Chrysomya chloropyga larvae meal as a protein source for broiler nutrition

by

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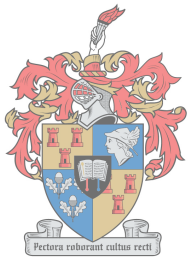

Thesis presented in fulfilment of the requirements for the degree of *Master of Science in the Faculty of AgriScience at Stellenbosch University*

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Declaration

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Summary

This study investigated the possibility of using *Chrysomya chloropyga* (copper bottom blow fly) larvae meal as a protein source in broiler nutrition. At first the chemical composition of the larvae meal was determined before three balanced diets were formulated containing 5%, 10% and 15% larvae meal, along with a maize-soya based control diet. The four diets were fed to 320 day-old Cobb 500 broiler chickens. A three phase feeding system were used i.e. starter (900g per bird), grower (1200g per bird) and finisher (1200g per bird). Each treatment was replicated eight times.

The effect of the copper bottom blow fly larvae on the production parameters of broilers were determined. Treatment had no effect on average intake per bird, feed conversion ratio (FCR), European production efficiency factor (EPEF) or livability. Broilers that were fed the 10% inclusion had a slightly higher live weight at slaughter and average daily gain (ADG) than the other three treatments.

The chemical, physical, carcass and sensory characteristics were then evaluated. Treatment had no effect on cold carcass weight, dressing percentage, thigh-, drumstick- wing and back yields. The 10% inclusion had a higher breast portion yield than the other treatments. No differences were observed regarding the yields of skin plus fat, muscle or bone percentages.

Treatment did not affect meat colour. No differences were observed regarding the initial pH (pH_i) of the breast and thigh meat, or the ultimate pH (pH_u) of the breast. The pH_u of the thigh of the 5% and 15% inclusion were lower than that of the Control and 10% inclusion.

The control meat had a higher moisture content and a lower ash content than the three treatment diets. Protein and fat did not differ. No differences regarding the amino acid composition and the mineral composition in the broiler meat were observed.

Few differences were observed for sensory attributes of the broiler meat. The 5% inclusion had the highest chicken aroma, the control had the lowest chicken aroma and the 10% and 15% were intermediate. The control had the highest initial juiciness, while the 10% and 15% had significant lower values for initial juiciness.

Lastly the organ, gut and tibia bone parameters were evaluated. Treatment had no effect on gizzard erosion and all gizzard scores were normal. Treatment had no effect on gizzard, liver, heart, bursa or spleen weights or the weight of these organs relative to live weight. Treatment had no effect on tibia bone breaking force and tibia bone breaking strength. Treatment had little effect on tibia bone mineral composition, with only a difference in the potassium (K) content; the control had a lower K content than the three larvae fed diets.

Overall the study concluded that copper bottom blow fly larvae meal can be used in broiler diets at inclusions levels of up to 15% without any negative effect while 10% inclusion yielded positive results in some instances.

Opsomming

Die doel van die studie was om die moontlikheid van die gebruik van *Chrysomya chloropyga* (CC) larwe meel in braaikuiken voeding te ondersoek. Die chemiese samestelling van die larwe meel was bepaal, en daarna is drie gebalanseerde diëte geformuleer wat 5%, 10% en 15% larwe meel bevat het. ’n Kontrole dieet is ook geformuleer. Die vier diëte is gevoer aan 320 dag-oud Cobb 500 braaikuikens. ’n Drie fase voersisteem is gevolg nl. aanvangs (900g per kuiken), groei rantsoen (1200g per kuiken) en afrond rantsoen (1200g per kuiken). Elke behandeling is ag keer herhaal.

Die effek van CC larwe meel op die produksie parameters van braaikuikens is bepaal. Behandeling het geen effek op voer inname, voeromsetverhouding, Europese produksie effektiwiteitsfaktor en kuiken oorlewing gehad nie. Die 10% insluiting het ‘n effe beter lewendige eindgewig en gemiddelde daaglikse toename (GDT) as die ander drie behandelings gehad.

Die chemiese, fisiese, karkas en sensoriese eienskappe was bepaal. Behandeling het geen effek op koue karkasgewig, uitslag persentasie, dy-, boud-, vlerk- en rugopbrengs gehad nie. Die 10% insluiting het ’n hoër borsopbrengs gehad as die ander behandelings. Geen verskille in terme van weefsel opbrengste was gevind nie.

Behandeling het geen effek op vleis kleur gehad nie. Geen verskille was gesien in die aanvanklike pH van die bors en dy asook die finale pH van die bors nie. Die finale pH van die dy van die 5% en 15% insluitings was effens laer as die van die kontrole en die 10% insluiting.

Die kontrole vleis het ‘n hoër vog inhoud gehad as die ander drie behandelings, asook ‘n laer as inhoud. Proteïene en vet het nie verskil nie. Geen verskille rakende aminosuur samestelling en die minerale samestelling van die vleis was aangemeld nie.

Min verskille wat sensoriese parameters betref is gesien. Die 5% insluiting het die hoogste hoender geur gehad, die kontrole het die laagste met die 10% en 15% intermediêr. Die kontrole het die hoogste aanvanklike sappigheid gehad, terwyl die 10% en 15% laer waardes vir aanvanklike sappigheid gehad het.

Laastens is die orgaan, maelmaag en tibia been parameters evalueer. Behandeling het geen effek op maelmaagerosie gehad nie, en die maelmaggies was geklassifiseer as normaal. Behandeling het geen effek op maelmaag, lewer, hart, bursa of milt gewigte, asook hulle verhoudings tot slagmassa gehad nie. Behandeling het geen effek op tibia been breekkrag of tibia been breeksterkte gehad nie. Behandeling het min effek op tibia been mineraalsamestelling gehad, met net verskille in kaliumvlakke (K). Die kontrole het ’n beduidende laer K-waarde gehad as die drie larwe behandelings.

Die gevolgtrekking kan gemaak word dat CC larwe meel in braaikuiken diëte gebruik kan word tot en met ’n insluiting van 15% sonder enige nadelige effek, die 10% insluiting het op ’n paar plekke die beter resultate gelewer.

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Lastly, my Jack Russel, Buks, who waited for hours outside the laboratory door every day for me to finish this impossible task.

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

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List of Abbreviations

a*	Red-green
AA	Amino acid
ADF	Acid detergent fibre
ADG	Average daily gain
Al	Aluminium
Ala	Alanine
ANOVA	Analysis of variance
Arg	Arginine
Asp	Aspartic acid
ATP	Adenosine triphosphate
b*	Blue-yellow
B	Boron
BSF	Black solder fly
BSM	Black soldier fly pre-pupae meal
Ca	Calcium
CC	*Chrysomya chloropyga*
CF	Crude fibre
cm	Centi-meter
CP	Crude protein
Cu	Copper
Cys	Cysteine
DAFF	Department of Agriculture, forestry and fisheries
DSA	Descriptive sensory analysis
EE	Ether extract
EPEF	European production efficiency factor
FC	Field cricket meal
FCR	Feed conversion ratio
Fe	Iron
FM	Fish meal
g	grams
GH	Grasshopper meal
Glu	Glutamic acid
Gly	Glycine
h	Hour
HFP	Housefly pupae mal
HFM	Housefly maggot
His	Histidine
IAAP	Ideal amino acid profile
Ile	Iso leucine
K	Potassium
kg	Kilogram
L*	Lightness
Leu	Leucine
Lys	Lysine

m	Meters
M	Maggot meal
Met	Methionine
Mg	Magnesium
min	Minutes
mL	Milliliter
mm	Milimeter
Mn	Manganese
N	Newton
Na	Sodium
NA	Not applicable
ND	Not detected
NDF	Neutral detergent fibre
P	Phosphate
PER	Protein efficiency ratio
Phe	Phenylalanine
Pro	Proline
SCM	Soya oil cake meal
SD	Standard deviation
Ser	Serine
SWH	Silkworm caterpillar
Thr	Threonine
Tyr	Tyrosine
µl	Micro litres
µm	Micro meters
Val	Valine
Zn	Zinc

Chapter 1: **General introduction**

The world human population is increasing rapidly and in 2050 there will be an estimated 9.7 billion people on earth (http://www.un.org/en/development/desa/news/population/2015-report.html). The need for more protein for human consumption is inevitable, and humans and animals are competing for the same protein sources. This has started a demand for alternative protein sources in animal nutrition. Capture fisheries have already reached maximum sustainable production and fishmeal will likely soon be diverted to direct human nutrition (Lim *et al*., 2008)

It is important that these alternative protein sources are sustainable and beneficial to the environment, as most organic waste pose a health risk if not managed correctly (Roberts & de Jager, 2004).

Most insects are rich in fat and protein, and are part of wild and free range birds' diets (Miao *et al*., 2005). The fact that insects form part of a large portion of wild birds' diets initiated this study.

The disposal of abattoir waste can pollute the environment (Mittal, 2006). Nutrients can possibly be recycled from abattoir waste by *Chrysomya chloropyga* (CC) and in this manner the risk to the environment can be reduced. South Africa produces approximately 19.5 million broilers per week (Astral, 2013). Broilers typically lose 30 % of their live weight of 1.9 kg as waste (Haitook, 2006). That means 19.5 million broilers will produce 11 115 tons of waste per week. Given the production potential of CC this can be converted to 500 000 tons of protein per annum while reducing the waste to feed in approximately three days.

Blood from slaughtered cattle in South Africa contains 20 000 tons of protein annually (Arvanitoyannis & Ladas, 2008) for which a new method of recycling needs to be investigated.

C. chloropyga (CC) is a carnivorous fly species and is of forensic importance (Richards *et al,.* 2011). The larvae of CC grow from a starting weight of 0.5 milligrams (mg) to a final weight of

61.5mg in 63 hours (De Souza et al., 1982). This make CC the ideal species to break down abattoir waste.

No research on the possible use of CC in broiler nutrition have been done to date. Therefore, a study was conducted to investigate the use of CC larvae meal fed on pork abattoir waste in broiler nutrition. The study included the evaluation of the chemical composition of CC larvae, production parameters, carcass characteristics, sensory attributes and organ, gut and tibia bone parameters of broilers fed CC larvae meal in diets at inclusions of 0%, 5%, 10% and 15%.

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Chapter 2: **Literature Review**

2.1 Introduction

With a fast-growing world human population, and limited protein sources which are becoming more expensive, it is important to find alternative protein sources for animal nutrition. The world human population grew exponentially from 1.5 to 6.1 billion between the years 1900 and 2000 (Ortiz-Ospina & Roser, 2016). The world population then grew further to more than 7.5 billion in 2016 (Ortiz-Ospina & Roser, 2016) and is expected to reach 9.7 billion in 2050 (http://www.un.org/en/development/desa/news/population/2015-report.html). Humans will soon compete with animals for the same protein sources, and a demand for alternative protein sources in animal nutrition will follow.

On the other hand, it is also important that the production of these alternative protein sources should be sustainable / renewable and beneficial to the environment. Most of the organic waste produced in South Africa can pose a health risk if not managed correctly (Roberts & de Jager, 2004). Organic waste streams such as abattoir waste, can possibly be used by insects, like the copper bottom blowfly (*Chrysomya chloropyga*) (CC), to produce an alternative protein source for animal nutrition. At the same time this can reduce the organic waste.

2.2 Organic waste products

Most of the organic waste produced come from the agricultural industry (El Boushy, 1991) and the nutrients in the waste products can be recycled using insects (El Boushy, 1991; Li *et al*., 2011). Agricultural waste include waste from farms, food retailers, the fermentation industry and abattoirs (El Boushy, 1991).

Abattoir waste can be buried in the ground to decompose, run off into fields, or the local authorities can dispose of it (Roberts & De Jager, 2004). This can pollute the environment with pathogens and contaminate ground water (Mittal, 2006). Nutrients can possibly be recycled from abattoir waste by CC and the risk to the environment can be reduced. In 2003 South

Africa produced approximately 19.5 million broilers per week (Astral, 2013). Broilers typically lose 30% of their live weight of 1.9kg as waste (Haitook, 2006). That means 19.5 million broiler will produce 11 115 tons of waste per week.

Abattoir waste include fat, feathers, rejected carcasses, dead on arrivals, hides, hooves, feet, heads, trimmings, intestines and blood (Roberts & De Jager, 2004). Intestines, hooves and heads can be sold as oval (Christoe, 2003) while feather meal is a protein source in animal nutrition (Dalev, 1994). Blood meal and carcass meal can serve as a great source of protein in animal nutrition, but are banned in most countries due to health risks (Act No 36 of 1947). Intestinal waste, blood and rejected carcasses have the biggest volume of waste products (Christoe, 2003). The blood from abattoir waste contains protein for which a new method of recycling needs to be investigated (Arvanitoyannis & Ladas, 2008). *C. chloropyga* (CC) is a carnivorous fly species and is of forensic importance (Richrads *et al*., 2011). The larvae of CC grow from a starting weight of 0.5 milligrams (mg) to a final weight of 61.5mg in 63 hours (De Souza *et al*., 1982). This make CC the ideal species to break down abattoir waste. Large scale abattoir waste break down and protein recovery means that CC must be mass reared. These insects mass rearing facilities should not experience high fly mortalities or low fertility and fecundity. Different fly species respond in different ways to mass rearing (Robinson, 2005). Increased stress due to overstocked or understocked cages can cause physical injury or reduced longevity as well as changes in interaction between sexes, leading to changes in fertility and fecundity (Rull *et al*,. 2011). Increased density can decrease male reproduction success due to decreased territoriality, changes in mating behavior and interrupted mating (Diaz-Fleischer *et al*., 2009; Gaskin *et al*., 2015). High densities was shown to lead to fewer territoriality and therefore fewer male interaction in *Drosophila melanogaster* (Hoffmann & Cacoyianni, 1990). *C. chloropyga* can produce a high number of eggs at a time and is a large mammal carcass specialist (Richards *et al*., 2009). Although the longevity of CC is lower as fly density increases, Parry (2014) found no clear trend in survival as an effect of increasing density. In a study on the mass rearing of four blow fly species, CC were the first to reach peak production and also produced the most eggs at day of peak production (Parry, 2014). At low density, CC had a low fertility rate, while at high density CC had a higher fertility rate than

three other blow fly species in the study (Parry, 2014). *C. chloropyga* congregates in large numbers on big carcasses and it may well be that there are too few interactions between the different sexes when the flies are present at low densities to stimulate mating (Rhainds, 2010). Ridley (1988) concluded that female CC will lay their eggs sooner and in higher quantities at higher densities as there are more opportunities to mate. This makes CC a good insect species to mass rear and to recycle protein from abattoir waste.

2.3 Insects, waste treatment and protein recovery

As early as 1919 the first reports of recovering protein from waste was described. Linder (1919) reared *Musca domestica* larvae on sewage and fed the dried larvae to rats. The next authors that used insects for waste treatment and protein recovery was Calbert & Martin (1969). Also, using *M. domestica* they used chicken manure as a substrate to grow the larvae in, and dried pupae were fed to chickens. Calbert & Martin (1969) concluded that dried house fly pupae provided sufficient protein for normal growth and development for broilers for the first two weeks of life.

