An evaluation of defatted black soldier fly (*Hermetia illucens*) larvae as a protein source for broiler chicken diets

by

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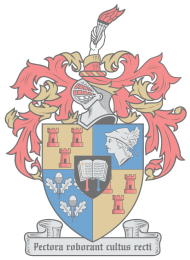

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Summary

The growth rate of the chicken meat production industry needs to match or exceed the growth rate of the human population to provide sufficient dietary protein for as many people as possible. Thus, alternative protein sources for animal feed are required to support the increasing demands on existing protein sources. Insect protein has recently been recognised as a potential protein source and feed ingredient for animal production systems. Black soldier fly (*Hermetia illucens*) larvae is one of the many insect protein sources being researched for its inclusion in fish, pig and poultry diets. Mass-rearing of larvae on various waste substrates acts simultaneously as a waste reduction and protein production system.

In the current study, the inclusion of defatted black soldier fly larvae (BSFL) in broiler chicken diets was evaluated. The study compared the inclusion of two different defatted BSFL treatments (namely dry-rendered (DR) and extruded (EX)), to that of a full-fat (FF) BSFL treatment and a control treatment. The protein source utilised in the control was soybean meal. The DR, EX and FF products were included at a 15% level in each of the three-phase treatment diets. The control treatment was found to have the significantly highest tibia bone calcium levels, as well as the most acidic ileal gut environment and heavier gizzards. No signs of gizzard erosion were found for any of the treatments tested. The DR treatment was found to be the least efficient larvae treatment tested. Although it had high intakes towards the end of the 28-day trial, this did not result in an increased growth rate. The digestibility trial DR diet was found to have approximately half the mineral concentrations of the EX treatment and a highly non-bioavailable energy content (AME of 8.84MJ/kg). The EX, DR and FF treatment had very high digestibility coefficients (above 90%) for all nutrients analysed. A microscopic evaluation found the DR treatment to have high levels of heat discolouration, yet no significant heat damage. Nonetheless, it was suggested that the palatability of the treatment may have been affected by the processing technique which may have played a role in the relatively inferior production performance. In contrast, the EX treatment performed relatively well within the production parameters with the highest level of breast meat crude protein. The treatment had the darkest breast meat, however did not fall outside of the parameter's normal. It also yielded the highest meat calcium levels amongst the treatments. The FF treatment yielded the highest calcium to phosphorus ratio, due to the significantly low phosphorus levels. The FF treatment boasted the highest resistance to bone breakage and was superior to all treatments in terms of average live weight, average daily gain, feed conversion ratio, European production efficiency factor and the protein efficiency ratio.

The DR treatment compared well with the control regarding production and carcass parameters with no adverse organ or bone limitations found for the DR treatment inclusion. The FF and EX treatments can both successfully be used as a viable protein source in broiler chicken diets at up to 15% inclusion to improve production efficiency.

Opsomming

Die groeikoers van hoendervleisproduksie moet die menslike bevolking se groeikoers ewenaar of oortref om vir soveel mense moontlik voldoende dieetproteïen te voorsien. Alternatiewe proteïenbronne word vir dierevoer benodig om die toenemende eise wat aan bestaande proteïenbronne gestel word, te ondersteun. Insekproteïen is onlangs as ’n potensiële proteïenbron en voerbestanddeel in diereproduksiestelsels erken. Larwes van die venstervlieg (*Hermetia illucens*) is een van die talle bronne van insekproteïen wat nagevors is vir insluiting in visse, varke en pluimvee se voeding. Larwes wat in massas op verskillende afvalsubstrate grootgemaak word, dien terselfdertyd as ’n stelsel vir afvalvermindering én ’n stelsel vir proteïenproduksie.

In hierdie studie is die insluiting van ontvette venstervlieglarwes (BSFL) in braaikuikens se dieet geëvalueer. Die studie het die insluiting van twee verskillende ontvette BSFL-behandelings (naamlik droë ontvetting (DR) en ekstrusie (EX)) met die gebruik van ’n volvet-(FF)-BSFL-behandeling en ’n kontrolebehandeling vergelyk. Die proteïenbron wat in die kontrole gebruik is, was sojameel. Die DR-, EX- en FF-produkte is in elk van die driefase-behandelingsdiëte teen ’n vlak van 15% ingesluit. Die kontrolebehandeling het die beduidend hoogste kalsiumvlakke in die tibia, die mees asidiese ileale ingewandsomgewing en swaarder kroppe gegee. Geen tekens van kroperosie is vir enige van die getoetste behandelings gevind nie. Die DR-behandeling was die mins doeltreffende larwebehandeling wat getoets is. Hoewel inname teen die einde van die 28 dae proeftyd hoog was, het dit nie die groeikoers verhoog nie. Die DR-proefdieet vir verteerbaarheid het nagenoeg die helfte van die mineraalkonsentrasies van die EX-behandeling en ’n hoogs nie-biobeskikbare energieinhoud (AME van 8.84MJ/kg) gehad. Die EX-, DR- en FF-behandelings het vir alle voedingstowwe wat ontleed is ’n uiters hoë verteerbaarheidskoëffisiënt (hoër as 90%) gehad. ’n Mikroskopiese evaluasie het getoon dat die DR-behandeling hoë vlakke van hitteverkleuring gehad het, maar geen betekenisvolle hitteskade nie. Die aanduiding is nietemin dat die verwerkingstegniek moontlik die smaaklikheid van die behandeling beïnvloed het, wat ’n rol kon gespeel het in die relatief swakker produksieprestasie. Daarteenoor het die EX-behandeling relatief goed gepresteer binne die produksieparameters, met die hoogste vlak van borsvleis-ruproteïen. Die behandeling het die donkerste borsvleis gelewer, maar dit het nie buite die parameter se normaal geval nie. Dit het van al die behandelings ook die hoogste vleiskalsiumvlakke gegee. Die FF-behandeling het weens die beduidende lae fosforvlakke die hoogste verhouding van kalsium tot fosfor gegee. Die FF-behandeling het die hoogste weerstand teen beenbreuke gelewer en het alle behandelings getroef wat betref gemiddelde lewende gewig, gemiddelde daaglikse gewigstoename, voeromsettingsverhouding, die Europese produksiedoeltreffendheidsfaktor en die proteïendoeltreffendheidsfaktor.

Die DR-behandeling het goed met die kontrole vergelyk wat produksie- en karkasparameters betref, met geen ongunstige orgaan- of beenbeperkings wat vir die DR-behandelingsinsluiting gevind is nie. Die FF- en

EX-behandelings kan albei met sukses tot 15%-insluiting gebruik word as ’n lewensvatbare bron van proteïen in braaikuikens se dieet om produksiedoeltreffendheid te verhoog.

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List of abbreviations

°C	Degrees Celsius
µL	Microliter
AA	Amino acid
ad lib	*ad libitum*
ADG	Average daily gain
ALASA	Agricultural Laboratory Association of Southern Africa
AME	Apparent metabolisable energy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BSE	Bovine Spongiform Encephalopathy
BSF	Black soldier fly
BSFL	Black soldier fly larvae
Ca	Calcium
CAF	Central Analytical Facility
CF	Crude fibre
Cl^-	Chloride
Co	Cobalt
CP	Crude Protein
CTTD	Coefficient of total tract digestibility
Cu	Copper
DM	Dry matter
DR	Dry-rendered
E. coli	*Escherichia coli*
EE	Ether extract
EPEF	European production efficiency factor
EX	Extruded
FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
Fe	Iron
FF	Full-fat
g	Grams
GE	Gross energy
GIT	Gastro-intestinal tract
GLM	General linear models
H_2SO_4	Sulphuric acid
IBD	Infectious bursal disease
K	Potassium
kg	Kilogram
L	Litres
LSM	Least square mean
m/m	Mass per mass
ME	Metabolisable energy
Mg	Magnesium
mg	Milligram
MJ	Megajoule

mL	Millilitre
mm	Millimetre
Mn	Manganese
N	Newton
N/g	Newton per gram
NaCl	Sodium chloride
NCD	New Castles disease
NPN	Non-protein nitrogen
NRC	National Research Council
P	Phosphorus
PER	Protein efficiency ratio
pH_i	Initial pH
pH_u	Ultimate pH
REC	Research Ethics Committee
S. enterica	*Salmonella enterica*
SAPA	South African Poultry Association
SE	Standard Error
Se	Selenium
spp	Species
TD	Tibial dyschondroplasia
TMA	Trimethylamine
USA	United States of America
Zn	Zinc

Notes

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

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CHAPTER 1

General introduction

The poultry industry makes up almost two thirds of the animal protein produced in South Africa which makes it, by far, the biggest contributor to the agricultural industry (SAPA, 2017). Within the broiler production industry, feed is the biggest cost. Protein is the most expensive component of broiler diets and therefore any alleviation in the price of protein will cause a significant financial relief on both producers and consumers.

The number of consumers eating animal protein to fulfil their dietary requirements is growing exponentially due to population explosion (Dar & Gowda, 2013). More people are choosing chicken meat as the relatively healthier and cheaper animal protein source (Yueng & Yee, 2002). The existing protein sources, both animal and plant derived, are not projected to meet future demands (Capper, 2013). Therefore, it is argued that the protein sources that are used directly by humans, should not also be shared as ingredients in animal production systems.

The increase in the world population will result in more waste being produced due to inefficient production systems and the discarding of nutrient-rich matter (Cordell *et al*., 2009). Making use of bioconversion, insects can be successfully reared on organic waste (Newton *et al*., 2005a). Insects can thus be mass-reared with relatively low water and space requirements and cheap inputs (such as waste). Insects also form part of the natural diet of chickens (DeFoliart, 1975), and therefore the inclusion of insects into broiler diets has been presented as a means of waste utilisation and nutrient recycling.

The *Hermetia illucens* (black soldier fly) are one of the several insects which have been investigated as a potential protein source in livestock diets. The inclusion of larvae and pre-pupae of the *H. illucens* have been researched in aquaculture diets (St-Hilaire *et al*., 2007; Sealey *et al*., 2011, Talamuk, 2016), swine diets (Newton *et al*., 2005b; Driemeyer, 2016) and poultry diets (De Marco *et al*., 2015; Uushona, 2015). Black soldier fly larvae (BSFL) are rich in both protein and lipids, and contain an amino acid profile suitable for several species (Newton *et al*., 2005b). These high levels of lipid dilute the level of crude protein content in BSFL.

The defatting of the BSFL would provide a product of a relatively higher crude protein content and results in a by-product of lipid, which has potential as a biofuel (Leong *et al*., 2016). However, various techniques of defatting are still under investigation and trial (Haasbroek, 2016; Surendra *et al*., 2016). The measure of success for defatting would be how well the process reduced the lipid content of the larvae, without adversely affecting the nutrient composition or nutrient bioavailability.

The aim of the study was to evaluate the use of defatted BSFL as a protein source in broiler chicken diets, using four assessment components:

I. Evaluation of the **production parameters** of broiler chickens fed 15% BSFL in their diets
II. Evaluate the **carcass characteristics,** including physical measurements and chemical meat analysis, of broilers provided with diets which include 15% defatted BSFL
III. Evaluate the effects of defatted BSFL treatments on the **organ, gut and bone parameters** of broiler chickens consuming diets with 15% BSFL inclusion
IV. Measure the **nutrient digestibility and apparent metabolisable energy** of the defatted BSFL treatments by young broiler chicks

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CHAPTER 2

Literature review

2.1 Introduction

The definition of "sustainable" has been given as "meeting the needs of the present without compromising the ability of future generations to meet their own needs" (Burton, 1987). As the 'future generation' grows larger and larger due to population explosion, incredible pressure is placed on the agricultural sector to provide enough food for everyone (Dar & Gowda, 2013). In order to provide sufficient protein for this growing population, the use of established protein sources (such as soybean meal) should not be shared between direct human consumption and indirect livestock consumption as this will further increase the cost of this commodity (Ravindran & Blair, 1993). The cost of fish meal is constantly rising, as natural fish stocks become depleted. Capper (2013) suggests that the existing agricultural production systems are not sustainable enough to meet future demands.

It is in this light, that efforts must be made toward the adjustment of the agricultural sector into an industry with more environmentally supportive components and practices. During food production, huge amounts of waste are produced and not recovered. This waste does, however, have potential in other industry sectors (Cordell *et al*., 2009). Insects have been recognized as viable decomposers of organic waste and can be used as a means of nutrient recovery from waste (Newton *et al*., 2005b). Insects have also been explored as an alternative source of protein in various animal feeds (Premalatha *et al*., 2011). Insects form part of the natural diet of many animals (DeFoliart, 1975) and therefore the concept of insect protein in animal diets is not completely novel.

Insects can be used as a protein source in livestock diets, and indirectly serve as a protein source for human consumption (Ramos-elorduy *et al*., 2002). Insects require much less space than crop production, utilise much less water than crops, do not depend on seasonal conditions and can therefore be produced on a continuous basis. Both larvae and pupae meal have been described as a valued source of essential amino acids and a well-balanced protein source for use in poultry diets (El Boushy, 1991; Pretorius, 2011).

The global consumption of poultry meat has increased considerably in the past decade (Ravindran, 2013). Poultry meat is believed to have increased in demand because of the increase in disposable income (Food and Agricultural Organisation (FAO), 2010) and because it is one of the cheaper and more readily available animal protein sources for consumers (Khusro *et al*., 2012). However, the increase in poultry products calls for an increase in the quantity and range of raw ingredients available for the production of broiler chickens (Premalatha *et al*., 2011).

Black soldier fly larvae (BSFL) and pupae have been mainly researched with regards to their application in aquaculture diets (Bondari & Sheppard, 1981, 1987; St-Hilaire *et al*., 2007b; Sealey *et al*., 2011), less so in

monogastric diets (Newton *et al*., 2005a) and minimally in broiler chicken diets. This chapter aims to review the use of the dipteran black soldier fly larvae in broiler diets and find the existing gaps in the literature requiring consideration and evaluation.

2.2 Global utilisation of insects

In developing countries where people experience a scarcity of available animal protein, people have, and are encouraged to, practice entomophagy as part of their daily lifestyles (Womeni *et al*., 2009; Riggi *et al*., 2013). 'Entomophagy' is the practice of consuming insects (Yen, 2009; Chakravorty *et al*., 2011; Ekpo, 2011). Insects are believed to provide as much as 10% of some ethnic populations protein, energy, vitamins and minerals (McEvilly, 2000). In some cases, insects are the preferred protein source over animal protein. For example, the Pedi clan of South Africa are found to choose to eat certain insects over consuming beef (DeFoliart, 1989).

Insects have also played a significant role in the practice of traditional healing and for medicinal purposes, referred to as 'Entomotherapy' (de Figueiredo *et al*., 2015). In this practice, certain substances believed to have valuable properties are extracted from insects and used in medicines (Dossey, 2010). Also in the medical field, the black soldier fly larvae are used in forensic science to estimate the post-mortem interval (PMI) in human corpses (Manzano-Agugliaro *et al*., 2012). The PMI helps to determine time of death and is defined as the time in which a dead body has been exposed to the environment (Turchetto & Vanin, 2004). This practice is referred to as forensic entomology (Lord *et al*., 1994).

Fly larvae, in general, are 'detritivores' (organisms that use organic waste as a food source), and are commonly found in compost heaps. Due to this trait, fly larvae have been studied as potential waste reducers (El Boushy, 1991). In expanding this valuable feature of larvae, the possibility of 'nutrient circulation' where these waste-reducing insects can be harvested and used as animal feed requires further investigation. Linder (1919) was the first to report on the production of larvae as a protein source for animal production from waste products, but this study was unfortunately not concluded.

2.2.1 Insects in animal feed

A wide variety of insect species have been studied and were found to provide a valuable protein source for a wide variety of livestock species (Awoniyi *et al*., 2003; Newton *et al*., 2005a; St-Hilaire *et al*., 2007b; Hopley, 2015). In this way, nature has provided a sustainable and efficient way of, not only controlling waste management, but also an environmentally friendly source of protein for animal feed (Bondari & Sheppard, 1987).

Insects do not require energy to regulate their bodily temperature as they are 'poikilothermic'. This term refers to an organism that has a body temperature that varies with the temperature of its surroundings. This allows insects to store more energy in their body mass and be excellent feed converters (Nijdam *et al*., 2012). Newton *et al*. (2005b) proposes that the essential amino acids provided by insects may help to minimize the

costs of animal production and lead to profit maximisation for animal producers. Therefore, insects are currently being considered as a cost-effective protein source for animal feeds (Premalatha *et al*., 2011).

2.2.2 Consumer perception

Wild birds, as well as free-range chickens, consume insects as part of their natural diet with no reported health problems experienced as a result of this (Miao *et al*., 2005). Studies undertaken in the United States of America (USA) and in Europe have found consumers are willing to pay more for animal products that are sourced from free-range production systems (Carlsson *et al*., 2003). Insects, specifically larvae, have been found to contain natural antibiotics that reduce the transmission of any possible pathogens (Sheppard *et al*., 2007). This may also help to alleviate the need for producers to include antibiotics in chicken feeds, which has also become a growing concern amongst consumers.

Pirvutoiu & Popescu (2013) found that the consumption of poultry meat increased amongst consumers with higher education and income levels. Yueng & Yee (2002) found consumers who were retired or did not hold degrees relied on food safety information used in product marketing to govern their food choices and the related concerns regarding health risks. These authors also found that chicken meat is perceived by consumers as the more healthy and popular option of choice in the United Kingdom. Therefore, if marketed as a speciality product supporting sustainability, insect protein fed to broiler chickens could potentially do very well in the market for a variety of consumers.

2.3 Insect protein

The environmental conditions in which insects are mass-reared affect the nutritional and physical qualities of the insect, and can therefore be optimised (Sealey *et al*., 2011). Insects suitable for mass production would need to feature specific traits regarding duration of larval stage, pupation synchronization, uniformity of larvae/pupae weight, conversion rates and daily biomass accumulation (Peters & Barbosa, 1977; Scriber & Slansky, 1981; Al-sharaby, 2010). Other attributes that would be favourable are protein quality, disease resistance and substrate cost and composition.

The studies regarding insect protein incorporation in animal feed are usually in comparison with existing protein sources, for example fish meal, soybean meal and groundnut oilcake. Most of the published literature reported the use of fly larvae as a protein source compared well as an ingredient in efficient broiler production with established protein sources. The common housefly larvae (*Musca domestica*) was studied by Calvert *et al*. (1969) using poultry waste as a larvae substrate and concluded that dried housefly larvae provided the protein needed by broilers for normal growth and development during the early stages of their lives. These authors were amongst the original experimentalists of using insect protein in animal feed.

Since then, other authors (Newton *et al*., 1977, 2005a; St-Hilaire *et al*., 2007a; Sealey *et al*., 2011; Finke, 2012) have concluded that BSFL presented a beneficial nutritional composition and could serve as a partial substitution for fish meal, as well as other protein sources used in animal nutrition.

2.3.1 Dipteran family

The Dipteran order of insects is known as the 'true flies' or 'two-winged flies' and this includes mosquitoes, black flies, midges, fruit flies and house flies (Resh & Carde, 2003). The two flies from this order that will be further discussed are the *M. domestica* and the *H. illucens.*

2.3.1.1 Common housefly (*M. domestica)* larvae and pre-pupae

The larvae of the common housefly have been shown to have great potential as a protein source in poultry nutrition (Teguia *et al*., 2002; Awoniyi *et al*., 2003; Zuidhof *et al*., 2003; Adeniji, 2007; Agunbiade *et al*., 2007; Hwangbo *et al*., 2009; Pretorius, 2011).

In a study conducted by Teguia *et al*. (2002), diets that contained the highest larvae inclusion allowed for a significantly higher weight gain than the diets that included fish meal. The breast muscles of the birds that consumed housefly larvae boasted higher lysine and tryptophan levels. However, Ocio & Vinaras (1979), Awoniyi *et al*. (2003) and Djordjevic *et al*. (2008) found that there were no significant differences in weight gain between birds that were fed diets with housefly larvae and those that were fed diets with good quality fishmeal. Furthermore, Hwangbo *et al*. (2009) believes the success of the housefly larvae meal is a result of the high protein content, high protein digestibility and optimal amino acid profile of the larvae meal. Ogunji *et al*. (2007) states that Spinelli *et al*. (1979) found the amino acid composition of larvae meal to be comparable with that of fish meal, including the essential amino acids. Housefly larvae meal has been reported as a very good source of lysine, methionine and arginine (El Boushy, 1991). Pretorius (2011) differentiated between the housefly larvae and housefly pupae on this subject and reported larvae as a good source of lysine and pupae a good source of arginine.

With regards to protein quantity, Awoniyi *et al*. (2003) reported housefly larvae as having a crude protein content of 55%. Pretorius (2011) found the crude protein to be slightly higher than this (60%). However, these are both in line with other authors' results that ranged between 39% and 70% (St-Hilaire *et al*., 2007a). Therefore, the housefly offers a superior crude protein to its dipteran relative the black soldier fly larvae that was reported to contain 42% crude protein, with relatively higher crude fat content of 38% (Newton *et al*., 1977).

2.3.1.2 Black soldier fly (*H. illucens*) larvae and pre-pupae

The black soldier fly (BSF) is known to reduce the prevalence and breeding of the housefly, which can help to reduce the possible spreading of disease by the housefly (Bradley & Sheppard, 1984). It is also believed that the BSF larvae are able to consume and digest organic waste at a faster and more efficient rate than the housefly larvae (Kim *et al*., 2011). Naturally, the BSF can be found all over South America and Asia, but is

native to Colombia (Canary & Gonzalez, 2012). They are able to survive and adapt to a wide array of environmental temperatures (McCallan, 1974). These flies fall under the Stratiomyidae family and, in the wild, are commonly found in habitats suitable for larval development such as marshlands and generally damp places with animal waste, rotten fruit or any decaying organic matter (Rozkošný, 1982; Li *et al*., 2011). The BSF is also not regarded as a pest species (Sheppard *et al*., 1994; Newton *et al*., 2005b) since the adult fly does not eat or look for food and thus does not enter areas where people live (Sheppard *et al*., 1994). The adult fly relies only on the energy stores accumulated during the larval stage.

2.3.1.2.1 Life cycle

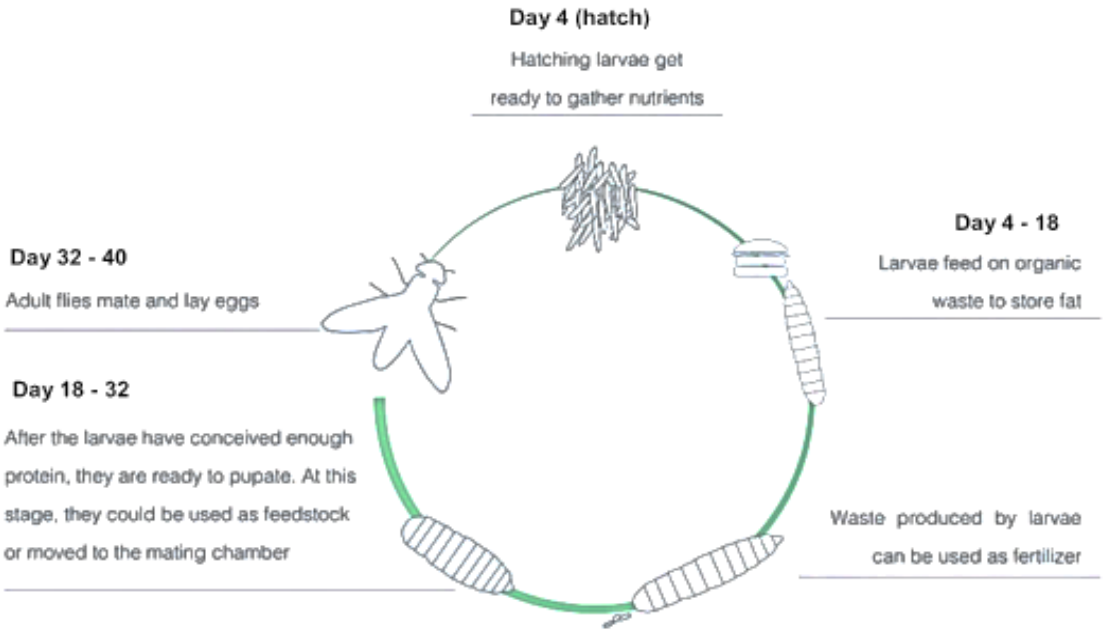

Figure 1 Life cycle of the black soldier fly, Hermetia illucens (Adapted from Fok, 2014)

Newton *et al*. (2005b) describes the BSF as having five distinct phases in its life cycle: egg, larvae, pre-pupae, pupae and adult. This life cycle is between 40 and 44 days (Fok, 2014). Fertilised eggs can take between 102 and 105 hours to hatch, at 24°C (Li *et al*., 2011). Newly hatched BSF larvae are creamy white in colour and actively crawl towards substrate where they vigorously feed during this life phase. In ideal environmental conditions, it takes the larvae approximately two weeks to reach maturity. However if the conditions are sub-optimal this period can last up to several months (Sheppard *et al*., 2002; Myers *et al*., 2008).

During the larvae and pupae phases, the BSF can convert organic waste into high protein and high fat biomass. During this bioconversion, waste is reduced and pathogens are minimised (Erickson *et al*., 2004), while nitrogen and phosphorus are also reduced (Sheppard, 1983; Sheppard *et al*., 1994; Newton *et al*., 2005b; Diener *et al*., 2009, 2011).

During the pre-pupae stage the mouth-part is changed into a hook mechanism used for moving around, which is why this phase is dubbed the ‘wondering phase’. After their mouth-part has changed form, they can no longer feed and therefore seek to escape their substrate and position themselves for pupation. It is this

behaviour during pre-pupation that can be used in mass-rearing as a self-collection method (Diener *et al*., 2011).

At maturity, the larvae weigh about 0.2g and are around 25mm in length and 6mm in diameter. Regardless of their small size they are tough and robust and are still able to survive extreme oxygen deprivation if need be (Sheppard *et al*., 2002). Within the larval stage there are a further 5 instar stages (Hall & Gerhardt, 2001).

2.3.1.2.2 Benefits

It has been reported that the use of black soldier fly larvae (BSFL) has led to the deactivation of *Escherichia coli* (*E. Coli)* O157:H7 when introduced into poultry manure (Erickson *et al*., 2004) but the same result was not found in bovine or pig manure in this study. This is contradictory to the finding by Liu *et al*. (2008), who found that BSFL did indeed reduce *E. Coli* O157:H7 activity in dairy manure (it is emphasized that the larvae to manure ratio needs to be carefully considered in order for this deactivation to be effective). Bondari & Sheppard (1987) reported that not only can the BSFL reduce the *E. Coli* pathogen count but also the *Salmonella enterica* pathogen count through the modification of manure microflora. The BSFL has also been found to significantly reduce *Salmonella* species (*spp*) present in human faeces (Lalander *et al*., 2013). In the same way that animals produce antibodies as part of a defence mechanism, lower forms of animals (including the BSFL) are able to chemically defend themselves from invasion of pathogens (Sheppard *et al*., 1994). These mechanisms involve antibacterial proteins or peptides, which can be produced in response to an attack or infection (Sheppard *et al*., 1994).

During the degradation and reduction of organic matter in BSFL substrate, the larvae's intestinal bacteria produce probiotic compounds. This was found to be true for three different diets and was attributed to the unique BSFL gut microflora (Jeon *et al*., 2011). During this degradation, the quantity of organic matter (in some cases manure) can be reduced significantly in quantity (Newton *et al*., 2005b), which leads to a 50-60% reduction in possible air pollution (Canary & Gonzalez, 2012). The BSFL are able to first utilize the nutrients in the manure and the remainder of this manure is then able to be used on crops as a fertilizer (Erickson *et al*., 2004).

Newton *et al*. (2005b) and Kim *et al*. (2011) both found BSFL to reduce organic matter by 60%, and in this process, achieve a bodily composition that is high in both energy and protein (Jeon *et al*., 2011). When 1248.6g of fresh manure was treated with 1200 BSFL, the larvae could produce 15.6g of biodiesel (1%), 54.4g of residual larvae (4%) and 96.2g of sugar (8%) (Li *et al*., 2011). Beyond this, the larvae harvested would be expected to have approximately 42% crude protein and 38% crude fat in bodily mass (Newton *et al*., 1977).

2.3.1.2.3 Chemical composition

The crude protein of the BSFL is lower than that of the housefly larvae and this is reported in a study by St-Hilaire *et al*. (2007b), who reported a crude protein content of 44% for BSF pre-pupae and a 70% crude protein content for the housefly larvae. Other authors (Table 1) have found protein contents that range from 35% crude protein (Haasbroek, 2016) to 44% protein (Surendra *et al*., 2016) for dried full-fat BSF larvae and pre-pupae (Newton *et al*., 1977; Bondari & Sheppard, 1981; St-Hilaire *et al*., 2007b; Diener *et al*., 2009, 2011; Kroeckel *et al*., 2012). A small portion of the protein in the BSF larvae and pre-pupae represents the chitinous cuticle, however this may be removed by additional fractionation in order to improve the amino acid profile (Newton *et al*., 2005a).

