force (N), breaking force per gram of bone weight (N/g) and breaking strength ( $\text{N}/\text{mm}^2$ ) were not ( $P>0.05$ ) influenced by treatment, even though treatment significantly influenced the Ca content of the tibia bones. Black soldier fly pre-pupae meal contains high Ca and P contents (Newton *et al.*, 2005). However the treatment diets were formulated for similar Ca levels, thus these results may indicate higher bioavailability of Ca in the BSM to broiler chicks. Furthermore, it was noted that the Ca content of tibia bones increased with the increase in the inclusion rate of BSM in the diets. Despite the increase in Ca content of the tibia bones causing a treatment effect ( $P=0.048$ ) on Ca:P ratio, no significant treatment differences were found between treatment diets when the differences were evaluated using the Bonferroni *post hoc* (least square) test. However, these differences (or lack thereof) are difficult to explain but might be accounted for by the low  $R^2=0.32$  observed, indicating the minimal extent at which the results are explained by the statistical model due to variations within treatments. Furthermore, Zinc was found to be the most abundant mineral present in broiler chicken tibia bone, followed by Pb, Ca, P and Na, while Mg and K were less abundant. No known literature could be sourced evaluating the effects BSM or any other insect meal on bone parameters in poultry.

According to Brenes *et al.* (2003), a deficiency in Ca and/or P in poultry diets causes an increase in mineral mobilization from bones resulting in porous and fragile bones that can break easily. The development of strong bones is essential in broiler production to avoid rapture and fractures of bones during growth and/or processing of the carcass. Moreover, birds are genetically selected for fast growth and attain higher weights than the skeletal frame can support, causing bone disorders in poultry production and mitigation strategies are vital to minimize this effect. The inclusion of BSM in broiler chicken diets supported normal bone growth and did not affect tibia bone breaking strength negatively. It was however noted that the increase in tibia bone Ca content with increase in BSM inclusion, might be attributed to higher bioavailability of the Ca in BSM to the birds. Therefore, it can be concluded that the addition of BSM to broiler chicken diets did not affect tibia bone development negatively but maintained normal bone formation.

## 5.5 Conclusion

The results in this study indicated that the inclusion of BSM in broiler chicken diets had no effect, positively or negatively, on organ weights, gizzard erosion score, pH of the small intestine, histomorphology of the duodenum and jejunum and tibia bone breaking force and strength. The results indicate that BSM did not affect the organ development of the broiler chicken nor their skeletal structure. BSM can therefore be added in broiler chicken diets up to 15% without affecting the growth, development of internal organs, small intestine and tibia bone. Future research should evaluate the effect of decreasing the amount of supplementary Ca in broiler chicken diets containing 15% BSM on broiler production, since an increase in BSM inclusion in the diet was observed to have led to an increase in Ca content of the tibia bones. The use of BSM in broiler chicken diets is observed to have supported normal bird growth and without affecting its organ, gut and skeletal parameters. However, noting the above indicating the viability of BSM for production of broiler chickens, its effects on the broiler carcasses meat quality is questioned.

## 5.6 References

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## Chapter 6

# Effect of black soldier fly (*Hermetia illucens*) pre-pupae meal on the carcass characteristics, physical measurements, chemical quality and sensory attributes of broiler chicken meat

### Abstract

Carcass characteristics, sensory, physical and chemical quality of broiler chicken meat fed black soldier fly (*Hermetia illucens*) pre-pupae meal (BSM) as a protein source were investigated. Four treatment diets based on BSM included at 0, 5, 10 and 15%, were used. Three hundred and twenty day-old Cobb 500 broiler chicks were randomly allocated to the treatment diets. These broiler chicks were raised to slaughter at day 35 of age. Treatment did not significantly affect slaughter live weight, cold carcass weight, dressing percentage and commercial portion yield of the breast, thigh, drumstick, wing or back. No treatment differences were observed for the physical characteristics of broiler carcasses regarding initial and ultimate pH of the breast and thigh muscles, thaw and cooking loss and colour. Neither did treatment have a significant effect on the sensory characteristics (flavour, aroma, tenderness and juiciness) or mineral content of the cooked broiler chicken meat. Treatment did not influence the fatty acids composition of the meat besides C14:0, which was high in the 15% treatment diet and low in the control. A significantly strong positive correlation was only found between sensory tenderness and sustained juiciness. No specific fatty acids were found to be correlated to chicken flavour, whilst the iron content of the cooked broiler meat did not influence the metallic flavour or aroma. Therefore, it can be concluded that the inclusion of BSM in broiler chicken diets did not compromise the quality of meat produced.

**Keywords:** Portion yield, breast muscle, flavour, tenderness, insect meal, maggots

### 6.1 Introduction

Production of consumable goods is focused on the ultimate satisfaction of consumer expectations (McIlveen, 1994). Tenderness, juiciness and flavour of meat and meat products are elements that contribute to consumer eating satisfaction. Meat tenderness is considered the main quality cue for the acceptability of cooked meat (Barbut, 1997), whilst juicy meat is desired than less juicy meat (Risvik, 1994) and flavour determines the overall product acceptability after consumption. Meat flavour is affected by water-soluble components (free sugars, sugar phosphates, peptides, nucleotides, bound sugars, free amino acids, peptides, nucleotides and other nitrogenous components) and fat, which are considered as the main precursors for flavour development (Mottram, 1998). Colour on the other hand plays a significant role in the purchasing intent of a consumer (Allen *et al.*, 1998; Fletcher, 1999; Qiao *et al.*, 2002; Hoffman & Cawthorn, 2012).

The fat content of meat determines the muscle tissue firmness, shelf life and flavour of the meat. The fatty acids contained in fat are responsible for flavour development in meat. Undesired meat flavours are a result of fat oxidation, with excessive oxidation leading to rancidity in meat which is not desirable (Song *et al.*, 2013). An increase in certain dietary fatty acids may lead to an increased rate of fat oxidation reducing the shelf life of meat. This may be attributed to the unstable double bond in some fatty acids, especially unsaturated fat (Hugo *et al.*, 2009). The rapid oxidation of meat is characterised by rancid odours and

flavours as a result of oxidative deterioration of meat post-mortem which may be minimised by the use of antioxidants in animal feed (resulting in deposition in meat), which slows down meat oxidation (Wood & Enser, 1997; Bou *et al.*, 2004).

In monogastric animals, fatty acid content of the diet pre-determines the fatty acid composition of the meat (O'Neill *et al.*, 1998; Coetzee & Hoffman, 2002; Barroeta, 2007; Cao *et al.*, 2012). Therefore, essential fatty acid content in the meat suitable for human consumption can be manipulated via enhancement of animal diets with feed ingredients containing the desirable fatty acids (Coetzee & Hoffman, 2002). However, reasonable feeding time before slaughter is required for the modification of dietary fatty acids into the meat. The dietary fatty acid modification into intramuscular fatty acid is less effective than its modification in the abdominal and subcutaneous fat (Lopez-Ferrer *et al.*, 1999).

Fat is essential in human diets providing energy required for optimal development and used as a carrier for fat soluble vitamins (Wiseman, 1997). In particular, n-3 fatty acids in human diets are found to modulate and prevent coronary heart diseases and cardiovascular diseases (Simopoulos, 1991; Connor, 2000). Black soldier fly pre-pupae meal (BSM) contains a desirable fatty acid composition despite being high in saturated fatty acids (Kroeckel *et al.*, 2012). Feeding broilers with BSM is therefore expected to produce meat with a desirable fatty acid profile, which will be beneficial to humans.

The benefits of feeding BSM to broilers can only be observed if the carcass and meat characteristics from these broilers are not adversely affected. However, studies on the effects of feeding broiler chickens with BSM on carcass and meat characteristics are lacking. The objective of this study was therefore, to investigate the effect of BSM (*Hermetia illucens*) on broiler chicken carcass characteristics (live slaughter weight, cold carcass weight, dressing percentage, carcass portion yield and the skin, bone and meat percentage of the breast portion), sensory attributes (aroma, flavour, initial juiciness, sustained juiciness and tenderness), physical measurements (pH, colour, thaw and cooking losses) and chemical composition (moisture, protein, fat, ash, minerals, amino acids and fatty acids).

## **6.2 Materials and methods**

### **6.2.1 Experimental layout, handling and management**

Detailed description of experimental layout, handling and chicken management procedures is outlined in Chapter 4 (section 4.2.1). The trial was carried out at Mariendahl Experimental Farm of Stellenbosch University (ethical clearance number SU-ACUM13-00026). The broiler chicks were raised to slaughter at day 35 of age.

