The nutritional potential of black soldier fly (Hermetia illucens) larvae for layer hens.

by

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Declaration

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Date: March 2018

Summary

The nutritional potential of black soldier fly (*Hermetia illucens*) larvae for layer hens

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Insect protein is becoming an increased area of interest because of the potential positive effects that it may have in animal feeds. Insect protein is believed to have beneficial nutritional components desirable for livestock while reducing the amount of environmental pollution due to their ability to be reared on bio-waste streams. Soya meal and fishmeal are the most commonly used protein sources in livestock diets. However, due to competition with human consumption and bio-fuel utilisation of soya and decreasing fish stocks for the production of fishmeal (making both these raw materials unsustainable), alternative protein sources in the form of insects are being investigated.

The black soldier fly (BSF) (*Hermetia illucens*) is regarded as the insect with the highest potential for waste recycling. There is limited research of the use of black soldier fly larvae (BSFL) incorporated into layer hen diets.

In this investigation, BSFL were processed with three different techniques: a full fat, dry rendered and an extruded meal. All three treatments were incorporated into three different layer diets at 15% inclusion levels. The diets were fed to layer hens for a period of 41 days and compared to a control maize soya diet.

Positive results as pertaining to production and egg quality parameters were found. The full fat and extruded meal had the highest egg lay percentage (amount of eggs laid throughout the duration of the trial per treatment) and differed ($P \leq 0.05$) from the control diet. No differences between treatments were found with regard to categorical data which included blood and meat spots, albumin spread and yolk colour and yolk membrane. With regard to egg quality parameters, a difference ($P \leq 0.05$) was found between the albumin weights. All three insect meals differed from the control diet with heavier albumin weights.

The results obtained in this study are in favour of the use of black solider fly larvae processed using any of the three techniques in poultry feeds.

Opsomming

Die voedings potensiaal van venstervlieglarwemeel (*Hermetia illucens*) vir lêhenne

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Insekproteïen word ŉ al groter veld van belangstelling vir die moontlike positiewe effek wat dit op dierevoere mag inhou. Dit word geglo dat insek proteïen voordelige voedingkundige voedings komponente bevat terwyl dit die hoeveelheid omgewingsbesoedeling verminder aangesien dit op voedsel afval geproduseer kan word. Soja meel en vismeel is die mees algemene proteïenbronne wat in die dierebedryf gebruik word. Die gebruik van hierdie bronne is egter in kompetisie met die voorsiening van mens voedsel en bio-brandstof. Die invloed wat soja verbouing het op biodiversiteit en die uitputting van vis reserwes dui daarop dat beide hierdie bronne nie volhoubaar is nie. ‘n Alternatiewe proteïenbron moet dus gevind word.

Die venstervlieg (BSF) (*Hermetia illucens*) word beskou as die insek met die grootste potensiaal vir afval hersirkulasie. Daar is beperkte navorsing op die gebruik van venstervlieg larwe (BSFL) meel in lêhenvoeding.

In hierdie ondersoek is die BSFL geprosesseer op drie verskillende maniere: ŉ volvet, droog ontvet en geëkstrueerde produk. Al drie behandelings is ingesluit in lêhen diëte teen ŉ insluitingspeil van 15%. Die voere is aan lêhenne vir ŉ periode van 41 dae voorsien en resultate is met ŉ standaard mielie soja dieet vergelyk.

Produksie en kwaliteitsparameters het positiewe resultate gelewer. Die volvet en geëkstrueerde meel het die hoogste eiersgelê persentasie gelewer (aantal eiers gelê gedurende die totale proef tydperk) en het van die kontrole dieet verskil ($P \leq 0.05$). Geen verskille tussen behandelings is gevind vir die kategoriese data soos insidensie van bloedspikkels, vleis spikkels, albumien verspreiding, geel kleur of membraan integriteit nie. Verskille ($P \leq 0.05$) was wel gevind vir albumien massa met al drie die insekdiëte wat hoër albumien massas gelewer het as die kontrole dieet.

Die resultate van hierdie studie het aangetoon dat meel van die venstervlieg larwes suksesvol vir lêhenproduksie gebruik kan word met besliste voordele ongeag van prosesserings metode.

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Abbreviations

a*	Redness
AA	Amino Acids
ADG	Average daily gain
Al	Aluminum
Ala	Alanine
AME	Apparent metabolisable energy
AMEn	Apparent metabolisable energy nitrogen corrected
ANOVA	Analysis of variance
Arg	Arginine
Asp	Aspartic Acid
b*	Yellowness
BSF	Black soldier fly
BSFL	Black soldier fly larvae
BW	Body weight
C	Celsius
Ca	Calcium
CF	Crude fibre
CP	Crude protein
Cu	Copper
Cys	Cysteine
DM	Dry matter
DR	Dry rendered

EAA	Essential Amino Acids
EX	Extruded
FAO	Food & Agriculture Organization
FCR	Feed conversion ratio
Fe	Iron
FF	Full fat
g	Grams
Glu	Glutamic Acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
K	Potassium
kg	Kilograms
L	Litres
L*	Lightness
Leu	Leucine
Lys	Lysine
ME	Metabolisable energy
Met	Methionine
Mg	Magnesium
mg	Mili grams
Mg	Magnesium
MJ	Mega joules

ml	Milliliters
Mn	Manganese
N	Nitrogen
Na	Sodium
NEAA	Non-Essential Amino Acids
NFE	Non-Fibre extract
NRC	National Research Council
P	Phosphorous
Phe	Phenylalanine
Pro	Proline
S	Sulphur
Ser	Serine
Thr	Threonine
Tmt	Treatment
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
Zn	Zinc

Chapter 1

General Introduction

The 21st century has brought along fundamental challenges to society especially with regard to socio-economic factors which have resulted in immense challenges to humans' well-being due to lack of food security. Hunger has become a major issue for today's global population with more than 1 billion people being affected. The world population is expected to grow from 6.8 billion to an estimated 9 billion people by 2050 (FAO, 2009). This increase in population and the effect that it will have on hunger and poverty needs to be addressed. The ability of agriculture to support this increased population has been a concern for generations and continues to be high on the global policy agenda. Global food security will continue to be a concern for the next 50 years and beyond (Godfray *et al.*, 2010). Food requirements will need to be increased by between 70% and 100% to relieve the problem of continuing hunger and malnutrition and to feed the additional 2 billion people as anticipated by the Food and Agriculture Organization of the United Nations (FAO, 2011).

Understandably, the rise in population has resulted in an increase in demand for livestock products (meat, fish and eggs) for human consumption. This increase has caused the attainability of raw materials for animal feed to be evermore challenging due to a rise in competition for human consumption and price. It is estimated that 1000 million tons of feed is produced globally each year. Feed for the poultry industry makes up the largest tonnage, followed by feeds for pig and cattle (FAO, 2002). Although global production for soya is on the rise (Ravindran, 2013), this popular protein source for animal feed will not be sustained due to global competition for the use thereof in human diets (Ravindran & Blair, 1992; Ravindran, 2013). The increased production of soya acreage also causes deforestation of areas of high biological value (Osava, 1999).

Energy and protein are the main limiting nutrients in feeds (Khan *et al.*, 2016). At present, ingredients for animal and fish feed include fishmeal, fish oil, soya beans, pure brewer's yeast and other grains (FAO, 2012). However, nutritionally animal protein sources such as fishmeal are preferred for the use in poultry diets over plant based proteins such as soya (Ravindran, 2013). This is due to the fact that protein sources from animal origins have a better balance of the essential amino acids as well as a good mineral and vitamin content which is beneficial for livestock (Akhter *et al.*, 2008). Fishmeal prices are on the rise due to their high demand. A decreased supply of industrially caught fish caused by the El Niño climatic cycle (resulting in a variable catch) and over exploitation, has led to this commodity being less feasible for smaller farmers (FAO, 2012). Plant based proteins are therefore used far more extensively due to their higher availability and lower price. Soya oilcake meal is one of these plant based protein sources. Soya oilcake is in high demand in the poultry industry due to its favourable amino acid composition and its high level of digestibility (Willis, 2003).

Another concern which arises from the ongoing use of soya is the detrimental effect that the use of these products will ultimately have on the environment. Increased soya production has led to the increased pressure on land availability and deforestation of areas responsible for many key ecosystem services (Foley *et al.*, 2011). The land used for soya production by just three of the major producers (Brazil, USA and Argentina) is estimated at 90 million hectares. These countries exhibit the common effects of mono-cultured crops which include a reduction of biodiversity, soil fertility and water resources proving the lack of sustainability of this protein source (Stamer, 2015). Crop yields are also on the decrease due to declining investment in research and infrastructure as well as water shortages (Godfray *et al.*, 2010). Besides the negative effects that current protein sources have on our environment, the use of these protein sources has also become far less economical.

Meeting the energy and protein requirement for poultry contributes largely to the cost of production (Skinner *et al.*, 1992). These two values are significant as feed is responsible for 70% of the production costs (Al-Qazzaz *et al.*, 2016). This cost will increase unless sustainable and cheaper protein rich alternative sources are found.

Along with the increase in population and heightened demand for animal products, is the increased amount of agricultural waste being produce. This accumulation of waste may have harmful effects on humans and the environment if not managed correctly (En & Sabiiti, 2011). A protein source which can be produced on organic waste streams while producing a product which is nutritionally rich is required to combat the pressure of unsustainable, conventional protein sources currently used in animal feeds.

Insects offer a possible solution. In nature, insects form a major biomass, as can be seen with insect pests (Ramos Elorduy *et al.*, 1997). There are roughly one million recognized species of insects, although it has been estimated that the global diversity is as high as 80 million (Erwin, 2004). Insects form part of the natural diet for fish and poultry (FAO, 2012). Wild poultry tend to consume a large variety of locusts, grasshoppers, crickets, termites, scale insects, beetles, caterpillars, pupa, fleas, bees, wasps and ants (Ravindran & Blair, 1992). For this reason, it comes as no surprise that insects in animal feed has been considered as a viable protein source since the 1970s (Finke, 2002).

Insects have a high feed efficiency while being reared on a bio-waste stream, meaning they are able to grow and reproduce on waste material. Furthermore, insects have a high protein and fat content (Ravindran, 2013) as well as high concentrations of vitamins and minerals (Khusro *et al.*, 2012). This is one of the primary reasons why insects are seen as a viable alternative protein source for animal feeds, together with the fact that they have a short life cycle (Ramos-Elorduy *et al.*, 2002; Ravindran, 2013).

Insects have a similar market to fishmeal as they are used in the feed for aquaculture and livestock as well as the pet industry (FAO, 2012). Several studies have shown that it is technically feasible to mass rear insects and use them as an alternative, protein rich ingredient in a poultry ration (Pretorius, 2011; Veldkamp *et al.*, 2012; Hopley, 2015; Van Schoor, 2017). The crude protein values for fly larvae and pupae can range from 53% to 63% on a dry weight basis compared with 44% to 48% in soya bean meal. These values for insect larvae add to the attractiveness of insects as a viable alternative to conventional protein sources (Defoliart, 1989).

Most academic research and industry applications have been focused on the common house fly (*Musca domestica*), the black soldier fly pre-pupae (*Hermetia illucens*), the mealworm (*Tenebrio molitor*), locusts (*Locusta migratoria, Schistocerca gregaria, Oxya spec*., etc) and silkworms (*Bombyx mori*) (Stamer, 2015). Black soldier fly (BSF) larvae have already been successfully formulated into diets for poultry as they are an efficient food waste recycler and support good growth (Hale, 1973; Hopley, 2015; Van Schoor, 2017).

It was generally concluded that BSF larvae can be a suitable protein source for animal feed. To differentiate between the pre-pupae and larvae; once completion of its larval development, the insect enters the pre-pupal stage. In this stage, the larva stops feeding and empties its digestive tract. Then, the pre-pupae migrate in search of a dry and protected site in preparation for metamorphosis (Spranghers *et al.*, 2016). Fully grown larvae can produce a biomass with a crude protein value between 40%-45% with a favourable amino acid composition and a fat value of up to 35% on a dry matter basis. This is highly favourable to livestock (Newton *et al.*, 1977; Stamer *et al.*, 2014).

The worldwide production of commercial layer hen eggs has increased in recent decades and exceeded 64 million tons in 2009. China is currently the largest producer, contributing 36% of the worlds' production (Miranda *et al.*, 2015). This is no surprise as eggs are an affordable, rich source of nutrients which can provide 18 vitamins and minerals. The composition can be affected by several factors such as hen diet, age and environmental factors (Samman *et al.*, 2009; Fraeye *et al.*, 2012). On average the macronutrient content of eggs include low amounts of carbohydrates and about 12g per 100g of protein and lipids, most of which are monounsaturated (Herron & Fernandez, 2004; Kassis *et al.*, 2010) and supply the diet with several essential nutrients. Another important factor which may raise egg consumption is the typical characteristics of modern life such as frequent travelling, busy schedules and less time to cook; increasing consumption of pre-cooked processed foods (Miranda *et al.*, 2015). The world is in need of affordable protein sources such as eggs, especially in developing countries, in which a third of the population are undernourished.

The purpose of this study is to determine whether the black soldier fly larvae (*H. illucens)* could be used as a replacement to fishmeal and soya meal as a viable protein source to layer hens. The larvae will be fed in different forms, namely a full fat, dry rendered or as an extruded meal. Different processing was done in order to determine whether processing methods may have an effect on nutritional composition of larvae meal and whether this may affect production and egg quality. Eggs from the layer hens fed the larvae will be collected daily and analyzed externally and internally to determine whether black solider fly larvae meal could truly be an alternative protein source for layer hens with no decrease in overall egg quality nor decrease in production and health of the bird.

References

Akhter, M., Khan, M.Z.U., Anjum, M.I., Ahmed, S., Rizwan, M., Ijaz, M. & Lahore, A.S., 2008. Investigation on the Availability of Amino Acids From Different Animal Protein Sources in Golden Cockerels. J. Anim. Plant Sci. 18(2–3): 53–56.

Al-Qazzaz, M.F.A., Ismail, D., Akit, H. & Idris, L.H., 2016. Effect of using insect larvae meal as a complete protein source on quality and productivity characteristics of laying hens. Rev. Bras. Zootec. 45(9): 518–523.

DeFoliart, G. R., 1989. The human use of insects as food and as animal feed. Bulletin of the ESA. 35(1): 22–35.

En, S. & Sabiiti, E., 2011. Utilising agricultural waste to enhance food security and conserve the environment. 11(6):1–7.

Erwin, T.L., 2004. The Biodiversity Question: How Many Species of Terrestrial Arthropods Are There? In Forest Canopies. Elserivier: 259–269. http://linkinghub.elsevier.com/retrieve/pii/B9780124575530500198. (Accessed 14 February 2017.)

Food and Agriculture Organization (FAO)., 2002. Protein sources for the animal industry: 1–25.

Food and Agriculture Organization (FAO)., 2009. Reform of the Committee on World Food Security - Final Version: 1–14.

