THE EVALUATION OF LARVAE OF *MUSCA DOMESTICA* (COMMON HOUSE FLY) AS PROTEIN SOURCE FOR BROILER PRODUCTION

by

Quinton Pretorius

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Department of Animal Sciences

Faculty of AgriSciences

Supervisor: Dr E. Pieterse

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DECLARATION

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Date: February 2011

SUMMARY

The evaluation of larvae of *Musca domestica* (common house fly) as protein source for broiler production

The objective of this study was to evaluate the use of *Musca domestica* (common house fly) larvae meal, as protein source, for broiler production. This was done by investigating the nutritive value of house fly larvae meal together with its total tract digestibility, potential toxicity and carcass characteristics of the broilers supplemented with house fly larvae meal. The proximate analysis of house fly larvae meal show that it contained, on a dry matter basis, a gross energy value of 20.10 MJ/kg, 60.38% crude protein, 14.08% crude fat and 10.68% ash and that the house fly pupae contained a gross energy of 20.42 MJ/kg, 76.23% crude protein, 14.39% crude fat and 7.73% ash. House fly pupae meal had the closest match of amino acid profile when compared with the ideal amino acid profile required by broilers and it has arginine relative to the lysine content closer to the ideal amino acid profile than the house fly larvae meal. The essential fatty acid, linoleic acid, was found at levels of 26.25 and 36.27% of the total fats for the house fly larvae and pupae meal respectively. House fly larvae meal supplementation did not induce gizzard erosion or showed toxicity (regarding the gastro intestinal tract, immune system and organ stress) in broilers.

Results revealed that house fly pupae meal had higher total tract digestibilities for most nutrients than of the house fly larvae meal. House fly larvae meal had a crude protein total tract digestibility of 69% and that of pupae meal was 79%. Both larvae and pupae meal had high amino acid total tract digestibilities of all the amino acids analysed. The house fly larvae and pupae meal had an apparent metabolizable energy (AME) value of 14.23MJ/kg and 15.15MJ/kg respectively. The larvae meal total tract crude fat and crude fibre digestibilities were 94% and 62% respectively. The pupae meal total tract crude fat and crude fibre digestibilities were 98% and 58% respectively.

House fly larvae meal supplementation in a three phase feeding system significantly increased average broiler live weights at slaughter, total feed intake, cumulative feed intake as well as average daily gain (ADG) when compared to commercial maize: soya oil cake meal diet. In direct comparison of larvae inclusion levels with fishmeal in isonitrogenous and isoenergetic diet, no significant differences were observed between a 10% house fly larvae and a 10% fish meal diets regarding performance characteristic. The 25% house fly larvae meal diet yielded significantly better average broiler live weights at slaughter, total feed intake, cumulative feed intake (from the second week until slaughter) as well as average daily gain when compared to the 25% fish meal diet in the growth phases.

Carcass characteristics of the 10% larvae, 10% fishmeal and commercial diets were compared. Chicks that received either the 10% house fly larvae meal or 10% fish meal supplementation produced significantly heavier

carcasses and breast muscle portions than the chicks that received the commercial maize: soya oil cake meal. No treatment differences were found regarding breast and thigh muscle colour or pH.

This study showed that house fly larvae meal can be regarded as a safe protein source that can be used to replace other protein sources and that has the ability to promote broiler performance without having any detrimental effects on carcass characteristics.

OPSOMMING

Die evaluasie van *Musca domestica* (gewone huisvlieg) larwe meel as ‘n proteien bron vir braaikuiken produksie

Die doel van die studie was om die effek van *Musca domestica* (gewone huisvlieg) larwe meel, as ‘n protein bron, in braaikuikens te evalueer. Dit was gedoen deur die nutrient waarde van huisvlieg larwe meel saam met die totale spysvertering verteerbaarheid, moontlike toksiesiteit en karkas-eienskappe van braai kuikens te evalueer. Laboratoruim analiese toon dat huisvlieg larwe meel 20.10 MJ/kg bruto energie, 60.38% ru- protein, 14.08% ru- vet en 10.68% as bevat en huisvlieg papie meel 20.42 MJ/kg bruto energie, 76.23% ru- protein, 14.39% ru- vet en 7.73% as bevat. Huisvlieg papie meel stem die meeste ooreen met die idiale amino suur profiel soos wat benodig word deur braaikuikens en dit het ‘n arginien tot lisien verhouding wat die meeste ooreenstem met die idiale amino suur profiel in vergelyking met huis vlieg larwe meel. Die essensiele vet suur, linolien suur, was geanaliseer teen vlakke van 26.25- en 36.27% van die totale vette onderskeidelik vir huisvlieg larwe- en papie meel. Huisvlieg larwe meel vervanging het nie spiermaag erosie of enige ander toksiese effekte te veroorsaak nie.

Resultate het getoon dat huisvlieg papie meel, in vergelyking met larwe meel, het ‘n hoër totale spysvertering verteerbaarheid vir meeste van die nutrient. Die huisvlieg larwe meel het ‘n totale ru- protein spysvertering verteerbaarheid van 69% en die van papie meel van 79%. Beide larwe en papie meel het hoë amino suur spysvertering verteerbaarheid. Larwe meel en papie meel het skynbare metaboliseerbare energie waardes van 14.23MJ/kg en 15.15%MJ/kg onderskeidelik. Die larwe meel het ‘n ru-vet en ru- vesel spysvertering verteerbaarheid van 94% en 62% onderskeidelik, waar die papies ‘n ru-vet en ru- vesel spysvertering verteerbaarheid van onderskeidelik 98% en 58% het.

Huisvlieg larwe meel vervanging in ‘n drie fase voer stelsel het getoon om die gemiddelde braaikuiken lewende gewigte by slag, totale voer iname, sowel as die gemiddelde daaglikse toename te verhoog waneer dit vergelyk word met ‘n kommersiele mielie- soya olie koek dieet. Geen mekwaardige verskille was waargeneem toe die 10% larwe meel dieet direk met die 10% vismeel diet vergelyk was rakende enige produksie einskappe gemeet nie. Die 25% larwe meel dieet het merkwaardig beter gemiddelde braaikuiken lewende gewigte by slag, totale voer iname, sowel as die gemiddelde daaglikse toename getoon wanneer vergelyk word met die 25% vismeel dieet gedurende die verskeie groei fases.

Karkas eienskappe van die 10% larwe meel, 10% vismeel en die kommersiele diete was gevergelyk. Kuikens wat 10% larwe meel en 10% vismeel in die diete ontvang het, het swaarder karkasse gelewer met swaarder borsie massas wanneer vergelyk word met die kommersiele mielie- soya olie koek dieet. Geen behandelings verskille was gevind rakende die borsie- en dy spier kleure of pH nie.

Die studie toon dat huisvlieg larwe meel as ‘n veillige protein bron kan beskou word, wat gebruik kan word om ander protein bronne te vervang. Huisvlieg larwe meel het ook die vermoë om braaikuiken produksie te verhoog sonder om enige negitiewe effekte rakende die karkas eienskappe te toon nie.

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LIST OF ABBREVIATIONS

ADF - Acid detergent fibre

ADG - Average daily gain

ANOVA - Analysis of variance

AME - Apparent metabolizable energy

ALASA- Agricultural laboratory association of Southern Africa

ATP- Adinosine triphosphate

CP - Crude protein

CSMA - Chemical Speciality Manufacturers Association's fly rearing medium

CTTD - Coefficient of total tract digestibility

DDS- Digital data systems

DGS- Distillers grains with solubles

DLM - Dehydrated larvae meal

DM- Dry matter

EE - Ether extract

EPEF - European production efficiency factor

FCR - Feed conversion ratio

FM- Fish meal

GC- Gas chromatography

HPLC- High performance liquid chromatography

LM- Larvae meal

LSD- Least significant difference

ME- Metabolizable energy

MUFA- Mono unsaturated fatty acids

NDF- Neutral detergent fibre

NRC- National Research Council

PER- Protein efficiency ratio

PUFA- Poly unsaturated fatty acids

S.E- Standard error

SFA- Saturated fatty acid

TMA- Trimethylamine

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 has been unavoidable.

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CHAPTER 1
General introduction

Broilers play a very important role in the nutrition of humans by providing a source of protein through their meat. By satisfying the nutrient requirements of broilers in order to ensure optimal productivity has resulted in the inclusion of high amounts of high quality protein sources especially in the starter and grower phases. These continuous demands for high quality proteins in the diets of broilers poses some challenges; firstly because the animals compete with humans for the same protein sources and secondly because there is a demand for renewable protein resources in animal nutrition.

Since nature has always provided insects as a feed source for wild animals, they have exceptional nutritional characteristics (Scholtz & Holm, 1985; Resh & Cardé, 2003). This creates potential to research and utilise potential insects available in nature for the animal feed industry which can be renewable and cost effective. The concept of insects for protein dates back almost a century, were Lindner (1919) was the first to report on the use of insects to produce a protein source. One of the advantages of using insects as alternative feed substrates is that some insects have the potential to be used in waste management as well as providing a useful protein feed source, for the purpose of this thesis this process will be referred to as the 'nutrient recirculation' process.

There are a number of suitable organisms that could be used in the nutrient recirculation process, but most research has shown that insects belonging to the order of Diptera demonstrated the most promising results (Calvert & Martin, 1969; Newton *et al*., 1977; Bondari & Sheppard, 1987; Inaoka *et al.*, 1999; Fasakin *et al*., 2003; Awoniyi *et al*., 2004; Newton *et al*., 2004; Aniebo *et al*., 2008). Insects from this order including the Muscidae and Stratiomyidae families are described as being ubiquitous, because they have the ability to colonize basically any habitat on earth (Scholtz & Holm, 1985; Resh & Cardé, 2003). The pupae are covered with a chitin layer (Ludwig *et al*., 1964) that might cause the pupae to be less suitable as feed source than the larvae, however there were no published results found for digestibility of housefly pupae meal. The uses of insect larvae meal as a protein source have been widely reported for pigs, poultry and fish (Newton *et al*., 1977; Bondari & Sheppard, 1987; Awoniyi *et al*., 2004).

In comparison with other Dipteran species *Musca domestica* (common house fly) has been the most widely studied as a potential feed source (Calvert & Martin, 1969; Inaoka *et al.*, 1999; Fasakin *et al*., 2003; Newton *et al*., 2004; Aniebo *et al*., 2008). The crude protein content of the house fly larvae reported in literature varies from 37.5% (Ogunji *et al*., 2006) to 63.1% (Calvert & Martin, 1969), this can be attributed to the nutrient composition being influenced by time of harvest (Calvert & Martin, 1969; Inaoka *et al.*, 1999; Newton *et al*., 2004; Aniebo *et al*., 2008), method of drying (Fasakin *et al*., 2003) and larval feed substrate (Newton *et al*., 1977). The objective of this study was to investigate the use of house fly larvae meal in broiler diets, by investigating apparent digestibility, production performance and carcass and meat quality characteristics. Potential toxic effects caused

by larvae meal supplementation were also evaluated by making use of various gut parameters and organ masses.

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CHAPTER 2
Literature review

2.1 Introduction

It is becoming increasingly important to find alternative good quality renewable protein sources that can replace or substitute current protein sources used in animal nutrition. This provides opportunities to explore other possible means of protein production in animal nutrition and such a possible means can come from various organisms that can also be beneficial to the environment, for example waste management. Nature has always provided many ways to manage the waste produced by organisms. The ways in which nature manages waste are through bacteria, fungi, protozoa and insects. This is a very important indicator that insects can be used to manage the waste produced by humans and animals and this also allows for opportunity to use the insects to produce useful protein sources. For the purpose of this review the process in which waste products are utilized by insects and in return create a useful protein source will be termed the 'nutrient recirculation' process. There are many suitable organisms that may be used in the nutrient recirculation and they belong to the orders of Diptera, Coleoptera and Haplotaxida. The use of insect larvae meal as a renewable protein source for pigs, poultry and fish has been widely reported (Newton *et al*., 1977; Bondari & Sheppard, 1987; Awoniyi *et al*., 2004).

Studies on the use of multicellular organisms to convert animal waste to useful products dates back nearly a century ago, where Linder (1919) was the first to report on the use of coprophagous insects, especially the housefly (*Musca domestica*) for the production of protein from waste. In his study he reared the fly larvae on sewage, harvested and dried the larvae and used it to feed rats. This project never really progressed very far and after the study that was done by Linder (1919) the next publication of interest was the work done by Calvert & Martin (1969) were they studied the use of insects to produce nutrients from poultry waste, the authors also used houseflies in their study. They concluded their study by indicating that dried housefly pupa provided sufficient protein for normal growth and development of broilers during the first two weeks of life.

Published information on the chemical composition of insects larvae meal and its suitability as protein source is variable, these differences can be attributed to differences in species, age at harvest (larvae versus pupae) (Calvert & Martin, 1969; Inaoka *et al*., 1999; Newton *et al*., 2004; Aniebo *et al*., 2008), method of drying (Fasakin *et al*., 2003) and larval feed substrate (Newton *et al*., 1977).

2.2 Suitable organisms for nutrient recirculation

The order of Diptera includes insects that are commonly called true flies or two-winged flies, insects that are familiar to this group include mosquitoes, black flies, midges, fruit flies and house flies (Resh & Cardé, 2003). Insects from this order are described as being ubiquitous, because they have the ability to colonize basically any habitat on earth (Scholtz & Holm, 1985; Resh & Cardé, 2003). For the purpose of this literature review only insects from the Muscidae families will be discussed.

2.2.1 Muscidae family

The common house fly, *Musca domestica,* belongs to the Muscidae family and can be found almost anywhere on earth including garbage heaps, faecal matter, decaying matter and discharges from wounds and sores (Scholtz & Holm, 1985; Resh & Cardé, 2003). The housefly larvae have shown to be used with great benefits as a potential protein source in poultry nutrition (Téguia *et al*., 2002; Awoniyi *et al*., 2003; Zuidhof *et al*., 2003; Adeniji, 2007; Agunbiade *et al*., 2007; Hwangbo *et al*., 2009).

2.2.1.1 Chemical composition of *Musca domestica* larvae

The basic life cycle of the housefly is illustrated in Figure 1. Due to the variation noted in chemical composition reported by various authors (Calvert & Martin, 1969; Inaoka *et al*., 1999; Newton *et al*., 2004; Aniebo *et al*., 2008; Fasakin *et al*., 2003; Newton *et al*., 1977) and the cause off this variation concluded as being age at harvest (larvae versus pupae) (Calvert & Martin, 1969; Inaoka *et al*., 1999; Newton *et al*., 2004; Aniebo *et al*., 2008), method of drying (Fasakin *et al*., 2003) and larval feed substrate (Newton *et al*., 1977).

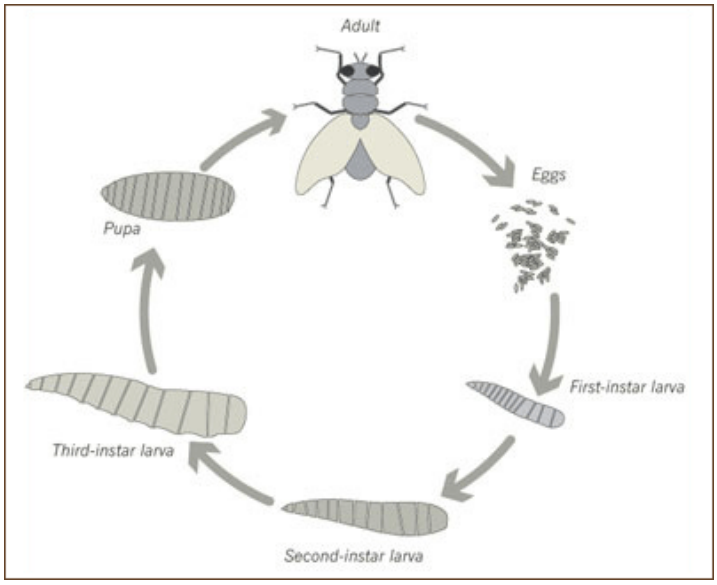

Figure 1 The life cycle of *Musca domestica* (Scholtz & Holm, 1985)

Differences observed in chemical composition (Table 1) and amino acid composition (Table 3) and it's relation to feed substrate and age at harvest (Table 5) as well as the influence of processing method is shown in Table 2.

Table 1 Comparison of house fly larvae and pupae composition (DM baisis) receiving different feed substrates

	Calvert & Martin, 1969	**Ogunji *et al*., 2006**	**Sogbesan *et al*., 2006**	**Aniebo *et al.,* 2008**
Feed substrate	Poultry manure	Poultry manure	Poultry manure	Cattle blood & bran
Stage at Harvest	Pupae (ground)	Larvae, did not state harvest stage	Larvae, did not state harvest stage	3rd day of larval formation
Crude Protein (%)	63.1	37.5	50.4	47.1
Crude Fibre (%)	-	-	1.6	7.5
Fat (%)	15.5	19.8	20.6	25.3
Ash (%)	5.3	23.1	11.7	6.6

Differences due to processing (Table 2) are mostly attributable to the dilution effect of either water or fat on remaining nutrients. In processing raw materials the chemical composition can be adjusted in order to make it more suitable for different species and developmental stages of livestock. With processing of larvae meal Fasakin *et al*. (2003) were able to vary crude protein contents from 47.35% to 50.52% dry matter basis. The defatted larvae meal showed a tendency to have higher crude protein values, because the removal of the oil caused the amount of the product to decrease with the same amounts of nutrients that resulted in a slight increase in the crude protein content (Shiau *et al*., 1990). This is also noted in the study done by Shiau *et al*. (1990) that with defatted soybean meal there is also a tendency for the crude protein and crude fibre to increase.

Table 2 Averages (± Standard error) of the moisture, crude protein, crude fat and ash of housefly larvae meal as influenced by processing methods (Fasakin *et al*., 2003)

Type Larvae meal	**Moisture (%)**	**Crude protein (%)**	**Crude fat (%)**	**Ash (%)**
Hydrolysed oven- dried	8.06 ± 0.05	45.60 ± 0.02	13.28 ± 0.03	13.20 ± 0.02
Hydrolysed sun- dried	8.40 ± 0.01	44.30 ± 0.03	13.65 ± 0.01	13.25 ± 0.01
Hydrolysed/defatted oven- dried	7.56 ± 0.02	46.70 ± 0.01	6.28 ± 0.01	13.30 ± 0.01
Hydrolysed/ defatted sun- dried	8.10 ± 0.01	45.65 ± 0.01	6.30 ± 0.01	12.32 ± 0.02
Defatted oven- dried	9.20 ± 0.01	45.75 ± 0.03	7.00 ± 0.02	13.35 ± 0.02
Defatted sun- dried	9.65 ± 0.04	45.10 ± 0.05	7.40 ± 0.01	13.45 ± 0.02
Full fat oven- dried	8.25 ± 0.02	43.45 ± 0.03	14.30 ± 0.03	14.35 ± 0.02
Full fat sun- dried	8.55 ± 0.04	43.30 ± 0.01	14.35 ± 0.03	14.65 ± 0.01

Table 3 summarizes the different amino profiles reported by different authors. Large variation is observed (Table 3) which could be attributable to laboratory processing methods used when analysing for these amino acids. Both Aniebo *et al*. (2008) and Ogunji *et al*. (2006) hydrolysed the samples before analyses, but Ogunji *et al*. (2006) used high performance liquid chromatography (HPLC) equipment and Aniebo *et al*. (2008) used Technicon Sequential Multi sample amino acid analyser to determine the specific amino acid content. Calvert & Martin (1969) used a Spinco amino acid analyser model 120C where the sample is deproteinized before analysis. From literature obtained, Ogunji *et al*. (2006) was the only researcher that could recover tryptophan in

their analysis and this is because they used an alkaline hydrolyses procedure that have a higher recovery rate for tryptophan than the acid hydrolysis procedure (Hugli & Moore, 1972).

Table 3 Amino acid profile of housefly larvae and pupae receiving different feed substrates

Amino Acid	Calvert & Martin, 1969 (% Protein)	Ogunji *et al*., 2006 (% Protein)	Aniebo *et al.*, 2008 (% Protein)
Feed substrate	Poultry manure	Poultry manure	Cattle blood & bran
Stage at Harvest	Pupae (ground)	Larvae, did not state harvest stage	3rd day of larval formation
Histidine	2.60	5.10	3.09
Arginine	4.20	4.60	5.80
Aspartic acid	8.50	4.50	8.25
Threonine	3.40	7.60	2.03
Serine	3.20	3.30	3.23
Glutamic acid	10.80	6.80	15.30
Proline	3.10	-	2.85
Glycine	3.90	0.90	4.11
Alanine	4.20	4.40	2.86
Cystine	0.40	-	0.52
Valine	3.40	1.30	3.61
Isoleucine	3.50	1.70	3.06
Leucine	5.30	5.60	6.35
Lysine	5.20	4.40	6.04
Tyrosine	4.90	2.50	2.91
Phenylalanine	4.20	10.20	3.96
Methionine	2.60	-	2.28
Tryptophan	-	1.50	-
Protein (% Dry Matter)	63.10	37.50	47.10

Table 4 reports the calculated ratio of indispensible amino acids to lysine. In practice methionine is regarded as the first limiting amino acid in poultry followed by lysine and by supplementing deficient diets with these amino acids increases the efficiency of protein utilization (Schutte & de Jong, 2004). In the ideal amino acid profile for broilers all the indispensible amino acids are expressed as a percentage of lysine, because the indispensible amino acids relative to lysine remains unaffected regardless environmental, dietary and genetic factors (NRC, 1994; Schutte & de Jong, 2004). The results reported by Calvert & Martin (1969) had the closest amino acid to lysine ratios when compared to the ideal amino acid profile. This indicates the importance of constant amino acid analysis regarding the different processing methods of house fly larvae meal and that other protein sources must be fed in conjunction with larvae meal in order to get the best amino acid profile for the animal.

Table 4 Calculated amino acid to lysine ratios of larvae meal in comparison to the ideal amino acid profile for broilers

Amino Acid	Calvert & Martin, 1969	Ogunji *et al*., 2006	Aniebo *et al.,* 2008	Ideal Amino Acid profile*
Lysine	100	100	100	100
Methionine + Cystine	58	-	46	75
Threonine	65	177	33	65
Arginine	81	105	96	110
Tryptophan	-	34	-	18
Valine	65	30	60	80
Isoleucine	67	39	51	70

(*) Ideal amino acid profile as determined by Schutte & de Jong, 2004

The most recently published study done by Aniebo & Owen (2010) shows that the nutritional value of house fly larvae meal is significantly influenced by the age at which the larvae is harvested as well as the method of drying (Table 5). Results of this study revealed that the protein content significantly ($P<0.05$) decreased with age. The authors observed a decrease in the protein values from 59.6, 54.2 to 50.8% DM respectively and an increase in the fat content that were found to be from 22.4, 23.9 to 27.3% dry mater respectively when the larvae were oven dried at two, three and four days of age (Table 5). This phenomenon could by be related to the fact that as the insect/larvae approached the pupa phase in metamorphosis the insect/larvae starts to store more energy in the form of lipids (Pearincott, 1960) and the insect/larvae utilizes the proteins in enzymatic reactions in the formation of the chitin layer (Kramer & Koga, 1986). Aniebo & Owen (2010) also reported that sun drying of larvae produced larvae with lower protein values than oven dried larvae and the fat content were higher in sun dried larvae than oven dried larvae.

Table 5 Average (± standard error) crude protein and fat content (DM basis) of larvae as affected by age and method of drying (Aniebo & Owen, 2010)

	Day 2 harvested	Day 3 harvested	Day 4 harvested
Oven dried			
Crude protein	$59.6^{a} \pm 0.05$	$54.2^{b} \pm 0.03$	$50.8^{a} \pm 0.04$
Fat	$22.4^{c} \pm 0.14$	$23.9^{b} \pm 0.14$	$27.3^{a} \pm 0.35$
Sun dried			
Crude protein	$55.3^{a} \pm 0.14$	$51.3^{b} \pm 0.04$	$45.5^{c} \pm 0.74$
Fat	$25.2^{a} \pm 0.14$	$28.0^{b} \pm 0.14$	$32.0^{a} \pm 0.35$

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

Only a number of authors have reported the mineral composition of larvae meal. Table 6 gives the mineral composition of larvae and pupae meal as reported by the various authors. Difference could, once again, be attributed to the differences in the stage of harvest (larvae vs. pupae), processing methods, feed substrates or vitamin/mineral premixes used in animal nutrition. Table 6 further shows that the pupae, if fed the same feed source, have a much higher mineral content than larvae, but that the larvae have a much higher Fe value (1317.34ppm vs. 465ppm). Fasakin *et al*. (2003) also found that processing had an effect on the mineral content of housefly larvae meal and their findings show that the process of hydrolysis and defatting of the larvae meal causes an increase in the levels of Ca, Mg and Mn. This is as mentioned previously, due to the fact that with the extraction of the oil the amount of product decreases with the same amount of minerals, thus concentrating the product that leads to a slight increase in all the minerals.

Table 6 Mineral compositions of processed housefly larvae and pupae

Minerals Analyzed	**Teotia & Miller., 1974**	**Fasakin *et al*., 2003**
Feed Substrate	Poultry manure	Poultry manure (Layer)
Stage of Harvest	Pupae	Larvae harvested after 96 hours
Processing method	Dried at 65ºC, overnight	Hydrolyzed oven dried
Ash (% DM[1])	11.90	13.20
P (% DM)	1.43	-
Ca (% DM)	0.93	0.31
K (% DM)	0.88	0.50
Na (% DM)	0.56	0.29
Mg (% DM)	-	0.25
Mn (ppm[2])	370.00	47.38
Cu (ppm)	34.00	25.71
Zn (ppm)	275.00	48.87
Fe (ppm)	465.00	1317.34

([1]) DM- Dry Matter ([2]) ppm- parts per million

Table 7 shows the fatty acids composition of larvae an pupae meal as reported by the different authors and it shows that the most acceptable fatty acid profile was obtained when the larvae were fed milk powder, sugar and layer manure (Hwangbo *et al*., 2009). These essential fatty acids will be sufficient for broiler growth, since broilers require the essential fatty acid, linoleic acid, at levels of less than 0.20% of the total diet (Zornig *et al*., 2001).