### **6.2.2 Slaughtering procedure**

At day 35 of age, birds were weighed to attain cage middle weight where one bird weighing around the mean was then selected for slaughter (32 sample birds were obtained). The broiler chickens were slaughtered at the Mariendahl experimental farm abattoir according to acceptable slaughtering standard methods used for

commercial chickens (Department of Agriculture, Forestry & Fisheries [DAFF], 2006). At slaughter the birds were rendered unconscious by electrical stunning (50-70 volts; 3-5 s), exsanguinated and allowed to bleed out for about 2 min. Thereafter, the birds were soaked in a rotating 60 °C water bath for 5 min, de-feathered and then eviscerated. Subsequently, 15 min after slaughter the carcass initial pH was measured from the right breast and thigh, and the carcass was immediately chilled at 4 °C for 24 h. The ultimate pH of the right breast and thigh was measured 24 h after chilling. After chilling the carcasses were transported to the meat laboratory at Stellenbosch University for further processing.

### **6.2.2 Carcass characteristics**

At the meat laboratory cold carcass weight was determined. The dressing percentage was calculated as the percentage of cold carcass weight to the live slaughter weight. The carcasses were portioned into commercial cuts (wing, breast, drumstick and thigh) using a meat slicing machine. The cutting procedure was as follows: firstly, the whole carcass was halved into two. Then the leg was removed by cutting above the thigh towards the acetabulum just behind the pubic bone. The leg was further cut perpendicular to the joints where the tibia, fibula and femur bones are attached together to obtain the drumstick and thigh portions. Then the wing was removed by cutting through the joint between the scapula and coracoid and the breast portion was separated from the wing.

The breast, wing, drumstick and thigh portions were then weighed in pairs and recorded. The right breast portion was skinned and deboned. The skin, muscle and bone of each breast portion were weighed separately for the determination of the bone, meat and skin (and subcutaneous fat) percentage. The left breast samples were vacuum-packed with their skins and bones attached, and frozen at -18 °C until further analyses.

### **6.2.3 Physical measurements**

#### **6.2.3.1 pH**

The pH measurements of each carcass were measured 15 min after slaughter and 24 h post-mortem. The pH was measured by means of a Crison pH 25 handheld portable pH meter (Lasec (Pty) Ltd, South Africa) with an automatic temperature adjuster. The Crison pH 25 was calibrated before pH measurements were taken with the standard buffers (pH 4.0 and pH 7.0) as provided by the manufacturer.

#### **6.2.3.2 Colour**

Breast meat colour was instrumentally measured at three randomly selected positions on the meat muscle surface of the fresh meat of each experimental unit, 24 h post-mortem. The colour was recorded using a Colour guide 45°/0° colorimeter (Catalogue no: 6805; BYK-Gardner, USA) to determine the L\*, a\* and b\* values. The L\* indicating lightness, a\* red-green range and b\* blue-yellow range of the meat muscle surface. The a\* and b\* values were used to calculate the hue angle ( $h_{ab}$ ) (°) and chroma value ( $C^*$ ) as outlined in Honikel (1998).

### **6.2.3.3 Thaw and cooking loss**

The thawed breast muscle weight was used to calculate for thaw loss as a percentage of the initial breast muscle weight before freezing. The weight of the cooked breast muscle as a percentage of the uncooked breast muscle weight was used to calculate the cooking loss. The cooking loss and thaw loss of the meat samples was determined according to the method described by Honikel (1998).

## **6.2.4 Chemical analysis**

### **6.2.4.1 Sample preparation**

The offcuts of the cooked chicken breast meat samples of each experimental unit after removal of DSA samples were used for chemical analyses. After completion of the DSA test, the meat offcuts per sample was homogenised separately then vacuum packed (separately for each analysis) and frozen at -18 °C until further analyses. Prior to each analysis the meat samples were removed and defrosted in a 4 °C refrigerator for ±12 h.

### **6.2.4.2 Proximate analysis**

The proximate analysis of the cooked meat samples was analysed according to acceptable standard methods as provided by the Association of Official Analytical Chemists International (AOAC) (2002). Refer to Chapter 3 for methodological analysis regarding dry matter, crude protein, ash content, amino acid analysis and mineral analysis under sections 3.2.4.1, 3.2.4.2, 3.2.4.4, 3.2.4.6, and 3.2.4.9, respectively. It should be noted that for dry matter analysis of meat, 2.5 g of the sample was utilized per subsample. Furthermore, protein analyses of meat was analysed on defatted meat samples weighing 0.15 g, with the Leco calibrated with EDTA (Leco Corporation). The protein percentage used was corrected for moisture and fat content. It should be noted that the minerals were eluted at wavelength of 2497 for B, Ca (317.933), Cu (324.754), Fe (259.94), K (766.49), Mg (285.213), Mn (257.61), Na (589.592), P (177.495) and Zn (213.856).

### **6.2.4.3 Crude fat**

The crude fat content of the meat sample was determined according to Lee *et al.* (1996); using 5 g homogenized cooked meat with chloroform/methanol (1:2 vol/vol). The solution was filtered through Whatman® No 1 into a separation funnel, following addition of 20 ml of 0.5% sodium chloride and allowed to separate. Thereafter, 5 ml of the fat solution was pipetted into a fat glass beaker and placed on a sand bath to allow the chloroform/methanol to evaporate. The results obtained were corrected with a 16.7 ml factor when total fat percentage was calculated.

### **6.2.4.4 Long chain fatty acid analysis**

The feed and meat samples fatty acid composition were determined. The fat content of the samples was extracted as described by Folch *et al.* (1957) using 2 g of the samples with addition of chloroform/methanol 2:1 solution containing 0.01% butylated hydroxytoluene as an anti-oxidant. A 0.5 ml heptadecanoic acid (C17:0) was added as an internal standard and the mixture was homogenised using a polytron mixer at speed setting C for 40 seconds. The samples were then trans-methylated with methanol/sulphuric acid 19:1 using 250 µl of the extracted fat solution for 2 h at 70 °C in a water bath. The solution was allowed to cool to

room temperature. Subsequently, water and hexane was added and the solution was vortexed, after allowing sufficient time for separation, the top hexane solution (fatty acid methyl esters [FAME]) was transferred to a clean Kimax tube and dried under nitrogen for  $\pm 30$  min. After drying, 50  $\mu\text{l}$  of Hexane was added and 1  $\mu\text{l}$  injected into the gas chromatography.

A Thermo Finnigan Focus Gas Chromatography apparatus (GC) (Thermo-electron Corporation, Rodano, Milan Italy) was used for determining the FAME. The GC column used was BPX70 (60 m  $\times$  0.25 mm; ID 0.25  $\mu\text{m}$ ; SGE International Pty Ltd, 7 Argent Place, Ringwood, Victoria 3134, Australia). An initial temperature of 60  $^{\circ}\text{C}$  for 5 min was used which was allowed to increase at a rate of 1:7  $^{\circ}\text{C}/\text{min}$  to a final temperature of 160  $^{\circ}\text{C}$ , injecting and detecting the sample was at 220  $^{\circ}\text{C}$  and 260  $^{\circ}\text{C}$ , respectively. The split flow of the GC was 20:120 with a Hydrogen carrier at 20 ml/min then a sample injection of 1  $\mu\text{l}$  with total run time of 45 min eluting the various fatty acids (FA). An internal standard injected into the GC of known FA enabled the identification of the FA contained in the samples and the results reported are expressed as a percentage of total fatty acids for the feed samples and for the meat samples in mg/g of meat.

## **6.2.5 Sensory analysis**

### **6.2.5.1 Sample preparation**

From the eight replicates only six replicates per treatment were used for sensory and subsequently chemical analysis. The left breasts of the birds were used for sensory analysis. Prior to conducting sensory analyses the samples were defrosted for  $\pm 12$  h at 4  $^{\circ}\text{C}$  and then blot-dried and weighed.

The blot-dried samples were deboned, skin plus fat removed and weighed. Subsequently, the muscle was placed inside a marked oven bag (Glad®). No salt (sodium chloride) nor seasoning additives nor preservatives were added to the breast meat used for the sensory analysis. Meat samples in the oven bags were placed on stainless steel grids fitted in oven roasting pans. Thermocouple probes attached to a hand operated digital temperature monitor (Hanna Instruments, South Africa) were placed in the centre of each of the meat samples and sealed in the oven bag to keep the probe in place.