Neither fish meal nor soybean meal can fully supply the broiler chicken with all the amino acids it requires (Table 4). The importance of the natural interaction between amino acids is not to be understated. To manage these interactions, it may be best to combine the BSFL with other protein sources to achieve the most optimal amino acid profile for the specific animal being fed. The amino acid (AA) profile of BSFL is closer to the ideal AA profile of broilers. However, according to the values reported in Table 4, BSFL would also need to be supplemented in some way to make up the difference in essential AA. Newton *et al*. (2005a) found BSFL had significantly higher levels of calcium, manganese and iron than soybean meal, although lower potassium levels. Kroeckel *et al*. (2012) reported BSFL to have 6.5% calcium and 0.7% phosphorus, which was higher calcium and lower phosphorus contents than those reported by Newton *et al*. (1977) at 5% for calcium and 1.5% for phosphorus (Table 2).

Table 1 Nutrient Composition of black soldier fly (on a dry matter basis (%))

	Tschirner & Simon, 2015	St Hilaire, 2007	Barroso, 2014	Bondari & Sheppard, 1981	Newton, 2005	Haasbroek, 2016	Surendra, 2016	Haasbroek, 2016	Kroeckel, 2012	Tschirner & Simon, 2015	Surendra, 2016	Surendra, 2016
Form	Full fat	Full fat	Full fat	Full fat	Full fat	Full fat	Full fat	Pressed	Pressed	Pressed	Pressed	Solvent extracted
Physiological Age	Young Larvae	Pre-pupae	Larvae	Larvae	Mixed age	Larvae	Pre-pupae	Larvae	Pre-pupae	Young Larvae	Pre-pupae	Pre-pupae
Gross Energy (MJ/kg)	-	-	-	-	-	-	24.1	-	21.1	-	21.9	19.3
Crude Protein (%)	37.2	43.6	36.2	38-40	43.2	35.10	43.7	38.05	47.6	49.2	53.1	63.9
Crude Fat (%) – Acid Hydrolysis	-	-	-	-	-	39.13	-	33.87	-	-	-	-
Crude Fat (%) – Ether Extract	30.8	33.1	18.0	18-28*	28.0	-	31.8	-	11.8	16.6	19.7	3.4
Crude Fibre (%)	-	-	-	-	-	-	10.1	-	9.6	-	10.9	13.2
Ash (%)	13.5	15.5	9.3	-	16.6	8.03	6.0	13.15	15.9	18.2	8.5	10.7

(*) – Method not mentioned

The relatively high crude fat content of the BSF larvae and pre-pupae can also be reduced using defatting techniques to achieve a higher crude protein content in the meal. The crude fat content of the full-fat BSF larvae and pre-pupae ranges between 18% (Bondari & Sheppard, 1981) and 39% (Haasbroek, 2016) (Table 1). However, during defatting, the crude protein content can be increased to as high as 64% (Surendra *et al*., 2016), using solvent extraction. Other authors have reported values of around 50% crude protein after pressing larvae (see Table 1).

The crude fibre content of the BSF pre-pupae are reported (Table 1) at around 10% for both the full-fat and solvent extracted pre-pupae (Kroeckel *et al*., 2012; Surendra *et al*., 2016). The crude fibre content of the pre-pupae is expected to be higher than the larvae crude fibre content, as the exoskeleton of the pre-pupae is more developed and is made up of chitin which presents itself in the form of fibre during analysis (Kroeckel *et al*., 2012).

Table 2 Mineral and ash content of dried black soldier fly pre-pupae (BSFPP) raised on poultry (PM) and swine manure (SM) (Newton *et al*., 2005b)

Mineral	Unit	*BSFPP-SM	**BSFPP-PM
Calcium	%	5.36	5.00
Phosphorus	%	0.88	1.51
Magnesium	%	0.44	0.39
Potassium	%	1.16	0.69
Manganese	mg/kg	348.00	246.00
Iron	mg/kg	776.00	1370.00
Boron	mg/kg	-	0
Zinc	mg/kg	271.00	108.00
Strontium	mg/kg	-	53.00
Sodium	mg/kg	1260.00	1325.00
Copper	mg/kg	26.00	6.00
Aluminium	mg/kg	-	97.00
Barium	mg/kg	-	33.00
Ash	%	16.60	14.60

*BSFPP-SM = Black soldier fly pre-pupae fed swine manure
**BSFPP-PM = Black soldier fly pre-pupae fed poultry manure

The ash content of BSFL and pre-pupae (Table 1) fall within the range of 6% (Surendra *et al*., 2016) and 17% (Newton *et al*., 2005b). With the defatted meals reaching as high as 18% (Tschirner & Simon, 2015). The gross energy of the BSF is reported as being slightly lowered by the defatting done by Surendra *et al*. (2016) from 24.1MJ/kg for full-fat to 19.3MJ/kg for solvent extracted.

2.3.1.2.4 Factors affecting chemical composition

Defatting is just one of the many factors which influence the chemical composition of the BSFL. Other factors include the age at which the BSF is harvested (larvae *versus* pre-pupae *versus* pupae) (Calvert *et al*., 1969; Newton *et al*., 2005b; Aniebo *et al*., 2009), the method in which it is dried (Fasakin *et al*., 2003), as well as the substrate with which it is provided (Newton *et al*., 1977). Another very important factor which may affect chemical composition values is the laboratory methods and type of analyses chosen by each author for the different nutrients, especially for the amino acid and fat determinations.

Table 3 Amino acid profile of BSF pre-pupae on a dry matter basis (g/100g)

	FAO (2015)	Newton (2005)	Newton (2005)	St Hilaire (2007)	Sealey (2011)
Substrate		Bovine Manure	Pig Manure	Pig Manure	Dairy manure & Fish Offal
Stage at harvest		Pre-pupae	Pre-pupae	Pre-pupae	Pre-pupae
Processing method		Dried at 70°C	Dried at 70°C	Dried at 80°C	Dried at 40°C
Analysis method		Not mentioned		AOAC approved	AOAC approved
Alanine	3.24	3.69	2.55	2.45	2.45
Arginine	2.36	2.24	1.77	1.78	-
Aspartic acid	4.63	4.56	3.04	4.09	4.09
Cysteine	0.04	0.06	0.31	-	-
Glutamic acid	4.59	3.81	3.99	4.34	-
Glycine	2.40	2.88	2.07	1.72	1.72
Histidine	1.26	1.91	0.96	0.76	0.76
Isoleucine	2.15	1.96	1.51	1.83	1.83
Leucine	3.33	3.53	2.61	2.66	2.66
Lysine	2.78	3.37	2.21	2.05	2.05
Methionine	0.68	0.86	0.83	0.77	0.77
Phenylalanine	2.19	2.20	1.49	1.83	1.83
Proline	2.78	3.26	2.12	-	-
Serine	1.31	0.12	1.47	1.37	
Threonine	1.56	0.55	1.41	1.58	1.58
Tryptophan	0.21	0.20	0.59	-	-
Tyrosine	2.90	2.51	2.38	2.22	2.22
Valine	3.45	3.41	2.23	2.99	2.99

FAO – Food and Agricultural Organisation

Aniebo *et al*. (2009) found the nutritional value of housefly larvae is significantly influenced by the age at which the larvae are harvested, as well as the method of drying used. The crude protein content of the housefly larvae significantly decreased with age and that the crude fat content significantly increased with age. Between two, three and four days of age, the crude protein dropped from 60% to 54% to 51% DM, respectively. The crude fat increased from 22% to 24% to 27% DM, respectively, during the three days of observation. The increase in crude fat may be due to the behaviour of storing energy before metamorphosing (Pearincott, 1960). The decrease in crude protein observed may be because larvae utilize protein in enzymatic reactions in the formation of the chitin layer (Kramer & Koga, 1986) or because of the dilution effect of the increased fat content. The sun drying method was found to provide larvae with a lower protein content and a higher fat content than the oven drying method (Aniebo *et al*., 2009). It was therefore concluded during this study that the best results were seen for maggots which were harvested at two days old, after being dried using the oven drying method (Aniebo *et al*., 2009).

2.4 Possible feed substrates

Ocio & Vinaras (1979) studied the use of larvae as a waste-management tool and concluded that larvae and pre-pupae can in fact be provided with municipal waste as a feed substrate and thereafter be

used successfully as a protein source in poultry diets. Diener *et al*. (2011) reported the quantity of waste reduction possible by the BSFL to range between 40% in swine manure and as much as 65-75% on household waste. Not only is the quantity of waste reduced, but also 43% of the nitrogen and 67% of the phosphorus is removed (Myers *et al*., 2008) from cow manure. Myers *et al*. (2008) concluded that larvae offer a possible key to agricultural waste and pollution reduction in their ability to bio-convert waste. Akpodiete *et al*. (1997) suggests the mixture of poultry manure together with palm oil for growing larvae.

The amount of waste estimated for consumers and in the food service in the USA alone, is 42.3 billion kilograms per year of which 26% is edible matter (Kantor *et al*., 1994). Products that are found to be sub-standard for human restaurants/markets, are left unbought or have past their sell-by dates and need to be discarded. This waste, amongst others, is the still perfectly acceptable for larvae substrate use.

2.4.1 Various waste

Waste was defined as the "wholesome edible material intended for human consumption, arising at any point in the food supply chain that is instead discarded, lost, degraded or consumed by pests" by the Food and Agricultural Organisation (FAO) in 1981 (Boland *et al*., 2013). It is important to note the term 'discard', as this should be done in a way that least harms the environment (Cheyne & Purdue, 1995). Landfills are harmful to the environment and have the potential to cause major pollutive issues for rivers, soil and the air (Seng *et al*., 2013). Seng *et al*. (2013) suggests that the increase in human population together with the increase in crop and livestock needed for this human population to survive, will pose an increasing challenge with regards to waste removal. In South Africa, waste originates from various sources and most of these carry a serious health risk to people if left unmanaged (Roberts & de Jager, 2004).

2.4.1.1 Agricultural waste

Manure can serve as a nutrient source for BSFL and there have been many reports on the success of waste being converted into a valuable protein source (Calvert *et al*., 1969; Newton *et al*., 2005b; St-Hilaire *et al*., 2007b; Sealey *et al*., 2011). It was found that the BSFL reduced layer hen manure by over 50%, whilst reducing the need for fly control (and the cost associated with this) (Sheppard *et al*., 2007). Sealey *et al*. (2011) found BSF pre-pupae to be raised successfully on dairy cow manure, whereas St-Hilaire *et al*. (2007b) effectively used pig manure as a substrate for BSF pre-pupae.

Newton *et al*. (2005b) reported that BSFL could reduce swine manure by 56% within two weeks. Other benefits associated with introducing larvae to manure is the moisture reduction (Calvert *et al*., 1969) and odour reduction (Miller *et al*., 1974). Poultry manure has been found to be a very inconsistent substrate for larvae, as it varies with the type of bird species, the age of the bird, the amount of feather

present in the manure as well just general variation in the chemical composition of the manure (El Boushy, 1991) The storage time of manure is also believed to significantly influence the chemical composition of the manure, as the crude protein has been found to drop from 30% to 12% between seven and 98 days of storing (Flegal *et al*., 1972). It would therefore be advisable to introduce larvae to fresh manure, so that the larvae can take advantage of the high energy and nutrient content available (Lalander *et al*., 2013).

Once the larvae have fed on the manure as a substrate, it is still possible to utilise the remainder of the substance as a soil amender (Newton *et al*., 2005b; Sheppard *et al*., 2007). Manure can be used as a compost and serves well as a fertilizer in gardens and on crops. Manure can also possibly be used as a biofuel (Leong *et al*., 2016).

2.4.1.2 Abattoir waste

In Nigeria, it has been found that approximately 46% of a cow, 48% of a sheep, 38% of a pig and 28% of a chicken is classified as waste and is discarded of by either dumping in landfills or in sewers (Adeyemi & Adeyemo, 2017). However, what is considered abattoir waste and what is edible matter differs from country to country. In South Africa, the intestines and heads of basically all animals are sold as offal or the 5th quarter (Christoe & House, 2003). Abattoir waste can include intestinal contents, excess fat, blood, feathers (in the case of chickens), hooves/feet and whole rejected carcasses (Roberts & de Jager, 2004). Abattoir waste is high in nutrients (Adeyemi & Adeyemo, 2017). Discarding abattoir waste has the potential to present serious health and environmental risks, specifically the contamination of ground and surface water with pathogens (Mittal, 2006) and the outbreak of food-borne diseases (Couillard & Zhu, 1993).

Any animal product that can be a source of bovine spongiform encephalopathy (BSE) is unacceptable as an animal feed source in terms of the Codex Alimentarius Commission (CAC/RCP 54-2004). The use of blood and carcass meal (ruminant-derived by-products) in pet foods is not prohibited in South Africa, but the use of these meals is deemed unacceptable for livestock consumption (Act No 36 of 1947 with adjustment to 2006). Animal-derived meal (bone, meat and blood) has also been used as soil fertilizer, providing a method of disposal and nutrient recovery (Ragályi & Kádár, 2012). Blood meal is extremely high in protein (approximately 89% DM) and boasts a good amino acid profile (Aniebo *et al*., 2009). This highly nutritious meal would be greatly advantageous as a larvae substrate and the restrictions on the direct use of abattoir by-products into livestock diets can be avoided by utilising larvae as an intermediate feed source.

2.4.1.3 Retail and household waste

Kitchen waste has its own health risks associated with inappropriate disposal. The high protein and fat levels can lead to ammonia and methane production and further cause volatile fatty acids to

accumulate as a result of anaerobic fermentation (Banks *et al*., 2011). Hypothetically, biogas could be produced from this anaerobic fermentation and the remainder of the decomposing material used as soil amender (Banks *et al*., 2011).

Retail industry waste includes uneaten and damaged goods from consumers and food service industry, as well as the losses that occur in fresh produce due to transportation spoil and passed due-dates (Kantor *et al*., 1994). It is believed that in the USA, 26% of all waste is edible matter and 20% of this is made up of fruit and vegetables (Kantor *et al*., 1994). Pieterse (2014) found that when BSFL where given 10kg of kitchen waste per square meter, the amount of (wet) larvae harvested was 1kg per square meter per day. With the exponential growth of the human population, the amount of retail and household waste is set to increase accordingly. The high bioconversion of this waste to nutrient-rich larvae biomass, allows the BSFL to be considered as a successful, and possibly cheaper, alternative protein source for animal feed.

2.5 Defatting

Soybeans, like BSFL, are naturally high in fat and are generally extruded to achieve a protein ingredient with around 46% crude protein, making it more suitable for broiler diets as the lipase enzyme in the chick only become fully colonised in the gut after approximately eight days post-hatch (Noy & Sklan, 1997). Similarly, various methods of defatting can be performed on BSF larvae and yield a lipid rich by-product, which then has the potential of being used as a biofuel (Surendra *et al*., 2016).

High fat levels in BSFL dilute the potential protein content, therefore any removal of the oil will increase the relative protein content left in the meal (Shiau *et al*., 1990). Sheppard *et al*. (2007) believes that in reducing the fat content of the meal, can increase the crude protein content to over 60% due to reduced dilution of the protein with lipids. Other advantages of defatting BSFL is that the risk of lipid oxidation is reduced (Zheng *et al*., 2013), allowing for a longer shelf life for the product.

Fats have an energy density two and a half fold that of carbohydrates, such as starch, and also offer a lower heat increment (van der Merwe & Smith, 1991). Chickens regulate their feed intake according to their energy intake (Leeson & Summers, 1997), given that all other dietary nutrients are balanced. Therefore, full-fat larvae meal may limit the intake of crude protein as the energy requirements are met sooner if diets are not formulated to be iso-energetic. Defatting the larvae may therefore allow for more BSFL crude protein substitution without the crude fat of the larvae limiting its inclusion in diets. The crude fat of BSFL has been found to range between 18% (Barroso *et al*., 2014) and 39% (Haasbroek, 2016) (Table 1). With previous defatting efforts allowing crude protein to increase to as high as 64% (Surendra *et al*., 2016), using solvent extraction. Other authors have used the pressing

method of defatting and have achieved a crude protein 48% (Kroeckel *et al*., 2012) and 49% (Tschirner & Simon, 2015) (Table 1).

It is suggested that processing methods can play a limiting role in the bio-availability of protein in feed for animals (Choct & Kocher, 2000). Boland *et al*., (2013) reports that any use of heat or acid treatment on an ingredient has the potential to cause protein denaturation, with lysine being the amino acid most affected by extreme heat processing and the Maillard reactions associated with this (Parsons, 1996). It is also believed that processing may lead to the total or partial destruction of cysteine, methionine and tryptophan (Castell, 1986). Therefore, in any investigation of heat or acid processing on feed ingredients, it is essential that the consequences on the nutrient digestibility are quantified. All other production, carcass and health parameters will be linked to the absorption and digestibility of the ingredient.

2.6 Black soldier fly larvae for animal nutrition

Both BSF pre-pupae and BSFL can be utilised as a feed ingredient in various animal's diets, and has been researched extensively in fish but not as vastly in monogastric and other animals (Bondari & Sheppard, 1981, 1987; Newton *et al*., 2005a; St-Hilaire *et al*., 2007a; Sealey *et al*., 2011). Fly larvae, in general, has been tested as a potential renewable protein source for pigs, fish and poultry (Newton *et al*., 1977; Bondari & Sheppard, 1987; Awoniyi *et al*., 2003).

2.6.1 Pigs

The amino acid profile (Table 3) of BSFL is believed to be well suited for use in pig diets (Newton *et al*., 1977). Newton *et al*. (1977) found BSFL to be a suitable protein source in grower pig diets, and gave credit to the BSFL for its calcium and lipid contents. However, the same study found BSFL to be inferior in its supply of threonine, methionine and cysteine. In this study, the larvae meal was replacing soybean oilcake meal, and the BSFL digestibility was found to be significantly lower than a conventional soybean based diet. Even so, the pigs used in that study did not discriminate against the BSFL in terms of palatability. Newton *et al*. (2005b) later tried the BSFL in early-weaned piglet diets and substituted plasma by 50% with BSF pre-pupae. This study revealed a superior production performance (better feed efficiency and weight gains) by the BSFL treatment compared with the control diet.

Driemeyer (2016) found no significant differences on average litter live weight, feed intakes and feed conversion ratio (FCR) ($P > 0.05$) when BSFL were supplemented into piglet creep diets at 3.5% inclusion. This study also concluded no immunological influence by BSFL inclusion.

2.6.2 Fish

Bondari & Sheppard (1981) evaluated the inclusion of BSFL into the diets of channel catfish and tilapia. No effect was found on the aroma and texture of the fish in this study and therefore was still acceptable to consumers. However, with regards to growth, Bondari & Sheppard (1987) tested a 10% BSFL substitution of fish meal and slowed growth rates were reported for caged channel catfish over a 15-week trial period. However, the diets used in this trial were not isonitrogenous or isoenergic and therefore the diets being compared were not providing equal nutrient levels. In contrast, Fasakin *et al*. (2003) found when defatted fly larvae was used, better overall performance was found than with full-fat fly larvae. No significant differences in growth were reported for rainbow trout given a diet with 50% BSF pre-pupae for a period of eight weeks, compared with the control diet (Sealey *et al*., 2011). This study also performed sensory analysis and no significant effect on fish fillet quality were found for BSF pre-pupae treatments, tested against a control. St-Hilaire *et al*. (2007a) also found the inclusion of BSFL into rainbow trout diets at 25% replacement of fish meal to have no effect on FCR or weight gain, this study did however have a low number of replicates and was performed over a short period of time. Similarly, juvenile turbot were reported to have accepted diets with 33% BSFL inclusion and no effects on feed intake and feed conversion were found (Kroeckel *et al*., 2012).

2.6.3 Poultry

Insects are included in the natural diet of wild birds and are consumed in their adult, pupal and larval forms in this way (Zuidhof *et al*., 2003). The feeding of BSFL to chickens is therefore not a completely original concept. Quail (*Coturnix japonica*) were fed a diet that included 50% BSFL and it was reported that this diet led to the quail having higher feed intakes and an improved FCR (Widjastuti *et al*., 2014)

Agunbiade *et al*. (2007) studied maggot (species was left unspecified) meal as a replacement for fish meal in layer hens. Fish meal is not commonly used in layer hen diets as the trimethylamine (TMA) oxide is believed to cause a fishy taint in eggs (Pearson *et al*., 1983). Regardless, the maggot meal supplementation lead to no differences in egg quality (egg shape and weight, yolk index and colour and Haugh units) when compared with the control (Agunbiade *et al*., 2007). Soybean meal based diets were also well substituted by BSFL in the diets of layer hens with no metabolic or health stress consequence (Maurer *et al*., 2016).

Pretorius (2011) studied the common housefly larvae as a protein source for broiler chickens using isonitrogenous and isoenergic treatments. No significant differences were found between the housefly larvae and fish meal in productive performance. When soybean meal and housefly larvae were compared each at 10% inclusion, superior average live weights, cumulative and weekly feed intakes as well as average daily gains were found for the larvae treatment. Similar work was done by Hwangbo *et al*. (2009), where it was reported that the weight gain in broilers due to housefly larvae

inclusion was superior to that of chickens on a basal control diet. Adeniji (2007) reported equally successful results in housefly larvae substitution, however the control in this study made use of groundnut oilcake, which was replaced at various levels by the housefly larvae. It was concluded that the larvae could effectively replace groundnut oilcake in broiler diets with no significant differences in weight gain, FCR or nutrient retention.

In a study on the inclusion of BSF pre-pupae in broiler chicken diets, Uushona (2015) reported that the production parameters of broiler chicken and quality of meat produced were not negatively influenced by a 15% inclusion level. No adverse sensory affects were found in cooked chicken breast of broilers who consumed BSF pre-pupae in that study. It was also found that an increase in BSF pre-pupae inclusion lead to an increase in tibia bone calcium content, indicating a high bioavailability of calcium from BSF pre-pupae. It was also found in this study that defatted BSF pre-pupae had a higher nutrient digestibility compared to the full-fat BSF pre-pupae (Uushona, 2015). Unfortunately, this author did not evaluate the nutrient composition of the specific pre-pupae used in the nutrient digestibility study, which limits the possible in-depth comparisons.

2.7 Broiler nutrition

The nutrient requirements of a bird depend on its species, age and type of production. Broiler performance (based on nutrient utilisation) is reported as being influenced by two things: metabolizable energy and crude protein of a diet (Zaman *et al*., 2008).

2.7.1 Requirements

Birds will regulate their intake according to their energy consumption (Leeson & Summers, 1997). Birds will therefore consume less feed when provided with a high energy diet (de Albuquerque *et al*., 2003), given the diet fed is balanced. However, chickens are unable to digest complex carbohydrates with insoluble fibre to supply themselves with energy, therefore chickens obtain their energy from simple carbohydrates, fats and sometimes protein as well (Hetland *et al*., 2004; Nalle *et al*., 2012).

The amino acid requirements differ for every animal species and even vary within species due to different physiological stages and needs (McDonald *et al*., 2002). Methionine is known to be the first limiting amino acid for poultry, followed by lysine, and adequate supply of these two amino acids will support optimised protein utilisation (Schutte & de Jong, 2004). In the ideal amino acid profile for broilers, all essential amino acids are expressed as a percentage of lysine, because the essential amino acids relative to lysine are unaffected regardless of genetics, dietary and environmental factors (National Research Council, 2004; Schutte & de Jong, 2004).

Table 4 Calculated amino acid to lysine ratios in comparison to the ideal amino acid profile for broiler chickens

Amino Acid	Black soldier fly larvae[3]	Soybean meal[1]	Fish meal[1]	Ideal amino acid profile for broilers[2]
Lysine	100.00	100.00	100.00	100.00
Methionine + Cysteine	-	47.54	48.00	*38.00
Threonine	62.66	63.93	54.67	74.00
Isoleucine	67.22	75.41	57.33	73.00
Valine	84.23	78.69	65.33	82.00

([1]) – Food and Agricultural Organisation (FAO) 2004
([2]) – National Research Council (NRC) 2004
([3]) – Pieterse *et al*., 2015
(*) – Only methionine

It can be seen in Table 4, that all three protein sources (BSFL, soybean meal and fish meal) chosen, need to be supplemented to different degrees in different amino acids to meet the broiler amino acid requirements. It would therefore be advised that BSFL be used in combination with another protein source, to make up for the lack of certain essential amino acids, or together with synthetic amino acids which make balancing diet formulations much easier. It is important to bear in mind the amino acid interactions when formulating diets as well.

2.7.2 Requirements of young chick

The purpose of the yolk during incubation, is to provide energy to the unhatched chick. Then prior to hatching (day 19 of incubation) the yolk is internalized into the abdominal cavity and continues to supply the chick with energy for several days post hatch (Uni *et al*., 1998). The intake of exogenous feed allows the gastrointestinal tract (GIT) of the chick to rapidly development, along with its vital organs (Uni *et al*., 1998). Very little intake is expected in the first few days post-hatch as the chick is adapting to its environment and is still being supplied with energy by the internalised yolk sac.

Calvert *et al*. (1971) studied growing chicks (first 14 days of life) and their growth response when supplemented with housefly pupae meal. Two treatments were defined in this study with the first only containing soybean meal as a protein source and the other only containing housefly pupae meal as a protein source. It was found that when larvae meal was supplied for the total trial period, it was beneficial to the weight gain per bird. However, this benefit was lost when the pupae meal was only supplied from day seven onwards. This gives the idea that the growth and development that occurs during the first week post-hatch can be optimised using insect protein.

It is vital that the requirements of the chick are met and digestion optimised, especially during the times where growth and development of the GIT is most rapid (Uni *et al*., 1999). Limited literature exists regarding the implementation of BSFL in young (from hatch to week two) broilers diets, including the nutrient digestibility quantification during this crucial stage of life.

2.7.3 Protein: degradation and digestibility

Protein quality is defined by the available dietary amino acids for animals to maintain their metabolic processes (Boland *et al*., 2013). Supplying excessive amino acids in the diet of animals is not only expensive, but also leads to the bird spending energy catabolizing these excess amino acids for excretion, which could lead to a loss of body weight depending on the level of oversupply (Kidd, 2004). This has been reported especially in the oversupply of lysine, threonine and methionine (Lewis *et al*., 1963). Therefore, the ratio of energy to protein in chicken diets is extremely important during formulation to avoid reduced growth rates (Aletor *et al*., 2000; Nalle *et al*., 2012).

Chicken diets are formulated on the availability of the amino acids in a feed ingredient (Lemme *et al*., 2004). According to the FAO (2010), supplementing diets with synthetic individual amino acids can help bring the necessary crude protein levels required in diets down by as much as 2%. Lysine is involved antagonistically with arginine, however this interaction is managed when these amino acids are supplied in equal amounts (Austic & Scott, 1975). Even so, if excessive arginine in found to be present in a diet, the addition of lysine has proven to help alleviate the consequent depression in growth rate (Chamruspollert *et al*., 2002). A proportion of the amino acids provided in diets, goes undigested (Lemme *et al*., 2004) and therefore the evaluation of any feedstuffs' amino acid digestibility is essential, if poultry are to be fed balanced diets (Short *et al*., 1999).

The digestibility potential of a protein source plays an important role in its value (Barroso *et al*., 2014). Measuring the digestibility of any nutrient in a feed requires the measuring of nutrient intake and faecal output for a specific nutrient in a specified period (Khan *et al*., 2003; Lemme *et al*., 2004). The digestibility of the protein source, as well as the amino acids that make up that protein source, also depend on the efficiency of the animal consuming it (Boland *et al*., 2013).

Zuidhof *et al*. (2003) reported on the total tract digestibility of dehydrated housefly larvae meal in turkey poults. The results from this study showed significantly higher total tract digestibility for crude protein, energy and all amino acids expect cysteine compared to a soybean based diet. Total tract digestibility of housefly maggot meal was also studied in broiler chickens by Hwangbo *et al*. (2009) and was found to have better digestibility coefficients than soybean meal. The crude protein digestibility was reported at 98%, and the essential amino acid digestibility at 95% (Hwangbo *et al*., 2009). In a more recent study by Pretorius (2011), total tract digestibilities were not found to be as high as Hwangbo *et al*. (2009) where the digestibility of crude protein of housefly larvae in broiler chickens was 69% (Table 5), with the housefly pupae meal being found to be 79%.