Food and Agriculture Organization (FAO)., 2011. World Livestock 2011. Livestock in food security: 1–130.

Food and Agriculture Organization (FAO)., 2012. Insects as animal feed. Edible Insects for the Future: 89–97.

Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology. 21(3): 269–285.

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D. & Zaks, D.P.M., 2011. Solutions for a cultivated planet. Nature. 478(7369): 337–342.

Fraeye, I., Bruneel, C., Lemahieu, C., Buyse, J., Muylaert, K. & Foubert, I., 2012. Dietary enrichment of eggs with omega-3 fatty acids: A review. Fd. Res. Int. 48(2): 961–969.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson,S., Thomas, S.M. & Toulmin, C., 2010. Food Security: The Challenge of Feeding 9 Billion People. Science. 5967(327): 812–818.

Hale, O., 1973. Dried *hermetia illucens* larvae (diptera: *Stratiomyidae*) as a feed additive for poultry. Ga Entomol Soc. J. http://agris.fao.org/agris-search/search.do?recordID=US201303264936. (Accessed 16 June 2017.)

Herron, K.L. & Fernandez, M.L., 2004. Are the current dietary guidelines regarding egg consumption appropriate? J. Nutr. 134(September 2003): 187–190.

Hopley, D., 2015. The evaluation of the potential *of Tenebrio molitor, Zophobas morio, Naophoeta cinerea, Blaptica dubia, Gromphardhina portentosa, Periplaneta americana, Blatta lateralis, Oxyhalao duesta* and *Hermetia illucens* for use in poultry feeds. MSc Diss. University of Stellenbosch, Stellenbosch.

Kassis, N., Drake, S.R., Beamer, S.K., Matak, K.E. & Jaczynski, J., 2010. Development of nutraceutical egg products with omega-3-rich oils. LWT - Food Sci. Technol. 43(5): 777–783.

Khan, S., Naz, S., Sultan, A., Alhidary, I.A., Abdelrahman, M.M., Khan, R.U., Khan, N.A., Khan, M.A. & Ahmad, S., 2016. Worm meal: a potential source of alternative protein in poultry feed. Worlds. Poult. Sci. J. 72(1): 93–102.

Khusro, M., Andrew, N. R. & Nicholas, A., 2012. Insects as poultry feed: A scoping study for poultry production systems in Australia. Worlds Poult. Sci. J. 68(3): 435–446.
Miranda, J.M., Anton, X., Redondo-Valbuena, C., Roca-Saavedra, P., Rodriguez, J.A.,

Lamas, A., Franco, C.M. & Cepeda, A., 2015. Egg and egg-derived foods: Effects on human health and use as functional foods. Nutrients. 7(1): 706–729.

Newton, G.L., Booram, C. V., Barker, R.W. & Hale, O.M., 1977. Dried *Hermetia Illucens* Larvae Meal as a Supplement for Pig. J. Anim. Sci. 44(3): 395–400.

Osava, M., 1999. Soy production spreads, threatens the amazon in Brazil. http://www.ipsnews.net/1999/09/environment-brazil-soy-production-spreads-threatens-amazon/. (Accessed 30 May 2017).

Pretorius, Q., 2011. The evaluation of larvae of *Musca domestica* (common house fly) as protein source for broiler production.MSc Diss. University of Stellenbosch, Stellenbosch.

Ramos Elorduy, J., Pino Moreno, J.M., Escamilla Prado, E., Alvarado Perez, M., Lagunez Otero, J. & Ladron de Guevara, O., 1997. Nutritional value of edible insects from the state of Oaxaca, Mexico. J. Food Compos. Anal. 10: 142–157.

Ramos-Elorduy, J., Gonzalez, E. A., Hernandez, A. R. & Pino, J. M., 2002. Use of *Tenebrio molitor* (coleoptera: *Tenebrionidae*) to recycle organic wastes and as feed for broiler chickens. J. Econ. Entomol. 95(1): 214–220.

Ravindran, V., 2013. Poultry Feed Availability and Nutrition in Developing Countries: Main Ingredients used in Poultry Feed Formulation. Poult. Dev. Rev. 2(10): 694–695.

Ravindran, V. & Blair, R., 1992. Feed resources for poultry production in Asia and the Pacific. II. Plant protein sources. Worlds. Poult. Sci. J. 48(3): 205–231.

Samman, S., Kung, F.P., Carter, L.M., Foster, M.J., Ahmad, Z.I., Phuyal, J.L. & Petocz, P., 2009. Fatty acid composition of certified organic, conventional and omega-3 eggs. Food Chem. 116(4): 911–914.

Skinner JT, Waldroup AL, Waldroup PW., 1992. Effects of Dietary Amino Acid Level and Duration of Finisher Period on Performance and Carcass Content of Broilers Forty-Nine Days of Age. J. Appl. Poult. Res. 71(7): 1207–1214.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S., 2016. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci. F Agric.(October): 1–19.

Stamer, A., 2015. Insect proteins — a new source for animal feed.EMBO reports. 16(6): 676–681.

Van Schoor, A. Van., 2017. The assessment of black soldier fly (*Hermetia illucens*) pre-pupae, grown on human faecal waste, as a protein source in broiler and layer diets. MSc Diss. University of Stellenbosch, Stellenbosch.

Veldkamp, T., 2012. Insects as a sustainable feed ingredient in pig and poultry diets: A feasibility study. Wageningen UR Livestock Research. 63(October):1–62.

Willis, S., 2003. The use of soybean meal and full fat soybean meal by the animal feed industry. 12th Australian soybean conference: 1–8.

Chapter 2

Literature review

The earth's remaining biodiversity is under threat due to the increase in the dedication of arable land and natural resources to food production. Over the next 50 years, a doubling in food demand is expected. This poses huge challenges for the sustainability of food production (Tilman *et al.*, 2002). A new tactic of food production such as sustainable intensification (large improvement in recycling available resources and waste) can be introduced to reduce the environmental impact and achieve higher yields with less waste per hectare of productive land (Stamer, 2015). Sustainability implies both high yields can be maintained, even during stressful environmental periods or major shocks (Tilman *et al.*, 2002) and the implementation of agricultural practices which have acceptable environmental impacts. Sustainable intensification could bring major improvements by further recycling available resources and waste. There is large potential for improvement in recycling. Around 90 Million tons of foods from private households, retailers and the food industry is discarded each year in the European Union alone (Stamer, 2015)**.** The livestock industry in the western world consumes 85% of global soya production. As a result, this decreases the amount of soya available for direct human consumption considerably.

Another ingredient which is also extensively used in the production of animal feeds is fishmeal, mainly for aquaculture. For the purpose of animal feed, 16 to 17 million tons of fish need to be caught as well as an additional five million tons of fish trimmings (Wijkström, 2009). Of this amount, 90% is used in the aquaculture sector for the production of fish for human consumption. Due to the ongoing decrease in fish stocks, it is a necessity to reduce the use of fish for fishmeal. This problem is partly alleviated by increasing soya in the diet of animals but, inevitably, a vicious cycle ensues as there is now competition for ingredients designated for the production of aquaculture feeds as well as human consumption. An alternative will, therefore, need to be found (Stamer, 2015).

During the past 40 years, global per capita meat production has increased by more than 60% (Tilman *et al.*, 2002). There has been a considerable increase in the demand for poultry products (Ravindran, 2013). This increase in demand for products causes increased pressure on the need for raw materials used for the production of poultry feeds, a commodity as mentioned earlier which is becoming more challenging to obtain (Premalatha *et al.*, 2011). The demand and challenge in obtaining these products have caused an increase in costs (Ravindran and Blair, 1992). Therefore a major problem which the poultry industry faces is supplying a sustainable feed ingredient which meets all the needs of poultry for production at a viable cost (Oyegoke *et al.*, 2006).

Along with the increase in population and demand for animal products, food security is at risk. Food security as defined by the Committee on World Food Security (FAO, 2009) as "exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life." The production of food has a considerable impact on the environment because it may lead to deforestation, soil erosion, desertification, reduction in plant biodiversity and water pollution (Steinfeld *et al.*, 2006). Adding to this environmental impact, livestock production is responsible for more than 14% of all greenhouse gas emissions (Gerber *et al.*, 2013). This impact needs to be reduced to ensure sustainable agriculture for future generations (Lang & Barling, 2013). Reducing the impact on the environment is crucial as meat demand globally is expected to rise by 76% from 2005/2007 to 2050 (Alexandratos & Bruinsma, 2003).

With time, a constant increase in the global population's mean income is expected due to urbanization. This global trend has caused an increase in the consumption of meat (Tilman & Clark, 2014). This increase means meat production will have to be maximized and improved so that it may be sustainable and safe for the environment. However, if dietary changes are not implemented, by the year 2050, global agricultural greenhouse gas emissions may increase by 80% (Tilman & Clark, 2014).

2.1 Alternative protein sources

Plant and animal based protein make up a large and important segment of an animal's diet. The usefulness of a protein depends on its ability to supply a sufficient amount of essential amino acids to the animal, particularly monogastric animals such as chickens, for production as well as the digestibility and toxicity of the protein (Scanes *et al.*, 2004). Proteins are made up of amino acids which are needed for the maintenance, reproduction and growth of the animal. The main protein ingredients used in poultry diets are fishmeal and soya. Soya beans are rich in oils (160-210g/kg) and contain all the essential amino acids needed by the animal for optimal performance; however cysteine and methionine are below the required concentrations. This is a potential problem as methionine is the first limiting amino acid, especially in diets rich in energy (Farkhoy *et al.*, 2012).

The decreased availability of essential amino acids for animals fed plant protein sources will have a detrimental effect on growth and production. This problem can however be alleviated by the addition of animal sourced proteins in small quantities to strengthen the amino acid concentration entering the animal from the feed (Beski *et al.*, 2015) such as blood meal and fish meal; or through the use of synthetic amino acids although these are expensive. These animal based protein sources are high in methionine, lysine, cysteine and tryptophan.

Fishmeal however has been included into diets in limited amounts due to reduced availability. Fishmeal prices vary due to availability, and due to the ongoing reduction in fish stocks, fishmeal prices are also on the rise. It is believed that the price of fishmeal has already doubled in the last five years due to the increased scarcity of the resource (Veldkamp *et al.*, 2012). This unfortunately affects the smaller scale farmers considerably as income does not always allow for expensive feed ingredients such as fishmeal (FAO, 2012).

As the ratio between the individual amino acids in protein concentrates varies significantly, supplementation with free synthetic amino acids would be successful. Especially when the variety of raw materials available makes it impossible to meet the requirements of the animal for all amino acids (Beski *et al.*, 2015).

A viable alternative to the above mentioned may be in the form of insects as a source of protein in animal feeds. This will contribute to the recycling of food waste but at the same time produce a feed ingredient which is high in protein and fat for livestock.

2.2 Entomophagy

Insects are the largest animal group on earth. Insects constitute more than 80% of the animal kingdom (Premalatha *et al.*, 2011). The class *insecta* harbours large amounts of biological and ecological diversity. It is well known that this class contains more species than all the other species of all other classes combined. 'Entomophagy' is the scientific term describing the consumption of insects by humans (Müller *et al.*, 2016). Insects have been eaten throughout the history of humanity and were one of many food sources that humans relied on before the development of technological ability and complex social structure. Although insects are now seen as being absent throughout western diets, insect consumption still remains popular on a global scale (Dossey *et al.*, 2016). It is estimated that edible insects form part of at least 2 billion peoples' diet and more than 1900 species of insect are currently used as food (FAO, 2013). The insects most consumed worldwide are beetles (Coleoptera, 31% of all insect species consumed), caterpillars (Lepidoptera, 18%), bees, wasps and ants (Hymenoptera, 14%). Moreover, grasshoppers, crickets, locusts (Orthoptera, 13%), cicadas, leafhoppers, planthoppers, scale insects and true bugs (Hemiptera, 10%) are also consumed. Termites (Isoptera), dragonflies (Odonata), flies (Diptera) and other insects each comprise less than 3% of insects consumed (FAO, 2013).

Since farming of insects for direct consumption by humans has only recently started, it is an area which needs more research. Human food waste, animal manure and human faecal matter are avenues which have been researched as feedstuffs for consumption by insects which are then used in animal feeds. This however is highly unlikely to be accepted as a way in which insects could be reared for human consumption (Jansson *et al.*, 2015). There has however been a shift in attitude about the consumption of insects in the developed world and an increased motivation to consume them (Ramos-Elorduy, 2009). One of the most compelling arguments about the consumption of insects is their high nutritional value that ensures a balanced diet for improved health and potential to aid the problems of food security (Durst *et al.*, 2010). This attitude is one for the future due to insects' high nutritive value and high resource efficiency so much so that rearing insects for consumption seems to fit with modern food production systems (Jansson *et al.*, 2015). Positive effects which the consumption of insects may have include the requirement for little space for farming of insects, they can be fed on by-products of various crops, have a high feed conversion efficiency, can quickly transform their feed to weight, many generations can be produced within a year (adding to the overall sustainability) and their high survival rate (Ramos-Elorduy, 2009).

2.3 Safety and disease from the use of- and rearing of insects

In the insects' habitats, they tend to become exposed to multiple pathogens, from different parasites that may regulate wild insect populations but may also have a wide impact on farmed species (Jansson *et al.*, 2015). The problematic effects of these diseases are not well known with regard to insect farming for food, however Weismann *et al.* (2012) found that the cricket *Acheta domesticus* is known to be affected by a densovirus and in the USA, the pet food industry was negatively affected by epizootic densovirus outbreaks. For this reason the FAO (2013) recommends maintaining a parent line if insect rearing on an industrial scale is practiced, regardless of the insect species, in case of any disease outbreak or bio-security risks.

Another potential danger posed to humans by unsafe insect rearing is zoonosis, an infection which can be passed between humans and animals (FAO, 2013). The farming and use of insects for humans' food and animal feed has not been going on long enough to truly understand the risk involved with these diseases and the transmission thereof (FAO, 2013). Since humans and insects are taxonomically distant compared to humans and conventional livestock, the chance of these zoonotic infections being transferrable may be low. However, consideration needs to be taken especially when livestock are the initial host of the pathogen, which then shifts to the preferred host: humans (FAO, 2013). This particular area of insect farming as a food or feed will need to be further investigated, in order to ensure safe and hygienic handling of insects, so that risk is kept to a minimum. However, a study by Van Schoor (2017) pertaining to the potential of rearing BSF pre-pupae on human faecal matter and then feeding to layer hens concluded that no food safety risks were found within the layer eggs and that the eggs were safe for human consumption. However, due to the high concentrations of faecal coli-forms ingested by the pre-pupae in the feed media, the pre-pupae were processed for safety by either using heating methods or chemical additives to remove faecal coli-forms. If left untreated, the pre-pupae could lead to major health problems for the animals.