The prepared samples were placed in a conventional oven (Defy, Model 835) connected to a computerized monitoring system responsible for regulation of the temperature (Viljoen *et al.*, 2001). The ovens were preheated to 160  $^{\circ}\text{C}$  (American Meat Science Association [AMSA], 1995). The meat samples were removed from the oven when a core temperature of 75  $^{\circ}\text{C}$  was reached for each sample (AMSA, 1995). The samples were cooled for 15 min allowing them to equilibrate to ambient temperature, blot dried and weighed. The cooked samples were each cut into 32 sample cubes of 1 cm  $\times$  1 cm  $\times$  1 cm. The cubes were then individually wrapped in aluminium foil (with the shiny side to the food) and placed into glass ramekins coded with a randomized three-digit code. The thirty two 1 cm  $\times$  1 cm  $\times$  1 cm cubes were given to eight judges each with four test samples per treatment. The coded ramekins, each containing four wrapped meat cubes were placed in a preheated industrial oven (Hobart, France) at 70  $^{\circ}\text{C}$  for 7 min and placed in a water bath preheated to 70  $^{\circ}\text{C}$  for the duration of the testing session.

### 6.2.5.2 Descriptive sensory analysis

Descriptive sensory analysis (DSA) was performed on four meat cubes per treatment with six consecutive replications, thus the experimental design: 4 treatments x 6 replications equals 24 breasts (experimental units). A panel of eight judges, with previous experience with sensory analysis of meat, were used. The panellists were trained according to the guidelines for sensory analysis of meat (AMSA, 1995) and the generic descriptive sensory analysis technique as described by Lawless & Heymann (2010) before the actual test analysis.

The panel undertook five training sessions and during each of these training sessions the panellists received four 1 cm x 1 cm x 1 cm cubes of meat from the four treatments. During the training sessions the panel decided on the following sensory attributes: chicken flavour, metallic flavour, chicken aroma, metallic aroma, initial and sustained juiciness and tenderness (evaluated on first bite). The definitions for each of the attributes are described in Table 6.1.

The re-test method was used for DSA, where the panellists received four 1 cm x 1 cm x 1 cm cubes of each of the four treatment samples in a completely randomized order. The panellist carried out DSA on the treatment samples while seated in individual tasting booths having computers fitted with Compusense® *five* (Compusense, Guelph, Canada) software programme. On Compusense® *five* the samples were analysed for the respective sensory attributes using an unstructured line scale with zero indicating “low intensity” and 100 “high intensity”. The sensory analysis sessions took place inside a temperature controlled room at 21 °C with artificial daylight in accordance to guidelines set by AMSA (1995). The panellists were availed with distilled water (21 °C), apple pieces and water biscuits in order to cleanse and refresh their palates between testing of samples. The DSA of the test samples was done over six sessions on 3 different consecutive days, with two testing sessions per day.

**Table 6.1** Definition and scale of each attribute used for the descriptive sensory analysis on breast portion

Sensory attribute	Description	Scale
Chicken aroma	Aroma associated with the chicken meat, as soon as the aluminium foil is removed	0 = None 100 = Prominent
Chicken flavour	Flavour associated with chicken prior to swallowing while chewing	0 = None 100 = Prominent
Metallic aroma	Aroma associated with raw meat and/or blood-like, as soon as the aluminium foil is removed	0 = None 100 = Prominent
Metallic flavour	Taste associated with raw meat and/or blood-like taste prior to swallowing while chewing	0 = None 100 = Prominent
Initial juiciness	Amount of fluid extruded on surface of meat sample when pressed between the thumb and forefinger (pressed perpendicular to fibres)	0 = Dry 100 = Extremely juicy
Sustained juiciness	Amount of moisture perceived during mastication, after the first 5 chews using the molar teeth	0 = Dry 100 = Extremely juicy
Tenderness	The impression of tenderness perceived after the first 5 chews using the molar teeth	0 = Tough 100 = Extremely tender

### 6.2.6 Statistical analysis

Statistical analysis of carcass, chemical and physical data were analysed using the general linear models (GLM) procedure of SAS (2009). The Shapiro-Wilk test for normality of data and homoscedasticity test was performed before proceeding with further analyses. The tests were considered significant at  $P > 0.05$ . A one-way analysis of variance (ANOVA) with Bonferroni's *post hoc* test was used for statistical analysis. Sensory analysis data was analysed by multivariate analyses using XLStat software (Version 2012, Addinsoft, New York, USA). In the event of significant non-normality values ( $P < 0.05$ ) in the sensory data, outliers were identified and residuals greater than three were removed. The correlations coefficients for the sensory, physical and chemical data was analysed using the Pearson's correlation coefficient ( $r$ ) using XLStat software (Version 2012, Addinsoft, New York, USA). The relationship between the sensory, physical and chemical data was indicated by performing a principal component analysis (PCA) with correlation matrix, combined with discriminant analysis (DA).

The 5% significance level was used for the statistical tests and treatment differences were declared at  $P < 0.05$ .

The statistical model for the ANOVA test is indicated by;  $Y_{ij} = \mu_i + \alpha_j + \epsilon_{ij}$ , where the terms in the model are defined as: the treatment effect response ( $Y_{ij}$ ), the overall mean ( $\mu_i$ ), treatment effect ( $\alpha_j$ ) and the unexplained error ( $\epsilon_{ij}$ ).

## 6.3 Results

### 6.3.1 Carcass characteristics

The inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets did not ( $P > 0.05$ ) influence live slaughter weight, cold carcass weight and dressing percentage (Table 6.2). Neither did treatment ( $P > 0.05$ ) influence the commercial portion cut yield of the breast, thigh, drumstick, wing and back of the broiler carcasses (Table 6.3). Treatment had an effect ( $P < 0.05$ ) on breast portion percentage yield regarding the skin plus fat and muscle, but did not ( $P > 0.05$ ) affect bone percentage (Table 6.4). The chicks receiving the 15% treatment diet attained a significantly higher skin plus fat percentage of the breast portion than the chicks receiving the control diet. However, the treatment Bonferroni's *post hoc* (least square means) test indicated no treatment differences regarding muscle percentage of the breast portion regardless of the ANOVA ( $P = 0.032$ ).



**Table 6.2** The means ( $\pm$  standard error) of live slaughter weight, cold carcass weight and dressing percentage of broilers as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets

Parameters	Treatments diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
Live slaughter weight (g)	2067.5 $\pm$ 10.65	2092.5 $\pm$ 24.48	2082.5 $\pm$ 34.52	2105.0 $\pm$ 28.72	0.776
Cold carcass weight (g)	1364.5 $\pm$ 11.32	1387.5 $\pm$ 19.15	1382.1 $\pm$ 25.36	1371.5 $\pm$ 21.77	0.849
Dressing percentage (%)	66.0 $\pm$ 0.50	66.3 $\pm$ 0.50	66.4 $\pm$ 0.50	65.2 $\pm$ 0.50	0.307

**Table 6.3** The means ( $\pm$  standard error) of broiler carcass portion yield (g) as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets

Portions	Treatments diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
Breast	545.6 $\pm$ 14.44	549.0 $\pm$ 12.75	520.8 $\pm$ 16.67	525.9 $\pm$ 12.05	0.409
Thigh	352.7 $\pm$ 5.27	374.2 $\pm$ 9.36	386.3 $\pm$ 10.86	364.2 $\pm$ 10.72	0.093
Drumstick	195.0 $\pm$ 4.82	202.2 $\pm$ 6.22	193.7 $\pm$ 6.08	209.4 $\pm$ 6.98	0.257
Wing	164.1 $\pm$ 4.69	166.9 $\pm$ 6.90	175.5 $\pm$ 2.58	174.5 $\pm$ 4.47	0.307
Back	93.3 $\pm$ 3.37	89.1 $\pm$ 3.46	96.9 $\pm$ 3.78	91.9 $\pm$ 3.90	0.509

**Table 6.4** The means ( $\pm$  standard error) for skin, muscle and bone percentage of broiler carcasses breasts as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets

Parameters	Treatments diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
Skin plus fat	5.7 <sup>b</sup> $\pm$ 0.38	6.6 <sup>ab</sup> $\pm$ 0.43	6.7 <sup>ab</sup> $\pm$ 0.32	7.5 <sup>a</sup> $\pm$ 0.43	0.028
Muscle	74.3 $\pm$ 0.91	74.2 $\pm$ 1.17	73.2 $\pm$ 1.30	69.5 $\pm$ 1.38	0.032
Bone	18.5 $\pm$ 0.77	17.1 $\pm$ 1.13	18.5 $\pm$ 1.42	21.6 $\pm$ 1.24	0.074

<sup>(a,b)</sup> Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

### 6.3.2 Physical measurements

The influence of BSM in the broiler diets on initial pH and ultimate pH of breast and thigh, colour, thaw loss and cooking loss of the breast muscle are summarised in Table 6.5. Treatment did not ( $P > 0.05$ ) affect initial and ultimate pH measurements of the breast and thigh muscles of the broiler carcasses. No treatment differences ( $P > 0.05$ ) on colour measurements were observed regarding the L\*, a\*, b\*, hue and chroma values of the broiler breast muscle. Furthermore, treatment did not affect ( $P > 0.05$ ) thaw loss nor cooking loss of the breast muscle.