Table 7 The fatty acid composition of housefly larvae and pupae

Fatty Acid (%†)	Hwangbo *et al*., 2009	Calvert & Martin, 1969	St-Hilaire *et al*., 2007
Stage of harvest	Larva (did not state age)	Pupae	Pupae
Feed substrate	Milk powder, sugar & layer manure	CSMA**	Cow manure
Lauric acid	-	-	0.18
Myristic acid	6.83	3.2	2.56
Palmitic acid	26.74	27.6	26.40
Palmitoleic acid	25.92	20.6	13.56
Stearic acid	2.32	2.2	4.77
Oleic acid	21.75	18.3	19.17
Linoleic acid*	16.44	14.9	17.83
Linolenic acid	-	2.1	
α-Linolenic acid*	-	-	0.87
Arachidonic acid	-	-	0.07
Eicosapentaenoic acid	-	-	0.05
SFA	35.89	-	-
UFA	64.11	-	-

(*) Essential Fatty Acids

(†) % of Fatty Acids

(**) CSMA- Chemical Specialities Manufactures Association's fly rearing medium

2.3 Waste products

In South Africa organic waste originates from many different sources of which most of them can pose a health risk if not managed properly (Roberts & de Jager, 2004). Organic waste that can potentially be utilized as a feed source by the nutrient recirculation organisms mostly comes from the agricultural sector, including abattoirs, fermentation industry and food retailers.

2.3.1 Agricultural waste

Waste produced by the different agricultural sectors includes: manure waste, harvest residues, and waste from processing plants (blood, whey, condemned food etc.). This waste is often turned into compost and used as fertilizers, but an increasing percentage is being used for biogas production (Abraham *et al*., 2007). Manure, especially poultry manure can serve as a potential source of nutrients for houseflies and has been reported on by a number of authors (Calvert & Martin, 1969; Teotia & Miller, 1974; Ogunji *et al*., 2006; Adeniji, 2007; St-Hilaire *et al*., 2007). The presence of housefly larvae in poultry manure decreases the moisture content (Calvert & Martin, 1969; Teotia & Miller, 1974), organic matter, (Calvert & Martin, 1969) odour (Teotia & Miller, 1974) and improves manure texture (Teotia & Miller, 1974). The chemical composition of poultry manure varies considerably, because the composition is dependent on the bird species, bird age, feeding ration of birds, amount of feed wastages in manure and amount of feathers present (El Boushy, 1991). Storage time of manure also have an influence on the chemical composition of the manure (Flegal *et al*., 1972), with a reduction in crude protein content of the manure from 30.3% to 18.3% with an increase in storage time from seven to 98 days

(Flegal *et al*., 1972). Because of these nutrient losses with increase in storage time it is necessary to start with nutrient capturing and nutrient binding from manure as quickly as possible.

2.3.1.1 Abattoir waste

Waste that originates from abattoirs includes; blood, intestines, intestinal contents, carcass trimmings, heads, hooves/feet, hides, dead on arrivals, rejected carcasses, feathers and fat (Roberts & de Jager, 2004). In South Africa there is a market for the intestines, heads and hooves. The intestines and heads of basically all animals are sold as offal or the 5th quarter (Christoe, 2003). Feathers that are produced from chicken abattoirs can be used in the household sector for the manufacturing of pillows and duvets. Feathers are also used in the feed industry as a protein source (Dalev, 1994). Hydrolysed feather meal is rich in proteins (about 810g/kg DM) and low in energy (9.87 MJ/kg ME) (NRC, 1994).

Blood from abattoirs can be used in the manufacturing of blood meal. Blood meal is a very rich source of proteins (approximately 889g/kg DM) with a good amino acid profile, but due to certain health risks it is banned or restricted as animal feed in many countries across the world. The condemned carcasses and dead on arrivals can be used in the manufacturing of carcass meal and used in the animal feed industry but is also banned in most countries over the world. In South Africa the feeding of blood and carcass meals are not banned but the use of certain meals are deemed an unacceptable practise (Act No 36 of 1947). In Africa, Asia, Europe, America, Southwest Pacific and in the East any animal product that can be a source of Bovine Spongiform Encephalopathy (BSE) are unacceptable in terms of the Codex Alimentarius Commission (CAC/RCP 54-2004) as a source of feed to animals. Abattoir waste can be disposed of by; municipal/local authority drainage, oxidation dams, run-off into the fields or buried and condemned carcasses can be placed into a trench or in a hole dug in the ground, to undergo decomposition (Roberts & de Jager, 2004). Risks associated with these practices include contamination of ground water or environmental pollution with pathogens (Mittal, 2006). By feeding blood to dipteran larvae the risk of contamination of the environment with blood could be reduced.

The largest volume of waste is however represented by the blood and intestinal content followed by rejected carcasses (Christoe, 2003) and therefore the emphasis will be on these waste products for nutrient recirculation. This subject has received attentions by other authors as well (Aniebo *et al*., 2008; Aniebo & Owen, 2010)

The nutrient recirculating process can have a positive impact on the environment and the animal feed industry, because there is a lot of unusable abattoir waste that can be utilized in the process. To put this into perspective there are three major broiler producers in South Africa namely; Rainbow, Early Bird and County Fair chickens. Rainbow chickens is the largest producer slaughtering 4 million broilers per week (http://www.rainbowchickens.co.za/about), followed by Early Bird chickens with 2.9 million broiler a week (http://www.earlybirdfarm.co.za) and lastly County Fair chickens with 1.2 million broiler per week (http://www.countyfair.co.za), giving a combined number of 8.1 million chickens a week. If a broiler loses up to

30% of their total body weight as waste (Haitook, 2006) the 8.1 million broilers weighing about 1.9kg each will thus produce up to 4 617 tonnes of waste per week.

2.3.3 Waste from the fermentation industry

The fermentation industry includes the brewery, distillery and further milk processing factories. By-products coming from the brewery include; malt culms, brewer's grain, spent hops and brewer's yeast (McDonald, 2002). Malt culms is rich in proteins (about 375g/kg DM), but is not high in energy and is a fibrous type of feed (NDF, 536.1g/kg DM; ADF, 176.8g/kg DM) (Brouns *et al*., 1995). Brewer's grain is a concentrate source of digestible fibre that is rich in proteins (24.2% DM) and high in phosphorous, but low in other minerals that is normally fed to ruminants, pregnant sows and growing pigs (Santos *et al*., 2003). Dried brewer's yeast is a by-product rich in proteins (about 420g/kg DM) that is highly digestible with a relative high nutritive value that is a valuable source of the B vitamins (except vitamin B_{12}) and phosphorous, but has a low calcium content that is favoured by all classes of farm animals (McDonald, 2002). Spent hops are very fibrous by-product of the brewery and rarely used as animal feed and mostly sold as fertilizer (Huszcza & Bartmanska, 2008).

By-products from the distilling industry include; distillers grain, distiller's soluble, distiller's dark grain and also malt culms where the composition of distillers grains (draff) vary, depending on the starter materials, but are usually high in unsaturated fatty acids and fibre with a low dry matter content (McDonald, 2002). Distiller's grains with soluble (DGS) is a valuable source of the B vitamins and protein (ranging from 23.4 to 28.7% DM), but there is a high degree of variability in the nutritional properties of DGS available to the feed industry (Cromwell *et al*., 1993).

Whey is a by-product from the cheese making industry and its composition varies according to the type of cheese produced (Thivend, 1977). Whey is a poor source of energy, fat-soluble vitamins, calcium and phosphorus and most of the whey protein is β-lactoglobulin that is of a very good quality and usually given to pigs in a liquid form (McDonald, 2002) or dried effectively and added to creep feeds of pigs (DeRouchey *et al.,* 2008).

2.3.4 Waste coming from retailers

There are also wastages coming from already produced products, which include food loses from the farm to the retailer (substandard food and transportation losses), retail losses (past due-date products) and consumer and food service losses (uneaten and rotten products) (Kantor *et al*., 1997). Kantor *et al*. (1997) estimated the food losses in America in their unpublished data and found that the retail stores produce about 2.5 billion kilograms of waste where less than 5% comes from edible material. Waste coming from the consumer and food service is estimated at about 42.3 billion kilograms of which 26% comes from edible material with fresh fruits and vegetables accounting for 20%.

2.4 The use of house fly larvae meal in animal nutrition

The on-going increase in feed prices, especially protein sources (e.g. fishmeal) has placed more emphasis on the exploitation of alternative protein sources not only in South Africa, but all over the world. In most documented studies the use of larvae meal was compared with other protein sources for the use in animal nutrition where the effect of larvae and pupae meal was evaluated as a replacement for other protein sources commonly used in animal feed (Newton *et al*., 1977; Awoniyi *et al*., 2003; Ogunji *et al*., 2006; Adeniji, 2007; Agunbiade *et al*., 2007). Newton *et al*. (1977), Awoniyi *et al*. (2003), Ogunji *et al*. (2006), Adeniji, (2007) and Agunbiade *et al*. (2007) concluded in their studies that house fly larvae meal has a suitable nutritional composition and can serve as a replacement for fish meal as well as other protein sources normally used in animal nutrition. Table 8 gives a comparison between fish meal, full fat soya meal and soya oilcake meal. It can be seen from this table that larvae meal is superior to some of the other traditional protein sources used in animal nutrition but also, in some cases, inferior (De Koning, 2005; Aniebo *et al*., 2008). In Table 8 it is seen that housefly larvae meal has a high crude protein content that compares to that of soya oil cake meal and is higher than that of sunflower oil cake meal and lower than fish meal. The housefly larvae meal has a higher crude fat content than any other protein source listed in Table 8. Housefly larvae meal has a superior amino acid composition to that of soya and sunflower oil cake meal and compares well to that of fish meal. However house fly larvae have higher histidine and methionine concentrations than fish meal.

Table 8 Comparison between the nutritional composition of housefly larvae meal and commonly used protein sources

	House fly larvae meal[†]	Fish meal (pilchard)*	Soya oil cake meal**	Sunflower oil cake meal**
Proximate composition (% Dry Matter Basis)				
Crude Protein	50.86	68.84	49.44	35.56
Ether Extract	27.32	5.66	0.45	1.22
Crude Fibre	8.10	1.07	7.87	26.67
Ash	6.75	20.38	7.64	
Amino acids				
Lysine	6.52	8.86	3.02	1.11
Histidine	3.34	2.88	1.31	0.61
Threonine	2.19	5.34	1.93	1.17
Arginine	6.26	7.04	3.53	2.56
Valine	3.90	6.83	2.33	1.78
Methionine	2.46	2.35	0.70	0.56
Isoleucine	3.30	5.55	2.20	1.11
Leucine	6.86	8.00	3.81	1.78
Phenylalanine	4.28	4.91	2.43	1.28
Tryptophan		1.07	0.83	0.50
Cystine	0.56	4.48	0.74	0.56
Tyrosine	3.14	4.70	2.15	

(*) de Koning. (2005), (**) NRC (1994), ([†]) Aniebo *et al*. (2008)

Although larvae meal, fish meal, soya and sunflower oil cake meal are excellent sources of protein, there are still differences regarding these protein substrates proximate analyses. Table 8 gives a clear indication of how these substrates differ according to their nutritive value and how these protein sources can be used together to complement each other in the animal feed industry. House fly larvae meal should probably be utilised in combination with other protein sources and the inclusion of possible crystalline amino acids in order to present a balanced amino acid profile to the animals.

2.4.1 Layer nutrition

There is little published literature on the use of larvae meal in the diets of laying hens and the only published literature of interest was the work done by Agunbiade *et al*. (2007), where they investigated the effect when fish meal was replaced with larvae meal in the diets laying hens. Fish meal inclusion in the diets of laying hens it is not common practice due to the trimethylamine (TMA) that is found in the form of TMA oxide in fish meal that lead to fishy taint in eggs (Pearson *et al*., 1983). Table 9 summarizes the effect of larvae meal supplementation in the diets of laying hens. In the study done by Agunbiade *et al*. (2007) the effect was studied when fish meal was replaced with larvae meal in a cassava based diet in two laying hen hybrids (50 weeks in lay). The larval species used to produce the larvae meal was unfortunately not specified. The authors investigated egg production and other egg quality attributes associated with this substitution.

Table 9 Experimental diet composition and performance of layers for diets comparing different levels of fish meal and larvae meal in a soya bean-, cassava leaf- and cassava root meal based diet (Agunbiade *et al*., 2007)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredients					
Soya bean meal	19.32	19.32	19.32	19.32	19.32
Cassava leaf meal	19.32	19.32	19.32	19.32	19.32
Cassava root meal	42.46	41.67	40.74	39.88	39.02
Fish meal	6.00	4.43	3.00	1.50	-
Larvae meal	-	2.36	4.72	7.08	9.44
Premix	0.25	0.25	0.25	0.25	0.25
Oyster shell	7.20	7.20	7.20	7.20	7.20
Bone meal	2.40	2.40	2.40	2.40	2.40
Methionine	0.15	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30
Vegetable oil	2.50	2.50	2.50	2.50	2.50
Nutritional composition (g/kg DM[3])					
Crude Protein	180.90	180.00	180.00	179.80	179.40
Crude Fibre	92.90	93.80	94.60	95.50	96.40
Ca	4.21	4.12	4.00	3.94	3.80
P	0.56	0.54	0.51	0.48	0.45
ME (MJ/kg)	10.40	10.39	10.38	10.37	10.36
Layer Performance					
Avr. Feed Intake (g/bird/day)	124.00	123.17	124.00	124.67	125.00
FCR (feed/kg egg)	3.04	3.20	2.78	3.05	3.83
Hen-day production	67.43[a]	62.95[a]	70.83[a]	63.68[a]	55.22[b]

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

([1]) FM- Fish meal, ([2]) LM- Housefly larvae meal, ([3]) DM- Dry Matter

From the results shown in Table 9 it can be seen that feed intake was not affected by the experimental treatments (P>0.05). Larvae meal also had no significant influence on feed conversion ratio (P>0.05), but the hen-day production was significantly affected (P<0.05) when 3.00% fish meal and 4.72% larvae meal were fed (diet 3). This effect can be due to the complimentary effect when larvae and fish meal (included at a level of 4.72% and 3.0% respectively of the total ingredients) are supplemented together which creates a better amino acid profile supplied to the animal (Agunbiade *et al*., 2007).

Larvae meal supplementation had no significant effect (P>0.05) regarding egg quality traits (egg shape index, yolk index, yolk colour, egg weight and haugh units when compared to the control diet receiving no larvae meal (Agunbiade *et al*., 2007). The authors findings showed that when larvae meal were supplemented at a level of 7.08% together with 1.50% fish meal (diet 4) and at a level of 9.44% with no fish meal (diet 5) a significant decrease (P<0.05) in shell thickness and shell weight were observed. These differences are related to the lower calcium content associated with larvae meal supplementation (Agunbiade *et al*., 2007) and not an inherent negative effect of larvae meal *per se*.

2.4.2 Broiler nutrition

Broilers are expected to grow to market weight in the shortest possible time for maximum profit. In most of the studies where house fly larvae meal was studied the effect were investigated when other protein sources were replaced. The replacement of fish meal (Awoniyi *et al*., 2003) and ground nut oil cake meal (Adeniji, 2007) in broiler nutrition are some protein sources investigated. There is also literature of showing where the effect of house fly larvae meal supplementation was investigated in the overall production performance of broilers (Awoniyi *et al*., 2003; Adeniji, 2007; Téguia *et al*., 2002) and the digestibility of house fly larvae meal in the diets of turkeys (Zuidhof *et al*., 2003) and broilers (Hwangbo *et al*., 2009).

2.4.2.1 The effect of house fly larvae meal on broiler growth performance and feed intake

The findings of Hwangbo *et al*. (2009) where the effect of larvae meal supplementation was investigated is summarised in Table 10. The diets were formulated to contain 0% (control), 5%, 10%, 15%, 20% larvae meal respectively and these diets were formulated to be isoenergetic and isonitrogenous with similar lysine and methionine inclusion levels.

Broilers receiving diets with larvae meal supplemented at 10 and 15% respectively had significantly higher ($P<0.05$) weight gains than the broilers receiving no larvae meal. The feed conversion ratio was also significantly lower ($P<0.05$) in all the diets supplemented with larvae meal when compared to the control (Table 10). Hwangbo *et al.* (2009) attributes these differences in weight gain, high crude protein digestibility and to the essential amino acid profile of the larvae meal. These differences can also be attributed the fact that the control diet had high levels of maize gluten meal (8.00%) that could have caused the lower performance (Afshar & Moslehi, 2000). These results differ from the findings of Awoniyi *et al*. (2003), Adeniji (2007) and Téguia *et al*. (2002) who found no significant effect ($P>0.05$) of larvae meal supplementation on weight gain and feed conversion ratio (FCR).

Table 10 Performance results of broilers receiving diets supplemented with various levels of housefly larvae meal (Hwangbo *et al*., 2009)

	Control	5% Larvae meal	10% Larvae meal	15% Larvae meal	20% Larvae meal
0-3 Weeks					
Live weight (g)	658	698	665	662	671
Feed intake (g)	925	931	928	919	941
FCR[1]	1.40	1.33	1.39	1.39	1.40
4-5 Weeks					
Live weight (g)	1020[b]	1077[b]	1113[a]	1123[a]	1107[b]
Feed intake (g)	1889	1861	1854	1852	1835
FCR	1.85[a]	1.72[b]	1.66[b]	1.65[b]	1.66[b]
0-5 Weeks					
Live weight (g)	1638[b]	1775[a]	1778[a]	1785[a]	1778[a]
Feed intake (g)	2814	2792	2782	2771	2776
FCR	1.71[a]	1.57[b]	1.56[b]	1.55[b]	1.56[b]

([1]) Feed Conversion Ratio (amount of feed needed to gain 1kg body weight)

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

Table 11 summarizes the findings of Téguia *et al*. (2002) where they studied the effect of larvae meal supplementation in broiler nutrition and its effect on performance and carcass characteristics in the starter, grower and finisher phases. The species of fly larvae used was not reported. All the treatment diets were formulated to have similar nutritional values, but the control diet contained no larvae meal. Results showed that there was no significant effect (P>0.05) regarding weight gain when 10% (diet 3) of the fish meal was replaced with larvae meal as compared to the control group (diet 1) in the starter phase. This may be attributed to the lower crude protein concentration (22.65%) as compared to the other treatment diets in the starter phase (Table 11). When 5% (diet 2) and 15% (diet 4) of the fish meal was replaced with larvae meal in the starter phase the weight gain was higher and this effect was found to be significantly better (P<0.05). During the finisher phase, Téguia *et al*. (2002) replaced 50% (diet 6) and 100% (diet 7) of the fish meal with larvae meal respectively. These authors found that there was no significant effect (P>0.05) on weight gain when 50% of the fish meal was replaced with larvae meal when compared to the control diet (diet 5). The weight gain was significantly better (P<0.05) when 100% of the fish meal was replaced with larvae meal when compared to the control diet. The overall inclusion levels of larvae meal were, however, very low and ranging between 0.23% and 2%.

Table 11 Diet composition and performance of broiler chickens when fish meal is replaced with larvae meal (Téguia *et al*., 2002)

	Starter Phase				Grower Phase		
Ingredients (% of diet)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**	**Diet 7**
Maize	62.50	62.50	62.50	62.50	56.00	56.00	56.00
Wheat middling	-	-	-	-	20.00	20.00	20.00
Soya bean meal	12.00	12.00	12.00	12.00	7.00	7.00	7.00
Cotton seed oil cake	10.00	10.00	10.00	10.00	4.00	4.00	4.00
Fish meal	4.50	4.28	4.05	3.83	2.00	1.00	-
Larvae meal	-	0.23	0.45	0.68	-	1.00	2.00
Premix	10.00	10.00	10.00	10.00	10.00	10.00	10.0
NaCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Nutritional composition (%DM[3])							
Metabolizable Energy (MJ/kg)	11.41	11.41	11.41	11.41	10.77	10.79	10.80
Crude Protein	23.19	23.61	22.65	23.05	20.88	21.23	20.99
Crude Fibre	4.52	4.51	4.44	4.48	4.86	5.00	4.95
Ash	2.75	3.83	3.70	3.75	8.50	8.42	8.43
Performance Results							
Live Weight Gain (g)	678.25^{c}	795.38^{ab}	717.50^{bc}	837.13^{a}	1062.19^{b}	1125.63^{ab}	1209.38^{a}
Feed Intake (g)	1356.50^{b}	1415.77^{ab}	1377.60^{b}	1456.58^{a}	2718.59^{b}	2972.81^{a}	2668.28^{b}
Feed Conversion	2.00	1.78	1.92	1.74	2.63	2.65	2.23

(^{abc}) Means with different superscript within the same column differ significantly (P<0.05), within a specific phase

(^{1}) LM- Larvae meal, (^{2}) FM- Fish meal, (^{3}) DM- Dry Matter

Adeniji (2007) also found that larvae meal had no significant effect (P>0.05) on feed intake and this agrees with the results found by Hwangbo *et al*. (2009). Results reported indicated that when 75% and 100% of groundnut oilcake meal was replaced by larvae meal, dry matter intakes were not influenced and this supports the data found by Awoniyi *et al*. (2003). These authors replaced fish meal with larvae meal at levels of 25, 50, 75 and 100% respectively with no significant effect on feed intake (P>0.05). The effect of larvae meal supplementation is more visible after three weeks of age and this may be due to the difference in which adults and young broiler chickens utilize the larvae meal protein (Awoniyi *et al*., 2003).

2.4.2.2 House fly larvae meal and broiler carcass characteristics

Results reported by Hwangbo *et al*. (2009) showed that larvae meal supplementation in the diets of broilers led to significantly better (P<0.05) carcass characteristics, such as; dressing percentage yield as well as breast muscle and thigh muscle yield as percentage of carcass weight. Table 12 shows how various carcass characteristics were influenced by the supplementation of larvae meal. It was noticed that broilers receiving larvae meal supplementation had a significantly higher (P<0.05) dressing percentage, breast muscle (% carcass weight) and thigh muscle (% carcass weight) yields when compared to the control group (Hwangbo *et al*., 2009). There was however no significant effect (P>0.05) with larvae meal supplementation on the amount of abdominal fat, as a percentage of the carcass weight and this supports the data found by Téguia *et al*. (2002).

Table 12 The influences of house fly larvae meal on broiler carcass characteristics (Hwangbo *et al*., 2009)

	Treatment diet (% Larvae Meal)				
	Control	**5%**	**10%**	**15%**	**20%**
Dressing Percentage	64.19[b]	66.07[a]	65.85[a]	65.87[a]	65.34[a]
Breast muscle (%CW)[1]	17.27[c]	18.84[b]	19.51[ab]	19.35[ab]	18.77[b]
Thigh muscle (%CW)	22.10[b]	23.74[a]	23.14[a]	23.74[a]	23.58[a]
Abdominal fat (%CW)	2.28	2.16	2.41	2.28	2.33

([1]) Percentage of Carcass Weight

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

Awoniyi *et al*. (2003) found that larvae meal supplementation had no significant influence on dressing percentage and breast muscle weights and this agrees with the findings of Téguia *et al*. (2002), but differs from the findings of Hwangbo *et al*. (2009). This contradictory literature could also be attributed to the trial design where Hwangbo *et al*. (2009) had 30 replicates per treatment in relation to the six replicates of Awoniyi et al., 2003 and the four replicates of Téguia *et al*. (2002).

Hwangbo *et al*. (2009) also studied the influence of larvae meal on broiler breast meat colour. Colour was determined by cutting of a piece of breast meat and allowing it to bloom for 30 minutes were after the colour of each sample was measured five times by a colorimeter to obtain the CIElab values (L*, lightness; a*, redness; b*, yellowness). The results showed that larvae meal supplementation had no significant effect (P>0.05) on meat colour regarding the CIElab L*, a* and b* values (Table 13).

Table 13 Effects of housefly larvae meal on meat colour of breast muscle from broiler chickens (Hwangbo *et al*., 2009)

	Treatment diet (% Larvae Meal)				
CIElab colour values	**0%**	**5%**	**10%**	**15%**	**20%**
L*	46.77	46.97	47.55	47.51	46.88
a*	5.80	5.25	5.73	6.01	5.78
b*	9.10	8.85	9.67	9.70	8.94

Means with different superscripts within the same row differ significantly (P<0.05)

2.4.2.3 House fly pupae meal in broiler nutrition

Calvert *et al*. (1971) studied the effect of growth response when housefly pupae meal is supplemented in the diets of growing chicks. In their study they tested two different treatment diets where the one treatment diet contained mainly soybean oil cake meal as a protein source and in the second treatment diet contained only dried house fly pupae meal as protein source. Results (Table 14) revealed that supplementation of the diet with larvae meal were beneficial in terms of weight gain per bird during the first 14 days if it was supplied for the total period whereas supplementation only from day seven onwards had no benefit.

Table 14 Performance of broiler chickens fed either a soybean diet or a house fly pupae meal diet (Calvert *et al*., 1971)

	Larvae meal diet	Pupae meal diet
Chicks fed from 7-14 days		
Weight gain (g/bird)	63	62
Feed intake (g/bird)	108	113
Feed conversion	1.71	1.82
Chicks fed from 1-14 days		
Weight gain (g/bird)	87[a]	96[b]
Feed intake (g/bird)	183	192
Feed conversion	2.10	2.00

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

These results show a similar pattern as results obtained in other studies (Hwangbo *et al*., 2009). Teotia & Miller (1974) studied the feeding value of housefly pupae for Single Comb White Leghorn chicks when compared to a diet containing soya bean meal from post-hatch until four weeks of age. Their findings showed no significant differences (P>0.05) regarding larvae meal supplementation on weight gain, feed intake and feed conversion ratio.

2.4.2.4 The digestibility of house fly larvae meal

There exists limited literature regarding the digestibility of housefly larvae meal in monogastric animals. Zuidhof *et al*. (2003) reported total tract digestibilities of dehydrated housefly larvae meal in turkey poults. These results are summarized in Table 15. The results show that there is a significant difference (P<0.05) regarding the coefficient of total tract digestibility in the dehydrated housefly larvae meal as compared to the commercial diet. The coefficients of total tract digestibility were significantly higher for gross energy, crude protein and all the amino acids except for cystine. Hwangbo *et al*., 2009 also reported that larvae meal had apparent digestibilities for crude proteins of 98% and the essential amino acids of 94.8%.