2.4 The viability of insect proteins

Insects have a high feed efficiency and a low feed conversion ratio, with the ability to be reared on a bio-waste stream; they are able to grow and reproduce on waste material (Collavo *et al.*, 2005). Insects are able to feed on waste material and can then be transformed into a feed highly desirable for livestock. The insects which have been studied intensively and have been found to be the most promising for industrial feed production are black soldier flies (*Hermetia illucens*), common housefly larvae (*Musca domestica*), silkworms *(Bombyx mori)* and yellow mealworms (*Tenebrio molitor*) (FAO, 2012). Table 2.1 summarizes the proximate analysis of these species whilst Table 2.2 summarizes the amino acid (AA) composition of these species relative to fishmeal and Table 2.3 compares the AA profile with other monogastric species and Table 2.4 the mineral composition of the selected insect species.

Table 2.1: Proximate nutritional values of common insect species (DM basis).

Insect	**Crude Protein (%)**	**Crude Fat (%)**	**Crude Fibre (%)**	**NDF (%)**	**ADF (%)**	**Ash (%)**	**Reference**
Coleoptera							
Tenebrio molitor	50.16	31.09	5.77	NA	NA	3.70	(Hopley, 2015)
Tenebrio molitor	49.80	37.10	NA	NA	NA	3.5	(Kim *et al.*, 2016)
Lepidoptera							
Bombyx mori (L)	53.75	8.09	NA	6.36	6.36	6.36	(Finke, 2002)
Bombyx mori (L)	53.00	19.20	NA	NA	NA	4.80	(Kim *et al.*, 2016)
Collosamia promethea (L)	49.40	10.00	10.8	NA	NA	6.90	(Landry *et al.*, 1986)
Manduca sexta (L)	58.10	20.70	9.4	NA	NA	7.40	(Landry *et al.*, 1986)
Spodoptera frugiperda (L)	57.80	20.20	6.7	NA	NA	5.60	(Landry *et al.*, 1986)
Pseudaletia unipuncta (L)	54.40	14.90	5.0	NA	NA	6.90	(Landry *et al.*, 1986)
Samia ricinii (PP)	54.20	26.20	3.26	NA	NA	3.80	(Longvah *et al.*, 2011)
Samia racinii (P)	54.60	26.20	3.45	NA	NA	3.80	(Longvah *et al.*, 2011)
Diptera							
Musca domestica (L)	78.17	7.50	NA	14.29	11.51	6.75	(Finke, 2013)
Musca domestica (L)	60.38	14.08	NA	NA	NA	10.68	(Pretorius, 2011)
Hermetia illucens	45.10	36.08	NA	9.79	7.73	9.02	(Finke, 2013)
Drosophila melanogaster (A)	68.00	19.00	NA	17.66	10.14	7.20	(Oonincx & Dierenfeld, 2011)
Blattodea							
Blatta lateralis	61.50	32.40	NA	9.06	7.12	3.90	(Finke, 2013)
Blatta lateralis (S)	76.05	14.45	NA	11.41	10.87	7.88	(Oonincx & Dierenfeld, 2011)
Blatta lateralis (M)	62.85	26.50	NA	12.76	12.75	6.89	Oonincx & Dierenfeld, 2011)
Soya oilcake meal	49.44	0.45	7.87	-	-	7.64	(NRC, 1994)

*L- larvae, A-Adult, PP-pre-pupae, P-pupae, S-small, M-medium

Crude protein (CP) values ranged from 45.10% to 78.17%. *M. domestica* reported by Finke (2013) and *B. lateralis* (S) reported by Oonincx & Dierenfeld (2011) had the highest CP values of 78.17% and 76.05%, respectively while *H. illucens* reported by Finke (2013) and *C. promethean* (L) reported by Landry *et al.* (1986) had the lowest values of 45.10% and 49.4%, respectively.

Fat values ranged from 7.5% to 37.10%. *T. molitor* reported by Kim *et al.* (2016) had the highest value of 37.10%. Whilst *M. domestica* and *B. mori* (L) had the lowest fat values of 7.5% (Finke, 2013) and 8.09% (Finke, 2002), respectively. The crude fat value of *M. domestica* reported by Pretorius (2011) was 14.08%. This value was higher than the crude fat values of *C. promethean* (L) recorded by Landry *et al.* (1986). *M. domestica* (L) recorded by Pretorius (2011) had the highest ash value of 9.02%. The lowest ash value was 3.5% which was from the *T. molitor* species (Kim *et al.*, 2016).

Table 2.2 shows the amino acid composition of a variety of insect species. Amino acids are the building blocks of proteins and therefore need to be present in cells for the successful formation of polypeptides (Wu, 2009). Nutritionally, essential amino acids (EAA) such as cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine are not synthesized in the animal's body and will therefore need to be supplemented in other forms to maintain physiological functioning of the animal (Baker, 2009; Wu, 2014). In addition to EAA, there are also non-essential amino acids (NEAA). The ratio between EAA and NEAA are critical for optimal production of the animal. Non-essential amino acids include glutamine, glutamate, proline, glycine and arginine (Wu *et al.*, 2013). These amino acids can be transaminated from other amino acids by the animal to meet requirements for maintenance, growth, development and health. Therefore the supplementation of these amino acids is not required. No compelling data, however, is available which suggests that NEAA are sufficiently transaminated for the maximum growth and optimal health between animals (Hou *et al.*, 2015). Animal studies and cell cultures show that the NEAA glutamine, glutamate and arginine have important roles to play in multiple signaling pathways regulating gene expression, intracellular protein turnover, nutrient metabolism and oxidative response (Yao *et al.*, 2008; Brasse-Lagnel *et al.*, 2009; Bruhat *et al.*, 2009) as well as nutrient metabolism which favours lean tissue growth and reduces white adipose tissue (Bauchart-Thevret *et al.*, 2010; Dai *et* *al.*, 2012; Wu *et al.*, 2012; San Gabriel & Uneyama, 2013). Nearly all of these amino acids are not supplied adequately in typical plant protein (soya bean meal) based diets for growing pigs (Wu *et al.*, 2010).

It is clear that animals have a dietary requirement for NEAA as well as EAA to ensure optimal performance, health, reproduction and lactation (Phang *et al.*, 2013; Wang *et al.*, 2013; Wu *et al.*, 2013). One of the main reasons why insect proteins are so highly desirable is due to their amino acid composition (Józefiak & Engberg, 2015). The nutritional value of a feedstuff is however not determined by the amount of AA, instead by the digestibility of the AA. Digestibility of the AA is determined by the source of protein and the specific animal species being fed (Boland *et al*., 2013). The protein source should contain an appropriate AA profile (Table 2.3) and be highly soluble with little or no anti-nutritional factors. Poor bio-availability of proteins to animals may be caused by processing methods (Choct & Kocher, 2000); heat and acid used during processing may lead to protein denaturation during processing (Boland et al., 2013). Lysine is the AA most affected by extreme heat processing as it is susceptible to Maillard reactions reducing its availability for use by the animal (Parsons, 1996). Any source of protein to animals should therefore be handled with care.

Table 2.2: Amino acid composition (g/100g) dry matter of some insect species.

Insect	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Ala	References
Lepidoptera																			
Bombyx mori	6.8	2.5	5.7	8.3	6.5	4.6	5.1	5.4	0.9	5.6	11.0	1.4	15.0	4.6	4.0	4.7	5.4	5.5	(Rao, 1994)
Cossus redtenbachi	6.0	1.6	5.1	7.9	4.9	2.1	9.3	4.7	0.6	6.1	11.0	1.3	17.0	5.5	5.5	5.9	6.2	6.5	(Ramos Elorduy *et al.*, 1982)
Coleoptera																			
Tenebrio molitor	2.7	1.5	2.5	5.2	2.7	0.6	1.7	2.0	0.4	2.9	4.0	0.4	5.5	2.7	3.4	2.5	3.6	4.0	(Finke, 2002)
Scyphophorous acupunctatus	4.4	1.5	4.8	7.8	5.5	2.0	4.6	4.0	0.8	6.2	9.1	2.2	16.0	6.1	5.4	6.6	6.4	6.5	(Ramos Elorduy *et al.*, 1982)
Diptera																			
Hermetia illucens	3.2	1.5	2.0	3.1	3.1	0.9	2.0	1.8	0.8	3.3	4.3	0.3	5.1	2.3	2.6	1.8	3.1	3.1	(Finke, 2013)
Musca domestica	5.2	2.9	4.4	7.8	7.3	4.6	13.0	4.4	0.6	5.1	11.1	2.4	13.0	5.8	4.8	3.7	7.0	6.5	(Ramos Elorduy *et al.*, 1982)
Orthoptera																			
Boopedon flaviventris	4.3	2.4	4.7	8.8	5.5	1.8	4.1	4.4	0.6	5.7	8.8	2.0	15.0	7.5	6.8	4.3	7.4	5.9	(Ramos Elorduy *et al.*, 1997)
Callipogon barbatum	5.9	2.2	5.8	10	5.7	2.0	4.7	4.0	0.7	7.0	9.1	2.0	10.0	9.2	6.2	3.7	4.8	8.0	(Ramos-Elorduy *et al.*, 2006)

Table 2.3: Dietary crude protein requirement (% dry matter) and ideal amino acid pattern (g/g lysine) of essential amino acids for growth of different animal species

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

(adapted from Boland *et al*., 2013)

Table 2.4 summarizes the mineral content of a few selected insect species. Minerals are the inorganic parts of the feed (NRC, 1994) and are necessary to ensure animals reach their full genetic growth and production potential. Calcium concentrations ranged from 1 to 24 g/kg, magnesium values ranged from 2.8 to 4.5 g/kg, iron values ranged from 39.7 to 171.65 mg/kg and manganese values ranged from 6.79 to 159.28 mg/kg. *H. illucens* had the highest concentration of all the previously mentioned minerals. However, *T. molitor* had the highest concentration of phosphorous. Values of phosphorous ranged from 9.2 to 14.2 g/kg between the insect species. Copper and zinc values ranged from 10.39 to 17.77 mg/kg and 131.02 to 177.46 mg/kg respectively. These values were highest in the *B. mori* species.

Calcium, phosphorous and magnesium are minerals which play an important role in bone development and maintenance. The calcium values mentioned above can be compared to that of milk which has a calcium concentration of 900 to 1300mg/kg (Kim *et al.*, 2016). The concentration of calcium found in *H. illucens* is significantly higher than that found in milk which is approximately 2400mg/kg. Zinc is responsible for at least a 100 enzyme functions which catalyze activation, cell division and elicit positive immune responses (King *et al.*, 2000). Copper is a valuable component of many oxidizing enzymes which are responsible for oxidation-reduction reactions (Halfdanarson *et al.*, 2008; Kim *et al.*, 2016). Iron is a component of haemoglobin which is responsible for the efficient transportation of oxygen in the blood and myoglobin and is also a co-factor for multiple enzymes (Beard & Han, 2009; Kim *et al.*, 2016). Manganese is responsible for the activation of certain enzymes such as hydrolases, transferases, kinases, and decarboxylases. Manganese also activates enzymes responsible for fatty acid metabolism and protein synthesis, and is important for balanced neurological functioning in the body (Watts, 1990).

Table 2.4: Mineral compositions of certain insect species

Insect	**Ca(1)**	**Mg(1)**	**P(1)**	**Cu(2)**	**Fe(2)**	**Mn(2)**	**Zn(2)**	**References**
Coleoptera								
T. molitor	1.2	2.8	14.2	17.8	39.7	6.8	131.0	(Barker *et al.*, 1998)
Zophobas morio	1.2	1.8	8.3	13.9	50.3	1.5	87.5	(Barker *et al.*, 1998)
Lepidoptera								
Bombyx mori (L)	1.0	3.0	14	20.8	95.4	24.9	177.5	(Finke, 2002)
Galleria mellonella	0.6	0.9	12	3.1	77.3	3.3	77.8	(Barker *et al.*, 1998)
Chilecomadia moorei	0.3	0.7	5.7	7.4	35.2	1.8	89.7	(Finke, 2013)
Orthoptera								
Acheta domestica	2.1	0.8	7.8	8.5	112.3	29.7	186.4	(Barker *et al.*, 1998)
Diptera								
H. illucens	24	4.5	9.2	10.4	171.7	159.3	144.9	(Finke, 2013)
Blattodea								
Blatta Lateralis	1.2	0.8	5.7	25.7	47.9	8.5	105.8	(Finke, 2013)
Eublaberus distanti	0.8	0.8	4.6	12.0	55.0	5.0	124.0	(Oonincx & Dierenfeld, 2011)

(1) g/kg,

(2) mg/kg

Yellow Mealworms (*T. molitor*) are already raised on an industrial scale and can be grown on low-nutritive waste products originating from fruit and vegetable processing plants. They can then be dried and fed to broiler chickens. Ramos-Elorduy *et al.* (2002) reared yellow mealworms on organic waste streams which were then fed to broilers. Different concentrations (0%, 5%, 10% DM) of mealworm larvae were used in a 19% protein content sorghum–soya bean meal basal diet. The effects on feed intake, feed efficiency and weight gain were evaluated. After 15 days, there were no significant differences between the treatments which allowed them to conclude that mealworms are in fact a viable, alternative and sustainable protein source which can be used in a broiler's diet. Hopley (2015) reared mealworms on a diet of wheat bran with water being supplied in the form of carrageenan gel (98% moisture) and carrots. Mealworms were included at a concentration of 10% with soya bean at a concentration of 13.17%. It was concluded that mealworms were able to be used in layer hen diets with a large positive effect on shell weight.

Maggots of the common housefly (*M. domestica*) which are found predominately in tropical environments are an important source of animal proteins for poultry. These maggots can have a dry matter (DM) content of 30% and a crude protein value of up to 54% (FAO, 2012). Maggots can be offered fresh, but for intensive farming they are more convenient as a dry product in terms of storage and transport. Studies have shown that the maggots of *M. domestica* were able to compare favourably to that of soya meal (Zuidhof *et al.*, 2003). Pretorius (2011) evaluated the ability of *M. domestica* in its larval and pupal forms as an alternative protein source for broilers and also found positive results. Chemical composition of the larvae meal showed that the larvae meal had a 60.4% crude protein value, 14.1% crude fat and a 10.7% ash value while the pupae meal had a 76.2% crude protein,14.4% fat and a 7.73% ash value respectively (Pretorius, 2011).

In fact, Pretorius (2011) found that common housefly larvae meal had significantly positive effects on the live weights, feed intake, cumulative feed intake as well as average daily gain of broilers when compared to a standard commercial broiler diet. Also, no detrimental effects were found on a gastro intestinal level; even with an inclusion level of up to 50%. This was not the case with the 50% inclusion level of fishmeal which had detrimental effects. Common housefly larvae was shown in his study to be a good quality alternative and renewable protein source which could be utilized to replace the conventional protein sources which are currently being used in the poultry industry.