**Table 6.5** The means ( $\pm$  standard error) of physical measurements of broiler carcasses as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in their diets

Parameters	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
pH <sub>i</sub> breast	6.38 $\pm$ 0.044	6.34 $\pm$ 0.063	6.32 $\pm$ 0.071	6.32 $\pm$ 0.044	0.887
pH <sub>u</sub> breast	6.13 $\pm$ 0.043	6.14 $\pm$ 0.045	6.15 $\pm$ 0.033	6.18 $\pm$ 0.047	0.830
pH <sub>i</sub> thigh	6.65 $\pm$ 0.056	6.48 $\pm$ 0.054	6.65 $\pm$ 0.061	6.63 $\pm$ 0.052	0.083
pH <sub>u</sub> thigh	6.65 $\pm$ 0.043	6.57 $\pm$ 0.047	6.66 $\pm$ 0.054	6.55 $\pm$ 0.065	0.390
L*	54.35 $\pm$ 0.705	53.93 $\pm$ 1.193	55.72 $\pm$ 1.439	54.16 $\pm$ 0.545	0.616
a*	0.45 $\pm$ 0.125	0.65 $\pm$ 0.192	0.60 $\pm$ 0.243	0.68 $\pm$ 0.167	0.844
b*	12.31 $\pm$ 0.269	12.86 $\pm$ 0.488	12.62 $\pm$ 0.738	11.37 $\pm$ 0.590	0.255
Hue	86.70 $\pm$ 1.251	87.02 $\pm$ 0.938	87.38 $\pm$ 0.945	86.75 $\pm$ 0.751	0.962
Chroma	12.35 $\pm$ 0.283	12.89 $\pm$ 0.483	12.72 $\pm$ 0.769	11.39 $\pm$ 0.596	0.258
Thaw loss	3.63 $\pm$ 0.613	3.79 $\pm$ 0.599	4.69 $\pm$ 0.567	3.15 $\pm$ 0.458	0.302
Cooking loss	42.20 $\pm$ 1.089	40.73 $\pm$ 0.771	37.96 $\pm$ 2.546	42.96 $\pm$ 1.340	0.157
pH <sub>i</sub> (Initial pH)					
pH <sub>u</sub> (Ultimate pH)					

### 6.3.3 Chemical analysis

The treatment diets chemical composition results including amino acids are shown in Chapter 4 (Tables 4.3-4.5), while the fatty acid composition results are depicted in Tables 6.8-6.10 for the starter, grower and finisher diets, respectively.

The inclusion of BSM in the broiler chicken diets did not ( $P>0.05$ ) influence the proximate composition of the cooked broiler breast meat regarding moisture, protein, fat and ash percentages (Table 6.6). Furthermore, no treatment differences were observed regarding the amino acids of the cooked broiler breast meat (Table 6.7). Treatment had no ( $P>0.05$ ) effect on the mineral composition of the cooked broiler breast meat regarding P, K, Ca, Mg, Na, Pb, Zn, Cu, Fe and B (Table 6.12). Amongst all the minerals analysed the cooked broiler breast meat was high in Na, K and P, and low in Fe, Zn and Ca, with traces of Cu, Mn and B. The fatty acid compositions of the cooked breast meat are shown in Table 6.11. Treatment did not ( $P>0.05$ ) influence the fatty acid composition of the cooked broiler breast meat besides C14:0. It was observed that the treatment diets fatty acid composition influenced the fatty acid deposition in the cooked broiler breast meat. The fatty acids C16:0 and C18:2n6cis were observed to be prominent in the breast meat whilst C14:0, C16:1, C18:3n3 were found in low concentrations. The feed was observed to have high polyunsaturated fatty acids (PUFA) content across all three feeding stages' diets. Trace concentrations of the "longer chain" fatty acids were deposited in minimal samples attaining insufficient statistical numbers and therefore were not included in the statistical analysis, and not reported on.

**Table 6.6** The means ( $\pm$  standard error) of the proximate analysis (g/100g) of broiler cooked breast meat as influenced by the inclusion of black soldier fly pre-pupae meal (BSM) in their diets

Parameters	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
Moisture	67.8 $\pm$ 0.37	67.4 $\pm$ 0.45	68.1 $\pm$ 0.77	67.7 $\pm$ 0.42	0.860
Protein	29.3 $\pm$ 0.65	29.2 $\pm$ 0.60	29.0 $\pm$ 0.85	29.4 $\pm$ 0.46	0.975
Fat	3.4 $\pm$ 0.32	3.4 $\pm$ 0.34	3.3 $\pm$ 0.41	3.7 $\pm$ 0.13	0.782
Ash	1.2 $\pm$ 0.02	1.2 $\pm$ 0.02	1.2 $\pm$ 0.02	1.2 $\pm$ 0.02	0.292

**Table 6.7** The means ( $\pm$  standard error) of the amino acid composition (g/100g) of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets

Amino acids	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
Histidine*	2.6 $\pm$ 0.11	2.7 $\pm$ 0.07	2.5 $\pm$ 0.10	2.6 $\pm$ 0.22	0.937
Serine	3.0 $\pm$ 0.17	3.0 $\pm$ 0.10	3.1 $\pm$ 0.09	3.2 $\pm$ 0.13	0.739
Arginine*	5.0 $\pm$ 0.25	5.1 $\pm$ 0.14	5.0 $\pm$ 0.20	5.3 $\pm$ 0.33	0.801
Glycine	3.3 $\pm$ 0.12	3.2 $\pm$ 0.06	3.4 $\pm$ 0.09	3.3 $\pm$ 0.20	0.945
Aspartic acid	6.0 $\pm$ 0.35	6.1 $\pm$ 0.32	6.5 $\pm$ 0.13	7.0 $\pm$ 0.23	0.066
Glutamic acid	9.6 $\pm$ 0.55	9.8 $\pm$ 0.48	10.2 $\pm$ 0.26	11.0 $\pm$ 0.35	0.135
Threonine*	3.3 $\pm$ 0.18	3.3 $\pm$ 0.12	3.4 $\pm$ 0.09	3.5 $\pm$ 0.14	0.623
Alanine	3.7 $\pm$ 0.16	3.8 $\pm$ 0.15	4.0 $\pm$ 0.07	4.1 $\pm$ 0.15	0.113
Proline	2.6 $\pm$ 0.11	2.6 $\pm$ 0.06	2.7 $\pm$ 0.07	2.7 $\pm$ 0.10	0.755
Cysteine	0.4 $\pm$ 0.04	0.4 $\pm$ 0.12	0.4 $\pm$ 0.02	0.4 $\pm$ 0.03	0.934
Lysine*	5.4 $\pm$ 0.34	5.6 $\pm$ 0.36	6.0 $\pm$ 0.15	6.4 $\pm$ 0.21	0.072
Tyrosine	2.7 $\pm$ 0.18	2.9 $\pm$ 0.13	2.8 $\pm$ 0.20	2.8 $\pm$ 0.25	0.922
Methionine*	2.1 $\pm$ 0.15	2.0 $\pm$ 0.06	2.0 $\pm$ 0.09	2.1 $\pm$ 0.12	0.890
Valine*	3.5 $\pm$ 0.14	3.5 $\pm$ 0.12	3.5 $\pm$ 0.09	3.7 $\pm$ 0.14	0.393
Isoleucine*	3.1 $\pm$ 0.12	3.2 $\pm$ 0.14	3.3 $\pm$ 0.10	3.5 $\pm$ 0.13	0.206
Leucine*	5.7 $\pm$ 0.27	5.8 $\pm$ 0.20	5.8 $\pm$ 0.19	6.1 $\pm$ 0.24	0.599
Phenylalanine*	3.4 $\pm$ 0.16	3.4 $\pm$ 0.09	3.3 $\pm$ 0.18	3.4 $\pm$ 0.20	0.910

\* Essential amino acids

**Table 6.8** The means of long chain fatty acid composition in Starter treatment diets percentage of fatty acids, as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets

Fatty acids	Treatment diets			
	Control	5% BSM	10% BSM	15% BSM
SFA <sup>1</sup>				
C14:0	0.3	0.5	4.6	2.8
C16:0	12.0	11.3	23.5	16.5
C18:0	5.4	5.0	5.5	4.6
C20:0	0.7	0.6	0.9	0.6
Total	18.4	17.5	34.4	24.5
MUFA <sup>2</sup>				
C16:1	0.2	0.3	2.0	1.7
C18:1n9 <i>cis</i>	23.6	23.1	23.6	25.2
C18:1n9 <i>trans</i>	-	-	0.6	-
C20:1	0.7	0.4	0.6	0.4
C22:1n9	0.3	0.4	0.3	0.4
Total	25.0	24.1	27.1	27.7
PUFA <sup>3</sup>				
C18:2n6 <i>cis</i>	51.5	52.8	35.7	44.1
C18:2n6 <i>trans</i>	-	-	0.3	-
C18:3n3	5.2	5.7	2.5	3.7
Total	56.6	58.5	38.5	47.8
PUFA: SFA	3.1	3.4	1.1	2.0
n-6	51.5	52.8	36.0	44.1
n-3	5.2	5.7	2.5	3.7
n-6: n-3	10.0	9.4	14.2	12.1