Table 15 Composition (Dry Matter basis, g/kg) and coefficient of total tract apparent digestibility of the dehydrated house fly larvae meal diet and a commercial diet for turkey poults (Zuidhof *et al*., 2003)

	Nutrient level in the diet				Coefficient of total tract apparent digestibility			
	DLM[1]	**S.E.[2]**	**CD[3]**	**S.E.**	**DLM**	**S.E.**	**CD**	**S.E.**
Gross Energy (MJ/kg)	23.1[a]	0.3	17.0[b]	0.0	0.777	0.005	0.775	0.004
AME[4] (MJ/kg)	17.9[a]	0.1	13.2[b]	0.1				
Crude Protein	593.0[a]	7.0	318.0[b]	8.5	0.988[c]	0.001	0.971[d]	0.001
Alanine	34.2[a]	0.2	14.2[b]	0.3	0.944[c]	0.001	0.846[d]	0.004
Arginine	28.7[a]	0.1	17.9[b]	0.2	0.917[c]	0.002	0.871[d]	0.005
Aspartic acid	50.2[a]	0.1	24.2[b]	0.1	0.932[c]	0.002	0.884[d]	0.003
Cystine	4.6[b]	0.1	5.4[a]	0.3	0.781[c]	0.006	0.779[c]	0.005
Glutamic acid	72.7[a]	0.9	56.6[b]	0.8	0.939[c]	0.002	0.932[d]	0.002
Glycine	24.9[a]	0.3	15.3[b]	1.0	0.880[c]	0.003	0.800[d]	0.005
Histidine	21.2[a]	0.2	9.1[b]	0.2	0.943[c]	0.002	0.859[d]	0.004
Isoleucine	22.1[a]	0.3	20.0[b]	0.1	0.939[c]	0.002	0.895[d]	0.003
Leucine	35.3[a]	0.3	23.4[b]	0.3	0.935[c]	0.002	0.924[d]	0.002
Lysine	38.7[a]	0.4	15.2[b]	0.2	0.969[c]	0.001	0.861[d]	0.004
Methionine	14.8[a]	0.1	5.0[b]	0.2	0.977[c]	0.001	0.903[d]	0.003
Phenylalanine	30.9[a]	0.3	13.9[b]	0.0	0.965[c]	0.001	0.902[d]	0.004
Proline	22.4[a]	0.3	20.4[b]	0.0	0.897[c]	0.003	0.894[c]	0.003
Serine	23.1[a]	0.3	14.2[b]	0.1	0.910[c]	0.004	0.860[d]	0.005
Threonine	23.7[a]	0.1	10.7[b]	0.1	0.913[c]	0.003	0.780[d]	0.006
Tryptophan	8.5[a]	1.0	4.9[b]	0.1	0.931[c]	0.002	0.876[d]	0.004
Tyrosine	34.7[a]	0.7	6.4[b]	0.1	0.980[c]	0.001	0.838[d]	0.009
Valine	29.0[a]	0.2	13.9[b]	0.1	0.938[c]	0.002	0.877[d]	0.000
Ca	4.4[b]	0.3	15.8[a]	0.6	0.448[d]	0.031	0.994[c]	0.001
P	10.9[b]	0.1	11.6[a]	0.1	0.804[d]	0.010	0.900[c]	0.004

([1]) DLM - Dehydrated Larvae meal ([2]) S.E. - Standard Error ([3]) CD - Commercial Diet

([4]) AME - Apparent Metabolizable Energy

([a,b]) Means in the rows within the nutrient level with different superscripts are significant different ($P < 0.05$)

([c,d]) Means in the rows within the coefficient of total tract apparent digestibility with different superscripts are significant different ($P < 0.05$)

2.5 House fly larvae meal and meat quality

The main factors that determine broiler meat quality can be divided into the appearance and physical characteristics and these factors are exclusively determined by the consumer. The appearance or colour of the meat is the first quality factor taken into account by the consumer and it determines if the meat will be purchased or not. The acidity of the meat is an important process that occurs especially when the muscle are converted to meat and by ensuring the pH of the meat gives an indication of the degree of meat acidification after slaughter (Allen *et al*., 1998; Qiao *et al*., 2001; Swatland, 2004). The rate and extend of the pH decline has an effect on the colour, water holding capacity as well as the tenderness of the meat (Van Laack *et al.*, 2000; Huff-Lonergan & Lonergan, 2005). There are numerous articles that demonstrate a significant relationship between the pH of the meat and the meat colour (Allen *et al*., 1998; Qiao *et al*., 2001; Swatland, 2004). Allen *et al*. (1998) reported that dark coloured broiler meat had higher pH values than lighter coloured meat, but the darker meat had a

reduced shelf-life that can be attributed to the increasing number of psychotropic bacteria that colonize the darker meat.

The water holding capacity is a physical characteristic that is an important factor in determining meat quality, because it influences the appearance of the meat prior to cooking as well as tenderness and juiciness during consumption (Huff-Lonergan & Lonergan, 2005). Cooking loss is another measure of the water holding capacity and during cooking the meat proteins denature and cellular structures are disrupted causing extra- and intracellular water to be released (Huff-Lonergan & Lonergan, 2005). During the process of *rigor mortis* when the muscle is converted to meat, the pH of the muscle declines until the major muscle proteins reaches the isoelectric point and this process leads to the expulsion of water into the extracellular space that is known as drip loss (Huff-Lonergan & Lonergan, 2005). The pH of the meat was shown to affect this process (Van Laack *et al*., 2000). If the pH is above the isoelectric point of the major proteins (pH= 5.3) it causes the water molecules to be more tightly bound, causing more light to be absorbed by the meat giving a paler colour (Van Laack *et al*., 2000).

2.6 Cost effectiveness of housefly larvae meal

Larvae meal production has the potential to be cost effective. Fashina-Bombata & Balogun (1997) completed a study were they compared the cost of the larvae meal production with that of fishmeal. These authors found that the cost of harvesting and processing the larvae meal was less than 20% of the cost of a similar weight in fishmeal. Ajani *et al*. (2004) in a later study reported that the replacement of fishmeal with 50% and 100% larvae meal has led to a reduction in cost of tilapia production by 18% and 28% respectively.

2.7 Conclusion

It is concluded from this literature review that insects belonging to the order Diptera show great potential as an alternative renewable protein source that can replace conventional protein sources used in animal nutrition. *Musca domestica* (common house fly) larvae meal has proven itself to be a suitable protein source that can be incorporated in the diets of broilers with no undesirable effects. House fly larvae meal has a high crude protein content ranging from 37.5% to 63.1% and a crude fat content ranging from 15.5% to 25.3%. The larvae meal also has a good amino acid profile that compares to that of fish meal. Differences were observed between larvae and fish meal when the ideal amino acid profile required by broilers were compared. These shortcomings can be overcome by adding crystalline amino acids or by feeding larvae meal in combination with other protein sources in broiler diets to obtain the ideal amino acid profile required. The performance of broilers where not effected when other protein sources (fish meal, soya and groundnut oil cake meal) were replaced with larvae meal in the diets of broilers. Some authors reported that performance (feed intake and live weight) of broilers were better with some degree of larvae meal supplementation. House fly larvae meal has a high total tract protein (98.8%) and amino acid (94.8%) digestibility that is higher than that of sunflower and soya oil cake meal. No adverse effects were found regarding carcass characteristics of larvae fed broilers.

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CHAPTER 3

Determining the nutritional composition of dried *Musca domestica* larvae and pupae meal produced under the same environmental conditions

3.1 Abstract

The nutritional composition of *Musca domestica*, common house fly, larvae and pupae meal was investigated using laboratory analysis. The proximate analysis of *M. domestica* larvae meal showed that it contained, on a dry matter basis, a gross energy value of 20.10 MJ/kg, 60.38% crude protein, 14.08% crude fat and 10.68% ash and that the house fly pupae contained a gross energy of 20.42 MJ/kg, 76.23% crude protein, 14.39% crude fat and 7.73% ash. The Arginine to Lysine ratio of larvae meal was calculated as 0.67 and 0.91 for pupae meal and the ratio of Isoleucine to Leucine was calculated as 0.68 for larvae meal and 0.64 for pupae meal. House fly pupae meal had the best amino acid profile compared to the ideal amino acid profile required by broilers and has an arginine relative to the lysine content closer to the ideal amino acid profile than the house fly larvae meal. The house fly pupae could serve as a good source of lysine and arginine in poultry nutrition. The amino acid composition compared well with other known protein sources (soybean oil cake meal and fish meal) used in broiler diets. The essential fatty acid, Linoleic acid, was found at levels of 26.25 and 36.27% of the total fats for the house fly larvae and pupae meal respectively. *M. domestica* larvae and pupae meal compared favourably to other protein sources used in animal nutrition.

Keywords- Nutritional composition, larvae meal, pupae meal, protein source

3.2 Introduction

House fly larvae meal is classified as a protein source, because the crude protein content varies between 37.5% and 63.1%. This variation is mostly due to differences in age at harvest (Calvert *et al*., 1970; Inaoka *et al*., 1999; Newton *et al*., 2004; Aniebo *et al*., 2008; Aniebo & Owen 2010), method of drying (Fasakin *et al*., 2003; Aniebo & Owen, 2010) and larval feed substrate (Newton *et al.*, 1977). The data reported by Fasakin *et al*. (2003) indicated that the different processing methods had an influence on the nutritive value of housefly larvae. Table 16 summarizes the nutritional composition of larvae and pupae protein sources as reported by various authors

A limited number of authors reported on the mineral composition of house fly larvae and pupae meal (Teotia & Miller 1974; Fasakin *et al*., 2003). House fly pupae were shown to have a much higher mineral composition than the house fly larvae, but the larvae had a much higher Fe content than the pupae (1317.34ppm vs. 465ppm), when maintained on the same feed source. House fly larvae meal compares well to other protein sources, such

as soybean oil cake and fish meal. House fly larvae meal has higher calcium, phosphorus, metabolizable energy (ME) and protein content when compared to soya oil cake meal (National Research Council, 1994). Fish meal has higher calcium and protein contents than larvae meal, but larvae meal has higher ME values due to its higher fat content than fish meal (National Research Council, 1994).

Table 16 Results obtained for proximate analysis (Dry matter basis) of the house fly larvae and pupae meal

	Zuidhof *et al*., 2003	**Ogunji *et al*., 2006**	**Aniebo *et al*., 2008**	**Hwangbo *et al*., 2009**	**St-Hilaire *et al*., 2007**	**Teotia & Miller., 1974**
Physiological Stage	Larvae	Larvae	Larvae	Larvae	Pupae	Pupae
Gross Energy (MJ/kg)	23.10	20.30	-	-	-	-
Crude Protein	59.30	38.90	50.81	67.98	79.91	61.40
Crude Fat	-	20.54	27.29	25.83	18.27	9.30
Crude Fibre	-	-	8.09	-	-	-
Ash	-	23.96	6.74	5.48	11.12	-

House fly larvae meal can be used successfully with other feed substrates providing the animal a balanced diet containing sufficient amounts of essential fatty acids, because house fly larvae contain both linoleic and linolenic acid (Hwangbo *et al*., 2009). Larvae was reported to contain higher percentages of palmitoleic acid (16:1n7), oleic acid (18:1n9), and linoleic acid (18:2n6) as essential fatty acids than the house fly pupae (Calvert *et al*.,1970). The fatty acid profile of the house fly larvae is largely influenced by nutrition with fatty acid composition being one of the first observed changes in the larvae in response to changes in nutrition (Hwangbo *et al*., 2009).

Due to the variation in nutritional composition reported by authors (Calvert *et al*., 1970; Teotia & Miller, 1974; Newton *et al*., 1977; Inaoka *et al*., 1999; Fasakin *et al*., 2003; Newton *et al*., 2004; Ogunji *et al*., 2006; Sogbesan *et al.*, 2006; Aniebo *et al*., 2008) it was decided to determine the nutritional composition of the house fly larvae and pupae. The objective of this study was to determine the nutritional composition of house fly pupae and larvae meal fed a milk powder, sugar and yeast diet, grown in a bran substrate to 36 hours post hatch.

3.3 Materials and methods

3.3.1 Larvae rearing and drying

Larvae were maintained on bran substrate and fed a standardised diet consisting of water, milk powder, sugar and yeast. Larvae were either harvested at 36 hours post hatch or allowed to pupate. Harvesting was done using a flotation method and killed by freezing at -20 ºC for 24 hours. Larvae and pupae were removed from the freezer and allowed to defrost at room temperature before drying in a ventilated oven at 65°C for 12 hours (pupae) and 24 hours (larvae). After drying the larvae and pupae were milled through a 3mm sieve using a

Christy and Norris junior laboratory mill. Milled samples were stored at -20°C until laboratory analyses were done.

3.3.2 Analytical methodologies

Analytical methodologies were performed at the Department of Animal Science, Stellenbosch University except for amino acid determinations where hydrolysis was done at the Stellenbosch University and amino acids analysis was done at the Institute of Animal Production, Western Cape Department of Agriculture.

3.3.2.1 Dry matter determination

The dry matter (DM) of the larvae and pupae meal was determined according to the Association of Official Analytical Chemists International (2002), Official Method 934.01. Two subsamples of each sample weighing 2g respectively were placed in a crucible drying for 24 hours at 100ºC. Thereafter the dry sample was weighed and the DM content was calculated using Equation 1:

Equation 1

$$\%\ \text{Moisture} = \frac{(\text{A} + \text{B}) - \text{C}}{\text{B}} \times \frac{100}{1}$$

$$\%\ \text{Dry Matter} = 100 - \%\ \text{Moisture}$$

Where:
A = Weight of empty and dry crucible
B = Weight of air dried test sample
C = Weight of crucible and moisture free test sample

3.3.2.2 Ash determination

The subsamples retained from the dry matter analysis were used for the determination of ash content. This method was followed as provided by the Association of Official Analytical Chemists International (2002), Official Method 942.05. These subsamples were combusted in a combustion oven for six hours at 500ºC. Thereafter the combusted subsamples were weighed and the Ash content was calculated using Equation **2**:

Equation 2

$$\%\ \text{Ash} = \frac{(\text{D} - \text{A})}{\text{Sample mass}} \times \frac{100}{1}$$

$$\%\ \text{Organic matter} = 100 - \%\ \text{Ash}$$

Where:
A = Weight of empty and dry crucible
D = Weight of crucible and ash

3.3.2.3 Crude protein determination

The crude protein content of the larvae and pupae meal subsamples were determined by measuring the total nitrogen (N) content according to the method described by Association of Official Analytical Chemists International (2002), Official Method 4.2.07, in the LECO FP528 apparatus. Two subsamples each weighing 0.1g were placed in a tin cup and then placed into the LECO FP528. Thereafter the N content was directly taken from the LECO FP528 and the Crude Protein (CP) content was calculated by using Equation 3:

Equation 3

$$\text{Crude Protein } (\%) = \text{Nitrogen } (\%) \times 6.25$$

3.3.2.4 Sample hydrolysis for amino acid determination

The amino acid profile was determined by the method described by Cunico *et al*., (1986). Firstly the samples were prepared trough hydrolysis and then the total amino acid profile was determined. During hydrolyses a sample weighing 0.1g was placed into a specialized hydrolysis tube. Six millilitres of a 6N Hydrochloric acid and a 15% Phenol solution were added to the respective samples. The samples were then placed under a vacuum by using a vacuum pump and N was added under pressure, hereafter the tubes were sealed off with a blue flame. These sealed samples were then left to hydrolyse for 24 hours at 110 ºC. After hydrolysis the samples were taken out of the tubes and placed into Eppendorf tubes and refrigerated until amino acid determination.

After hydrolysis the samples underwent a pre-column derivatisation of the amino acids and were separated using High Performance Liquid Chromatograph. This procedure was completed by the detection of the amino acids using a fluorescence detector.

3.3.2.5 Crude fat determination

The Crude Fat or Ether Extract (EE) content was determined by making use of the diethyl ether reagent method using the Tecator Soxtec System HT 1043 Extraction Unit according to Association of Official Analytical Chemists International (2002), Official Method 920.39. Two subsamples of each sample weighing 2g respectively were placed in a soxhlet fat beaker. Thereafter 50ml of diethyl ether was added to the subsample and placed into the Tecator Soxtec System HT 1043 Extraction Unit. The subsamples were placed in a drying oven for 2 hours at 100ºC. The Crude Fat content was then calculated by using Equation 4:

Equation 4

$$\%\ \text{Crude Fat} = \frac{(\text{Mass of soxhletbeaker} + \text{Fat}) - (\text{Mass of soxhletbeaker})}{\text{Mass of Sample}} \times \frac{100}{1}$$

3.3.2.6 Gross energy determination

The determination of the gross energy was performed using the CP 500 isothermal bomb calorimeter as described by the digital data system (DDS) CP 500 operating manual. Two subsamples of each sample weighing 0.5g respectively were pelletized. The pelletized subsample was then placed in the bomb and filled with pure oxygen until 3000 kPa was reached. The bomb was then placed into the CP 500 Bomb Calorimeter and the gross energy was directly taken from it measured in MJ/kg and standardized with benzoic acid.

3.3.2.7 Crude fibre determination

The crude fibre determination was performed according to Association of Official Analytical Chemists International (2002), Official Method 962.09. Two subsamples of each sample weighing 1g were placed into a glass crucible and thereafter into the Fibertec/Dosifiber extrusion apparatus. Boiling 0.128M H_2SO_4 was added and the samples were left to cook for 30 minutes where after the subsamples were washed three times with distilled water. Thereafter 0.313M sodium hydroxide was added and the samples were left to cook for 30 minutes and then the subsamples were again washed three times with distilled water. After the completion of this procedure the sample were dried at 100ºC for 24 hours and then combusted in a combustion oven for 6 hours at 500ºC. The crude fibre content was then calculated by using Equation **5**:

Equation 5

$$\text{Crude Fibre}\ (\%) = \frac{\text{A} - \text{B}}{\text{Sample mass (g)}} \times \frac{100}{1}$$

Where:
A = Sample and crucible after drying
B = Sample and crucible after ashing

3.3.2.8 Fatty acid determination

Fatty Acid composition was determined according to the method as described by Van Jaarsveld *et al*. (2000) and Kovacs *et al*. (1979) using the thermo Finnigan Focus gas chromatograph (GC). This method works on the basis of lipolysis, because the lipid bonds are broken and the fatty acids are extracted from the samples. Thereafter the extracted fatty acids were methylated and then analysed by gas chromatography. During the methylation procedure 2g of the sample was weighed into an extraction tube. Thereafter 20ml of

Chloroform:Methanol (2:1) and an internal standard were added. The samples were left to polytron for one minute and were then transferred to an extraction funnel and afterwards the contents were dried by using a vacuum filter. The flask was again filled up with 50ml Chloroform:Methanol (2:1) solution and mixed then 250µl was transferred to a Kimax tube and dried under nitrogen in a water bath at 45°C. Thereafter 2ml transmethylating reagent was added and left in the water bath at 70°C for two hours. After the samples were left to cool, 1ml of distilled water and 2ml of hexane-vortex were added and the top phase was transferred to the Kimax tube. The samples were again dried under nitrogen in a water bath at 45°C and the tube was sealed and stored at 4°C. The samples were then analysed by gas chromatography to determine the fatty acid content of the samples.

3.3.2.9 Mineral analyses

Mineral composition was determined using the combustion method as described by the Agricultural laboratory association of Southern Africa (ALASA) handbook of feeds and plant analysis volume 1, method no. 6.1.1 for feeds and plants. Two grams of the dry larvae and pupae meal samples was combusted for eight hours at 480ºC. After combustion, 5ml of a 1:1 Hydrochloric acid solution was added to the sample and made up to 40ml by distilled water. The results of the test samples were then directly taken from the Inductively Coupled Plasma. In this method the minerals: P, K, Ca, Mg, Na, Cu, Mn, Fe, Al, Zn and B were determined.

3.4 Results and discussion

Table 17 summarizes the composition of house fly larvae obtained through the different laboratory methods. Current results were compared with published results and it was noted that the literature values for the crude protein content of house fly larvae meal ranged between 38.9% (Ogunji *et al*., 2006) and 67.98% (Hwangbo *et al.*, 2009). Crude protein values obtained in the current study (60.38%) is comparable to that reported by Zuidhof *et al*. (2003) but was higher than reported by Ogunji *et al*. (2006) and Aniebo *et al.* (2008) and lower than reported by Hwangbo *et al*. (2009) (Table 16). The high crude protein content reported by Hwangbo et al., 2009 could be related to the larval growth medium, because these authors maintained their larvae on a mixture of milk powder and sugar in poultry manure, the high urea concentration in the poultry manure could attribute to the higher nitrogen (McDonald, 2002) values in the larvae, hence higher crude protein values. Although blood is rich in proteins Aniebo *et al*. (2008) still obtained lower crude protein values when their larvae were maintained on a mixture of cattle blood and bran. These low crude protein values could be related to the fact that these authors dried their larvae at 105ºC that could lead to some nitrogen becoming volatile (Papadopoulos, 1989), giving the lower protein values.

Crude fat content of the house fly larvae meal ranges from 14.44% (Fasakin *et al*., 2003) to 27.29% (Aniebo *et al*., 2008). The crude fat values obtained in the current study (14.08%) are comparable to the oven dried larvae analysed by Fasakin *et al*. (2003), but is below that reported by Aniebo *et al*. (2008). Although these authors

used similar laboratory methods, the diethyl ether reagent method, the differences here can probably be related to larvae feed substrate, since Fasakin *et al*. (2003) fed poultry manure and Aniebo *et al.* (2008) fed a mixture of cattle blood and bran. Few authors reported on the gross energy values of house fly larvae meal, but it was noted that some literature values for gross energy ranges from 20.30 MJ/kg (Ogunji *et al.*, 2006) to 23.10 MJ/kg (Zuidhof *et al*., 2003). The gross energy value of the house fly larvae meal obtained in the current study (20.10 MJ/kg) is comparable to the values reported by Ogunji *et al*. (2006), but Zuidhof *et al*. (2003) reported higher gross energy values. Aniebo *et al*., 2010 reported that the fat content increases significantly ($P<0.05$) with age, hence a higher gross energy value.

A comparison of results obtained for the composition of pupae meal in the current study with published literature showed that values for the crude protein concentration of house fly pupae meal ranges between 61.4% (Teotia & Miller, 1974) and 79.91 % (St-Hilaire *et al*., 2007). Crude protein values obtained for the house fly pupae meal in the current study (76.23%) is comparable to the results of St-Hilaire *et al.* (2007), but was higher than the results reported by Teotia & Miller (1974). Teotia & Miller (1974) maintained their larvae on poultry manure and also dried their larvae using the same technique as in the current study. Variation observed in results might be attributable to differences in analysis with standard methods changing from 1970 to date (Horowitz, 1965: Association of Official Analytical Chemists International, 2002)

The reported values for the crude fat content of the house fly pupae meal ranged between 9.3% (Teotia & Miller, 1974) and 18.27% (St-Hilaire *et al.*, 2007). Crude fat content of the house fly pupae meal obtained in the current study (14.39%) is comparable to the results of Calvert *et al*. (1970) but is lower than the results of St-Hilaire *et al.* (2007), but higher than that reported by Teotia & Miller (1974). The literature values for the crude fibre content of house fly pupae meal ranged between 9.3% (Teotia & Miller, 1974) and 16.1% (St-Hilaire *et al.*, 2007). Crude fibre content of the house fly pupae meal obtained in the current study is comparable to the results by St-Hilaire *et al*. (2007) and is higher than the results of Teotia & Miller (1974). The crude fibre content of the house fly larvae meal obtained in the current study (8.59%) is comparable to the values reported by Aniebo *et al*. (2008) of 8.09%. By reviewing these research articles there are no reports on the age of these larvae after pupation, it could have been that metamorphisms in some pupae are in more advance stages (Williams & Birt, 1972) than other pupae which could have an influence on the overall results obtained.

Table 17 shows that the main difference observed between the house fly larvae and pupae meal is that the pupae has high crude protein content (76.23% against 60.38%). This difference can be explained by the fact that the pupae are covered with a chitin layer that consist of nitrogen- hydrogen bonds (Kramer & Koga, 1986). This higher N content of the pupae led to an increase in the calculated protein content, due to the method of analyses used (Association of Official Analytical Chemists International (2002), Official Method 4.2.07).

Table 17 also show how both house fly larvae and pupae meal compares to other protein sources (National Research Council, 1994). House fly larvae and pupae meal is well comparable to other known protein sources, although they do not have as high crude proteins content as found in blood meal (86.28%) it however is higher than soybean oil cake meal (49.44%). The house fly pupae meal has higher crude protein content than dehydrated fish meal whereas the house fly larvae meal has lower crude protein content. Both house fly larvae and pupae meal has a higher crude fat content than found in blood meal (1.70%), dehydrated fish meal (10.11%) and soya oil cake meal (0.90%).