Table 5 Coefficient of total tract digestibilities (CTTD) of various larvae and pupae meal for broiler chickens

	Pretorius (2011)	**de Marco (2015)**	**Uushona (2015)**
Insect and phase	Common housefly larvae	Black soldier fly larvae	Black soldier fly pre-pupae
Fat Processing	Full-fat	Full-fat	Defatted
Nutrient			
AME	14.23	17.38	16.85
Dry matter	0.81	0.53	-
Ash	0.83	0.34	0.92
Crude fibre	0.62	-	0.81
Ether extract	0.94	0.99	1.01*
Crude protein	0.69	0.51	0.97
Arginine	-	0.83	0.99
Histidine	0.87	0.81	0.96
Isoleucine	0.91	0.45	0.96
Leucine	0.92	0.76	0.94
Lysine	0.95	0.46	0.98
Methionine	0.95	0.42	0.98
Phenylalanine	0.91	0.63	0.97
Threonine	0.93	0.75	0.96
Valine	0.91	0.62	0.95
Alanine	0.90	0.86	0.96
Aspartic acid	0.93	0.61	0.96
Glutamic acid	0.91	0.74	0.95
Cysteine	0.92	0.82	0.88
Glycine	0.83	0.67	0.94
Proline	0.91	0.89	0.94
Serine	0.86	0.82	0.95
Tyrosine	0.96	0.43	0.98

(*) – Acid hydrolysis method used
AME – Apparent metabolisable energy

Table 5 compared the coefficient of total tract digestibilities (CTTD) reported by various authors for larvae meal in broiler chickens. It is understood that the defatting processing (not specified) implemented by Uushona (2015) did not adversely affect the digestibility of the BSFL. Higher CTTD values are found in the defatted BSFL, compared with those values reported by De Marco *et al*. (2015). The crude protein digestibility coefficients for full-fat housefly larvae (Pretorius, 2011) and full-fat BSFL (De Marco *et al*., 2015) are relatively low at 69% and 51%, respectively. The high fat digestibility values given by all authors cited in Table 5 may be due to the fact that triglycerides are the major food reserve for larvae and these are stored in large quantities as future energy source during pupation (Chapman, 1971).

McDonald *et al*. (2002) suggests that the fibre content of food has the largest influence on the digestibility of feed, mentioning the relevance of both the quantity and composition of the fibre used. However, this author did keep the literature focus on ruminants and not monogastric animals. However, within the exoskeleton of insects is a polymer of N-acetyl glucosamine and glucosamine, which represents a non-protein nitrogen (NPN) portion of the larvae or pre-pupae (Chapman, 1971).

Chitin often presents itself in the form of fibre during chemical analysis and, because of its structure, slightly elevates the nitrogen estimation of the insect meal at hand (Lindsay *et al*., 1984).

Okine & Mathison (1991) have reported that livestock digest a larger portion of nutrient, when fed in a restricted fashion instead of *ad libitum*. The higher the intake, the lower the apparent digestibility because of the rapid movement through the digestive tract which limits the time available for enzymatic activity and therefore digestion (McDonald *et al*., 2002). Precision feeding would then allow digestibility to be optimised with a balanced diet being provided, without having to be provided *ad lib*.

2.7.4 Effects on intestines, organs and skeleton

Development of the chicken, along with its feed utilisation and digestive capacity are largely affected by the intestinal microbiota and the metabolic activities associated with this (Rehman *et al*., 2008). Fasting during the first two weeks of the chicks' life can cause damage to the chicks' gut, as the antibody response through the B and T lymphocytes have late colonization and therefore an intestinal antibody response is delayed (Shira *et al*., 2005). If fasting occurs in the first 48 hours post-hatch fewer cells are produced per villus and a smaller surface area will mean limited nutrient absorption throughout the chicks' life (Geyra *et al*., 2001). Therefore, there can be an emphasis placed on palatability and balance of a diet in the first two weeks of the chicks' life (Geyra *et al*., 2001). A healthy gut enables high nutrient absorption and further strengthens the immune status through an increase in plasma immunoglobulin levels in the animal (Salim *et al*., 2013). The lymphoid organs of the chicken are essential in ensuring the resistance to pathogens, as well as fighting infection all whilst maintaining productivity (Fasina *et al*., 2006).

Modern broiler chickens in intensive production systems are genetically coded for fast growth and optimal productivity. The consequence of this fast growth however, is that the bone development is not as fast as the weight gain causing bone and leg problems (Hocking *et al*., 2009; Garcia *et al*., 2013). Among these problems is osteoporosis which has negative financial consequences for producers (Rubin *et al*., 2007). During osteoporosis, a decline in mineral content and overall bone mass is experienced causing the micro structure of the bone to change and become more fragile which may lead to fracturing (Gregory & Wilkins, 1989; Peck *et al*., 1993; Bishop *et al*., 2000; Rubin *et al*., 2007).

Bones that fragment during the slaughter or deboning process causes a discolouration of meat due to 'leaching', which results in consumer rejection (Gregory & Wilkins, 1989; Rath *et al*., 2000; Garcia *et al*., 2013). Bone defects can be avoided through the correct inclusion of calcium and phosphorus in diets, and more importantly in the correct ratio (Leeson & Summers, 1997). A ratio of 2:1 for calcium to phosphorus is recommended by the National Research Council (2004). Many animal feed ingredients contain phosphorus which is bound by phytate and is unavailable to the animal for

absorption. Newton *et al*. (1977) and Newton *et al*. (2005a) reported that the BSFL contain high levels of both calcium and phosphorus, which may help in the development of the skeletal system. However, this would contradict the finding by Williams *et al*. (2000) who found that the dietary levels of calcium and phosphorus not to affect the bone reabsorption of calcium and phosphorus. The role of chloride (Cl) within the dietary electrolyte balance is reported to affect bone development (Vieira *et al*., 2003), as well as the levels of magnesium (Mg) and Vitamin D_3 (Garcia *et al*., 2013). The flexibility and breaking strength of chicken bones has also been reported to be negatively influenced by the inclusion of mycotoxins such as aflatoxins and ochratoxins in broiler diets (Huff *et al*., 1980).

Mycotoxins and the presence of gizzerosine are known to result in high levels of gizzard erosion (Johnson & C. Pinedo, 1971). Pretorius (2011) found the common housefly to cause a slight discolouration on the gizzard of broiler chickens, however this was not related to erosion and therefore did not affect the health or productivity of the birds. Uushona (2015) found no signs of gizzard erosion or adverse effects on organs, gut histomorphology or tibia bone parameters when evaluating the inclusion of BSF pre-pupae at up to 15% inclusion levels. It was concluded that BSF pre-pupae was safe to include into broiler chicken diets up to 15% inclusion.

2.7.5 Effects on growth and intake

The presence of mycotoxins, aflatoxins, ochratoxins (Huff *et al*., 1980; Awad *et al*., 2009), protease and trypsin inhibitors (Clarke & Wiseman, 2000), as well as soluble non-starch polysaccharides (NSP) (Rebolé *et al*., 2010) all have the capability of negatively affecting the growth performance of animals.

Several studies have tested housefly larvae meal as a replacement for fishmeal has the potential to introduce gizzard erosion inducers. Teguia *et al*. (2002) studied the substitution of fish meal with housefly larvae meal with no significant differences being reported for both the weight gain or carcass characteristics at inclusion levels of 0%, 5%, 10% and 15%. It was also concluded that housefly larvae contained a higher metabolisable energy content than fish meal. Awoniyi *et al*. (2003) also reported on the use of housefly larvae in broiler diets and concluded that at 4% inclusion, housefly larvae could completely replace fish meal (in an iso-nitrogenous diet) without affecting the feed consumed, weight gained or feed efficiency. This study concluded that 25% replacement of fishmeal was optimal, with higher replacement levels causing a reduction in feed intake. Okah & Onwujiariri (2012) replaced as much a 50% of fishmeal with housefly larvae meal and found significantly higher weight gain and dressing percentage than the control, however the feed intake was also hindered in treatments with over 20% inclusion levels, due to nutrient imbalances. Hwangbo *et al*. (2009) also tested iso-nitrogenous and iso-energetic diets (with similar lysine and methionine levels in each) of between 0% and 20% larvae inclusion. The treatment groups that received 10% and 15% had significantly higher weight gains, at 1.778kg for both the 10% and 20% inclusion groups after 5 weeks, than the 0%

inclusion group at 1.638kg. All housefly larvae meal treatments had significantly lower FCR (Hwangbo *et al*., 2009).

Pretorius (2011) tested the common housefly larvae as an alternative protein source and compared these larvae with fish meal and soybean meal. In this study, 10% inclusion of larvae meal yielded significantly higher average live weights, cumulative and weekly feed intakes and average daily gains (ADG) than when soybean was used as a main protein source. This inclusion level was concluded as the optimal in this study. At the same inclusion level (10%), no significant differences were found compared to 10% fish meal inclusion, however at higher levels of inclusion the larvae meal outperformed the fish meal. Miller *et al*. (1974) found no significant differences in single comb white leghorn chicks weight gain, feed intake and feed conversion when compared to a soybean meal control diet performance.

When BSF pre-pupae was evaluated as a replacement for soybean meal in broiler diets, no significant differences were found in the growth parameters for diets that included up to 15% BSF pre-pupae inclusion against the control which had soybean meal as its protein source (Uushona, 2015). With the protein efficiency ratio (PER) in this study showing the 5% inclusion level of BSF pre-pupae to be significantly lower than the control, and the 10% and 15% inclusion treatments. However, no adverse effects were found, with up to 15% of the diet being BSF pre-pupae, regarding the European production efficiency factor (EPEF), ADG and FCR (Uushona, 2015).

2.7.6 Effects on meat quality and carcass characteristics

When muscle, which has been accumulated during the growth of an animals' life, is converted to meat, in a process known as *rigor mortis* (Allen *et al*., 1998). The pH of the muscle drops until the major proteins isoelectric point is reached which results in the expulsion of water into the extracellular space, which is known as drip loss (Huff-lonergan & Lonergan, 2005). Therefore, the initial pH of the meat affects the drip loss process (van Laack *et al*., 2000). The rate and extent to which the pH drops, effects the tenderness of the meat as well as the water holding capacity of meat (van Laack *et al*., 2000; Huff-lonergan & Lonergan, 2005). There is believed to be a definite relationship between meat colour and meat pH (Allen *et al*., 1998; Qiao *et al*., 2002; Swatland, 2004). Allen *et al*. (1998) reported that darker meat was coupled with a higher pH than lighter coloured meat; however darker meat was found to have a reduced shelf-life which was suggested to be a result of psychotropic bacteria colonizing darker meat.

In a study by Hwangbo *et al*. (2009), the inclusion of housefly larvae meal in broiler diets was found to have no effect on the colour of the breast portion and was found to yield significantly higher dressing percentage as well as heavier breast and thigh portion yields (as a % of carcass weight) compared with the control. Teguia *et al*. (2002) reported no significant differences in abdominal fat (as a percentage

of carcass weight) in a study using housefly larvae in broilers as well. In agreeance with the results found by Teguia *et al*., (2002) was a study that also found no significant differences between treatments and the control for dressing percentage and breast muscle weights (Awoniyi *et al*., 2003). Although both these results differ from those by Hwangbo *et al*. (2009), Hwangbo *et al*. (2009) had much higher replicates than Awoniyi *et al*. (2003), which would allow for a better statistical accuracy.

Pieterse (2014) found housefly larvae, included at 10%, yielded significantly better live weights and carcass weights than the control or the treatments containing fish meal. This study also reported higher breast and thigh (percentage of carcass weight) muscle yields than the control diet. No sensory differences were reported between the control and housefly larvae treatments with regards to aroma, initial juiciness, flavour and tenderness (Pieterse, 2014).

A sensory analysis was also done for the inclusion of BSF pre-pupae in broiler chicken diets (Uushona, 2015). It was concluded that BSF pre-pupae inclusion did not affect the meat eating quality of meat produced, with no taste discrimination. The same study tested the carcass characteristics of BSF pre-pupae inclusion, with no significant differences being found in the portion yields, live weight, carcass weight or dressing percentage of treatments that consumed diets containing 0% (control), 5%, 10% and 15% BSF pre-pupae inclusion (Uushona, 2015). Therefore, it was concluded that the inclusion of BSF pre-pupae can be implemented up to a 15% level without adverse effects on the meat quality or carcass yields.

2.8 Cost effectiveness and feasibility

As much as 70% of broiler production costs are allocated to feed costs. The production of BSF larvae meal has the potential to provide a cost-effective, sustainable protein source, which could contest with industry standard protein sources on a performance level as well. With an increase in the consumption of poultry meat in many cultures (Bolan *et al*., 2010), the demand for cheaper poultry meat is expected to rise (Nalle *et al*., 2012).

The replacement of fish meal by 50% and 100% using larvae meal, led to a reduction of tilapia production costs by 18% and 28%, respectively (Ajani *et al*., 2004). Fashina-Bombata & Balogun (1997) also compared the cost of larvae production with that of fish meal and found that the cost of larvae meal production was less than 20% of the same quantity fish meal production. Even so, this was concluded 20 years ago, before the fish stocks in the oceans were further depleted. Since then, the price of fish meal has risen due to the decline in possible supply (IMF, 2010). As the world population grows, competition between humans and animals for protein sources will become more evident and prices will reflect the higher demand (Ravindran & Blair, 1993; Nalle *et al*., 2012). Therefore,

alternative protein sources must be established for animal feeds in order to keep all protein prices as low as possible.

It is possible that insect protein, specifically defatted BSF larvae meal, could be approached and marketed as a speciality feed, with the removed oil serving as a biofuel, resulting in the monetary value and consumer perception of the ingredient being much higher and better. However, the legal limitations regarding the use of certain substrates for the larvae and the trade restrictions between countries would need to also be addressed for this infant industry to succeed financially.

2.9 Conclusion and motivation for study

From the information provided in this chapter, it can be concluded that research efforts into the use of sustainable, alternative protein sources are necessary for the long-term continuation and improved success of the broiler industry. The use of the dipteran species, common housefly larvae and BSF pre-pupae in animal feeds has thus far proven to provide promising productive and digestive performance in several animal species. It would therefore be of great interest to evaluate the BSFL, both full-fat and using various defatting techniques, as a possible replacement of soybean meal in broiler chicken diets. It is crucial to measure and know the health risks and effects associated with any novel feed ingredient, if there are any. The quantification of nutrient digestibility for both full-fat and defatted BSFL products would be required to realize the chemical composition and assist in the improved formulation of diets involving this feed ingredient. Ultimately, the inclusion of BSFL (full-fat and defatted) in broiler diets has no known literature published regarding its effect on the growth parameters and carcass characteristics of broilers and could potentially provide improved outcomes as a novel protein source in broiler chicken diets.

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CHAPTER 3

An evaluation of defatted black soldier fly (*Hermetia illucens*) larvae for broiler chicken diets: nutritional composition and production parameters

Abstract

The effects of defatted *Hermetia illucens* (black soldier fly) larvae inclusion in broiler chicken diets on production parameters were tested using 240 Cobb-500 chicks. Two defatting techniques and treatments were tested, namely dry rendering (DR) and extrusion (EX). The larvae used in the trial had relatively high gross energy contents and higher protein contents post-defatting. The full-fat (FF) and defatted *H. illucens* larvae products were included at 15% inclusion over a period of 28 days. The control of the study included both full fat soya and soya oilcake as a protein source. Treatments were allocated to cages in a randomised block design. Significant differences were found in the following parameters: average live weight, weekly feed intake, cumulative feed intake, average daily gain (ADG), feed conversion ratio (FCR), European production efficiency factor (EPEF) and protein efficiency ratio (PER). No significant differences were found for the liveability parameter or week three's feed intake. The EX treatment outperformed the alternative defatted treatment (DR treatment), however the FF treatment outperformed all treatments. Both FF and EX *H. illucens* larvae meal can be successfully used as a protein source and will improve broiler performance at up to 15% inclusion level.

Keywords: - Gross energy, crude protein, dry-rendering, extrusion, ADG, FCR, PER, EPEF

3.1 Introduction

In the broiler production industry over 70% of the costs involved are for feed (Teguia & Beynan, 2005). Protein sources are the most expensive ingredients in these diets (Ellinger, 1958). Therefore, any reduction in the price of the protein source being used will ultimately be carried through the entire supply-chain quite significantly. Black soldier fly has proven to be a very promising additional protein source to soybean meal, which contains anti-nutritional factors, as well as fishmeal, which is increasingly unavailable and thus expensive (Bondari & Sheppard, 1987; Newton, 2005; Uushona, 2015).

The objective of the broiler industry is to achieve heavier live weights in the least possible time (Longo *et al*., 2007), which requires improved feed conversion ratios. Superior growth performance would require higher intakes as soon as possible after hatching (Uni *et al*., 1998). This, in turn, allows for

higher average daily gains throughout the entire growth period. The development, health and ultimate growth potential of the birds depends not only the protein component of the diet (Zaman *et al*., 2008), but more specifically the amino acid contents and ratios of the specific protein at hand. Protein quality is defined by a protein's amino acid ratio (Ellinger, 1958; Boland *et al*., 2013), and under this definition BSFL has been classified as a good quality protein (Tschirner & Simon, 2015).

In order to quantify the efficiency with which a bird can convert this high quality protein into deposited bodily protein, the protein efficiency ratio (PER) is used (Bender, 1956). It has been suggested that a PER of 3:1 for broiler production is expected (Wilding *et al*., 1968). A more inclusive measure of production for worldwide producers is the European production efficiency factor (EPEF), which allows for the liveability of the flock to also be accounted for, and therefore overall health status of the chickens is made a consideration in flock performance (Butcher & Nilipour, 1998). Full-fat BSF pre-pupae was found to have a PER of 2.5 and a EPEF of 431 when included at a level of 15% of the diet (Uushona, 2015). Good performances were also reported by (Pretorius, 2011a) for 10% inclusion levels of common housefly (*Musca domestica*) larvae, namely a very high PER of 3.68 and an EPEF of 377.90. With such good production potential being demonstrated by both the BSF pre-pupae and the housefly larvae, it is imperative that the BSFL also be tested with regards to its effect on productive performance in broiler chickens.

The first of the two study aims was to compare the various effects of the two defatting techniques on the production parameters of broiler chickens with full-fat BSFL and soybean meal. The production parameters used for this comparison were weekly and cumulative intakes, weight gains, average daily gains, FCR, PER and EPEF. The second aim of the trial was then to further investigate the viability of the various BSF larvae meal treatments as protein sources and compare these with soybean meal using production parameters of broiler chickens. The initial investigation, however, needed to be the quantification of the nutrient compositions of the two defatted BSFL treatments and full-fat BSFL treatment.

3.2 Nutritional composition of full-fat black soldier fly larvae, dry rendered black soldier fly larvae and extruded black soldier fly larvae

A single batch of BSF larvae was reared on kitchen waste at the AgriProtein Technologies Pty Ltd. facilities (Philippi, Cape Town) and the entire batch was processed as full-fat BSF whole dried BSFL. The batch was then divided into three equal parts, of which one part remained full fat whole dried larvae and was simply ground (as is), before being included in the trial as the 'FF' treatment. The second part of the batch was processed as dry rendered larvae meal, which was then used as the 'DR' treatment, and the third part was processed through an extruder to provide the product used as 'EX'

treatment. All three treatments were then analysed by proximate analysis, amino acid analysis and mineral analysis to provide an overall understanding of the differences and composition of each treatment.

Analytical methodologies were performed at the Department of Animal Sciences, Stellenbosch University. This, however, excludes the amino acid determinations, where the initial hydrolysis was done at Stellenbosch University and thereafter amino acids analysis was done at the Central Analytical Facilities (CAF), Stellenbosch. The mineral analysis was done at the Western Cape Department of Agriculture's Institute for Plant Production at Elsenburg. Analytical methodologies on the dry matter, ash, crude protein, crude fat and crude fibre content were performed. This constitutes the proximate analysis.

3.2.1 Dry matter determination

The dry matter (DM) of the larvae and pupae meal was determined in accordance with the Association of Official Analytical Chemists (AOAC) International (2002), Official Method 934.01. Two sub-samples of each sample, each weighing 2g, were placed in a crucible to dry for 24 hours at 100°C. Thereafter, the dry sample was weighed and the DM content was calculated using Equation 1:

Equation 1

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

% Dry Matter = 100 - % Moisture

Where:

A = Weight of dry, empty crucible

B = Weight of air-dried test sample

C = Weight of crucible and moisture-free test sample

3.2.2 Ash determination

The sub-samples retained from the dry matter analysis were used for the determination of ash content. This method was followed as provided by the AOAC Official Method 942.05. These sub-samples were combusted in a combustion oven for six hours at 500°C. Thereafter the combusted sub-samples were weighed and the ash content was calculated using Equation 2:

Equation 2

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

Organic Matter (%) = 100 - % Ash

Where:

A = Weight of dry, empty crucible

D = Weight of crucible and ash

3.2.3 Crude protein determination

The crude protein content of the various larvae meal sub-samples was determined by measuring the total nitrogen content in accordance with the method described by AOAC, Official Method 4.2.07, in the LECO FP528 apparatus. Two sub-samples each weighing 0.1g were placed in a tin cup and then placed into the LECO FP528. Thereafter the nitrogen content was directly taken from the LECO FP528 and the Crude Protein (CP) content was calculated by using Equation 3:

Equation 3

Crude Protein (%) = Nitrogen (%) x 6.25

3.2.4 Crude fat determination

Fat content determination was done per the AOAC (2002) Method 954.02 that makes use of acid hydrolysis. A clean, dry fat beaker from the oven (100°C) was placed in a desiccator for 30 minutes to cool down. Hereafter the beaker was accurately weighed. Two grams of sample, in duplicate, were weighed into test tubes and 2mL of ethanol was added to the tubes to moisten the samples. Hereafter, 10mL of a HCl solution (38%) was added to the tubes. The test tubes were then boiled in a water bath for 35 minutes. Tubes were removed from the water bath and left for 30 minutes to return to room temperature. The boiled samples were emptied into individual separator funnels. After which, 25mL of diethyl ether was added to the separator funnel and the funnels were shaken for one minute each. The top see-through liquid part was carefully transferred into a designated fat beaker. Then 15mL of diethyl ether was added to the funnel and each funnel was again shaken for one minute, followed by the addition of 15mL of petroleum ether. The funnel was shaken for one minute. The resultant top, separated layer was carefully transferred to the fat beaker. The steps of adding the diethyl ether and petroleum ether were repeated once more. Hereafter, the fat beakers were placed in the sand bath to ensure that all the ether had evaporated. The beakers were then placed in a desiccator to cool down for 30 minutes before accurate weighing for further calculation. Thereafter the crude fat content was calculated using Equation 4:

Equation 4

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

3.2.5 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's operating manual. Two sub-samples weighing 0.5g of each sample were pelletized. A pelletized sub-sample was then placed in the bomb and filled with pure oxygen until 3000kPa 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 the calorimeter was standardized in between the sample testing with benzoic acid.

3.2.6 Crude fibre determination

The crude fibre content of the samples was determined with the aid of an ANKOM$_{200/220}$ Fibre Analyzer (Ankom® Technology Corp. Fairport, New York, USA), in accordance with the method suggested by the manufacturers. The filter bags were soaked in acetone to remove any impurities, air-dried and then oven dried at 100°C for two hours. Samples of 0.95 - 1.0g were weighed into individual bags and the bags were heat sealed and marked with a solvent and acid resistant marker pen. The samples in their individual bags were washed with petroleum ether as a defatting procedure. Once completely air-dried, the sample bags were placed on the appropriate bag suspender trays in the ANKOM$_{200/220}$ Fibre Analyzer along with the blank bags which served as correction factors. The machine was filled with a 1.9L of an acid detergent solution until all the bags were covered. They were processed for precisely 40 minutes at 100°C, after which the solution was drained from the analyser. Samples were then rinsed with distilled hot water and the rinse was repeated once. Afterwards, the machine then filled with 1.9L of a base solution until all the bags were covered and once again processed by the machine for 40 minutes at 100°C. Three distilled hot water rinses were then performed after the base solution had been drained from the machine. The bags were then placed on paper towels and lightly pressed to remove excess water. They were soaked in acetone for three minutes and allowed to air dry before being transferred to a 100°C oven to dry completely (approximately 2-4 hours). Once dry, they were cooled in a desiccator and then weighed. Each bag was then allocated a clean, dried and weighed crucible. Crucibles and bags were placed in the furnace and allowed to ash for 2 hours at 600°C. The crucibles were then cooled to ambient temperature in the desiccators and the remaining matter was weighed for each sample for further calculations. Crude fibre was then determined using Equation 5:

Solutions used

Sulfuric acid solution — 0.255 ± 0.005N. 1.25g H_2SO_4/100mL.

Sodium hydroxide solution — 0.313 ± 0.005N. 1.25g NaOH/100mL.

Equation 5

$$\text{Crude Fibre (\%)} = \frac{100 \times (W3 - (W1 \times C1))}{W2}$$

Where:

W1 = Bag Weight (g)

W2 = Sample Weight (g)

W3 = Weight of Organic Matter (Loss of weight on ignition of bag and fibre)

C1 = Blank bag correction factor

3.2.7 Amino acid determination

The samples were prepared through hydrolysis and then the total amino acid profile was determined (Association of Official Analytical Chemists (AOAC) International, 2002). During hydrolysis, a sample weighing 0.1g was placed into a specialized hydrolysis tube. Six millilitres of a 6N HCl and a 15% Phenol solution were added to the respective samples. The samples were then placed in a vacuum 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°C.

After hydrolysis, the samples were taken out of the tubes and placed into Eppendorf tubes and refrigerated until the amino acid determination phase. The hydrolysed sample was neutralized with 6M NaOH, diluted accordingly with water and internal standard (Norvaline) was added. After which, 10uLof the neutralized sample was then derivatized with 70uL of a sodium borate buffer (0.2M, pH 8.8) and 20uL AccQ-Tag derivatizing agent (prepared with dry acetonitrile). This was done at 55°C for 10 minutes. The subsequent chromatographic analysis then included amino acid separation and detection performed using a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) with a Waters Ultra Tag C18 column (2.1 x 50mm x 1.7um) held at 60°C. The mobile phase was also supplied in the AccQ-Tag Ultra amino acid kit from Waters.

Data acquisition of analysis was performed by MassLynx software, which integrated the peaks at the retention times and plots calibration curves based on peak response (peak area/internal standard peak area) against concentration. Samples were processed and concentrations were calculated based on the calibration curve for each amino acid. With sample processing, the weight of the original sample is accounted for, along with the 6ml HCl and any other dilutions used during analysis preparation. Results obtained are in mg/kg. These values are then divided by 10 000 to report results as % m/m.

3.2.8 Mineral determination

The mineral analysis was done at the Western Cape Department of Agriculture's Institute for Plant Production at Elsenburg. Mineral content was determined on 0.5g of dried and finely ground samples. Each sample was incinerated at 460-480°C for 6 hours and left to cool down. After cooling, 5mL of 6M HCl was added. The sample was then placed in an oven for 30 minutes at 50°C. Subsequently 35mL of distilled water was added and the solution was filtered into a brown bottle and made up to a final volume of 50mL with distilled water (Agricultural Laboratory Association of Southern Africa (ALASA), 1996). Minerals were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Mineral concentrations were calculated using iTEVA Analyst software. Argon gas flow rate was 2-5mL/min and instrument settings were as follows: camera temp -27°C, generator temp 24°C, optics temp 38°C, RF power 1150W, pump rate 50rpm, aux gas flow 0.5L/minute, nebulizer 0.7L/minute, coolant gas 12L/minute and normal purge gas flow. Wavelengths for the minerals were as follows: Al 167.079nm, B 249.773nm, Ca 317.933nm, Cu 324.754nm, Fe 259.940nm, K 766.490 nm, Mg 285.213nm, Mn 257.610nm, Na 589.592nm, P 177.495nm and Zn 213.856nm. After 11 samples were processed, standards with a high, medium and low range were analysed for quality control.

Table 6 Nutrient composition (DM basis) of the whole dried full-fat BSFL, dry-rendered BSFL and extruded BSFL

Proximate analysis	Units	FF treatment	DR treatment	EX treatment
Gross Energy	MJ/kg	26.18	26.14	25.22
Crude Protein	%	36.64	48.43	43.69
Crude Fat	%	42.37	30.48	29.47
Crude Fibre	%	8.45	8.13	8.77
Mineral Content				
Phosphorous	%	0.72	0.73	0.84
Potassium	%	1.16	1.28	1.53
Calcium	%	4.27	3.82	4.96
Magnesium	%	0.31	0.23	0.36
Sodium	mg/kg	1251.87	1978.91	1508.52
Iron	mg/kg	606.49	1176.80	1313.95
Copper	mg/kg	15.80	15.20	15.86
Zinc	mg/kg	122.35	132.65	212.13
Manganese	mg/kg	99.57	91.52	159.09
Boron	mg/kg	4.33	4.20	3.85
Amino Acid Content				
Lysine	% m/m	1.46	1.83	2.24
Aspartic Acid	% m/m	3.16	4.18	3.90
Glutamic Acid	% m/m	4.08	5.99	5.30
Serine	% m/m	1.92	2.32	2.26
Histidine	% m/m	1.16	1.10	1.12
Glycine	% m/m	0.73	0.96	0.78
Threonine	% m/m	1.33	1.87	1.79
Arginine	% m/m	1.98	2.55	2.28
Alanine	% m/m	2.78	3.48	3.56
Tyrosine	% m/m	2.91	2.56	2.65
Valine	% m/m	1.91	2.44	2.32
Methionine	% m/m	0.65	0.93	0.71
Phenylalanine	% m/m	2.48	2.71	2.51
Isoleucine	% m/m	1.25	1.66	1.52
Leucine	% m/m	2.61	3.46	3.18
Proline	% m/m	2.53	2.75	2.74
Cysteine	% m/m	0.09	0.05	0.12
Hydroxyproline	% m/m	0.06	0.32	0.09

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

3.3 Materials and methods

3.3.1 Birds and housing system/layout

The trial was conducted at chicken house C of the poultry section of the Mariendahl experimental farm (33° 51’ 0 S; 18° 49’ 60 E) situated outside of Stellenbosch in the Western Cape. Ethical clearance was obtained from the research ethics committee (REC) of animal care and use (ACU) under the protocol number SU-ACUD16-00013. Two hundred and forty Cobb 500, day-old chicks were collected

from County Fair Anysrug Hatchery H5 already vaccinated against New Castle disease (NCD) and Infectious bursal disease (IBD) prior to collection. Upon arrival on Mariendahl, the chicks were then randomly allocated to cages and cages to treatments. Using a random block design, four treatments were each allocated to six replications, with 10 chicks per replication. Each wire cage (0.9 x 0.6m) held ten birds (in accordance to the SAPA code of conduct), and each cage was assigned a bell drinker and a pan feeder. During the adaption period, the chicks were gradually encouraged to use nipple drinkers and tube feeders. Each cage was equipped with one tube feeder and two nipple drinkers.