<sup>1</sup> Saturated fatty acid<sup>2</sup> Monounsaturated fatty acid<sup>3</sup> Polyunsaturated fatty acid

**Table 6.9** The means of long chain fatty acid composition in Grower treatment diets percentage of fatty acids as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets

Fatty acids	Treatment diets			
	Control	5% BSM	10% BSM	15% BSM
SFA <sup>1</sup>				
C14:0	0.2	1.3	5.7	5.9
C16:0	11.4	13.1	32.0	26.2
C18:0	5.0	5.0	9.4	5.9
C20:0	0.6	0.6	1.3	0.8
Total	17.2	20.0	48.4	38.8
MUFA <sup>2</sup>				
C16:1	0.1	0.5	0.5	1.4
C18:1n9 <i>cis</i>	21.6	24.0	18.1	21.6
C18:1n9 <i>trans</i>	-	-	4.6	1.8
C20:1	0.2	0.3	-	0.3
C22:1n9	0.3	0.3	0.6	0.4
Total	22.3	25.0	23.8	25.5
PUFA <sup>3</sup>				
C18:2n6 <i>cis</i>	53.5	50.1	25.0	32.6
C18:2n6 <i>trans</i>	-	-	1.0	0.9
C18:3n3	7.0	4.9	1.9	2.1
Total	60.5	55.0	27.8	35.7
PUFA: SFA	3.5	2.8	0.6	0.9
n-6	53.5	50.1	26.0	33.5
n-3	7.0	4.9	1.9	2.1
n-6: n-3	7.6	10.1	14.0	15.7

<sup>1</sup> Saturated fatty acid<sup>2</sup> Monounsaturated fatty acid<sup>3</sup> Polyunsaturated fatty acid

**Table 6.10** The means of long chain fatty acid composition in Finisher treatment diets percentage of fatty acids, as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets

Fatty acids	Treatment diets			
	Control	5% BSM	10% BSM	15% BSM
SFA <sup>2</sup>				
C14:0	2.7	0.4	1.9	2.4
C16:0	16.1	12.0	14.9	16.1
C18:0	4.0	4.2	4.3	4.0
C20:0	0.5	0.7	0.5	0.5
Total	23.3	16.8	21.5	23.3
MUFA <sup>3</sup>				
C16:1	1.4	0.3	0.9	1.3
C18:1n9cis	23.7	20.8	22.2	23.2
C20:1	-	-	-	0.3
C22:1n9trans	0.2	-	0.3	0.3
Total	25.2	21.1	23.4	25.1
PUFA <sup>4</sup>				
C18:2n6cis	47.1	56.0	49.7	46.9
C18:3n3	4.4	6.1	5.3	4.8
Total	51.5	62.1	55.1	51.6
PUFA: SFA	2.2	3.7	2.6	2.2
n-6	47.1	56.0	49.7	46.9
n-3	4.4	6.1	5.3	4.8
n-6: n-3	10.8	9.3	9.3	9.8

<sup>1</sup> Saturated fatty acid<sup>2</sup> Monounsaturated fatty acid<sup>3</sup> Polyunsaturated fatty acid

**Table 6.11** The means ( $\pm$  standard error) of long chain fatty acid composition of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets (mg/g meat)

Fatty acids	Treatment diets				P-value
	Control	5% BSM	10% BSM	15% BSM	
SFA <sup>1</sup>					
C14:0	0.3 <sup>b</sup> $\pm$ 0.09	0.4 <sup>ab</sup> $\pm$ 0.06	0.7 <sup>ab</sup> $\pm$ 0.12	1.0 <sup>a</sup> $\pm$ 0.21	0.013
C16:0	9.5 $\pm$ 1.92	7.4 $\pm$ 1.40	10.3 $\pm$ 1.85	9.6 $\pm$ 1.51	0.655
C18:0	4.2 $\pm$ 0.35	3.2 $\pm$ 0.18	5.3 $\pm$ 0.93	4.5 $\pm$ 0.47	0.134
Total	13.3 $\pm$ 1.45	10.4 $\pm$ 1.00	16.2 $\pm$ 2.74	14.3 $\pm$ 1.49	0.173
MUFA <sup>2</sup>					
C16:1	0.3 $\pm$ 0.11	0.6 $\pm$ 0.16	0.5 $\pm$ 0.20	0.8 $\pm$ 0.17	0.307
C18:1n9cis	5.7 $\pm$ 1.14	7.7 $\pm$ 1.35	6.1 $\pm$ 1.54	7.8 $\pm$ 1.45	0.607
C18:1n9trans	0.8 $\pm$ 0.36	0.5 $\pm$ 0.23	1.0 $\pm$ 0.53	0.7 $\pm$ 0.36	0.794
Total	6.8 $\pm$ 1.14	8.7 $\pm$ 1.32	7.6 $\pm$ 1.49	9.3 $\pm$ 1.38	0.571
PUFA <sup>3</sup>					
C18:2n6cis	7.9 $\pm$ 2.71	9.9 $\pm$ 2.16	6.1 $\pm$ 1.72	8.3 $\pm$ 1.94	0.681
C18:3n3	0.6 $\pm$ 0.25	0.6 $\pm$ 0.20	0.4 $\pm$ 0.13	0.4 $\pm$ 0.18	0.801
Total	8.5 $\pm$ 2.75	10.5 $\pm$ 2.11	6.5 $\pm$ 1.67	8.7 $\pm$ 1.99	
PUFA: SFA	0.7 $\pm$ 0.27	1.1 $\pm$ 0.21	0.5 $\pm$ 0.18	0.7 $\pm$ 0.19	0.399
n-6	7.9 $\pm$ 2.71	9.9 $\pm$ 2.16	6.1 $\pm$ 1.72	8.3 $\pm$ 1.94	0.681
n-3	0.6 $\pm$ 0.25	0.6 $\pm$ 0.20	0.4 $\pm$ 0.13	0.4 $\pm$ 0.18	0.801
n-6: n-3	3.9 $\pm$ 1.70	5.9 $\pm$ 2.22	4.2 $\pm$ 2.00	5.8 $\pm$ 2.75	0.882
TFA <sup>4</sup>	28.5 $\pm$ 3.25	29.6 $\pm$ 3.49	30.3 $\pm$ 3.38	32.2 $\pm$ 2.44	0.867

<sup>(a,b)</sup> Means with different superscripts within the same row differ significantly ( $P < 0.05$ )

<sup>1</sup> Saturated fatty acid

<sup>2</sup> Monounsaturated fatty acid

<sup>3</sup> Polyunsaturated fatty acid

<sup>4</sup> Total fatty acids

**Table 6.12** The means ( $\pm$  standard error) of mineral composition of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets

Parameters	Units	Treatment diets				P-value
		Control	5% BSM	10% BSM	15% BSM	
Phosphorous	%	0.67 $\pm$ 0.021	0.68 $\pm$ 0.017	0.68 $\pm$ 0.021	0.65 $\pm$ 0.021	0.793
Potassium	%	0.69 $\pm$ 0.026	0.68 $\pm$ 0.024	0.66 $\pm$ 0.024	0.66 $\pm$ 0.018	0.759
Calcium	%	0.03 $\pm$ 0.002	0.03 $\pm$ 0.004	0.03 $\pm$ 0.003	0.03 $\pm$ 0.006	0.976
Magnesium	%	0.11 $\pm$ 0.004	0.11 $\pm$ 0.003	0.11 $\pm$ 0.004	0.11 $\pm$ 0.005	0.961
Iron	mg/kg	28.11 $\pm$ 3.037	25.64 $\pm$ 1.871	27.85 $\pm$ 2.146	25.99 $\pm$ 2.180	0.841
Copper	mg/kg	0.46 $\pm$ 0.023	0.69 $\pm$ 0.085	0.48 $\pm$ 0.060	0.52 $\pm$ 0.068	0.106
Zinc	mg/kg	29.15 $\pm$ 1.940	29.76 $\pm$ 1.515	26.75 $\pm$ 0.803	26.21 $\pm$ 1.241	0.249
Manganese	mg/kg	0.83 $\pm$ 0.065	0.72 $\pm$ 0.030	0.92 $\pm$ 0.055	0.93 $\pm$ 0.059	0.069
Boron	mg/kg	0.71 $\pm$ 0.049	0.70 $\pm$ 0.046	0.63 $\pm$ 0.062	0.71 $\pm$ 0.078	0.739
Sodium	mg/kg	768.17 $\pm$ 64.953	678.50 $\pm$ 32.146	657.67 $\pm$ 41.277	655.67 $\pm$ 48.912	0.333

### 6.3.4 Descriptive sensory analysis and correlations

Treatment did not ( $P > 0.05$ ) affect chicken aroma and flavour, metallic aroma and flavour, initial and sustained juiciness and tenderness of cooked broiler breast muscles (Table 6.13). Minimal relevant correlations were found amongst the proximate, physical and chemical results with sensory attributes

(Table 6.15). Therefore, for interpretation of the results only relevant positive correlations will be focused on as outlined in Table 6.14. Sustained juiciness was found to be positively correlated to tenderness and moisture percentage, while the correlation between chicken flavour and protein percentage of the meat samples was not significant. The Figures 6.1-6.3 illustrate the DA plots between chemical, proximate and sensory attributes as per treatment effects.