Table 17 Nutritional composition (DM basis) of housefly larvae, pupae meal, fish meal, soya oil cake meal and blood meal

	Larvae Meal dried at 65ºC	Pupae Meal dried at 65ºC	Fish meal (dehydrated)**	Soya oil cake meal**	Blood meal (spray dried)**
Proximate analysis					
Gross Energy (MJ/kg)	20.10	20.42	18.57	18.92	21.08
Crude Protein (%)	60.38	76.23	69.13	49.44	86.28
Crude Fat (%)	14.08	14.39	10.11	0.90	1.70
Crude Fibre (%)	8.59	15.71	0.54	7.87	0.53
Ash (%)	10.68	7.73	-	5.90	-
Mineral Content					
Phosphorus (%)	2.40	1.72	1.77	0.73	0.45
Potassium (%)	1.27	1.25	0.40	2.25	0.19
Calcium (%)	0.41	0.52	1.34	0.33	0.59
Magnesium (%)	1.15	0.82	0.33	0.30	0.17
Sodium (mg/kg)	8243.79	5718.18	3260.87	1123.60	3404.26
Iron (mg/kg)	275.26	257.54	326.09	134.83	2148.94
Copper (mg/kg)	18.18	37.51	-	24.72	10.64
Zinc (mg/kg)	325.36	363.42	82.61	44.94	4.26
Manganese (mg/kg)	348.57	415.93	54.35	32.58	5.32
Boron (mg/kg)	0.68	0.86	-	-	-
Aluminium (mg/kg)	20.62	7.03	-	-	-
Amino Acid Content					
Lysine*	3.43	4.92	3.57	3.05	7.5
Aspartic acid	3.92	6.64	-	-	-
Glutamic acid	6.35	9.16	-	-	-
Serine	1.58	2.56	2.20	2.60	3.34
Histidine*	0.58	0.86	2.37	1.33	3.53
Glycine	2.25	3.13	6.40	2.15	4.88
Threonine*	1.93	2.31	1.47	1.95	3.35
Arginine*	2.31	4.50	3.12	3.56	3.86
Alanine	3.48	3.11	-	-	-
Tyrosine	2.50	4.06	0.85	2.16	2.20
Valine*	2.76	3.37	2.41	2.35	7.74
Methionine*	0.47	1.37	1.09	0.70	0.59
Phenylalanine*	2.58	3.61	1.61	2.45	6.02
Isoleucine*	1.92	2.63	2.11	2.22	1.01
Leucine*	2.84	4.14	3.43	3.84	11.20
Recovery rate	64.61	73.97	-	-	-

(**) NRC, 2004

(*) Essential Amino Acids

A limited number of authors reported on the mineral content (ash) of larvae meal and it were reported that the ash content ranged between 5.16% (Aniebo *et al*., 2008) and 23.96% (Ogunji *et al*., 2006), but unfortunately these authors did not report on the actual mineral composition of larvae diets. Ash values of the house fly larvae meal obtained in the current study are not comparable to any published results. Since similar methods were employed the lower ash content in comparison to the findings of Hwangbo *et al*. (2009) may be related to age (Kramer & Koga, 1986) or feed substrate.

Table 18 Calculated amino acid to lysine ratios in comparison to the ideal amino acid profile for broiler chicks

Amino Acid	Larvae Meal dried at 65ºC	Pupae Meal dried at 65ºC	Fish meal (dehydrated)	Soya oil cake meal	Blood meal	Ideal Amino Acid profile*
Lysine	100	100	100	100	100	100
Threonine	56	47	41	64	45	65
Arginine	67	91	87	117	51	110
Valine	80	68	68	77	103	80
Isoleucine	56	53	59	73	13	70
Methionine	14	28	31	23	8	38

(*) Ideal amino acid profile as determined by Schutte & de Jong, 2004

It is seen from these results that amino acid level of pupae meal is constantly higher than that of larvae meal. This may be related to the fact that the insects need this high amino acid concentration for the process of metamorphoses (Williams & Birt, 1972). The total tract amino acid digestibility of house fly larvae meal in turkeys was shown to range between 78% and 98% (Zuidhof *et al.*, 2003). No published results for digestibility of house fly pupae meal were found. Table 18 illustrates how the various protein sources compare to the ideal amino acid profiles. The ideal amino acid profiles for broilers were determined by expressing all the indispensible amino acids as a percentage of lysine (NRC, 1994; Schutte & de Jong, 2004). It is seen in Table 18 that house fly larvae meal were the best comparable to the ideal amino acid profile when compared to the house fly pupae meal, but the arginine relative to the lysine content were closer to the ideal amino acid profile for the pupae meal. The calculated indispensible amino acids to lysine ratios for the house fly pupae meal were comparable to the amino acid ratios of dehydrated fish meal. The soya oil cake meal had the best amino acid profile of all the protein sources listed in Table 18, but the phytate present in soya oil cake meal leads to a decrease in the bioavailability of the amino acids (Thompson & Serraino, 1986). Thompson & Serraino (1986) reported that the complex that is formed between the proteolytic enzymes, phytate and proteins within the animal's stomach could lead to a decrease in amino acid and protein digestibilities. The amino acid content of house fly larvae and pupae meal are comparable to other protein sources used in animal nutrition where the house fly pupae meal had noticeably higher lysine, arginine, tyrosine, valine, phenylalanine and leucine levels when compared to house fly larvae meal, soya oil cake meal and dehydrated fish meal.

The Arginine to lysine ratio of larvae meal and pupae meal were calculated as 0.67 and 0.91 respectively. Since birds are susceptible to lysine-arginine antagonism larvae and pupae meal can be fed with other protein sources

to ensure the optimum ratio of arginine to lysine of 1:1 in the diet. The interaction of lysine and arginine in animal nutrition is a complex process, but excess lysine has three basic consequences namely; lysine competes with arginine in the renal tubules causing a reduction in arginine retention (Jones *et al*., 1966), levels of lysine in the diet of poultry causes an increase in renal arginase activity that cause an increase in the oxidation of arginine (Leeson & Summers, 1997; Austic & Nesheim, 1970) and smaller amounts of excess lysine can cause a depression of the hepatic glycine transamidinase activity in chicks (Jones *et al*., 1966). By increasing the amounts of lysine in the diet can cause an increase urea excretion and slightly increase arginine excretion (Austic & Scott, 1975). In the current study the house fly larvae and pupae meal contains relatively high lysine concentrations calculated at 2.01% and 3.75% respectively. When lysine concentration in the diet exceeds 3% it has been shown to cause arginine degradation by renal arginase, depression of hepatic glycine transamidinase, depression of appetite and arginine loss through urine (Austic & Scott, 1975). Due to the high protein content of the meals under investigation it can be accepted that it will not be suitable as sole feed source for poultry and that the high lysine levels observed would not be detrimental but that the meals could serve as lysine source in animal feed mixtures. House fly pupae meal could also serve as a source of arginine in animal feed mixtures.

The ratio of isoleucine to leucine is calculated as 0.68 for larvae meal and 0.64 for pupae meal. This ratios can be considered good, but not optimal, because too high concentrations of leucine can lead to a reduction in the utilization of isoleucine (Leeson & Summers, 1997). Burnaham *et al*. (1992) found in their study that a severe decrease in the ratio of isoleucine to leucine in the diets of poultry depresses food intake and thus weight gain as well, but if the isoleucine concentration is sufficient to meet the requirement of the bird then a relative oversupply of leucine will not depress growth. The isoleucine requirements of broilers are 0.89% (National Research Council, 1994) and the house fly larvae meal can supply 0.7% isoleucine in the diets of broilers. Because house fly larvae and pupae meal will be fed together with other feed substrates containing isoleucine, thereby providing the animals need for isoleucine and preventing poor broiler performances.

Table 19 summarizes the results obtained by analysing the fatty acid profile of the house fly larvae and pupae meal. The essential fatty acid, linoleic acid, was found at a level of 36.27% of the total fat in pupae meal and 26.25% of the total fat in larvae meal. The house fly larvae meal has a better fatty acid profile than the house fly pupae meal, one such an explanation is that the higher Poly unsaturated fatty acid (PUFA) content of house fly pupae meal inhibit lipid synthesis and in return causes an increase in fatty acid oxidation within the insect body (Shimomura *et al*., 1990), hence the lower fatty acid values. The higher levels of unsaturated fatty acids present in house fly pupae meal might lead to decrease energy losses and higher ME values (Crespo & Esteve-Garcia, 2001) then when house fly larvae meal if used in the diets of broilers.

Table 19 The Long Chain Fatty Acid compositions of House fly larvae meal

Symbol	Common Name	Systemic name	Larvae meal dried at 65ºC (% of total fat)	Pupae meal dried at 65ºC (% of total fat)
Saturated Fatty acids (SFA)				
C14:0	Myristic	Tetradecanoic acid	4.08	2.70
C15:0	Pentadecylic	Pentadecanoic acid	0.86	1.06
C16:0	Palmitic	Hexadecanoic acid	38.01	34.85
C18:0	Stearic	Octadecenoic acid	4.39	2.75
C20:0	Arachidic	Eicosanoic acid	0.09	0.14
C21:0			0.11	0.05
C22:0	Behenic	Docosanoic acid	0.05	0.08
C24:0	Lignoceric		0.03	0.07
Monounsaturated Fatty Acid (MUFA)				
C14:1	Myristoleic	cis-9-Tetradecanoic acid	0.00	1.57
C15:1		cis-10-Pentadecanoic acid	0.00	1.44
C16:1	Palmitoleic	cis-9-Hexadecenoic acid	8.26	5.59
C18:1 n-9c	Oleic	cis-9-Octadecenoic acid	22.02	22.40
C18:1 n-9t	Elaidic		0.60	0.43
C20:1	Gondoic	cis-11-Eicosenoic acid	0.34	0.37
C22:1 n-9	Erucic	13-Docosenoic acid	0.05	0.00
C24:1	Nervonic	Tetracosanoic acid	0.03	0.03
Polyunsaturated Fatty Acid (PUFA)				
C18:2 n-6c*	Linoleic	cis-9,cis-12-Octadecadienoic acid	26.25	36.27
C18:2 n-6t	Linolelaidic		0.12	0.08
C18:3 n-6	γ-Linolenic	6,9,12-Octadecatrienoic acid	1.99	2.73
C18:3 n-3*	α-Linolenic	9,12,15-Octadecatrienoic acid	0.03	0.07
C20:2		11,14-Eicosadienoic acid	0.11	0.06
C20:3 n-3		cis-11,14,17-Eicosatrienoic acid	0.02	0.29
C20:3 n-6	Homo-g-Linolenic	cis-8,11,14-Eicosatrienoic acid	0.59	0.03
C20:4 n-6	Arachidonic	cis-5,8,11,14-Eicosatetraenioc acid	0.10	0.05
C20:5 n-3	Eicosapentaenoic acid	cis-5,8,11,14,17-Eicosapentaenoic	0.03	0.08
C22:2		cis-13,16-Docosadienoic acid	0.00	0.00
C22:5 n-3	Docosapentaenoic acid	cis-7,10,13,16,19-Docosapentaenoic	0.00	0.15
C22:6 n-3	Docosahexaenoic acid	cis-4,7,10,13,16,19-Docosahexaenoic	0.03	0.03
SFA			**47.62**	**41.71**
MUFA			**30.71**	**31.40**
PUFA			**29.14**	**39.85**
PUFA:SFA			**0.66**	**1.09**
(n-6)/(n-3)			**279.84**	**70.09**

(*) Essential Fatty Acids

3.5 Conclusion

The study revealed that *Musca domestica* larvae and pupae meal are comparable to other conventional already known protein sources, such as soya oil cake meal, blood meal and fish meal used in animal nutrition. Although the house fly pupae meal has much higher crude protein content than the larvae meal the digestibility of the pupae meal could be of concern. Digestibility trials are essential to investigate to what extent the nutrients of house fly larvae and pupae are digested by the animal body. Although pupae meal has higher amino acid level the extent to which this is digestible will determine its value in relation to larvae meal. House fly larvae meal represents the ideal amino acid ratio more closely than do pupae meal. House fly pupae meal had an arginine relative to the lysine content closer to the ideal amino acid profile than the larvae meal. The house fly pupae could serve as a good source of lysine and arginine in poultry nutrition. The findings of the nutritional composition in the current study will enable animal nutritionists to formulate diets containing house fly larvae and pupae meal to ensure a balanced diet. It is also reported that various factors can have an influence on the composition of house fly larvae and pupae meal respectively, it is therefore important to constantly monitor quality of these protein sources depending on their individual production system.

3.6 References

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CHAPTER 4

Evaluation of *Musca domestica* larvae meal in terms of possible toxicities, organ stress and immune suppression

4.1 Abstract

In the first part effects of *Musca domestica* larvae and pupae meal on the gizzard of a hundred Ross 308 day old broiler chicks were investigated in a fully randomized trial design consisting of four treatment diets (pupae meal dried at 65ºC and larvae meal dried at either 45ºC, 65ºC or 85ºC). None of the four treatment diets significantly induced gizzard erosion in the chicks. In the second part the effect of *Musca domestica* larvae meal were compared with diets containing comparable fish meal levels and a control diet. Birds were slaughtered at 14, 28 and 35 days of age and the gastro intestinal tracts were investigated and organ weights (heart, spleen bursa and liver) measured and liver colour was also measured. No differences were observed for any of the parameters and therefor it is concluded that *M. domestica* larvae meal supplementation in diets at rates of up to 50% had no influence on immune function (spleen: bursa ratio) or organ stress (detoxification). *Musca domestica* larvae meal can therefore be regarded as a safe product comparable to standard maize soya (control) and fish meal based diets.

Keywords- Gizzard erosion, gastro intestinal tract, organ stress, fish meal, larvae meal, broilers

4.2 Introduction

Gizzard erosion is a major problem in the poultry industry all over the world (Johnson, 1971). This disease also known as “black vomit” is characterized by low mortalities (Itakura *et al*., 1981), listlessness and a reduction in feed intake. *Post mortem* signs associated with gizzard erosion are the black watery content present in the crop (due to acid hydrolysis of blood), proventriculus and gizzard with the gizzard lining being eroded away and ulceration of the gizzard musculature (Johnson, 1971). In some severe cases the ulceration can perforate the gizzard muscle which can lead to peritonitis (Johnson, 1971). The most common factors associated with gizzard erosion are those that can lead to excessive secretion of the parietal glands (Itakura *et al*., 1981) which causes a decrease in gastric acid pH (Miyazaki & Umemura, 1987) and subsequent erosion and bleeding (Johnson, 1971).

The nature of the diet and certain minerals has the ability to induce gizzard erosion in poultry (Fisher *et al*., 1973; Ross, 1979). Ross (1979) found that pelleted feed brought about the formation of gizzard erosion when compared to a mash feed. The cause of this was unclear to the author, but it was believed to be the method of pelleting itself in combination with various other factors that could have been responsible. Copper sulphate is sometimes used in broiler nutrition as a growth promoter or to increase feed conversion ratios. Fisher *et al*.

(1973) found that gizzard lining damage was closely related to the copper concentration in the diet, but the severity of the damage differs with sex and individual.

Stress is another factor that can cause gizzard erosion in poultry (Grabarević *et al*., 1993; Džaja *et al*., 1996). Grabarević *et al*. (1993) and Džaja *et al.* (1996) both found that when broiler chicks were exposed to stressful circumstances they were more likely to suffer gizzard erosion. Stress increases the levels of aspartate aminotransferase and creatine kinase activities in the proventriculus of stressed chicks (Džaja *et al.*, 1996). This increase in enzymatic activity leads to an increase in stomach acidity that is responsible for gizzard erosion and ulceration in broilers under stress.

There are also natural causes of gizzard erosion associated with adenoviral infections (Abe et al., 2001; Ono *et al*., 2003). Adenoviral gizzard erosion was shown to be exacerbated by the infectious bursal disease virus and chicken anaemia virus, Tanimura *et al*. (1993) and Abe *et al*. (2001) isolated the group I avian adenovirus as a causative agent to induce gizzard erosion in broiler chickens.

Fishmeal is a high quality protein source with an excellent amino acid composition mostly used in the diets of broiler chickens in the starter and grower phases. Histamine (Džaja *et al*., 1996) in fish meal (Harry *et al*., 1975; Itakura *et al*., 1981; Itakura *et al*., 1982; Shimasaki *et al*., 2006) is an important factor that can cause gizzard erosion in broilers. There are a number of naturally occurring bacteria that exists on fishmeal that can bring about the formation of histamine by causing decarboxylation of especially the histidine amino acid present in fish meal (Ferencik, 1970). During processing, overheating of fishmeal brings about the formation of gizzerosine, (S)-2-amino-9-(4-imidazolyl)-7-azanonanoic acid (Shimasaki *et al*., 2006) that is formed by the reaction of histamine or histidine and lysine (Okazaki *et al.*, 1983). Gizzerosine acts as a potent antagonist of the H_2 receptor present in the acid secreting parietal cells of the glandular epithelium in the proventriculus. This causes excessive gastric acid secretions of especially pepsin and hydrochloric acid (Masumura *et al*., 1985) which are responsible for gizzard erosion.

Mycotoxins are also responsible for inducing gizzard erosion in poultry (Hoerr *et al.*, 1982; Dorner *et al*., 1983; Diaz & Sugahara, 1995). Broiler diets commonly mixed in a tropical environment which is normally associated with high temperatures and humidity may contain mycotoxins, especially aflatoxin B1 (Reddy, 1992). Diaz & Sugahara (1995) found synergysteic effects of mycotoxins and gizzerozine and reported that a combination of aflatoxin B1 and gizzerosine in high doses were more likely to induce severe incidences of gizzard erosion than when aflatoxin and gizzerosine was supplemented independently.

There is very limited published literature available on any possible toxic effects of housefly larvae meal. Téguia *et al.* (2002) investigated various organs weights at 49 days of age, but found no significant difference ($P>0.05$) regarding the heart, liver and gizzard mass relative to the body weight when they replaced 50% and 100% of the fishmeal with larvae meal in the finisher diets. These results however showed that as the inclusion level of

larvae meal increased there was an increase in the gizzard and liver masses suggesting that there could be toxic effects associated with larvae meal feeding. Since Téguia *et al*., (2002) had only four replications and may be the reason for the varying results found regarding the increase in gizzard and liver masses. It is therefore important to investigate in detail the potential toxic effects that may be associated with house fly larvae meal as part of this study.

The objective of this study was to investigate the effect of *M. domestica* larvae and pupae meal on gizzard erosion, organ stress and possible immune suppression of broilers.

4.3 Materials and methods

4.3.1 Gizzard erosion trial

Animals

One hundred day-old Cobb 500 broiler chicks as hatched were used. The chicks were vaccinated against Newcastle disease and Infective bronchitis at one day old. Mortalities were subjected to *post mortem* investigation with special attention given to the gizzard.

Housing system

The trials were performed at the Poultry section of the Mariendahl Experimental farm of Stellenbosch University. During the first six days the chicks were kept in a temperature controlled house according to the management practices described by Cobb International (2008).

After day six the chicks were moved to the bioassay unit. This unit comprises a temperature controlled room equipped with wire cages. Artificial lighting was provided at a pattern of 18 hours of light altering with 6 hours of darkness. Ventilation in the house was set to provide a minimum of six air changes per hour. The chicks had *ad libitum* access to feed and water during the duration of the experimental period.

Experimental diet

During the first seven days the chicks were maintained on a commercial starter diet formulated to produce marketable chickens weighing 1.9kg according to the nutrient specifications provided by Cobb International (2008) (Table 20). Hereafter the chicks were switched over onto one of the four treatment diets shown in Table 20.

To produce the respective meals the house fly larvae and pupae were harvested and immediately placed in a freezer at -20ºC. Larvae and pupae were removed from the freezer after 24 hours, defrosted at room temperature and dried in a ventilated oven at either 45°C, 65°C or 85°C depending on the treatment until a

constant mass was reached. After drying the larvae and pupae were milled through a 3 mm sieve using a Christy and Norris junior laboratory mill. Milled samples were stored at -20°C until mixed into the trial feeds.

Table 20 Ingredient composition (%) of the various diets used for determination of gizzard erosion potential in broilers fed larvae and pupae meal

	Commercial Starter	Treatment 1 (45ºC LM[1])	Treatment 2 (65ºC LM)	Treatment 3 (85ºC LM)	Treatment 4 (65ºC PM[2])
Maize	60.16	50.00	50.00	50.00	50.00
Soybean Full Fat	17.84				
Soybean Oil Cake meal	9.38				
Fish meal	10.00				
Housefly Larvae meal (45ºC)			50.00		
Housefly Larvae meal (65ºC)				50.00	
Housefly Larvae meal (85ºC)					50.00
Housefly Pupae meal (65ºC)		50.00			
DL-Methionine	0.21				
L-Threonine	0.03				
Premix*	0.15	0.15	0.15	0.15	0.15
Limestone	1.06				
Salt	0.04				
Monocalcium Phosphate	0.82				
Sodium Bicarbonate	0.28				

(*) Vitamins and minerals are included according to the levels provided by the National Research Council (1994)

([1]) LM- Larvae meal, ([2]) PM- Pupae meal

Experimental design and trial procedure

Chicks were randomly allocated to pens and treatment in the bioassay unit with five chicks per pen and five cages per treatment. At the end of the trial period the chicks were killed by cervical dislocation and the gizzards removed for scoring. Gizzards were scored on an ordinal scale, 1-5 (Table 21).

Table 21 Gizzard Erosion scoring description

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

4.3.2 Toxicity testing

Six broiler chicks of the each treatment group were slaughtered (one chick per replicate) on day 14, 28 and 36. Chicks were killed by cervical dislocation and the various organs were removed for weighing and the intestines were scored on an ordinal scale. The chicks were maintained on a commercial diet and diets supplemented with 10% *M. domestica* larvae meal, 10% fish meal, 25% *M. domestica* larvae meal, 25% fish meal, 50% *M. domestica* larvae meal and 50% fish meal respectively. Diet composition and calculated nutritional values are presented in Table 30, Table 31 and Table 32 in Chapter 6.

Data collection and analysis

The liver colour was measured using the BYK- Gardner Colour Guide. The CIElab colour system was used (Commition International de L'Eclairage, 1976) with three measurements; L* (lightness), a* (redness) and b* (yellowness). Positive a* values are a measure of redness and negative a* values are a measure of greenness. Positive b* values are a measure of yellowness and negative b* values indicates blueness.

The liver, heart, spleen, bursa and gizzard were removed by carefully cutting them out with a scalpel and care was taken not to cut any of the organs during dissecting. The following organs ratios were calculated; spleen: body weight, spleen: bursa, spleen: liver and bursa: body weight. The intestines were removed from each chick and a two centimetre piece of small intestine immediately anterior to the pancreas was removed and the pH measured, using a Crison pH25 Meter, by inserting the probe into the cut end of the intestine. The pH meter probe was placed directly into the piece of small intestine and the instrument was given time to stabilize before the pH reading was taken and the probe was rinsed with distilled water. The probe was rested in a KCL 3M electrolytic solution between each treatment. The piece of intestine immediately adjacent to the section used for pH measurement was removed and the various scores were done, as summarized in Table 22.

Statistical analysis was done by using one-way analysis of variances (ANOVA) with Fisher least significant difference (LSD) *post hoc* test of STATISTICA (data analysis software system), Version 9, by StatSoft Inc. (2009).

Table 22 Gastro intestinal tract scoring description

Score	Description
GIT* before cut	
Colour	
1	Small intestine has a healthy pinkish colour
2	The small intestine has a pale or redder discolouration
3	The small intestine are severely discoloured (very pale/red)
Gas	
1	No gas
2	Moderate gas build up
3	Severe gas build up
Liquid	
1	The intestinal contents are of a normal consistency
2	Moderate amounts of liquid present in the intestinal contents
3	Intestine filled with a large amount of liquid
Intestinal wall thickness	
1	Intestinal wall thickness is of normal thickness
2	Intestinal wall is moderately thinner or thicker
3	Intestinal wall substantially thinner or thicker
GIT tone after cut	
Tone C	
1	When small intestine is cut transversely it curls immediately
2	When small intestine is cut transversely it curls, but takes time
3	When small intestine is cut transversely no curling occurs
Tone L	
1	Upon exertion the small intestine has a high tensile strength (doesn't break easily if under pressure)
2	Upon exertion the small intestine has a moderate tensile strength (break if pulled moderately)
3	Upon exertion the small intestine has a very low tensile strength (break very easily under stress)
Tone Cut	
1	When small intestine is cut in length, it curls immediately
2	When small intestine is cut in length, it curls but takes some time
3	When small intestine is cut in length, no curling takes place
GIT inside surface	
Mucous amount	
1	Healthy mucous layer covering the small intestinal villi
2	Moderate amount of mucous/discoloured mucous in the small intestine
3	Excessive amount of mucous/discoloured
Villi	
1	Villi are long and wavey
2	Villi are short
3	Villi are severely damaged or eroded away
Blood Spots	
1	Inside surface of the small intestine has no blood spots
2	Moderate amount of blood spots occur in the inside surface of the small intestine
3	The small intestine is severely covered with blood spots

(*) GIT- Gastro intestinal tract

4.4 Results and discussion

4.4.1 Gizzard erosion study

Table 23 shows the different gizzard erosion scores obtained after macroscopic evaluation of the gizzards. No significant differences (P>0.05) were found between treatments for the gizzard parameters measured in either treatment. Macroscopic evaluation revealed a yellow discolouration of the gizzard lining caused by the larvae meal treatments. This discoloration was not associated with erosion and did not influence health status or weight gain and it is thus concluded that this discoloration is due to nontoxic pigmentation. During the trial one mortality was observed with the chicks receiving the 65ºC larvae meal diet, there were no indications that the specific treatment was responsible. It can be confirmed that neither *M. domestica* larvae nor pupae meal caused gizzard erosion in broiler chicks under the conditions explained and it is concluded that there was no gizzerosine formation caused by the histidine- lysine interaction induced by high drying temperatures (Okazaki *et al*., 1983).

Table 23 Number of observations per category of gizzard erosion scores recorded for the different treatments groups

GE*- Score	Pupae dried at 65ºC	Larvae dried at 45ºC	Larvae dried at 65ºC	Larvae dried at 85ºC
1	20	22	17	15
2	3	3	6	5
3	2	0	1	3
4	0	0	0	2
5	0	0	0	0

(*) GE- Gizzard Erosion

4.4.2 Toxicity testing

Table 24 summarizes the organ weights and various organ ratios resulting from the different treatments. The organ weights give an indication of oxidative stress while the spleen: bursa, spleen: body weight, bursa: body weight and spleen: liver ratios indicated immune stress (Cooper *et al*., 1966; Collett, 2005). The age by treatment effect did not significantly alter (P>0.05) the results obtained regarding the organ masses and ratios (Table 24), but where treatment effects where the only variable significant differences (P<0.05) were found regarding the liver, heart and bursa relative to body weights as well as bursa: body weight, spleen: liver and spleen: bursa ratios.