Another alternative is the *B. mori,* more commonly referred to as the silk worm or mulberry silk moth, which forms part of the family *Lepitdoptera*. This species of insect may be the most domesticated insect species in the world, therefore, it comes as no surprise that it has a high popularity as a human food source as well as a source of nutritional feed for animals (Defoliart, 1995)**.** The Nutrition Division of the Thai Ministry of Public Health (1987) include silkworm pupae among the local foods that can be used in the supplementary food formulae it developed for malnourished infants and pre-school children. A study in Sri-Lanka by Wijayasinghe & Rajaguru (1977) tested the ability of *B. mori* as an effective replacement to fishmeal fed to poultry. They tested the concentration of the silk worm at a 12% inclusion level and concluded that the silk worm at this level was able to replace fishmeal for broilers.

2.5 Insects suitable for nutrient recirculation

There are three specific orders in the insect world which have been noted to be the most efficient with regard to nutrient recirculation. These are namely *Diptera*, *Coleoptera* and *Haplotaxida* (Bondari & Sheppard, 1987). The order of *diptera* includes insects that are regularly recognized as true flies or two-winged flies such as the fruit flies, black soldier flies (BSF), house flies, midges and mosquitoes (Resh & Carde, 2003). Within the order *diptera,* BSF fall under the family *stratiomyidae*. The BSF adults are located in areas which are suitable for their larvae to flourish such as wetlands, moist places in soil and under bark, in decomposing organic matter and animal manure.

In terms of waste recycling the most promising of these are the BSF (*H. illucens*), Common housefly (*M. domestica*), and yellow mealworm (*T. molitor*) (Veldkamp *et al.*, 2012). These species have received the most attention by researchers as it is believed that together they can valorize organic waste, which amounts globally to 1.3 billion tons per year (Veldkamp *et al.*, 2012). The BSF is however the species this thesis will be focused on.

2.6 The Black Soldier Fly

The Black soldier fly (BSF) (*H. illucens*), is a common fly of the *stratiomyidae* family. It is native to south-eastern United States and is commonly found throughout the Western hemisphere and Australian region. It is a resilient insect and is able to live through harsh environmental conditions such as droughts, food shortage and even oxygen deficiency (Diener *et al.*, 2011). Adult BSF do not possess a stinger, mouthpart or digestive organs and therefore cannot bite or feed. Healthy adults tend to range from 15 to 20 mm in length (Sheppard *et al.*, 2002) and will live, breed and lay eggs in areas which are close to an environment which is suitable for the flourishing of their larvae. Adult BSF are not seen as pests as they are not attracted to human residences or food (Furman *et al.*, 1959). Adult BSF do not feed; instead they live off their fat reserves which are stored during their larval stage. Larvae of BSF are omnivorous; they also mainly feed on kitchen waste, decaying organic matter and manure.

Females deposit approximately 350 to 700 eggs in their short life of five to eight days (Fok, 2014). The larvae which hatch from these eggs have attracted the attention of researchers as they are able to digest varying types of waste and produce a feedstuff which is highly nutritious for livestock. This healthy biomass contains approximately 40% protein and 30% fat, all whilst feeding on waste and manure (Newton *et al.,* 2005). The BSF’s brief life cycle enables the production of these flies on a large scale and in a sustainable manner thereby allowing the certainty of a viable food source due to their frequent reproduction (Park, 2016).

2.6.1 Breeding and life cycle of the black soldier fly

The BSF has a life cycle between 40 to 44 days (Fok, 2014). It takes only a few days once the fly becomes an adult after emerging from the pupal case for the female to find a mate. Shortly after mating, the female fly will deposit her eggs in an environment suitable for the larvae to thrive. Areas which are suitable are usually close to decaying organic matter such as kitchen waste or manure. Eggs are a creamy white colour. The eggs will hatch into larvae in approximately four days (Sheppard, 1992).

Once the eggs have hatched, the larvae will find any waste which they may immediately start to consume. After two weeks the larvae will reach full maturity provided that they are in a favourable environment. However, if environmental conditions are not favourable, this process may take as long as several months (Sheppard *et al.*, 2002). The ability of the BSF larvae to lengthen its life cycle when in a less favourable environment is an important reason why it may be used for waste disposal processing (Sheppard, 1992). Black soldier fly larvae pass through four stages, namely the egg, larvae (five instars), pupae and adult stage (Hall & Gerhardt, 2002). At maturity they are about 25 mm in length, 6 mm in diameter and weigh approximately 0.2 g. These larvae and pupae are very tough and robust and can survive under conditions of extreme oxygen deprivation (Sheppard et al., 2002). The larvae are a pale white colour and have a small black head containing their mouthparts (Newton *et al.,* 2005).

2.6.2 Social, economic and environmental benefits of the black soldier fly

2.6.2.1 Biomass conversion

The black solider fly larvae (BSFL) are able to handle a large variety of waste material, such as animal manure, municipal organic waste, fresh human faeces and decaying vegetables, just to name a few. Different studies have produced differing values of waste reduction potential. Newton *et al.* (2005) found a reduction of 56% and Diener *et al.* (2011) found 65-75% reduction. These values were however calculated on different waste streams.

2.6.2.2 Odour Reduction

Odour reduction is another benefit derived from these insects. This is accomplished by their abundant densities on waste material combined with their avid appetite, causing the waste material to be processed at a fast rate, while the larvae are processing this waste; they aerate and dry the material suppressing bacterial growth. The combination of all these characteristics causes a reduction in odours (Diener *et al.*, 2011).

2.6.2.3 Pollution Reduction Potential

The principal food for many insects, especially for the BSFL, is manure (Newton *et al.*, 2005)**.** Not only do these insects possess the potential to produce a desirable feed from waste biomass, but the insect larvae reduce the nutrient concentration and the amount of manure residue, leading to a reduction in the amount of pollution, possibly by 50-60% or more (Newton *et al.*, 2005). Black soldier fly larvae can also be responsible for the reduction of harmful bacteria, unwanted odours and housefly populations. They are able to cause this reduction by making manure more liquid, causing it to be less favourable to the house fly larvae (Veldkamp *et al.*, 2012).

2.6.2.4 Housefly Control

The common housefly (*M. domestica*) tends to come into more contact with humans for a number of reasons. The common house fly feeds throughout its life due to its physiology of having functional feeding parts. This causes the fly to always be on the lookout for edible organic matter, such as human food, making interaction between the fly and human more common. The BSF's physiological traits of having no functional feeding parts cause it to have no attraction to homes, consequently reducing any pest like behaviour and living its life apart from humans (Barry, 2004). However, the BSF has a strong ability of reducing the number of house flies by preventing the house fly from ovipositing (the act of depositing eggs). The reduction of house flies will be a large benefit as they are prominent disease vectors, adding to the importance of their population control. The ability of colonization by BSF was reported by Sheppard *et al.* (1994) who discovered that BSF had the ability of colonizing poultry and pig manure causing a reduction in common housefly populations by 94-100%.

2.6.3 Potential of Black soldier flies for use as Sources of Nutrients

Black soldier flies, as discussed previously, have large nutritional benefits; including a rich source of lipids, proteins, polysaccharides and calcium. All these nutritional factors allow the BSF to be a potential feed source for animals (Popa & Green, 2012). Black soldier fly pre-pupae are composed of approximately 40% protein and 30% fat (Sheppard *et al.*, 1994). Their high protein concentration is comparable to that of fishmeal which is why they may be such an advantageous protein source in the animal feed industry.

2.6.4 Processing affecting nutritional quality of a feed ingredient

There is much literature suggesting that chemical composition of the insect meal can be manipulated by a number of factors. The processing method may cause a variation between samples of larvae meal (Fasakin *et al.*, 2003) (Table 2.5). Knowing that the processing method may have an effect on the final chemical composition can prove very advantageous; this will allow one to produce a product using the correct processing method which will be perfectly suitable for a certain type of animal in its respective stage of life (Driemeyer, 2016). As example, the variation in crude protein values between 43.30% and 46.70% on a dry matter basis due to processing methodology (Table 2.5) illustrates the potential of manipulating the nutritional value of the larvae.

Aniebo & Owen (2010) reported that the age at which the larvae are processed as well as the method of drying used causes variation between the chemical composition of the insect meal. Results revealed that protein content of the house fly larvae decreased as the age of the larvae increased (Table 2.6). At two days of age the crude protein content was measured at 55.4%; this concentration decreased to 50.2% at three days old. At four days old, the crude protein dropped to 47.1% (Aniebo & Owen, 2010). The opposite was found with regard to fat content. At two days of age, the fat content was measured at 20.8%. This increased to 22.2% after three days of age and a 25.3% fat value was observed after four days of age. The change in chemical composition was explained by Pearincott (1960) as that when the larvae approach the pre-pupae phase in metamorphosis, they start to store more energy in the form of lipids. The decrease in protein over time was caused by the larvae using the proteins for important enzymatic reactions such as the formation of the chitin layer. As the larvae move closer to the pre-pupae stage and eventually the pupation stage, chitin levels start to increase (Kramer & Koga, 1986). Insect proteins are highly digestible (between 77% and 98%) (Ramos Elorduy *et al.*, 1997), however insects which contain higher amounts of chitin have lower protein digestibility values. For this reason, larvae are preferred to be used as a feed ingredient. It was also observed by Aniebo & Owen (2010) that oven-dried maggots had mean higher protein content (50.9%) and less fat (22.8%) than sun dried maggots (47% and 26.4%). This proves that processing methods and drying techniques have a large influence on the nutritional outcome of an ingredient.

Table 2.5: Averages (± Standard error) of the moisture, crude protein, crude fat and ash of housefly larvae meal as influenced by processing methods (adapted from Fasakin *et al*., 2003).

Type of Larvae meal	**Moisture (%)**	**Crude Protein (%)**	**Crude fat (%)**	**Ash (%)**
Hydrolysed oven-dried	8.1 ± 0.05	45.6 ± 0.02	13.3 ± 0.03	13.2 ± 0.02
Hydrolysed sun-dried	8.4 ± 0.01	44.3 ± 0.03	13.7 ± 0.01	13.3 ± 0.01
Hydrolysed/defatted oven-dried	7.6 ± 0.02	46.7 ± 0.01	6.3 ± 0.01	13.3 ± 0.01
Hydrolysed/defatted sun-dried	8.1 ± 0.01	45.7 ± 0.01	6.3 ± 0.01	12.3 ± 0.02
Defatted oven-dried	9.2 ± 0.01	45.8 ± 0.03	7.0 ± 0.02	13.4 ± 0.02
Defatted sun-dried	9.7 ± 0.04	45.1 ± 0.05	7.4 ± 0.01	13.5 ± 0.02
Full fat oven-dried	8.3 ± 0.02	43.5 ± 0.03	14.3 ± 0.03	14.4 ± 0.02
Full fat sun-dried	8.6 ± 0.04	43.3 ± 0.01	14.4 ± 0.03	14.7 ± 0.01

Table 2.6: Average (± standard error) crude protein and fat content (DM basis) of house fly larvae as affected by age and method of drying (adapted from Aniebo & Owen, 2010).

	Day 2 harvested	**Day 3 harvested**	**Day 4 harvested**
Crude Protein			
Oven dried	$55.4^{a} \pm 0.05$	$50.2^{a} \pm 0.03$	$47.1^{b} \pm 0.04$
Sun-dried	$51.0^{a} \pm 0.14$	$47.7^{b} \pm 0.14$	$42.3^{c} \pm 0.74$
Fat			
Oven-dried	$20.8^{a} \pm 0.14$	$22.2^{b} \pm 0.14$	$25.3^{c} \pm 0.35$
Sun-dried	$23.4^{a} \pm 0.14$	$26.0^{a} \pm 0.14$	$29.7^{b} \pm 0.35$

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

2.6.5 Chemical compositions of the black soldier fly larvae

As depicted in Table 2.7, Newton *et al* (2005) found protein levels of 43.2% of BSF pre-pupae reared on pig manure while a value of 42.1% was found when reared on poultry manure (Newton *et al.*, 1977). St-Hilaire *et al.* (2007) reported a protein value of 43.6% when reared on pig manure. The amount of fat which the black soldier fly contains is dependent on the diet it is given; therefore the fat content is highly variable. It is believed that the fat content for soldier fly larvae was 28.0% on pig manure, 35% on cattle manure (Newton *et al.*, 1977) and 34.8% on poultry manure (Newton *et al.*, 2005). Results found by Oonincx *et al.* (2015) reported crude protein values ranging between 38% and 46%, and fat values between 21% and 35%. Crude protein and fat values of larvae in a trial conducted by Driemeyer (2016) were 35.9% and 48.1% respectively.

Ash content varied between different samples of BSF pre-pupae depending on their feed substrate (Table 2.7); Newton *et al.* (2005) found an ash content of 16.6% when the BSF pre-pupae were reared on pig manure. When reared on poultry manure a value of 14.6% was found (Newton *et al.*, 1977). Similarly, an ash content of 15.5% was found by St-Hilaire *et al.* (2007) when pre-pupae were fed pig manure. However, a low ash value of 7.8% was recorded by Driemeyer (2016) when BSF pre-pupae were reared on pig manure.

Table 2.7: Proximate values recorded for *H. illucens* (DM basis).

Crude Protein	Crude fat	Ash	Reference
43.2%	28.0%	16.6%	Newton., 2005
43.6%	33.1%	15.5%	St-Hilaire *et al.*, 2007
38.0-46.0%	21.0-35.0%	-	Oonincx *et al.*, 201
42.1%	34.8%	14.6%	Newton *et al.*, 1977
35.9%	48.1%	7.8%	Driemeyer, 2016

2.6.6 Chitin benefit

Apart from having a desirable (soluble) protein content, insect species also contain high amounts of chitin, which is the main constituent in the insect exoskeleton. Chitin is a non-toxic, biodegradable linear polymer. Recent studies confirmed that chitin has effects on innate and adaptive immune-response, including the ability to recruit and activate innate immune cells and induce cytokine and chemokine production via a variety of cell surface receptors including macrophage mannose receptor, toll-like receptor 2 (TLR-2), and Dectin-1 (Lee *et al.*, 2008). Chitin has the ability to improve the immune status of livestock and therefore may play a role in decreasing the amount of antibiotics used in the feed industry. This can prove highly beneficial as high density animal production operations can increase livestock disease incidences (Tilman *et al.*, 2002). However an increase in chitin causes a decrease in digestibility of the insect protein as reported by Belluco *et al.* (2013). Other uses of chitin from insects is a new research area which could be investigated. Chitin can be processed into chitosan, where many applications are possible: it can be used in the cosmetic, pharmaceutical, textile, paper or waste water industries. These uses for insect chitin is worth considering as a high value side product (Veldkamp *et al.*, 2012).