**Table 6.13** The means ( $\pm$  standard error) of sensory attributes as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chickens diets

Parameters	Treatment diets				P-value	LSD
	Control	5% BSM	10% BSM	15% BSM		
Chicken aroma	68.0 $\pm$ 1.43	71.6 $\pm$ 1.16	68.4 $\pm$ 1.95	70.1 $\pm$ 2.15	0.592	5.30
Chicken flavour	68.6 $\pm$ 1.83	70.5 $\pm$ 1.53	68.4 $\pm$ 0.82	70.7 $\pm$ 2.16	0.395	4.74
Metallic aroma	1.8 $\pm$ 0.58	1.9 $\pm$ 0.70	1.9 $\pm$ 0.53	2.2 $\pm$ 0.39	0.880	1.78
Metallic flavour	6.5 $\pm$ 0.82	5.8 $\pm$ 0.70	5.6 $\pm$ 1.32	6.7 $\pm$ 1.85	0.836	3.92
Initial juiciness	73.3 $\pm$ 1.63	73.1 $\pm$ 1.96	72.5 $\pm$ 2.03	72.0 $\pm$ 1.07	0.211	4.45
Sustained juiciness	70.2 $\pm$ 1.36	72.2 $\pm$ 1.58	72.6 $\pm$ 2.37	71.9 $\pm$ 1.90	0.839	5.84
Tenderness	81.7 $\pm$ 2.08	83.1 $\pm$ 1.90	81.1 $\pm$ 3.00	84.3 $\pm$ 2.36	0.941	7.83

**Table 6.14** Relevant positive correlation of sensory, physical and chemical attributes of cooked breast meat as influenced by inclusion of black soldier fly pre-pupae meal (BSM) in broiler chicken diets

Parameters	Tenderness		Protein %		Moisture %		Metallic flavour		pH <sub>i</sub> <sup>3</sup> breast	
	r <sup>1</sup>	P-value	r <sup>1</sup>	P-value	r <sup>1</sup>	P-value	r <sup>1</sup>	P-value	r <sup>1</sup>	P-value
Initial juiciness									0.557	0.005
Sustained juiciness	0.838	<0.001			0.598	0.002	0.495	0.014		
Chicken flavour			0.553	0.001						
Chicken aroma			0.463	0.023						
Moisture %	0.474	0.019								
C18:1n9c MUFA <sup>2</sup>							0.411	0.046		
							0.458	0.024		

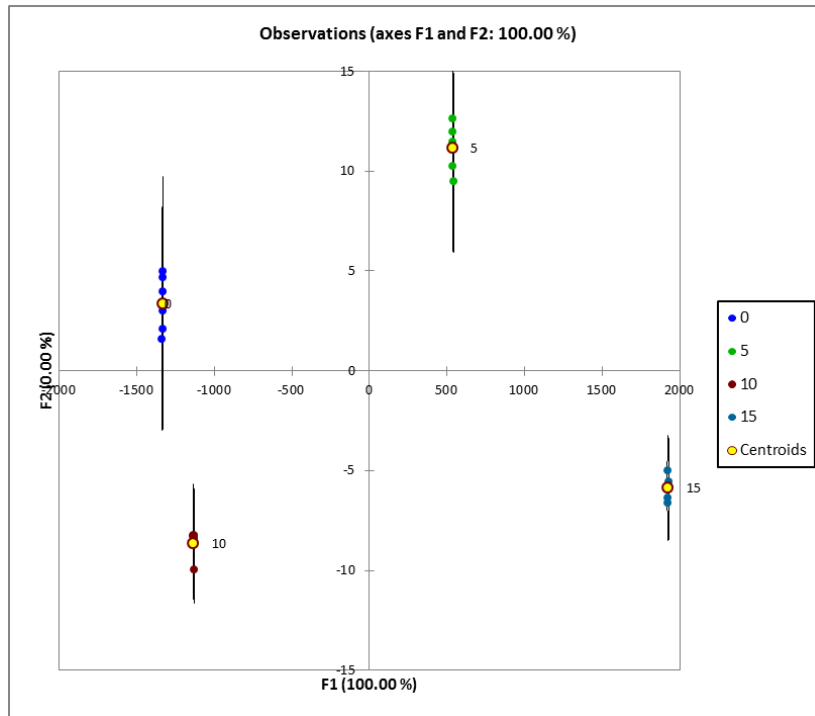
<sup>1</sup> Pearson correlation coefficient

<sup>2</sup> Mono-unsaturated fatty acids

<sup>3</sup> Initial pH

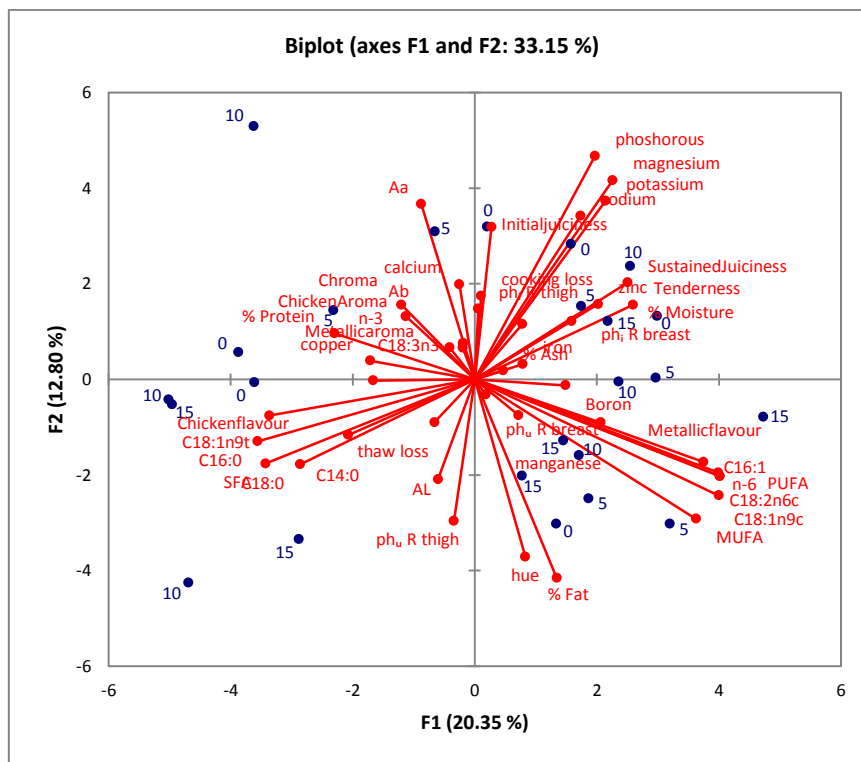






**Figure 6.1** Descriptive analysis (DA) plot illustrating the classification of treatments based on the tested parameters

**Figure 6.2** Descriptive analysis (DA) plot illustrating prominent parameters as per treatment based on observation DA plot Figure 6.1



**Figure 6.3** Principle component analysis (PCA) bi-plot indicating the means for each analysed parameter with the sensory attributes as per treatment replications

## 6.4 Discussion

### 6.4.1 Carcass characteristics

Carcass characteristics are essential attributes determining portion yields for retail. The inclusion of BSM in broiler chicken diets did not ( $P>0.05$ ) influence carcass characteristics regarding live slaughter weight, cold carcass weight and dressing percentage. Pieterse *et al.* (2014) found inclusion of 10% house fly (HF) larvae meal in broiler chicken diets had significantly influenced live slaughter and cold carcass weight, with chicks fed larvae meal attaining higher weights than chicks on the control diets, but dressing percentage was not significantly influenced by treatment. Similarly, Hwangbo *et al.* (2009) found higher dressing percentage, breast muscle and thigh muscle of broiler chickens fed HF meal (included at 5, 10, 15 and 20%). The studies by Pieterse *et al.* (2014) and Hwangbo *et al.* (2009) are not comparable to this study due to differences in insect species used. Even though the studies are not comparable, their results indicate insect meals to be potential feed sources yielding meat of similar size portions as those fed diets containing traditional feed ingredients. Overall, insect meals have proven to contain nutrients that sustain broiler production.