The treatment effect showed in Table 24 indicates that the liver weight relative to body weight of the chicks that received the 50% larvae meal diet differ significantly (P<0.05) from the chicks that received the other diets. The chicks that received the 10% fish meal diet had significant lower (P<0.05) heart weights relative to body weight when compared to the control group. The chicks that received the 50% larvae meal diet had the highest heart weights relative to the body weight it was significantly higher (P<0.05) than the chicks that received the 10% fish and larvae meal diets. The bursa weights relative to body weight of the chicks that received the 25% larvae diet

were significantly higher (P<0.05) than the chicks that received the 10% fish meal, 10% larvae meal and the control diets. The bursa: body weight of the chicks that received the 25% and 50% larvae meal diets were significantly higher (P<0.05) than the rest of the treatment groups. The chicks that received the 10% larvae meal diet had the highest spleen: liver ratio and it was significantly higher (P<0.05) than the chicks that received the 25% fish meal, 25% and 50% larvae meal diets. The chicks that received the 10% fish and larvae meal diet had significantly higher spleen: bursa ratios than the chicks that received the 25% and 50% larvae meal diets, but they did not differ significantly (P>0.05) from the chicks that received the 25% fish meal and control diets.

Chicks that received the 50% larvae meal supplementation had significantly (P<0.05) larger livers than the rest of the treatment diets and this may be related to the fact that the high protein diet caused the excess amino acid to be catabolised more by the liver to form ammonia and keto-acids (Baker, 1996). This increase in liver activity could be responsible for the increase in liver size. The bursa weights relative to the body weight of the chicks that received the 25% larvae and 50% larvae meal diets were the highest, these high levels of larvae meal was significant higher (P<0.05) than the other treatment diets, but they did not differ from the chicks that received the 25% fish meal diet. This may be related to the fact that these high protein diets caused more undigested proteins to reach the caeca and these undigested proteins have been shown to be inflammatory and thus further reduce feed efficiency (Collett, 2005; Sturkie & Benzo, 1976; Davidson *et al*, 2008). The bursa forms part of the bursa- dependant lymphoid system responsible for immunocompitance in the body (Cooper *et al*., 1966) and this inflammatory response caused by excess proteins led to an increase in bursa activity, hence increasing its size. The data reported regarding toxicity of house fly larvae meal feed supplement in the current study is comparable to the results reported by Téguia *et al*. (2002). These authors reported no significant difference (P>0.05) regarding the heart, liver and gizzard mass relative to the body weight when larvae meal was supplemented in the diets of broilers. There is evidence in literature suggesting improved lymphoid (spleen and bursa) organ growth when the protein concentration increases above 18% (Roa *et al*., 1999), it is therefore accepted that the deviations observed here were due to the nutrient density of the various diets and not to the house fly larvae meal supplementation *per se*.

Table 24 Averages (± standard error) of liver, heart, spleen and bursa weights together with organ ratios of broilers receiving different treatment diets (in g)

Day 14	Diet (control)	Diet 2 (10% fish meal)	Diet 3 (10% larvae meal)	Diet 4 (25% fish meal)	Diet 5 (25% larvae meal)	Diet 7 (50% larvae meal)
Liver (% BW*)	3.7300^{a} ± 0.74	3.9800^{ac} ± 0.57	4.2100^{bc} ± 1.05	3.8700^{ac} ± 1.56	4.2000^{bc} ± 0.97	4.2600^{bc} ± 0.64
Heart (% BW)	0.8720^{ab}± 0.18	0.7970^{b} ± 0.10	0.8920^{ab} ± 0.13	0.9180^{ad} ± 0.59	1.0120^{cd} ± 0.38	0.9720^{ad} ± 0.16
Spleen (% BW)	0.0910 ± 0.03	0.0820 ± 0.03	0.0930 ± 0.04	0.0920 ± 0.07	0.0910 ± 0.06	0.0850 ± 0.04
Bursa (% BW)	0.2530^{ab}± 0.08	0.2030^{b} ± 0.09	0.2590^{ab} ± 0.06	0.3120^{ac} ± 0.25	0.3010^{ac} ± 0.10	0.3830^{c} ± 0.11
Spleen: BW	0.0009 ± 0.00	0.0008 ± 0.00	0.0009 ± 0.00	0.0009 ± 0.00	0.0009 ± 0.00	0.0009 ± 0.00
Bursa: BW	0.0025^{ac}± 0.00	0.0020^{ab}± 0.00	0.0026^{ae} ± 0.00	0.0031^{ae}± 0.00	0.0030^{cde}± 0.00	0.0038^{d} ± 0.00
Spleen: Bursa	0.3600^{ab}± 0.05	0.4500^{a} ± 0.07	0.3700^{ac} ± 0.05	0.2900^{ac} ± 0.03	0.3200^{ac} ± 0.06	0.2300^{bc}± 0.03
Spleen: Liver	0.0250 ± 0.00	0.0210 ± 0.00	0.0220 ± 0.00	0.0240 ± 0.00	0.0220 ± 0.00	0.0210 ± 0.00
Day 28						
Liver (% BW)	2.6500^{a} ± 1.44	2.6300^{a} ± 2.37	2.5600^{a} ± 1.78	2.7500^{a} ± 2.35	2.8500^{a} ± 1.68	3.2900^{b} ± 1.77
Heart (% BW)	0.7270 ± 0.52	0.6500 ± 0.29	0.6770 ± 0.30	0.6900 ± 0.69	0.6650 ± 0.60	0.7280 ± 0.36
Spleen (% BW)	0.1250^{a} ± 0.06	0.1310^{a} ± 0.14	0.1210^{a} ± 0.17	0.0890^{b} ± 0.08	0.1130^{ab} ± 0.16	0.1010^{ab}± 0.13
Bursa (% BW)	0.3080^{ab}± 0.26	0.2380^{a} ± 0.23	0.3020^{ab}± 0.35	0.2380^{a} ± 0.16	0.3470^{b} ± 0.45	0.3030^{ab}± 0.14
Spleen: BW	0.0012 ± 0.00	0.0013 ± 0.00	0.0012 ± 0.00	0.0009 ± 0.00	0.0011 ± 0.00	0.0010 ± 0.00
Bursa: BW	0.0031^{ab}± 0.00	0.0024^{a} ± 0.00	0.0030^{ac}± 0.00	0.0024^{a} ± 0.00	0.0035^{bce}± 0.00	0.0031^{ae}± 0.00
Spleen: Bursa	0.4200^{ab}± 0.04	0.5700^{a} ± 0.07	0.4100^{ac}± 0.04	0.3900^{ac}± 0.03^{ac}	0.3400^{bc} ± 0.04	0.3500^{bc}± 0.06
Spleen: Liver	0.0480^{ac}± 0.00	0.0500^{a} ± 0.01	0.0470^{ad}± 0.00	0.0330^{be}± 0.00	0.0400^{cde}± 0.01	0.0300^{be}± 0.00
Day 35						
Liver (% BW)	2.8500^{ac} ± 2.75	2.5000^{ab} ± 1.38	2.4500^{bd} ± 2.43	2.7200^{ab} ± 2.21	2.6800^{abd} ± 1.90	3.1200^{c} ± 2.17
Heart (% BW)	0.7420^{ab} ± 0.95	0.6750^{a} ± 1.07	0.6350^{a} ± 0.83	0.7330^{ab} ± 1.49	0.6430^{a} ± 0.60	0.8180^{b} ± 0.93
Spleen (% BW)	0.1190 ± 0.22	0.1390 ± 0.26	0.1500 ± 0.19	0.1240 ± 0.23	0.1340 ± 0.06	0.1230 ± 0.18
Bursa (% BW)	0.2390^{a} ± 0.50	0.2550^{a} ± 0.85	0.2570^{a} ± 0.80	0.2790^{ab} ± 0.61	0.3530^{b} ± 0.64	0.3130^{ab} ± 0.82
Spleen: BW	0.0012^{ac} ± 0.00	0.0014^{a} ± 0.00	0.0015^{ad} ± 0.00	0.0013^{bde} ± 0.00	0.0014^{ae} ± 0.00	0.0012^{cde} ± 0.00
Bursa: BW	0.0024^{a} ± 0.00	0.0025^{a} ± 0.00	0.0026^{a} ± 0.00	0.0028^{ac} ± 0.00	0.0035^{bc} ± 0.00	0.0032^{ac} ± 0.00
Spleen: Bursa	0.5700^{abcd} ± 0.13	0.5800^{ac} ± 0.06	0.6200^{a} ± 0.07	0.4800^{acd} ± 0.08	0.4000^{d} ± 0.05	0.4300^{cd} ± 0.07
Spleen: Liver	0.0420^{a} ± 0.00	0.0560^{bc} ± 0.00	0.0610^{b} ± 0.00	0.0460^{a} ± 0.01	0.0500^{ac} ± 0.00	0.0400^{a} ± 0.00
Treatment effect						
Liver (% BW)	3.0770^{a} ± 3.86	3.0380^{a} ± 3.53	3.0710^{a} ± 3.41	3.1130^{a} ± 3.12	3.2450^{a} ± 3.10	3.5560^{a}± 2.62
Heart (% BW)	0.7800^{ab} ± 1.05	0.7070^{c} ± 1.10	0.7340^{ac}± 0.96	0.7810^{ab}± 0.99	0.7730^{abc} ± 0.77	0.8390^{b}± 0.75
Spleen (% BW)	0.1120 ± 0.19	0.1170 ± 0.26	0.1200 ± 0.27	0.1020 ± 0.18	0.1130 ± 0.19	0.1030 ± 0.14
Bursa (% BW)	0.2660^{a} ± 0.37	0.2320^{a} ± 0.51	0.2730^{a} ± 0.50	0.2760^{ab}± 0.39	0.3340^{b} ± 0.54	0.3330^{b}± 0.36
Spleen: Body weight	0.0011 ± 0.00	0.0012 ± 0.00	0.0012 ± 0.00	0.0010 ± 0.00	0.0011 ± 0.00	0.0010 ± 0.00
Bursa: Body weight	0.0027^{a} ± 0.00	0.0023^{a} ± 0.00	0.0027^{a} ± 0.00	0.0028^{a} ± 0.00	0.0033^{b} ± 0.00	0.0034^{b}± 0.00
Spleen: Liver	0.0380^{abc} ± 0.00	0.0420^{ac} ± 0.00	0.0440^{a} ± 0.00	0.0340^{bd}± 0.00	0.0370^{cb} ± 0.00	0.0300^{d}± 0.00
Spleen: bursa	0.4500^{abc} ± 0.05	0.5350^{b} ± 0.04	0.4670^{ab}± 0.04	0.3860^{ad}± 0.03	0.3520^{cd} ± 0.03	0.3290^{d}± 0.04

(*) BW- Body Weight

(^{a,b,c,d,e}) Means with different superscripts within the same row differ significantly (P<0.05)

Table 25 summarizes the CIElab colour values (L*, a* and b*) of the livers as affected by the various dietary treatments. There were no significant liver colour L* differences (P>0.05) between the chicks that received the 10% fish and larvae meal diets on the three slaughter dates. No significant differences (P>0.05) were observed for liver colour L* for the chicks that received the 25% fish and larvae meal diets at day 14 and day 28. At day 35 the chicks that received the 25% fish meal diet had significant lighter (P<0.05) liver colour L* than the chicks that received the 25% larvae meal diet. The liver colour a* of the chicks that received the 50% larvae meal diet was significantly lower (P<0.05) than that of the chicks on the control diet while no treatment differences (P>0.05) were observed at day 14. The liver colour a* of the chicks that received the 10% larvae meal diet was significantly lower (P<0.05) than that of the chicks in the control diet while no treatment differences (P>0.05) were observed at day 28. The liver colour a* of the chicks that received the 25% larvae meal diet was significantly higher (P<0.05) than that of the chicks that received the 10% larvae meal diet, while no treatment differences (P>0.05) were observed at day 35. No treatment differences (P>0.05) were observed regarding the liver colour b* at day 14 and 28, but at day 35 the chicks that received the 50% larvae meal diet had significantly lower (P<0.05) liver colour b* than the chicks that received the 25% fish meal diet.

Light livers in the broiler chicken under optimum growing conditions represent a normal physiological condition of the bird (Trampel *et al*., 2005). This lightness of the liver (L* colour value) observed with the chicks that received the 50% house fly larvae meal diet can be related to nutritional stress. There was no literature found that reported on the optimum liver colour of broilers, but there is however literature that report that a yellow discolouration of the liver may be caused by ulcerative enteritis (Grist, 2006). The liver colour b* at day 14 is significant yellower (P<0.05) for all the treatments when compared to the liver colour b* at day 28 and 35. This is due to the fact that in young birds the yellow discolouration of the liver is due to the presence of absorbed yolk (Grist, 2006).

Table 25 Average liver colour values (± Standard error) obtained due to the effects of different treatments

	Diet 1 (control)	Diet 2 (10% fish meal)	Diet 3 (10% larvae meal)	Diet 4 (25% fish meal)	Diet 5 (25% larvae meal)	Diet 7 (50% larvae meal)
Day 14						
L*	31.79^{a} ± 2.15	35.95^{acd} ± 2.31	36.29^{acd} ± 1.31	36.79^{bd} ± 1.92	36.29^{acd} ± 0.56	32.49^{ac} ± 0.64
a*	14.82^{a} ± 0.45	13.97^{ab} ± 0.40	13.54^{ab} ± 0.67	13.48^{ab} ± 0.55	13.61^{ab} ± 0.56	12.72^{b} ± 0.80^{b}
b*	14.98 ± 0.65	14.58 ± 1.94	15.04 ± 0.99	15.03 ± 1.22	15.11 ± 0.95	12.24 ± 0.95
Day 28						
L*	28.09 ± 1.64	30.22 ± 1.39	30.81 ± 0.60	30.11 ± 1.17	27.76 ± 1.23	27.57 ± 1.38
a*	14.47^{a} ± 0.93	12.99^{ab} ± 0.38	12.29^{b} ± 0.35	13.67^{ab} ± 0.61	13.00^{ab} ± 0.87	12.43^{ab} ± 0.65
b*	10.46 ± 1.82	10.96 ± 0.96	11.19 ± 0.58	12.84 ± 0.79	10.28 ± 0.94	10.38 ± 0.84
Day 35						
L*	28.98^{ad} ± 1.22	27.08^{abcd} ± 2.70	28.74^{abcd} ± 1.60	29.16^{ad} ± 0.69	24.55^{c} ± 2.10	24.59^{dc} ± 0.65
a*	15.65^{ab} ± 1.21	15.27^{ab} ± 0.95	14.45^{a} ± 0.79	16.21^{ab} ± 0.59	16.45^{b} ± 0.27	15.24^{ab} ± 0.34
b*	11.52^{abc} ± 1.43	11.84^{abc} ± 1.35	10.23^{ac} ± 1.54	14.02^{b} ± 0.80	12.44^{abc} ± 0.66	9.21^{c} ± 2.00

L*- lightness, a*- redness, b*- yellowness

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

Table 26 Frequency of the gastro intestinal parameters and average pH (± standard error) of the jejunum of broilers receiving varying amounts of larvae meal in comparison with fish meal

Day 14	Diet 1 (control)	Diet 2 (10% fish meal)	Diet 3 (10% larvae meal)	Diet 4 (25% fish meal)	Diet 5 (25% larvae meal)	Diet 7 (50% larvae meal)
GE – score	1	1	1	2	1	1
Gastro Intestines before cut						
Colour	1	1	1	1	1	1
Gas	1	2	2	2	2	2
Liquid	1	1	1	2	2	2
Membrane thickness	1	1	1	1	1	1
Gastro Intestinal tone						
Tone C	1	1	1	1	1	1
Tone L	1	1	1	1	1	1
Tone Cut	1	1	1	1	1	1
Inside Surface of Gastro intestines						
Mucous amount	1	1	1	1	1	1
Villi	1	1	1	1	1	1
Blood Spots	1	1	1	1	1	1
Day 28						
GE – score	2	1	1	2	1	1
Gastro Intestines before cut						
Colour	1	1	1	1	1	1
Gas	1	1	1	1	1	1
Liquid	1	1	1	1	1	1
Membrane thickness	1	1	1	1	1	1
Gastro Intestinal tone						
Tone C	1	1	1	1	1	1
Tone L	1	1	1	1	1	1
Tone Cut	1	1	1	1	1	1
Inside Surface of Gastro intestines						
Mucous amount	1	1	1	2	1	1
Villi	1	1	1	1	1	1
Blood Spots	1	1	1	1	1	1
pH	6.08±0.05[a]	6.03±0.07[a]	6.07±0.03[a]	6.17±0.08[a]	6.05±0.12[a]	6.09±0.11[a]
Day 35	1	1	1	2	1	1
GE – score						
Gastro Intestines before cut	1	1	1	1	1	1
Colour	1	1	1	1	1	1
Gas	1	1	1	1	1	1
Liquid	1	1	1	1	1	1
Membrane thickness	1	1	1	1	1	2
Gastro Intestinal tone						
Tone C	1	1	1	1	1	1
Tone L	1	1	1	1	1	1
Tone Cut	1	1	1	1	1	1
Inside Surface of Gastro intestines						
Mucous amount	2	1	1	1	1	1
Villi	1	1	1	1	1	1
Blood Spots	1	1	1	1	1	1
pH	6.10±0.05[a]	6.08±0.09[a]	6.16±0.04[a]	6.38±0.13[bc]	6.29±0.05[acd]	6.43±0.04[bd]

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

Table 26 Table 26 summarizes the results obtained by the various gastro intestinal parameters, scored on an ordinal scale (1-5). There were no significant differences (P>0.05) observed between any gastro intestinal parameter for the different slaughter dates. No significant differences (P>0.05) were observed for pH at day 28. At day 35 significant differences (P<0.05) for gut pH were observed of the chicks that received the 25% fish meal and 50% larvae meal diets compared to the chicks that received the control, 10% fish meal and 10% larvae meal diets. There were no significant differences regarding any gastro intestinal parameter scored. At a 50% larvae meal supplementation pigmentation of the gizzard and intestines were observed, since the health of these broilers were not affected it is accepted that the pigmentation was caused by pigmentation in the larvae meal. This was however not measured and no further discussion is possible.

4.5 Conclusion

The results obtained from this study showed that neither the use of *M. domestica* larvae or pupae meal nor the temperature of drying of larvae meal (45 – 85ºC) induced gizzard erosion. Although pigmentation of the gizzards was observed in some chicks this pigmentation did not appear to be detrimental. Since *M. domestica* larvae and pupae meal did not induce gizzard erosion in broilers in any way it can therefore be regarded as a safe product. These results also showed that the use of the *M. domestica* larvae meal in the diets of broilers did not have any detrimental effect in any of the gastrointestinal and organ parameters measured, even with a 50% *M. domestica* larvae meal supplemented diet. *Musca domestica* larvae and pupae meal have the potential to be used as a safe and renewable insect protein source in poultry diets as a replacement for commercially available protein sources including fish meal, soya oil cake meal and sunflower oil cake meal.

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CHAPTER 5

Determination of the total tract digestibilities of *Musca domestica* larvae and pupae meal in the diets of broiler chickens

5.1 Abstract

The total tract digestibilities of *Musca domestica* (common house fly) larvae and pupae meal were investigated in one hundred and twenty 21 day old broilers in a fully randomized trial design consisting of three treatment diets (maize meal, house fly larvae and pupae meal). Acid insoluble ash markers were used to determine the total tract apparent digestibilities of the three treatment diets. House fly larvae meal had crude protein total tract digestibility of 69% and that of pupae meal was 79%. Both larvae and pupae meal had high amino acid total tract digestibilities of all the amino acids analysed. The house fly larvae and pupae meal had a calculated apparent metabolizable energy (AME) value of 14.23MJ/kg and 15.15MJ/kg respectively. The total tract digestibilities of the crude fat and crude fibre were determined at 94% and 62% respectively for the house fly larvae and 98% and 58% respectively for the house fly pupae. Digestibility results indicated that the nutrients available in house fly larvae and pupae meal can be utilized efficiently by broilers.

Keywords- Total tract digestibility, markers, larvae meal, pupae meal, broilers

5.2 Introduction

In order to get an understanding of what substances in feed sources are digested and taken up by the animal it is usual to undertake digestibility trials. Digestibility is a measure of how efficiently an animal utilizes its feed substrates (Goodwin, 2009) and this is important from an animal nutritionist point of view to ensure maximum animal productivity. House fly larvae and pupae meal showed potential to be used as a renewable protein source in the diets of poultry. There is limited published data of interest available that reported on the total tract digestibilities of these insect protein sources. It is therefore important to understand the extent of digestion of the house fly larvae and pupae meal.

The digestibility of larvae meal was found to be high when investigated in the diets of turkey poults (Zuidhof *et al*., 2003) and broiler chickens (Hwangbo *et al*., 2009). Zuidhof *et al*. (2003) reported on the nutritional value of dehydrated housefly larvae in the diets of turkey poults and also on the total tract digestibilities of the nutritional components of the house fly larvae, by making use of an acid insoluble ash marker (Celite™). Zuidhof *et al*. (2003) reported that house fly larvae meal had significantly higher total tract digestibilities for ($P<0.05$) energy, crude protein and all the amino acids (except for cystine) in turkeys when compared to the soya oil cake meal commercial diet. Zuidhof *et al*. (2003) and Hwangbo *et al*. (2009) reported a crude protein total tract digestibility of 98% and Hwangbo *et al*. (2009) reported total tract digestibilities of essential amino acids to be 94.8%.

Inert markers for determining digestibility are regularly used in digestibility trials in animal nutrition; in cattle (Thonney *et al*., 1979), tilapia (Goddard & McLean, 2001) and various avian species (Sales & Janssens, 2003). The recovery of acid insoluble ash markers were reported to be as high as 99.9% when used to determine nutrient digestibility in pigs (Kavanagh *et al*., 2001). Scott & Boldaji, (1997) reported that the inclusion level of an acid insoluble ash marker, Celite™, in the diets of broilers can have an influence on the apparent metabolizable energy (AME) value. These authors reported that when Celite™ was included at a level of 0.5% or 1.0% less variation occurred when determining the AME of the feed under investigation.

The aim of this experimental trial was to investigate the total tract digestibilities of various nutritional components of *Musca domestica* larvae and pupae meal as a feed source by making use of an acid insoluble ash marker, Celite™.

5.3 Materials and methods

5.3.1 Digestibility trial

Animals

One hundred and twenty day-old Cobb 500 broiler chicks as hatched were used. The chicks were vaccinated against newcastle disease and infective bronchitis at day old. Mortalities were subjected to a full *post mortem* investigation.

Housing system

During the first twenty days the chicks were kept in a temperature controlled house at the Poultry section of the Mariendahl Experimental farm of Stellenbosch University according to the management practices described by Cobb International (2008).

After day twenty the chicks were moved to the experimental house on the farm. This unit comprises of a temperature controlled room equipped with 120 metabolic wire cages measuring 0.9m x 0.6m each containing one tube feeder and two nipple drinkers. Artificial lighting was provided at a pattern of 18 hours of light altering with 6 hours of darkness. Ventilation in the house was set to provide a minimum of six air changes per hour. The chicks had *ad libitum* access to feed and water during the duration of the experimental period.

Experimental diets

During the first twenty one days the chicks were maintained on a commercial starter diet formulated to produce marketable chickens weighing 1.9kg at 35 days according to the nutrient specifications provided by Cobb

International (2008). Hereafter the chicks were switched over onto one of the four treatment diets which are shown in Table 27.

Table 27 Ingredient composition of the commercial starter diet with the different treatment diets (% of the diet)

	Commercial Starter	Treatment 1 (Larvae meal)	Treatment 2 (Maize meal)	Treatment 3 (Pupae meal)
Maize	60.16	50.0	100	50.0
Soybean full fat	17.84			
Soybean oil cake meal	9.38			
Fish meal	10.00			
Housefly larvae meal (65ºC)		50.0		
Housefly pupae meal (65ºC)				50.0
DL-Methionine	0.21			
L-Threonine	0.03			
Premix*	0.15	0.15	0.15	0.15
Acid insoluble ash (Celite™)**	-	1.00	1.00	1.00
Limestone	1.06			
Salt	0.04			
Monocalcium phosphate	0.82			
Sodium bicarbonate	0.28			

(*) Vitamins and minerals are included according to the levels provided by the (National Research Council, 1994)

(**) Celite included at a level of 1% (Scott & Boldaji, 1997)

Experimental design and trial procedure

Chicks were randomly allocated to pens and treatment in the experimental house with five chicks per cage and eight cages per treatment. Digestibilities of larvae and pupae meal dried at 65°C were done using the Acid Indigestible Assay as described by Scott & Hall (1998).

The broiler chickens were moved to the metabolic cages on the 20th day. From day 20 to 24 the chickens were left to adapt to their new environment, during this time the chickens were fed a commercial grower diet. From day 25 to 27 the chicks were adapted to the various treatment diets, during this time the individual group *ad libitum* intakes were determined. From day 28 to 31 the actual digestibility trial (data collection period) was conducted.

Data collection and analysis

At the onset of the trial a 500 gram representative sample of each treatment diet was collected and frozen at -20ºC until used in laboratory analyses.

The broiler chickens were weighed on the beginning (20th day) and at the end (31st day) of the digestibility trial. During the time when the chickens were left to adapt to the environment, no measurements were done or data collected. From the 25th to 27th day daily feed intakes and feed refusals were measured and the feed offered were adjusted to adapt to the *ad libitum* feed intakes. On the 28th day of the data collection period the faecal

trays were placed under the metabolic cages and faeces was collected and weighed until the 31st day. During the data collection period daily feed intakes and feed refusals were determined. All procedures were conducted at 08:00 in the morning.

5.3.2 Analytical methodologies

Analytical methodologies were performed at the Department of Animal Science, Stellenbosch University except for amino acid analysis where the sample hydrolysis was done at the Stellenbosch University and the amino acid content analysed at the Institute of Animal Production, Western Cape department of agriculture.

Analytical methodologies on the dry matter (3.3.2.1 Dry matter determination), ash (3.3.2.2 Ash determination), crude protein (3.3.2.3 Crude protein determination), crude fat (3.3.2.5 Crude fat determination) and crude fibre (3.3.2.7 Crude fibre determination) content were performed as described in Chapter 3. The samples were hydrolysed (3.3.2.4 Sample hydrolysis for amino acid determination) before being sent for further analysis.