2.4 The use of insect meal in animal nutrition

The increase in the price of especially protein sources made it important to produce alternative protein sources for animal nutrition all over the world. The use of house fly larvae meal was in most studies compared with conventional protein sources and the aim was to replace the conventional protein sources with larvae meal (Newton *et al*., 1977; Awoniyi *et al*., 2003; Ogunji *et al*., 2006; Adeniji, 2007; Agunbiade *et al*., 2007). All these researchers concluded that house fly larvae meal has the right nutritional values to replace conventional protein sources such as fishmeal. Studies on the value of insect meal in animal nutrition were performed on many occasions. Among these insects are *Musca domestica* (House fly) (Zuidhof *et al*., 2003; Aniebo *et al*., 2008; Pretorius, 2011; Pieterse & Pretorius, 2013), *Hermetia illucens* (Black Soldier fly) (Hale, 1973; Newton *et al*., 1977; Bondari & Sheppard, 1987; Kroeckel *et al*., 2012, Uushona, 2016), *Tenebrio molitor* (Mealworm) (Klasing *et al*., 2000; Ng *et al*., 2001; Ramos-Elorduy *et al*., 2002, Hopley, 2016), *Zophobas morio* (Giant Mealworm) (Barker *et al*.,

1998; Jabir *et al*., 2012; Finke, 2013), *Acrida cinerea* (Grasshopper) (Wang *et al*., 2007), *Cirina forda* (Pallid Emperor Moth) (Oyegoke *et al*., 2006) and *Anaphe infracta* (Silkworm) (Ijaiya & Eko, 2009).

Some of this studies include the comparison of fish meal (Téguia *et al*., 2002; Awoniyi *et al*., 2003; Ogunji *et al*., 2006; Agunbiade *et al*., 2007; Pretorius, 2011), groundnut meal (Adeniji, 2007) and soya bean oil cake meal (Hwangbo *et al*., 2009) with common house fly (*M. domestica*) larvae meal.

2.4.1 Chemical composition of insects

The comparison of the nutritional values of insect meals with that of fish meal and soya oil cake meal (Animals have specific amino acid requirements (Teles *et al*., 2011). Therefore amino acid composition of a protein source is important (Conconi *et al*., 1984). Table 2.3 compare the amino acid composition of various insect species with that of fish meal. Fish meal has a higher lysine value than all the insect species in this study. The ideal amino acid profile (IAAP) is a concept where the pattern of amino acids (defined as a percentage of lysine) maximizes growth in animals (Baker, 1996). When the amino acids provided in the ration are out of balance, the excess of the least limiting amino acids will be de-aminated and used as energy. This de-amination will also result in higher nitrogenous excretions (http://www.puyallup.wsu.edu). The oversupply of protein causes more Nitrogen excretion via uric acid; a significant amount of energy is required for this process (Macleod, 1997). An estimated six molecules of adenosine triphosphate (ATP) are used to excrete one gram of Nitrogen (Macleod, 1997).

The amino acid composition of black soldier fly larvae (BSF), house fly and alkali fly meet and in some instances, exceed the amino acid profiles of humans, pigs, broilers and Nile tilapia (

Table 2.4). When evaluating these amino acid profiles it can be assumed that BSF could be used as a protein source in the diets of the above. In some countries humans might not want to eat BSF larvae as different cultures use different food sources.

When evaluating the nutrient composition of mopane worms, it was found that the average protein content was 48.3% and that the amino acid composition compare favourably with that of soya (Glew *et al*., 1999; Greyling & Potgieter, 2004). The only amino acid which is really in short supply in Mopane worms is Methionine for humans (Table 2.5).

Table 2.1) show that insect meals have favorable chemical compositions. In most cases crude fat will be the factor that limit the insect meal inclusion. The differences in chemical composition suggest that insect meals should probably be used in combination with other protein sources to formulate balanced diets.
Various authors have determined the proximate composition of various insect species. The results and the variation in nutritive values are shown in Table 2.2. The crude protein (CP) content of the majority of the insect species evaluated in these tables is higher than 45%. This suggests that the CP of most insect species is higher than that of soya. This is in agreement with Ramos-Elorduy *et al*. (1981) and de Guevara *et al*. (1995), who concluded that insect species in general have high CP contents.

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Table 2.1 Comparison of the nutritional value of insect meals with that of fish and soya bean meal

Parameters	**HFM[1,a]**	**M[2,b]**	**FC[3,c]**	**GH[4,d]**	**SWC[5,e]**	**FM[6,f]**	**SCM[7,f]**	**HFP[8,g]**	**BSM[9,h]**
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

[a] Aniebo *et al*. (2009)
[b] Awoniyi *et al.* (2003)
[c] Wang *et al.* (2005)
[d] Hassan *et al*. (2009)
[e] Ijaiya & Eko (2009)
[f] NRC (2004)
[g] Pieterse & Pretorius (2014)
[h] Newton *et al*. (2005a)

[1] HFM (Housefly maggot, blood & wheat meal)
[2] M (Maggot meal)
[3] FC (Field cricket meal)
[4] GH (Grasshopper meal)
[5] SWC (Silkworm caterpillar)
[6] FM (Fish meal dehydrated)
[7] SCM (Soya oil cake meal)
[8] HFP (Housefly pupae meal dried)
[9] BSM (Black soldier fly pre-pupae meal dried)

Table 2.2 Proximate compositions of various insect species

	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Neutral Detergent Fibre (%)	Acid Detergent Fibre (%)	Ash (%)	References
Lepidoptera							
Achroia grisella (L)	33.97	60.00	NA	19.50	8.19	1.40	Finke, 2002
Bombyx mori (L)	53.75	8.09	NA	6.36	6.36	6.36	Finke, 2002; Frye & Calvert, 1989
Chilecomadia moorei (L)	38.94	73.86	NA	6.53	3.52	2.01	Finke, 2012
Hyalophora cecropia (L)	54.70	10.20	14.70	NA	NA	5.90	Landry *et al*., 1986
Collosamia promethean (L)	49.40	10.00	10.80	NA	NA	6.90	Landry *et al*., 1986
Manduca sexta (L)	58.10	20.70	9.40	NA	NA	7.40	Landry *et al*., 1986
Spodoptera frugiperda (L)	57.80	20.20	6.70	NA	NA	5.60	Landry *et al*., 1986
Pseudaletia unipuncta (L)	54.40	14.90	5.00	NA	NA	6.90	Landry *et al*., 1986
Spodoptera eridania (L)	54.70	13.90	7.10	NA	NA	9.80	Landry *et al*., 1986
Samia ricinii (PP)	54.20	26.20	3.26	NA	NA	3.80	Longvah *et al*., 2011
Samia racinii (P)	54.60	26.20	3.45	NA	NA	3.80	Longvah *et al*., 2011
Cirina forda	20.00	12.50	8.70	NA	NA	NA	Osasona & Olaofe, 2010
Antherea pernyi	71.90	20.10	NA	NA	NA	4.00	Zhou & Han, 2006
Coleoptera							
Z. morio (A)	68.05	14.25	NA	50.14	32.06	6.16	Oonincx & Dierenfeld, 2012
Z. morio	46.79	42.04	NA	9.26	6.41	2.38	Finke, 2002
T. molitor	49.08	35.17	NA	14.96	6.56	2.36	Finke, 2002

	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Neutral Detergent Fibre (%)	Acid Detergent Fibre (%)	Ash (%)	References
Cotinis ntida	51.75	5.41	19.3	NA	NA	12.34	Rakashantong *et al*., 2010
Hymenoptera							
Oecophylla smaragdina	53.46	13.46	15.38	NA	NA	6.55	Rakashantong *et al*., 2010
Orthoptera							
Acheta domesticus	66.56	22.08	NA	22.08	10.39	3.57	Finke, 2002; Bernard *et al*., 1997
Microcentrum rhombifolium (A)	77.80	9.00	NA	41.14	19.39	9.10	Oonincx & Dierenfeld, 2012
Anurogryllus arboreus	48.69	20.60	11.61	NA	NA	9.36	Rakashantong *et al*., 2010
Diptera							
H. illucens	45.10	36.08	NA	9.79	7.73	9.02	Finke, 2012
Musca domestica (L)	78.17	7.50	NA	14.29	11.51	6.75	Finke, 2012
D. melanogaster (A)	68.00	19.00	NA	17.66	10.14	7.20	Oonincx & Dierenfeld, 2012; Barker *et al*., 1998
Blattodea							
Blatta Lateralis	61.50	32.40	NA	9.06	7.12	3.90	Finke, 2012
B. lateralis (S)	76.05	14.45	NA	11.41	10.87	7.88	Oonincx & Dierenfeld, 2012
B. Lateralis (M)	62.85	26.50	NA	12.76	12.75	6.89	Oonincx & Dierenfeld, 2012

	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Neutral Detergent Fibre (%)	Acid Detergent Fibre (%)	Ash (%)	References
Eublaberus distanti	52.10	43.10	NA	NA	NA	2.98	Oonincx & Dierenfeld, 2012
Gromphadorhina portentosa	63.35	20.30	NA	36.54	13.12	8.49	Oonincx & Dierenfeld, 2012
Periplaneta americana	53.90	28.40	NA	NA	9.40	3.30	Bernard *et al*., 1997

A – Adullt, M – medium, S- small, L- larvae, P- pupae, PP- pre-pupae, NA- not applica

Table 2.3 Amino acid composition (g/100g) dry matter of some insect species and fish meal.

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Ala	Reference
Lepidoptera																			
Samia ricinii	4.40	2.7	1.4	6.6	6.5	2.3	5.2	4.8	NA	5.4	9.9	0.50	13.0	4.9	6.5	5.3	6.4	6.1	Longvah *et al*. (2011)
Bombyx mori	6.80	2.5	5.7	8.3	6.5	4.6	5.1	5.4	0.9	5.6	11.0	1.40	15.0	4.6	4.0	4.7	5.4	5.5	Rao (1994)
Cossus redtenbachi	6.00	1.6	5.1	7.9	4.9	2.1	9.3	4.7	0.6	6.1	11.0	1.30	17.0	5.5	5.5	5.9	6.2	6.5	Ramos-Elorduy *et al*. (1982)
Coleoptera																			
Scyphophorous acupunctatus	4.40	1.5	4.8	7.8	5.5	2.0	4.6	4.0	0.8	6.2	9.1	2.20	16.0	6.1	5.4	6.6	6.4	6.5	Ramos-Elorduy *et al*. (1997)
Zophobos morio	2.30	1.4	2.2	4.5	2.4	0.5	1.6	1.9	0.4	2.4	3.8	0.35	5.7	2.3	2.6	2.2	3.3	3.4	Finke (2002)
Tenebrio molitor	2.70	1.5	2.5	5.2	2.7	0.6	1.7	2.0	0.4	2.9	4.0	0.40	5.5	2.7	3.4	2.5	3.6	4.0	
Hymenoptera																			
Vespa basalis	1.70	1.1	2.6	3.5	1.9	0.9	1.9	1.8	NA	2.6	3.4	ND	7.5	3.6	3.7	1.9	2.5	3.4	Ying *et al*. (2010)
Polistes sagittarius	1.60	1.1	2.0	2.8	1.6	0.5	1.8	1.5	NA	2.4	3.0	ND	6.2	2.5	3.2	1.6	1.8	2.6	
Orthoptera																			
Boopedon flaviventris	4.30	2.4	4.7	8.8	5.5	1.8	4.1	4.4	0.6	5.7	8.8	2.0	15.0	7.5	6.8	4.3	7.4	5.9	Ramos-Elorduy *et al*. (1997)
Gryllus testaceus	3.70	1.9	3.1	5.5	4.8	1.9	2.9	2.8	NA	4.4	6.3	1.0	9.1	3.6	4.5	3.7	3.9	5.6	Wang *et al.* (2005)
Sphenarium histrio	6.60	1.1	5.3	8.7	5.7	2.0	12.0	4.0	0.6	5.1	9.3	1.3	4.3	5.3	7.2	5.1	7.3	7.7	Ramos-Elorduy & Pino (1982)

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Ala	Reference
Callipogon barbatum	5.90	2.2	5.8	10.0	5.7	2.0	4.7	4.0	0.7	7.0	9.1	2.0	10.0	9.2	6.2	3.7	4.2	8.0	Ramos-Elorduy *et al*. (2006)
Diptera																			
Musca domestica	5.2.	2.90	4.4	7.8	7.3	4.60	13.0	4.4	0.60	5.1	11.1	2.40	13.0	5.8	4.8	3.7	7.0	6.5	Ramos-Elorduy & Pino (1982)
Ephydra hians	2.7.	1.00	5.0	8.0	5.8	3.80	10.0	4.6	0.40	5.6	11.0	2.20	16.0	4.9	6.5	3.8	5.1	12.0	
Hermetia illucens	3.17	1.52	2.0	3.1	3.1	0.87	2.0	1.8	0.77	3.3	4.3	0.25	5.1	2.3	2.6	1.8	3.1	3.1	Finke (2012)
Blattodea																			
Blatta lateralis	4.5.	1.80	2.5	3.9	4.2	1.10	2.5	2.5	0.55	3.9	4.9	0.45	7.4	3.9	3.6	2.7	4.5	5.5	Finke (2012)
Fishmeal	6.14	3.60	4.8	7.8	7.9	2.50	4.1	4.4	1.00	5.2	9.0	1.00	13.0	6.2	4.5	4.0	3.2	6.3	Lall & Anderson (2005)

NA- not applicable, ND- not detected

Table 2.4 Comparison of the ideal amino acid profiles of humans, pigs, broilers and Nile tilapia with that of three Diptera species (Black soldier fly, House fly and Alkali fly).

	IAAP humans (FAO/WHO/UNU 2007, 1)	IAAP pigs (NRC 1998)	IAAP broilers (NRC 1994)	IAAP Nile Tilapia (NRC 1993)	Black soldier fly larvae (Newton *et al*., 2005)	House fly Ramos-Elorduy & Pino (1982)	Alkali fly Pino (1982)
Lysine	100	100	100	100	100	100	100
Methionine	33	27	38	52	36	63	66
Threonine	50	64	74	73	64	60	79
Leucine	130	100	109	66	118	107	138
Isoleucine	67	54	73	61	68	60	86
Valine	87	68	82		101	70	97
Histidine	33	32	32	34	43	40	17
Arginine		38	110	82	80	71	47

Table 2.5 Comparison of the amino acid profile of mopane worms to the ideal amino acid profile for broilers and humans (values calculated as a percentage of lysine)

	Threonine (%)	Valine (%)	Isoleucine (%)	Methionine (%)
Mopane worm[1]	65	80	70	38
Ideal amino acid profile for broilers[2]	98	87	64	32
Ideal amino acid profile for humans[3]	50	87	67	50

([1]) Ohiokpehai *et al*. (1996), ([2]) Schutte & de Jong (2004), ([3]) World Health Organization (2007)

The mineral content of some insect species are shown in **Error! Not a valid bookmark self-reference.**. While some insect species are low in calcium (Ca), insects are good sources of Copper (Cu), Iron (Fe) and Zinc (Zn) (Oliveira *et al*,. 1976). Glew *et al*. (1999) also concluded that mopane worms contain minerals such as magnesium, calcium, zinc and manganese. No literature regarding the minerals of CC in animal nutrition was found.