Although the chicks receiving the 15% treatment diets attained a higher ( $P>0.05$ ) skin plus fat percentage of the breast portion than the chicks receiving the control diet, this might be attributed to increased level of BSM in the diet but is not conclusive due to the wide variations found ( $R^2=0.27$ ) within each treatment. The ANOVA results for muscle percentage of the breast portion indicated treatment differences ( $P=0.032$ ), but the Bonferroni's *post hoc* (least square means) test showed no treatment differences. There is no biological

explanation to these statistical results obtained but these may be attributed to the cutting method, causing differences in cut portions since a desk deboning method was used. The deboning method might have led to more meat remaining on the bone or skin plus fat bringing about the huge variations within each treatment as indicated by the low  $R^2$  value of 0.27. No treatment differences were obtained regarding the bone percentage of the breast portion ( $R^2=0.22$ ). The coefficient of determination ( $R^2$ ) value indicates the extent at which the results are explained by the statistical model with a  $R^2 \geq 75\%$  considered as a good fit for the model. The lack of treatment differences attained regarding the carcass characteristic parameters investigated might be attributed to an adequate amino acid content of the BSM (Table 2.8) supporting normal meat development in broiler chickens. The use of BSM have been researched mainly in fish, with Sealey *et al.* (2011) reporting no significant treatment influence on fish muscle ratio and Kroeckel *et al.* (2012) noting a decrease in slaughter body weight with increased levels of BSM in the diets fed to juvenile turbot. There is, however no supporting literature found on effect of BSM or larvae meal on carcass characteristics when fed to poultry.

#### **6.4.2 Physical measurements**

Treatment did not ( $P>0.05$ ) influence the physical measurements analysed. Meat colour is considered by consumers as an important quality cue at point of purchase (Qiao *et al.*, 2002; Hoffman & Cawthorn, 2012). The paleness of meat is indicated by the  $L^*$  value of the meat, with a high  $L^*$  value being an indicator of poor meat quality (Chen *et al.*, 2013). Meat colour is defined by the hue angle while the Chroma value indicates extent of colour intensity and saturation of a muscle. An increased hue angle will mean less red colour in the meat, while an increased Chroma value will mean redder meat colour. In this study, the  $L^*$ ,  $a^*$ ,  $b^*$ , hue and Chroma values were not significantly affected by treatment; attaining similar colour to muscles yielded from chicks fed the control diet which resemble commercially sold chicken meat. According to Barbut (1997) and Allen *et al.* (1998), dark broiler breast fillets have a high muscle pH with a low lightness ( $L^*$ ) value and a high water-holding capacity (low drip loss and low cooking losses) than light breast fillets. The  $L^*$  value of the raw breast meat was observed to be above 53.93, just meeting the threshold value of pale soft exudate (PSE) of  $L^* \geq 54$  set by Barbut (1997), while Van Laack *et al.* (2000) states normal broiler meat colour have  $L^*$  values ranging between 50 and 56. Therefore, according to Van Laack *et al.* (2000), the  $L^*$  values indicate that no PSE or dark firm dry meat was found which was further confirmed by the absences of the sensory attributes of mealiness and toughness from the meat samples as tested by DSA.

Research has shown that there is a positive correlation between water-holding capacity and pH values of fresh meat (Barbut, 1997; Allen *et al.*, 1998). The initial pH value of the breast muscle in this study decreased 24 h later to reach an ultimate pH of 6.13-6.18 with no treatment differences found ( $P>0.05$ ). Van Laack *et al.* (2000) reported normal broiler breast meat to have an ultimate pH of 5.96, which is lower than that obtained in this study. The initial and ultimate pH of the thigh muscle for birds receiving the control diet remained the same while that of the 10% BSM increased with 0.01 units attaining an ultimate pH of 6.66. The ultimate pH measured was slightly higher than the normal range as indicated by Laack *et al.* (2000) but this was however acceptable and did not affect the water holding capacity (thaw and cooking loss) and colour of the meat. The 5% BSM treatment diet broiler chickens thigh portion's ultimate pH value increased

while that of the 15% decreased 24 h later. However, no treatment differences were found ( $P>0.05$ ) regarding initial and ultimate pH of the thigh muscle. Despite the slightly high ultimate breast pH obtained, it was not observed to affect colour, water retention and texture of the meat, which were in optimal ranges and also further substantiated by the lack of correlations found (Figure 6.3). No treatment differences were found regarding thaw loss, cooking loss, initial and sustained juiciness and tenderness, which are indications of water retention of the meat samples. The meat samples attained thaw loss of  $\pm 3.5\%$  and  $\pm 40\%$  cooking loss, translating into high initial and sustained juiciness (both above 70%) and high tenderness (above 80%) as generated by DSA. Therefore, the use of BSM in broiler diets did not influence the physical measurements of the meat.

### 6.4.3 Chemical analysis

Treatment effects on cooked breast meat proximate analysis and amino acids results revealed no ( $P>0.05$ ) differences. The broiler cooked meat was observed to contain percentage moisture, protein, fat and ash similar to that of commercial broilers as analysed by Geldenhuys *et al.* (2013) and the control utilised in this study. This was expected due to the adequate nutritional content of BSM (Chapter 3) and its proven effects on the meat quality of various fish species investigated (St-Hilaire *et al.*, 2007; Sealey *et al.*, 2011; Kroeckel *et al.*, 2012).

The identified fatty acid content of the cooked breast meat was directly influenced by the fatty acid content of the treatment diets. This is confirmed by various authors, reporting fatty acid composition of the feed to pre-determine the fatty acid composition of the meat produced in monogastric animals (Coetzee & Hoffman, 2002; Cao *et al.*, 2012). The feed was found to be particularly high in all the identified fatty acids deposited in the meat. The cooked breast meat was found to be high in palmitic acid (C16:0) and linoleic acid (C18:2n6*cis*) and low in palmitic acid (C16:1) and myristic acid (C14:0). Furthermore, the treatment diets were high in C18:2n6*cis* and contained traces of C18:1n9*trans*, C20:1, C22:1n9 and C18:2n6*trans*.

The meat samples were observed to be high in SFA followed by PUFA then MUFA, while the feed samples were high in PUFA; over 50% across all three feeding stages diets. The recommended PUFA/SFA for humans is  $>0.45$  (Raes *et al.*, 2004). The ratios of PUFA/SFA in the cooked meat were 0.52-1.05, higher than that recommended, although they were not significantly different from each other as pertaining to diet. The PUFA/SFA was noted to be lower than that found by others in cooked broiler chicken meat (Geldenhuys *et al.*, 2013). Meat fatty acid content results however, are not entirely comparable as they are pre-determined by diet and may differ significantly. Polyunsaturated fatty acids are considered vital in human diets as they are responsible for various functions in the body as precursors of cellular function molecules especially those involved in reproduction, blood coagulation, inflammation and cardiac physiology (Durand *et al.*, 2005). Kroeckel *et al.* (2012) observed BSM to be high in SFA (Table 2.7) which might have led to the high SFA content of the meat observed in this study (Table 6.11). The C14:0 fatty acid compound of the breast meat was influenced ( $P<0.05$ ) by treatment, with the 15% diet being significantly higher than the control diet. A similar trend was observed in the C14:0 content of the feed where it was observed to be higher in the 15% treatment diets and lower in the control diets, which may explain the significant differences observed in the

meat content. However, the reason for this specific fatty acid being higher in this diet is unclear as the same raw materials were used during the mixing of the diets.

The cooked broiler chicken meat as influenced by inclusion of BSM was found to be low in the omega-3 and omega-6 fatty acids, which is expected in poultry meat. The omega-3 fatty acids are important in human diets as mammals cannot convert omega-6 to omega-3, which is found to modulate and prevent coronary heart diseases and cardiovascular diseases (Simopoulos, 2002). A dietary omega-6/omega-3 ratio value not >5 is recommended for human consumption (Legrand, 2002), as an increase intake of omega-6 in human diets can cause an increase in high-density lipoprotein cholesterol causing potential health risks (Simopoulos, 2002). Therefore, poultry meat contain an amount of n-6/n-3 sufficient to sustain daily requirements without concerns of consuming more than required for health related reasons.