5.3.2.1 Acid insoluble ash determination

This procedure was performed according to the method as described by Van Keulen & Young (1977). Two subsamples each weighing 0.5 g of each sample was placed into an 80cm^3 crucible and combusted in a combustion oven for 12 hours at 500ºC. Thereafter the combusted subsamples were quantitatively transferred to a 500 cm^3 Erlenmeyer flask and 100 ml of a 2M hydrochloric acid solution was added. This mixture was then boiled for five minutes on a hotplate and then filtered through a Whatman® No 41 filter paper. The flask was rinsed with hot distilled water and the filter paper was washed free of acid. The filter paper with the ash residue was then placed in the previously weighed crucible and combusted in a combustion oven for 12 hours at 500ºC. Thereafter the combusted subsamples were weighed and the acid- insoluble ash content was calculated by using Equation 6:

Equation **6**:

Equation 6

$$\text{Acid insoluble ash } (\%) = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times \frac{100}{1}$$

Where:
W1 = Mass of crucible, in g
W2 = Mass of crucible and sample, in g
W3 = Mass of crucible and ashed sample after final ashing, in g

5.3.2.2 Gross energy determination

The gross energy (GE) values were determined (3.3.2.6 Gross energy determination) and then used to calculate the apparent metabolizable energy (AME) of each treatment diet using Equation 7 as described by Scott & Boldaji (1997).

Equation 7

$$\text{Apparent Metabolizable Energy (AME)} = \text{GE}_{\text{diet}} - [\text{GE}_{\text{excreta}} \times \left(\frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{excreta}}}\right)]$$

5.3.2.3 Acid detergent fibre (ADF) determination

The acid detergent fibre (ADF) determination was performed according to the method described by ANKOM Technology Corporation (2006a). Two subsamples of each sample weighing 0.5g each were placed into the filter bag and sealed with a heat sealer. These subsamples were then placed into the ANKOM^{200} apparatus and after the addition of the ADF solution the apparatus was left to run for 60 minutes. Thereafter the samples were rinsed three times with distilled water and then soaked in acetone. These subsamples were left to air dry and then dried at 100ºC for 12hours. The ADF of the subsamples was calculated using Equation 8:

Equation 8

$$\%\ \text{ADF(as is basis)} = \frac{[\text{W3} - (\text{W1} \times \text{C1})]}{\text{W2}} \times \frac{100}{1}$$

Where:
W1 = Bag Weight (g)
W2 = Sample Weight (g)
W3 = Dried weight of bag with fibre after extraction process (g)
C1 = Blank bag correction factor

5.3.2.4 Neutral detergent fibre (NDF) determination

The neutral detergent fibre (NDF) determination was performed according to the method described by ANKOM Technology Corporation (2006b). Two subsamples of each sample weighing 0.5g were placed into the filter bag and sealed with a heat sealer. The subsamples were then placed into the ANKOM^{200} apparatus and NDF solution was added and the apparatus left to run for 75 minutes. Thereafter the subsamples were rinsed four times with distilled water. Alpha-amylase was added to the first and second rinse of distilled water. Thereafter the samples were soaked in acetone and left to air dry and then dried at 100ºC for 12hours. The NDF content of the subsamples was calculated using

Equation **9**:

Equation 9

$$\%\ NDF\ (as\ is\ basis) = \frac{[W3 - (W1 \times C1)]}{W2} \times \frac{100}{1}$$

Where:
W1 = Bag Weight (g)
W2 = Sample Weight (g)
W3 = Dried weight of bag with fibre after extraction process (g)
C1 = Blank bag correction factor

5.3.3 Coefficient of total tract digestibility

The coefficients of total tract digestibility (CTTD), of each analysed nutrient were calculated by using the basic Equation 10.

Equation 10

$$\text{Nutrients consumed (g/trial)} = \text{Nutrient}_{\text{analysed in feed}} \times \text{Dry Matter}_{\text{Intake}}\ \text{(g/trial)}$$

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

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

$$\text{Coefficients of Total Tract Digestibility (g/kg)} = \frac{\text{Digested Nutrient}}{\text{Nutrients consumed}}$$

The total tract apparent digestibility obtained for the 100% maize meal diet were used to correct for digestibility of the larvae & maize and pupae & maize diets in order to obtain total tract apparent digestibility of the 50% larvae and 50% maize meal as well as the 50% pupae and 50% maize meal diet to calculate the exact total tract apparent digestibilities of only the larvae and pupae meal.

5.4 Results and discussion

Statistical analyses were done by using STATISTICA (data analysis software system), Version 9, by StatSoft Inc. (2009). Because age did not have any effect on the data the statistics were done by using one-way Analysis of Variances (ANOVA) with Fisher least significant difference (LSD) post hoc test.

Table 28 summarizes the nutrient composition of the different treatment diets as determined by the various laboratory analyses.

Table 28 The analysed nutrient composition of the treatment diets

	Units	Treatment 1 (Larvae meal)	Treatment 2 (Maize meal)	Treatment 3 (Pupae meal)
Gross energy	MJ/kg	19.54	17.19	19.72
Crude protein	%	31.50	8.35	37.19
Ash	%	8.97	4.27	7.25
Ether extract	%	7.88	3.16	7.06
Crude fibre	%	5.69	2.71	8.99
NDF[1]	%	13.85	9.61	21.59
ADF[2]	%	5.35	3.41	10.78
Alanine	%	1.51	0.33	1.57
Threonine*	%	0.88	0.16	1.38
Serine	%	0.91	0.23	1.85
Glutamic acid	%	0.00	0.46	1.40
Valine*	%	2.15	0.44	5.02
Histidine*	%	1.21	0.36	1.33
Aspartic acid	%	0.64	0.45	0.74
Arginine	%	2.35	0.23	4.16
Lysine*	%	1.68	0.24	2.28
Proline	%	1.56	0.80	1.63
Methionine*	%	0.47	0.05	0.55
Tyrosine	%	1.27	0.39	1.80
Cysteine	%	0.08	0.04	0.12
Isoleucine*	%	1.06	0.32	1.15
Phenylalanine*	%	1.32	0.62	1.64
Leucine*	%	2.18	1.28	2.61
Glycine	%	1.03	0.21	1.19
Hydroxy proline	%	0.07	0.01	0.07

(*) Essential amino acids

([1]) NDF- Neutral detergent fibre, ([2]) ADF- Acid detergent fibre

Table 29 summarizes the coefficient of total tract digestibility (CTTD) for the different treatment diets. It is noted in Table 29 that there were no CTTD value of arginine for the larvae meal treatment diet, as the arginine was below the detection point of 5 nmol/ml when the amino acids were analysed. In Table 29 it is indicated that there were differences between the CTTD for the house fly larvae and pupae meal. The CTTD for the dry matter for the larvae and pupae meal differ significantly ($P<0.05$) when compared to each other. The pupae meal had significantly higher ($P<0.05$) AME values when compared to the larvae meal. The CTTD for the crude protein, ether extract and ADF were significantly higher ($P<0.05$) for the pupae meal when compared to the larvae meal. The CTTD for the ash and NDF did not differ significantly ($P>0.05$) between the larvae and pupae meal, but the CTTD for the crude fibre was significant higher ($P<0.05$) in the larvae meal when compared to the pupae meal.

The CTTD for the essential amino acids threonine, lysine, methionine, isoleucine, phenylalanine and leucine in the pupae meal were significantly higher ($P<0.05$) than the dried larvae meal. There were no significant difference ($P>0.05$) of the CTTD for the essential amino acids valine and histidine between the larvae and pupae

meal. Results indicated that the pupae meal has significantly higher ($P<0.05$) CTTD for all the non-essential amino acids analysed than the larvae meal, except for the non-essential amino acid tyrosine which showed no significant difference ($P>0.05$). Over processing, especially overheating, is considered to be a primary cause of a reduced amino acid bioavailability to broilers (Parsons, 1996) and this could result in the lower amino acid digestibilities reported for the larvae meal when compared to pupae meal. Although the temperature of drying was kept constant the house fly larvae were dried for longer due to the higher moist content which could have led to heat damage. This is especially true for the CTTD for lysine, because lysine is the amino acid most easily affected by over processing due to its susceptibility to the Maillard reaction (Parson, 1996)

Results found in the current study revealed that house fly pupae meal has a higher coefficient of total tract digestibility, given the drying procedures followed, as the pupae are covered with a chitin layer (Ludwig *et al*., 1964; Kramer & Koga, 1986) that may have the potential to be less suitable as feed source than the larvae. This is because of the higher digestibilities of most nutrients of the pupae meal. There was however no published results found for the digestibility of pupae meal to support this. The crude protein total tract digestibilities of the pupae meal (79%) are comparable to that of soya oil cake meal (80.7%) (Sebastian *et al*., 1997), whereas the crude protein total tract digestibility (69%) of the larvae meal were reported to be lower. Results indicated that house fly larvae and pupae meal have higher total tract digestibilities for all the essential amino acid analysed when compared to soya oil cake meal in poultry diets (Sebastian *et al*., 1997; Hwangbo *et al*., 2009). House fly larvae meal is not comparable to house fly pupae meal, although these two meals did not differ significantly regarding the dry matter digestibility. The house fly pupae meal, had in most cases, significantly better total tract digestibility of most nutrients compared to house fly larvae meal. The crude protein total tract digestibility of house fly larvae and pupae reported in the current study are not comparable to that reported by Zuidhof *et al*. (2003) and Hwangbo *et al*. (2009). These authors reported crude protein total tract digestibility of house fly larvae meal at 98.8% and 98% respectively. The reported essential amino acid total tract digestibilities of the house fly larvae and pupae meal were comparable to that reported by Zuidhof *et al*. (2003) and Hwangbo *et al*. (2009), but were higher than that of soya oil cake meal (Sebastian, *et al*., 1997). There was no comparable literature found that reported on the total tract digestibility of the ash, ether extract, crude fibre, NDF and ADF for either the house fly larvae or pupae meal.

Table 29 Average (with standard errors) coefficient of total tract digestibility (CTTD) of larvae and pupae meal and the apparent metabolizable energy (AME) for broilers by making use of an acid insoluble ash marker (Celite™)

	Larvae meal		Pupae meal	
	CTTD**	SE[3]	CTTD	SE
AME (MJ/kg)	14.23[a]	20.94	15.15[b]	19.29
Dry Matter	0.81[a]	0.005	0.83[a]	0.005
Crude Protein	0.69[a]	0.009	0.79[b]	0.007
Ash	0.83[a]	0.004	0.85[a]	0.005
Ether Extract	0.94[a]	0.004	0.98[b]	0.003
Crude Fibre	0.62[a]	0.012	0.58[b]	0.013
NDF[1]	0.87[a]	0.005	0.87[a]	0.004
ADF[2]	0.35[a]	0.020	0.67[b]	0.010
Alanine	0.90[a]	0.007	0.86[b]	0.008
Threonine*	0.93[a]	0.010	0.97[b]	0.005
Serine	0.86[a]	0.027	1.00[b]	0.015
Glutamic acid	0.91[a]	0.006	0.99[b]	0.003
Valine*	0.91[a]	0.006	0.91[a]	0.005
Histidine*	0.87[a]	0.005	0.87[a]	0.004
Aspartic acid	0.93[a]	0.006	1.00[b]	0.004
Arginine	-	-	0.93	0.012
Lysine*	0.95[a]	0.005	0.99[b]	0.004
Proline	0.91[a]	0.005	0.91[a]	0.005
Methionine*	0.95[a]	0.004	0.99[b]	0.003
Tyrosine	0.96[a]	0.005	0.96[a]	0.004
Cysteine	0.92[a]	0.010	0.96[b]	0.009
Isoleucine*	0.91[a]	0.005	0.95[b]	0.004
Phenylalanine*	0.91[a]	0.005	0.95[b]	0.003
Leucine*	0.92[a]	0.005	0.96[b]	0.003
Glycine	0.83[a]	0.009	0.89[b]	0.010
Hydroxy proline	0.97[a]	0.018	1.00[b]	0.016

(*) Essential Amino Acids, (**) CTTD- Coefficient of total tract digestibility

([1]) NDF- Neutral detergent fibre, ([2]) ADF- Acid Detergent fibre, ([3]) SE- Standard error

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

One of the main differences observed between the house fly larvae and pupae meal in the current study was the difference in crude protein total tract digestibility. This difference could be related to the fact that the milling process of the house fly pupae increased the surface area for digestion (McDonald, 2002) that could have made substances in the chitin layer more available to the animal. The total tract digestibility for ADF was significantly lower (P<0.05) for the house fly larvae meal when compared to the pupae meal. Dietary fibre was reported to influence passage rate, as it cause a reduction in digestion in the upper digestive tract and an increase digestion in the lower digestive tract of almost all nutrients (Wenk, 2001). The crude fibre of the pupae meal diet (8.99%) were higher than the crude fibre of the larvae meal diet (5.69%) and this could cause a slower rate of passage,

hence longer time for digestion in the lower digestive tract. The lower AME values reported for the larvae meal compared to the pupae meal could be related to the higher ADF content of larvae meal.

5.5 Conclusion

The total tract digestibility of the housefly larvae and pupae meal for broilers reported in the current study will be of importance for animal nutritionists to ensure effective formulation of broiler diets where these respective meals are used. Housefly pupae meal has significantly higher total tract digestibilities of most nutrients when compared to housefly larvae meal, indicating that the house fly pupae meal is a better quality protein source when compared to housefly larvae meal under the current drying regime. The amino acids present in housefly larvae and pupae meal have a high bioavailability that can be utilized efficiently by broilers. Housefly larvae and pupae meal are both comparable to other conventional protein sources and have the potential to replace these protein sources that is renewable.

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CHAPTER 6

Comparison of the production parameters of broiler chicks grown on a diet containing either *Musca domestica* larvae meal, fish meal or soya oil cake meal as the main protein source

6.1 Abstract

The effects of *Musca domestica* (common house fly) larvae meal supplementation on broiler performance of four hundred and twenty broiler chicks were investigated in a stratified block design consisting of seven treatments (a commercial diet and diets supplemented with 10% *M. domestica* larvae meal, 10% fish meal, 25% *M. domestica* larvae meal, 25% fish meal, 50% *M. domestica* larvae meal and 50% fish meal). *M. domestica* larvae meal supplementation in a three phase feeding system significantly increased average broiler live weights, feed intake, cumulative feed intake as well as average daily gain (ADG) when compared to a commercial broiler feed. There were no significant differences between a 10% *M. domestica* larvae and a 10% fish meal supplementation regarding any performance characteristic. The 25% *M. domestica* larvae meal supplementation had significantly better average broiler live weights, feed intake, cumulative feed intake as well as ADG when compared to the 25% fish meal supplementation diet in the growth phases. *M. domestica* larvae meal can be used to replace other protein sources that have the ability to promote broiler performance.

Keywords- Production, larvae meal, fish meal, soy oil cake meal, broilers

6.2 Introduction

Protein is a very important ingredient required in broiler nutrition and for decades a lot of emphasis has been placed on the quality of proteins especially the amino acid composition of these protein sources (Ellinger, 1958). The cost of the particular protein plays a very important role in the selection of appropriate protein sources used in animal nutrition. With the lack of renewable protein sources together with the rise in protein feed costs, it is becoming more important to find good quality alternative and sustainable protein sources (Téguia *et al.*, 2002). Alternative protein sources that are renewable and affordable need to be developed or discovered for animal nutrition. Such a potential protein source can be supplied by *Musca domestica* larvae. Many studies have reported on the evaluation of fly larvae meal as a complete or partial replacement of other protein sources i.e. groundnut meal (Adeniji, 2007), fishmeal (Téguia *et al*., 2002; Awoniyi *et al*., 2003; Ogunji *et al.*, 2006; Agunbiade *et al*., 2007) and soya bean oil cake meal (Hwangbo *et al*., 2009) in broiler nutrition.

Hwangbo *et al.* (2009) reported that when broilers received larvae meal at levels of 10 and 15% of the total diet during their starter phase, significantly better ($P<0.05$) weight gains and feed conversion ratios (FCR) were

achieved until five weeks as compared to the soya oil cake meal control diet. These results are not comparable to that reported by Awoniyi *et al*. (2003), Adeniji (2007) and Téguia *et al.* (2002), because these authors reported that larvae meal supplement did not significantly influence (P>0.05) weight gain and FCR. This controversy in literature could be attributed to the trial design where Téguia *et al*. (2002) only had four replications in relation to the three replications of Adeniji (2007) and Hwangbo *et al*. (2009), whereas Awoniyi *et al*. (2003) had no replications. Although some authors had the same amount of replicates, the fewer number of birds used in the treatments by some authors could have led to a higher variation in the results obtained. These differences can also be attributed the fact that Hwangbo *et al*. (2009) included relatively high levels of maize gluten meal (>5%) that could have influenced broiler performance especially feed intake (Afshar & Moslehi, 2000).

The protein efficiency ratio (PER) is a measure of the protein quality and one of the simplest methods in determining the nutritive value of proteins (Bender, 1956). Wilding *et al.* (1968) reported that the optimum protein efficiency ratio to be 3:1 for broiler production. Broiler producers and integrators in Europe, Africa and Asia use the European production efficiency factor (EPEF) to compare live bird performances within the flock (Butcher & Nilipour, 2009). There was no literature found that reported on the EPEF and PER when house fly larvae meal were supplemented in the diets of broilers.

The aim of this trial was to investigate the effect of *Musca domestica* larvae meal supplementation on production parameters of broilers.

5.3 Materials and methods

Animals and housing system

Four hundred and twenty day-old Ross 308 broiler chicks as hatched were used. Mortalities were subjected to full *post mortem* investigation. During the duration of the experimental period the chicks were kept in a temperature controlled house at the Poultry section of the Mariendahl Experimental farm of Stellenbosch University. Management practices described by Ross International (2009) were followed. This unit comprises a temperature controlled room equipped with 120 wire cages measuring 0.9m x 0.6m, each containing a tube feeder and two nipple drinkers. Ventilation in the house was set to provide a minimum of six air changes per hour. The chicks had *ad libitum* access to feed and water during the duration of the experimental period.

Experimental diets

The chicks were assigned to seven different treatment diets. Treatment diets are shown in Table 30, Table 31 and Table 32. The diets were formulated so that the chicks were maintained on the minimum nutrient specifications as provided by Ross International (2009), but the 25% and 50% larvae and fish meal diets had an oversupply of proteins. The treatment diets were allocated so that the chicks received 900g starter, 1200g grower and 1200g finisher per bird.

Table 30 Ingredient and calculated nutrient composition of trial starter diets

	Unit	Diet 1 (Control)	Diet 2 (10% FM[2])	Diet 3 (10% LM[3])	Diet 4 (25% FM)	Diet 5 (25% LM)	Diet 6 (50% FM)	Diet 7 (50% LM)
Ingredients								
Maize	%	51.68	54.55	47.81	60.48	45.40	45.80	33.01
Soybean full fat	%	32.98	24.18	21.12	3.78	21.81		10.65
Soybean	%	7.99	8.77	15.70	10.43	4.42		
Fish meal	%	3.32	10.00		25.00		50.00	
Housefly larvae meal (65º)	%			10.00		25.00		50.00
L-Lysine	%	0.14		0.04				
DL-Methionine	%	0.34	0.24	0.35	0.05	0.36		0.36
L-Threonine	%	0.07						
Premix*	%	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Limestone	%	1.61	1.02	1.98		2.40		2.35
Salt	%	0.20	0.05	0.27		0.36	3.95	0.37
MCP[1]	%	1.26	0.82	0.95				
Sodium bicarbonate	%	0.16	0.12	0.13				
Oil- soya	%			1.14				3.02
Calculated nutritional value								
Dry matter	%	88.55	88.44	88.94	88.35	89.15	89.77	90.36
AMEn** chick	MJ/kg	12.60	12.60	12.60	12.60	13.41	12.60	14.94
Crude protein	%	22.97	24.40	25.00	27.77	28.14	36.64	34.74
Ether extract	MJ/kg	11.48	11.49	10.65	11.47	10.00	10.92	9.25
Ash	%	4.69	4.94	5.62	5.42	7.02	8.30	8.77
Crude fibre	%	3.39	2.97	3.66	2.06	4.07	1.01	4.62
Crude fat	%	8.77	7.68	8.62	5.66	8.98	6.74	12.52
Calcium	%	1.00	1.00	1.00	1.05	1.00	2.01	1.00
Lysine	%	1.43	1.47	1.41	1.81	1.52	2.64	1.85
Methionine	%	0.69	0.69	0.67	0.70	0.68	0.99	0.67
Cystine	%	0.38	0.38	0.38	0.38	0.37	0.39	0.37
Methionine + Cystine	%	1.07	1.07	1.06	1.08	1.05	1.39	1.04
Threonine	%	0.93	0.96	0.92	1.11	0.97	1.50	1.11
Tryptophan	%	0.26	0.27	0.28	0.29	0.29	0.37	0.34
Arginine	%	1.53	1.57	1.52	1.66	1.45	2.09	1.45
Isoleucine	%	1.03	1.10	1.05	1.24	1.05	1.65	1.14
Leucine	%	1.98	2.10	1.95	2.37	1.92	2.93	1.93
Histidine	%	0.62	0.66	0.58	0.74	0.52	0.96	0.45
Phenylalanine	%	1.04	1.05	1.13	1.08	1.23	1.28	1.45
Tyrosine	%	0.81	0.84	0.95	0.88	1.07	1.04	1.35
Phenylalanine + Tyrosine	%	1.85	1.89	2.08	1.96	2.29	2.32	2.80
Valine	%	1.14	1.24	1.22	1.45	1.32	1.92	1.56
Glycine + Serine	%	2.12	2.31	2.12	2.71	2.14	3.70	2.32
Phosphorous	%	0.81	0.80	0.80	0.88	0.78	1.38	1.16
Available phosphorous	%	0.50	0.50	0.50	0.60	0.53	1.12	0.96
Sodium	%	0.16	0.16	0.16	0.24	0.16	1.99	0.16
Chloride	%	0.22	0.22	0.22	0.40	0.26	3.13	0.25
Potassium	%	0.90	0.82	0.98	0.66	0.99	0.51	1.07
Linoleic acid	%	4.54	3.61	3.53	1.79	3.24	1.07	2.57

([1]) MCP- Monocalcium phosphate, ([2]) FM- Fish meal, ([3]) LM- Larvae meal

(*)Vitamins and minerals are included according to the levels provided by the (National Research Council, 1994)

(**)AMEn- Nitrogen-corrected apparent metabolizable energy value

Table 31 Ingredient and calculated nutrient composition of trial grower diets

	Unit	Diet 1 (Control)	Diet 2 (10% FM[2])	Diet 3 (10% LM[3])	Diet 4 (25% FM)	Diet 5 (25% LM)	Diet 7 (50% LM)
Ingredients							
Maize	%	43.47	52.50	44.51	63.01	51.10	32.86
Soybean full fat	%	49.02	33.78	37.86	10.93	12.43	
Soybean	%	2.17	2.45	4.99	0.71	9.13	11.38
Fish meal	%	2.88	10.00		25.00		
Housefly larvae meal (65ºC)	%			10.00		25.00	50.00
DL-Methionine	%	0.30	0.25	0.35	0.10	0.38	0.33
Premix*	%	0.25	0.25	0.25	0.25	0.25	0.25
Limestone	%	0.41		0.93		1.32	0.39
Salt	%	0.24	0.08	0.31		0.30	0.32
MCP[1]	%	1.19	0.61	0.73			4.15
Sodium bicarbonate	%	0.07	0.09	0.08		0.09	0.32
Calculated nutritional value							
Dry matter	%	88.70	88.42	88.82	88.26	88.86	90.30
AMEn** chick	MJ/kg	13.20	13.20	13.20	13.20	13.20	13.20
Crude protein	%	25.01	24.88	25.95	26.20	27.34	36.00
Ether extract	MJ/kg	11.95	12.05	11.10	12.12	9.86	7.17
Ash	%	3.95	4.02	4.75	5.22	5.85	6.99
Crude fibre	%	3.76	3.14	3.97	2.02	3.92	4.60
Crude fat	%	11.04	9.28	10.03	6.93	7.53	7.73
Calcium	%	0.60	0.60	0.60	1.04	0.60	1.00
Lysine	%	1.49	1.51	1.46	1.70	1.45	1.92
Methionine	%	0.69	0.70	0.69	0.73	0.68	0.66
Cystine	%	0.40	0.39	0.39	0.36	0.37	0.39
Methionine + Cystine	%	1.10	1.09	1.08	1.09	1.05	1.04
Threonine	%	0.98	0.98	0.96	1.05	0.94	1.16
Tryptophan	%	0.29	0.28	0.29	0.27	0.28	0.36
Arginine	%	1.71	1.61	1.60	1.53	1.37	1.54
Isoleucine	%	1.15	1.12	1.09	1.16	1.01	1.20
Leucine	%	2.11	2.13	2.02	2.26	1.89	2.03
Histidine	%	0.68	0.68	0.61	0.70	0.50	0.48
Phenylalanine	%	1.15	1.08	1.18	1.00	1.19	1.52
Tyrosine	%	0.88	0.84	0.96	0.80	1.05	1.42
Phenylalanine + Tyrosine	%	2.03	1.91	2.14	1.80	2.23	2.94
Valine	%	1.25	1.26	1.26	1.36	1.28	1.64
Glycine + Serine	%	2.34	2.36	2.22	2.56	2.06	2.43
Phosphorous	%	0.79	0.76	0.76	0.87	0.78	2.21
Available phosphorous	%	0.45	0.45	0.45	0.60	0.52	2.00
Sodium	%	0.16	0.16	0.16	0.23	0.16	0.23
Chloride	%	0.23	0.23	0.23	0.39	0.23	0.23
Potassium	%	1.00	0.84	1.02	0.58	0.95	1.13
Linoleic acid	%	5.73	4.46	4.72	2.48	2.48	0.99

([1]) MCP- Monocalcium phosphate, ([2]) FM- Fish meal, ([3]) LM- Larvae meal

(*)Vitamins and minerals are included according to the levels provided by the (National Research Council, 1994)