Table 2.6 Selected mineral content of various insect species

	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Reference
Lepidoptera								
Galleria mellonella	0.6	0.9	12.0	3.06	77.27	3.28	77.78	Barker *et al*. (1998)
Bombyx mori	1.0	3.0	14.0	20.81	95.38	24.86	177.46	Finke (2002)
Chilecomadia moorei	0.3	0.7	5.7	7.40	35.18	1.78	89.70	Finke (2012)
Coleoptera								
Tenebrio molitor	1.2	2.8	14.2	17.77	39.70	6.79	131.02	Barker *et al*.
Zophobas morio	1.2	1.8	8.3	13.94	50.34	1.54	87.50	(1998)
Rhynchophorous phoenicis	2.1	1.3	6.9	16.00	158.00	35.00	158.00	Rumpold & Schlüter (2013)
Orthoptera								
Acheta domestica	2.1	0.8	7.8	8.50	112.33	29.65	186.36	Barker *et al*. (1998)
Diptera								
Hermetia illucens	24.0	4.5	9.2	10.39	171.65	159.28	144.85	Finke (2012)
Drosophila melanogaster	1.7	1.7	13.2	16.00	400.50	16.50	223.00	Oonincx & Dierenfeld (2011)
Blattodea								
Blatta Lateralis	1.2	0.8	5.7	25.66	47.89	8.54	105.83	Finke (2012)
Gromphadorhina portentosa	2.5	2.4	9.3	22.50	153.50	10.00	202.00	Oonincx & Dierenfeld (2011)
Eublaberus distanti	0.8	0.8	4.6	12.00	55.00	5.00	124.00	Oonincx & Dierenfeld (2011)

2.4.2 Production parameters, growth performance and feed intake

When broilers received a diet with 10 or 15% house fly larvae meal, Hwangbo *et al*. (2009) concluded that live weight gain was significantly higher than the soya oil cake control groups at five weeks from the start of the trail. Feed conversion ratio (FCR) and feed intake did not differ significantly from the control diet (P>0.05). When feeding a ratio with 10% house fly larvae meal, Pretorius (2011) reported significantly higher live weights, feed intake and average daily gain (ADG) for broilers than the soya oil cake control diet.

No significant effect (P>0.05) on weight gain and FCR were observed by Awoniyi *et al*. (2003), Adeniji (2007), Aniebo *et al*. (2008) and Téguia *et al*. (2002) when broiler diets were supplemented with *M. domestica* larvae meal. Adeniji (2007) reported that the inclusion of *M. domestica* larvae meal in broiler diets had no significant effect on feed intake. Hwangbo *et al*. (2009) reported similar results. When fish meal was replaced by *M. domestica* larvae meal at various different levels, no significant differences (P>0.05) were found by Awoniyi *et al*. (2003).

When comparing a diet with *M. domestica* pupae meal as the only protein source with a diet containing soybean oil cake as the main protein source, Calvert *et al*. (1971) found significant better weight gain in broilers from day one to day 14 fed the pupae meal. When these two diets were fed from day seven to 14, no significant differences were found. This pattern is in line with the findings of Hwangbo *et al*. (2009). Teotia & Miller (1974) found no significant differences (P>0.05) in weight gain, FCR and feed intake when feeding a diet with *M. domestica* pupae meal to white leghorn chicks from day one to day 28.

When Uushona (2016) compared the production parameters of broilers fed a soya based control diet with that of broilers receiving diets with black soldier fly pre-pupae meal at rates of 5%, 10% and 15%, no significant differences (P>0.05) were found regarding ADG, FCR, EPEF or liveability. Uushona (2016) however concluded that 5% inclusion level had a lower (P≤0.05) PER than the other diets in her study.

2.4.3 Carcass characteristics and meat quality

Hwangbo *et al*. (2009) reported significantly better (P≤0.05) carcass characteristics when *M. domestica* larvae meal were included in broiler diets. Dressing Percentage, thigh muscle weight and breast muscle weight as a percentage of carcass weight were significantly higher. This is in contrast with Awoniyi *et al*. (2003) and Téguia *et al*. (2002) who found no significant influence of *M. domestica* larvae meal on breast muscle weight and dressing percentage. The differences can possibly be attributed to the fact that Téguia *et al*. (2002) and Awoniyi *et al*. (2003) had four and six replicates per treatment respectively, while Hwangbo *et al*. (2009) had 30 replicates per treatment. No significant differences in abdominal fat were observed by Téguia *et al*. (2002) when broilers that received the different ratios were evaluated.

When Uushona (2016) compared the carcass characteristics of broilers that received diets with black soldier fly pre-pupae meal at rates of 0%, 5%, 10%, and 15%, no differences were observed regarding live slaughter weight, cold carcass weight, dressing percentage or carcass portion yields (breast, thigh, drumstick, wing and back). Uushona (2016) also did not observe significant differences in the initial and ultimate pH of the breast or thigh or in the colour of the meat (L*, a*, b*).

Hwangbo *et al*. (2009) reported no significant differences in meat colour when comparing a soya bean meal control diet with treatment diets containing 5%, 10%, 15% and 20% house fly larvae meal.

2.4.4 Organ, gut and bone parameters

For the poultry industry all over the world, gizzard erosion is a huge problem (Johnson, 1971). High mortalities, low feed intake and listlessness characterizes this phenomenon (Itakura *et al*., 1981). Gizzard erosion can by diagnosed *post mortem* by the presence of a black watery content in the crop, proventriculus and gizzard, with the lining of the gizzard eroded and ulcers on the gizzard muscles (Johnson, 1971). Gizzard erosion can be caused by a number of dietary factors which include minerals such as copper (Fisher *et al*,. 1973; Ross, 1979), form of the diet (pellet vs. mash)(Ross, 1979), presence of certain bacteria (Ferencik, 1970), toxins like gizzerozine (Okazaki *et al*., 1983), the presence of mycotoxins (Hoerr *et al*., 1982; Dorner *et al*., 1983; Diaz & Sugahara, 1995) or stress (Grabarevic *et al*., 1993; Dzaja *et al*., 1996).

Although copper sulphate is used in broiler diets as a growth promoter, Fisher *et al*. (1973) concluded that gizzard erosion was related to the copper concentration in the diet. Chicks fed pelleted feed had more gizzard erosion than those fed mash feed (Ross, 1979). The author believed the method of pelleting induced gizzard erosion. Bacteria that occur naturally on fish meal cause the formation of histamine by the decarboxylation of histidine in fish meal (Ferencik, 1970). Gizzerosine is formed when histamine or histidine reacts with lysine during overheating of fish meal in the processing of fish meal (Okazaki *et al*., 1983). Excessive secretions of hydrochloric acid and pepsin are secreted in the stomach when gizzerosine is present, causing gizzard erosion (Masumura *et al*., 1985). The presence of mycotoxins in broiler diets also causes gizzard erosion (Hoerr *et al*., 1982; Dorner *et al*., 1983; Diaz & Sugahara, 1995). When broiler chicks are exposed to stressful environments, more gizzard erosion occur (Grabarevic *et al*., 1993; Dzaja *et al*., 1996). The levels of aspartate aminotransferase and creatine kinase in the proventriculus are increased by stress in broilers chicks (Dzaja *et al*., 1996). This lead to lower pH in the stomach and gizzard erosion.

There is limited literature available regarding toxic effects of insect meals in broiler nutrition. Teguia *et al*. (2002) found no significant differences ($P \leq 0.05$) in the weights of the liver, gizzard and hearts of broilers when replacing 50% and 100% of the fish meal in the diet with house fly larvae meal. Pretorius (2011) reported no significant differences ($P \leq 0.05$) in the weights of the liver, gizzard and heart relative to chick weight even when 50% of the diet was house fly larvae meal. Uushona (2016) reported no significant differences ($P \leq 0.05$) in the weights of gizzard, liver, heart, bursa, and spleen when supplementing a soya based diet with black soldier fly pre-pupae at rates of 5%, 10% and 15%.

Pretorius (2011) concluded that neither house fly larvae meal nor pupae meal caused gizzard erosion when dried at 45°C, 65°C and 85°C. When Uushona (2016) included black soldier fly pre-pupae in broiler diets at rates of 0%, 5%, 10% and 15%, no differences ($P > 0.05$) were observed. Uushona (2016) concluded that gizzard erosion scores of the broilers fed the BSF pre-pupae did not exceed two and can be classified as acceptable gizzard erosion scores.

This result is similar to results of studies where house fly larvae meal (Teguia *et al*., 2002) were used in broiler diets. In contrast, Okah & Onwujiariri (2012) reported lower heart weights and higher gizzard weights in broilers fed house fly larvae meal, while treatment did not affect

liver weights. The broilers used in the study by Okah & Onwujiariri (2012) were older than 35 days, and this might have influenced their results.

When BSF pre-pupae was included in broiler diets at rates of 0%, 5%, 10% and 15%, no differences (P>0.05) were found regarding the pH of the duodenum, jejunum or ileum (Uushona, 2016). The normal gut pH range in healthy poultry are 5.5-6.2 in the duodenum, 5.8-6.9 in the jejunum and 6.3-8.0 in the ileum (Van der Klis & Jansman, 2002).

When Uushona (2016) evaluated the mineral content of the tibia bones of broilers fed BSF pre-pupae meal at rates of 0%, 5%, 10% and 15% she observed that the 15% inclusion had a higher calcium (Ca) content than the 5% inclusion, while the control and the 10% inclusion were intermediate. Black soldier fly pre-pupae contains high P and Ca contents (Newton *et al*., 2005), but the diets in Uushona's (2016) study were formulated for similar Ca levels. The higher Ca content in the tibia bones of the 15% BSM pre-pupae inclusion may indicate higher bioavailability of Ca in it (Uushona, 2016).

2.5 Conclusion

The fast-growing world human population requires an increase of protein for human consumption (meat). Broiler meat could fulfill that need because of its high growth rate and good feed conversion ratio. The need for more broiler meet pose another problem for the farmers; more nutrients will be needed to produce more broiler meat. Fish meal and soya meal are the most used protein sources in broiler nutrition. With the growing demand for these products it has become very expensive.

The production of fish meal is not sustainable, and this could leave the biodiversity of the ocean out of balance. The demand for more soya could also impact nature negatively, as more land would be needed for soya production.

In many studies insect meals proved to be suitable protein sources for animal nutrition. The production of CC larvae meal could be another sustainable insect protein source for animal production. At the same time abattoir waste could be reduced by CC, making it both sustainable and beneficial to the environment.

Because no literature on CC larvae meal in animal nutrition was available, a study was conducted to evaluate the possibility of using CC larvae meal as a protein source in broiler nutrition.

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Chapter 3: **Comparison of the Production Parameters of broilers grown on a diet containing either *Chrysomya chloropyga* or soya as the main protein source**

3.1 Abstract

This study evaluated the possibility of using larvae of the copper bottom blow fly (*Chrysomya chloropyga*) (CC) as a protein source in broiler nutrition. Proximate and amino acid analyses of the larvae were used for formulation of iso-nitrogenous and iso-energetic diets with 0%, 5%, 10% and 15% CC larvae meal. The effects of the larvae meal in the ratios were tested on three hundred and twenty broiler chickens. A three phase feeding system were used i.e. starter (900g per bird), grower (1200g per bird) and finisher (1200g per bird). Each treatment was replicated eight times. Results of the proximate and amino acid analyses showed that copper bottom blow fly larvae are suitable to use as a protein source in broiler nutrition. The 10% inclusion rate increased rate of gain, live weight and European production efficiency factor when compared to the soya based control diet. No differences ($P>0.05$) were found between the four treatment diets for cumulative weekly intake per bird, average weekly intake per bird, average weekly gain per bird, feed conversion efficiency or acceptability. It is thus concluded that the larvae of the copper bottom blow fly is a viable protein source for broiler production.

Keywords- Larvae meal, copper bottom blow fly, broiler, poultry

3.2 Introduction

Protein and the quality of protein is very important in broiler nutrition. The amino acid composition of protein sources can have a great influence on broiler performance. Conventional protein sources such as fish meal and soya meal are expensive (Agunbiade *et al*., 2007) and not always sustainable. Overfishing and deforestation (to clear land for planting of soya) have negative impacts on the environment. Over the years agriculture has had a negative impact on the world's ecosystems. Agriculture is also damaging the earth's biodiversity, causing certain insect species to multiply and others to be eliminated

(Premalatha *et al*., 2011). Deforestation also cause floods to occur more frequently; Bradshaw *et al*., (2007) found a positive correlation between flood occurrence and non-natural forest cover. However, flood occurrences decrease when natural forest cover is present.

Alternative protein sources therefore need to be investigated to supplement conventional protein sources in a world where there is not enough sustainable protein sources to feed a growing human population. Such sustainable protein sources for animal nutrition can possibly be supplied by the mass rearing of insects, and in this case *C. chloropyga* (copper bottom blow fly). Although no studies have been reported on using CC meal in broiler rations, studies on the complete or partial replacement of conventional protein sources in broiler diets with insect meals or larvae meal have been reported. Insects that have been evaluated include *Musca domestica* (House fly) (Zuidhof *et al*., 2003; Aniebo *et al*., 2008; Pretorius, 2011; Pieterse & Pretorius, 2013), *Hermetia illucens* (Black Soldier fly) (Hale, 1973; Newton *et al*., 1977; Bondari & Sheppard, 1987; Kroeckel *et al*., 2012), *Tenebrio molitor* (Mealworm) (Klasing *et al*., 2000; Ng *et al*., 2001; Ramos-Elorduy *et al*., 2002; Hopley, 2016), *Zophobas morio* (Giant Mealworm) (Barker *et al*., 1998; Jabir *et al*., 2012; Finke, 2013), *Acrida cinerea* (Grasshopper) (Wang *et al*., 2007), *Cirina forda* (Pallid Emperor Moth) (Oyegoke *et al*., 2006) and *Bombyx mori* (Silkworm) (Ijaiya & Eko, 2009).

Some of this studies include the replacement of fish meal (Téguia *et al*., 2002; Awoniyi *et al*., 2003; Ogunji *et al*., 2006; Agunbiade *et al*., 2007; Pretorius, 2011), groundnut meal (Adeniji, 2007) and soya bean oil cake meal (Hwangbo *et al*., 2009; Pretorius, 2011) with common house fly (*M. domestica*) larvae meal.

The mass production of CC can possibly have a positive effect on commercial broiler production, either by lowering the cost to rear a broiler, or by enhancing the productivity on such farms. The supplementation of other protein sources with CC larvae meal can also have a positive impact on the environment, as CC production is sustainable and can utilize abattoir waste to produce protein for animal feed.

By supplementing conventional protein sources with CC larvae meal, less pressure is put on soya production and fishing while decreasing the negative effect of food production waste.

This study was conducted to investigate the value of CC larvae meal as a protein source in broiler feeds.

3.3 Materials and Methods

3.3.1 Larvae production and treatment

Larvae used in this study were produced and treated on the Mariendahl Experimental farm of the University of Stellenbosch. Abattoir waste were transported in a sealed container from Winelands pork, a commercial pig abattoir in Cape Town to Mariendahl, before being fed to the larvae.

The copper bottom blow fly (*C. chloropyga*) (CC) eggs were collected from the breeding colony (maintained on the experimental farm) daily, and placed on abattoir waste prior to hatching. The eggs hatched within three hours. Larvae were allowed to grow until crawl off which was around the sixth day. After crawling off, the larvae were collected and killed by freezing at -20°C prior to drying in an oven at 60°C for 24h. After drying the larvae were milled and stored in the freezer at -20°C until the feed was mixed.