No published data was found documenting the chemical composition of broiler chicken meat as affected by inclusion of BSM in their diets. However, Pieterse *et al.* (2014) reported the use of HF in broiler chicken diets produced meat with an acceptable chemical composition comparable to those fed soya bean and fish meal diets. Therefore, it can be concluded that insect meals used in broiler chickens have the potential to produce meat with similar chemical composition as those fed diets containing traditional feed ingredients.

#### **6.4.4 Descriptive sensory analysis and correlations**

No treatment differences were observed ( $P>0.05$ ) regarding sensory attributes analysed for chicken aroma and flavour, metallic aroma and flavour, initial and sustained juiciness and tenderness of broiler meat. Similar effects of BSM on fish meat were observed by Bondari & Sheppard (1981) and Sealey *et al.* (2011). In a study by Pieterse *et al.* (2014), broiler chickens fed HF larvae and pupae meal produced breast meat that was high in metallic flavour compared to those fed soya-bean and fishmeal based diets and warranted further research on iron uptake of insect larvae and pupae. In this study the use of BSM in broiler chicken diets led to no treatment differences regarding cooked breast meat iron content (Table 6.12), metallic flavour and metallic aroma (Table 6.13). Furthermore, no correlations were found regarding metallic flavour and aroma to the iron content of the cooked breast meat (Table 6.15).

The results obtained from the physical, chemical and proximate analysis were analysed for correlations with sensory attributes, however minimal significant correlations were found (Table 6.15). Due to minimal meaningful significant correlations, those to be discussed are shown in Table 6.14. The principle component analysis (PCA) bi-plot and descriptive analysis (DA) plot illustrates the respective correlation of the physical, chemical, proximate and sensory attributes as affected by treatment. The DA plot (Figure 6.2) further classified into treatment effects by Figure 6.1 illustrating the specific attributes that were found dominant in each treatment. Firstly, the DA plot indicates the 15% treatment diet chicken's yielded meat associated with C14:0, C16:1, ash percentage, metallic aroma and flavour and Iron. Secondly, the 10% treatment diet samples are associated with SFA, C18:1n9*trans*, Chroma, cooking loss and moisture percentage, whilst the 0% treatment diet (control) yielded breast portions associated with omega-3 fatty acids, sodium, calcium, phosphorous, L\* and initial juiciness. Lastly, the chicks receiving the 5% treatment diet produced meat

associated with chicken aroma and flavour, PUFA, MUFA, hue and tenderness. The association of attributes found in each treatment samples illustrated by the DA plot, were however not significant as no treatment differences were observed.

The PCA bi-plot illustrates the sensory attributes associated with each sample. The extent of each line as indicated in Figures 6.2 and 6.3 show the correlation ( $r$ ) strength and weakness, which are clearly weak. The PCA bi-plot generated is complex to explain as no general trend in treatment groupings was obtained as the observations were scattered across the plot. It can, however be observed that fat percentage is positively correlated to most of the fatty acids but negatively correlated to protein percentage, chicken aroma and metallic aroma. It is expected for the intramuscular fat in meat to be positively correlated to tenderness (Warriss, 2000) and flavour (Geldenhuys *et al.*, 2014), although no strong correlations were found for fat percentage to tenderness and flavour in this study; this may be attributed to the low fat content of the meat (Table 6.6). Tenderness was rather observed to be positively correlated to moisture percentage and sustained juiciness. The correlations were both significant and stronger ( $r=0.838$ ) in sustained juiciness and weak ( $r=0.474$ ) in moisture percentage. No specific fatty acid was correlated to flavour. Lack of literature on effects of BSM on meat sensory attributes of broiler chickens resulted in minimal comparison to substantiate the results obtained. However, the results obtained are promising to the broiler industry confirming BSM to be a viable protein source in broiler chickens producing meat equivalent in sensory quality to the control birds.

## 6.5 Conclusion

The viability of BSM as an alternative protein source in broiler chickens is not only proven on basis of its ability to maintain growth but also on its meat quality. The inclusion of BSM in broiler diets did not influence the quality of the meat produced nor compromise the meat eating quality as no taste discrimination was observed by DSA utilising trained panellists. However, it is of interest to study the acceptability of meat protein sources produced from non-traditional feed sources to consumers. This is of significance as some consumers might regard the use of insect meals as feed sources in animal diets to be organic and some not, which can be a major determinant of the commodity price and thus a possible marketing strategy. Furthermore, the larvae of black soldier fly has the advantage of re-utilising waste products and is therefore more “green” and thus suitable for consumers’ demands. This will enable reduction of organic waste and in the process producing pre-pupae usable for broiler chicken production that is proven to not compromise the quality of meat produced.

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## Chapter 7

### General conclusion and recommendations

The objectives of this study were fourfold, strategized into investigating the potential of black soldier fly pre-pupae meal (BSM) as a protein source in the production of broiler chickens. Firstly, the coefficients of total intestinal tract digestibility (CTTD) values of nutrients in various processed BSM's in broiler chickens were obtained. The results indicated that BSM's were digestible in broiler chickens attaining CTTD's higher than 70%. The removal of fat from BSM significantly increased digestibility of nutrients as compared to the full fat BSM dried at 65 °C (BSM65) diet. In this study the nutrient composition of the BSM used was not analysed which would have shed more light on its specific nutritional composition. Further research is also recommended to investigate the effects of the defatted BSM dried at 65 °C (DF-BSM) meal since it attained the highest CTTD of analysed nutrients. This will enable the quantification of the bioavailability of nutrients in DF-BSM for broiler production.

In the second part of the study BSM dried at 65 °C (BSM) was tested for its effects on production of broiler chickens as a protein source included at 0, 5, 10 and 15%. The results of the production study indicated BSM inclusion in broiler chickens diets as a protein source sustained and support normal bird growth, since no major treatment differences were observed. The use of insect meals in animal diets will also lead to an increase in organic waste vermi-composting, thereby minimizing waste effects, and in the process yield larvae, pupae or pre-pupae which will increase protein availability for animal use. This will contribute to food security as it will minimize usage of specific crop products, also used for human consumption, in broiler diets.

Thirdly, the study investigated the effects of BSM on organ, gut and skeletal parameters of broiler chickens. The organ size, small intestine pH and histomorphology measurements were analysed and no key treatment differences were observed. Furthermore, the breaking strength, ash percentage and mineral composition of the tibia bone were evaluated and no treatment differences were observed as compared to the control. Treatment differences were, however found between the 5% and 15% treatment diets regarding Ca content of the tibia bones; indicating an increase in BSM inclusion to have led to an increase in tibia bone Ca content. This indicates that the Ca content within the BSM might have a high bioavailability to the birds given that the treatment diets were formulated for equal Ca levels. Therefore, it is recommended to research further the effects of different supplementation levels of Ca in diets containing 15% BSM on broiler chicken production to quantify the bioavailability of Ca in BSM to broiler chickens and their effects on bone strength.

The last objective was to determine the quality of meat produced from broiler chickens fed BSM included at 0, 5, 10 and 15%. The quality of the meat of the chickens fed the different diets was determined by physical measurements (colour, pH, thaw and cooking loss), carcass portion yields, nutrient composition (proximate, amino acids and fatty acids), mineral content and sensory analysis (flavour, tenderness and juiciness) with no significant treatment differences observed. Therefore, it can be concluded that the inclusion of BSM in broiler chickens diets produced meat without compromising its quality. It was of interest to determine the

effect of the BSM iron content on the meat metallic flavour and aroma as suggested by previous authors based on the high iron uptake by insect larvae. However, no treatment differences were observed regarding all sensory attributes investigated. Further research is warranted on iron uptake of various insects' larvae fed different sources of organic waste. This will enable the quantification of iron intake by insects which can be used to produce meat and products with low or high iron content, depending on nutritional requirements in various geographical regions across the globe. Research is warranted on the consumer perception and acceptability of meat products produced from animals fed diets containing insect meal as a feed ingredient.

It can, therefore, be concluded that BSM is a viable protein source suitable for use in broiler chicken production, without compromising the production efficiency. Also, the study showed that BSM has the ability to sustain broiler chickens growth and subsequently produce meat without compromising its physical, eating and chemical quality. Depending on the economies of scale and availability of BSM, it can be included in broiler chicken diets as a protein source up to 15% (the maximum inclusion level tested in these experiments), without affecting production of broiler chicken or quality of meat produced. The use of BSM in broiler chicken diets can enhance the broiler feed industry by contributing to the reduction of protein shortage for use in poultry diets. The challenge is therefore to produce BSM in sufficient quantities and of similar quality utilisable in broiler chicken production. Further research is recommended on the cost-benefit analysis of producing insect meals and their use in animal diets on both small and large scale production.

Lastly, the effects of BSM on the production efficiency of layers, aquaculture, the production of pigs and ruminant animals are lacking and research is required to quantify these effects.