(**)AMEn- Nitrogen-corrected apparent metabolizable energy value

Table 32 Ingredient and calculated nutrient composition of trial finisher diets

	Unit	Diet 1 (Control)	Diet 2 (10% FM[2])	Diet 3 (10% LM[3])	Diet 4 (25% FM)	Diet 5 (25% LM)
Ingredients						
Maize	%	44.90	55.61	54.41	69.67	55.89
Soybean full fat	%	51.45	32.54	30.82		9.85
Soybean	%			1.40	4.84	6.24
Fish meal	%		10.00		25.00	
Housefly larvae meal (65ºC)	%			10.00		25.00
DL-Methionine	%	0.27	0.18	0.32	0.03	0.32
L-Threonine	%			0.04		
Premix	%	0.25	0.25	0.25	0.25	0.25
Limestone	%	1.36	0.76	1.74	0.21	2.05
Salt	%	0.31	0.08	0.30		0.30
MCP[1]	%	1.31	0.49	0.63		
Sodium bicarbonate	%	0.07	0.09	0.09		0.09
Calculated nutritional value						
Dry matter	%	88.68	88.35	88.52	88.04	88.74
AMEn** chick	MJ/kg	13.20	13.25	13.25	12.86	13.25
Crude protein	%	23.12	23.49	22.54	24.55	25.41
Ether extract	MJ/kg	12.02	12.18	11.35	11.90	10.02
Ash	%	4.45	4.59	5.09	5.20	6.32
Crude fibre	%	3.82	3.01	3.62	1.77	3.74
Crude fat	%	11.23	9.13	9.05	5.22	7.19
Calcium	%	0.85	0.85	0.85	1.10	0.85
Lysine	%	1.34	1.42	1.22	1.58	1.31
Methionine	%	0.61	0.62	0.62	0.64	0.61
Cystine	%	0.39	0.37	0.36	0.34	0.35
Methionine + Cystine	%	1.00	0.99	0.97	0.98	0.95
Threonine	%	0.90	0.93	0.85	0.98	0.86
Tryptophan	%	0.27	0.26	0.24	0.24	0.25
Arginine	%	1.60	1.50	1.32	1.40	1.22
Isoleucine	%	1.06	1.05	0.91	1.07	0.91
Leucine	%	1.98	2.04	1.80	2.16	1.76
Histidine	%	0.64	0.64	0.52	0.66	0.45
Phenylalanine	%	1.09	1.01	1.01	0.92	1.09
Tyrosine	%	0.82	0.78	0.82	0.75	0.97
Phenylalanine + Tyrosine	%	1.91	1.79	1.84	1.68	2.07
Valine	%	1.15	1.19	1.09	1.29	1.19
Glycine + Serine	%	2.15	2.23	1.89	2.41	1.88
Phosphorous	%	0.75	0.72	0.70	0.85	0.76
Available phosphorous	%	0.42	0.42	0.42	0.60	0.52
Sodium	%	0.16	0.16	0.16	0.23	0.16
Chloride	%	0.23	0.23	0.23	0.40	0.23
Potassium	%	0.98	0.79	0.87	0.52	0.87
Linoleic acid	%	5.98	4.39	4.23	1.59	2.32

([1]) MCP- Monocalcium phosphate, ([2]) FM- Fish meal, ([3]) LM- Larvae meal

(*)Vitamins and minerals are included according to the levels provided by the (National Research Council, 1994)

(**) AMEn- Nitrogen-corrected apparent metabolizable energy value

The treatment diets were formulated in three phases to contain 0% larvae meal (control) and 10%, 25% and 50% larvae and fish meal respectively. Diets containing up to 10% larvae and fish meal are representative of diets used commercially. Diets containing in excess of 10% larvae meal oversupply protein and amino acids but was used in direct comparison with similar fish meal inclusion levels in order to test the effect of larvae meal with a known and accepted comparable protein source. Fish meal is used as a comparison diet, because fish meal is already accepted as a protein source in the animal feed industry while a maize: soya diet is used as control diet, because it is an internationally accepted mixture suitable for poultry production. Due to the high mortality rate observed in the 50% fish meal diet, it was decided to discontinue the treatment.

Experimental design and trial procedure

Four hundred and twenty broiler chicks were divided into 42 cages using a stratified block design representing seven treatments with six replications per treatment and 10 chicks per replicate.

Data collection and analysis

Body weights of broilers were determined at day old and weekly thereafter. Feed was supplied *ad libitum* and weekly intake was determined. Data were used for the calculation of feed conversion ratio (FCR), average daily gains (ADG), protein efficiency ratio (PER) (Boling-Frankenbach *et al*., 2001) and the European production efficiency factor (EPEF) (Awad *et al*., 2009). The formulae used are showed in Equation 11, Equation 12, Equation 13 and Equation 14.

Statistical analyses were done by using STATISTICA (data analysis software system), Version 9, by StatSoft Inc. (2009). Where age effects were not a variable the statistics were done by using one-way analysis of variance (ANOVA) with Fisher least significant difference (LSD) *post hoc* test. Where age and treatment effects were variables the statistics were done using mixed model repeated measures of ANOVA with Fisher LSD *post hoc* test.

Equation 11

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

Equation 12

$$\text{Average Daily Gain} = \frac{\text{Average Live Weight per Chick (g)}}{\text{Age (days)}}$$

Equation 13

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

Equation 14

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

6.4 Results and discussion

Table 33 summarizes the results reported during the broiler growth performance trial. No treatment differences (P>0.05) were observed at day 14 regarding the average live weights, weekly feed intakes and cumulative feed intakes.

At 21 days, there were no treatment differences (P>0.05) between the chicks that received the 10% fish meal and 10% larvae meal diet regarding average live weights and weekly feed intakes. Data reported in the current study showed that the chicks that received either the 10% fish meal or 10% larvae meal diet had significantly (P<0.001) greater average live weights when compared to the chicks that received the control diet and the chicks that received the 50% larvae meal diet, but the average live weight was only significantly better (P<0.05) than the chicks the received the 25% fish meal diet. No treatment differences (P>0.05) were observed between the chicks that received the 25% fish meal and 25% larvae meal diet regarding average live weights and weekly feed intakes. Chicks that received the 10% fish meal diet had significantly better (P<0.05) weekly feed intakes than the chicks that received the control diet. The chicks that received the 50% larvae meal diet were not comparable to any treatment diet, because this treatment had significantly lower (P<0.05) average live weights, feed intakes and cumulative feed intakes when compared to the other treatments.

At 28 days, the chicks that received the 50% larvae meal diet had significantly lower (P<0.001) average live weight, weekly feed intakes and cumulative feed intakes when compared to the other treatment diets. Chicks that received the 25% larvae meal diet had significantly higher (P<0.05) average live weights compared to the chicks that received the 25% fish meal and the control diet, but no treatment differences (P>0.05) were found when compared to the chicks that received the 10% fish meal diet. The chicks that received the 10% larvae meal diet had the significant higher (P<0.001) average live weights when compared to chicks that either received the 25% larvae meal, 25% fish meal or the control diet, no treatment differences (P>0.05) were found when compared to chicks that received the 10% fish meal diet. No treatment differences (P>0.05) were found between the chicks that either received the 10% fish meal, 10% larvae meal or the 25% larvae meal diet regarding the weekly feed intakes and cumulative feed intakes, but they had significantly higher (P<0.001) weekly feed intakes and cumulative feed intakes when compared to the chicks that received the 25% fish meal and the control diets.

Table 33 Averages (± standard error) of weekly live weight (g), weekly feed intake (g) and cumulative feed intake (g) and production ratios of broilers receiving varying amounts of larvae meal in comparison with fish meal

	Diet 1 (control)	Diet 2 (10% FM[5])	Diet 3 (10% LM[6])	Diet 4 (25% FM)	Diet 5 (25% LM)	Diet 7 (50% LM)
Day 7						
Average Live Weight	108.1 ± 4.45	114.50 ± 3.12	126.9 ± 2.61	109.3 ± 2.42	121.8 ± 2.32	110.5 ± 2.90
Weekly Feed Intake	103.2 ± 3.45	105.01 ± 2.21	108.1 ± 1.46	104.5 ± 3.53	104.5 ± 1.39	97.8 ± 3.47
Cumulative Feed intake	103.2 ± 3.45	105.01 ± 2.21	108.2 ± 1.46	104.5 ± 3.53	104.5 ± 1.39	97.8 ± 3.47
Day 14						
Average Live Weight	294.7 ± 3.88	337.6 ± 7.06	367.2 ± 7.22	298.4 ± 11.53	334.5 ± 5.65	280.1 ± 8.58
Weekly Feed Intake	297.3 ± 9.86	320.4 ± 8.30	327.2 ± 2.89	307.4 ± 8.85	333.9 ± 16.08	292.3 ± 21.72
Cumulative Feed intake per Chick	400.4 ±12.46	425.4 ± 9.19	435.3 ± 3.61	411.9 ± 6.90	438.4 ± 15.51	390.1 ± 21.56
Day 21						
Average Live Weight	546.5^{a} ± 32.93	678.7^{b} ± 23.70	702.1^{b} ± 25.65	582.4^{ac} ± 17.48	660.2^{bc} ± 10.44	443.5^{d} ± 25.13
Weekly Feed Intake	410.2^{a} ± 15.67	482.6^{b} ± 17.74	468.1^{abc} ± 22.64	418.6^{ac} ± 11.55	454.3^{ab} ± 6.80	292.7^{d} ± 21.02
Cumulative Feed intake	810.5^{a} ± 27.07	908.0^{a} ± 25.55	903.4^{a} ± 25.32	830.5^{a} ± 14.00	892.6^{a} ± 15.98	682.9^{b} ± 39.26
Day 28						
Average Live Weight	1024.4^{a} ± 62.78	1216.1^{bc} ±41.03	1270.4^{b} ± 39.54	1024.6^{a} ± 24.81	1152.4^{c} ± 24.29	803.3^{d} ± 48.43
Weekly Feed Intake	667.0^{a} ± 33.05	793.7^{b} ± 33.43	777.3^{b} ± 24.89	657.3^{a} ± 13.33	762.1^{b} ± 17.75	536.7^{c} ± 34.61
Cumulative Feed intake	1477.5^{a} ± 59.07	1701.7^{b} ± 58.14	1680.7^{b} ± 50.07	1487.7^{a} ± 27.01	1654.7^{b} ± 18.43	1219.6^{c} ± 72.02
Day 35						
Average Live Weight	1635.6^{a} ± 90.14	1908.5^{b} ± 66.01	1941.3^{b} ± 49.80	1598.4^{a} ± 29.47	1792.4^{c} ± 42.46	1230.5^{d} ± 79.06
Weekly Feed Intake	951.7^{a} ± 36.68	1064.6^{b} ± 34.08	1071.9^{b} ± 28.00	957.5^{a} ± 21.22	1069.4^{b} ± 24.27	779.3^{c} ± 51.91
Cumulative Feed intake	2429.3^{a} ± 89.98	2766.3^{b} ± 90.09	2752.6^{b} ± 74.92	2445.3^{a} ± 45.52	2724.3^{b} ± 25.06	1998.9^{c} ±123.36
ADG (g)[1]	46.70^{a} ± 2.58	54.53^{bc} ± 1.89	55.47^{b} ± 1.42	45.67^{a} ± 0.84	51.21^{c} ± 1.21	35.16^{d} ± 2.26
FCR[2]	1.48^{ab} ± 0.00	1.43^{a} ± 0.02	1.42^{a} ± 0.02	1.52^{b} ± 0.02	1.53^{b} ± 0.03	1.62^{c} ± 0.02
EPEF[3]	315.40^{a} ±23.77	376.50^{b} ±15.01	377.90^{b} ± 10.71	293.50^{a} ± 7.32	317.60^{a} ± 26.68	182.90^{c} ± 13.61
PER[4]	3.62^{a} ± 0.08	3.57^{a} ± 0.03	3.68^{a} ± 0.05	3.19^{b} ± 0.02	3.12^{b} ± 0.04	2.43^{c} ± 0.03

([1]) ADG- Average daily gain, ([2]) FCR- Feed conversion ratio, ([3]) EPEF- European production efficiency factor, ([4]) PER- Protein efficiency ratio, ([5]) FM- Fish meal, ([6]) LM- Larvae meal.

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

At the end of the trial, the chicks that received the 50% larvae meal diet had significantly lower (P<0.001) average body weight, weekly feed intakes, cumulative feed intakes, ADG, FCR, EPEF and PER when compared to the other treatment diets. No treatment differences (P>0.05) were found between the chicks that received the 10% fish meal and the 10% larvae meal diet regarding the average body weights, weekly feed intakes and cumulative feed intakes. The chicks that received the 10% fish meal and the 10% larvae meal diet had significantly higher (P<0.001) average body weights, weekly feed intakes and cumulative feed intakes when compared to the chicks that received either the 25% fish meal, 25% larvae meal or the control diets. The data showed that the chicks that received the 25% larvae meal diet had significantly better (P<0.05) average body weights, weekly feed intakes and cumulative feed intakes when compared to the chicks that received the 25% fish meal diet. No treatment differences (P>0.05) were found regarding the ADG, FCR, EPEF and PER between the chicks that received the 10% larvae and fish meal diets. The chicks that received either the 10% larvae or fish meal diets had significantly higher (P<0.001) ADG when compared to the chicks that received the 25% fish meal and control diets. The chicks that received the 25% fish meal diet had significantly lower (P<0.001) ADG than the chicks that received the 25% larvae meal diet. No treatment differences (P>0.05) were found between the chicks that received either the 25% larvae or 25% fish meal diets regarding the FCR, EPEF and PER. Figure 2, Figure 3 and Figure 5 gives a visual summary of the effects on all the respective performance characteristics over the entire experimental period.

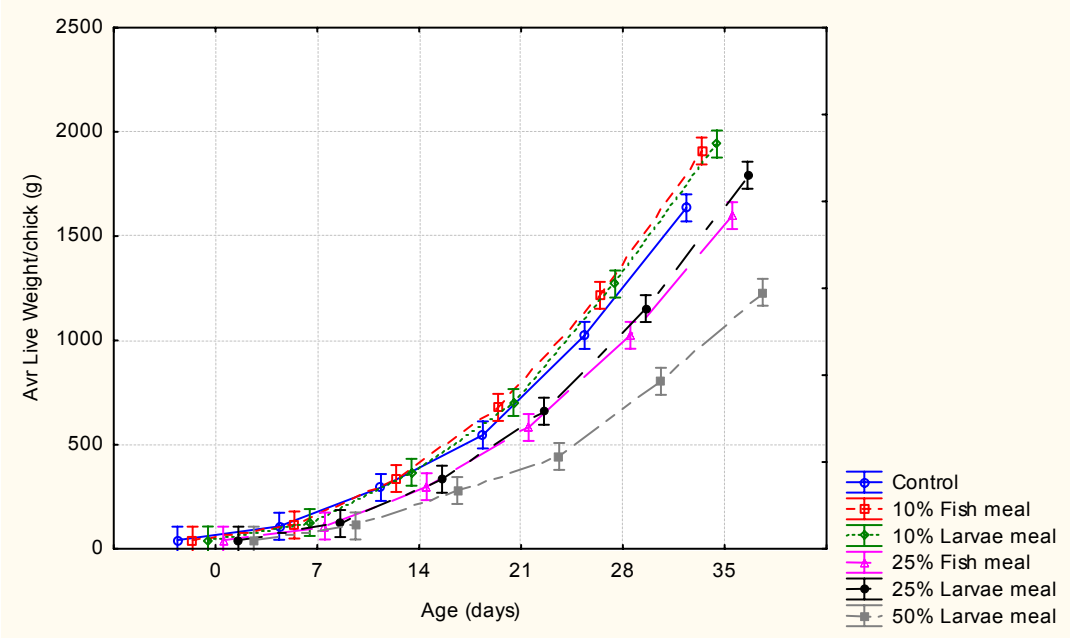

Figure 2 Least square means with error bars for the average live weights caused by age and treatment interaction (P<0.001, 95% confidence interval)

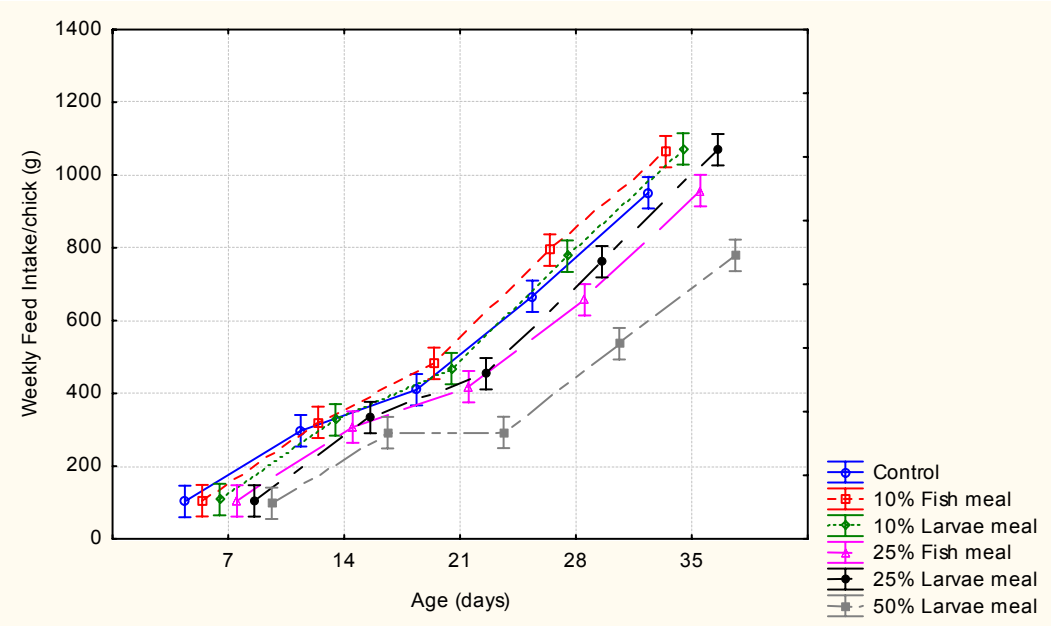

Figure 3 Least square means with error bars for the average weekly feed intakes caused by age and treatment interaction (P<0.001, 95% confidence interval)

Data reported in the current study revealed that the chicks that received a 50% house fly larvae meal supplementation had the lowest average live weight, feed intakes, cumulative feed intakes, ADG and FCR (Figure 2, Figure 3 and Figure 4). This poor performance reported could be explained by the fact that the very high crude protein content of the diet due to a 50% larvae meal inclusion level together with amino acids imbalances that existed in the diet. This is because a balanced amino acid profile is required in the feed for broiler maintenance and growth (Jacob *et al*., 1994). The high crude protein content of the diet also caused an increase in the nitrogen (N) excretion, as uric acid, from the excess amino acids and this process requires a lot of energy (Macleod, 1997). Macleod (1997) estimates that six moles of adinosine triphosphate (ATP) molecules are required to excrete 1g of N. High protein diets lead to high amounts of undigested proteins reaching the caeca and these undigested proteins were shown to cause an inflammatory response and thus further reducing feed efficiency (Collett, 2005). These facts could also be responsible for the significant better (P<0.05) average live weight, feed intakes and cumulative feed intakes of the chicks that received ether the 10% fish meal or the 10% larvae meal diet when compared to the chicks that received either the 25% fish meal or the 25% larvae meal diet.

The treatment differences observed could be because the arginine to lysine ratio for the chicks that either received the control, 10% fish meal or 10% larvae meal diets were calculated to be above 1:1 as compared to the chicks that either received the 25% fish meal, 25% larvae meal or 50% larvae meal diets that were calculated to be below 1:1. Low arginine to lysine ratios were reported to cause a deficiency of the essential amino acid arginine (Austic & Nesheim, 1970; Leeson & Summers, 1997). The interaction of lysine and arginine in animal nutrition is a complex process where data reported that excess lysine has three basic consequences.

Firstly lysine competes with arginine in the renal tubules causing a reduction in arginine retention (Jones *et al*., 1966) and secondly high levels of lysine in the diet of poultry causes an increase in renal arginase activity that cause an increase in arginine oxidation (Austic & Nesheim, 1970; Leeson & Summers, 1997). Thirdly moderate amounts of lysine excesses can cause a depression of the hepatic glycine transamidinase activity in chicks (Jones *et al*., 1967). Austic & Scott, (1975) reported that as the amount of lysine increased in the diet the amount of urea excretion also increased with a slight increase in arginine excretion in the urine.

It was found in the current study that the starter, grower and finisher treatment diet that contained either 25% fish meal, 25% larvae meal or 50% larvae meal had low arginine content. Austic & Scott, 1975 reported that when the lysine content of the diet increased above 3% it caused arginine degradation by renal arginase, depression of hepatic glycine transamidinase, depression of appetite and arginine loss through urine. These authors also reported that when the lysine content of the diet increased above 2% there was a significant urinary loss of arginine. In the current study the lysine content of the treatment diets did not increase above 2%, except in the grower diet that contained 50% larvae meal (1.92% lysine). Data reported by Austic & Scott (1975) also found that if the lysine levels increased to 1.5%, the feed consumption started to decrease significantly. This could also be a factor that contributed to the overall significant lower feed intakes observed with the chicks that received the 50% larvae meal supplementation.

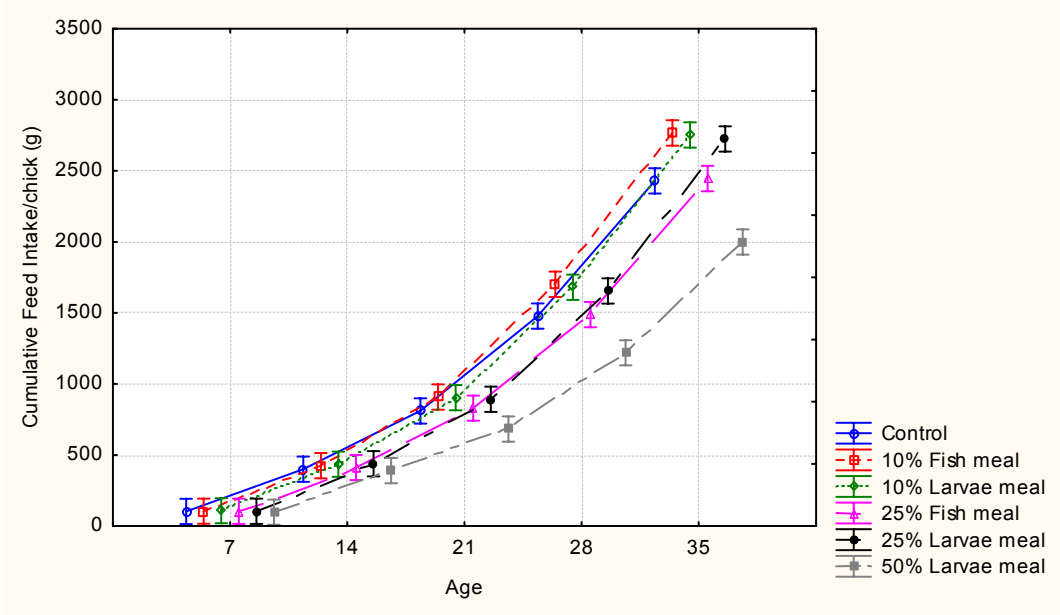

Figure 4 Least square means with error bars for the cumulative feed intakes caused by age and treatment interaction (P<0.001, 95% confidence interval)

In the current study there was no treatment differences (P>0.05) regarding any performance parameter measured between the broilers that were supplemented with either 10% house fly larvae or 10% fish meal. Data

reported however that the chicks that received the 25% house fly larvae meal diet had significantly better (P>0.05) average live weights, feed intakes, cumulative feed intakes and ADG from 28 days until the end of the experimental period than the chicks that received the 25% fish meal diet. These differences observed could be related to the energy content of the diet, because the AMEn of the diet supplemented with 25% house fly larvae meal was higher than the diet that contained 25% fish meal (13.25 MJ/kg vs. 12.86 MJ/kg), leaving more energy available for maintenance and for deamination of excess protein (Macleod, 1997).

The results obtained in the current study for a 10% larvae meal supplementation are comparable to the results reported by Hwangbo *et al*. (2009). These authors reported that weight gains and FCR were significant better (P<0.05) when 10% and 15% larvae meal was supplemented in the total diet of broilers over the whole experimental period when compared to a commercial diet containing soya oil cake meal as protein source.