3.3.2 Animals and housing

Three hundred and twenty day-old Cobb 500 chicks were used in this study. The broilers were housed at Stellenbosch University, Mariendahl experimental farm, Stellenbosch. The unit at the poultry section is temperature controlled and equipped with 120 wire cages. The cages measure 0.9m x 0.6m, and each cage has two nipple drinkers and a tube feeder. Broilers were stocked 10 chicks per cage, with a density of 18.5 chicks/m^2. Thirty two cages were used in this study. From day one till day four the broiler chicks were fed a commercial starter diet to allow adaptation to the new environment. At four days of age, broilers were placed in the cages and remained there until day 35 when they were slaughtered. A randomized block design was used. Lighting and temperature were set according to the Cobb 500 management guide (Cobb-Vantress, 2012). Ventilation were set to provide a minimum of six air changes per minute. The broilers had *ad libitum* access to water and feed.

Ethical clearance was obtained from Stellenbosch University, with ethical clearance number SU-ACUM13-00026.

3.3.3 Experimental diets

Four different ratios of CC were fed to the broilers. The control contained 0% CC larvae meal, with soya as the main protein source. The three treatment diets contained 5%, 10% and 15% CC larvae meal respectively.Table 3.1, Table 3.3 and Table 3.4 show the four diets fed to the chickens. The treatments were formulated to meet the minimum requirement of the Cobb 500 broiler chick (Cobb-Vanress, 2012). Amino acid composition were formulated according to the ideal amino acid requirements for broilers (NRC, 1944).

Table 3.1 Dietary treatments containing 0%, 5% 10% or 15% *Chrysomya chloropyga* (copper bottom blow fly) (CC) larvae meal.

Diet	Inclusion level	Description
Control	0%	Standard maize soya based diet
CC larvae	5%	Standard maize soya based diet with 5%(CC)
CC larvae	10%	Standard maize soya based diet with 10% CC
CC larvae	15%	Standard maize soya based diet with 15% CC

Table 3.2 Formulated ingredient (%) and calculated nutrient composition of broiler starter diets containing copper bottom blowfly (*Chrysomya chloropyga*) *(*CC) larvae

		Control	5% CC	10% CC	15% CC
Formulated ingredient composition					
CC	%		5.000	10.000	15.000
Fine Yellow maize	%	50.091	50.330	49.642	56.936
Full fat soya bean meal	%	15.392	15.130	15.865	11.240
Soya oilcake meal (50% CP)	%	30.138	25.396	20.068	12.026
L-lysine HCl	%	0.136	0.177	0.214	0.471
DL methionine	%	0.125	0.107	0.090	0.123
Vitamin and mineral premix	%	0.150	0.150	0.150	0.150
Limestone	%	1.660	1.660	1.657	1.702
Salt	%	0.129	0.219	0.114	0.064
Monocalcium phosphate	%	1.639	1.639	1.639	1.649
Sodium bicarbonate	%	0.540	0.193	0.561	0.640
Sunflower oil	%				
Calculated ingredient composition					
AMEn_chick	MJ/kg	11.900	11.921	11.900	11.900
Crude protein	%	20.691	20.247	19.882	16.425
Lysine	%	1.525	1.521	1.519	1.497
Methionine	%	0.496	0.498	0.499	0.518
Cysteine	%	0.419	0.420	0.420	0.390
Methionine+cysteine	%	0.915	0.917	0.919	0.909
Threonine	%	0.968	0.968	0.968	0.861
Tryptophan	%	0.303	0.303	0.304	0.266
Arginine	%	1.728	1.728	1.731	1.518
Isoleucine	%	1.161	1.118	1.076	0.895
Leucine	%	2.162	2.086	2.006	1.758
Crude fibre	%	3.003	3.084	3.178	3.058
Crude fat	%	5.203	6.000	6.937	7.146
Calcium	%	1.000	1.000	1.000	1.000
Available phosphorous	%	0.500	0.500	0.500	0.500
Sodium	%	0.220	0.160	0.220	0.220
Chloride	%	0.160	0.220	0.160	0.160

AMEn – Apparent metabolisable energy, CP – Crude protein

Table 3.3 Formulated ingredient (%) and calculated nutrient composition of broiler grower diets containing copper bottom blowfly (CC) larvae

		Control	5%CC	10%CC	15%CC
Formulated ingredient composition					
CC	%		5.000	10.000	15.000
Fine Yellow maize	%	45.682	47.108	48.533	49.959
Full fat soya bean meal	%	44.172	39.086	34.000	28.914
Soya oilcake meal (50% CP)	%	6.147	4.764	3.380	1.996
L-lysine HCl	%	0.045	0.095	0.145	0.195
DL methionine	%	0.145	0.123	0.101	0.079
Vitamin and mineral premix	%	0.150	0.150	0.150	0.150
Limestone	%	1.632	1.641	1.649	1.658
Salt	%	0.275	0.260	0.246	0.231
Monocalcium phosphate	%	1.632	1.634	1.636	1.638
Sodium bicarbonate	%	0.118	0.139	0.160	0.180
Calculated nutrient composition					
AMEn_chick	MJ/kg	12.901	12.745	12.590	12.434
Crude protein	%	19.533	18.953	18.372	17.792
Lysine	%	1.394	1.389	1.383	1.378
Methionine	%	0.492	0.489	0.487	0.484
Cysteine	%	0.396	0.397	0.398	0.400
Methionine+cysteine	%	0.888	0.887	0.885	0.884
Threonine	%	0.915	0.912	0.909	0.907
Tryptophan	%	0.280	0.280	0.281	0.281
Arginine	%	1.627	1.622	1.616	1.611
Isoleucine	%	1.078	1.034	0.991	0.947
Leucine	%	2.019	1.947	1.875	1.802
Crude fibre	%	3.650	3.608	3.567	3.526
Crude fat	%	10.000	10.000	10.000	10.000
Calcium	%	1.000	1.000	1.000	1.000
Phosphorous	%	0.832	0.831	0.829	0.828
Available phosphorous	%	0.500	0.500	0.500	0.500
Sodium	%	0.160	0.160	0.160	0.160
Chloride	%	0.220	0.220	0.220	0.220

AMEn – Apparent metabolisable energy, CP – Crude protein

Table 3.4 Formulated ingredient (%) and calculated nutrient composition of broiler finisher diets containing copper bottom blowfly (CC) larvae

		Control	5%CC	10%CC	15%CC
Formulated ingredient composition					
CC[1]	%		5.000	10.000	15.000
Fine Yellow maize[2]	%	47.164	47.099	47.035	47.837
Full fat soya bean meal[3]	%	48.887	43.987	39.088	33.286
L-lysine HCl	%				0.022
DL methionine	%	0.134	0.099	0.063	0.032
Vitamin and mineral premix	%	0.150	0.150	0.150	0.150
Limestone	%	1.640	1.640	1.639	1.644
Salt	%	0.290	0.284	0.279	0.268
Monocalcium phosphate	%	1.634	1.634	1.634	1.634
Sodium bicarbonate	%	0.100	0.106	0.113	0.126
Calculated nutrient composition					
AMEn_chick	MJ/kg	13.128	12.944	12.760	12.564
Crude protein	%	18.219	18.334	18.448	18.251
Lysine	%	1.286	1.285	1.284	1.281
Methionine	%	0.465	0.458	0.450	0.444
Cysteine	%	0.382	0.390	0.399	0.404
Methionine+cysteine	%	0.848	0.848	0.849	0.848
Threonine	%	0.870	0.892	0.914	0.926
Tryptophan	%	0.263	0.272	0.282	0.287
Arginine	%	1.538	1.582	1.627	1.650
Isoleucine	%	1.017	1.006	0.995	0.970
Leucine	%	1.936	1.905	1.874	1.827
Crude fibre	%	3.726	3.711	3.696	3.650
Crude fat	%	10.836	10.835	10.834	10.698
Calcium	%	1.000	1.000	1.000	1.000
Phosphorous	%	0.824	0.828	0.832	0.833
Available phosphorous	%	0.500	0.500	0.500	0.500
Sodium	%	0.160	0.160	0.160	0.160
Chloride	%	0.220	0.220	0.220	0.220

AMEn – Apparent metabolisable energy, CP – Crude protein

3.3.4 Data collection and analysis

Total body weights of all birds per cage were measured on day four (at placement) and weekly thereafter. The last weight measured was at day 32 before slaughtering. Cage weights were used to determine average live weights. To determine weekly intake, the initial feed weight were added to the additional feed supplied during that week, whilst weekly refusals were subtracted. Mortalities were recorded daily.

This data was used to determine feed conversion ratio (FCR), average daily gain (ADG), protein efficiency ratio (PER) (Boling-Frankenbach *et al*., 2001) and the European production efficiency factor (EPEF)(Awad *et al*., 2009). Average daily gain was estimated by fitting a linear model to the live weight data with the slope representing the rate of change and therefore average

daily gain (ADG). The formulae used to calculate these production parameters are showed in Equation 1, Equation 2and Equation 3.

Equation 1

$$\text{Feed Conversion Ratio} = \frac{\text{Cumulative Feed Intake (g)}}{\text{Average Live Weight per Chick (g)}}$$

Equation 2

$$\text{Protein Efficiency Ratio} = \frac{\text{Weight Gain (g)}}{(\text{Weekly Feed Intake (g)} \times \text{Protein \% of Diet})/100}$$

Equation 3

$$\text{European Production Efficiency Factor} = \frac{\text{Liveability \%} \times \text{Live Weight (g)}}{\text{Age (days)} \times \text{FCR}} \times \frac{100}{1}$$

On day 32, two birds per pen of the middle weight group were randomly selected and their weight recorded. Thereafter these birds were slaughtered using standard commercial practices; electrical stunning followed by exsanguination.

3.3.5 Analytical and mathematical methodologies

Larvae were submitted for chemical analyses before the trial began. Larvae were grown over a period of seven days for the trial. Each batch was sampled and these samples were mixed and pooled before a subsample was taken for analysis. Dry matter (DM) of the larvae was determined according to the Official Method 934.01 of the AOAC (Feldsine *et al*., 2002). The subsamples used to determine DM were thereafter used to determine the ash content of larvae meal (Official Method 942.05, Feldsine *et al*., 2002). Crude fibre (CF) was determined using Official Method 962.09 (Feldsine *et al*., 2002). Ether extract (EE) was determined using the Foss Tecator Soxtec HT 1043 Extraction Unit (Höganäs, Sweden) while total fat was determined using an acid hydrolysis (method 954.02 AOAC International 2000). Crude protein (CP) were determined by measuring the total nitrogen content of the larvae meal, according to Official Method 4.2.07 (Feldsine *et al*., 2002) in a LECO FP 528 Protein / Nitrogen Determinator and multiplying the nitrogen value by 6.25. Amino acids (excluding tryptophan and cysteine) were determined using the method described by Cunico *et al*., (1986). The

samples were prepared through hydrolysis and then the total amino acid profile was determined through pre-column derivatisation of the amino acids, which were then separated using high performance liquid chromatography. This procedure was completed by the detection of the amino acids using a fluorescence detector. Mineral composition was determined using the combustion method as described by Method no. 6.1.1 for feeds and plants of the Agriculture Laboratory Association of Southern Africa (AgriLASA) (2007). The concentrations of P, K, Ca, Mg, Na, Cu, Fe, Al, Zn and B were determined.

3.3.6 Statistical analysis

The study consisted of a randomised design with four treatments; larvae meal inclusion levels of 0%, 5%, 10% and 15%. Data was subjected to an analysis of variance (ANOVA) utilising SAS Statistical software (Version 9.2) (SAS, 2009). The Shapiro-Wilk test was performed to test for normality (Shapiro; Wilk, 1965). All of the outliers were identified and removed before final analysis of the ANOVA's. Differences were deemed to be significant at $P \leq 0.05$.

3.4 Results and Discussion

The nutritional composition of dried, ground CC larvae is depicted in

Table 3.5. When the amino acid (AA) composition is compared with the ideal amino acid requirements of pigs, broilers, Nile tilapia and humans (

Table 3.6), it can be seen that it meets or exceeds the amino acid requirements of broilers (NRC. 1994), Nile tilapia (National Research Council (US). Committee on Animal Nutrition, 1993), and pigs (NCR, 1998). Except for leucine, it also meets the amino acid requirements of humans (Joint WHO/FAO/UNU Expert Consultation, 2007).

A comparison of the proximate analysis between insect meals and that of fish and soya bean meal (Table 3.7) indicates that CC has a much higher crude fat content than fish meal and soya oil cake meal, but is lower in crude protein. This is because fish meal and soya oil cake meal are byproducts and the most of the crude fat content were removed, leaving a product with lower crude fat and higher crude protein to use in animal nutrition. The partial removal of crude fat of CC will also increase the crude protein content of CC, making it more comparable with fish meal and soya oil cake meal. The partial removal of crude fat in CC will also allow easier and longer storage before the product goes rancid.

The proximate analysis of the different species of insects differ possibly due to species, diets of the insects and stage of harvesting.

Table 3.5 Analysed nutritional composition on an as is basis of larvae of the copper bottom blowfly (*Chrysomya chloropyga*) (CC)

	CC larvae	
Proximate composition (%)		
Moisture		63.81
	As is	**DM**
Ash	11.65	32.20
Crude protein (CP)	13.21	48.01
Crude fibre (CF)	2.23	6.16
Crude fat (EE)	6.90	19.07
Amino acid composition (g/kg CP)		
Arginine		3.60
Serine		1.92
Aspartic acid		0.99
Glutamic acid		1.96
Glycine		2.08
Threonine		1.90
Alanine		1.69
Tyrosine		1.96
Proline		1.89
HO-Proline		0.02
Methionine		1.04
Valine		1.95
Phenylalanine		2.80
Isoleucine		1.55
Leucine		2.21
Histidine		1.43
Lysine		2.32

Table 3.6 Comparison of the ideal amino acid requirements of humans, pigs, broilers and Nile tilapia with that of the copper bottom blow fly (CC) (*Chrysomya chloropyga*) larvae

	IAAP[1] humans (FAO/WHO/ UNU. 2007)	IAAP pigs (NCR. 1998)	IAAP broilers (NRC. 1994)	IAAP Nile Tilapia (NRC. 1993)	CC larvae meal
Lysine	100	100	100	100	100
Methionine	33	27	38	52	45
Threonine	50	64	74	73	82
Leucine	130	100	109	66	95
Isoleucine	67	54	73	61	67
Valine	87	68	82		84
Histidine	33	32	32	34	62
Arginine		38	110	82	155

IAAP – Ideal amino acid profile

Table 3.7 Comparison of the nutritional value of insect meals with that of fish and soya bean meal

Parameters	**HFM[1,a]**	**BSM[2,b]**	**FC[3,c]**	**GH[4,d]**	**SWC[5,e]**	**FM[6,f]**	**SCM[7,f]**	**HFP[8,g]**	**CC[9]**
Proximate analysis (%)									
Crude protein	47.60	43.20	58.30	53.58	50.30	69.13	49.44	76.23	48.01
Crude fat	25.30	28.00	10.30	26.52	16.43	10.11	0.90	14.39	19.07
Crude fibre	7.50	-	8.70	9.21	10.90	0.54	7.87	15.71	6.16
Ash	6.25	16.60	2.96	4.31	12.03	-	5.90	7.73	32.20
Amino acids (%)									
Lysine	6.04	2.21	4.79	-	5.02	3.57	3.05	4.92	2.32
Methionine	2.28	0.83	1.93	-	3.02	1.09	0.70	1.37	1.04
Threonine	2.03	-1.41	2.75	-	4.50	1.47	1.95	2.31	1.90
Mineral content (%)									
Ca	-	5.36	-	-	1.05	1.34	0.33	0.52	-
P	-	0.88	-	-	2.77	1.77	0.73	1.72	-