On the first night in the facility, no hours of darkness were given, after-which one hour of darkness per 24-hour cycle was provided until the birds weighed an average of 100g each (which ensued on day four). Birds were given *ad libitum* access to food and water throughout the trial. The lighting and temperature schedules were aligned with the specifications set out by the primary breeder (Cobb 500 standard). Ventilation was set at a minimum of six changes per hour. Birds were checked a minimum of every 2 hours during the first week of the trial and thereafter a minimum of every 4 hours, except during the dark hours of the lighting schedule. Birds were monitored for cannibalism, abnormal behaviour in terms of water and feed consumption, as well as activity levels and any signs of illness or dysfunction. All mortalities were subjected to post-mortem inspection and the cause of death was established and recorded. The feed allocated for the pens was corrected with each mortality.

3.3.2 Experimental treatments and formulations

All birds were weighed and received the same control starter diet for a four-day adaption period, after which the various treatments commenced. Each bird was provided with 100g of the adaption starter diet, 800g of treatment starter diet (Table 8), 1200g of treatment grower diet (Table 9) and 1200g of treatment finisher diet (Table 10). Each pen was then allowed to move into the next feeding phase as their allocated portions of each phase were completed. The trial starter diets were eaten in approximately 15 days, grower diets were finished within 7 days and finisher diets were finished in 6 days. Diets were mixed on the experimental farm at room temperature (28°C) and were fed in a mash form.

Table 7 Primary protein source per treatment

Treatment	Protein Source
FF	15% Full fat BSF larvae meal
DR	15% Dry rendered BSF larvae meal
EX	15% Extruded BSF larvae meal
Control	Soybean meal

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

For this study, four treatments were evaluated. One of the treatments was allocated as a control diet, whilst the other three all contained various BSF larvae as a protein source. In contrast, the control diet contained a protein source of soybean meal. All three additional treatments included 15% BSF larvae meal. Treatment 1 contained full fat whole dried larvae meal. Treatment 2 contained defatted dry-rendered larvae meal and Treatment 3 was defatted extruded larvae meal. Each treatment was allocated 6 cages (60 individual chickens) at the commencement of the trial. The trial was run for 28 days. All feed was weighed, weekly refusals were recorded and chickens were weighed once a week. Their feed was removed during the last dark hour to ensure all birds were weighed in a starved condition when the lights resumed and weights were recorded. Diets were formulated using the minimum nutrient specifications provided by Cobb 500. The ingredients used to formulate the treatment diets are shown in Table 8, Table 9 and Table 10 for the starter, grower and finisher diets, respectively.

The treatments were processed using different techniques. The extruded larvae were killed and cleaned in 70°C water for the duration of 10 minutes, using a device that was engineered by AgriProtein Technologies Pty Ltd. This design is a proprietary design of the company. After euthanasia, the larvae were then dried using a Gryphon dryer, for 15 minutes at 170°C. Before cooling, these larvae were immediately transferred into a blue press/extruder, where the extruders' barrel was heated using LPG gas burners at approximately 170°C. The heating of the barrel ensured the larvae retained heat as passing through the screw and therefore resulting in the highest quantity lipid removed from the larvae. This method has been refined and tested by AgriProtein Technologies Pty Ltd. The dry rendered larvae were also killed and cleaned in water for 10 minutes at 70°C by the same design established by AgriProtein Technologies Pty Ltd. as the extruded larvae treatment mentioned above. However, after the euthanasia the cleaned larvae were frozen at -20°C and then defrosted prior rendering. Once defrosted, the larvae were heated to 130°C for one hour at atmospheric pressure (1-bar). Pressure was then increased to 3-bar for a period of 30 minutes, causing the product to hydrolyse. This hydrolysation was done using a 4 ton, steam jacketed Windmeul. A twin-screw press was then used to remove the free oil from the cooked larvae. The final step after hydrolysation was then to sieve the larvae through a shaker to remove larger particles.

Table 8 Ingredient and calculated nutrient composition of trial starter diets

Ingredients	Units	FF	DR	EX	Control
Maize	%	44.97	44.69	47.38	40.36
Soya bean (Full fat)	%		6.55	7.85	20.00
Soya bean (46%)	%	36.53	26.18	26.11	31.78
L-lysine (HCl)	%	0.20	0.27	0.26	0.12
DL methionine	%	0.25	0.22	0.23	0.41
L-threonine	%	0.05	0.06	0.06	0.08
Premix	%	0.45	0.45	0.45	0.45
Limestone	%	0.97	0.99	1.01	1.76
Salt	%	0.20	0.20	0.14	0.25
Mono-dicalcium phosphate	%	1.20	1.21	1.38	1.64
Sodium bicarbonate	%	0.18	0.19	0.16	0.14
Sunflower oil	%				3.01
Hermetia illucens larvae (Full fat)	%	15.00			
Hermetia illucens larvae (Dry rendered)	%		15.00		
Hermetia illucens larvae (Extruded)	%			15.00	
Calculated nutrient composition (DM basis)					
Dry matter	%	89.08	89.74	88.63	89.22
AMEn chick	MJ/kg	12.76	12.65	12.65	12.65
Crude fat	%	8.00	8.18	6.44	8.69
Crude fibre	%	4.22	4.14	4.53	3.58
Crude protein	%	26.00	26.00	26.00	26.00
Ash	%	5.47	5.24	4.72	5.12
Calcium	%	1.05	1.05	1.05	1.05
Lysine	%	1.60	1.60	1.60	1.60
Methionine	%	0.66	0.64	0.64	0.77
Cysteine	%	0.44	0.47	0.46	0.34
Methionine + Cysteine	%	1.21	1.22	1.22	1.19
Threonine	%	1.08	1.08	1.08	1.08
Tryptophan	%	0.34	0.34	0.34	0.32
Arginine	%	1.83	1.79	1.80	1.81
Isoleucine	%	1.20	1.16	1.17	1.20
Histidine	%	0.62	0.61	0.61	0.63
Phenylalanine	%	1.07	1.04	1.04	1.08
Tyrosine	%	1.03	0.98	0.98	1.00
Phenylalanine + Tyrosine	%	2.01	1.93	1.93	1.98
Valine	%	1.20	1.19	1.18	1.14
Leucine	%	2.16	2.14	2.14	2.16
Total Phosphorous	%	0.70	0.67	0.71	0.77
Available phosphorous	%	0.50	0.50	0.50	0.50
Sodium	%	0.16	0.16	0.16	0.16
Chloride	%	0.23	0.23	0.23	0.23
Potassium	%	0.99	0.90	0.94	1.10

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae
AMEn - Nitrogen-corrected apparent metabolizable energy value

Table 9 Ingredient and calculated nutrient composition of trial grower diets

Ingredients	Units	FF	DR	EX	Control
Maize	%	56.44	56.86	55.70	50.92
Soya bean (Full fat)	%	3.30	15.23	16.42	20.00
Soya bean (46%)	%	22.07	9.80	9.66	20.59
L-lysine (HCl)	%	0.32	0.31	0.30	0.22
DL methionine	%	0.22	0.18	0.19	0.37
L-threonine	%	0.08	0.06	0.06	0.10
Premix	%	0.45	0.45	0.45	0.45
Limestone	%	0.71	0.71	0.74	1.50
Salt	%	0.19	0.21	0.14	0.24
Mono-dicalcium phosphate	%	1.02	1.02	1.18	1.46
Sodium bicarbonate	%	0.21	0.19	0.16	0.17
Sunflower oil	%				3.98
Hermetia illucens larvae (Full fat)	%	15.00			
Hermetia illucens larvae (Dry Rendered)	%		15.00		
Hermetia illucens larvae (Extruded)	%			15.00	
Calculated nutrient composition (DM basis)					
Dry matter	%	88.64	89.41	88.29	88.90
AMEn chick	MJ/kg	13.20	13.20	13.20	13.20
Crude fat	%	8.83	9.85	8.10	9.88
Crude fibre	%	3.93	3.98	4.37	3.25
Crude protein	%	21.63	22.36	22.31	21.81
Ash	%	4.65	4.52	3.99	4.32
Calcium	%	0.90	0.90	0.90	0.90
Lysine	%	1.38	1.38	1.38	1.37
Methionine	%	0.58	0.55	0.56	0.69
Cysteine	%	0.40	0.43	0.42	0.30
Methionine + Cysteine	%	1.07	1.09	1.09	1.06
Threonine	%	0.93	0.94	0.94	0.93
Tryptophan	%	0.27	0.28	0.28	0.25
Arginine	%	1.47	1.50	1.50	1.47
Isoleucine	%	0.96	0.97	0.97	0.98
Histidine	%	0.52	0.52	0.52	0.53
Phenylalanine	%	0.87	0.87	0.87	0.89
Tyrosine	%	0.83	0.81	0.81	0.82
Phenylalanine + Tyrosine	%	1.62	1.60	1.59	1.63
Valine	%	1.16	1.19	1.18	1.08
Leucine	%	1.88	1.90	1.89	1.88
Total Phosphorous	%	0.70	0.69	0.72	0.76
Available phosphorous	%	0.45	0.45	0.45	0.45
Sodium	%	0.16	0.16	0.16	0.16
Chloride	%	0.23	0.23	0.23	0.23
Potassium	%	0.80	0.74	0.78	0.91

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae
AMEn - Nitrogen-corrected apparent metabolizable energy value

Table 10 Ingredient and calculated nutrient composition of trial finisher diets

Ingredients	Units	FF	DR	EX	Control
Maize	%	61.42	61.83	60.68	56.41
Soya bean (Full fat)	%	4.65	16.57	17.77	20
Soya bean (46%)	%	16.02	3.76	3.60	15.21
L-lysine (HCl)	%	0.27	0.26	0.26	0.19
DL methionine	%	0.17	0.12	0.14	0.32
L-threonine	%	0.07	0.06	0.06	0.10
Premix	%	0.45	0.45	0.45	0.45
Limestone	%	0.65	0.66	0.68	1.45
Salt	%	0.21	0.22	0.16	0.25
Mono-dicalcium phosphate	%	0.90	0.90	1.07	1.35
Sodium bicarbonate	%	0.19	0.18	0.15	0.16
Sunflower oil	%				4.12
Hermetia illucens larvae (Full fat)	%	15.00			
Hermetia illucens larvae (Dry Rendered)	%		15.00		
Hermetia illucens larvae (Extruded)	%			15.00	
Calculated nutrient composition (DM basis)					
Dry matter	%	88.44	89.20	88.09	88.70
AMEn chick	MJ/kg	13.4	13.4	13.4	13.4
Crude fat	%	9.18	10.20	8.44	10.15
Crude fibre	%	3.81	3.86	4.25	3.10
Crude protein	%	19.675	20.41	20.36	19.73
Ash	%	4.36	4.23	3.70	4.01
Calcium	%	0.85	0.85	0.85	0.85
Lysine	%	1.21	1.22	1.22	1.21
Methionine	%	0.50	0.48	0.49	0.61
Cysteine	%	0.38	0.41	0.40	0.28
Methionine + Cysteine	%	0.98	0.99	0.99	0.96
Threonine	%	0.85	0.86	0.86	0.85
Tryptophan	%	0.25	0.25	0.25	0.22
Arginine	%	1.32	1.35	1.35	1.30
Isoleucine	%	0.86	0.87	0.87	0.87
Histidine	%	0.47	0.48	0.48	0.48
Phenylalanine	%	0.79	0.79	0.79	0.80
Tyrosine	%	0.67	0.65	0.65	0.65
Phenylalanine + Tyrosine	%	1.46	1.44	1.43	1.46
Valine	%	1.06	1.10	1.09	0.98
Leucine	%	1.60	1.61	1.61	1.59
Total Phosphorous	%	0.53	0.52	0.55	0.60
Available phosphorous	%	0.42	0.42	0.42	0.42
Sodium	%	0.16	0.16	0.16	0.16
Chloride	%	0.23	0.23	0.23	0.23
Potassium	%	0.71	0.66	0.70	0.82

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae
AMEn - Nitrogen-corrected apparent metabolizable energy value

3.3.3 Data collection and analysis

The following hypothesis was proposed:

H_o: There is no statistically significant difference amongst the production parameters of broiler chickens fed different BSF larvae products as a protein source.

The statistical analysis was done using statistical analysis software (STATISTICA, version 13). Normality and homoscedasticity tests were run on data before the means were tested and the significance was set to $P \leq 0.05$. Where age effects were not a variable the statistics were done by using one-way analysis of variance (ANOVA) with a Fisher least significant difference (LSD) P*ost hoc* test. Where age and treatment effects were variables, the statistics were also done using mixed model repeated measures of ANOVA with a Fisher LSD *Post hoc* test. Data was 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 ADG was determined by means of fitting simple linear regression of the weight over time. The slope of the resulting regression function is ADG and was used to compare animals between treatments The formulae used are shown in Equation 6, Equation 7, Equation 8 and Equation 9:

Equation 6

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

Equation 7

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

Equation 8

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{(Weekly feed intake (g) x protein \% of diet)/100}}$$

Equation 9

$$\text{European production efficiency factor} = \frac{\text{Liveability \% x live weight (g)}}{\text{Age (days) x FCR}} \times \frac{100}{1}$$

The liveability of the birds was defined as the percentage of birds that survived the trial over the total number of birds at the start of the trial.

3.4 Results and discussion

3.4.1 Nutritional composition of tested treatments: full-fat, dry-rendered and extruded

In reducing the fat contents of the DR and EX treatments, these treatments exhibit higher crude protein levels (Table 6), due to the concentration effect. This makes it possible for the DR and EX products to be used at higher substitution rates, as the crude fat limitations, which exist in young chick diets, can be adhered to. The amino acids concentrations are higher in the DR and EX treatments, and this is explained using the same concentration effect logic as the crude protein above. Not all of the individual amino acid concentrations, however, increased proportionally with the protein content increase in the EX and DR treatments. The limiting amino acids for poultry are lysine (for muscle growth) and methionine (for feather growth) (Fisher *et al*., 1981; Schutte & de Jong, 2004). Therefore, it is imperative that these amino acids be given extra consideration when formulating broiler chicken diets and whilst studying suitable protein sources. If the limiting amino acids can be supplied in the appropriate concentrations, the protein utilization and further protein efficiency can be optimized (Schutte & de Jong, 2004). In the ideal amino acid profile (Table 4) for broiler chickens, all the essential amino acids are expressed as a percentage of lysine, because the essential amino acids relative to lysine remains unaffected regardless of environmental, dietary and genetic factors (National Research Council, 2004; Schutte & de Jong, 2004). The methionine and lysine levels of the current studies treatments are higher (FF and DR) and in line (EX) with those reported by other authors (Table 11).

Both the study done by Haasbroek (2016) and Surendra *et al*. (2016) used liquid chromatography according to the AOAC(2002), method 982.30 E (a,b,c), as a means of determining the amino acid content of various BSF meals. This allows us to compare these results with those found in the current study which also followed the same amino acid determination methods. The current study found the FF treatment to be relatively low in glycine, while several of the other amino acids (serine, arginine, glutamic acid, aspartic acid, alanine, proline, tyrosine, phenylalanine and leucine) were relatively higher than the other studies' results found in Table 11.

Therefore, with the exception of glycine, all amino acids exceed or are similar to those found by Haasbroek (2016) and Surendra *et al*. (2016) relative to lysine. Therefore, the larvae substrate and defatting techniques of the current study were found to not change the amino acid levels of the treatments.

Table 11 Amino acid profile of black soldier fly (relative to lysine) and the ideal amino acid profile of broiler chickens

	FF treatment	DR treatment	EX treatment	Full fat larvae [1]	De-fatted larvae [2]	Full Fat pre-pupae [3]	De-fatted pre-pupae [4]	Ideal amino acid profile for broiler chickens [5]
His	79	60	50	54	49	77	78	32
Ser	132	127	101	85	89	70	79	114
Gly	50	52	35	159	134	112	121	
Arg	136	139	102	88	87	101	109	114
Glu	279	327	237	218	213	130	144	-
Thr	91	102	80	72	75	68	72	73
Ala	190	190	159	131	154	122	121	-
Pro	173	150	122	122	131	96	102	55
Cys	6	3	5	3	4	51	77	36
Lys	100	100	100	100	100	100	100	100
Tyr	199	140	118	111	107	108	116	56
Met	45	51	32	28	28	40	38	46
Val	131	133	104	116	116	111	116	82
Ile	86	91	68	72	84	69	77	73
Leu	179	189	142	128	137	107	114	109
Phe	170	148	112	72	69	68	72	66
Crude Protein (%)	36.64	48.43	43.69	35.10	38.05	43.70	53.1	-

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae
(1) and (2) (Haasbroek, 2016)
(3) and (4) (Surendra *et al*., 2016)
(5) NRC (2004)

The DR and EX treatments presented similar results to the FF treatment, but had relatively higher threonine relative to the compared defatted meals in Table 6. Interestingly, only DR was found to have higher arginine, glutamic acid, aspartic acid, alanine and leucine (relative to lysine) when compared to the defatted meals by Haasbroek (2016) and Surendra *et al*. (2016). Therefore, the defatting technique of DR appears to allow for higher amino acid retention post-processing compared to the EX treatment. The digestibility of the amino acids and protein would still need to be assessed in order for this to be deemed beneficial. It is also important to bear in mind during comparison, that the pre-pupae used by Surendra *et al*. (2016) would naturally have a higher protein content (Aniebo *et al*., 2009) compared to the larvae used in the current study and by Haasbroek (2016) (Table 1). This is because pre-pupae have additional (non-protein) nitrogen in their relatively more developed exoskeleton. This would also directly affect the amino acid levels, which make up crude protein. The crude protein levels for the

current studies' treatments were found to be in line with other authors (Table 1). The FF crude protein content was 37%, the DR 48% and the EX 44%.

The crude fat contents of the treatments were a core focus in the current study. Haasbroek (2016) found full-fat larvae crude protein to be 35% and the defatted larvae 38%. A higher crude protein value is seen in the EX treatment (44%) and the DR treatment (48%) than seen by the defatting done by Haasbroek (2016). The lower crude fat reduction achieved by Haasbroek (2016) was using a self-constructed grinding and heating machine as a defatting technique. The crude protein levels recorded by Surendra *et al*. (2016) were higher than the current study. Within the current study, the crude fat content of the FF treatment (42%) was slightly higher than that found by other authors for full-fat BSF pre-pupae (Table 1). Other substrates such as swine manure (Newton, 2005; St Hilaire 2007) and chicken manure (Bondari & Sheppard, 1981) were used to rear BSF larvae and these substrates both yielded larvae with lower crude fat values.

Another largely contributing factor to larvae composition is the age at which the larvae are harvested (Aniebo *et al*., 2009). St-Hilaire *et al*. (2007a), as well as Newton (2005), used pre-pupae that were slightly older than the larvae used in the current study. The pre-pupae are believed to have the higher crude fat and crude protein contents relative to larvae (St-Hilaire *et al*., 2007b), however this is contrary to the findings of the current study, thus other causative factors for these higher crude fat values must be present. For instance, the ether extract (crude fat) method (AOAC method 920.39 or AOAC method 945.16) for fat determination fails to include the polar and bound lipids found in substances and the solvent used needs to be chosen specifically for certain samples as no solvent is appropriate for all samples when performing ether extraction (AAFCO, 2014). This method would therefore run the risk of supplying potentially inaccurate, partial crude fat values. The alternative fat determination method is acid hydrolysis prior ether extraction (AOAC method 954.02), which extracts otherwise omitted fatty acids from glycerides, glycolipids, phospholipids and sterol esters (AAFCO, 2014). The acid hydrolysis method is commonly used when samples have high fat content, which is why this method is most suitable for BSFL. This may also explain the relatively low crude fat values given by Surendra *et al*. (2016) and other authors (Table 1) where the ether extract method was used, compared with those given by Haasbroek (2016) and the current study where acid hydrolysis was done prior to ether extraction.

Ash is the inorganic residue which remains after water and organic matter has been removed by heating. The ash values recorded by various authors shown in Table 1, vary considerably amongst authors. However, the ash content of the FF, DR and EX (12%, 10% and 12%, respectively) treatments (Table 6) do fall within the average range of the values found in the abovementioned studies. The values in Table 1 suggest that defatting of BSFL, generally, allows for the ash content to increase. The

Ca and P dietary levels are of great importance in the broiler industry as the growth and development of strong bones in meat birds is a prerequisite for successful production (Hocking *et al*., 2009). Calcium values of BSFL are notoriously higher than many other insect protein candidates, as well as boasting a superior calcium to phosphorus ratio (Makkar *et al*., 2014). In comparison with the full-fatted 5% Ca content recorded by (Newton *et al*., 2005) and the full-fatted 3% Ca content recorded by Haasbroek (2016),the FF treatment Ca content (4%) of this study is a good average between the aforementioned studies and affirms the composition of the BSFL to be a good source of Ca.

The fibre content of the three tested treatments of the current study are very similar and therefore this would suggest that the fibre content of BSF larvae is largely unaffected by the defatting processing tested. In the comparative results provided by Surendra *et al*. (2016), the same concept is held true as the fibre content is also seemingly unaffected by the various defatting techniques under testing. The fibre content determined however does account for chitin, a long-chain polymer of acetylglucosamine, which has been suggested to reduce intake, and furthermore growth, in fish species (Kroeckel *et al*., 2012). Chitin is a primary component of the exoskeleton of the BSFL (Ng *et al*., 2001), yet it is more significant in pre-pupae which have a more developed exoskeleton than the larvae. The crude fibre for the current study treatments is therefore predictably lower than those stated by other authors for BSF pre-pupae (Surendra *et al*., 2016).

3.4.2 Live weight and average daily gain

Table 12 summarizes the results obtained from the broiler production parameters trial undertaken, with all tested parameters having significant differences excluding the liveability and week three's weekly feed intake. The live weights recorded from the trial revealed that the control group performed significantly worse than all the other treatment groups receiving various BSF larvae meals, throughout the trial. It is evident that regardless of the fat content of the BSF larvae or the defatting technique, inclusion of BSF larvae compared to soybean meal inclusion yields higher live weights during and after a 32-day growth period. It was also found that the FF achieved significantly higher live weights than all other treatments during the second and final week of the growth trial. The FF also significantly outperformed DR throughout the entire trial period (Figure 2).

Table 12 Averages (± standard error) of weekly live weight (g), weekly feed intake (g) and cumulative feed intake (g) and production ratios of broilers receiving Whole Dried Full fat BSF larvae, Dry Rendered BSF larvae and Extruded BSF larvae

Production Days	Treatment				P-Value
	Control	FF	DR	EX	
Day 11 (Week 1)					
Average Live Weight	210.0^{c} ± 10.00	305.0^{a} ± 2.82	276.0^{b} ± 7.40	285.0^{ab} ± 4.62	0.000
Weekly Feed Intake	151.8^{b} ± 2.44	157.9^{b} ± 3.71	161.3^{ab} ± 5.51	173.4^{a} ± 4.65	0.013
Cumulative Feed Intake	151.8^{b} ± 2.44	157.9^{b} ± 3.71	161.3^{ab} ± 5.51	173.4^{a} ± 4.65	0.013
Day 18 (Week 2)					
Average Live Weight	451.9^{c} ± 24.91	689.5^{a} ± 15.62	617.7^{b} ± 13.58	632.1^{b} ± 18.33	0.000
Weekly Feed Intake	419.3^{b} ± 29.53	578.3^{a} ± 9.92	552.9^{a} ± 22.57	575.9^{a} ± 43.22	0.002
Cumulative Feed Intake	571.2^{b} ± 30.25	736.2^{a} ± 10.81	714.2^{a} ± 25.81	749.4^{a} ± 43.65	0.001
Day 25 (Week 3)					
Average Live Weigh	1028.5^{c} ± 41.08	1301.2^{a} ± 19.36	1147.6^{b} ± 25.95	1251.0^{ab} ± 50.95	0.000
Weekly Feed Intake	854.0 ± 39.73	827.7 ± 19.23	856.5 ± 13.00	882.0 ± 41.23	0.675
Cumulative Feed Intake	1425.2^{b} ± 50.25	1563.9^{ab} ± 14.31	1570.7^{a} ± 31.13	1631.4^{a} ± 73.24	0.039
Day 32 (Week 4)					
Average Live Weight	1610.5^{c} ± 48.22	2046.8^{a} ± 22.94	1791.9^{b} ± 40.40	1879.3^{b} ± 31.28	0.000
Weekly Feed Intake	1115.2^{b} ± 44.18	1186.3^{ab} ± 14.34	1257.0^{a} ± 30.27	1122.8^{b} ± 26.43	0.013
Cumulative Feed Intake	2540.4^{b} ± 71.28	2750.1^{a} ± 18.84	2827.8^{a} ± 43.34	2754.1^{a} ± 58.93	0.006
ADG (g) [1]	47.37^{b} ± 1.42	60.20^{ac} ± 0.67	52.70^{bc} ± 1.19	55.27^{abc} ± 0.92	0.000
FCR [2]	1.69^{a} ± 0.06	1.41^{c} ± 0.02	1.67^{ab} ± 0.06	1.55^{b} ± 0.02	0.001
EPEF [3]	277.45^{c} ± 17.18	427.33^{a} ± 10.63	317.67^{b} ± 15.95	351.56^{b} ± 8.09	0.000
PER [4]	2.72^{b} ± 0.11	3.23^{a} ± 0.05	2.68^{b} ± 0.08	2.87^{b} ± 0.04	0.000
Liveability (%)	98.33 ± 4.08	100.00 ± 0.00	100.00 ± 0.00	98.33 ± 4.08	0.582

(^{1}) – Average Daily Gain (ADG)
(^{2}) – Feed Conversion Ratio (FCR)
(^{3}) – European Production Efficiency Factor (EPEF)
(^{4}) – Protein Efficiency Ratio (PER)
(^{a,b}) – Means with different superscripts within the same row differ significantly (P <0.05)
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

The formulated crude fat values for FF treatment starter, grower and finisher were lower than that of the actual diet fed (Table 13). The FF starter diet was formulated as 8% crude fat (Table 8), however was found to be 12% crude fat after proximate analysis. The FF grower diet was formulated as 9% crude fat (Table 9), but the diet contained 12% crude fat. Also, the FF finisher diet was formulated to have 9% crude fat (Table 10) and the diets contained 13% crude fat. Contrary to the proposed theory that young chicks have an inability to digest fats from a young age (Noy & Sklan, 1997), the highest performing treatment in this trial for live weight gain was that which had the highest crude fat value throughout all diet phases. The success of the full-fat BSFL fat could be due to the fat portion of the FF treatments providing other benefits, apart from only being an energy source. Tsushima & Ina (1978) as well as Nandeesha *et al*. (1989), both found that the defatting of larvae resulted in lipophilic growth stimulants being lost. The observed tolerance for BSFL fat may be because insects form part of the natural instinctual diet of chickens, which might suggest that the young chicks are not as negatively affected by BSFL fat, as they are by other fat sources. Future studies would need to compare the tolerance and digestibility of BSFL fats.

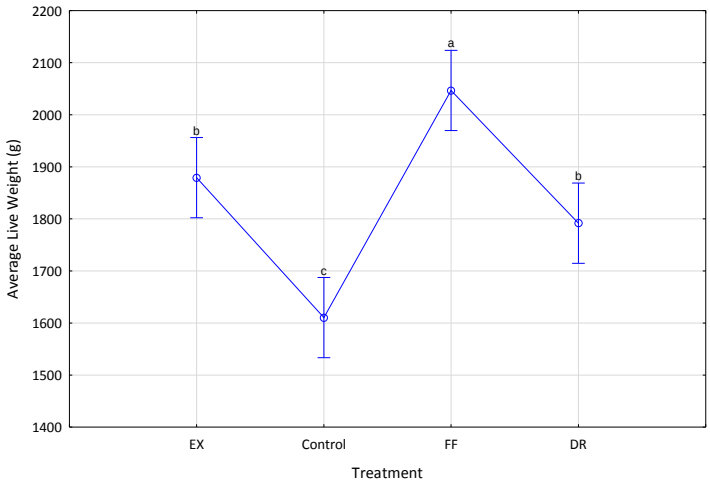

Figure 2 Least square means with error bars for average live weight day 32 (week 4) per treatment (P <0.00, 95% confidence interval)

The reports that defatting causes a loss of growth stimulants contradicts the current study comparison of ADG (Equation 7) between treatments. The FF (60.20g), DR (53.70g) and EX (55.25g) treatments all were reported as having significantly higher ADG than the control (47.37g) (Figure 3). Pretorius (2011b) reported ADG values of 55.47g for 10% housefly larvae inclusion and 51.21g for 25% housefly inclusion in broiler diets. Butcher & Nilipour (2009) reported that an ADG of 50g supported efficient broiler growth. Therefore, all treatments except for the control exceeded this standard. Uushona (2015) reported an ADG of 65.8g for 15% BSF pre-pupae inclusion in broiler diets. The reason for the ADG values found by Uushona (2015) were higher, could be because their birds were only slaughtered at 35 days of age where the current study slaughtered at 32 days of age. These additional days of production, when the birds feed intake increases exponentially, could explain the inflated ADG.