2.6.7 Nutritional benefits to other livestock species

2.6.7.1 Fish

Farmed fish are in high demand due to their favourable protein quality. Fishmeal is also a major supplier of protein in diets for farmed fish. Although there are few studies regarding insect protein in fish diets, the interest is increasing. Experiments have been conducted using BSFL as a feed for a number of different fish species. These fish species include the Channel catfish *(Ictalurus punctatus*), Yellow catfish (*Pelteobagrus fulvidraco*), Blue tilapia (*Oreochromis aureus*), Rainbow trout (*Oncorhynchus mykiss*) and the Atlantic salmon (*Salmo salar*). In all the above species it was conclusive that BSFL are a viable substitute to fishmeal in the diet, either partially or fully. However, in some cases, not all results were positive. Results for the channel catfish showed that chopped BSFL grown on hen manure resulted in similar performance (body weight and total length) to that of the control diet. However, in tank grown fish, feeding 100% larvae meal did not provide a high enough DM or CP value for good growth (Bondari & Sheppard, 1987). The chopping of the BSFL was not recommended as it decreased feed efficiency and increased feed waste. Chopping was only done as young catfish refused the whole larvae. Studies by Stamer *et al.* (2014) concluded that rainbow trout fed BSFL at an inclusion level of up to 50% showed no negative effects on feed conversion ratio, body weight gain and protein retention ratio. Studies by St-Hilaire *et al.* (2007) also concluded that BSF was a viable alternative protein source for rainbow trout; it was found that a replacement of fishmeal up to 25% had no effects on the feed conversion ratio.

2.6.7.2 Pigs

Experiments by Newton *et al.* (1977) using BSFL meal were conducted on growing pigs, with the larvae meal used as an alternative to soya oilcake meal. It was concluded that BSFL meal can be used as a suitable ingredient in grower diets. The main reason for this is the desirable amino acid composition, lipid and Ca contents. However, supplementation of certain amino acids, such as methionine, cysteine and threonine, will need to be added in order to obtain a balanced diet. The ash content of the diet was also high. During this study it was also found that there was no difference between the palatability of the insect meal and the palatability of the soya meal diet (Newton *et al.*, 1977). A study by Driemeyer (2016) on pigs being fed a creep diet containing BSFL as an alternative protein source at an inclusion level of 3.5% found that the production parameters, which included live weights, average daily gains and feed intakes, were not affected negatively by the inclusion of larvae meal in the creep feeds. Blood haematology results further showed that the inclusion had no negative or positive effects on the blood parameters of the animal.

2.6.7.3 Poultry

Insects in their adult, pupal and larval forms are part of the diet of wild chickens (Zuidhof *et al*., 2003). The feeding of insects to fowls is therefore not an original idea. Black soldier fly meal as a protein source has not been extensively researched amongst layer hens; however a study by Al-Qazzaz *et al.* (2016) found numerous positive results. Al-Qazzaz *et al.* (2016) concluded that BSF larvae were a viable protein source for layer hens. Cullere *et al.* (2016) reported that BSF at an inclusion level of up to 15% as a replacement for soya bean meal and soya bean oil had no problematic effects on digestibility, productive performance as well as carcass and meat quality of quail broilers. Hopley (2015) tested the ability of layer hens to be reared on BSFL and BSF pre-pupae meal and whether this had any effect on the production parameters and egg quality and concluded that the production parameters were favourable and that the chickens on larvae meal had a lower FCR. Also, the latter found that the egg quality parameters were either comparable or superior to that of the control treatment. Van Schoor (2017) tested the effect that BSF pre-pupae meal reared on human waste would have on layer production parameters and egg quality. Results were also positive; egg quality was not affected by the inclusion of the pre-pupae meal and at the inclusion of 10%, production parameters were also not affected. The rate of degradation (shelf-life) of the eggs was also not affected by the inclusion of the pre-pupae meal. Therefore, Van Schoor (2017) concluded that BSF pre-pupae meal may be used as an alternative protein source in layer hen diets with no significant effects on the egg quality, shelf life and production parameters.

2.7 Intensive insect production

If producers would like to incorporate insects into animal feed, the production of insects would need to meet the requirements needed for this inclusion. Some systems have been developed for the mass production of insects. However, these systems have only been developed for a limited number of species due to the lack of demand (Sánchez-Muros *et al.*, 2014). Although most of the edible insects used today for human consumption are collected from the wild, insect farming has been going on for at least 7000 years. Insect farming was mostly done for the production of sericulture (silk), the production of shellac and eventually apiculture (honey). Medicinal products were also produced through the farming of insects. Research has also gone into using insects as biological weapons for the control of unwanted pests via the sterile insect technique (SIT) (Singh, 1982; Singh & Moore, 1985).

With an on-going increase in the development of modern mass-rearing and harvesting techniques in combination with post-harvest processing technologies, the production of insects (protein) has reduced production costs while increasing insect mass (Rumpold & Schlüter, 2013). This increases the attractiveness of insect farming as a whole.

For an insect to be a viable alternative for commercial mass rearing, many factors have to be taken into consideration. Housing standards will need to be considered such as ventilation, humidity and temperature. It is also important to be aware of potential insect diseases, the possibility of environmental impact by rearing invasive species as well as bio-security risks. In terms of space needed for mass rearing, insects are able to add simplicity to the production system as insects can be reared in 3-Dimentional systems allowing one to fully utilize a building. Species of insect will also need to be considered, knowledge of life cycle and growth rate of the insect can be used to determine which insect fits the profile best, for example it is possible to produce over 180 kg of live weight of Black soldier fly in 42 days from 1m^2 compared to only 30 kg of adult crickets in the same dimensions (Józefiak & Engberg, 2015).

Temperature plays a role in a number of important factors regarding insect farming. The optimal temperature for many insect species is between 27°C to 30°C. Sub-optimal temperatures can cause a decrease in feed intake and therefore growth rates.

Insects are most sensitive to humidity fluctuations; for example mealworms will die under high humidity conditions (>70%) (Józefiak & Engberg, 2015).

Consumer acceptance for the use of insects via direct consumption by humans as food or incorporated into animal feeds has to be taken into account. Insects chosen for rearing are selected on a number of desired traits such as size, social behaviour, safety, reproductive potential measured as egg production rate and high hatchability. Nutritional remuneration, storage potential and marketability should also be considered (Schabel, 2010). Production parameters will also need to be considered as these parameters include duration of larval stage and synchronization of pupation. Other factors to consider include tolerance to disease, feed costs and more importantly, quality of the protein obtained (Peters & Barbosa, 1977; Scriber & Slansky, 1981; Sharaby *et al*., 2010).

2.8 Commercial table egg quality

The ability for one to produce an egg of a high quality and standard from farm to table is essential for the success of a farm or layer unit. Many factors are involved in the production of a high quality egg. A high quality egg which is externally and internally suitable for the consumer is important worldwide (Roberts, 2004). Factors which may affect the egg may come in the form of management, disease, nutrition and genetics, just to name a few.

2.8.1 Factors affecting commercial table egg external quality

The egg shell is of importance whether it is for the packaging of a nutritious food product or whether it is for the overall protection of the embryo from contamination or mechanical damage. The egg shell is necessary to ensure that the egg reaches the consumer free of any potential health risks such as bacteria and viruses. Any possible abnormalities found on the egg shell compromising the shell’s protective ability causes a reduction in egg value whether it be for hatching or for a food product. Farmers and egg producers place great emphasis on this point as any reduction in shell value may have huge economic effects. Once the shell has formed around the egg, the nutrient investment from the hen’s perspective has been made. A death of an embryo or the loss of nutritive value will decrease any possible profits (Hunton, 2005). It is well known that macro minerals (Ca and P) act as eggshell structural components and play essential roles in eggshell function (Mabe *et al.*, 2003). The trace elements Zn, Mn, and Cu also influence the organic matrix of eggshells and therefore can influence the rigidity of the eggshell (Stefanello *et al.*, 2014). Other factors which have an influence on egg quality include bird strain (Curtis *et al.*, 1985), bird age (Nys, 1986; Roland, 1988), management (Etuk *et al.*, 2004), mycotoxins (Resanoviã *et al.*, 2009), heat stress and diseases (Roberts, 2004).

2.8.2 Hen nutrition affecting egg weight and egg internal and external qualities

Certain nutrients and therefore the dietary feed formulation, is known to have an effect on egg quality. Poor quality eggs can be due to either an excess or insufficient amount of nutrients in the feed. Nutritional approaches aiming to minimize the incidence of egg defects have been developed. Another area of interest is the use of specific dietary nutrients such as canola to successfully enrich the egg for human nutrition. With production of eggs with multiple beneficial properties, nutritional strategies have achieved success in enhancing a healthy lifestyle and to increase the psychological well-being of the consumers (Wang *et al.*, 2017).

Increasing levels of protein (which insect meals are high in) (Keshavarz & Nakajima, 1995, Leeson, 1989), methionine (Keshavarz, 1995) and lysine (Zimmerman, 1997) have resulted in improvements in production parameters, especially in improving egg weights. Methionine is the first limiting amino acid for egg weight (Al-Saffar & Rose, 2002) and an increase in methionine significantly increases the egg weight (Harm & Russell, 1993).

The diet of the hen plays an important role in terms of providing adequate nutrition for the production of high quality eggs. Calcium and P are important minerals with regard to the rigidity and quality of the hens' eggs. This is understandable as eggs contain up to 3 g of Ca (Roberts, 2004). The ratio between P and Ca also needs to be managed effectively. If P is undersupplied in the diet, the hen will start withdrawing P from her skeletal structure. This is a process known as demineralization which will cause a weakening of the hen’s skeleton which will in turn have problematic effects on overall production of the hen. On the other hand, an oversupply of P may lead to the restriction of absorption of Ca from the gut leading to decreased shell quality (Boorman & Gunaratne, 2001). The balance between the Ca and P requirements can be affected by a number of factors. However, Bar *et al.* (2002) and Sohail & Roland (2002) found that age plays an important role in determining this nutritional balance. Dietary supplementation of trace elements, such as Zn, Cu and Mn, were also found to improve eggshell quality, although the independent effect of Mn, Zn, or Cu was not clarified (Mabe *et al.*, 2003). *H. illucens* has a high Ca content (24 g/kg) when compared to many other insect species (Table 2.3); this will be very beneficial to ensuring a strong egg shell.

Two studies have investigated the mechanism of dietary Mn and Zn addition on eggshell quality. These showed that dietary Mn supplementation could improve eggshell quality by enhancing the glycosaminoglycans and uronic acid synthesis in the eggshell glands, which can affect the ultrastructure of eggshells, promote the process of eggshell calcification, and also improve the layer's physiological status (Xiao *et al*., 2014). Zinc has an important role to play in the formation of healthy eggs. Zinc deficiency affects the quality of the epithelium due to the role of Zn in protein synthesis. Zinc also indirectly affects epithelial secretions, by affecting the structure of the epithelium or directly during the synthesis of egg shell membranes. Zinc plays a role in the magnum during the deposition of albumen and in the isthmus where egg shell membranes are produced (Tabatabaie *et al.*, 2007). In another study, Zn was found to promote calcium deposition in the eggshell gland by the elevation of carbonic anhydrase and osteopontin mRNA expression and increasing carbonic anhydrase activity, which contributed to the improvement of eggshell quality (Zhang, 2013).

Vitamins such as vitamin D and C also play important roles in the production of a high quality egg. Vitamin D_3 has been found to play an essential role in the absorption and metabolism of Ca and P (Liem, 2009).

Water has also been found to have effects on the quality of eggs. Water containing high amounts of electrolytes (2000 mg NaCl/L) tend to have a negative long term effect on the egg shell quality (Balnave & Yoselewitz, 1987). Sodium chloride (NaCl) can have a negative effect on the metabolism of laying hens; effects cause birds to be unable to deposit a shell on the egg during its passage through the oviduct. Moreover, an excessive chloride intake may limit Ca transportation to the shell gland and reduces the bicarbonate concentrations in the shell gland lumen (Pourreza *et al.*, 1994; Chen & Balnave, 2001).

Phytase in the diet as found by Hatten *et al.* (2001) and Lim *et al.* (2003) had positive effects on the egg shell quality. Phytase is normally added to poultry diets in order to catalyze the release of P from phytate (Pandey *et al.*, 2001). Phytase can be derived from a number of sources including plants, animals and micro-organism. Recent research has shown that microbial sources are more promising for the production of phytase on a commercial level (Pandey *et al*., 2001). Exogenous phytase is added to diets not only to enhance phytate P utilization, but also to reduce potential environmental pollution by phytate P and also reduce dietary costs (Waldroup, 1999). A too high or low level of available P in a laying hens diet may adversely affect the bird´s performance and reduce the eggshell quality (Harms, 1982). Feeding during times of heat stress may also have an effect on shell quality. Panting during high temperatures results in respiratory alkalosis due to a loss of CO_2 from the blood and involves an increase in blood pH. The level of ionized calcium in the blood therefore decreases reducing the amount of calcium available for egg shell formation. As a management tool farmers often add 1 g of limestone grit “on top” of their ration, as this helps alleviate the negative effect panting has on Ca in the body to ensure good egg shell quality.

The internal components of an egg consist of the yolk and the albumin. There are many ways in which to measure the internal components of the egg; however the internal components should be free of any meat spots, blood spots or any abnormal pigmentation. Yolk is also judged to determine whether it is of good quality. A good yolk needs to be the correct colour (generally yellow-orange) and a score of 8 or 9 is most desirable amongst consumers and needs to have a strong perivitelline membrane. If the perivitelline membrane is weak, this indicates an old egg and the yolk will break more easily (Kirunda & McKee, 2000). The quality of the albumin or white of the egg is generally measured by the height of the albumin at a distance of 1 cm from the yolk (Roberts, 2004). Many factors have been found to have an effect on the internal qualities of the egg and include storage time (Samli *et al.*, 2005), hen strain

(Curtis *et al.*, 1985) bird age Roland (1988), management (Etuk *et al.*, 2004), mycotoxins (Resanoviã *et al.*, 2009), heat stress and diseases (Roberts, 2004).

Nutritional manipulation of the diet of the layer has also been shown to have effects on the yolk and albumin of the layer hen's eggs (Galea, 2011). Studies by House *et al.* (2002) and Leeson & Caston (2003) found that an increase in the concentration of feed of a variety of vitamins such as riboflavin, folic acids, niacin, thiamine, pyridoxine, panthotenic acid, biotin, vitamin B12 all caused an increase in the amount of these vitamins found in the albumin. These vitamins are all beneficial to human health which increases the quality of the egg. According to Finke (2013), BSFL contain the carotenoids beta-carotene (<0.20 mg/kg), lutein (0.59 mg/kg) and zeaxanthin (1.28 mg/kg). These carotenoids are generally responsible for the yellow pigmentation in feedstuffs thereby contributing to the yellow pigment of the yolk.

The supplementation of hens' diets with fatty acids has also received increased interest due to the beneficial effects of certain fatty acids to human health (Baucells *et al.*, 2000; González-Muñoz *et al.*, 2009; Oliveira *et al.*, 2010). Many of these studies have highlighted the effect of poly unsaturated fatty acids (PUFA) of the n-3 series, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) having health benefits to humans. The n-3 fatty acids are responsible for normal growth and development and cause a decrease in the amount of lipid serum, anti-thrombotic, anti-arrythmic, and anti-inflammatory effects (Hu *et al.*, 1999; Li *et al.*, 1999). Documentation by Pandalai *et al.* (1996) concluded that the dietary intake of n-3 PUFA had an inhibitory effect on the development of prostate and breast cancer.