[a] Aniebo *et al*. (2009)
[b] Newton *et al*. (2005)
[c] Wang *et al.* (2005)
[d] Hassan *et al*. (2009)
[e] Ijaiya & Eko (2009)
[f] NRC (2004)
[g] Pieterse & Pretorius (2014)

[1] HFM (Housefly maggot, blood & wheat meal)
[2] BSM (Black soldier fly pre-pupae meal dried)
[3] FC (Field cricket meal)
[4] GH (Grasshopper meal)
[5] SWC (Silkworm caterpillar)
[6] FM (Fish meal dehydrated)
[7] SCM (Soya oil cake meal)
[8] HFP (Housefly pupae meal dried)
[9] CC (Copper bottom blow fly larvae meal dried)

As pertaining to the broiler production data (Table 3.8), the live weights of the chicks at the start of the trail (day 4 of age) did not differ (P=0.24). It is therefore accepted that any

differences in live weights later in the trial are due to the dietary treatments. After one week, the live weights did not differ (P>0.05), but from 18 days of age until slaughter at day 35 the live weights (P≤0.05) differed. Live weighs at 18 days of age of the birds that were fed the 10% inclusion level were higher than that of the birds fed the control diet. The 5% inclusion level showed statistically similar live weights to the 10% inclusion, while the 15% inclusion level showed results similar to the control diet. At 25 days of age, the 10% inclusion level had higher live weights (P≤0.05) than the 15% and control diets. The 15% grower and finisher diets had slightly lower energy levels due to the fact that it was difficult to formulate a ration with 15% of full fat CC larvae meal. This could explain the lower growth rate in comparison with the 5% and 10% diets. The 5% inclusion level was intermediate and did not differ (P>0.05) from any of the other diets. On 35 days of age the 10% inclusion level still had the highest live weights, but did not differ (P>0.05) from the 5% and 15% inclusion levels. Although the control diet had the lowest live weights on these ages, it did not differ (P>0.05) from the 5% and 15% inclusions, but did differ from the 10% inclusion level (P≤0.05). Hwangbo *et al*., (2009) also measured higher live weights in broilers fed a 5% and 10% house fly larvae meal diet compared to a soya oil cake based control diet at five weeks into the trail, while Pretorius (2011) also reported higher live weights in broilers fed a 10% house fly larvae meal compared to a soya oil cake control diet. However no significant differences in live weights were observed by Uushona (2016) when black soldier fly pre-pupae were included in broiler diets at 5%, 10% and 15% compared to a soya based control diet.

The replacement (Teguia *et al*., 2002; Awoniyi *et al*., 2003) and partial replacement (Pretorius, 2011) of fishmeal with house fly larvae meal had no significant differences on live weight in broilers. Pretorius (2011) also noted higher mortalities in broilers fed high levels of fish meal. Although growth rates were low, Pretorius (2011) did not observe any mortalities in broilers fed a 50% house fly larvae meal inclusion diet. The low growth rate could be attributed to the oversupply of protein in the diet. Such high inclusion levels of protein leads to a high cost of deamination to the birds and subsequent energy shortage. The oversupply of protein also causes more nitrogen excretion via uric acid; a significant amount of energy is required for this process (Macleod, 1997). An estimated six molecules of adenosine triphosphate (ATP) are used to excrete one gram of Nitrogen (Macleod, 1997). Undigested proteins reaching the caeca also cause an inflammatory response, and this can reduce feed efficiency further (Collet,

2005). An imbalanced amino acid profile of the diet could also have attributed to the low growth rate reported by Pretorius (2011).

When a sorghum-soybean meal based diet was replaced by *T. molitor* at rates of 0%, 5% and 10% in broiler starter diets, Ramos-Elorduy *et al*., (2002) did not find significant differences in weight gain. Hopley (2016) also observed no significant differences in average live weight when a soya based control diet was compared to a 10% inclusion level op *T. molitor* in layer hens.

During the first two weeks of the trail there were no differences (P>0.05) in average feed intake per bird. During the third week the 10% inclusion level had a higher (P≤0.05) intake per bird than the 15% inclusion. Although the 5% inclusion level and the control had slightly lower intakes per bird than the 10%, and slightly higher intakes than the 15% inclusion level, no differences were found (P>0.05). During the last week, there were no differences (P>0.05) in average weekly intake per bird between the four treatments. Hwangbo *et al*. (2009) also reported no differences in feed intake when a 5% and 10% inclusion level of house fly larvae meal was compared to a soya based control diet, while Pretorius (2011) reported higher feed intakes in broilers fed the 10% inclusion of house fly larvae meal in comparison with a soya based control diet. When comparing black solder fly pre-pupae meal at inclusion levels of 5%, 10% or 15% with a soya based broiler diet, Uushona (2016) reported no differences for feed intake. The replacement of fishmeal with house fly larvae meal had no effect on feed intake (Awoniyi *et al*., 2003; Teguia *et al*., 2002).

When a sorghum-soybean meal based diet was replaced by *T. molitor* at rates of 0%, 5% and 10% in broiler starter diets, Ramos-Elorduy *et al*. (2002) did not find significant differences in feed intake. Hopley (2016) also observed no significant differences in weekly feed intake or cumulative feed intake when a soya based control diet was compared to a 10% inclusion level op *T. molitor* in layer hens. Ijaiya & Eko (2009) reported no significant differences in feed intake when fish meal was replaced by *A. infracta* at ratios of 0%, 25%, 50%, 75% and 100% in broiler diets containing 20% crude protein. At the end of the first week of the trail, the 5% inclusion level had a higher (P≤0.05) feed conversion ratio (FCR) than the control and 10% inclusion diets. There were no differences (P>0.05) between the 15% inclusion and the other diets regarding FCR in the first week of the trail. At the end of week two, the 10% inclusion lever

had a higher (P≤0.05) FCR than the control diet, but did not differ (P>0.05) from the 5% and 15% inclusion levels. The total FCR of the control, 5% and 15% inclusions did not differ (P>0.05) at the end of week two. At the end of week three, the 5% inclusion level had a significantly higher total FCR than the control diet, but did not differ significantly from the 5% and 15% diets. At the end of week four there were no differences (P>0.05) between the FCR's of the four diets. This was expected, as there were also no differences in FCR in the partial (Hwangbo *et al*., 2009) or complete (Pretorius, 2011) replacement of soya oil cake based diets with house fly larvae meal in broiler diets. Uushona (2016) also reported no differences in FCR when a soya based control diet was replaced by black soldier fly pre-pupae meal at 5%, 10% and 15% inclusion levels.

As pertaining to the growth rates, at the end of the second week the 10% inclusion level had a higher (P≤0.05) average weekly gain than the control and the 15% inclusion level, but did not differ (P>0.05) from the 5% inclusion diet. During week three the 10% inclusion diet had a higher (P≤0.05) average weekly gain than the 15% ratio, but did not differ (P>0.05) from the control and 5% diets. No differences (P>0.05) were observed between the control, 5% and 15% inclusion levels during this period. The 10% inclusion level had a higher (P≤0.05) average daily gain (ADG) than the control and 15% inclusion diets over the duration of the trail. The ADG did not differ (P>0.05) between the 10% and 5% inclusion diets. The ADG of the control, 5% and 15% inclusion levels also did not differ (P>0.05). When comparing a soya oil cake control diet with a 10% house fly larvae meal inclusion diet, Pretorius (2011) reported significant higher ADG in broilers fed the trail diet, while Uushona (2016) reported no differences in ADG when a 10% black soldier fly pre-pupae meal diet was compared to a soya based control diet.

No significant differences (P>0.05) were found between the liveability of the broilers fed any of the four ratio's. Liveability was constantly high (>96%). Uushona (2016) also found no differences in liveability when a soya oil cake based control diet was compared to black soldier fly diets at inclusions of 5%, 10% and 15%.

Table 3.8 Means and standard deviations (SD) of production parameters of broilers grown for 32 days receiving varying amounts of *Chrysomya chloropyga* larvae meal in comparison with a maize soya based diet

Day	Parameter	Control		5%		10%		15%		P Value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
4	Live weight (g)	106.03	3.41	109.00	3.43	109.41	3.20	108.64	4.12	0.2424
11	Live weight (g)	271.02	7.03	268.48	12.67	278.63	4.80	266.69	13.55	0.1197
	Average weekly intake per bird (g)	224.51	5.60	231.61	10.71	230.63	9.56	220.70	12.19	0.1084
	Average cumulative intake per bird (g)	224.51	5.60	231.61	10.71	230.63	9.56	220.70	12.19	0.1084
	FCR for total period	1.34^{a}	0.04	1.41^{b}	0.08	1.32^{a}	0.03	1.36^{ab}	0.07	0.0272
	Average weekly gain per bird (g/week)	165.00	6.07	159.48	10.49	169.21	4.28	158.05	10.36	0.0422
18	Live weight (g)	612.11^{a}	16.38	624.63	32.71	656.50	22.21	603.50^{a}	39.23	0.0060
	Average weekly intake per bird (g)	497.75	21.50	490.50^{ab}	24.09	508.25^{b}	24.18	476.25	27.82	0.0883
	Average cumulative intake per bird (g)	722.26	23.51	722.11	31.54	738.88	32.33	696.95	37.19	0.0883
	FCR for total period	1.42^{a}	0.03	1.39^{ab}	0.04	1.34^{b}	0.02	1.40^{ab}	0.08	0.0128
	Average weekly gain per bird (g/week)	341.09^{a}	13.69	356.15^{ab}	28.60	377.88^{b}	20.11	336.81^{a}	29.41	0.0077
25	Live weight (g)	1122.39^{a}	26.07	1152.50^{ab}	56.19	1206.00^{b}	52.12	1101.25^{a}	74.10	0.0043
	Average weekly intake per bird (g)	764.24^{ab}	28.61	774.75^{ab}	32.58	803.00^{a}	30.65	733.25^{b}	53.73	0.0092
	Average cumulative intake per bird (g)	1486.50^{ab}	44.90	1496.86^{ab}	62.55	1541.88^{a}	55.57	1430.20^{b}	88.38	0.0172
	FCR[1] for total period	1.46^{a}	0.02	1.43^{b}	0.04	1.40^{ab}	0.04	1.44^{ab}	0.05	0.0349
	Average weekly gain per bird (g/week)	510.28^{ab}	18.42	527.88^{ab}	24.73	549.50^{a}	34.83	497.75^{b}	36.17	0.0092
32	Live weight (g)	1758.33^{a}	46.37	1829.97^{ab}	50.11	1880.00^{b}	98.83	1790.42^{ab}	78.01	0.0130
	Average weekly intake per bird (g)	1033.68	49.17	1100.78	81.15	1068.75	44.58	1098.19	102.42	0.2456
	Average cumulative intake per bird (g)	2520.18	70.71	2597.64	77.57	2610.63	73.22	2528.39	64.45	0.0323
	FCR for total period	1.52	0.02	1.51	0.03	1.47	0.07	1.50	0.05	0.2537
	Average weekly gain per bird (g/week)	635.94	28.20	677.47	60.76	674.00	58.53	689.17	75.52	0.3104
35	Live weight (g)	2033.42^{a}	67.31	2110.14^{ab}	56.78	2165.50^{b}	121.39	2077.19^{ab}	93.97	0.0398
	Average weekly intake per bird (g)	480.33	28.45	469.17	40.57	473.75	25.31	454.14	107.56	0.8464
	Average cumulative intake per bird (g)	3000.51	93.59	3066.81	73.26	3084.38	72.69	2982.53	92.70	0.0575
	FCR for total period	1.56	0.02	1.53	0.04	1.50	0.08	1.51	0.08	0.3195
	Average weekly gain per bird (g/week)	275.08	30.38	280.17	24.09	285.50	34.05	286.78	22.89	0.8333
	EPEF[2]	411.07^{a}	20.46	439.74^{ab}	21.02	468.09^{b}	50.53	426.95^{ab}	36.72	0.0190
	Liveability	97.50	4.63	98.75	3.54	100.00	0.00	96.25	5.18	0.2720
	ADG[3]	64.22^{a}	1.94	66.89^{ab}	1.90	68.76^{b}	4.02	65.47^{a}	3.09	0.0050

The 10% inclusion level had a higher (P≤0.05) European Production Efficiency Factor (EPEF) than the control diet, but did not differ (P>0.05) from the 5% and 15% inclusion levels. No significant differences in EPEF were observed between the control, 5% and 15% inclusion levels. Pretorius (2011) observed no differences in EPEF when a 10% inclusion of house fly larvae meal diet was compared to a 10% fish meal diet, nor did Pretorius (2011) reported differences in EPEF when House fly larvae meal and fish meal diets were compared both at 25% inclusion levels. When comparing black soldier fly pre-pupae diets at inclusions of 5%, 10% and 15% with a soya oil cake control diets, Uushona (2016) observed no differences in EPEF.

3.5 Conclusion

The study revealed that commercially produced larvae meal from *C. chloropyga* (CC) raised on abattoir waste was superior compared with that of standard maize soya based diets for commercial broiler production regarding live weight, EPEF and ADG. The proximate composition as well as the amino acid composition of CC compared favorably to that of the ideal amino acid requirements of humans, broilers, pigs and tilapia. The use of these larval meals in broiler diets when fed on an iso-nutritional basis resulted in production parameters similar to, or exceeding that, of a standard maize soya based broiler diet. All production parameters were superior with the 10% inclusion level resulting in the highest live weight at slaughter, the highest ADG and the best EPEF. It can thus be concluded that the use of these products will have a positive effect on productivity of broiler farms. The results of the current study warrant further research into the use of different species of larval meals as well as the use of larvae meals in the nutrition of other high producing farm species. Further processing, and especially defatting of the larvae meal could add value to the products since the high fat content (19.07%) is the single most limiting factor when formulating diets for monogastric animals. Also it is recommended that this research be expanded into the field of ruminant nutrient with the evaluation of the meal as a potential bypass protein for high producing ruminants.

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Chapter 4: **The effect of copper bottom blow fly (*Chrysomya chloropyga*) larvae meal on chemical, physical, sensory and carcass characteristics of broiler chicken meat**

4.1 Abstract

The effects of copper bottom blow fly (CC) larvae meal when used as a protein source in broiler diets on the sensory, chemical, physical and carcass characteristics of broilers were investigated. Four formulated diets consisting of 0%, 5%, 10% and 15% larvae meal were used. Three hundred and twenty day-old chicks were randomly allocated to the diets and slaughtered on day 35. Diet had no significant effect on live slaughter weight, cold carcass weight or dressing percentage. Of the carcass portion yields, diet had an effect only on breast portion yield and no effect on the thigh, drumstick, wing or back portion yields. Also, diet did not influence skin plus fat, muscle or bone yields. Diet influenced the ultimate pH of the thigh, but had no influence on the initial pH of the breast nor thigh, nor on muscle colour. Diet also had no effect on the protein, fat and ash content of the meat, but differences in moisture contents were observed; the control had a higher moisture content than the CC treatment diets. No differences in amino acids and mineral contents of the meat were observed. When comparing the sensory attributes, the 5% inclusion level had the most intense chicken aroma. The control had the least intense chicken aroma, while the 10% and 15% were intermediate. The cooked breast meat of the control birds had the highest initial juiciness, while the 10% inclusion level had the lowest.