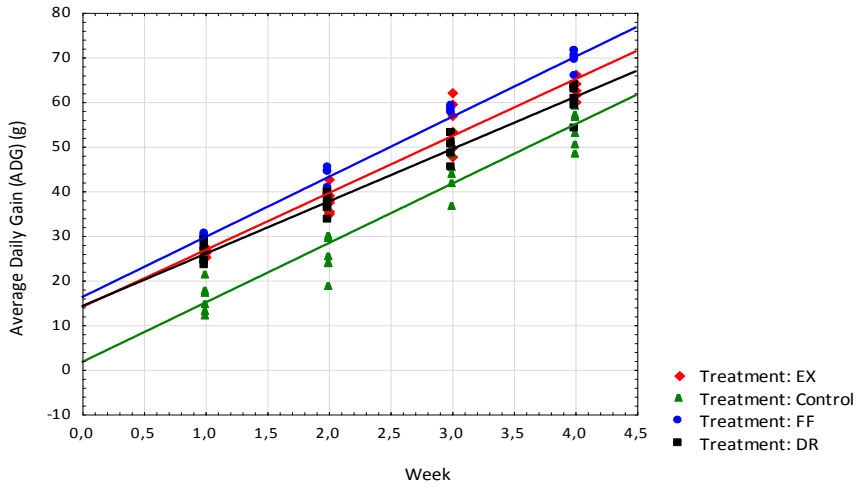

Figure 3 Linear regression for the average daily gain (ADG) in grams per treatment over time (weeks) (P <0.00, 95% confidence interval)

3.4.3 Feed intake, feed conversion ratio and protein efficiency ratio

The highest (significantly higher than the FF and the control) weekly intake during the first week of the trial was EX at 173.4g per chick (Table 12). This is lower than the 210.7g per chick intake found on day 11 for 15% full-fat BSF pre-pupae inclusion by Uushona (2015) , yet no significant differences were found against this authors' control treatment. With regards to palatability, it may be possible the current studies larvae could be more palatable than the pre-pupae. Young chicks should be encouraged to begin consuming feed as soon after hatch as possible, in order to allow gut development and enzyme stimulation to take place (Noy & Sklan, 1997). This has been found to improve the overall performance of broiler chickens (Pretorius, 2011b). The stress of the environmental changes and absorption of the yolk into the abdomen mean that the chicks naturally take longer to settle into feeding regimes and locating water and feed sources (Noy & Sklan, 1997). Therefore, diets achieving higher consumption in early post-hatch period can provide ongoing benefits throughout production. Sheppard *et al*. (2007) found that the BSFL contain natural antibiotics which would not only help chicks progress from passive immunity to active immunity in the first few days of life, but also allow for cheaper, antibiotic-free meat product, which addresses a growing concern about the use of chemicals in meat products among consumers.

In the second week of the trial, the control group consumed significantly lower feed (419.3g per chick) than all other treatments ($P < 0.05$), where EX treatment groups consumed 575.9g, DR treatment groups consumed 552.9g and FF treatment groups consumed 578.2g per chick. Nandeesha *et al*. (1989) suggests that the fat of the insect larvae contains appetite/palatability stimulants which would allow insect included treatment diets to be consumed at a higher rate. It is suggested that these stimulants would also then be reduced with any reduction of fat content in larvae. The defatted treatments however, did not have significantly lower intakes in the second week compared to the full-fat treatment. Therefore, the reduction in fat seems not to have affected the high palatability of the BSFL, relative to the control treatment.

In the fourth and final week of the trial, DR treatment intake (1257.0g per chick) was significantly higher than all treatments, except FF treatment. Contrary to the comparison in week one, all treatments of the current study achieved higher intakes than all BSF pre-pupae treatments observed by Uushona (2015). If chickens which consume significantly more feed, are simultaneously accruing more body mass than other treatments, this would be a positive finding as the feed conversion ratio would be low and this would directly indicate a higher efficiency in productivity and overall cost savings. However, regardless of the significantly higher intake recorded by DR in the final week of the trial, the live weight gains are not a positive image of such excessive intake.

Butcher & Nilipour (2009) reported that an FCR of 1.85 or less were required for the efficient production of broiler chickens. The feed conversion ratio (Equation 6) allows for both the cumulative intake, and the average live weight gain, to be taken into consideration simultaneously. It essentially demonstrates the use of the diet consumed by the chicken in accumulating body mass. Ultimately, the biggest concern for broiler producers is the cost of production and therefore the aim would be to find a diet which allows for the highest efficiency in growth, using ingredients which are as cost-effective as possible. Marsman *et al*. (1997) suggests that processing, specifically toasting, of soybeans improves the FCR in broiler chickens consuming soybean protein. It is therefore of interest to discover if the same principles hold true for the processing of BSFL. In the current study, the FF treatment (FCR of 1.41) was found to have a significantly better FCR to all other treatments tested, with the worst performing treatment being the control (FCR of 1.69) (Figure 4). Nonetheless, all treatments in the current study achieved better FCR's (including the control treatment) than the industry standard provided by Butcher & Nilipour (2009). Uushona (2015) found no significant differences with regards to FCR between their treatments and found the FCR of 1.6 for their control, as well as for the 5% and 10% BSF pre-pupae inclusion level treatments. However, in the same study, the 15% BSF pre-pupae inclusion level treatment was found to have a FCR of 1.5, which is still not as good as the current studies FF treatment (at 15% larvae inclusion). Additionally, the control of the current study which included soybean meal, was found to have the significantly highest/worst FCR compared with the FF and EX treatments. This is a very positive finding in support of the viability of BSFL protein inclusion for production performance.

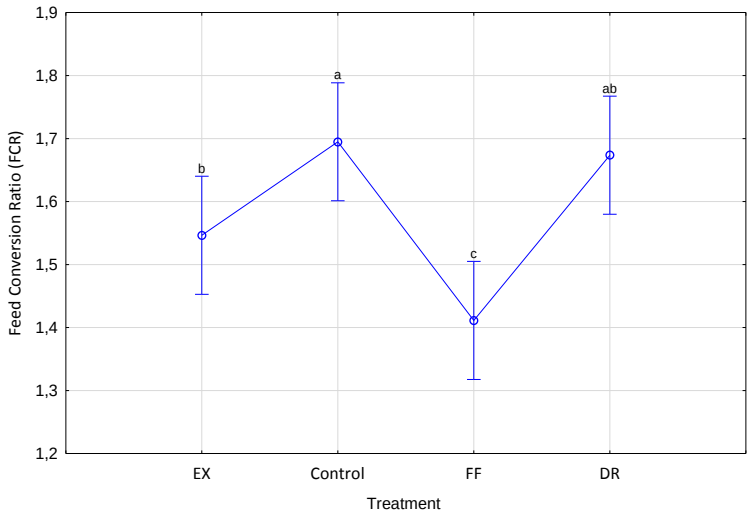

Figure 4 Least square means with error bars for feed conversion ratio (FCR) per treatment (P <0.00, 95% confidence interval)

The higher intake of feed and corresponding lower body mass accretion by the DR treatment a big disadvantage when using this treatment for broiler production. Chickens adjust their intake to their

energy requirements (Leeson & Summers, 1997). It is possible that the energy found in the DR treatment was not as bioavailable, and therefore the fats in the DR are less digestible than that of the EX and FF treatments. In view of this finding, additional microscopic evaluation was performed by Interlab© to assess the level and consequence of the DR and EX processing with regards to potential over-processing and possible heat damage. The FF treatment was found to contain 10-15% heat discolouration with 5-10% of the product showing signs of heat damage. The EX treatment was found to contain approximately 15-20% heat discolouration and only 5-10% of the product showed signs of heat damage. Both FF processing and EX processing were declared as 'good' and non-consequential. However, the DR treatment was found to show signs of over-processing with at least 60% of the treatment showing discolouration due to heat treatment, with 5-10% of the product declared as having heat damage. Incorrect heat processing techniques can cause proteins to denature (Camire, 1991). Even though the DR treatment was found to have little heat damage, the production parameters results from dry rendering included poorer live weight gains and a relatively worse FCR that the other defatted treatment. It is proposed, that a possible low energy bioavailability of the DR treatment may have caused the birds to increase their intake to fulfil their energy requirements.

With regards to the recorded cumulative intakes for the trial, the same pattern is found as the weekly intakes, in that the control achieved significantly lower intakes than all other treatments in week 2 and week 4 of the trial. While EX was found to have significantly higher cumulative intake than FF in the first week. This may have to do with the palatability of the EX treatment. However, the two treatments (FF and EX) did not differ significantly from one another in the weeks that followed this, we therefore cannot make assumptions and compare general palatability between the two treatments, relying only on this finding.

Consumers are showing preference toward lean carcasses of late and are deterred by excess fat accumulation on carcasses in the retail markets (Troy & Kerry, 2010). Therefore, the production objective is now to produce heavier but leaner carcasses to satisfy consumer demands. The use of the protein efficiency ratio (PER) (Equation 8) allows for the quantification of the carry-over of dietary protein to bodily protein in the birds. Wilding *et al*. (1968) reported that the optimum protein efficiency ratio to be 3:1 for broiler production. Significant differences were found by Uushona (2015) whilst evaluating the PER of various BSF pre-pupae dietary inclusion levels. It was found that the 5% inclusion level (PER of 2.4) had a significantly lower PER than the control, the 10% inclusion level, as well as the 15% inclusion level (which all had a PER of 2.5). In the current study, the FF treatment yielded a significantly better protein efficiency at 3.23 in comparison with the control at 2.72, as well as the two defatted larvae meals (DR at 2.68 and EX at 2.87). In comparison to the findings by Uushona (2015) and the other treatments of the study, the FF treatment displayed an exceptionally high PER. The current study however produced values closer to those mentioned by Wilding et al. (1968), with

the FF treatment being the only treatment to exceed this standard. The protein efficiency may therefore be hindered by the reduction of crude fat from the other larvae meals (DR and EX), or other processing consequences. A comparative digestibility trial would need to be performed to validate such assumptions.

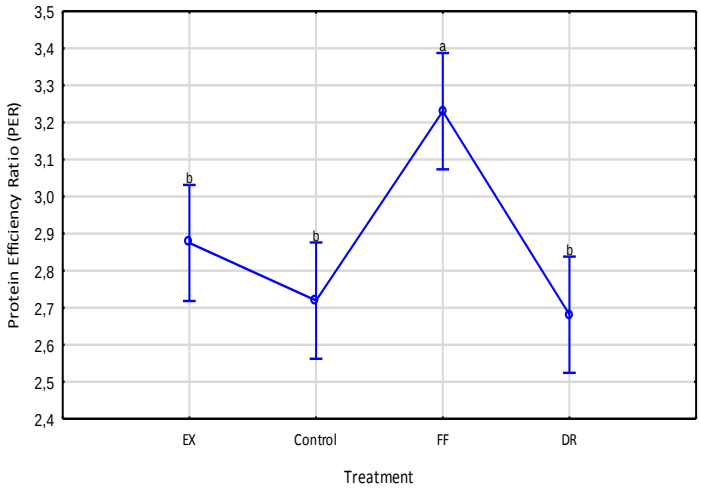

Figure 5 Least square means with error bars for the protein efficiency ratio (P <0.00, 95% confidence interval)

Another possible contributor to the differences in PER, might be the variations in crude protein consumed between treatments (Table 13). The crude protein of the FF treatment starter diet was found to be 6% higher than the original formulation (Table 8). The FF treatment starter diet that was provided during the trial (Table 13) was 32% crude protein, and therefore treatment diets were not iso-protonic. Similarly, the FF finisher diet was tested (Table 13) and found to contain the highest crude protein (23%) of all the finisher treatments, and slightly higher than the formulation stated (Table 10). These relatively higher crude protein values of the starter and finisher FF diets may have contributed to the PER differences stated above. This would only be a contributing factor if the crude protein requirements of the birds receiving DR and EX treatment diet were not being met.

Table 13 Determined proximate analysis of treatment diets

	Dry matter (%)	Ash (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)
FF Treatment					
Starter	89.08	9.26	32.06	4.58	12.48
Grower	88.85	6.62	19.73	4.61	12.11
Finisher	89.35	6.91	22.77	4.10	13.10
DR treatment					
Starter	89.09	7.75	25.96	4.54	8.87
Grower	89.87	5.73	24.65	5.07	12.95
Finisher	89.53	5.20	20.66	4.26	10.30
EX treatment					
Starter	88.66	8.05	26.86	5.02	8.78
Grower	89.53	6.70	23.80	4.84	10.59
Finisher	89.33	5.37	19.20	4.42	9.98
Control treatment					
Starter	89.53	6.55	27.44	5.95	10.09
Grower	89.34	5.74	25.22	4.15	10.11
Finisher	89.53	7.66	17.94	4.26	11.17

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

3.4.4 European production efficiency factor and liveability

The liveability parameter of the current trial yielded no significant differences between treatments. The overall health and animal welfare was kept a priority throughout the trial and consequently only one bird needed to be withdrawn from the trial throughout the entire duration. Regular monitoring, balanced diets and optimum environmental conditions are key factors in the success of the liveability parameter in the trial. Liveability is just one of the considerations in the European production efficiency factor (EPEF) (Equation 9) parameter, along with the live weight, FCR and age of the bird. The EPEF value given for efficient broiler production is anything above 260 units (Butcher & Nilipour, 2009). The FF treatment was found to have a significantly better EPEF at 427.33 than all other treatments (DR at 317.67, EX at 351.56 and the control at 277.45), and the control was found to perform significantly worse than all other treatments in this parameter specifically. However, all treatments tested were above the standard given by Butcher & Nilipour (2009) for efficient broiler production. The differences between treatments for EPEF stem from the significant differences primarily found in the live weights and FCR components of the formulation for EPEF. As no differences were found in liveability parameter and all the birds used in the trial were of the same age, these

components of the EPEF formulation are assumed to have little to no impact on the differences found in EPEF.

Uushona (2015) found no significant differences between various inclusion levels of full-fat whole dried BSF pre-pupae with regards to EPEF, these treatments did not differ significantly from the control group. Nevertheless, the 15% inclusion level of the study yielded an EPEF of 431.0 which is in line with the result found in the current study for the FF treatment at 15% BSFL inclusion (427.33). However, other contributing factors (such as management, diet, environment and breed) in these two compared studies may have played a role in their respective results and cannot allow for a direct comparison. The inclusion of common housefly in broiler diets was evaluated by Pretorius (2011b), and significant differences were found in this study with regards to the EPEF between treatments. The 10% inclusion level of fishmeal and the 10% inclusion level of housefly larvae meal outperformed all other inclusion levels tested (25% and 50%) which could probably be attributed to the fact that protein was totally oversupplied at these inclusion levels. In the current trial, which utilised a 15% inclusion level, the FF treatment outperformed the defatted BSFL meals (DR and EX), as well as the control group. No proximate analysis was performed on the soya-based protein sources used in the trial. It would be worth evaluating in further research whether the same results would be found at higher or lower levels of inclusion of each BSFL treatment tested, as those found for 15% inclusion.

3.5 Conclusion

The DR treatment had the highest intake in the final week of the trial. However, this was not reflected in the weight gains. The treatment was assessed by microscopic evaluation and no excessive over-processing was found, only high levels of discolouration. The processing of the DR treatment may have caused the energy content to be less bioavailable which would explain the high levels of intake, as the birds' attempted to meet their energy requirements. The other defatted treatment, EX, also had high intakes in the first week of the trial. Of the two defatted treatments, the EX treatment boasted the better PER, ADG, EPEF and FCR. The EX treatment was found to be superior to the control treatment in all these abovementioned production parameters, as well. The FF treatment yielded the best production parameter results in the current trial. It achieved the highest live weights at slaughter, the highest ADG and the best FCR, PER and EPEF amongst treatments. Both the crude fat and crude protein of this treatment were higher in the diets than in the formulations. Since insects would form part of the natural diet of chickens, it is possible that their tolerance to insect derived fats is higher than plant-based fats. Also, it is suggested that lipophilic growth stimulants, as well as appetite and/or palatability stimulants, play a role in the production parameter success of this treatment.

All treatments in the current study provided superior FCR and EPEF to industry standards. Improving the production performance of broiler chickens using BSFL also provides an additional protein

ingredient for animal feed and helps reduce the demand for other protein sources, which are directly consumed by humans. Higher growth rates and superior protein conversion allow slaughter weights to be reached quicker, minimising the production costs for producers and supporting the continuation of the broiler industry.

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CHAPTER 4

Evaluation of defatted black soldier fly (*Hermetia illucens*) larvae for broiler production: carcass characteristics

Abstract

The effects of defatted *Hermetia illucens* (black soldier fly) larvae inclusion in broiler chicken diets on carcass characteristics were tested using 240 Cobb-500 chicks. Two defatting techniques were tested, namely dry rendering (DR) and extrusion (EX). The full-fat (FF) and defatted *H. illucens* larvae products were included at 15% inclusion over a period of 28 days. No significant differences were found between FF, EX, DR or the control treatment regarding: dressing percentage, breast portions, wing portions, drum portions, tissue yields, breast and thigh pH, as well all colour parameters except lightness (L*). The control had the lowest dry matter content and highest calcium content in the breast meat. The EX treatment had the lowest L* readings and highest crude protein content in the breast meat. The DR treatment had significantly lower potassium levels and lower breast meat crude protein. The mineral levels and breast colour readings were in the normal range for all treatments. The FF treatment was found to produce significantly heavier live, warm and cold carcass weights and thigh portions than the control and DR treatment. The FF treatment would therefore be superior in maximising the carcass quantity; the EX treatment would additionally allow for higher meat crude protein levels.

Keywords: - Dry-rendering, extrusion, L*, calcium, live weight, thigh portion, pH

4.1 Introduction

The animal protein market experiences extensive demands from consumers regarding meat quality, meat preferences and ethical consciousness. Many demands stem from consumers becoming increasingly health conscious and animal production welfare conscious (Mcilveen, 1995). The industry would suffer great losses if these concerns were not addressed by producers or if standards were not upheld. The modern way of life involves greater access to information (Biehal & Chakravarti, 1983). Information regarding production practices and animal well-being puts animal producers under a constant spotlight, which increases the demand for transparency of processing and traceability of meat products (Mora & Menozzi, 2008).

The demands for quality meat, meat preferences and ethical consciousness are also coupled with the limitations of household food budgets. Consumers in the marketplace usually find a chicken carcass that fits their budget where, tighter budgets only allow for smaller chicken carcasses. However, consumers are also concerned with the 'value for money' concept (Sproles & Kendall, 1986), which only applies to heavier whole carcasses. Heavier carcasses experience higher protein deposition than smaller carcasses (Fisher, 2013). Heavier carcasses therefore result in meatier carcasses in comparison to smaller carcasses, which have higher bone tissue yield (Shahin & Abd Elazeem, 2005). The dressing percentage attained by a carcass determines the amount of tissue that can be sold in exchange for money whilst the remainder of the carcass can be considered as waste (Haitook, 2006).

Broiler genetics have improved over the years and are constantly being bettered as selection takes place in broiler breeder systems (Rostagno *et al*., 2007). These genetic improvements code for protein deposition to be initiated sooner and for longer thus resulting in meatier carcasses that reach slaughter weight sooner; also reducing production costs and ultimately providing a higher value product to consumers. Higher dressing percentages ultimately mean more meat product per live animal produced. Uushona (2015) found no significant dressing percentage differences when BSF pre-pupae were included in broiler chicken diets at various inclusion levels. The same conclusion was found when common housefly (*Musca domestica*) larvae was tested against fishmeal and soybean meal alternatives (Pretorius, 2011). However, both chapter 3 and Pretorius (2011) found positive protein efficiency ratios (PER) in their respective studies with the inclusion of insect meal.

Not all consumers opt for buying whole carcasses in the marketplace, and prefer to choose from the various portions of the carcass, namely: wings, breasts, drums or thighs. Due to the varying degree of meat available on each of these portions, the prices of these portions are not equal and therefore it is important for researchers to test the effects of treatments on the various portions and their respective masses. Uushona (2015) found no significant differences with regards to portion cut yields with varying inclusion levels of BSF pre-pupae. However, the carcass portions in that study included a 'back portion', which the current study did not recognize as a commercial cut. Therefore, these studies cannot be directly compared with regards to this parameter.

Meat quality can be reduced to two factors that consumers deem important: meat appearance and physical characteristics (Allen *et al*., 1998; van Laack *et al*., 2000; Qiao *et al*., 2002; Swatland, 2004; Huff-lonergan *et al*., 2014). The meat colour is the first thing that consumers use to vouch for the product and thus ultimately determines purchasing intent (Allen *et al*., 1998; Qiao *et al*., 2002; Huff-

lonergan *et al*., 2014). Therefore, any deviation from the generally accepted colours norms in meat, will result in consumer rejection (Qiao *et al*., 2002). Meat colour and meat pH are closely linked (Allen *et al*., 1998; Swatland, 2004; Mancini & Hunt, 2005). If the pH value of meat is found to be above the isoelectric point (pH 5.3) of major proteins, it causes the water molecules to be more tightly bound, which in turn absorbs more light causing the meat to appear lighter or paler (van Laack *et al*., 2000). The meat colour is measured using lightness (L*) has been correlated with the pH reading in raw breast fillets (Qiao *et al*., 2002). The pH parameter plays a vital role as an indicator of the process that occurs following *rigor mortis*, where the muscle is converted into meat. Therefore, pH is a key indicator of meat quality.

Muscle pH does not only have an influence on the colour of the meat and the muscle to meat conversion, but also tenderness and juiciness (van Laack *et al*., 2000; Huff-lonergan *et al*., 2014). The water-holding capacity of meat affects the appearance of the meat on store shelves (Huff-lonergan *et al*., 2014). Risvik (1994) found that consumers prefer juicier meat, and Barbut (1997) found that tenderness is also a key component in the determination of meat quality by consumers. The rate at which the muscle pH drops after slaughter influences the tenderness and water-holding capacity of the meat. When the isoelectric point of the protein is reached, water is expelled from the meat as the negative and positive protein molecules form stronger bonds (van Laack *et al*., 2000).

In addition, Mottram (1998) found that the flavour associated with meat is largely to do with the fat content of the meat, as fat plays a role in muscle tissue firmness. However, high fat contents in meat may cause excessive oxidation that can lead to a decreased shelf life as meat may become rancid (Song *et al*., 2013). This oxidation can be slowed down by the inclusion of antioxidants in animals' feed which can then successfully be carried through to the meat for market (Wood & Enser, 1997; Bou *et al*., 2017). Fortunately, BSFL are found to possess rich antioxidants in their composition (Makkar *et al*., 2014). This would come in as a great benefit in the prevention of rancidity while still being able to provide flavour-filled, tender meat products. With defatted BSFL providing less fat per unit fed, the antioxidants should be present at higher levels than in the full fat BSFL meals. Chickens, which belong to the monogastric family, are known to mimic the fatty acid composition of their diet, in their own composition (O'neill *et al*., 1998; Barroeta & Barroeta, 2015; Cao *et al*., 2017). Thus another way to improve the meat quality of broilers with BSFL is to expose chickens to the desirable fatty acid composition of BSFL in diets so that broilers will acquire improved fatty acid profiles (Kroeckel *et al*., 2012) and improved meat quality.

The aims of the current study were to evaluate the meat quality that arises when full fat and defatted BSFL protein sources are provided in broiler chicken diets. Meat quality can be measured using the pH reading of the meat and the colour readings of specific cuts. These parameters were then evaluated. The overall growth and consequential dressing percentage was also tested to determine which treatment allows for the highest meat yield per carcass, as well as the various portion cut yields and composition of the breast portion yield. A meat proximate analysis and mineral analysis was undertaken to determine the complete composition of the meat for each treatment.

4.2 Methods and materials

A detailed description of experimental layout, handling and chicken management procedures is outlined in chapter 3 (3.3.1). Briefly, 240 day-old chicks (as hatched) were randomly allocated to four treatments (FF, EX, DR and control), replicated six times in a completely randomized design. The trial was carried out at Mariendahl Experimental Farm of Stellenbosch University (ethical clearance number SU-ACUD16-00013). The broiler chicks were raised to slaughter at 32 days of age. The starter, grower and finisher diets provided are shown in chapter 3 (Table 8, Table 9 and Table 10). 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.

4.2.1 Slaughter processing and physical measurements

At 32 days of age, one bird per pen (six per treatment) was selected with a body weight close to the mean weight of its pen and its live weight was recorded. These birds were slaughtered according to standard commercial practice, including electrical stunning followed by exsanguination. The broilers were scalded, defeathered and eviscerated (this included the removal of all the internal organs, feet and neck). Initial muscle pH (pH_i) of the breast and thigh were determined 15 minutes *post mortem* using a calibrated portable Crison pH25 meter (Crison Instrument Sa, Alella, Barcelona, Spain) by means of a small incision in the centre of both the thigh and breast muscle. Following the pH measurement, the carcasses were hung in cold storage at 4°C for 24 hours. Ultimate muscle pH (pH_u) was determined 24 hours *post mortem* in the same manner and position as described for pH_i.

Live weight, hot carcass weight and cold carcass weight were recorded 24 hours *post mortem* as well. Dressing percentage was calculated as the percentage difference between the live weight of the chicken and the weight of the cold carcass. Commercial portion yields were determined by first cutting the cold carcasses in half using a portion cutter. Subsequently, the thigh and drumstick were removed by cutting above the thigh towards the acetabulum and behind the pubic bone. The drumstick and thigh were separated by cutting perpendicular towards the joint connecting these two cuts. The wings

were removed from the carcass by cutting through the joint between the scapula and the coracoid. The separate portions were weighed using a Mettler PC 4400 scale (Mettler-Toledo, Switzerland). Percentage component yields were then calculated by expressing these weights as a percentage relative to cold carcass weight. Subsequently, the breast was dissected into muscle, skin and subcutaneous fat combined and bone. These fractions were weighed and expressed as a percentage relative to the total breast weight.

The dissected breast muscle was placed on a flat surface and allowed to bloom for 30 minutes (Warriss, 2000) at 8°C. According to Warriss (2000) a blooming period between 15 to 60 minutes is adequate. Meat colour (L*, a*, b* measurements) were measured using a CIE-Lab colour meter (BYK-Gardner GmbH, Gerestried, Germany) where L* represents lightness, a* represents redness and b* represents yellowness (Nollet Leo, 2007). 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 a* and b* values used to calculate the hue angle (h_{ab}) (°) and chroma value (C^*) as outlined in Honikel (1998). The hue angle defines the meat colour while the chroma defines the colour intensity and colour saturation. Higher hue values are linked with less of a red colour in meat, whilst higher chroma values are found for meat that is redder in colour. Measurements were taken in quadruplets over the total area of the muscle and the average of the measurements was calculated.

4.2.2 Chemical analysis

The meat component of the samples measured during the breast component analysis (described above) were homogenised separately and then vacuum packed and frozen at -18°C until further analyses. Prior to each analysis the meat samples were removed and defrosted in a 4°C refrigerator for ± 24 hours.

4.2.2.1 Proximate analysis and mineral determination: meat

The proximate analysis of the meat samples (analysed in duplicate) was analysed according to acceptable standard methods as provided by the Association of Official Analytical Chemists (AOAC) International (2002). Refer to chapter 3 for methodological analysis regarding dry matter (3.2.1), crude protein (3.2.3), ash content (3.2.2) and mineral determination (3.2.8) . It should be noted that for the dry matter analysis of the meat, 2.5g of the sample was utilized per sub-sample. Furthermore, protein analysis of the meat was analysed on defatted meat samples weighing 0.15g with the Leco machine calibrated with EDTA (Leco Corporation). A sub-sample of the defatted meat samples was used for mineral determination. The protein percentage used was corrected for moisture and fat content.

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

The mineral analysis was done at the Western Cape Department of Agriculture's Institute for Plant Production at Elsenburg (3.2.8 Mineral determination). Refer to chapter 3 for full methodological explanation. It should be noted that the minerals were eluted at wavelength of 2497 for B, Ca (317.933), Cu (324.754), Fe (259.94), K (766.49), Mg (285.213), Mn (257.61), P (177.495) and Zn (213.856).

4.2.3 Statistical analysis

The following hypotheses was proposed:

H_1: There is no statistical difference between the analysed meat quality attributes and carcass characteristics of broiler carcasses as influenced by defatting BSFL.

H_0: There is a statistical difference between the analysed meat quality attributes and carcass characteristics of broiler carcasses as influenced by defatting BSFL.