The vitelline membrane, as mentioned, is the membrane responsible for maintaining the functional shape of the egg yolk. This membrane is responsible for keeping the yolk whole and prevents the contamination of the egg yolk with the egg white, causing economical losses. A strong vitelline membrane allows one to separate the yolk from the white easily. Similarly to the yolk composition, the fatty acid composition of the vitelline membrane is determined by the fatty acid composition of the diet (Watkins *et al.*, 2003). These fatty acids then also determine the permeability and elasticity of the membrane. Aydin *et al.* (2001) found that the permeability of the membrane was affected by the concentration of saturated fatty acids in the diet.

Although nutrition plays an important role in the strength of this membrane, the age of the bird as well as storage time and temperature as mentioned earlier, also play a role. The older the hen is, the weaker the vitelline membrane. Eggs that are stored for a longer period also have weaker vitelline membranes as do eggs stored at higher temperatures (Galea, 2011).

2.9 Conclusion

This chapter provided an overview of the literature on how insects could be a viable source of proteins for human and animal consumption. After reviewing the potential use of insects in animal feed, the chapter focused on the black soldier fly larvae. By evaluating the above information, it is noticeable how the BSFL may have the potential to be used as an alternative protein source to the conventional soya meal and very expensive fishmeal. The main attraction of using the BSF as an animal feed is its high nutritive value due to its desirable amino acid composition and fat content. The larvae are able to produce these components while being reared on a variety of waste streams, such as manure or kitchen waste, thereby reducing environmental pollution.

Also discussed above is the fact that the processing method of the larvae influences its chemical composition, and thus its nutritional value. However, knowledge on the effects of processing on the nutritional values is still scarce. This thesis will thus be evaluating the effect of the BSFL processed using three different methods, namely a full fat, or a dry rendered or as an extruded meal. The effects on the growth and health of layer hens as well as the effect these insect species may have on the quality of the layer hens' eggs will be assessed. The overall aim of this trial is to determine whether BSF meal could be used as an alternative protein source for layer hens.

2.10 References

Al-Qazzaz, M.F.A., Ismail, D., Akit, H. & Idris, L.H., 2016. Effect of using insect larvae meal as a complete protein source on quality and productivity characteristics of laying hens. Rev. Bras. Zootec. 45(9): 518–523.

Alexandratos, N. & Bruinsma, J., 2003. World agriculture: towards 2015/2030: An FAO perspective. Land use policy. 20(4): 1–375.

Al-Saffar, R.S.P., 2002. The response of laying hens to dietary amino acids. Wrld's. Poult. Sci. 58: 209–234.

Aniebo, A.O. and Owen, O.J., 2010. Effects of age and method of drying on the proximate composition of housefly larvae (*Musca domestica Linnaeus*) meal (HFLM). Pakistan J. Nutr. 9(5): 485–487.

Aydin, R., Pariza, M.W. & Cook, M.E., 2001. Olive oil prevents the adverse effects of dietary conjugated linoleic acid on chick hatchability and egg quality. J. Nutr. 131(3): 800–806.

Baker, D.H., 2009. Advances in protein–amino acid nutrition of poultry. Amino Acids. 37(1): 29–41.

Balnave, D. & Yoselewitz, I., 1987. The relation between sodium chloride concentration in drinking water and egg-shell damage. Br. J. Nutr. 58(3): 503–509.

Bar, A., Razaphkovsky, V. & Vax, E., 2002. Re-evaluation of calcium and phosphorus requirements in aged laying hens. Br. Poult. Sci. 43(2): 261–269.

Barker, D., Fitzpatrick, M.P. & Dierenfeld, E.S., 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17(2): 123–134.

Barry, T., 2004. Evaluation of the economic, social, and biological feasibility of bioconverting food wastes with the black soldier fly (*Hermetia illucens*). Thesis (D.Sc.). University of North Texas, Texas, USA.

Baucells, M.D., Crespo, N., Barroeta, A.C., López-Ferrer, S. & Grashorn, M.A., 2000.

Incorporation of different polyunsaturated fatty acids into eggs. Poult. Sci. 79(1): 51–59.

Bauchart-Thevret, C., Cui, L., Wu, G. & Burrin, D.G., 2010. Arginine-induced stimulation of protein synthesis and survival in IPEC-J2 cells is mediated by mTOR but not nitric oxide. AJP Endocrinol. Metab. 299(6): 899–909.

Beard, J. & Han, O. 2009. Systemic iron status. Biochim. Biophys. Acta - Gen. Subj. 1790(7): 584–588.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C.C., Paoletti, M.G. & Ricci, A., 2013. Edible insects in a food safety and nutritional perspective: a critical review. Comprehensive Reviews in Food Science and Food Safety. 12: 296–313.

Beski, S.S.M., Swick, R.A. & Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. Anim. Nutr. 1(2): 47–53.

Bondari, K. & Sheppard, D.C., 1987. Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). Aquacult. Res. 18(3): 209–220.

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

Boorman, K.N. & Gunaratne, S.P., 2001. Dietary phosphorus supply, egg-shell deposition and plasma inorganic phosphorus in laying hens. Br. Poult. Sci. 42(1): 81–91.

Brasse-Lagnel, C., Lavoinne, A. & Husson, A., 2009. Control of mammalian gene expression by amino acids, especially glutamine. FEBS J. 276(7): 1826–1844.

Bruhat, A., Chérasse, Y., Chaveroux, C., Maurin, A.C., Jousse, C. & Fafournoux, P., 2009. Amino acids as regulators of gene expression in mammals: Molecular mechanisms. BioFactors. 35(3): 249–257.

Chen, J. & Balnave, D., 2001. The influence of drinking water containing sodium chloride on performance and eggshell quality of a modern, colored layer strain. Poult. Sci. 80(1): 91–94.

Choct, M. & Kocher, A., 2000. Non-starch carbohydrates: Digestion and its secondary effects in monogastrics. Proceedings-Nutrition Society of Australia. 24: 31–38.

Collavo, A., Glew, R.H., Huang, Y.S., Chuang, L.T., Bosse, R. & Paoletti, M.G., 2005. House cricket small-scale farming: 519–544.

Cullere, M., Tasoniero, G., Giaccone, V., Miotti-Scapin, R., Claeys, E., De Smet, S. & Dalle Zotte, A., 2016. Black soldier fly as dietary protein source for broiler quails: apparent digestibility, excreta microbial load, feed choice, performance, carcass and meat traits. Animal: an international journal of animal bioscience. 2010(August): 1–8.

Curtis, P.A., Gardner, F.A. & Mellor, D.B., 1985. A Comparison of Selected Quality and Compositional Characteristics of Brown and White Shell Eggs: I. Shell Quality. Poult. Sci. 64(2): 297–301.

Dai, Z.L., Li, X.L., Xi, P.B., Zhang, J., Wu, G. & Zhu, W.Y., 2012. Regulatory role for l-arginine in the utilization of amino acids by pig small-intestinal bacteria. Amino Acids. 43(1): 233–244.

DeFoliart, G. R., 1995. Edible insects as mini livestock. Biod. & Conserv. 4: 306–321.

Diener, S., Zurbrügg, C., Gutiérrez, F.R., Nguyen, D.H., Morel, A., Koottatep, T. & Tockner, K., 2011. Black Soldier Fly Larvae for Organic Waste Treatment – Prospects and Constraints. Proc. Of the Wastesafe. Conf. on solid waste management in developing countries; Khulna, Bangladesh:1–8.

Dossey, A.T., Morales-Ramos, J.A. & Rojas, M.G., 2016. Insects as sustainable food ingredients: production, processing and food applications. 3(1):61–84.

Driemeyer, H., 2016. Evaluation of black soldier fly (*Hermetia illucens*) larvae as an alternative protein source in pig creep diets in relation to production, blood and manure microbiology parameters .MSc Diss. University of Stellenbosch, Stellenbosch.

Durst, P.B., Johnson, D. V, Leslie, R.N. & Shono, K., 2010. Forest insects as food: humans bite back. RAP Publication 2010/02: 1–231.

Etuk, E.B., Okoli, I.C. & Uko, M.U., 2004. Prevalence and Management Issues Associated with Poultry Coccidiosis in Abak Agricultural Zone of Akwa Ibom State, Nigeria. Int. J. Poult. Sci. 3(2): 135–139.

Farkhoy, M., Modirsanei, M., Ghavidel, O., Sadegh, M. & Jafarnejad, S., 2012. Evaluation of Protein Concentration and Limiting Amino Acids Including Lysine and Met + Cys in Prestarter Diet on Performance of Broilers. Vet. Med. Int. 2012: 1–7.

Fasakin, E.A., Balogun, A.M. & Ajayi, O.O., 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish (*Clarias gariepinus*) fingerlings. Aquac. Res. 34(9): 733–738.

Finke, M.D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21(3): 269-285.

Finke, M.D., 2013. Complete Nutrient Content of Four Species of Feeder Insects. Zoo Biol. 32(1): 27–36.

Fok, G., 2014. Black soldier fly larvae composting. Guillermo Fok. Utubersidad.com. (Accessed 22 September 2017.)

Food and Agriculture Organization (FAO)., 2009. Reform of the Committee on World Food Security - Final Version: 1–14.

Food and Agriculture Organization (FAO)., 2012. Insects as animal feed. Edible Insects: Future Prospects for food and Feed Security: 89–97.

Food and Agriculture Organization (FAO)., 2013. Edible insects: Future prospects for food and feed security: 1–201.

Furman, D.P., Young, R.D. & Catts, P.E., 1959. *Hermetia illucens* (Linnaeus) as a Factor in the Natural Control of *Musca domestica* Linnaeus. J. Econ. Entomol. 52(5):

917–921.

Galea, F., 2011. Nutrition and food management and their influence on egg quality. http://www.wpsaaeca.com/aeca_imgs_docs/4nutrition_and_food_management_and_their_influence_on_egg_qualit.pdf. (Accessed 8 March 2017.)

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, G., 2013. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome.

González-Muñoz, M.J., Bastida, S., Jiménez, O., Lorenzo de, C., Vergara, G., Sánchez-Muniz, F.J. & Sánchez-Muniz, F.J., 2009. The effect of dietary fat on the fatty acid composition and cholesterol content of Hy-line and Warren hen eggs. Grasas y Aceites. 60(4): 350–359.

Halfdanarson, T.R., Kumar, N., Li, C.Y., Phyliky, R.L. & Hogan, W.J., 2008. Hematological manifestations of copper deficiency: a retrospective review. Eur. J. Haematol. 80(6): 523–531.

Hall D. C. & Gerhardt, R. R., 2002. Medical and Veterinary Entomology. Academic Press. San Diego, California. Flies (*Diptera*): 127–161.

Harm RH & Russell GB., 1993. Optimizing egg mass with amino acid supplementation of a low protein diet. Poult. Sci. 72: 1892–1896.

Harms, R.H., 1982. Influence of nutrition on eggshell quality. 2. Phosphorus. Poult. Advis. http://agris.fao.org/agris-search/search.do?recordID=US201302575469. (Accessed 9 May 2017.)

Hatten, L.F., Ingram, D.R. & Pittman, S.T., 2001. Effect of Phytase on Production Parameters and Nutrient Availability in Broilers and Laying Hens: A Review. J. Appl. Poult. Res. 10(3): 274–278.

Hopley, D., 2015. The evaluation of the potential *of Tenebrio molitor, Zophobas morio, Naophoeta cinerea, Blaptica dubia, Gromphardhina portentosa, Periplaneta* *americana, Blatta lateralis, Oxyhalao duesta* and *Hermetia illucens* for use in poultry feeds. MSc Diss. University of Stellenbosch, Stellenbosch.

Hou, Y., Yin, Y. & Wu, G., 2015. Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. Exp. Biol. Med. 240(8): 997–1007.

House, J.D., Braun, K., Ballance, D.M., O’Connor, C.P. & Guenter, W., 2002. The enrichment of eggs with folic acid through supplementation of the laying hen diet. Poult. Sci. 81(9): 1332–1337.

Hu, F.B., Stampfer, M.J., Manson, J.E., Rimm, E.B., Wolk, A., Colditz, G.A. & Hennekens, C.H., 1999. Dietary intake of a-linolenic acid and risk of fatal ischemic heart disease among women. Am. J. Clin. Nutr. 69: 890–897.

Hunton, P., 2005. Research on eggshell structure and quality: an historical overview. Rev. Bras. Ciência Avícola. 7(2): 67–71.

Jansson, A., Berggren, Å., Editors, S., Rydhmer, L. & Johnsson, P., 2015. Insects as Food – Something for the Future? A report from Future Agriculture. Uppsala, Swedish University of Agricultural Sciences (SLU).

Józefiak, D. & Engberg, R.M., 2015. Insects as poultry feed. Proc. 20th Eur. Symp. Poult. Nutr.

Kassis, N.M., Beamer, S.K., Matak, K.E., Tou, J.C. & Jaczynski, J., 2010. Nutritional composition of novel nutraceutical egg products developed with omega-3-rich oils. LWT – Fd. Sci. Technol. 43(8): 1204–1212.

Keshavarz K., 1995. Further investigations on the effect of dietary manipulations of nutrients on early egg weight. Poult. Sci. 74: 62–74.

Keshavarz K. & Nakajima S., 1995 The effect of dietary manipulations of energy, protein, and fat during the growing and laying periods on early egg weight and egg components. Poult. Sci. 74: 50–61.

Kim, S.K., Weaver, C.M. & Choi, M.K., 2016. Proximate composition and mineral content of five edible insects consumed in Korea. J. Fd: 15(1): 143–146.

King, J.C., Shames, D.M. & Woodhouse, L.R., 2000. Zinc and Health : Current Status and Future Directions Zinc Homeostasis in Humans 1. J. Nutr. 130: 1360–1366.

Kirunda, D.F.K. & McKee, S.R., 2000. Relating Quality Characteristics of Aged Eggs and Fresh Eggs to Vitelline Membrane Strength as Determined by a Texture Analyzer. Poult. Sci. 79(8): 1189–1193.

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

Landry, S. V, Defoliart, G.R. & Sunde, M.L., 1986. Larval Protein Quality of Six Species of Lepidoptera (*Saturniidaeo, Sphingidae, Noctuidae*). J. Econ. Entomol. 79(198): 600–604.

Lang, T. & Barling, D., 2013. Nutrition and sustainability: an emerging food policy discourse. Future food and health. 72: 1–12.

Lee, C.G., Ph, D., Silva, C.A. Da, Lee, J. & Elias, J.A., 2008. Chitin Regulation of Immune Responses: An Old Molecule With New Roles. Current Opinion in Immunology, 20(6): 684–689.

Leeson, S. 1989. Energy intake and layer performance. In proc. Califonia Nutr. Conf., California Grain and Feed Association, Fresno, CA: 72–79.