Keywords – larvae meal, tenderness, yield, flavor

4.2 Introduction

The use of *Chrysomya chloropyga* (copper bottom blow fly) (CC) larvae meal in broiler diets can possibly impact on the carcass, chemical, physical and sensory characteristics of their meat. In response to these changes the consumer could be influenced in his choice to

purchase, or not to purchase, meat. The consumer's initial selection is influenced by the appearance of the product (Fletcher 2002; Huff-Lonergan and Lonergan, 2005) while the repurchase decision is based on sensory attributes (Fletcher, 2002).

No literature regarding the use of CC larvae meal in broiler nutrition is available. However, many studies were conducted using other insect species in broiler nutrition. When Pieterse *et al*. (2014) used house fly larvae meal in broiler diets, the treatments had a positive influences on slaughter weight and cold carcass weight, but had no influence on dressing percentage. Teguia *et al.* (2002) and Awoniyi *et al*. (2003) reported similar results on dressing percentage, while Hwangbo *et al*. (2009) found an increase in dressing percentage. Uushona (2016) used black soldier fly pre-pupae in her trails and did not report any differences in live slaughter weight, cold carcass weight or dressing percentage.

The use of house fly larvae meal (Pieterse *et al*., 2014) and black soldier fly pre-pupae (Uushona, 2016) had no influence on the colour of broiler breast meat, and in both studies the colour was classified as normal. Sealy *et al*. (2011) found high levels of moisture and fat in trout when black soldier fly larvae was used in the diets. Uushona (2016) reported no differences in the mineral content of broiler meat when black soldier fly pre-pupae were used in the diets. House fly larvae meal (Pieterse *et al*., 2014) and black soldier fly pre-pupae meal (Uushona, 2016) had no influence on the sensory attributes of broiler meat.

Although studies on using insect meal as a protein source in broiler nutrition did differ in some occasions regarding chemical, physical, sensory and carcass characteristics of broiler chicken meat, the influence were in no occasion negative. Therefore the use of CC larvae meal can possibly be used as an alternative protein source in broiler nutrition.

4.3 Materials and methods

4.3.1 Experimental layout, handling and management

This section is described in detail in Chapter 3 (Section 3.3.1).

4.3.2 Slaughtering procedure

At 35 days of age, the birds were weighed individually to determine the average live weight of all the birds in each cage. One broiler with a live weight closest to this average weight was then selected for slaughter.

The broilers 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 ~2 min. Thereafter, the birds were soaked in a rotating 60 °C water bath for 5 min, de-feathered and then eviscerated.

4.3.3 Carcass characteristics

After slaughter, the carcasses were refrigerated (~4 °C) overnight where after the 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 cut into commercial portions (thigh, wing, drumstick and breast) using a portion cutter. The cutting procedure was as follows: firstly, the whole carcass was halved. 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 using a Mettler PC 400 scale (Mettler-Toledo, Switzerland) 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. Percentage component yields were calculated by expressing these weights as n percentage of cold carcass weight. The left breast samples were vacuum-packed with their skins and bones attached, and frozen at -18°C for sensory analyses.

4.3.4 Physical measurements

4.3.4.1 pH

Subsequently, 15min after slaughter the carcass initial pH (pH_i) was measured from the right breast and thigh, where after the carcass was chilled at ~4 °C for 24 h. The ultimate pH (pH_u) of the right breast and thigh was measured 24 h after chilling. The pH was measured by means of a small incision in the centre of both the breast and the thigh muscle with 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. After chilling the carcasses were transported (15 mins transport time) to the meat laboratory at Stellenbosch University for further processing.

4.3.4.2 Colour

Breast meat colour was measured 24 h post-mortem. The skin and subcutaneous fat on the thigh and breast was removed, and the exposed thigh and breast muscle were placed on a flat surface. Colour was measured at three randomly selected positions on the meat muscle surface after a 20 min blooming period. The colour was recorded using a Colour guide 45°/0° colorimeter (Catelogue 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 calculated the hue angle (h_{ab}) (°) and chroma value (C^*) as outlined in Honkel (1998).

4.3.5 Chemical analysis

4.3.5.1 Sample preparation

Six replicates (birds) per treatment were used for sensory and also chemical analysis. After the breast sub-samples for descriptive sensory analysis (DSA) were removed, the remainder were used for chemical analysis. These cuts were homogenised and vacuum packed separately and frozen at -18°C until analysed. Before analysis the samples were removed from the -18°C freezer and defrosted in a refrigerator at 4°C for approximately 12h before being homogenised.

4.3.5.2 Proximate analysis

For proximate analysis the homogenised cooked breast meat samples were analysed using the methods described by the Association of Analytical Chemists International (AOAC) (2002). Official method 934.01 was used to determine dry matter (DM). Samples were dried for 24 h at 100˚C. Crude protein was determined by using the LECO FP528 machine to measure the total nitrogen content. Method 992.15 was used to determine the nitrogen content, which was then multiplied by 6.25 to determine crude protein. Retained samples from the dry matter determination were used for further analysis to determine the ash content of the homogenised cooked breast meat samples. Official method 942.05 was used; combustion of the samples occurred at 500°C for 6 h.

The amino acid composition was determined using official method 994.12. Six mm of 6N hydrochloric acid containing 15% phenol solution was added to the cooked breast meat samples and then sealed under nitrogen and left in an 110˚C oven. After 24 h, the samples were removed, left to cool down and then frozen in Eppendorf tubes at -18°C until further analysis. Amino acid analysis was done using a Water API Quattro Micro instrument with the sample 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, 760 µl of Water and 200 µl Internal standard. To prepare the samples, 20 µl AccQ Tag reagent and 70 µl Borate buffer were added to a 10 µl sample. After the sample was vortexed, it was allowed to stand for 1 min at room temperature and then placed in a heating block for 10 min at 55°C. To determine the amino acid composition, 1 µl was then injected in the apparatus.

The mineral composition of the cooked breast meat samples were determined at the institute of Animal Production, Western Cape Department at Elsenburg by using the combustion method No. 6.1.1 as described by Agriculture Laboratory Association of South Africa (AgriLASAS) (2007).

To determine the crude fat content, 5 g of cooked breast meat were homogenised with 50 mL chloroform/methanol (1:2 vol/vol) where after the solution was filtered into a separation funnel through Whatman® No 1. Thereafter, 20 mL of 0.5% sodium chloride was added and

the solution was allowed to separate. Then 5 mL of the lower extract was placed into a fat glass beaker and dried on a sand bath (~85°C) to allow the chloroform/methanol to evaporate. A 16.7 mL factor were used to correct the results as total percentage of fat (Lee *et al*., 1996).

4.3.6 Sensory analysis

4.3.6.1 Sample preparation

The left breasts of the birds were used for sensory analysis. Prior to conducting sensory analyses the samples were defrosted for ±12 h at 4°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. The ovens were pre-heated to 160 °C (American Meat Science Association [AMSA],1995). The meat samples were removed from the oven when a core temperature of 75°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 x 1 cm x 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 32 sample 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°C for 7 min and placed in a water bath preheated to 70°C for the duration of the testing session.

4.3.6.2 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 = 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 4.1.

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 the 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 three different consecutive days, with two testing sessions per day.

Table 4.1 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

4.3.7 Statistical analysis

The following hypothesis was proposed:

H_1: There is no statistical difference amongst the analysed meat quality attributes and carcass characteristics of broiler carcass as influenced by CC

H_0: There is a statistical difference amongst the analysed meat quality attributes and carcass characteristics of broiler carcasses as influence by CC

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). 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, $Y_{ij} = \mu_i + \alpha j + \varepsilon_{ij}$ where the terms in the model are defined as; the treatment effect response (Y_{ij}), the overall mean (μ_i), treatment effect (αj) and the unexplained error (ε_{ij}).

4.4 Results and Discussion

4.4.1 Carcass characteristics

Table 4.2 indicates that the inclusion of *C. chloropyga* (CC) larvae meal in broiler diets at rates of 0%, 5%, 10% and 15% had no influence (P>0.05) on live weight, cold carcass weight and dressing percentage. Pieterse *et al*. (2014) reported higher (P≤0.05) live slaughter weights and cold carcass weights in broilers fed a 10% inclusion of house fly larvae meal compared to a soya based control diet, but did not report any differences in dressing percentage. Téguia *et al*. (2002) and Awoniyi *et al*. (2003) reported similar results for dressing percentage when using house fly larvae meal, but Hwangbo *et al*. (2009) noted that the use of larvae meal increased dressing percentage (P≤0.05). When comparing slaughter weight, cold carcass weight and dressing percentages in broilers fed on diets containing a soya based control diet, and treatment diets containing 5%, 10% and 15% black soldier fly pre-pupae meal, Uushona (2016) also reported no differences (P>0.05).

It can be noted that CC larvae meal had little influence on carcass portion yields. No differences (P>0.05) were found in thigh, drumstick, wing and back weights. However, the inclusion of CC larvae meal at a 10% level had higher (P≤0.05) right breast weight than the control diet. The 5% and 15% inclusion levels were intermediate. Pieterse *et al*. (2014) noted differences (P≤0.05) in the carcass portion yield of broilers fed on a 10% house fly larvae meal based diet compared to the soya based control diet. Pieterse *et al*. (2014) found higher (P≤0.05) yields of breast and thigh muscle as a percentage of carcass weight of the broilers

that were fed the treatment diets when compared to the control-diet broilers. However, the leg and wing weights did not differ (P>0.05) in the carcasses of the 10% house fly larvae meal and the soya based control (Pieterse *et al*., 2014). Uushona (2016) reported no differences (P>0.05) in breast, thigh, drumstick, wing or back yields when broiler diets containing 5%, 10% and 15% black soldier fly pre-pupae meal were compared to a soya based control diet. Table 4.2 shows no differences (P>0.05) regarding breast skin plus fat percentage, breast muscle percentage and breast bone percentage. This differs from Uushona (2016) who reported higher (P≤0.05) skin plus fat yields from broilers fed 15% black soldier fly pre-pupae diets compared to the soya based control diets, while the 5% and 10% inclusion levels were intermediate. No differences (P≤0.05) were observed by Uushona, (2016) regarding bone percentages of broilers as influenced by feeding black soldier fly pre-pupae meal.

Table 4.2 The means (± standard error) of live slaughter weight, cold carcass weight, dressing percentage, carcass portion yields (g) and skin, muscle and bone percentages of broilers as influenced by inclusion of *Chrysomya chloropyga* meal (CC) in their diets

Parameters	**Treatments diets**				***P*-value**
	Control	**5% CC**	**10% CC**	**15% CC**	
Live slaughter weight (g)	2067.5 ±10.65	2047.5 ± 0.04	2172.5 ± 0.04	2095.0 ± 0.04	0.052
Cold carcass weight (g)	1364.5 ±11.32	1388.73 ±30.99	1472.27 ±38.55	1399.5 ±25.36	0.067
Dressing percentage (%)	66.0 ± 0.50	67.86 ± 0.01	67.78 ± 0.01	66.83 ± 0.01	0.525
Right Breast	267.58^{b}± 8.12	277.45^{ab}± 5.18	299.35^{a}± 9.33	270.91^{ab}± 7.68	0.031
Thigh	352.65 ± 5.27	348.93 ±11.63	373.36 ±10.44	365.10 ± 10.72	0.290
Drumstick	195.04 ± 4.82	209.65 ± 6.52	213.20 ± 9.76	205.55 ± 5.38	0.293
Wing	172.60 ± 9.41	178.15 ± 7.96	178.73 ± 5.62	179.19 ± 5.78	0.091
Back	93.29 ± 3.37	94.61 ± 2.78	99.61 ± 4.76	98.00 ± 5.56	0.704
Skin plus fat	5.73 ± 0.38	6.93 ± 0.55	6.28 ± 0.35	6.87 ± 0.48	0.212
Muscle	74.25 ± 0.91	74.21 ± 1.39	72.79 ± 1.56	71.33 ± 1.35	0.366
Bone	18.54± 0.77	17.55 ± 1.02	20.00 ± 1.61	20.72 ± 0.87	0.205

$^{(a,b)}$ Means with different superscripts within the same row differ significantly (P≤0.05)

4.4.2 Physical measurements

Table 4.3 indicates that the inclusion of CC larvae did not have an influence (P>0.05) on the initial pH of the breast or thigh, nor on the ultimate pH of the breast. However, there were differences in the ultimate pH of the thigh with that of the control being higher (P≤0.05) than the 5% and 15% CC larvae inclusion levels. The 10% CC larvae inclusion level was intermediate. Similarly, no differences (P>0.05) were observed in the pH of breast and thigh meat of broilers fed a 10% house fly larvae meal and the soya based control diet (Pieterse *et al*., 2014). Nor were any differences (P>0.05) reported regarding initial and ultimate pH of both breast and thigh meat of broilers that received diets with 5%, 10% or 15% black soldier fly pre-pupae meal, compared to a soya based control diet (Uushona, 2016). No differences (P>0.05) were observed regarding the L*, a*, b*, hue and chroma measurements of the colour of the breast (

Table 4.3) and the ordinate values are very similar to that reported in other investigations where insect larvae were fed to broilers. For example, when 10% house fly larvae meal were fed to broilers, the lightness of both the breast meat of the experimental broilers and that of the soya based control diet were classified as normal, and did not differ ($P>0.05$) from each other (Pieterse *et al*., 2014). However, in the same study, Pieterse *et al*. (2014) concluded that the breast meat of the soya based control broilers were redder ($P\leq0.05$) than the breast meat of the 10% house fly larvae meal treatment. Pieterse *et al*. (2014) noted no difference in the yellowness of the breast meat of the 10% house fly larvae meal treatment compared to that of the soya based control broilers. In the thigh, no differences ($P>0.05$) were observed in the colour (L*, a* and b*) between the 10% house fly larvae meal treatment broilers and the soya based control broilers (Pieterse *et al*., 2014). Similar to this investigation, when diets containing 5%, 10% and 15% black soldier fly pre-pupae meal were compared to a soya based control diet, Uushona (2016) observed no significant differences ($P>0.05$) regarding L*, a*, b*, Hue and Chroma of the breast or thigh.