The 5% significance level was used for the statistical tests and treatment differences were declared at $P \leq 0.05$. The statistical model for the ANOVA test is indicated by, $Yij = \mu i + \alpha j + \varepsilon ij$ where the terms in the model are defined as: the treatment effect response (Yij), the overall mean (μi), treatment effect (αj) and the unexplained error (εij).

Statistical analysis was done using STATISTICA (data analysis software system), Version 13, by StatSoft Inc. (2009). Where age effects were not a variable, the statistical calculations were done by using one-way analysis of variances (ANOVA) with the Fisher least significant difference (LSD) post hoc test.

4.3 Results and discussion

4.3.1 Dressing percentage and breast components

Table 14 summarizes the influence of treatment on dressing percentage and tissue yields. Dressing percentage gives an indication of muscle, fat and bone growth as well as visceral growth up until slaughter. The dressing percentage is lower when the mass of the visceral organs and the fat percentage is higher. Therefore, these components are inversely related. However, a higher dressing percentage is advantageous as more meat is yielded from each bird in production. The dressing percentage is calculated as cold carcass weight divided by the live weight. The current study found

that the FF treatment was higher (P <0.05) than the control for the live weight component of the dressing percentage. In addition, the FF treatment was higher (P <0.05) than the control and the DR treatment for the cold carcass weight component of the dressing percentage formulation. The dressing percentage parameter was found to have no treatment differences (P >0.05). No significant differences were recorded either by Uushona (2015) for black soldier fly pre-pupae treatments with regards to dressing percentage. Furthermore, Uushona (2015) found no significant findings in the live weight and cold carcass weight parameters as well. The significant findings in the current study for live weight, warm carcass weight and cold carcass weight (see Table 14) cannot go unmentioned as these results highlight the FF treatment as being at least as good with regards to growth and increased muscle growth compared with those of the control treatment that represents the industry standard protein source.

Uushona (2015) did, however, find significant differences in the breast tissue components yields, namely finding the control to have significantly lower skin and subcutaneous fat on the breast portion than the 15% BSF pre-pupae inclusion level treatment. The current study found no significant differences for the breast tissue component yields (Table 14). While the percentages of skin and subcutaneous fat for the current study were higher for all treatments compared with the percentages recorded by Uushona (2015) and van Emmenes (2014). This is therefore not related to the treatment effects, as all treatments were collectively higher than other studies, but rather a result of possible environmental or genetic factors.

Table 14 Average (± standard error) broiler carcass measurements as influenced by treatment diet

Parameters	FF	DR	EX	Control	P-Value
Live weight (g)	2031.0^{a} ± 140.72	1817.2^{ab} ± 197.91	1843.3^{ab} ± 203.28	1719.5^{b} ± 202.34	0.06
Warm weight (g)	1403.4^{a} ± 125.86	1231.9^{b} ± 115.70	1239.0^{ab} ± 163.74	1151.7^{b} ± 146.97	0.04
Carcass weight (CW) (g)	1385.8^{a} ± 123.43	1214.5^{b} ± 117.25	1227.6^{ab} ± 163.19	1139.1^{b} ± 143.79	0.04
Dressing percentage (%)	68.2 ± 1.69	66.9 ± 1.29	66.9 ± 1.72	66.2 ± 2.16	0.25
Portion yield					
Drumstick (g)	181.75 ± 20.56	171.53 ± 29.65	163.07 ± 18.18	150.76 ± 16.57	0.12
Wing (g)	212.73 ± 23.61	187.89 ± 32.17	190.06 ± 28.21	180.53 ± 29.74	0.27
Thigh (g)	423.07^{a} ± 85.21	348.01^{b} ± 42.97	364.68^{ab} ± 45.96	331.08^{b} ± 37.60	0.05
Breast (g)	567.31 ± 131.23	505.30 ± 42.46	507.36 ± 93.91	473.21 ± 72.07	0.36
Drumstick (% of CW)	6.55 ± 0.28	7.05 ± 0.92	6.68 ± 0.54	6.63 ± 0.27	0.44
Wing (% of CW)	7.67 ± 0.47	7.72 ± 0.86	7.80 ± 1.12	7.92 ± 0.80	0.96
Thigh (% of CW)	15.32 ± 3.14	14.30 ± 0.53	14.89 ± 0.94	14.57 ± 0.68	0.76
Breast (% of CW)	20.27 ± 3.44	20.86 ± 1.22	20.54 ± 1.34	20.72 ± 1.18	0.96
Breast tissue yield					
Skin & subcutaneous fat (%)	8.78 ± 1.03	7.85 ± 2.76	8.29 ± 1.47	7.37 ± 1.28	0.56
Bone (%)	25.27 ± 6.97	32.15 ± 4.88	33.35 ± 6.53	28.17 ± 7.97	0.17
Meat (%)	65.95 ± 7.43	60.00 ± 6.74	58.36 ± 7.06	64.47 ± 8.74	0.28

(^{a,b}) – Means with different superscripts within the same row differ significantly (P <0.05)
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae
CW – Carcass Weight

4.3.2 Carcass component yield

The various carcass components are marketed at varying prices to one another. This makes some components assume higher economic importance than others for a processor. The breast component has the highest proportion of meat to bone and the breast component of the carcass obtains the highest price by consumers for this reason (Husak *et al*., 2017). In the current study, no significant differences were found for the tissue yield between treatments (Table 14). Other components that were also found to have no significant differences between treatments were the wing component and the drum component. All components were compared by physical mass (grams) and as a percentage of the cold carcass weight (Table 14). The fact that these parameters had no significant differences is a positive finding in the light of alternative environmentally-sustainable protein sources as the performance with regards to meat production is not reduced or hindered by using BSFL protein sources (defatted or not).

The thigh portion also showed no significant differences when compared on a percentage of cold carcass weight basis to thigh component. However, there were significant differences between treatments when comparing the thigh component in terms of physical mass (grams). The FF treatment thigh mass was significantly higher than that of the control, as well as the DR treatment. Similar findings were given by Hwangbo *et al*. (2009) whilst studying the housefly (*M. domestica*) as a protein source. All inclusion levels of housefly larvae tested yielded significantly heavier thigh components to the control treatment. However, Pretorius (2011) who also studied the housefly, found the control to have a significantly heavier thigh component to the insect protein treatments. Uushona (2015) found no significant differences in any portion yields whilst studying various inclusion levels of BSF pre-pupae. Even with such a wide range of results found amongst maggot studies for this parameter, one could still relate these results to the protein efficiency ratios (PER) found for this study. The FF treatment had significantly better PER than all other treatments (control, DR and EX). This may directly influence the thigh mass as this is major muscle in the bird's body and therefore a higher protein efficiency will result in higher protein deposition and ultimately bigger and heavier muscles.

Lysine is the first amino acid to be affected by heat processing (Parsons, 1996) and the first limiting amino acid with regards to muscle growth (Schutte & de Jong, 2004). It would seem the use of heat on the FF, DR and EX treatments did not adversley affect the lysine content of the treatments, as the treatments were all able to yield high carcass portions and parallel dressing percentages to the control treatment. However, a digestibility trial would be need to accurately assess the bioavailability of lysine in the processed larvae treatments.

4.3.3 pH and CIE-lab measurements

The current study found no significant differences for a*, b*, hue and chroma (Table 15). However, significant differences were found with regards to the L* parameter. The EX treatment was found to have a significantly lower L* than all other treatments. Pale soft exudate (PSE) categorized meat has a L* range of 50 to 56 (van Laack *et al*., 2000). While Barbut (1997) believes that an L* value equal to or above 54 is indicative of PSE. All treatments tested fall below both theories' ranges (Table 15) and thus cannot be used as a supportive argument for EX treatment meat quality being superior. Any deviation from the norm in terms of meat colour may result in consumer rejection and a decrease in purchasing intent (Qiao *et al*., 2002). A higher L* value is said to be an indication of poor meat quality (Chen *et al*., 2013). However, this alone does not assume the EX treatment will yield a better meat quality. Though, if the darker breasts of the EX treatment are coupled with a higher muscle pH, then they have the potential to have better water-holding capacity allowing for juicier meat than lighter coloured breasts (Barbut, 1997; Allen *et al*., 1998). Furthermore, Allen *et al*. (1998) found that a darker meat colour was due to a higher pH, which in turn, could cause the shelf life of meat to drop as this darker colour would lead to an increased psychotropic bacterial activity. It is therefore imperative to correlate meat colour and meat pH to accurately determine the treatment effects as positive or negative.

Table 15 The means (± standard error) of physical measurements of broiler carcasses as influenced by inclusion of defatted black soldier fly larvae in broiler chicken diets

	Treatment				
	FF	**DR**	**EX**	**Control**	**P-Value**
Breast Colour					
L*	$50.09^{a} \pm 3.51$	$49.83^{a} \pm 2.72$	$46.56^{b} \pm 1.56$	$50.90^{a} \pm 2.38$	0.05
a*	2.98 ± 0.75	2.80 ± 0.88	3.48 ± 0.76	2.46 ± 0.25	0.12
b*	11.19 ± 1.42	11.95 ± 2.35	11.62 ± 1.59	11.69 ± 1.44	0.90
Hue	74.95 ± 4.40	76.64 ± 4.43	73.16 ± 4.11	78.00 ± 1.71	0.18
Chroma	11.60 ± 1.38	12.30 ± 2.34	12.16 ± 1.53	11.95 ± 1.41	0.90
pH					
Breast($_i$)	5.89 ± 0.30	5.78 ± 0.15	6.02 ± 0.19	5.98 ± 0.23	0.28
Breast ($_u$)	6.07 ± 0.46	6.20 ± 0.50	6.13 ± 0.18	5.97 ± 0.21	0.75
Thigh ($_i$)	6.10 ± 0.17	5.99 ± 0.14	6.14 ± 0.14	6.20 ± 0.17	0.16
Thigh ($_u$)	6.23 ± 0.25	6.36 ± 0.10	6.30 ± 0.11	6.31 ± 0.20	0.67

u – ultimate
i – initial
(^{a,b}) – Means with different superscripts within the same row differ significantly (P <0.05)
EX – Extruded black soldier fly larvae
DR – Dry rendered black soldier fly larvae
FF – Full fat black soldier fly larvae

For the current study, 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 current study found no significant differences between treatments with regards to initial and ultimate pH readings in both the breast and thigh (Table 15). van Laack *et al*. (2000) reported normal broiler breast meat as having a pH of 5.96. The values recorded for all four treatments tested in the current study have ultimate breast pH readings slightly higher than this figure (5.96) presented by van Laack *et al*. (2000) (Table 15), but they are still relatively similar. Lower ultimate breast pH readings have been associated with low-water holding capacity and paleness (Barbut, 1997). The ultimate pH reading of the meat, when meat is usually also paler, should be lower than the initial pH reading, as after slaughter the glycogen, glucose and glucose-6-phosphate reserves are converted to lactate. This in turn decrease the pH of the meat (van Laack *et al*., 2000).

Contrarily, in the current study, both the breast and thigh portions had lower initial pH readings (more acidic) than ultimate pH readings in all treatments, except for those in the control treatments breast portion which remained relatively constant. Therefore, one may suggest a correlation to the presence of BSFL (full fat or defatted) in the chicken diets but Uushona (2015) found that the pH did indeed drop between the initial and ultimate readings when testing BSF pre-pupae in broiler diets. Nonetheless, the initial breast pH readings of the current study differ largely from those reported by Uushona (2015), whilst these ultimate pH readings for the breast portion are more aligned between the two studies. van Emmenes (2014) also found the pH of the ultimate readings to be lower (more acidic) than those of the initial readings when studying broiler chickens' carcass parameters. Meat found to have a lower ultimate pH (such as the studies mentioned above) may be expected to contain more lactate than meat with a higher pH, but the correlation between pH and lactate was not found to be significant (van Laack *et al*., 2000). No plausible explanation can be found to explain why the current study's initial pH readings were lower than the ultimate pH readings.

As previously mentioned in the chapter, it is believed that a strong correlation lies between meat pH and meat colour, as Fletcher (1999) found darker colours to have a higher pH and lighter muscles to have a lower pH reading. Yet this is contrary to the findings described by Barbut (1997) and Allen *et al*. (1998) above. The EX treatment of the current study had the (significantly) darkest breast colour amongst treatments, yet it did not have the lowest or highest ultimate breast pH reading amongst treatments. Therefore, despite the EX treatment having a significantly lower L* (darker colour) breast value, there seems to be no correlation to the meat's pH and consequently conclusions regarding meat quality could not be drawn.

4.3.4 Breast meat proximate analysis and mineral determination

In the meat proximate analysis performed, the control treatment was found to have lower ($P < 0.05$) dry matter (%) than the FF treatment and the EX treatment. With regards to crude protein, the DR

treatment was found to have significantly lower crude protein than the FF and EX treatment, while the EX treatment was found to have significantly higher crude protein reading than the control treatment. The crude protein comparisons place the control treatment in an inferior performance position compared to one of the defatted BSFL EX treatments. This result is very beneficial in the argument for the use of defatted EX BSFL protein sources in broiler diets instead of soybean meal. However, the same cannot be said for the other defatted BSFL treatment tested. The DR treatment processing needs to be reassessed as the same batch of larvae were exposed to the same substrate for all BSFL treatments tested but the protein carry-over to muscle was negatively affected. This was possibly caused by processing factors. It also cannot be due to the reduction in crude fat content as the success of the EX treatment in this parameter contradicts that line of reasoning. High crude protein in meat is essential to the nutrient attractiveness of meat by consumers. The control treatment portraying a natural higher moisture percentage than all other treatments means that the meat may be significantly juicer than the meat of the BSFL treatments. However, Uushona (2015) found no significant differences for initial or sustained juiciness between treatments of between 0%, 5%, 10% and 15% BSF pre-pupae inclusions where, the same 15% inclusion level being the same inclusion level as the current study was used.

Table 16 The means (± standard error) of the proximate analysis and mineral composition (DM basis) of breast meat as influenced by inclusion of defatted black soldier fly larvae in broiler chicken diets

	Treatment				
Parameters	**FF**	**DR**	**EX**	**Control**	**P-Value**
Dry Matter (%)	24.58^{a} ± 0.89	24.08^{ab} ± 0.96	24.81^{a} ± 0.65	23.46^{b} ± 0.87	0.06
Protein (%)	21.03^{ab} ± 0.53	19.37^{c} ± 0.88	22.03^{a} ± 1.13	20.09^{bc} ± 1.04	<0.01
Fat (%)	9.62 ± 1.93	11.53 ± 3.24	8.64 ± 1.77	10.31 ± 2.33	0.23
Ash (%)	4.48 ± 2.06	4.20 ± 1.50	5.39 ± 2.62	4.21 ± 1.50	0.70
Mineral					
Phosphorus (%)	3.14 ± 0.17	3.08 ± 0.17	3.04 ± 0.15	3.13 ± 0.37	0.86
Potassium (%)	3.41^{ab} ± 0.20	3.26^{b} ± 0.22	3.72^{a} ± 0.49	3.67^{a} ± 0.32	0.08
Calcium (%)	0.11^{ab} ± 0.02	0.09^{b} ± 0.02	0.09^{b} ± 0.02	0.12^{a} ± 0.02	0.04
Magnesium (%)	0.53 ± 0.03	0.53 ± 0.07	0.55 ± 0.04	0.53 ± 0.05	0.81
Iron (mg/kg)	226.07 ± 32.12	243.87 ± 39.99	231.01 ± 46.26	253.02 ± 18.98	0.59
Copper (mg/kg)	2.99 ± 1.76	2.95 ± 1.36	2.30 ± 0.54	3.83 ± 1.75	0.36
Zinc (mg/kg)	119.71 ± 10.39	117.57 ± 12.52	113.08 ± 9.74	126.99 ± 19.92	0.38
Manganese (mg/kg)	5.75 ± 1.36	5.71 ± 1.70	4.82 ± 0.91	6.48 ± 2.15	0.38
Boron (mg/kg)	3.70 ± 0.85	3.56 ± 0.72	3.41 ± 0.71	4.47 ± 1.77	0.37
Aluminium (mg/kg)	98.98 ± 78.12	67.07 ± 15.01	102.90 ± 45.11	88.84 ± 35.04	0.60

$(^{a,b})$ – Means with different superscripts within the same row differ significantly (P <0.05)
EX – Extruded black soldier fly larvae
DR – Dry rendered black soldier fly larvae
FF – Full fat black soldier fly larvae

No significant differences were found in the crude fat content and ash components of the proximate analysis of the current study. As previously mentioned, fat in meat lends itself to flavour and thus may be necessary for consumer acceptance. However, excessive fat content in meat can lead to oxidation,

causing rancidity, and may result in consumers shying away from repurchasing chicken meat. The current study's breast meat fat values were almost three fold those reported by Uushona (2015). However, that study was performed on cooked meat and therefore cannot be directly compared with raw meat, as fat rendering would have taken place. Uushona (2015) also found no significant differences in any of the meat proximate analysis components whilst investigating BSF pre-pupae at different inclusion levels for broiler diets. Although, Uushona's study was done on cooked meat samples, whilst the current study used raw meat samples, which could influence the composition of the meat. The significant differences found in the current study for investigating defatted BSFL in broiler diets, is a good motivation for further research efforts in the use of defatting BSFL as an improved protein source.

Further mineral analysis (Table 16) was undertaken on the breast meat after defatting of samples highlighted two minerals with significant differences. The potassium levels in the DR treatment were significantly lower than those found in the EX treatment and the control treatment. The potassium percentage levels recorded in (Table 6) for the treatments (FF, EX and DR) were nonetheless similar to one another. Although the potassium levels in the meat were statistically different, there may be little biological difference between the values and their differences may not necessarily affect the consumer in any way or have an influence on the birds' functioning. Also, the calcium levels of the control treatment were found to be significantly higher than the levels found in the DR treatment and the EX treatment. Even though high calcium levels are reported for BSFL by Newton *et al*. (2005), calcium absorption is regulated both nutritionally and physiologically (Adedokun & Adeola, 2013) and therefore absorption cannot necessarily be increased by higher calcium levels in the diet if the requirements of the bird are already met. This is shown by the success of choice feeding of calcium aside from the broilers basal diet so that chickens may regulate their own calcium intake per their requirements (Wilkinson *et al*., 2014). Choice feeding of calcium may alleviate some of the skeletal disorders associated with insufficient calcium supply and intake.

The meat samples were tested for other minerals, namely: P, Mg, Al, B, Fe, Cu, Zn and Mn but there were no significant differences found between treatments and therefore no treatment effects were found for these parameters. It is suggested that future research include a vitamin analysis of the BSFL to broaden the understanding and permit further insight into the larvae composition and nutrient interactions.

4.4 Conclusion

The control treatment was found to have the lowest dry matter breast meat composition. However, past studies did not find the juiciness of meat to be compromised by BSF pre-pupae protein sources

used at the same inclusion levels as those of the current study. The EX treatment meat composition was found to have the significantly higher crude protein content than the control treatment though. This is a very good argument for the use of EX treatment in providing sustainable protein sources for a growing world population with increasing dietary protein needs. As for the other defatted treatment (DR treatment), a significantly lower crude protein content and significantly lower potassium content was found. The FF treatment was found to have significantly heavier live weights as well as warm weights and cold weights to the control treatment and the DR treatment (for warm and cold weights only), as well as significantly heavier thigh portions to the other two treatments. It is suggested that a vitamin analysis be performed on BSFL in future research for additional knowledge of the BSFL composition.

The 15% inclusion of the DR, EX or FF treatment into broiler chickens' diets allows for as good, if not significantly better, carcass parameter results compared with the control treatment (soybean meal inclusion). Similar tissue yields, portion yields, dressing percentages and meat compositions mean that all BSFL treatments tested can be used a viable alternative protein source in the broiler industry.

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CHAPTER 5

Evaluation of defatted black soldier fly (*Hermetia illucens*) larvae for broiler production: organ and bone parameters

Abstract

The effects of defatted *Hermetia illucens* (black soldier fly) (BSF) larvae inclusion in broiler chicken diets on carcass characteristics were tested using 240 Cobb-500 chicks. Two defatting techniques were tested, namely dry rendering (DR) and extrusion (EX). The full-fat (FF) and defatted *H. illucens* larvae were included at 15% inclusion over a period of 28 days. Treatments were allocated to cages in a randomised block design. The control had the significantly shortest tibia bones, most acidic ileums, heaviest gizzards and lowest levels of bone dry matter and bone fat. The FF treatment had the significantly heavier tibia bones, spleens and heart weights. The FF treatment had the significantly lowest bone P levels and the control had the highest bone Ca levels. The FF treatment had the significantly highest Ca:P ratio and it outperformed all treatments in terms on bone breakage and withstood the highest breaking force. The heavier hearts of the FF treatment may be due to the heavier live weight. The FF treatments resistance to breakage allows the use of this treatment to minimise bone deformity risks. No adverse effects on the organs or tibia bones of the broilers were found for the 15% inclusion of defatted or full-fat larvae.

Keywords: - Tibia bone, Ca:P, bone breakage, bioavailability, deformity

5.1 Introduction

Climate change has negatively affected the production yield of plant based animal feeds, resulting in a decreased supply and in turn causing prices to rise (Dar & Gowda, 2013). These increased prices filter down through the production chain and result in end-products, such as meat, that are more expensive for consumers. In an attempt to achieve food security for a growing world population, alternative feed ingredients to those which are plant based, need to be explored and added to livestock diets (Teguia & Beynan, 2005). These diets need to be balanced, taking the nutritional quality of the combined ingredients into account (Ensminger, 1992), to bring about optimal growth with efficient physical development. Any nutrient imbalances will affect the broiler chicken's growth and development (Awad *et al*., 2009). For optimal production of broilers, digestion and absorption of nutrients should have minimal wasted nutrients (Awad *et al*., 2009). Moreover, the growth and development of the

birds' vital organs (namely the hearts and livers) are necessary for the many body functions and processes needing maintenance in the production of healthy birds.

Under-developed organs can be a result of low arginine in broiler chicken diets (Kwak *et al*., 1999). Likewise, insufficient trace minerals (namely Zn, Se, Mn and Cu) in their diets may influence avian immunity (Kidd, 2004). Immunity and appropriate immune responses are primarily possible through the supply of adequate nutrients, among other influences. When birds are exposed to pathogens, the ability to resist infection and maintain productivity throughout pathogenic invasion, is dependent on the birds' lymphoid organs. The bursa of Fabricius and the spleen are both lymphoid organs part of the avian immune system (Yegani & Korver, 2008). The bursa is the only lymphoid organ that acts as both a primary and secondary lymphoid organ in avian species. The B-cells produced by the bursa are responsible for anti-body production and fighting antigens present in the birds' bodies (Glick, 1991). It is therefore of importance to evaluate the measurement of the bursa mass and the spleen mass, as well as the ratio between these two, to determine the level of infectious activity the bird has been exposed to throughout its lifetime (Kwak *et al*., 1999). Liver colour can also be used in the evaluation of the poultry health. A light-coloured liver is considered normal (Trampel *et al*., 2005), despite a paucity of research about ideal liver colours. Nevertheless, as the bird ages the naturally-occurring yellow colour resulting from the absorbed yolk is slowly lost, and thus if yellow discolouration is still found by slaughter age in birds, this may be an indication of ulcerative enteritis (Grist, 2004).

Gizzard erosion is another health problem that is prevalent in the poultry industry (Johnson & C. Pinedo, 1971). Gizzard erosion is believed to have several causes, including certain dietary minerals inclusions (Fisher *et al*., 1973), the form (pellet *versus* mash) of feed provided (Ross, 1979), stress (Džaja *et al*., 1996), and mycotoxins (Hoerr *et al*., 1982), but most commonly the presence of gizzerosine associated with fishmeal implementation in diets (Harry *et al*., 1975; Itakura *et al*., 1981). It is also known as 'black vomit' and includes symptoms of listlessness and decreased intake (Itakura *et al*., 1981). The 'black vomit', which is a black watery substance, resides in the gizzard and proventiculus, and is formed through the acid hydrolysis of blood (Johnson & C. Pinedo, 1971). Fishmeal can potentially cause gizzerosine formation when incorrectly processed (Okazaki *et al*., 1983). Gizzerosine works antagonistically on the H_2-receptors of the proventiculus' parietal cells, which causes excessive gastric acid secretion (Masumura *et al*., 1985), ultimately resulting in gizzard erosion. The gizzard weight, gizzard pH readings and the gizzard erosion scoring will allow for a thorough investigation into the level of gizzard erosion. The digestive tract pH would also need to be measured to assess the condition of the gastric juices with regards to microbial growth and colonisation. In healthy birds, the normal pH range for the intestinal sections are given as: duodenum (5.5-6.2), jejunum (5.8-6.9) and the ileum as 6.3-8.0 (van Der Klis & Jansman, 2002).

Calcium, phosphorus and magnesium form vital components of the skeleton (Pond *et al*., 2005). When insufficient mobilisation of calcium and phosphorus from the bone occurs, the mineral metabolism cannot be supported. These minerals (Ca and P) are also often lacking in many animal feed ingredients and therefore may lead to an increased prevalence of leg defects and bone breakage (Orban *et al*., 1999; Brenes *et al*., 2003). These outcomes pose a major animal welfare issue to the broiler industry, as well as increased costs for producer. In addition, if these breakages occur during processing, the meat is usually downgraded due to bloody breast meat (Driver *et al*., 2006). This bloody breast meat is caused by blood leaching and later results in consumer rejection of market meat (Rath *et al*., 1999). Black soldier fly larvae (BSFL) are known for high levels of calcium and phosphorus in their composition (Newton, 2005), which might help maintain improved mineral metabolism and furthermore support bone development. The bioavailability of the calcium and phosphorus sources provided in broiler diets is of utter importance and needs to be kept as high as possible for correct formulations to be made. Uushona (2015) found the calcium and phosphorus digestibility levels in BSF pre-pupae to be above 80% for both full-fat and defatted treatments, which means the BSF pre-pupae minerals responsible for skeletal development were found to be highly bioavailable. Bone status can therefore be used to evaluate the dietary mineral adequacy in broiler production (Pond *et al*., 2005).

There is limited literature on the effect of BSFL dietary inclusion on broiler chicken bone and organ parameters and there is even less literature regarding the defatted BSFL inclusion in broiler chicken diets. Therefore, the aims of this study are to quantify the effects of defatted and full fat BSFL inclusion in broiler chicken diets with regards to bone and organ parameters. It is essential to evaluate the level of toxicity risk of any new animal feed ingredient (Teguia & Beynan, 2005). Thus, the organ parameters tested in the study would include the measurement of organ weights, digestive tract pH measurements, liver colour and gizzard erosion scoring. The tibia bone parameters would then include chemical and mineral analysis of the tibia bone, the physical measurements of the tibia bone, as well as bone breakage strength.

5.2 Methods and materials

A detailed description of experimental layout, the handling of the birds and management procedures are outlined in Chapter 3 (3.3.1). Briefly, 240 day-old chicks (as hatched) were randomly allocated to four treatments (FF, EX, DR and control), replicated six times in a completely randomized design. The trial was carried out at Mariendahl Experimental Farm of Stellenbosch University (ethical clearance number SU-ACUD16-00013). The broiler chicks were raised to slaughter at 32 days of age. The starter, grower and finisher diets provided are stipulated in Table 8, Table 9 and Table 10).

At 32 days of age, one bird per pen (six per treatment) was randomly selected that represented the mean weight of the chickens in each pen. At slaughter, the birds were rendered unconscious by electrical stunning (50-70 volts; 3-5 seconds) then exsanguinated and allowed to bleed out for approximately two minutes. Thereafter, the organs: heart, spleen, liver, gizzard and bursa of Fabricius were immediately removed from the carcass with care to avoid any damage. The organs were then weighed using a PC 400 Mettler laboratory scale (Mettler-Toledo, Switzerland). The organ weights were recorded and further calculated as organ weight relative to body weight of the live bird. The weight ratio of spleen to bursa of Fabricius was also calculated. The gizzards were cut open, rinsed with clean water and scored for gizzard erosion all by the same scorer, using an ordinal scale according to Johnson & C. Pinedo (1971) shown in Table 17. The pH of the gizzards was also recorded.

Table 17 Gizzard erosion scoring

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

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 a* and b* values were used to calculated the hue angle (h_{ab}) (°) and chroma value (C*) as outlined in (Honikel, 1998). The hue angle defines the specific colour of the liver while the chroma defines the colour intensity.

After the removal of the organs, gut samples were taken of the duodenum (on the gizzard side of the duodenum at the start of the pancreas), jejunum (approximately in the centre) and the ileum (5mm from Meckel's diverticulum to the ileocecal junction) within 15 minutes post-mortem. The pH of the duodenum, jejunum, ileum, proventiculus and cecum were measured using a calibrated (standard buffers pH 4.0 and 7.0 at 25°C) portable Crison pH25 meter (Alella, Barcelona) by inserting the pH electrode into the centre of the digestive tract region being measured. The probe was thoroughly rinsed with distilled water between each reading. The probe was rested in a KCl 3M electrolytic solution when not being used or rinsed.