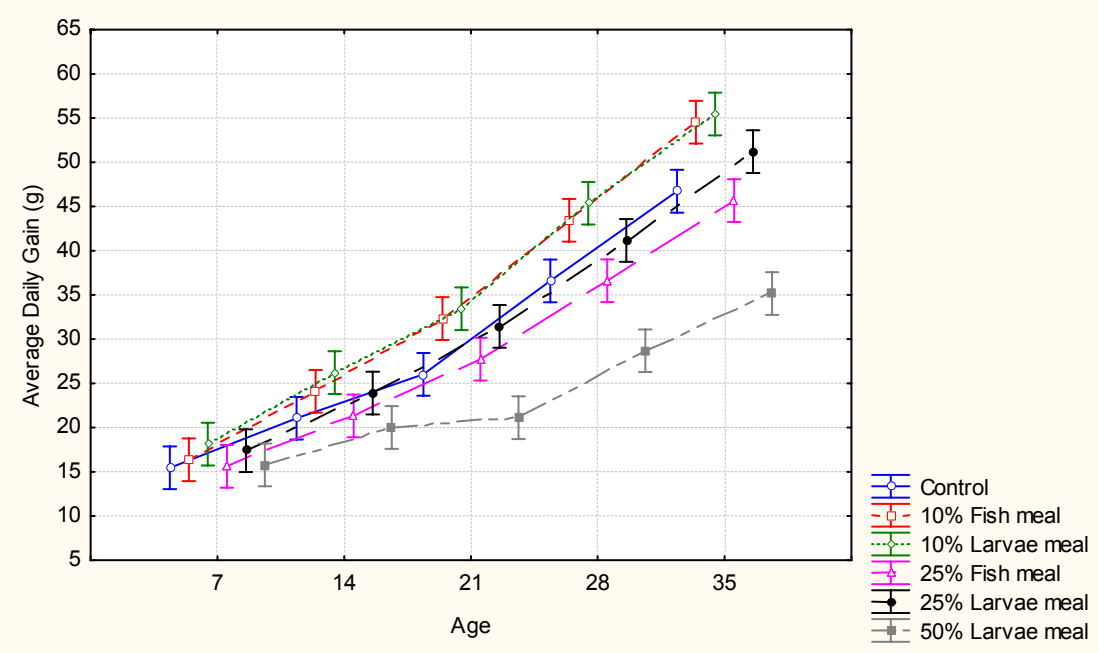

Figure 5 Least square means for the ADG caused by the age and treatment interaction (P<0.001, 95% confidence interval)

Data reported in the current study showed that the diets containing 50% larvae meal had significantly lower (P<0.05) calculated EPEF and PER values when compared to the other treatment diets. The diets containing either 10% larvae meal or 10% fish meal had significantly higher (P<0.05) calculated PER than the diets containing either 25% larvae meal or 25% fish meal. This indicated that the chicks that received either the 10% larvae meal or 10% fish meal utilized their dietary proteins more efficiently. These observed differences could be explained by the fact that when the diet contained high crude protein values the PER differences correspond to the differences in lysine (Buamah & Singsen, 1975). This is because imbalances in the lysine content could

cause reduction in feed intake (Austic & Scott, 1975), leading to a lower PER value. All the calculated PER and EPEF values of the different treatment diets except for the 50% larvae meal supplementation in the current study were above the optimum PER value as reported by Wilding *et al*. (1968) of 3:1 and a EPEF value above 260 units (Butcher & Nilipour, 2009). In Europe a flock regarded to have acceptable growth and liveability parameters should attain an EPEF above 260 units (Butcher & Nilipour, 2009). Data reported that the chicks that received either the 10% larvae meal or the 10% fish meal diet had significant better ($P<0.05$) calculated EPEF values than the chicks that received either the 25% larvae meal or the 25% fish meal diet. Butcher & Nilipour (2009) reported that an average FCR of 1.85 and an ADG of 50g were required for normal broiler production. In the current study it is indicated that the chicks that received either the 10% fish meal, 10% larvae meal, 25% fish meal or the 25% larvae meal had an FCR and ADG above the required values as reported by Butcher & Nilipour (2009).

6.5 Conclusion

The results reported in the current study revealed that *Musca domestica* larvae meal supplementation (at a level of 10% of the total diet) in a three phase feeding system had significantly better performance using the performance parameters measured. When house fly larvae meal was included at a level of 10% of the total diet the average live weights, feed intake, cumulative feed intake as well as ADG were significantly higher compared to a commercial broiler diet that contained soya oil cake meal as the main protein source. Results of the study revealed that at a 10% larvae meal supplementation in the diet was the optimum inclusion level for maximum broiler performance. House fly larvae meal are comparable to fish meal and no treatment differences were found regarding any performance parameter measured between the chicks that received either the 10% larvae meal or the 10% fish meal in the diet. It was however noted that at high inclusion levels of larvae meal or fish meal, the larvae meal were superior to a fish meal diet. Firstly because the chicks that received the 25% larvae meal supplementation had significantly better average live weights, feed intake, cumulative feed intake as well as ADG when compared to the chicks that received the 25% fish meal supplemented diet. Secondly because the chicks that received the 50% larvae meal supplementation survived through the entire experimental period although their performances were poorer, whereas the 50% fish meal supplementation diet was terminated due to welfare reasons. It is thus concluded that *Musca domestica* larvae meal is a good source of protein that it had no detrimental effect on broiler production, even at excessive inclusion levels, and that it has the potential to replace other conventional protein sources (fish meal, soybean oil cake meal) used in the diets of broiler.

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CHAPTER 7

Comparison of the carcass characteristics of broilers chicks grown on a diet containing either *Musca domestica* larvae meal, fish meal or soya bean meal as the main protein source

7.1 Abstract

The effects of *Musca domestica* (common house fly) larvae meal on the meat quality of thirty six broiler chickens were investigated in a fully randomized trial design consisting of three treatment diets consisting either 10% fish meal, 10 % *M. domestica* larvae meal or a maize- soya bean meal diet. Chicks that received either the 10% *M. domestica* larvae meal or 10% fish meal produced significantly heavier carcasses than the chicks that received the maize- soya diet. No treatment differences were found regarding breast and thigh muscle colour as well as the breast and thigh muscle pH. Chicks that received either the 10% larvae meal or 10% fish meal diets had significant higher breast muscle portions relative to carcass weight than the chicks that received the maize- soya diet. House fly larvae meal can be incorporated in to the diets of broilers that have the ability to produce a heavier carcass without negatively affecting specific carcass characteristics.

Keywords- Carcass characteristics, larvae meal, fish meal, soya oil cake meal, broilers

7.2 Introduction

The main factors that determine broiler meat quality can be divided into the appearance and physical characteristics of the meat and these factors are exclusively determined by the consumer (Allen *et al*., 1998; Van Laack *et al.*, 2000 Qiao *et al*., 2001; Swatland, 2004; Huff-Lonergan & Lonergan, 2005). Because consumers are first exposed the appearance (colour, drip etc.) of meat it is the determining factor as to whether the product will be purchased or not (Huff-Lonergan & Lonergan, 2005). Muscle pH is an important factor that has an influence on meat colour, tenderness and water holding capacity (Van Laack *et al.*, 2000; Huff-Lonergan & Lonergan, 2005). The acidification of the meat is an important process that occurs especially when the muscle is converted to meat during the process of *rigor mortis* (Allen *et al*., 1998; Qiao *et al*., 2001; Swatland, 2004). Measuring the pH of the meat gives an indication of the degree of meat acidification after slaughter and an indirect measure of the meat quality.

Because poultry meat colour is a critical food quality attribute, determining the consumer's initial selection of a raw meat product in the marketplace (Huff-Lonergan & Lonergan, 2005) and for the consumer's final evaluation and ultimate acceptance of the cooked product upon consumption (Van Laack *et al.*, 2000). Hwangbo *et al*. (2009) evaluated broiler carcass colour of chicks that received larvae meal as a protein source. The results

reported by Hwangbo *et al*. (2009) revealed that house fly larvae meal supplementation had no significant effect (P>0.05) on breast meat colour. The relationship between the pH of the meat and the meat colour are well established (Allen *et al*., 1998; Qiao *et al*., 2001; Swatland, 2004). Allen *et al.* (1998) reported that dark coloured broiler meat had higher pH values than lighter coloured meat, but the darker meat had a reduced shelf-life that could be attributed to the increased number of psychotropic bacteria that colonize the darker meat.

Water holding capacity is a physical characteristic that is an important factor in determining meat quality, because it influences the appearance of the meat prior to cooking as well as tenderness and juiciness during consumption (Huff-Lonergan & Lonergan, 2005). During the process of *rigor mortis* when the muscle is converted to meat, the pH of the muscle declines until the major muscle proteins reaches the isoelectric point that causes negative and positive proteins to be attracted to each other and water to be expelled to the extracellular space (drip loss) (Van Laack *et al.*, 2000). Muscle pH was shown to affect the degree of drip loss and it is therefore important to measure the muscular pH of the broiler carcass to get an indication of the meat quality. If the pH is above the isoelectric point of the major proteins (pH= 5.3) it causes the water molecules to be more tightly bound, causing more light to be absorbed, leading to a paler meat colour (Van Laack *et al.*, 2000).

Published results indicate that larvae meal supplementation has a significant influence on various carcass characteristics, such as; dressing percentage as well as breast muscle and thigh muscle yield as percentage of the carcass weight (Hwangbo *et al*., 2009). The results reported by Hwangbo *et al*. (2009) revealed that when larvae meal was supplemented in the diets of broilers the dressing percentages, breast muscle (% carcass weight) and thigh muscle (% carcass weight) yields were significantly higher (P<0.05) when compared to the control group with soya bean meal as protein source. The results reported by Hwangbo *et al*. (2009) are not comparable to that reported by Téguia *et al*. (2002) and Awoniyi *et al.* (2003). Téguia *et al*. (2002) and Awoniyi *et al*. (2003) who reported that larvae meal supplementation in broiler diets had no significant effect (P>0.05) on dressing percentage and leg muscle yields (% carcass weight). The data reported by of Awoniyi *et al*. (2003) also showed that larvae meal supplementation in broiler diets had no significant influence (P>0.05) on the breast muscle yields (% carcass weight). This controversy could be attributed to inclusion levels or the lack of repetitions observed in some trials. Data reported that larvae meal supplementation in the diets of broilers had no significant effect (P>0.05) on the amount of abdominal carcass fat, as a percentage of the carcass weight (Téguia *et al*., 2002; Hwangbo *et al*., 2009).

The aim of this experimental trial was to compare carcass characteristics of broilers chicks that were grown on a diet containing either house fly larvae meal, fish meal or soya bean meal as the main protein source.

7.3 Materials and methods

Animals and experimental procedure

Thirty six Ross 308 broiler chicks 36 days of age were slaughtered. These chicks were obtained from the experimental trial described in Chapter 6. Only chicks that received the control, 10% fish meal and 10% house fly larvae meal treatment diets were slaughtered and evaluated. The treatment diets used are shown in Table 34. Two chicks per pen were selected from the middle weight group, rendering 12 chicks per treatment. Chicks were slaughtered according to acceptable commercial standards through immobilization by electrical stunning, followed by exsanguinations.

Data collection

Before and after slaughter broiler live and carcass weights were recorded. The breast and thigh muscles pH was measured by using a Crison pH25 Meter 15 minutes after slaughter. The pH meter probe was placed directly into the left breast muscle and the instrument was given time to stabilize before the pH reading was taken. Between each measurement the probe was rinsed with distilled water and rested in a 3M KCl electrolytic solution. The carcass was portioned to obtain commercial cut yields by weighing the different and the breast, thigh, leg and wing.

The breast muscles were removed by cutting from the *clavicale furcula* bone alongside the carina (keel) bone. The breast muscles were cut up into six pieces and the skins of the thigh muscles were removed and left to bloom for an hour in order to measure the colour. The colour measurements were taken with a BYK- Gardner Colour Guide and for the purpose of this study, the CIElab colour system was used (Commition International de L'Eclairage, 1976) with three measurements; L* (lightness), a* (redness) and b* (yellowness). Positive a* values are a measure of redness and negative a* values are a measure of greenness. Positive b* values are a measure of yellowness and negative b* values indicates blueness.

Statistical analysis were done by using STATISTICA (data analysis software system), Version 9, by StatSoft Inc. (2009). Where age effects were not a variable the statistics were done by using one-way analysis of variances (ANOVA) with Fisher least significant difference (LSD) *post hoc* test. For the purpose of this study the body portions on the right side were used to investigate the treatment effects on the various carcass characteristics and the body portions on the left side were used for meat colour assessment and pH as affected by the treatments.

Table 34 Ingredient and calculated composition of the treatment diets

		Starter			Grower			Finisher		
	Unit	**Diet 1 (C[2])**	**Diet 2 (10% FM[3])**	**Diet 3 (10% LM[4])**	**Diet 1 (C)**	**Diet 2 (10% FM)**	**Diet 3 (10% LM)**	**Diet 1 (C)**	**Diet 2 (10% FM)**	**Diet 3 (10% LM)**
Ingredients										
Maize	%	51.68	54.55	47.81	43.47	52.50	44.51	44.90	55.61	54.41
Soybean full fat	%	32.98	24.18	21.12	49.02	33.78	37.86	51.45	32.54	30.82
Soybean	%	7.99	8.77	15.70	2.17	2.45	4.99			1.40
Fish meal	%	3.32	10.00		2.88	10.00			10.00	
Housefly larvae meal (65º)	%			10.00			10.00			10.00
L-Lysine	%	0.14		0.04				0.27	0.18	0.32
DL-Methionine	%	0.34	0.24	0.35	0.30	0.25	0.35			
L-Threonine	%	0.07								0.04
Premix*	%	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Limestone	%	1.61	1.02	1.98	0.41		0.93	1.36	0.76	1.74
Salt	%	0.20	0.05	0.27	0.24	0.08	0.31	0.31	0.08	0.30
MCP[1]	%	1.26	0.82	0.95	1.19	0.61	0.73	1.31	0.49	0.63
Sodium bicarbonate	%	0.16	0.12	0.13	0.07	0.09	0.08	0.07	0.09	0.09
Oil- soya	%			1.14						
Calculated nutritional value										
Dry matter	%	88.55	88.44	88.94	88.70	88.42	88.82	88.68	88.35	88.52
AMEn** chick	MJ/kg	12.60	12.60	12.60	13.20	13.20	13.20	13.20	13.25	13.25
Crude protein	%	22.97	24.40	25.00	25.01	24.88	25.95	23.12	23.49	22.54
Ether extract	MJ/kg	11.48	11.49	10.65	11.95	12.05	11.10	12.02	12.18	11.35
Ash	%	4.69	4.94	5.62	3.95	4.02	4.75	4.45	4.59	5.09
Crude fibre	%	3.39	2.97	3.66	3.76	3.14	3.97	3.82	3.01	3.62
Crude fat	%	8.77	7.68	8.62	11.04	9.28	10.03	11.23	9.13	9.05
Calcium	%	1.00	1.00	1.00	0.60	0.60	0.60	0.85	0.85	0.85
Lysine	%	1.43	1.47	1.41	1.49	1.51	1.46	1.34	1.42	1.22
Methionine	%	0.69	0.69	0.67	0.69	0.70	0.69	0.61	0.62	0.62
Cystine	%	0.38	0.38	0.38	0.40	0.39	0.39	0.39	0.37	0.36
Methionine+ Cystine	%	1.07	1.07	1.06	1.10	1.09	1.08	1.00	0.99	0.97
Threonine	%	0.93	0.96	0.92	0.98	0.98	0.96	0.90	0.93	0.85
Tryptophan	%	0.26	0.27	0.28	0.29	0.28	0.29	0.27	0.26	0.24
Arginine	%	1.53	1.57	1.52	1.71	1.61	1.60	1.60	1.50	1.32
Isoleucine	%	1.03	1.10	1.05	1.15	1.12	1.09	1.06	1.05	0.91
Leucine	%	1.98	2.10	1.95	2.11	2.13	2.02	1.98	2.04	1.80
Histidine	%	0.62	0.66	0.58	0.68	0.68	0.61	0.64	0.64	0.52
Phenylalanine	%	1.04	1.05	1.13	1.15	1.08	1.18	1.09	1.01	1.01
Tyrosine	%	0.81	0.84	0.95	0.88	0.84	0.96	0.82	0.78	0.82
Phenylalanine+ Tyrosine	%	1.85	1.89	2.08	2.03	1.91	2.14	1.91	1.79	1.84
Valine	%	1.14	1.24	1.22	1.25	1.26	1.26	1.15	1.19	1.09
Glycine + Serine	%	2.12	2.31	2.12	2.34	2.36	2.22	2.15	2.23	1.89
Phosphorous	%	0.81	0.80	0.80	0.79	0.76	0.76	0.75	0.72	0.70
Available phosphorous	%	0.50	0.50	0.50	0.45	0.45	0.45	0.42	0.42	0.42
Sodium	%	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chloride	%	0.22	0.22	0.22	0.23	0.23	0.23	0.23	0.23	0.23
Potassium	%	0.90	0.82	0.98	1.00	0.84	1.02	0.98	0.79	0.87
Linoleic acid	%	4.54	3.61	3.53	5.73	4.46	4.72	5.98	4.39	4.23

([1]) MCP- Monocalcium phosphate, ([2]) C- Control, ([3]) FM- Fish meal, ([4]) LM- Larvae meal

(*) Vitamins and minerals are included according to the levels provided by the National Research Council (1994)

(**) AMEn- Nitrogen-corrected apparent metabolizable energy value

7.4 Results and discussion

Table 35 summarizes the influence of treatment on carcass characteristics. The chicks that received the 10% larvae meal diet had significantly higher ($P<0.05$) live and carcass weights when compared to the chicks that received the control diet (Figure 6), but no significant differences ($P>0.05$) were found when compared to the chicks that received the 10% fish meal diet. Chicks that received either the 10% larvae meal or 10% fish meal diets had significantly higher ($P<0.05$) breast and thigh muscle yields as a percentage of carcass weight than the chicks that received the control diet. The chicks that received the 10% larvae meal diet had significantly lower ($P<0.05$) leg muscle yields as a percentage of carcass weight than the chicks that received either the control or 10% fish meal diets. No treatment differences ($P>0.05$) were found regarding the wing muscle yields as a percentage of the carcass weight. The breast muscle colour L* was significantly higher ($P<0.05$) for the chicks that received the 10% fish meal diet when compared to the other treatment diets. Results indicated that the chicks that received the 10% larvae meal diet had significantly lower ($P<0.05$) breast muscle colour a* when compared to the chicks that received the control diet and had significantly lower ($P<0.05$) thigh muscle colour b* than the chicks that received the 10% fish meal diet. No treatment differences ($P>0.05$) could be found regarding the breast and thigh muscle pH.

No treatment differences ($P>0.05$) were found regarding the dressing percentages. The data reported in the current study are comparable to that reported by Téguia *et al*. (2002) and Awoniyi *et al.* (2003), but it however differ from that reported by Hwangbo *et al.* (2009). Hwangbo *et al.* (2009) reported that chicks that received larvae meal in their diets had significant better ($P<0.05$) dressing percentages than chicks that received a soy bean meal diet.

Table 35 Average (± standard error) broiler carcass measurements as influenced by treatment

	Diet 1 (control)	Diet 2 (10% FM[1])	Diet 3 (10% LM[2])
Live weight (G)	1845.5[a] ± 51.56	2013.8[ab] ± 45.87	2076.7[b] ± 40.03
Carcass weight (g)	1389.3[a] ± 59.87	1508.5[ab] ± 41.75	1545.2[b] ± 33.78
Dressing percentage (%)	75.3 ± 1.26	74.9 ± 2.67	74.4 ± 1.54
Body portion masses (% carcass weight)			
Right side			
Breast muscle	25.83[b] ± 0.53	27.69[a] ± 0.42	28.09[a] ± 0.72
Thigh muscle	9.75[b] ± 0.39	8.21[a] ± 0.21	8.44[a] ± 0.32
Leg muscle	7.20[a] ± 0.09	6.69[b] ± 0.18	7.17[a] ± 0.17
Wing muscle	4.44 ± 0.14	4.88 ± 0.07	4.58 ± 0.14
Colour and pH measurements			
Breast muscle			
L*	50.86[a] ± 0.57	52.64[b] ± 0.57	50.24[a] ± 0.67
a*	4.67[a] ± 0.30	4.32[ab] ± 0.25	3.77[b] ± 0.29
b*	14.03 ± 0.49	15.17 ± 0.45	14.14 ± 0.56
pH	6.14 ± 0.06	6.26 ± 0.05	6.15 ± 0.02
Thigh muscle			
L*	58.33 ± 0.75	58.36 ± 0.91	57.52 ± 0.64
a*	4.44 ± 0.33	4.45 ± 0.48	3.89 ± 0.31
b*	12.70[ab] ± 0.39	13.23[a] ± 0.47	11.42[b] ± 0.60
pH	6.05 ± 0.04	6.07 ± 0.04	6.13 ± 0.05

L*- lightness, a*- redness, b*- yellowness

([1]) FM- Fish meal, ([2]) LM- Larvae meal

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

Van Laack *et al*. (2000) reported that the pH of normal meat is 5.96 and that the normal meat colour of the CIElab L*, a* and b* measurements were 55.1, 2.2 and 9.6 respectively. The data reported in the current study showed that the breast muscle colour of all the treatments fall below the normal where the chicks that received either the control diet or the 10% house fly larvae meal diet had lower breast muscle colour L*. This reported data revealed that the chicks that received the 10% fish meal diet produced meat of lighter colour than described by Van Laack *et al*. (2000). All the chicks from the different treatment diets had higher thigh muscle colour L* than described by Van Laack *et al*. (2000) and this indicated that the meat from the thigh region for all the treatments are darker in colour. All the chicks from the different treatment diets had lower breast and thigh muscle colour a* values described by Van Laack *et al*. (2000) and this indicated that all the diets produce a redder colour meat. Van Laack *et al*. (2000) reported that meat with a lower ultimate pH (the pale breast) could be expected to contain more lactate than meat with a higher pH, because after slaughter the glycogen, glucose, and glucose-6-phosphate reserves are converted into lactate that decreases meat pH. (Fletcher, 1999) concluded in their study that there is a strong correlation between pH and meat colour where darker muscles had a higher pH and lighter muscles had a lower pH value. The pH values reported in the current study was similar to that described by Van Laack *et al*. (2000).

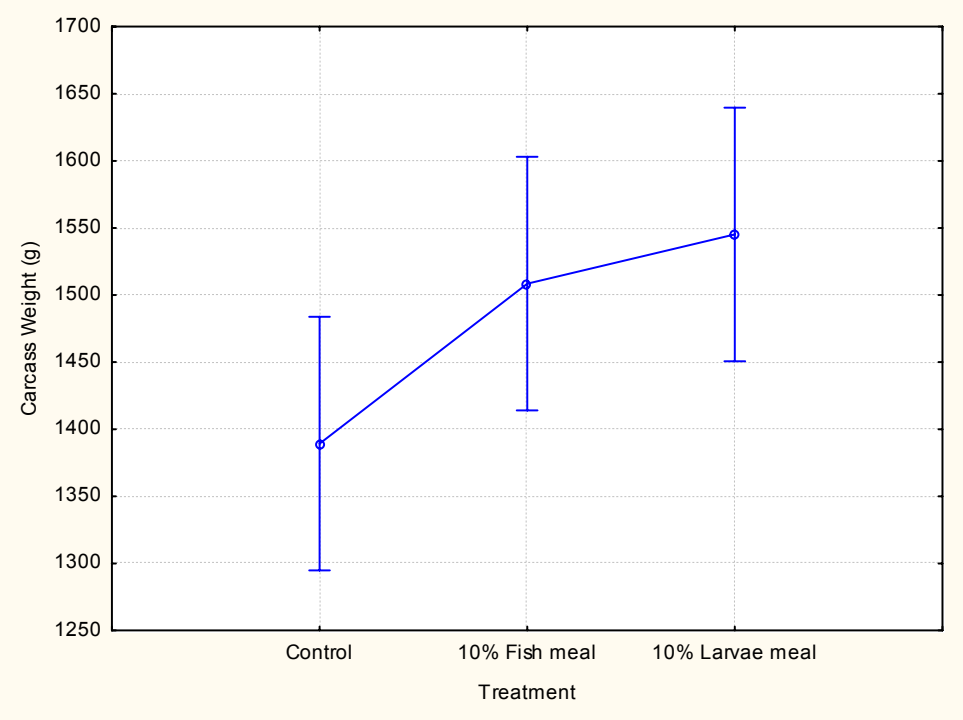

Figure 6 Least square means with error bars for the carcass weights caused by the various treatment (P<0.05, 95% confidence interval)

7.5 Conclusion

Results of the current study revealed that there was no significant treatment differences found between chicks that either received the 10% larvae meal, 10% fish meal or the control treatment diet regarding dressing percentages as well as breast and thigh muscle pH. Chicks that received either the 10% larvae meal or the 10% fish meal produced heavier carcasses when compared to a soya bean meal diet. No treatment differences were found regarding the breast, thigh and wing muscle portions as a percentage of the carcass weight between the chicks that received either the 10% larvae meal or the 10% fish meal diets. Chicks that received the 10% larvae meal diet had significantly lower thigh muscle and significantly higher breast muscle portions as a percentage of body weight than the chicks that received the soya oil cake meal diet. Data reported by the current study indicated that house fly larvae meal can be incorporated into the diets of broilers that produce heavy birds without significantly affecting specific carcass characteristics.

7.6 References

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CHAPTER 8
General conclusion

Musca domestica (common house fly) was proven in this study to be a good quality renewable protein source that can be efficiently utilized to replace conventional protein sources currently used in the diets of broilers. The proximate analysis of house fly larvae meal shows that it contains a gross energy value of 20.10 MJ/kg, 60.38% crude protein, 14.08% crude fat and 10.68% ash while that of the house fly pupae contains a gross energy of 20.42 MJ/kg, 76.23% crude protein, 14.39% crude fat and 7.73% ash.

Data reported show that house fly larvae meal supplementation had a significant influence on average broiler live weights, feed intake, cumulative feed intake as well as ADG when compared to a commercial broiler diet. It is reported in the current study that house fly larvae meal had no detrimental effect on any of the gastro intestinal and organ parameters measured, even at an inclusion level of 50% of the total diet which was not the case with a 50% fish meal diet. A 10% house fly larvae meal inclusion level in broiler diets produced broiler with heavier carcasses than chicks that received soya bean meal as the main protein source without having any detrimental effects on carcass characteristics measured.

Data reported regarding the total tract digestibilities indicated that the total tract crude protein digestibility of house fly pupae meal is significantly better (79%) when compared to the house fly larvae meal (69%). It was also reported that all the analysed amino acids, especially the essential amino acids had total tract digestibilities in excess of that of soy bean meal and comparable to that of fishmeal. In the current study it was found that pupae meal had higher digestibilities and could be used more efficiently than house fly larvae meal in broiler nutrition. The lower digestibilities could, however, be attributed to the longer drying time of larvae meal which could have damaged protein and decreased digestibility

Further Research

In the current study the digestibility of larvae meal was found to be lower than that of pupae meal where this could be attributed to heat damage during drying. It is proposed that the influence of different drying times and temperatures on digestibility of larvae meal be determined in order to establish optima.

In the current study only the use of house fly larvae meal was investigated in the diets of broilers, but much more research is needed on the use of especially house fly pupae meal in broiler diets. In the current research data reported that house fly pupae meal was utilized more efficiently by broilers.

Research is also required on the use of house fly larvae and pupae meal in the diets of laying hens to determine the effect of these meals on hen day production as well as on egg quality.

Research in other species including pigs, companion animals, aquaculture and ruminants is also warranted. This research should focus on bio-availability of minerals, palatability, susceptibility to heat damage and rumen degradability.