Table 4.3 The means (± standard error) of physical measurements of broiler carcasses as influenced by the inclusion of *Chrysomya chloropyga* meal (CC) in their diets

Parameters	Treatment diets				*P*-value
	Control	5% CC	10% CC	15% CC	
pH_i breast	6.38 ± 0.044	6.35 ± 0.059	6.38 ± 0.056	6.41 ± 0.034	0.890
pH_μ breast	6.13 ± 0.043	6.06 ± 0.037	6.13 ± 0.032	6.06 ± 0.020	0.261
pH_i thigh	6.65 ± 0.056	6.57 ± 0.061	6.58 ± 0.042	6.52 ± 0.044	0.302
pH_μ thigh	6.65^{a} ± 0.043	6.46^{b} ± 0.025	6.52^{ab} ± 0.043	6.47^{b} ± 0.039	0.006
L*	54.35 ± 0.705	53.35 ± 1.261	53.94 ± 1.223	54.03 ± 0.873	0.922
a*	0.74 ± 0.304	-0.35 ± 0.227	0.22 ± 0.318	0.14 ± 0.331	0.105
b*	12.31 ± 0.259	9.97 ± 0.706	10.54 ± 0.760	12.15 ± 1.037	0.085
Hue	86.70 ± 1.251	92.88 ± 2.075	89.33 ± 1.443	90.36 ± 1.774	0.093
Chroma	12.35 ± 0.283	10.01 ± 0.680	10.57 ± 0.774	12.18 ± 1.032	0.083

$^{(a,b)}$ Means with different superscripts within the same row differ significantly (P≤0.05)

4.4.3 Chemical analysis

The moisture content of cooked broiler breast meat of the control was higher (P=0.002) than the three treatments (Table 4.4). There were no significant differences between the four treatments for protein and fat, but the 15% CC had a higher ash content (P=0.054) than the control, while the 5% and 10% were intermediate (Table 4.4). When Sealey *et al*. (2011) partially replaced fishmeal with black soldier fly larvae meal at rates of 25% and 50% of the protein content of the diets of rainbow trout, they found a higher (P≤0.05) moisture content in the muscles of both treatment groups. Sealey *et al*. (2011) also found lower (P≤0.05) fat levels in the muscles of both treatment groups, while the protein and ash content did not differ (P>0.05). When a soya based control diet was compared to diets containing black soldier fly pre-pupae meal at inclusion levels of 5%, 10% and 15%, Uushona (2016) observed no differences (P>0.05) regarding moisture, protein, fat and ash of the cooked breast meat of broilers receiving different levels of black soldier fly pre-pupae meal.

Table 4.4 The means (± standard error) of the proximate analysis (g/100g; meat) of broiler cooked breast meat as influenced by the inclusion of *Chrysomya chloropyga* meal (CC) in their diets

Parameters	Treatment diets				*P*-value
	Control	**5% BSM**	**10% BSM**	**15% BSM**	
Moisture	68.9 ± 0.58^{a}	66.1 ± 0.50^{b}	66.2 ± 0.50^{b}	66.0 ± 0.50^{b}	0.002
Protein	28.4 ± 0.57	28.9 ± 0.50	29.3 ± 0.50	29.4 ± 0.50	0.568
Fat	2.1 ± 0.24	2.8 ± 0.21	2.8 ± 0.21	2.7 ± 0.21	0.090
Ash	1.1 ± 0.09^{b}	1.2 ± 0.08^{ab}	1.2 ± 0.08^{ab}	1.4 ± 0.08^{a}	0.054

[a,b] Means with different superscripts within the same row differ significantly (P≤0.05)

Table 4.5 The means (± standard error) of the amino acid composition (g/100g) of cooked breast meat as influenced by inclusion of *Chrysomya chloropyga* meal (CC) in broiler chickens diets

Amino acids	Treatment diets				*P*-value
	Control	5% BSM	10% BSM	15% BSM	
Histidine*	2.5 ± 0.09	2.5 ± 0.08	2.5 ± 0.09	2.5 ± 0.08	0.995
Serine	3.0 ± 0.08	3.0 ± 0.07	3.1 ± 0.08	3.0 ± 0.07	0.860
Arginine*	5.2 ± 0.12	5.3 ± 0.11	5.3 ± 0.11	5.4 ± 0.10	0.883
Glycine	3.3 ± 0.07	3.5 ± 0.06	3.3 ± 0.07	3.4 ± 0.06	0.266
Aspartic acid	7.2 ± 0.18	7.0 ± 0.15	7.2 ± 0.16	7.2 ± 0.14	0.916
Glutamic acid	11.3 ± 0.24	11.1 ± 0.21	11.3 ± 0.23	11.4 ± 0.20	0.865
Threonine*	3.6 ± 0.09	3.5 ± 0.08	3.5 ± 0.08	3.5 ± 0.07	0.945
Alanine	4.2 ± 0.08	4.3 ± 0.07	4.2 ± 0.07	4.2 ± 0.07	0.782
Proline	2.8 ± 0.06	2.8 ± 0.05	2.7 ± 0.05	2.8 ± 0.05	0.542
Cysteine	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02	0.683
Lysine*	7.5 ± 0.55	7.6 ± 0.48	7.5 ± 0.51	6.7 ± 0.45	0.543
Tyrosine	3.1 ± 0.11	3.0 ± 0.10	3.1 ± 0.11	3.1 ± 0.09	0.723
Methionine*	2.0 ± 0.10	2.3 ± 0.08	2.3 ± 0.09	2.3 ± 0.08	0.086
Valine*	4.2 ± 0.09	4.2 ± 0.07	4.1 ± 0.08	4.1 ± 0.07	0.466
Isoleucine*	3.7 ± 0.08	3.8 ± 0.07	3.7 ± 0.07	3.7 ± 0.06	0.758
Leucine*	6.4 ± 0.13	6.3 ± 0.12	6.3 ± 0.12	6.3 ± 0.11	0.946
Phenylalanine*	3.5 ± 0.12	3.4 ± 0.10	3.5 ± 0.11	3.4 ± 0.10	0.717

* Essential amino acids

There were no treatment differences (P>0.05) regarding the amino acid composition in the cooked broiler breast meat (

Table 4.5). Uushona (2016) also reported no significant differences in the amino acid composition of cooked breast meat of broilers fed a soya based control diet and 5%, 10% and 15% black soldier fly pre-pupae meal, respectively. Sealey *et al*. (2011) also found no significant differences (P≤0.05) in the amino acid compositions when a fish meal based diet was compared to diets containing 25% and 50% of black soldier fly larvae meal as a percentage of total protein in the diet of rainbow trout.

Table 4.6 shows no differences (P>0.05) in the mineral composition between the four treatments of cooked broiler breast meat. Uushona (2016) also found no differences (P>0.05) in the mineral composition of cooked breast meat of broilers receiving either a soya based control diet or a treatment diet containing black soldier fly pre-pupae meal at inclusions of 5%, 10% or 15%.

Table 4.6 The means (± standard error) of mineral composition of cooked breast meat as influenced by inclusion of *Chrysomya chloropyga* meal (CC) in broiler chickens diets

Parameters	Units	Treatment diets				
		Control	5% BSM	10% BSM	15% BSM	P-value
Phosphorous	%	0.69 ± 0.022	0.67 ± 0.019	0.64 ± 0.019	0.68 ± 0.019	0.428
Potassium	%	0.73 ± 0.030	0.71 ± 0.026	0.68 ± 0.026	0.70 ± 0.026	0.606
Calcium	%	0.03 ± 0.005	0.03 ± 0.004	0.03 ± 0.004	0.03 ± 0.004	0.930
Magnesium	%	0.12 ± 0.004	0.11 ± 0.004	0.11 ± 0.004	0.12 ± 0.004	0.373
Iron	mg/kg	28.11 ± 3.037	25.64 ± 1.871	27.85 ± 2.146	25.99 ± 2.180	0.841
Copper	mg/kg	0.46 ± 0.023	0.69 ± 0.085	0.48 ± 0.060	0.52 ± 0.068	0.106
Zinc	mg/kg	29.15 ± 1.940	29.76 ± 1.515	26.75 ± 0.803	26.21 ± 1.241	0.249
Manganese	mg/kg	0.83 ± 0.065	0.72 ± 0.030	0.92 ± 0.055	0.93 ± 0.059	0.069
Boron	mg/kg	0.71 ± 0.049	0.70 ± 0.046	0.63 ± 0.062	0.71 ± 0.078	0.739
Sodium	mg/kg	717.33 ± 33.503	593.88 ± 29.014	602.25 ± 29.014	613.88 ± 29.014	0.041

4.4.4 Descriptive sensory analysis

The results of the sensory analysis are shown in Table 4.7. No differences were observed for chicken flavour, metallic aroma, metallic flavour, sustained juiciness and tenderness in the cooked chicken breast meat. However, the 5% inclusion level of CC had a higher chicken aroma (P=0.008) than the control, while the 10% and 15% were intermediate. The control had the highest initial juiciness, but did not differ (P>0.05) from the 5% inclusion level. The 15% inclusion of CC had an initial juiciness similar to the 5% CC, but significantly lower than that of the control. The 10% inclusion level had the lowest initial juiciness of the four treatment diets which differed from that of the control and 5% CC. When Pieterse *et al*. (2014) compared the

sensory attributes of meat of broilers fed a 10% house fly larvae meal treatment diet compared to a soya based control diet, no differences (P>0.05) were observed for chicken aroma, initial juiciness, chicken flavour, sustained juiciness, toughness and mealiness. However, the meat of the 10% house fly larvae treatment broilers had a significant higher (P≤0.05) score for metallic aroma and metallic aftertaste (Pieterse *et al*., 2014). Although both did not differ in the present investigation, the metallic flavor (P=0.067) could indicate possible differences with a larger samples size; an aspect that warrants further research. When Uushona (2016) compared the sensory attributes of cooked breast meat of broilers that received either a soya based control diet or a diet containing black soldier fly pre-pupae meal at inclusion levels of either 5%, 10% or 15%, she observed no significant differences regarding chicken aroma, chicken flavour, metallic aroma, metallic flavour, initial juiciness, sustained juiciness or tenderness.

Table 4.7 The means (± standard error) of sensory attributes as influenced by inclusion of *Chrysomya chloropyga* meal (CC) in broiler chickens diets

Parameters	Treatment diets				*P*-value
	Control	5% CC	10% CC	15% CC	
Chicken aroma	64.8 ^{b} ± 7.65	69.7 ^{a} ± 9.27	67.8^{ab} ± 7.50	66.2^{ab} ± 8.45	0.008
Chicken flavour	63.5 ± 7.28	67.1 ± 8.61	67.4 ± 8.28	66.5 ± 9.67	0.183
Metallic aroma	2.4 ± 4.76	1.1 ± 3.14	1.5 ± 3.63	1.8 ± 4.35	0.262
Metallic flavour	4.0 ± 5.95	2.6 ± 4.89	3.5 ± 4.77	2.3 ± 4.27	0.067
Initial juiciness	76.0 ^{a} ± 9.79	73.5 ^{ab} ± 11.04	70.1^{c} ± 11.08	71.4 ^{bc} ± 9.59	0.012
Sustained juiciness	69.4 ± 9.10	66.2 ± 7.87	66.5 ± 8.53	67.6 ± 9.08	0.059
Tenderness	84.4 ± 11.97	82.5 ± 9.24	83.4 ± 11.67	82.4 ± 9.84	0.525

4.5 Conclusion

The carcass characteristics and meat quality of broilers fed diets containing CC larvae meal at rates of 5%, 10% and 15% meet and in some cases, exceed that of broilers fed a ~~m~~ soya based control diet. It can thus be concluded that CC larvae meal can successfully be used in broiler nutrition regarding carcass characteristics and meat quality. Although no differences were observed in the colour and metallic flavor of the meat, more researched should be done regarding this aspect. Copper bottom blow flies are raised on abattoir waste, which include blood which has a high iron (Fe) content. This Fe in broiler meat can possibly be utilized by anemic humans that consume the meat. The fact that no literature regarding meat colour, Fe

content of meat or metallic flavour of meat of broilers raised on a diets containing CC larvae meal to compare the results of this study with make it impossible to make a recommendation regarding Fe in the meat. Further studies need to be conducted regarding Fe availability from the blood feed to the broilers. Before broiler meat produces from CC diets can be sold to the consumer, a survey should be done to make sure the consumer will buy broiler meat from such unconventional protein source.

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Chapter 5: **The effect of copper bottom blow fly (*Chrysomya chloropyga*) (CC) larvae meal on organ, gut and tibia bone parameters of broiler chickens**

5.1 Abstract

This study investigated the effect of *Chrysomya chloropyga* larvae meal as a protein source in broiler diets. The effect on gizzard erosion and organ weights, intestinal pH and tibia bone parameters were evaluated. Treatment had no effect on gizzard erosion score, and the gizzard erosion scores of all the treatments were described as normal. Treatment also did not influence organ weights and organ weights relative to live weights. The pH of the duodenum of the control diets were significant lower than that of the 5%, 10% and 15% inclusion of CC larvae meal. Treatment did not influence jejunum pH. The pH of the ileum of the control diets were significant lower than that of the 5%, 10% and 15% inclusion of CC larvae meal. Treatment had no effect on tibia bone breaking force (N) and tibia bone breaking strength (N/mm^2). Treatment did not influence tibia bone minerals, except for potassium (K). The control diet had a lower K level than the treatment diets. It can be concluded that the inclusion of CC in broiler diets has no negative effect on any of the parameters evaluated.

Keywords: tibia, bone breaking strength, minerals,

5.2 Introduction

For the poultry industry all over the world, gizzard erosion has always been a huge problem (Johnson, 1971). Gizzard erosion are normally characterized by a rough inner lining of the gizzard, and by erosions in the inner muscle layer of the gizzard (Wessles & Post, 1989). High mortalities, low feed intake and listlessness characterizes this disease (Itakura *et al*., 1981). Gizzard erosion can by diagnosed *post mortem* by the presence of a black watery content in the crop, proventriculus and gizzard, with the lining of the gizzard eroded and ulcers forming on the gizzard muscles (Johnson, 1971).

Gizzard erosion can be induced by the minerals in the diet, or the diets itself (Fisher *et al*,. 1973; Ross, 1979). Chicks fed pelleted fed have more gizzard erosion than those fed mash feed (Ross, 1979). The authors believed the method of pelleting induced gizzard erosion. Although copper sulphate is used in broiler diets as a growth promoter, Fisher *et al*. (1973) concluded that gizzard erosion was related to the copper concentration in the diet.

When broiler chicks are exposed to stressful environments, more gizzard erosion occur (Grabarevic *et al*., 1993; Dzaja *et al*., 1996). The levels of aspartate aminotransferase and creatine kinase in the proventriculus are increased by stress in broilers chicks (Dzaja *et al*., 1996). This lead to a lower pH in the stomach and gizzard erosion.

Bacteria that occur naturally on fish meal cause the formation of histamine by the decarboxylation of histidine in fish meal (Ferencik, 1970). Gizzerosine is formed when histamine or histidine reacts with lysine during overheating of fish meal in the processing of fish meal (Okazaki *et al*., 1983). Excessive secretions of hydrochloric acid and pepsin are secreted in the stomach when gizzerosine is present, causing gizzard erosion (Masumura *et al*., 1985).

The presence of mycotoxins in broiler diets also causes gizzard erosion (Hoerr *et al*., 1982; Dorner *et al*., 1983; Diaz & Sugahara, 1995). Raw materials from a hot and humid environment may contain mycotoxins (Reddy, 1992).

There are limited literature available regarding toxic effects of insect meals in broiler nutrition. Teguia *et al*. (2002) found no significant differences ($P \leq 0.05$) in the masses of the liver, gizzard and hearts of broilers when replacing 50% and 100% of the fish meal in the diet with house fly larvae meal. Pretorius (2011) reported no significant differences ($P \leq 0.05$) in the masses of the liver, gizzard and heart relative to chick weight even when 50% of the diet were house fly larvae meal. Uushona (2016) reported no significant differences ($P \leq 0.05$) in the masses of gizzard, liver, heart, bursa, and spleen when supplementing a soya based diet with black soldier fly pre-pupae at rates of 5%, 10% and 15%.