Both tibias were removed from the carcass and frozen at -20°C for further analysis. The left tibias were later thawed, cleaned of adherent tissue and weighed. The left tibias were measured in length and mid-diaphyseal diameter using a Vernier calliper with accuracy of 0.1mm, and the radius was later calculated from the diameter reading. The breaking strength was determined using the three-point destructive bending test prescribed by Fleming *et al*. (1998) using an Instron 3345 material testing machine (model 2519-107) fitted with a 3-point-bend rig with a load cell capacity of 5000N and crosshead of 30mm/min. During the bone breakage assessments, each bone was placed onto the machine in a stable position, with the mid-diaphyseal diameter at the centre of the breaking probe. The centre point was marked on the diaphysis with ink and placed between the two 14mm retaining bars, set 38mm apart. The 18mm diameter crosshead probe approached the anterior side of the tibia at 30mm/min until the bone was broken. The breaking strength (N) was recorded as the point of maximum load before failure occurred. The breaking force (N/g) was later calculated using the breaking strength over the weight of the tibia bone, see Equation 10 below:

Equation 10 :

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

5.2.2 Chemical analysis

The right tibia was thawed, cleaned of adherent tissue and cartilage before the weight was recorded. Dry matter of the tibias was determined according to the Official Method 934.01 of the Association of Official Analytical Chemists (AOAC) (2002). Tibias were placed in a dry porcelain crucible and dried at 100°C for 24 hours. Next, the tibia and crucible were placed in the desiccator for 30 minutes to cool and then weighed. Tibias were then defatted in petroleum ether for 48 hours (Rama Rao & Reddy, 2001) and were also broken in half beforehand to facilitate fat extraction. The defatted dry bone weight was determined by firstly drying the tibias at 100°C for 24 hours. Then the dry defatted bones were left to cool in desiccators and their weights were recorded. Lastly, the fat free bone ash percentage was determined after placing the defatted tibia in a furnace for 24 hours at 600°C (Zhang & Coon, 1997). The crucibles and ash were removed from the furnace before it had completely cooled down and placed in desiccators before being weighed. All the weight measurements of the bone were determined using a Mettler AE 200 scale (Mettler-Toledo, Switzerland) with 0.0001g accuracy.

Mineral analysis was performed at the Western Cape Department of Agriculture's Institute for Plant Production at Elsenburg. Mineral composition was determined according to the combustion method (method no. 6.1.1) in (ALASA, 1996). The tibia ash samples had 5ml of 6M hydrochloric acid added to each sample individually. The samples were placed in an oven for 30 minutes at 50°C, after which 35ml distilled water was added and the solution filtered into a bottle and made up to a final volume of 50ml

with distilled water. Elements were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Element concentrations were calculated using iTEVA Analyst software.

5.2.3 Statistical analysis

The hypotheses of this chapter were given as:

H_0: There is no statistical difference amongst organ and tibia bone parameters investigated in broiler chickens fed full fat or defatted BSFL.

H_1: There is a statistical difference amongst organ and tibia bone parameters investigated in broiler chickens fed full fat or defatted BSFL.

Statistical analysis for all the parameters were analysed using the general linear models (GLM) procedure of SAS (2009). Parameters were tested for normality and homoscedasticity before analysis. Welch's variance-weighted ANOVA test was applied when the assumption for homoscedasticity was rejected. Means were separated with a Bonferroni post hoc test (SAS, 2009). Significance was declared at $P \leq 0.05$. The statistical model for ANOVA is indicated by; $Yij = \mu i + \alpha j + \varepsilon ij$ where the terms in the model are defined as; the treatment effect response (Yij), the overall mean (μi), treatment effect (αj) and the unexplained error (εij).

5.3 Results and discussion

5.3.1 Organ weights and liver colour

In Table 18 the organs weights (in grams) are found where the bursa, gizzard and liver were found to have no significant differences amongst treatments. However, significant differences were found between treatments for the heart weight parameter. Broiler chickens are bred for fast growth rates, and therefore run the risk of getting ascites as the physical demand on the vital organs to support the body growth is too high (Julian, 1998). Nonetheless, no birds died of ascites during the current study from any of the treatment groups. The FF treatment was found to have a significantly heavier heart than other treatments, however this was not the case when the heart was taken as a percentage of the body weight. Therefore, because the FF treatment chickens were found in Table 12 to have significantly higher live weights it would make sense that the heart grew relatively larger with the rest of the body, allowing the FF treatments hearts to be heavier in grams compared with the other treatments.

Likewise, the spleen of the FF treatment was also found to be significantly heavier (in grams) than the DR treatment and control treatment. Once again, this could simply be explained by the relatively larger

live weights of the FF treatment. If the spleen or bursa as a percentage of the body weight were found to be significantly heavier for the FF treatment, this would open a discussion of immune status indication, however no significant differences were found for these lymphoid organs when taken as a percentage of the body weight or the spleen to bursa ratio, which allow for an assumption of relatively similar immunity amongst treatments groups. All organs for all treatments tested in the current study were in line with the organs weights (in grams) reported by Uushona (2015) for BSF pre-pupae inclusion in broiler diets.

Table 18 Mean (± standard error) of organ weights in grams (g) of broiler chickens fed full fat or defatted black soldier fly larvae in their diets

	Treatment				
	FF	**DR**	**EX**	**Control**	**P-value**
Organ					
Gizzard (g)	31.62 ± 3.53	29.44 ± 3.69	31.98 ± 4.28	30.57 ± 3.32	0.64
Heart (g)	11.38^{a} ± 2.18	9.35^{b} ± 1.32	9.38^{b} ± 1.09	8.91^{b} ± 1.51	0.05
Spleen (g)	2.94^{a} ± 0.72	2.03^{b} ± 0.67	2.49^{ab} ± 0.61	2.06^{b} ± 0.66	0.09
Liver (g)	44.30 ± 9.34	40.43 ± 6.97	39.91 ± 3.43	37.77 ± 3.81	0.37
Bursa (g)	4.25 ± 0.75	3.93 ± 0.96	4.54 ± 2.11	3.32 ± 1.34	0.48

$(^{a,b})$ – Means with different superscripts within the same row differ significantly (P <0.05)
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

All organ weights, when calculated as a percentage of body weight, and spleen to bursa ratio in the current study were also similar to those recorded by Uushona (2015) for BSF pre-pupae inclusion into broiler diets. However, even though Uushona (2015) found no significant treatment effects at hand, a significant difference was found for the current studies' gizzard weight parameter. The control treatment was found to produce a significantly heavier gizzard (1.85%) than the FF treatment (1.51%) when compared as a percentage of the body weight (Table 19). This does not agree with Okah & Onwujiariri (2012) who stated that the insect protein included diets produced heavier gizzards. There authors reported a gizzard weight of 1.51% for the fish meal included control treatment and 1.94% for 20% replacement of fish meal with maggot. This was nevertheless during an investigation of common house fly larvae inclusion in broiler chicken diets and not full fat BSFL, as well as differences in respective studies' control treatments. The control diet of the current study did have the highest crude fibre content amongst starter treatment diets at 6%, however all other treatments had crude fibre levels above 5%. This difference is quite small and the same pattern is not seen for the grower and finisher diets. Chitin presents itself as part of the crude fibre content of insects (Kroeckel *et al*., 2012), as it makes up the exoskeleton of the maggot (Ng *et al*., 2001). Chitin levels are higher in pre-pupae as they have a more developed exoskeleton than larvae. Chitin is not believed to be detrimental to poultry digestion (Ravindran & Blair, 1993). The BSFL included treatments (FF, DR and EX) may have

had similar crude fibre levels to the control, but only also included a chitin component within this crude fibre content. This explains the more developed gizzard of the control as the crude fibre in the control did not include chitin, and could have required relatively more gizzard development and function to digest its relatively higher true fibre content.

Table 19 Mean (± standard error) of organ weights as a percentage of body weight for broiler chickens fed full fat or defatted black soldier fly larvae in their diets

	Treatment				
	FF	**DR**	**EX**	**Control**	**P-value**
Organ					
Gizzard (% of BW)	1.51^{b} ± 0.20	1.64^{ab} ± 0.10	1.73^{ab} ± 0.26	1.85^{a} ± 0.13	0.02
Heart (% of BW)	0.54 ± 0.06	0.52 ± 0.06	0.50 ± 0.04	0.54 ± 0.08	0.56
Spleen (% of BW)	0.14 ± 0.03	0.11 ± 0.03	0.13 ± 0.03	0.12 ± 0.04	0.53
Liver (% of BW)	2.09 ± 0.32	2.25 ± 0.23	2.15 ± 0.15	2.29 ± 0.12	0.41
Bursa (% of BW)	0.20 ± 0.03	0.22 ± 0.04	0.24 ± 0.10	0.20 ± 0.09	0.74
Spleen:Bursa	0.71 ± 0.22	0.53 ± 0.18	0.59 ± 0.13	0.69 ± 0.33	0.46

$(^{a,b})$ – Means with different superscripts within the same row differ significantly (P <0.05)
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae
BW – Body weight

With regards to the liver colour, no significant differences were found between treatments for any of the liver colour parameters (a*, b*, L*, hue and chroma) tested (Table 20). The L* values found for the treatments in the current study are relatively higher than those reported by Pretorius (2011) when evaluating common housefly liver colour parameters, however due to the lack of significant differences no effect can be found with regards to a treatment effect. Rather, these differences may be due to genetic or environmental factors which all the treatments were equally exposed to. However, as mentioned above (5.1), a lighter liver colour is considered normal (Trampel *et al*., 2005). The current study treatments (slaughtered at day 32) all had liver colours lighter than those found by Pretorius (2011) in birds slaughtered at an age of 28 days and 35 days, with higher levels of inclusion of housefly yielding lower L* values. Both the current study and Pretorius (2011) used the same colour guide and system. Similar to the liver colour, the liver weights were not found to reveal significant differences between treatments either.

Table 20 Mean (± standard error) of liver colour parameters for broiler chickens fed full fat or defatted black soldier fly larvae in their diets

	Treatment				
	FF	**DR**	**EX**	**Control**	**P-value**
Parameter					
L*	35.65 ± 3.66	35.58 ± 3.94	34.29 ± 2.29	36.56 ± 1.64	0.56
a*	11.99 ± 1.14	12.89 ± 0.84	12.38 ± 1.30	13.16 ± 0.98	0.27
b*	13.15 ± 1.09	13.22 ± 2.58	12.89 ± 1.64	14.47 ± 1.25	0.49
Hue	47.49 ± 4.59	45.29 ± 4.56	46.05 ± 3.22	47.68 ± 3.07	0.67
Chroma	17.84 ± 1.71	18.52 ± 2.30	17.90 ± 1.85	19.58 ± 1.20	0.33

$(^{a,b})$ – Means with different superscripts within the same row differ significantly (P <0.05)
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

5.3.2 Gizzard erosion

Table 21 gives a summary of the various scores obtained by the treatments with regards to gizzard erosion. No significant differences were found between treatments for the gizzard erosion scoring parameter. This is a positive finding for the use of sustainable protein source alternatives such as the FF, DR and EX treatments, which will therefore a safer animal protein source option compared with fishmeal, which is known as one of the causes of gizzard erosion as discussed above (5.1). The exception of one relatively high score in the FF and two in both the DR and control treatments were not seen as a trend in the other observations for the treatments. Therefore, we cannot assume treatment effects were involved in these occurrences.

Table 21 Number of observations per gizzard erosion category recorded per treatment group

	Treatment Observations			
Score	**FF**	**DR**	**EX**	**Control**
0	2	2	2	1
1	3	2	4	3
2	1	2	0	2
3	0	0	0	0
4	0	0	0	0
P-value	0.683			

FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

5.3.3 Intestinal pH measurements

Several digestive tract section pH readings were measured post-slaughter and recorded in Table 22. The bird's health, the type of nutrients in the digesta, along with the gut microflora, influence the intestinal pH (Rahmani *et al*., 2005). The pH in the various regions of the GIT affect the specific nutrient digestion and absorption which occurs in that region (Rahmani *et al*., 2005). The gizzard and proventiculus, which are situated in the highest part of the digestive tract were found to show no treatment effects, as no significant differences were found. The readings for these sections

(proventiculus and gizzard) were expected to, and were found to be, much lower and more acidic than the rest of the digestive tract, as the breakdown of food particles is initiated with the addition of hydrochloric acid in preparation for the absorption of nutrients later in the digestive processing (Svihus, 2014).

Table 22 Mean (± standard error) of intestinal pH readings for broiler chickens fed full fat or defatted black soldier fly larvae in their diets

	Treatment				
	FF	**DR**	**EX**	**Control**	**P-value**
Organ/Section					
Cecum	6.89 ± 0.38	7.00 ± 0.55	7.09 ± 0.33	6.54 ± 0.42	0.16
Proventiculus	2.74 ± 0.60	3.81 ± 1.33	3.67 ± 1.42	3.34 ± 0.84	0.36
Duodenum	6.03 ± 0.13	6.08 ± 0.24	5.90 ± 0.44	5.87 ± 0.25	0.50
Jejunum	6.05 ± 0.25	6.08 ± 0.16	6.05 ± 0.15	5.87 ± 0.18	0.24
Ileum	6.54^{a} ± 0.63	6.55^{a} ± 0.45	6.78^{a} ± 0.23	5.86^{b} ± 0.62	0.02
Gizzard	2.77 ± 0.75	3.55 ± 1.73	3.63 ± 1.75	3.27 ± 0.67	0.67

$(^{a,b})$ – Means with different superscripts within the same row differ significantly (P <0.05)
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

Further down the digestive tract is the duodenum and jejunum. Once again, no significant differences were found in these digestive tract sections and these readings are very similar to those recorded by Uushona (2015), when investigating BSF pre-pupae inclusion level effects. Uushona (2015) reported no significant differences between inclusion levels for the duodenum, jejunum or the ileum. The current study, though, found treatment effects in the pH readings of the ileum section of the digestive tract. The control treatment was found to have a significantly more acidic ileum than all the other treatments. The BSFL included treatments (FF, EX and DR) had similar ileum pH readings than reported by Uushona (2015).

All the GIT pH readings for all treatments of the current study were found within the ranges given by van der Klis & Jansman (2002) for healthy chickens, except for the significantly acidic ileum of 5.86 of the control treatment. Other authors have reported the pH of the ileum as between 6.41 and 6.80 for control diets which include soybean meal (van Emmenes, 2011; Uushona, 2015). Therefore, all treatments ileum pH readings fall within this range except for the control treatment (Table 22). Without a healthy gut, even the most balanced diet cannot be utilised correctly for optimal growth performance (Yegani & Korver, 2008). The ileum is thought to play a role as a site for water and mineral absorption, although some digestion and absorption of fat, protein and starch may occur there as well. The passage rate through the ileum is much slower than other GIT sections. However, the cecum which is located after the ileum in the GIT, had no significant differences for the pH readings with a mean of 6.88 (with a standard error of 0.45), which is very close to a neutral pH reading of seven. Therefore, the acidic ileum environment of the control treatment does not carry through into the cecum and

allow for acidic litter to be excreted, or further acidity problems in the large intestine. The mean (6.88) found for the cecum pH in the current study is in line with the control treatment of van Emmenes (2014), which was tested under the same experimental conditions. It is suggested that the BSFL provided an improved gut health which may have in turn stabilised the ileal pH.

5.3.4 Bone parameters

The skeletal support of a broiler chicken is necessary for optimum animal welfare conditions, maximised production performance and ultimately financial soundness for the producer. Therefore, the evaluation of the bone parameters is essential in the bigger picture of improving the broiler industry for all role players. Table 23 is a summary of the tibia bone parameters evaluated for the current study.

5.3.4.1 Bone strength

Meat birds are subject to the development of tibial dyschondroplasia (TD), osteochondritis, rickets and epiphyseal separation. These are a few of the rapid growth problems which result in pain and often lameness in broiler chickens. During TD birds move in a 'creeping' fashion on their hocks and struggle to reach feed and water sources (Julian, 1998). Tibial dyschondroplasia is likely caused by a lack of specific nutrients required by proliferating chondrocytes (Julian, 1998). No birds were found to develop TD, or any of the abovementioned skeletal deformities, in the current study and this may be attributed to the balanced diets provided to all treatments. The FF treatment was found to have heavier ($P < 0.05$) tibia bones than the EX treatment and the control treatment. Heavier bones have a higher density and this suggests the FF treatments heavier bones are a good finding in the argument for the FF treatment, as low bone density is believed to be a risk factor for bone fracture (Julian, 1998).

A further very positive finding in the argument for the use of the FF treatment is that this treatment was found to stand the significantly highest breaking force (N) above all other treatments tested (EX, DR and control). The strength exhibited by the FF treatment could either be attributed to something associated with the fat content of the treatment, or this was a consequence of processing the other treatments (DR and EX). A digestibility study would be able to distinguish if the calcium and phosphorus bioavailability of the EX and DR treatments is still equivalent to the FF treatment, after processing. Uushona (2015) found no significant differences in breaking force (N) between inclusion levels of BSF pre-pupae in broiler diets and the mean of the results recorded for that study are in line with that found in the current study.

Table 23 Mean (± standard error) of bone parameters for broiler chickens fed full-fat and defatted black soldier fly larvae in their diets

	Treatment				
	FF	**DR**	**EX**	**Control**	**P-value**
Parameter					
Tibia breaking force (N)	420.02^{a} ± 62.53	325.71^{b} ± 37.81	350.34^{b} ± 46.41	321.16^{b} ± 40.70	<0.01
Tibia bone breakage (N/g)	39.15 ± 3.86	34.74 ± 3.71	38.05 ± 4.40	38.32 ± 4.79	0.31
Weight (g)	10.73^{a} ± 1.12	9.48^{ab} ± 1.53	9.21^{b} ± 0.71	8.40^{b} ± 0.60	<0.01
Radius (mm)	4.21 ± 0.27	4.26 ± 0.42	4.01 ± 0.22	3.99 ± 0.29	0.36
Length (mm)	87.75^{a} ± 3.95	85.06^{a} ± 3.82	85.09^{a} ± 2.08	80.36^{b} ± 2.34	<0.01
Moisture %	51.27^{b} ± 0.74	50.51^{b} ± 1.14	50.11^{b} ± 1.06	53.36^{a} ± 1.88	<0.01
Fat %	12.97^{a} ± 1.26	13.61^{a} ± 2.22	13.34^{a} ± 2.88	3.79^{b} ± 1.59	<0.01
Ash %	22.59 ± 0.52	22.14 ± 0.60	22.68 ± 0.80	22.71 ± 1.81	0.77
As a % of bone ash:					
Calcium (%)	46.42^{ab} ± 5.24	41.14^{b} ± 30.49	51.56^{ab} ± 21.48	59.40^{a} ± 12.34	0.08
Phosphorus (%)	8.71^{c} ± 1.85	12.87^{b} ± 8.33	20.91^{a} ± 5.31	20.61^{a} ± 4.44	<0.01
Ca:P	5.36^{a} ± 0.40	3.35^{ab} ± 1.50	2.54^{b} ± 0.26	2.91^{b} ± 0.50	<0.01
Potassium (%)	0.90^{b} ± 0.07	0.97^{a} ± 0.15	0.97^{a} ± 0.07	1.02^{a} ± 0.14	<0.01
Magnesium (%)	0.85 ± 0.05	0.86 ± 0.06	0.88 ± 0.05	0.92 ± 0.13	0.25
As mg/kg in bone ash:					
Iron (mg/kg)	222.60 ± 48.14	212.84 ± 35.57	198.91 ± 37.80	202.17 ± 52.99	0.16
Copper (mg/kg)	2.65 ± 0.59	2.94 ± 1.00	4.14 ± 6.19	3.25 ± 1.19	0.39
Zinc (mg/kg)	395.15 ± 108.38	350.03 ± 73.04	387.53 ± 83.35	437.33 ± 86.75	0.13
Manganese (mg/kg)	10.39 ± 3.91	11.55 ± 4.53	11.80 ± 4.38	11.39 ± 3.00	0.40
Boron (mg/kg)	6.18^{c} ± 3.34	10.53^{ab} ± 5.65	8.25^{bc} ± 5.55	13.38^{a} ± 3.50	<0.01
Aluminium (mg/kg)	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{a} ± 0.00	0.01^{b} ± 0.00	<0.01

(^{a,b}) – Means with different superscripts within the same row differ significantly (P <0.05)
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

The control treatment was found to have a significantly shorter tibia bone to all other treatments. This may be correlated with the overall inferior growth experienced by this treatment as explained in chapter 3 (3.5), where it describes the control treatment as having the lowest live weight compared with all treatments. Therefore, in relation to the body weight, tibia length differences are linked to body weight differences and therefore no justification required regarding treatment effect. No significant treatment effects were found with regards to radius or bone breakage (N/g) of the tibia bone. The bone breakage (N/g) values recorded for the current study are in line with those reported by Uushona (2015).

5.3.4.2 Ash and mineral content

No treatment effects were found in the ash percentages of the tibia bone in the current study (Table 23). These means were found in line with those recorded by Uushona (2015), who also recorded no significant differences in ash percentage, those treatments all were found to have an ash value of roughly 22%. The control treatment was found to have significantly more bone moisture, however less bone fat and bone aluminium levels to all other treatment groups tested. Although statistically different, the bone aluminium levels for all treatments did not differ largely in actual units and were not expected to influence broiler production. The control had significantly higher levels of boron in bone than the EX and FF treatments, whilst, the FF treatment contained significantly lower levels of boron (6.18) in the bone ash relative to the DR treatment (10.53) and the control treatment (13.38). Boron, when supplemented into broiler diets, has successfully increased tibial calcium and phosphorus levels (Bozkurt *et al*., 2012), however this may not be the case regarding the boron levels in bone ash.

All treatments in the current study where found to have high (between 41 and 59%) levels of calcium. The control treatment was found to have significantly higher bone calcium levels than the DR treatment (Table 23). Due to the high calcium levels in BSFL (Newton, 2005), it was expected that the control would have lower calcium levels in the bone compared to BSFL. The values are slightly higher compared with Uushona (2015) where levels were reported at just above 40%. van Emmenes (2014) reported levels slightly above 30%, but this study was regarding phytase enzyme supplementation in broiler chickens' diets and did not include insect protein. These differences are equally high amongst all current studies treatments and the vast difference from other authors cannot be described as a treatment effect. It may be to do with the more modern lines of broiler genetics or the bioavailability of other ingredients included in all treatments (for example limestone or monocalcium phosphate). The bioavailability of the DR treatment calcium may have been affected by the dry rendering processing though. The FF treatment was found to have a significantly higher calcium to phosphorus ratio than the control and the EX treatment. The FF treatment Ca:P was 5.36, this is much higher than

the 2.1 reported by Uushona (2015) for all pre-pupae treatments. The other treatments (DR, EX and control) were more in line with this authors' findings. The high difference in this ratio is not a result of a high calcium content, rather due to the low phosphorus levels relative to the other treatments. The FF treatment had the significantly lowest phosphorus levels (Table 23) than the other treatments and were found to be much lower than those reported by other authors (Uushona, 2015).

A calcium or phosphorus deficiency may lead to an increase in bone breakage and bone defects (Brenes *et al*., 2003). The EX treatment was found to have higher ($P < 0.05$) phosphorus levels relative to the FF and DR treatments. The FF treatment mineral levels would make it seem at risk of inferior bone development, as calcium together with dietary vitamin D_3 and phosphorus are believed to aid bone development (Rath *et al*., 2000). However, the results for the breaking force (N) parameter discussed above revealed the FF treatment to be significantly more resistant to bone breakage than all other treatments. Therefore, the correlation between bone breakage and these mentioned minerals responsible for bone development may only hold true if the minerals are observed as an interacting group. It would also suggest that according to the findings in the current study, a larger Ca:P ratio could be beneficial to bone strength. No significant differences were found between treatments for Mg levels nor for Fe, Cu, Zn or Mn levels.

Other minerals that were discovered to have treatment effects was potassium. Supplemented dietary potassium is said to reduce heat stress as well as have a positive relationship with blood calcium levels in broilers (Ait-Boulahsen *et al*., 1995). Calcium is mobilised from the bones to maintain a healthy mineral metabolism and support rapid growth. Therefore, more calcium is found in the blood when potassium is supplemented into the water/feed of chickens (Ait-Boulahsen *et al*., 1995). The FF treatment was found to have significantly lower potassium than all other treatments, even though throughout the trial the different treatments dietary levels of potassium were not different (Table 6). The relatively lower bone potassium levels of the FF treatment (0.9) did not appear to have any adverse effect on bone strength or development. The potassium levels in the FF treatment may have been utilised to deal with stress levels, possibly resulting in lower potassium deposition in bones. Although no other results indicated higher stress levels in the FF treatment. The potassium level (1.0) recorded by Uushona (2015) for 15% BSFL pre-pupae inclusion was similar to the findings in the current study for all treatments tested.

5.4 Conclusion

The feasible use of insect protein alternatives can only be accepted if the inclusion does not negatively affect the health status of the birds in production and, if possible, aid in the alleviation of the current problems associated with broiler production. No toxicity signs were found in the lymphoid organ

parameters. No significant gizzard erosion differences or levels were found for any of the treatments tested. Chitin components included in the BSFL treatments crude fibre are suggested to have allowed for seemingly equal levels of dietary crude fibre amongst treatments. However, the higher actual crude fibre of the control treatment may have resulted in a relatively more developed (and thus heavier) gizzards. Because chitin was not measured in the current study, this cannot be confirmed. The liver colours of all the treatments resembled a healthy birds' liver colour. The FF treatment was found to have significantly heavier tibia bones, spleens and heart weights (in grams), however these could simply be explained by the treatments relatively heavier live weights at slaughter. Although this treatment was also found to have the significantly lowest phosphorus levels amongst all the treatments and significantly largest Ca:P, it did outperform all other treatments regarding resistance to bone breakage. This finding would indicate a minimisation in skeletal deformity risk for chickens receiving this treatment. A digestibility study would provide insight into the bioavailability of the defatted BSFL nutrients and the consequences of these digestibilities could then relate to other parameters. Even so, none of the BSFL included treatments tested adversely affected broiler chickens when included at a dietary level of 15%. The study found 15% BSFL broiler diet inclusion (both full-fatted and defatted) to maintain normal gut environments as well as support skeletal strength and normal vital organ development.

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CHAPTER 6

Evaluation of the total tract digestibilities of defatted black soldier fly (*Hermetia illucens*) larvae in the diets of broiler chickens

Abstract

The total tract digestibilities of *Hermetia illucens* (black soldier fly) larvae were investigated in 240, seven-day-old, broiler chicks allocated in a randomized block design consisting of four treatments (maize meal, dry-rendered (DR) larvae, extruded (EX) larvae and full-fat (FF) larvae). Ten birds were used per replication, and six replications were used per treatment. The AME values for the FF, DR and EX were 16.65MJ/kg, 8.84MJ/kg and 15.79MJ/kg, respectively. The crude protein and amino acid digestibilities for FF, DR and EX larvae were all found to be very high (above 90%). The crude fibre, ash, phosphorus and calcium values of the current study were higher than those reported by other authors, however all other values were in line with other authors findings. All similar digestibility studies previously done were however performed on older, more mature chickens. The mineral concentrations of the DR treatment were negatively influenced by the defatting technique used. The very low intakes and suggested low bioavailability of the DR treatments energy content resulted in an extremely low AME. Both the EX and FF treatments can be used in broiler chicken diets and can be expected to be highly digested and absorbed, allowing nutrient requirements to be sufficiently met.

Keywords: - Dry-rendering, extrusion, AME, crude protein, amino acids, bioavailability

6.1 Introduction

During diet formulation, it is essential that the nutrients provided to broilers match the requirements of the broilers as closely as possible, to ensure optimal growth and production. This can be a difficult task to perform throughout the rapid growth and development that modern broilers undergo from hatch to slaughter. Optimal absorption and digestibility ultimately minimises the waste of nutrient, and therefore could also be seen as a cost-saving practice for producers. Animals that consume nutrients which have a high digestibility coefficient, attain higher growth rates compared to those fed low digestibility nutrients (Thang *et al*., 2010).

A digestibility study is a method of quantitatively measuring the digestion of certain nutrients in a feed ingredient, which are consumed by an animal (Scott & Boldaji, 1997). The digestibility of the nutrients in a protein source is an important indication of the quality of that protein source (Manzano-Agugliaro *et al*., 2012). The evaluation of amino acid digestibility of feed ingredients is essential if poultry are to

receive balanced diets (Sánchez-Muros *et al*., 2014). The present practice of using roosters as a representative subject in digestibility studies (McNab, 1994) is not appropriate for all feed ingredients (Huang *et al*., 2006). In general, modern broilers are capable of higher digestibility than both layers and roosters (Huang *et al*., 2006). Not only is digestibility dependent on the breed, but also on the age of the bird. Some authors reported that digestibility increased with increasing age (Wallis & Balnave, 1984; Doeschate *et al*., 1993), whilst others reported a reduced digestibility with increasing age (Zelenka & Lisk, 1986). More specifically, it is believed that protein absorption and consequent digestibility are also influenced by the age of the bird (Tarvid, 1995).