Leeson, S. & Caston, L.J., 2003. Vitamin Enrichment of Eggs. J. Appl. Poult. Res. 12(1): 24–26.

Li, D., Sinclair, A., Wilson, A., Nakkote, S., Kelly, F., Abedin, L., Mann, N. & Turner, A., 1999. Effect of dietary a-linolenic acid on thrombotic risk factors in vegetarian men. American J. of Clin. Nutr. 69: 872–882.

Liem, A., 2009. Dietary factors influencing calcium and phosphorous utilization by broiler chicks. PhD Diss. University of Georgia., Athens.

Lim, H.S., Namkung, H. & Paik, I.K., 2003. Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. Poult. Sci. 82(1): 92–99.

Longvah, T., Mangthya, K. & Ramulu, P., 2011. Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricinii*) prepupae and pupae. Fd. Chem. 128(2): 400–403.

Mabe, I., Rapp, C., Bain, M.M. & Nys, Y., 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult. Sci. 82(12): 1903–1913.

Müller, A., Evans, J., Payne, C.L.R. & Roberts, R., 2016. Entomophagy and Power. J. Insects as Food Feed. 2(2): 121–136.

National Research Council (NRC)., 1994. Nutrient Requirements of Poultry (NRP).

Newton, G.L., Booram, C. V., Barker, R.W. & Hale, O.M., 1977. Dried *Hermetia Illucens* Larvae Meal as a Supplement for Pig. J. Anim. Sci. 44(3): 395–400.

Newton, L., Sheppard, C., Watson, D., Burtle, G. & Dove, R., 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of pig manure. University of Georgia, Tifton, G.A. (available at www.cals.ncsu.edu/ waste_mgt/smithfield_projects/phase2report05/cd,web%20files/A2.pdf)

Nys, Y., 1986. Relationships between age, shell quality and individual rate and duration of shell formation in domestic hens. Br. Poult. Sci. 27(2): 253–259.

Oliveira, D.D., Baião, N.C., Cançado, S. V, Grimaldi, R., Souza, M.R., Lara, L.J.C. & Lana, A.M.Q., 2010. Processing,products and food safety. Effects of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks. Poult. Sci. 89(11): 2484–2490.

Oonincx, D.G.A.B. & Dierenfeld, E.S., 2011. An investigation into the chemical composition of alternative invertebrate prey. Zoo Biol. 29: 1–15.

Oonincx, D.G.A.B., Van Broekhoven, S. Van Huis, A. & Van Loon, J.J.A., 2015. Feed Conversion, Survival and Development, and Composition of Four Insect Species on Diets Composed of Food By-Products. PLoS ONE. 10(12): 1–20.

Oyegoke, O.O., Akintola, a. J. & Fasoranti, J.O., 2006. Dietary potentials of the edible larvae of *Cirina forda* (westwood) as a poultry feed. African J. Biotechnol. 5(October): 1799–1802.

Pandalai, P.K., Pilat, M.J., Yamazaki, K., Naik, H. & Pienta, K.J., 1996. The effects of omega-3 and omega-6 fatty acids on in vitro prostate cancer growth. Anti-cancer Res. 16(2): 815–820.

Pandey, A., G. Szakacs, C.R. Soccol, J.A. Rodriguez-Leon, & V.T. Soccol., 2001. Production, purification and properties of microbial phytases. Bioresource Technol. 77(3): 203–214.

Park, H.H., 2016. ScholarWorks@UMass Amherst. University of Massachusetts-Amherst. Black Soldier Fly Larvae Manual: 1–13.

Parsons, C.M., 1996. Digestible amino acids for poultry and pig. Anim. Feed Sci. Technol. 59(1): 147–153.

Pearincott, J.V., 1960. Changes in the lipid content during growth and metamorphosis of the house fly. Musca domestica Linnaeus. J. Cell. Comp. Physiol. 55: 167–174.

Peters, T.M. & Barbosa, P., 1977. Influence of population density on size, fecundity, and developmental rate of insects in culture. Annu. Rev. Entomol. 22(1): 431–450.

Phang, J.M., Liu, W. & Hancock, C., 2013. Bridging epigenetics and metabolism: role of non-essential amino acids. Epigenetics. 8(3): 231–236.

Popa, R. & Green, T.R., 2012. Using black soldier fly larvae for processing organic leachates. J. Econ. Entomol. 105(2): 374–378.

Pourreza, J., Nili, N. & Edriss, M.A., 1994. Relationship of plasma calcium and phosphorus to the shell quality of laying hens receiving saline drinking water. Br. Poult. Sci. 35(5): 755–762.

Premalatha, M., Abbasi, T., Abbasi, T. & Abbasi, S.A., 2011. Energy-efficient food production to reduce global warming and ecodegradation: The use of edible insects. Renew. Sustain. Energy Rev. 15(9): 4357–4360.

Pretorius, Q., 2011. The evaluation of larvae of *Musca domestica*(common house fly) as protein source for broiler production.MSc Diss. University of Stellenbosch, Stellenbosch.

Ramos-Elorduy, J., 2009. Anthropo-entomophagy: Cultures, evolution and sustainability. Entomol. Res. 39(5): 271–288.

Ramos-Elorduy, J., González, E.A., Hernández, A.R. & Pino, J.M., 2002. Use of *Tenebrio molitor* (*Coleoptera*: Tenebrionidae) to recycle organic wastes and as feed for broiler chickens. J. Econ. Entomol. 95(1): 214–220.

Ramos-Elorduy, J., Neto, E. M. C., dos Santos, J. F., Moreno, J. M. & Torres, I. L., 2006. Estudio comparativo del valor nutritivo de varios coleoptera comestibles de méxico y pachymerus nucleorum de brasil. Interciencia: Revista De Ciencia y Tecnología De América. 31(7): 512–516.

Ramos-Elorduy, J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L. & de Guevara, O. L., 1997. Nutritional value of edible insects from the state of Oaxaca, Mexico. J. fd. Comp. Analy. 10(2): 142–157.

Ramos Elorduy de Conconi, J., Borgues Rodríguez, H. & Pino Moreno, J.M., 1982. Valor nutritivo y calidad de la proteína de algunos insectos comestibles de México. Folia Entomol. Mex. 53(53): 111–118.

Rao, P.U., 1994. Chemical Composition and Nutritional Evaluation of Spent Silk Worm Pupae. J. Agric. Fd. Chem. 42(10): 2201–2203.

Ravindran, V., 2013. Poultry Feed Availability and Nutrition in Developing Countries: Main Ingredients used in Poultry Feed Formulation. Poult. Dev. Rev. 2(10): 694–695.

Ravindran, V. & Blair, R., 1992. Feed resources for poultry production in Asia and the Pacific. II. Plant protein sources. Worlds. Poult. Sci. J. 48(3): 205–231.

Roberts, J.R., 2004. Factors Affecting Egg Internal Quality and Egg Shell Quality in Laying Hens. J. Poult. Sci. 41(3): 161–177.

Resanoviã, M., Nesic, D. & Nesia, D., 2009. Mycotoxins in poultry production. Zbornik Matice srpske za prirodne nauke 116: 7–14. https://doi.org/10.2298/ZMSPN0916007R

Resh, V. H. & Carde, R.T., 2003. Encyclopedia of insects.Academic Press San Diego, CA.

Roland, D.A., 1988. Research Note: Egg Shell Problems: Estimates of Incidence and Economic Impact. Poult. Sci. 67(12): 1801–1803.

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

Samli, H.E., Agma, A. & Senkoylu, N., 2005. Effects of Storage Time and Temperature on Egg Quality in Old Laying Hens. J. Appl. Poult. Res. 14(1): 548–553.

San Gabriel, A. & Uneyama, H., 2013. Amino acid sensing in the gastrointestinal tract. Amino Acids. 45(3): 451–461.

Sánchez-Muros, M.J., Barroso, F.G. & Manzano-Agugliaro, F., 2014. Insect meal as renewable source of food for animal feeding: a review. J. Clean. Prod. 65: 16–27.

Scanes, C.G., Brant, G. & Ensminger, M.E., 2004. Poultry science(4th Edition). Pearson Prentice Hall.

Schabel, H.G., 2010. Forest insects as food: a global review. Forest insects as food humans bite back. Proc. a Work. Asia-Pacific Resour. their potential Dev. Chiang Mai, Thailand, 19-21 February, 2008: 37–64.

Scott, T.A., Kampen, R. & Silversides, F.G., 1999. The effect of phosphorus, phytase enzyme, and calcium on the performance of layers fed corn-based diets. Poult. Sci. 78(12): 1742–1749.

Scriber, J. M. & Slansky Jr, F., 1981. The nutritional ecology of immature insects.

Annu. Rev. Entomol. 26(1): 183–211.

Sharaby, A., Montaser, S. A., Mahmoud, Y. A. & Ibrahim, S. A., 2010. The possibility of rearing the grasshopper *Heteracris littoralis* (R.) on semi synthetic diets. J Agric. Fd. Techn. 1(1): 1–7.

Sheppard, D.C., 1992. Large-scale Feed Production from Animal Manures with a Non-Pest Native Fly. University of Georgia. http://www.hollowtop.com/finl_html/manureflies.htm. (Accessed 7 June 2017.)

Sheppard, C. D., Newton, L. G., Thompson, S. A. & Savage, S., 1994. A value added manure management system using the black soldier fly. Bioresour. Technol. 50(3): 275–279.

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

Simopoulos, A.P., 2000. Human Requirement for n-3 Polyunsaturated Fatty Acids. Poult. Sci. 79(7): 961–970.

Singh, P., 1982. The rearing of beneficial insects. New Zealand. Entomol. 7(3): 304–310.

Singh P & Moore, R.F., 1985. Handbook of insect rearing. Vol. I and 2. Elsevier Science Publishers, Auckland, New Zealand.

Sohail, S.S. & Roland, D.A., 2002. Influence of Dietary Phosphorus on Performance of Hy-Line W36 Hens. Poult. Sci. 81(1): 75–83.

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P. & De Smet, S., 2016. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci. Fd. Agric.(October): 1–19.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W. & Sealey, W., 2007. Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aquac. Soc. 38(1): 59–67.

Stamer, A., 2015. Insect proteins — a new source for animal feed. EMBO reports. 16(6): 676–681.

Stamer, A., Wesselss, S., Neidigk, R. & Hoerstgen-Schwark, G., 2014. Black Soldier Fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients' class in aquaculture diets. Org. World Congr. Istanbul, Turkey: 13–15.

Stefanello, C., Santos, T.C., Murakami, A.E., Martins, E.N. & Carneiro, T.C., 2014. Productive performance, eggshell quality, and eggshell ultrastructure of laying hens fed diets supplemented with organic trace minerals. Poult. Sci. 93(1): 104–113.

Steinfeld, H., Gerber, P., Wassenaar, T. & Castel, V., 2006. Livestock's long shadow: environmental issues and options. Food and Agriculture Organisation, Rome.

Tabatabaie, M.M., Aliarabi, H., Saki, A.A., Ahmadi, A. & Hosseini Siyar, S.A., 2007. Effect of different sources and levels of zinc on egg quality and laying hen performance. Pakistan J. Biol. Sci. 10(19): 3476–3478.

Tilman, D. & Clark, M., 2014. Global diets link environmental sustainability and human health. Nature. 515(7528): 518–522.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. & Polasky, S., 2002. Agricultural sustainability and intensive production practices. Nat. 418(6898): 671–677.

Van Schoor, A. Van., 2017. The assessment of black soldier fly (*Hermetia illucens*) pre-pupae , grown on human faecal waste , as a protein source in broiler and layer diets. MSc Diss. University of Stellenbosch, Stellenbosch.

Veldkamp, T., 2012. Insects as a sustainable feed ingredient in pig and poultry diets: A feasibility study. Wageningen UR Livestock Research. 63(October): 1–62.

Waldroup, P.W. 1999. Nutritional approaches to reducing phosphorus excretion by poultry. Poult. Sci. 78(5): 683–691.

Wang, W., Wu, Z., Dai, Z., Yang, Y., Wang, J. & Wu, G. 2013. Glycine metabolism in animals and humans: implications for nutrition and health. Amino Acids. 45(3): 463–477.

Wang, J., Yue, H., Wu, S., Zhang, H. & Qi, G. 2017. Nutritional modulation of health, egg quality and environmental pollution of the layers. Anim. Nutr. 3(2): 91–96.

Watkins, B.A., Feng, S., Strom, A.K., DeVitt, A.A., Yu, L., & Li, Y. 2003. Conjugated Linoleic Acids Alter the Fatty Acid Composition and Physical Properties of Egg Yolk and Albumen. J. Agric. Fd. Chem. 51(23): 6870–6876.

Watts, D.L., 1990. The nutritional relationships of manganese. J. Orthomol. Med. 5(4): 219–222.

Weismann, D.B., Gray, D.A., Pham, H.T. & Tijssen, P., 2012. Billions and billions sold: Pet-feeder crickets (Orthoptera: *Gryllidae*), commercial cricket farms, an epizootic densovirus, and government regulations make for a potential disaster. Zootaxa.Magnolia Press. 3504: 67–88.

Wijayasinghe, M.S. & Rajaguru, A.S.B., 1977. Use of silkworm (*Bombyx mori L.*) pupae as protein supplement in poutry rations. J. Nat. Sci.Coun. Sri-lanka. 5(2): 95–104.

Wijkström, U.N., 2009. The use of wild fish as aquaculture feed and its effects on income and food for the poor and the undernourished. Fish as Feed inputs Aquac. Pract. Sustain. Implic. (518): 400–407.

Wu, G., 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids. 37(1): 1–17.

Wu, G., 2014. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. J. Anim. Sci. Biotechnol. 5(1): 1–34.

Wu, G., Bazer, F.W., Burghardt, R.C., Johnson, G.A., Kim, S.W., Knabe, D.A., Li, X.L., Satterfield, M.C., Smith, S.B. & Spencer, T.E., 2010. Functional amino acids in pig nutrition and production. Dyn. Anim. Nutr. Wageningen Acad. Publ. Netherlands: 69–98.

Wu, G., Wu, Z., Dai, Z., Yang, Y., Wang, W., Liu, C., Wang, B., Wang, J. & Yin, Y., 2013. Dietary requirements of “nutritionally non-essential amino acids” by animals and humans. Amino Acids. 44(4): 1107–1113.

Wu, Z., Satterfield, M.C., Bazer, F.W. & Wu, G., 2012. Regulation of brown adipose tissue development and white fat reduction by L-arginine. Curr. Opin. Clin. Nutr. Metab. Care. 15(6): 529–538.

Xiao, J.F., Zhang, Y.N., Wu, S.G., Zhang, H.J., Yue, H.Y. & Qi, G.H., 2014. Manganese supplementation enhances the synthesis of glycosaminoglycan in eggshell membrane: A strategy to improve eggshell quality in laying hens. Poult. Sci. 93(2): 380–388.