Weight gain in modern broilers occur faster than bone development, and as a result the bones are too weak to support the weight of the bird (Hocking *et al*., 2009; Garcia *et al*., 2013). During

the slaughtering procedure these porous bones can fracture and bleed, causing the discoloration of meat close to the bone as well as welfare concerns (Rath *et al*., 2000; Brenes *et al*., 2003; Garcia *et al*., 2013). The development of avian skeletal structure mainly depend on calcium (Ca), phosphorus (P) and dietary vitamin D (Rath *et al*., 2000). Although no literature is available on the effect of CC on broiler tibia bone parameters, it might have an effect.

This study was conducted to investigate the possible influence of copper bottom blow fly (*C. chloropyga*) (CC) larvae meal could have on gizzard erosion, organ stress and tibia bone parameters.

5.3 Materials and methods

The materials and methods used for the treatments, layout, housing, bird handling and management as well as the diets are described in Chapter 3 under materials and methods. In summary, eight replicas of four treatment diets were fed to 320 Cobb 500 day-old chicks until 35 days of age. Four different inclusion levels of CC larvae meal defined the feeds. One bird of average weight per cage were selected at day 35 to slaughter. Slaughtering methods used in commercial abattoirs were used as described by the 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.

5.3.1 Organ Sample

Immediately after exsanguinations and de-feathering, the organs were removed from the carcasses. Organs used in this study were the spleen, heart, gizzard, liver and the *bursa of fabricius.* After the gizzard was cut open and rinsed, it was evaluated for gizzard erosion using an ordinal scale as described by Johnson & Pinedo (1971). The gizzard erosion scoring description are shown in Table 5.1. Immediately after removal, the organs were weighed using a PC Mettler laboratory scale (Mettler-Toledo, Switzerland).

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)

5.3.2 Intestinal Samples

Gut samples were retained from the duodenum, jejunum and ileum within 15 minutes after slaughter. The duodenum was removed at the start of the pancreas on the gizzard side of the duodenum. Jejunum sample were retained from the center of the jejunum. Ileum samples were taken 5mm from the Meckel's diverticulum to the ileocecal junction. The pH of this three intestinal samples were measured using a Crison pH 25 meter. The pH electrode was placed into the center of the intestinal sample to be measured. Between each reading the probe was rinsed with distilled water.

5.3.3 Tibia bone samples

After the organs and intestines were removed, the tibia bones were removed from the rest of the carcass. While the skin and muscles were still attached to the tibia bone, it was stored at -18°C until analysis. Before analysis the samples were thawed at 4°C for approximately 12 h. The right tibia was then cleaned of all visible tissue. The length of the tibia was then measured with a Vernier caliper. The tibia was weighed and bone breaking strength was measured with an Instron (Fleming *et al*., 1998).

5.3.4 Tibia bone strength and bone mineral content

An Instron tensile/compression machine with a 50kg load cell was used to determine bone strength. Bones was set into the machine one at a time, with their mid-diaphyseal diameter in the middle of the probe. The bending force of the bone was measured using a 10mm probe that moved at a constant speed of 30mm/s. the Instron is controlled by a computer and this system recorded the force in Newton. This methodology was used as described by Fleming *et al*. (1998). Bone strength was measured in Newton force per square millimeter (N/mm^2) and this strength indicates the rigidity of the bone (Rath *et al*., 1999; Baird *et al*., 2008).

The remains after bone breaking was weighed, dried at 100°C for 48 h and weighed again to determine dry matter. After that the dried bone were placed in a furnace at 600°C for 24 h and weighed gain to determine the ash content of the bones. The ash content was sent to the Institute of Animal Production, Western Cape Department of Agriculture at Elsenburg for mineral analysis. The minerals were 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.

5.3.5 Statistical analysis

The hypotheses for this chapter is as follows:

> H_0: There are no statistical differences between organ, gut and tibia bone parameters investigated in broilers fed four different levels of CC.
>
> H_1: There are statistical differences between organ, gut and tibia bone parameters investigated in broilers fed four different levels of CC.

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 $P \leq 0.05$. 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.

5.4 Results and discussion

5.4.1 Gizzard erosion and organ weight

The inclusion of CC at the rates of 0%, 5%, 10% and 15% in broiler diets did not affect the gizzard erosion score (

Table 5.2). A gizzard erosion score of two and below is acceptable (Johnson & Pinedo, 1971). In this study there were no gizzard erosion scores of more than two in the treatment diets. It can therefore be concluded that no gizzerosine formation occurred (caused by histidine-lysine interaction at high temperatures) as a result of drying temperatures used in this study (Okazaki *et al*., 1983). It can also be concluded that no harmful minerals such as high levels of copper (Fisher *et al*., 1973; Ross, 1979), no harmful bacteria (Ferencik, 1970) and no mycotoxins (Hoerr *et al*., 1982; Dorner *et al*., 1983; Diaz & Sugahara, 1995) were present in the treatment diets.

When CC was included in broiler diets at rates of 0%, 5%, 10% and 15%, no differences ($P>0.05$) were found in the weights of their gizzard, liver, heart, *bursa*, spleen or spleen:*bursa* (Table 5.3). This result is similar to results of studies where house fly larvae meal (Teguia *et al*., 2002) and black soldier fly pre-pupae meal (Uushona, 2016) were used in broiler diets. In contrast, Okah & Onwujiariri (2012) reported lower heart weights and higher gizzard weights in broilers fed house fly larvae meal, while treatment did not affect liver weights. The broilers used in the study by Okah & Onwujiariri (2012) were older than 35 days, and this might affect their results.

Table 5.2 Gizzard erosion scores as influenced by inclusion of *Chrysomya chloropyga* larvae meal (CC) in broiler chicken diets

Treatment diets	**Gizzard erosion score**			
	0	1	2	3
0% (Control)	0	2	5	1
5% CC	4	0	3	0
10% CC	2	3	3	0
15% CC	4	2	2	0
Chi-Square *P*-value	0.362			

Table 5.3 Mean (± standard deviation) of organ weight and organ weight relative to body weight as influenced by inclusion of *Chrysomya chloropyga* meal (CC) in broiler chicken diets

Parameters	**Treatment diets**				***P*-value**
	Control	**5% CC**	**10% CC**	**15% CC**	
Organ weight (g)					
Gizzard	32.7 ± 3.66	35.0 ± 2.46	34.0 ± 3.70	35.6 ± 5.17	0.480
Liver	35.5 ± 4.82	36.5 ± 5.06	36.1 ± 3.43	37.6 ± 6.02	0.855
Heart	10.6 ± 1.26	10.8 ± 1.59	11.2 ± 1.37	10.7± 1.04	0.801
Bursa	3.5 ± 1.64	4.3 ± 0.71	3.8 ± 1.78	3.9 ± 0.80	0.693
Spleen	2.6 ± 0.60	2.7 ± 0.73	2.6 ± 0.57	2.6 ± 0.88	0.978
Spleen: *Bursa*	0.9 ± 0.57	0.7 ± 0.25	0.9 ± 0.57	0.7 ± 0.24	0.554
Organ weight relative to body weight (%)					
Gizzard	1.6 ± 0.07	1.7 ± 0.07	1.6 ± 0.07	1.7 ± 0.04	0.326
Liver	1.7 ± 0.07	1.8 ± 0.07	1.7 ± 0.07	1.8 ± 0.07	0.553
Heart	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	0.932
Spleen	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.890
Bursa	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.572
Bursa: spleen	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.573

Treatment also did not influence (P>0.05) organ weight relative to body weight. It is important to evaluate the *bursa* weight relative to live weight. An increase in *bursa* weight relative to live weight indicates the possibility of increased immunosuppression of the bird's immune system (Pope, 1991).

5.4.2 Intestinal pH

The inclusion of CC larvae meal in broiler diets did influence (P≤0.05) intestinal pH. The pH of the duodenum of birds of the 5%, 10% and 15% CC inclusion diets was higher (P<0.0001) than

the control (Table 5.4). Treatment did not affect the pH of the jejunum. The ileum of the control birds had a higher (P<0.0001) pH than the treatment diets. In this study, the pH of the duodenum of the 5%, 10% and 15% birds were higher than the normal duodenum pH (5.5-6.2) of broilers, while the control birds had duodenums with normal pH. The pH of the jejunum and ileum of all the birds in this study were within the normal pH range. Normal pH ranges for broiler jejunum and ileum are 5.8-6.9 and 6.3-8.0, respectively (Van der Klis & Jansen, 2002).

Table 5.4 Mean (± standard deviation) of small intestine pH as influenced by inclusion of *Chrysomya chloropyga* meal (CC) in broiler chicken diets

Parameters	Treatment diets				*P*-value
	Control	5% CC	10% CC	15% CC	
Duodenum	$6.0^{b} \pm 0.62$	$6.9^{a} \pm 0.23$	$6.9^{a} \pm 0.19$	$6.8^{a} \pm 0.35$	0.000
Jejunum	6.3 ± 0.14	6.5 ± 0.37	6.5 ± 0.14	6.4 ± 0.19	0.178
Ileum	$6.8^{a} \pm 0.25$	$6.1^{b} \pm 0.35$	$6.0^{b} \pm 0.31$	$6.3^{b} \pm 0.11$	<.0001

$^{(a,b)}$ Means with different superscripts within the same row differ significantly (P≤0.05)

5.4.3 Tibia bone parameters

The results of the tibia bone breaking force and breaking strength are shown in Table 5.5. Treatment die not affect bone breaking force (N) or bone breaking strength (N/mm^2). This is in line with Uushona (2016) who compared black soldier flies pre-pupae with a maize-soya control diets.

Table 5.5 Mean (± standard error) of tibia breaking force and strength of broiler chickens fed different levels of copper bottom blow fly larvae meal (CC) in their diets

Parameters	Units	Treatments				*P*-value
		Control	5% BSM	10% BSM	15% BSM	
Breaking force	N	357.8 ± 29.09	375.1 ± 29.09	359.9 ± 25.20	391.2 ± 26.95	0.805
Breaking strength	N/mm^2	75.4 ± 5.40	68.9 ± 5.40	66.1 ± 4.68	71.9 ± 5.00	0.615

The inclusion of CC in broiler diets had no effect (P>0.05) on the calcium (Ca), phosphate (P), Magnesium (Mg) sodium (Na), copper (Cu), zinc (Zn) and Iron (Fe) content of broiler tibia bones (Table 5.6). The tibia bone of the control diet had higher (P<0.0001) Potassium (K) levels than the treatment diets. This similar tibia bone mineral composition was expected because all the diets were formulated to have the same specifications.

Table 5.6 Mean (± standard error) of tibia bone ash percentage and mineral content of broiler chickens fed different levels of copper bottom blow fly larvae meal (CC) in their diets

Parameters	Units	Treatment diets				*P*-value
		Control	5% BSM	10% BSM	15% BSM	
Calcium	%	41.0 ± 1.14	38.6 ± 1.14	37.4 ± 1.00	38.1 ± 1.05	0.126
Phosphate	%	19.6 ± 0.44	19.3 ± 0.44	18.1 ± 0.38	18.8 ± 0.40	0.083
Potassium	%	1.0[b] ± 0.04	1.6[a] ± 0.04	1.5[a] ± 0.04	1.4[a] ± 0.04	<0.0001
Magnesium	%	0.7 ± 0.01	0.7 ± 0.01	0.7 ± 0.01	0.7 ± 0.01	0.080
Sodium	mg/kg	12.8 ± 0.78	28.3 ± 0.78	14.8 ± 0.68	13.7 ± 0.72	0.890
Copper	mg/kg	1.9 ± 1.53	1.7 ± 1.53	2.8 ± 1.33	4.2 ± 1.42	0.618
Zinc	mg/kg	303.1 ± 10.21	325.1 ± 10.21	322.8 ± 8.85	306.0 ± 9.46	0.287
Iron	mg/kg	234.0 ± 14.17	288.5 ± 14.17	263.6 ± 12.28	239.9 ± 13.12	0.043

[a,b] Means with different superscripts within the same row differ significantly (P≤0.05)

5.5 Conclusion

The results in this study show that the larvae of the copper bottom blow fly (CC) had no influence on organ weights and gizzard erosion. The CC larvae also did not influence tibia bone parameters. To investigate the repeatability of the intestinal pH measurements, more studies should be done. It can therefore be concluded that CC larvae meal can be included in broiler diets without influencing their immune system or the intestinal organ development.

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Chapter 6: **General Conclusion**

This study was conducted to investigate the possibility of using *Chrysomya chloropyga* (copper bottom blow fly) larvae meal as a protein source in broiler nutrition.

Treatment had no effect on intake per bird, feed conversion ratio (FCR), European production efficiency factor (EPEF) or liveability. Broilers that were fed the 10% inclusion had a slightly higher slaughter weight and average daily gain (ADG) than the other three treatments.

Treatment had no effect on cold carcass weight, dressing percentage, thigh-, drumstick- wing and back yields. The 10% inclusion had a higher right breast portion yield than the other treatments. No differences were observed regarding the yields of skin plus fat, muscle or bone percentage.

Treatment did not affect meat colour. No differences were observed regarding the initial pH (pH_i) of the breast and thigh meat, or the ultimate pH (pH_u) of the breast. The ph_u of the thigh of the 5% and 15% inclusion were lower than that of the Control and 10% inclusion.

The control meat had a higher moisture content and a lower ash content than three treatment diets. Protein end fat did not differ. No differences regarding the amino acid composition and the mineral composition in the broiler meat were observed.

Few differences were observed for sensory attributes of the broiler meat. The 5% inclusion had the highest chicken aroma, the control had the lowest chicken aroma and the 10% and 15% were intermediate. The control had the highest initial juiciness, while the 10% and 15% had significant lower values for initial juiciness.

Treatment had no effect on gizzard erosion and the gizzard scores were classified as normal. Treatment had no effect on gizzard, liver, heart, bursa or spleen weights or the weight of these organs relative to live weight. Treatment had no effect on tibia bone breaking force and tibia bone breaking strength. Treatment had little effect on tibia bone mineral, with only n difference in the potassium (K) value. The control had n lower K composition than the three treatment diets.

Overall the study concluded that copper bottom blow fly larvae meal can be used in broiler diets, with the 10% inclusion getting slightly better results than the rest on a few occasions such as live slaughter weight and ADG.

More research needs to be considered regarding this study to investigate the repeatability of these parameters evaluated. Especially the pH of the intestines were expected not to differ. Research should be done on the histology of the broiler intestines and organs fed CC larvae meal. Differences in histology of the intestines may declare the pH differences observed in the intestine in this study.

The four treatments in this study were formulated to have identical specifications regarding nutrients. This may explain the few differences in parameters observed in this study. However, the fatty acid composition of the larvae was not determined prior to the study. This may have led to some differences observed. Research should be conducted to investigate the fatty acid composition of both the larvae and the broiler meat to make a better conclusion regarding the suitability of CC in broiler nutrition.

To complete the research, the digestibility of CC larvae meal should be researched. Digestibility results may explain the differences in live slaughter weight and ADG in this study.

The objective of this study was not to prove that CC larvae meal is superior to soya in broiler diets, but rather that it can be used as an additional protein source in the broiler industry. It can be concluded that CC larvae meal is a suitable protein source for broiler nutrition.

Research regarding CC larvae meal in animal nutrition is very limited. Studies to investigate the suitability of CC larvae meal in other animal nutrition (pigs, layers, fish and ruminants) should be considered.