Soybean meal is the industry standard protein source in broiler production. However, due to the prevalence of anti-nutritional factors in soybeans, the use of heat processing has been found to increase the digestibility of soybean meal, but this comes at a risk of over-heating (Marsman *et al*., 1997). Over-heating during processing results in an increase in Maillard reactions which causes proteins to denature (Parsons, 1996). This ultimately results in a reduction of sugars and ultimately a lowered protein and energy digestibility (Qin & van der Poel, 1998). Considering the risks associated with soybean meal processing, alternative protein sources need to be found and their digestibility coefficients quantified.

Black soldier fly larvae, a relatively new protein source in animal feed, has limited literature regarding its digestibility potential. Uushona (2015) and De Marco *et al*. (2015) both have reported total tract digestibilities on various processed BSF treatments (including that of a defatted meal). However, De Marco *et al*. (2015) performed their trial using only roosters and all birds were 32 days of age, using BSF larvae. Uushona (2015) used experimental subjects as-hatched, however used BSF pre-pupae and started the trial period later in the bird's life, when chickens were already 43 days old.

It is therefore the objective of the study to evaluate the general nutrient, amino acid and mineral digestibility and apparent metabolisable energy (AME) of different (namely full-fat BSFL, dry rendered BSFL and extruded BSFL) processed BSFL in young broiler chicks.

6.2 Methods and materials

6.2.1 Experimental animals, layout and diets

Ethical clearance (SU-ACUD16-00013) was obtained from the Research Ethics Committee (REC) of Animal Case and Use (ACU). The experimental trial was conducted at the poultry section (chicken house C) of Mariendahl Experimental farm (33° 51' 0 S; 18° 49' 60 E). A total of 240 day-old Cobb 500 broiler chicks as hatched were collected from County Fair Anysrug Hatchery H5 having been vaccinated against New Castles disease (NCD) and Infectious bursal disease (IBD) prior to collection. After arrival on Mariendahl experimental farm, the chicks were then randomly allocated to cages and

cages randomly allocated to treatments. Using a random block design, four treatments were each allocated to six replications, with 10 chicks per replication. Chicks were kept in a temperature controlled house according to the management practices described by Cobb-vantress (2015) until the end of the study. Each wire cage (0.9 x 0.6m) held ten birds (in accordance to the SAPA code of conduct), and each cage was assigned a bell drinker and a tube feeder. Artificial lighting was provided at a pattern of 18hrs of light altering with 6 hours dark. Ventilation in the house was set to provide a maximum of six air changes per hour. The chicks had *ad libitum* access to a commercial starter diet (Table 24) and water from arrival until day four, thereafter they were given two days for adaption to their treatment diets. The trial then ran from day seven until day 12.

Table 24 Ingredient composition of the commercial starter diet and the different treatment diets (% of the diet)

	Commercial Starter Diet	Treatment 1 (FF)	Treatment 2 (DR)	Treatment 3 (EX)	Treatment 4 (Maize meal)
Maize	38.37	50.00	50.00	50.00	100.00
Soybean (Full fat)	24.44				
Full fat BSFL		50.00			
Dry rendered BSFL			50.00		
Extruded BSFL				50.00	
Soybean 46	23.73				
L-lysine HCl	0.48				
DL methionine	0.49				
L-threonine	0.03				
Premix*	0.15	0.15	0.15	0.15	0.15
Limestone	1.77				
Salt	0.08				
Monocalcium Phosphate	1.67				
Sodium Bicarbonate	0.65				
Sunflower Oil	5.35				

(*) – Vitamin and mineral premix included according to (National Research Council, 2004) requirements
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

The treatment diets were as follows: a 100% maize diet with vitamin and mineral premix; a 50% full-fat BSFL (FF) and 50% maize meal diet with vitamin and mineral premix; a 50% dry rendered BSFL (DR) and 50% maize meal diet with vitamin and mineral premix; and a 50% extruded BSFL (EX) diet and 50% maize meal with vitamin and mineral premix (see Table 24). Vitamin and mineral premixes were included according to the NRC (2004). All BSFL used in the trial were derived from the same batch from AgriProtein Technologies Pty Ltd. (Phillipi, Cape Town) having been exposed to the same conditions and substrates, thereafter split into three parts and processed according to the treatment descriptions given above (3.2). The treatment diets are shown in Table 24 below. The diets were mixed and administered to birds as mash diets. Thereafter, the birds were grown out to commercial slaughter size of about 1.9kg (32 days old) on commercial grower and finisher diets and sold to a commercial abattoir, who collected the birds directly from the experimental centre.

Table 25 The analysed nutrient composition of the treatment diets

	Units	Treatment diets			
		Treatment 1 (FF)	**Treatment 2 (DR)**	**Treatment 3 (EX)**	**Treatment 4 (Maize meal)**
Gross energy	MJ/kg	20.54	21.28	20.15	16.86
Dry matter	%	88.56	89.67	89.10	86.01
Crude protein	%	19.00	26.72	24.38	7.72
Ash	%	5.60	5.42	5.97	1.45
Crude fat (acid hydrolysis)	%	20.01	17.72	16.62	4.20
Crude fibre	%	4.89	5.31	5.96	1.77
Histidine*	% m/m	0.63	0.54	0.63	0.13
Serine	% m/m	1.21	1.29	1.31	0.18
Arginine	% m/m	1.33	1.29	1.29	0.21
Glycine	% m/m	1.42	1.50	1.44	0.17
Aspartic acid	% m/m	1.79	2.28	2.20	0.20
Glutamic acid	% m/m	2.81	3.61	3.25	0.63
Threonine*	% m/m	0.90	1.02	0.98	0.14
Alanine	% m/m	1.78	1.55	1.65	0.32
Proline	% m/m	1.73	1.55	1.65	0.32
Cysteine	% m/m	0.06	0.02	0.06	0.02
Lysine*	% m/m	1.14	0.94	1.22	0.07
Tyrosine	% m/m	1.47	1.19	1.50	0.19
Methionine*	% m/m	0.38	0.46	0.41	0.07
Valine*	% m/m	1.25	1.23	1.29	0.15
Isoleucine*	% m/m	0.92	0.71	0.82	0.14
Leucine*	% m/m	1.97	1.95	1.93	0.43
Phenylalanine*	% m/m	1.16	1.52	1.54	0.28
Phosphorus	%	0.46	0.51	1.00	0.29
Potassium	%	0.76	0.86	1.88	0.48
Calcium	%	1.32	1.17	3.52	0.05
Magnesium	%	0.19	0.17	0.40	0.11
Sodium	mg/kg	535.00	950.00	1482.00	84.00
Iron	mg/kg	370.00	665.00	1404.00	67.96
Copper	mg/kg	10.46	7.51	15.62	2.29
Zinc	mg/kg	151.38	171.40	343.60	121.50
Manganese	mg/kg	127.76	146.14	253.40	100.82
Boron	mg/kg	3.48	3.51	6.72	3.15
Aluminium	mg/kg	40.00	220.00	600.00	8.00

(*) – Essential amino acids
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

6.2.2 Data collection

After arrival, the birds were all given a commercial starter diet from day zero to day five on an *ad libitum* basis. The birds were introduced to their treatment diets and provided with the recommended daily feed intakes specified by Cobb-vantress (2015) from day five. Daily feed intakes and refusals were measured from day five to day seven in order to estimate the *ad lib* intake as closely as possible and achieve precision feeding. Feed intakes and refusals were weighed and recorded from day seven to

day 11. Faecal collection and weighing took place from day eight to day 12. Faecal collection trays were placed beneath the cages and were covered in new plastic sheets each day ensuring accurate collection. Each day during the trial, if the feed was completely depleted the amount of feed offered was increased on following day to ensure non-restricted intakes. The faeces was collected and weighed at the same time (16:00) each day. All five days refusal collections and faecal collections were pooled per cage. Representative samples of 500g were taken of the diets and each cages' refusals and these were stored in a cold dry room in airtight bags until further analyses were done. Faecal collections were frozen at -20°C daily immediately after weighing to minimise bacterial contamination. Representative samples of faecal collections were taken for each cage.

6.2.3 Analytical methodologies

Analytical methodologies on the dry matter (3.2.1), ash (3.2.2), crude protein (3.2.3), crude fat (3.2.4), mineral determination (3.2.8) and crude fibre (3.2.6) content were performed as described in chapter 3. The samples were then hydrolysed (3.2.7) before being sent for further amino acid analysis. Chemical analysis for collected faecal, refusal and feed samples for the study were all conducted at the department of Animal Sciences, Stellenbosch University, except for the determination of amino acid and the mineral determinations. Amino acid determination was done at the Central Analytical Facility, Stellenbosch University. The mineral determination was done at the Western Cape Department of Agriculture's Institute for Plant Production at Elsenburg.

6.2.3.1 Gross energy and apparent metabolisable energy

The gross energy (GE) was determined according to methods described in chapter 3 (3.2.5). This value was then used to determine the apparent metabolisable energy (AME) for each treatment diet, using the following Equation 11 described by Nalle *et al*. (2012) and De Marco *et al*. (2015):

Equation 11:

$$\text{AME}_{\text{diet}}\,(\text{MJ/kg}) = \frac{(\text{Feed Intake (kg)} \times \text{GE of Diet}) - (\text{Excreta output (kg)} \times \text{GE of excreta})}{\text{Feed Intake(kg)}}$$

Equation 12:

$$\text{AME}_{\text{test ingredient}}\,(\text{MJ/kg}) = \frac{\text{AME of diet} - (\text{AME of maize} \times 0.50)}{0.50}$$

6.2.3.2 Coefficient of total intestinal tract digestibility

The coefficients of total tract digestibility (CTTD), of each analysed nutrient were calculated by using the following basic equations described by (Ravindran *et al*., 2005) and (De Marco *et al*., 2015):

Equation 13:

$$\text{Nutrient offered (g/trial)} = \text{Nutrient}_{\text{Analysed in feed}} \times \text{DM}_{\text{Intake}}\ (\text{g/trial})$$

Equation 14:

$$\text{Nutrient excreted (g/trial)} = \text{Nutrient}_{\text{Analysed in faeces}} \times \text{DM}_{\text{Excreta}}\ (\text{g/trial})$$

Equation 15:

$$\text{Nutrient refused (g/trial)} = \text{Nutrient}_{\text{Analysed in refusal}} \times \text{DM}_{\text{Refusal}}\ (\text{g/trial})$$

Equation 16:

$$\text{Nutrient consumed (g/trial)} = \text{Nutrient}_{\text{consumed}} - \text{Nutrient}_{\text{Refused}}$$

Equation 17:

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

The 100% maize meal diet CTTD obtained was then used for the correction of the 50% BSFL/50% maize meal diets, according to Aksnes *et al*. (1996) and De Marco *et al*. (2015), using Equation 18:

Equation 18:

$$\text{Coefficient of total tract digestibility}_{\text{test ingredient}}\ (\text{g/kg}) = \frac{\text{CTTD}_{\text{diet}} - (\text{CTTD}_{\text{maize}} \times 0.5)}{0.5}$$

6.3 Statistical analysis

Statistical analyses were done by using STATISTICA (data analysis software system), Version 9, by StatSoft inc. (2010). As the age of the birds 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. An outlier replication for the statistical interpretation of the amino acid analysis was removed from the treatment 3 (EX). The means were calculated accordingly.

The following hypothesis was proposed:

H_1: There is no statistical difference amongst the CTTD of nutrient composition and AME of full-fat BSFL and various defatted BSFL meals in in young broiler chick diets

H_0: There is a statistical difference amongst the CTTD of nutrient composition and AME of full-fat BSFL and various defatted BSFL meals in young broiler chick diets

The model for the one-way ANOVA is indicated by, $Yij = \mu i + \alpha j + \varepsilon ij$ where the terms in the model are defined as; the treatment effect response (Yij), the overall mean (μi), treatment effect (αj) and the unexplained error (εij)

6.4 Results and discussion

Table 26 summarizes the coefficient of total tract digestibility (CTTD) and apparent metabolisable energy (AME) for the different treatments tested. There were differences found for all nutrients and minerals, as well as cysteine and lysine. The DR treatment was found to have a significantly low apparent metabolisable energy (AME) of 8.84MJ/kg (Table 26). The FF and EX treatments had AME findings of 16.58MJ/kg and 15.79MJ/kg, respectively. In the determination of AME, feed intake plays a significant role within the formulation (Equation 11: The feed intake of the DR treatment was 1096.53g per cage during the trial and significantly lower than the FF treatment (2018.23g) and the EX treatment (2135.76g), by almost half. Therefore, both the low voluntary intakes and low AME value of the diet means the DR treatment may not allow chickens to grow and develop efficiently. In Figure 6**,** we see the relationship between AME and feed intake described by Leeson & Summers (1997).

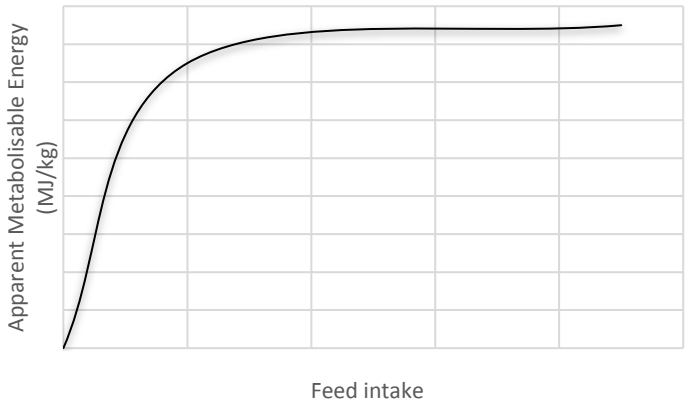

Figure 6 Relationship between feed intake and apparent metabolisable energy (AME) (modified from Leeson & Summers, 1997)

Leeson & Summers (1997) suggest that chickens vary their intake according to the energy content of the diet being consumed. However, this was not the case in for the current studies' DR treatment. Therefore, it is suggested that one or more additional factors played a role in the intake of the DR treatment birds. For instance, the DR treatments' low intakes could be due to a high unpalatability of the ingredient. Three cultivars of white lupins were investigated by Nalle *et al*. (2012) with regards to their AME. The cultivars all had crude protein levels of around 35% and crude fat values of around 13%. Both values are lower relative to the DR treatment, however their AME mean was between 8.05MJ/kg and 9.6MJ/kg, which was similar to the DR treatment. The low AME of these cultivars is reported to have been caused by the high level of anti-nutritional factors in the lupins. Donkoh &

Attoh-Kotoku (2009) reported processed cotton seed cake AME to be 7.8MJ/kg and processed soybean meal AME as 9.90MJ/kg, both coupled with high amino acid digestibilities. Treatment intakes were not reported for this study. Together AME and protein dietary contents are believed to have a large influence on the performance of birds (Zaman *et al*., 2008).

Table 26 Average (± standard errors) coefficient of total tract digestibility (CTTD) of full fat, dry rendered and extruded black soldier fly larvae and their apparent metabolizable energy (AME) for young broilers

	Treatment diets			
	Treatment 1 (FF)	**Treatment 2 (DR)**	**Treatment 3 (EX)**	**P-value**
AME (MJ/kg)	16.58^{a} ± 1.069	8.84^{b} ± 1.019	15.79^{a} ± 1.154	<0.01
AME intake (MJ)	33.53^{a} ± 3.607	9.77^{b} ± 2.167	33.73^{a} ± 2.627	<0.01
Dry matter	0.96^{a} ± 0.004	0.95^{b} ± 0.007	0.96^{a} ± 0.003	<0.01
Crude protein	0.95^{b} ± 0.002	0.96^{a} ± 0.003	0.95^{b} ± 0.004	<0.01
Ash	0.93^{a} ± 0.002	0.96^{b} ± 0.004	0.94^{c} ± 0.004	<0.01
Crude fat (acid hydrolysis)	0.95^{a} ± 0.004	0.98^{b} ± 0.004	0.96^{c} ± 0.006	<0.01
Crude fibre	0.93^{b} ± 0.009	0.95^{a} ± 0.006	0.93^{b} ± 0.005	<0.01
Histidine*	0.99 ± 0.007	0.97 ± 0.015	0.96 ± 0.053	0.32
Serine	0.99 ± 0.010	0.97 ± 0.009	0.96 ± 0.044	0.39
Arginine	0.99 ± 0.006	0.98 ± 0.007	0.97 ± 0.030	0.24
Glycine	0.98 ± 0.010	0.96 ± 0.017	0.93 ± 0.081	0.29
Aspartic acid	0.99 ± 0.010	0.97 ± 0.010	0.97 ± 0.038	0.27
Glutamic acid	0.99 ± 0.010	0.98 ± 0.007	0.97 ± 0.032	0.39
Threonine*	0.99 ± 0.010	0.97 ± 0.010	0.96 ± 0.049	0.32
Alanine	0.99 ± 0.009	0.98 ± 0.007	0.97 ± 0.041	0.35
Proline	0.99 ± 0.007	0.97 ± 0.009	0.96 ± 0.055	0.39
Cysteine	0.99^{a} ± 0.008	0.95^{b} ± 0.020	0.97^{a} ± 0.026	<0.01
Lysine*	0.99^{a} ± 0.010	0.97^{b} ± 0.012	0.98^{ab} ± 0.026	0.02
Tyrosine	0.99 ± 0.007	0.97 ± 0.016	0.96 ± 0.053	0.29
Methionine*	0.99 ± 0.007	0.97 ± 0.009	0.96 ± 0.048	0.34
Valine*	0.98 ± 0.011	0.97 ± 0.010	0.96 ± 0.054	0.31
Isoleucine*	0.98 ± 0.009	0.96 ± 0.015	0.95 ± 0.067	0.27
Leucine*	0.99 ± 0.009	0.97 ± 0.009	0.96 ± 0.047	0.29
Phenylalanine*	0.99 ± 0.007	0.97 ± 0.011	0.96 ± 0.048	0.38
Phosphorus	0.94^{a} ± 0.002	0.97^{b} ± 0.003	0.97^{c} ± 0.002	<0.01
Potassium	0.94^{a} ± 0.003	0.95^{b} ± 0.004	0.97^{c} ± 0.003	<0.01
Calcium	0.92^{a} ± 0.011	0.95^{b} ± 0.008	0.97^{c} ± 0.006	<0.01
Magnesium	0.92^{a} ± 0.004	0.95^{b} ± 0.006	0.96^{c} ± 0.003	<0.01
Sodium	0.95^{a} ± 0.005	0.96^{a} ± 0.003	0.97^{b} ± 0.004	<0.01
Iron	0.92^{a} ± 0.005	0.95^{b} ± 0.004	0.94^{c} ± 0.003	<0.01
Copper	0.94^{a} ± 0.002	0.94^{a} ± 0.006	0.96^{b} ± 0.003	<0.01
Zinc	0.91^{a} ± 0.005	0.95^{b} ± 0.004	0.96^{c} ± 0.006	<0.01
Manganese	0.92^{a} + 0.005	0.95^{b} + 0.004	0.95^{b} + 0.005	<0.01
Boron	0.93^{a} ± 0.011	0.94^{b} ± 0.004	0.96^{c} ± 0.005	<0.01
Aluminium	0.91^{a} ± 0.003	0.94^{b} ± 0.016	0.93^{b} ± 0.009	<0.01

(^{a,b}) – means with different subscripts in the same row differ significantly (P <0.05)
(*) – Essential amino acids
FF – Full fat black soldier fly larvae
DR – Dry rendered black soldier fly larvae
EX – Extruded black soldier fly larvae

Even though the DR treatment displayed dismal intakes and significantly low AME figures, it was in fact found to have the significantly highest CTTD of crude protein, crude fat, ash and crude fibre. However, very small actual percentage differences were seen between treatments for these parameters, and would therefore not necessarily have a biological impact. Considering the inferior growth performance recorded in Table 12, where the DR treatment was tested as a potential alternative protein source for production parameters, higher digestibility in this treatment for digestibility parameters clearly to not translate into superior production performance. Due to the over-processing acknowledged in the DR treatment in Chapter 3 (3.2), it is likely that the DR treatment was highly unpalatable, which played a role in the reduced intake.

The lack of bioavailable energy in the DR treatment is also assumed to be a consequence of the dry rendering process, as the full-fat (FF) treatment did not reveal a similarly low AME. The AME of the FF treatment (16.58MJ/kg) and of the EX treatment (15.79MJ/kg) were very much in line with those AME values reported by De Marco *et al*. (2015) at 17.38MJ/kg for full-fat BSF larvae and Uushona (2015) at 16.85MJ/kg for defatted BSF pre-pupae. The CTTD values for crude fat in all the treatments were the same as those reported by De Marco *et al*. (2015) and Uushona (2015). The crude protein, ash and dry matter CTTD values in the current study were high, relative to De Marco *et al*. (2015), however the current studies' findings were still in line with those found by Uushona (2015) as well as those found by Pretorius (2011), whilst evaluating a different maggot, the common housefly (*Musca domestica*). The substrate of the larvae used by De Marco *et al*. (2015) only consisted of cereal by-products, were as the current study larvae substrate was made up of assorted kitchen waste, which may have provided a more balanced and higher protein substrate to cereal by-products alone. The other noteworthy methodological difference between the current study and De Marco *et al*. (2015), was the sex and age of the broilers used. The current study used as-hatched seven day old chicks, whilst De Marco *et al*. (2015) used only roosters of 43 days old. Therefore, the factor of age may well be an influence on CTTD values of broilers for crude protein, as suggested by (Tarvid, 1995).

All nutrient, mineral and amino acid CTTD values for all three treatments (FF, DR and EX) in the current study were relatively high (all above 90%) and showcase an extremely high production potential for both full fat or defatted BSFL treatments. It has been suggested that using the total tract excreta collection method in a digestibility study, can overestimate the amino acid digestibility due to the varying microflora effects in the caeca (Huang *et al*., 2006). However, Huang *et al*. (2006) did not find any differences when comparing both the total tract digestibility and the apparent ileal nutrient digestibility methods. In order to better account for endogenous losses, it is believed that the true ileal nutrient digestibility method is most accurate (Huang *et al*., 2006), though the total tract digestibility method is still cheaper, faster and more samples are able to be collected (McNab &

Boorman, 2002), resulting in more accurate means. Additional analysis with additional samples can be financially straining.

With regards to amino acid CTTD, the DR treatment was also found to have the significantly lowest CTTD of cysteine (0.95) as well as, the second limiting amino acid in chickens, lysine at 0.97 (relative to the FF treatment). Uushona (2015) reported lower digestibility coefficients for cysteine (0.77 for full fat and 0.88 for defatted) than the current study, however the lysine values (0.94 for full fat and 0.98) for defatted were very similar. Lysine has one of the slowest absorption rates of all amino acids (Webb, 1990). Certain factors that can reduce the digestibility potential of feed by the animal, these include the use of heat and acid treatment, which may lead to protein denaturation during processing (Boland *et al*., 2013). Lysine is the most affected AA by extreme heat processing as it is susceptible to Maillard reactions reducing its availability for use by the animal (Parsons, 1996). The DR treatment was analysed by microscopic evaluation (3.4) and was found to have high levels of heat discolouration, which may well be the reason for the relatively lower digestibility of lysine for this treatment. Regardless of the statistical difference, the percentage difference between the DR treatment and the other treatments is small enough that it may not necessarily carry any biological impact.

Regarding mineral comparisons, the EX treatment had the significantly highest CTTD for potassium and copper, whilst the FF treatment had the significantly lowest CTTD for magnesium, iron, zinc, boron, manganese, aluminium as well as calcium and phosphorus. The DR treatments mineral contents were all approximately half of those provided by the EX treatment. Therefore, the dry rendering of BSFL could hinder the mineral content of the larvae.

All the minerals tested were found to significantly differ from each other but all the significant differences were based on very few percentage points difference between treatments. No literature regarding the digestibility of minerals from any BSFL included diets can be found, except for calcium and phosphorus. All treatments in the current study were found to have relatively higher CTTD values for calcium (between 0.92 and 0.97) and phosphorus (between 0.94 and 0.97) than those reported by Uushona (2015) at 0.80 to 0.90 for calcium and 0.63 and 0.93 for phosphorus, after evaluating BSF pre-pupae digestibility in 32 day old broilers. The National Research Council (2004) states that the calcium and phosphorus requirements for chicks between zero and three weeks is higher than the requirements for broilers of any older age. Therefore, the digestibility ability of young chicks may be naturally more able to digest higher levels of calcium and phosphorus in order to support rapid growth and development.

The current studies chicks' high requirement for these minerals may therefore be the reason a higher digestibility coefficient was achieved, instead of being a reflection on the treatments digestibility

potential. The current study would seem in line with the suggestion made by (Zelenka & Lisk, 1986) that chicks have a high digestibility capability that decreases with age, however still contradict results reported by authors who found general digestibility in broilers to increase with age (Wallis & Balnave, 1984; Doeschate *et al*., 1993).

6.5 Conclusion

The DR treatment diet provided sufficient crude protein and amino acids that were of a high quality with regards to digestibility. However, the treatment had very low intakes which suggested low palatability and the possibility of low bioavailable energy in the treatment. The relative parts these factors played is unknown. The mineral concentrations found for the DR treatment diet used were approximately half those of the EX treatment diet. The treatment was suspected of over-processing and microscopic evaluation found the treatment to have high heat discolouration. However, low levels of heat damage were evident. The FF and EX treatments both displayed excellent digestibility coefficients of all nutrients tested in young broiler chicks, as well as both having favourable AME value.

Other than AME and AME intake, significant differences were found for lysine, cysteine, dry matter, crude protein, crude fibre, crude fat, ash and all the minerals (all CTTD values were above 0.90), however these statistical differences may not carry much biological value. Maggots (both housefly and BSF) have previously been found to have very high amino acid CTTD values, in line with those found for the current study. The crude fibre, ash, phosphorus and calcium values of the current study were higher than those reported by other authors.

These differences could be associated with the differences in chosen methodology between previous authors and the current study. For example, using total tract *versus* ileal digestibility methods, various larvae substrate, sex of the chicks and age of the chicks. No other authors have previously tested the digestibility coefficients of BSFL in young chicks. Future research should be aimed at isolating the age factor in digestibility of insect protein sources in order to equate its influence with other factors standardized, resulting in more accurate comparisons.

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CHAPTER 7

General Conclusion

The study found the use of environmentally-sustainable protein sources in broiler diets to help the long-term combat of increasing consumer protein demands and supplying alternative protein sources to those directly consumed by humans. The use of BSFL bioconversion not only helps reduce organic waste, but turns such waste into a highly valued protein source for broiler chicken diets.

The study aimed to assess defatted black soldier fly larvae (BSFL) as an alternative protein source in broiler chicken diets. Two defatting techniques were assessed, namely a dry-rendering (DR) technique and an extrusion (EX) technique. Other treatments included in the study were a control, which utilised soybean meal as a protein source, and a full-fat (FF) BSFL treatment. The digestibility of all nutrients tested, including amino acids and minerals, for all three BSFL treatments (FF, DR and EX) tested were all reported at above 90%, which greatly supports the argument for the use of BSFL protein in general.

The only exception was the apparent metabolisable energy (AME) of the DR treatment which was found to be much lower than the other treatments. The defatting technique used for this treatment is suggested to have hampered the palatability of the ingredient and bioavailability of the fat. The product was tested for over-processing and was not found to have high heat damage however high levels of heat discolouration was found and this may have affected the palatability of the treatment. The vitamin composition of the BSFL should be investigated and the digestibility of these vitamins quantified. This could help to understand the results found for other parameters, such as bone mineralisation and strength, on a deeper level. The calcium bioavailability of the DR treatment was questioned during the organ and bone parameter evaluation. However, the calcium digestibility of the DR treatment was found to be 95% in the digestibility trial, bearing in mind the calcium concentration of the DR treatment was less than half of that found in the EX treatment. All the mineral concentrations of the DR treatment were very low in the digestibility study diets. The DR treatment was found to yield lower growth rates even though the treatment was found to have the highest intake at certain points of the trial. High intakes were not found when the DR treatment was provided at 50% dietary inclusion during the digestibility study, instead very low intakes were recorded in young broiler chicks for this treatment.

Both the FF and EX treatments were found to be a viable protein source at 15% dietary inclusion and offered superior growth rates, carcass yields, bone strength and exceptionally high digestibility of all nutrients tested. The defatting of the EX treatment allowed for a higher crude protein composition within the 15% inclusion providing chickens with more crude protein than the FF treatment. The FF

treatment outperformed the EX treatment with regards to growth rates and carcass characteristics, as well as resistance to bone breakage. The DR treatment yielded higher live weights to the control treatment, with comparable production parameters and carcass characteristics. None of the BSFL treatments were found to cause adverse organ or bone limitations.

Although the study found all three BSFL to compare very well to soybean meal, further research is required to continually improve defatting techniques and the consequences thereof. The digestibility of insects by young chicks is a highly unpublished topic and considering the positive results found in the current study, requires more attention. The possible benefits (for example, lipophilic growth stimulants and appetite stimulants) linked to the fat content of BSFL should be confirmed and quantified through further research efforts. The three BSFL treatments tested in the current study all compare well with or in fact exceed the production yields offered by the industry standard, soybean meal, at 15% inclusion levels. The use of BSFL as a protein source in the broiler industry may act as a beacon of fruitful change in the agricultural sector, where much transformation is required in order to sustain or expand production levels in the future.