Yao, K., Yin, Y.L., Chu, W., Liu, Z., Deng, D., Li, T., Huang, R., Zhang, J., Tan, B., Wang, W. & Wu, G., 2008. Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. J. Nutr. 138(5): 867–872.

Y.N. Zhang., 2013. Effects of dietary zinc on eggshell quality and antioxidant status of old laying hens. MSc Diss. Chinese Academy of Agricultural Sciences.

Zimmerman, R. A., 1997. Management of egg size through precise nutrient delivery. J. Appl. Poult. Res. 6: 478–482.

Zuidhof, M., Molnar, C., Morley, F., Wray, T., Robinson, F., Khan, B., Al-Ani, L. & Goonewardene, L., 2003. Nutritive value of house fly (*Musca domestica*) larvae as a feed supplement for turkey poults. Anim. Feed Sci. Technol. 105(1–4): 225–230.

Chapter 3

Nutrient composition of full fat, dry rendered or extruded black soldier fly larvae (*Hermetia illucens*)

3.1 Abstract

Different processing techniques on the nutritional value of black soldier fly larvae (BSFL); namely a full fat larvae meal (FF), or a dry rendered larvae meal (DR) or an extruded larvae meal (EX), were investigated. Proximate composition, amino acid composition and mineral content of the larvae meal from the different processing techniques were determined. The dry rendered process yielded the highest crude protein (CP) value of 48.18% which was comparable to that of soya oilcake meal. The full fat meal had the highest fat value of 42.90% whilst the extruded meal had the lowest fat value (30.24%). The larvae were a good source of amino acids; the extruded meal had the most comparable amino acid profile to the ideal amino acid profile for poultry. Arginine-lysine ratios were favourable (between 1.01 and 1.39) for all three techniques. This is important as birds are susceptible to lysine-arginine antagonism if this ratio falls below one. The larvae proved to be a good source of macro and micro minerals. To conclude, all three processing techniques should be suitable in the production of a desirable feed which can be supplemented into layer hen diets.

Keywords: Nutritional value, Black soldier fly larvae, amino acid profile, minerals, proximate composition, production

3.2 Introduction

The increased demand for poultry products has placed immense pressure on the acquisition of raw materials used in the poultry industry (Khusro *et al.*, 2012). Poultry diets are mainly comprised of energy sources fortified with plant and animal protein sources. Globally, the main energy source used is maize (corn) whilst soya oilcake meal is used as the plant protein source. The most widely used animal protein source is fishmeal and meat/carcass meal. The majority of developing countries are net importers of these raw materials (Ravindran, 2013). Maize is the most commonly used feed grain due to its rich energy source, starch. Starch is highly digestible for poultry and is a high-density source of readily available energy. Maize, however is also used for human consumption, making maize a very sought after commodity. The increase in human population and therefore the increase in consumption of grain by people as well as chickens, along with variable harvests due to drought, has caused an increase in the price of maize. This can have increasingly large effects on the poultry industry. This large effect has encouraged the poultry industry to look into alternative sources of raw materials (Khusro *et al.*, 2012).

Once energy rich raw materials have been established in the diet, protein based raw materials are required. The most widely used plant based protein source used in poultry diets is soybean meal. Plant based protein sources supply the major source of dietary protein requirements (Ravindran, 2013). The use of soya as a protein for animals is however not sustainable. As human consumption of soya increases, competition for this valued commodity increases (Stamer, 2015).

Plant protein sources may be nutritionally imbalanced in terms of essential amino acids, particularly lysine which is seen as the first limiting amino acid in cereals. This brings in the importance of animal based protein sources such as fishmeal. Unless these plant based protein sources are supplemented with an animal based protein source and/or certain synthetic amino acids, plant based diets will not meet the requirements for essential amino acids for optimum meat and egg production. Due to their high prices, animal based proteins are usually only used to balance out amino acid profiles rather than to be major sources of proteins (Ravindran, 2013).

Fishmeal is a rich source of protein which is highly digestible and contains valuable essential amino acids, minerals such as calcium and phosphorous, and essential fatty acids (Miles & Jacob, 1997). Fishmeal is an important and sometimes, the only source of animal protein in developing countries. It is either produced locally or imported. The correct amount of fishmeal to be used in a diet depends on the type of cereals used as well as the price. In general, the average inclusion of fishmeal is 8% for young birds and 4% for older meat birds and layers (Ravindran, 2013). Future expansion of fishmeal production is limited as marine over-exploitation has caused a reduction in the amount of small pelagic forage fish which is typically used for the production of fishmeal and fish oil (Veldkamp & Bosch, 2015). Production has not seemed to increase over the past 20 years. This is highly unlikely to change due to the ever increasing pressure on fish stocks (Ravindran, 2013). This increased demand for this raw material has led to prices doubling over the past five years. This is an issue as feed ingredients are responsible for 60 to 70% of production costs in poultry systems (Veldkamp & Bosch, 2015).

A viable alternative may be in the form of insects as a source of protein in animal feeds. Insects have been proposed as a high quality, sustainable protein source which could contribute to global food security as an animal feed (Finke, 2002; Premalatha *et al.*, 2011) or directly when fed to humans (Veldkamp & Bosch, 2015). Insects are a viable alternative protein source as they are able to be produced by being fed on organic waste streams (Veldkamp *et al.*, 2012). The feeding of insects to poultry will be beneficial in developing agricultural waste recycling systems, thereby reducing waste and environmental pollution (Khusro *et al.*, 2012).

Also, the ratio of essential amino acids to lysine is important to ensure a high quality protein is fed to poultry for optimal production. The essential amino acid composition of the black soldier fly pre-pupae is thought to be comparable to that of the ideal amino acid profile required for poultry. However, it is postulated that the processing of the fly pre-pupae/larvae may influence this nutritional profile. Therefore the aim of this study was to determine the nutrient quality of the black soldier fly larvae (BSFL) which has been processed by three different methods, namely full fat, dry rendered and extruded. The full fat process was the same method as that of Driemeyer (2016), the dry rendered process was done in a similar manner to the production of poultry by-product and the extruded meal involved the process of extrusion. The rendering process of poultry by-product is done to convert the by-products of meat and poultry processing into marketable products which include edible and inedible fats and proteins which have multiple agricultural and industrial uses (Jayathilakan *et al.*, 2012). By-product materials include viscera, meat scraps such as fat, bone, blood, feathers, hatchery by-products (infertile eggs, dead embryos, etc.) and dead animals. Edible fats include lard and food grade tallow. Inedible rendering products include; industrial and animal feed grade fats, meat and poultry by-product meals, feather meal and dried blood. There are two processes for inedible rendering, namely the dry rendering and wet rendering process. The wet rendering process involves adding water to the raw material and using steam to cook and separate the fat. The dry rendering process is continuous and uses the raw materials' own moisture and grease for cooking; no water is added. The raw material is cooked with dry heat in open steam jacketed drums until all the moisture has evaporated. Once all the moisture has evaporated and the raw material has dehydrated effectively, fat is removed by draining. The material is then passed through a screw press and any excess fat and moisture is removed. The material is then granulated or ground up into a meal (Jayathilakan *et al.*, 2012).

The extrusion-cooking process combines the effect of heat and extrusion. Heat is added to the dough like feed in one of many processes. The first process being direct heat from steam, the second being indirect transfer of heat by steam or electrical heaters surrounding the barrel and thirdly, the heat could come from the build-up of mechanical energy coming from the shaft of the screw. During the cooking process, temperatures may reach up to 200°C; however this temperature is only sustained for very short periods (5 to 10 seconds). The extrusion process can be wet or dry depending on the use of water or not (Serrano, 1997).

The nutrient compositions of the processed BSFL were analyzed to see if these three processing methods were viable to be used as layer hen feed.

3.3 Materials and methods

3.3.1 Insect rearing and processing

The BSFL evaluated had been processed using three different methods: full fat, dry rendered or extruded. All larvae were reared on a feed medium of kitchen waste. Fly eggs were collected from breeding holds and placed into a hatchery area which was kept at 32°C and a relative humidity of 80%. After 24 hours, the larvae were transferred onto their growth medium (kitchen waste). Kitchen waste consisted of food that had passed their sell-by date, transportation losses, damaged products, etc. Larvae were left on the growth medium for 36 hours and harvested. Once harvested, the three different processes were carried out.

The full fat process (Treatment 1) was the same method used by Driemeyer (2016), and included killing and drying. Killing was by freezing; larvae were placed in a freezer at -20°C for 24 hours. Larvae were then removed from the freezer and allowed to defrost at room temperature before being dried in a ventilated oven for 24 hours at 65°C until a constant weight was obtained. Samples were then milled using a Foss Knifetec 1095 (Höganäs, Sweden) and stored in a fridge until mixing of treatments.

Treatment 2, which was the dry rendered process, was done in a similar manner to the production of poultry by-product. The dry rendering was done by Agriprotein (Pty) Ltd as follows: BSFL were killed and cleaned by placing in 70°C water for 10 minutes. Dead BSFL were then frozen for up to 30 days, at -20°C. Typically, the BSFL were thawed 24 hours before rendering. The BSFL were heated to 130°C for 1 hour at atmospheric pressure (1-bar). The pressure was then increased to 3 bar for 30 minutes. This step ensures the product had been hydrolyzed (the vessel used was a 4 ton, steam jacketed WINDMEUL). A twin-screw press was then used to remove the free oil from the cooked BSFL. The process hydrolysed the product, the final step was to shake the hydrolysed product through a shaker to remove large particulates. A Haarslev batch cooker was used for the dry rendering process.

Treatment 3 involved the process of extrusion which manipulates the material shape and was done as follows by Agriprotein (Pty) Ltd: the BSFL were killed and cleaned at 70°C for 10-minutes in water. The dead BSFL were then dried using a Gryphon dryer at 170°C for 15 minutes. The still warm whole dried larvae (WDL) were then immediately transferred to a single screw press/extruder. The screw vane was made from a 20mm thick, Q345 low alloy steel plate. It had a specialised compression ratio to match the requirements of the raw material and had the following dimensions: 3.0 x 1.0 x 1.2 m. The barrel of the extruder was heated using LPG gas burners to approx. 170°C.

3.3.2 Analysis of feed samples

Samples of the processed BSFL were obtained from Agriprotein in 15kg bags. Two kilograms of each sample was milled using a Foss Knifetec 1095. Before the samples were analyzed they were sub-sampled by quartering on a clean surface; the feed was placed onto a flat surface and spread out evenly, the evenly distributed feed was then divided into quarters. Two diagonally opposite portions were then rejected. The remainder of the material was then subsequently mixed and the process is repeated until a sample of 200-300g is obtained. To ensure that the sample is homogenous, all the samples were ground using a hammer mill with a 1.5 mm sieve before the proximate analysis.

3.3.3 Methods for chemical analysis

3.3.3.1 Determination of dry matter

The dry matter (DM) of all insects evaluated was determined by methods described by the Association of Official Analytical Chemists (2002), official method 934.01.

Method

Two samples, weighing 2 g each, were placed in a porcelain crucible and dried at 100°C for 24 hours. Once samples were dried, they were weighed and the DM content was calculated using Equation 3.1

Equation 3.1:

$$\% \text{ Moist} = \frac{(A + B) - C}{B} \quad \text{x} \quad 100$$

$$\% \text{ Dry matter} = 100 - \% \text{ Moist}$$

A=Weight of empty crucible

B=Weight of air dried sample

C=Weight of crucible and dry sample

3.3.3.2 Determination of ash content

Samples retained from the dry matter analysis were used for the determination of ash content. Ash content was determined by using methods described by the Association of Official Analytical Chemists International (2002), official method 942.05.

Method

Samples were combusted in a ash furnace at 500°C for approximately six hours. Once combustion was complete, the samples were placed into a desiccator for 30 minutes in order to cool off. Once the samples were cool, they were weighed and the ash content calculated using Equation 3.2.

Equation 3.2:

$$\%\text{Ash} = \frac{D - A}{\text{sample mass}} \times 100\%\text{Organic matter} = 100 - \%\text{Ash}$$

A = weight of empty and dry crucible

D= weight of crucible and ash

3.3.3.3 Determination of crude protein

The CP content of all samples were determined by measuring the total nitrogen (N) content according to the methods described by Association of Official Analytical Chemists International (2002), Official Method 4.2.07, in the LECO FP528 apparatus.

Method

Two aliquots of each feed sample weighing 0.1 g were placed in a foil cup and then placed into the LECO FP528. The N content was directly taken from the LECO FP528 and the CP content calculated using Equation 3.3.

Equation 3.3:

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

3.3.3.4 Determination of crude fibre

Crude fibre analysis was done using the Filter Bag Technique and the ANKOM Fibre Analyser. A sulphuric (H_2SO_4) acid solution (0.255N) and a sodium hydroxide (NaOH) solution (0.313N) were used as reagents.

Method

Approximately 1 g of sample was weighed into a weighed ANKOM filter bag and heat sealed using a heat sealer. Bags were then soaked in petroleum ether for 10 minutes in order to extract all fat. Once fat had been extracted, the bags were air dried. Bags were then placed in the ANKOM fibre analyser and agitated at 100°C in 1.9 L of the previously mentioned H_2SO_4 solution for 40 minutes. At the end of the extraction, samples were rinsed twice with hot water while still in the fibre analyser. Samples were then agitated at 100°C in 1.9 L of the previously mentioned (NaOH) solution for 40 minutes, after which samples were again rinsed twice. Once samples were removed and air dried, they were soaked in acetone for 5 minutes. Samples were then air dried before being place in the oven to dry for 2 to 4 hours. Once samples were removed from the oven and cooled down, they were weighed. Samples were then incinerated to ash in a pre-weighed crucible at 500°C for five hours. Ash samples were then also weighed and the percentage crude fibre determined using Equation 3.4. One blank containing no sample was also included in the run in order to determine the blank bag correction factor.

Equation 3.4:

$$\%\text{Crude fibre} = 100 \times \frac{W_3 - (W_1 \times C_1)}{W_2}$$

Where:

W_1 = bag tare weight

W_2 = sample weight

W_3 = weight of organic matter (loss of weight on ignition of bag and fibre)

C_1 = Ash corrected blank bag factor (loss of weight on ignition of blank bag/original blank bag).

3.3.3.5 Determination of crude fat

Crude fat was determined using the acid hydrolysis method. This method involves acid hydrolysis using HCL followed by removal of lipids with mixed ethers. Ether is evaporated and the remainder of sample is expressed as crude fat.

Method

Fat cups were prepared by being heated in an oven at 100°C to ensure that they were free of moisture. These fat cups were then placed into a desiccator and allowed to cool for 30 minutes until room temperature. Two samples of 2 g each are weighed off and placed into a test tube. Ethanol (2 ml) was measured out and placed into the test tubes in order to wet the sample. After the ethanol had been added, 10 ml of HCL was added. These test tubes were then placed in a boiling water bath for approximately 30 min, removed from the water bath and allowed to cool for 30 min under an extraction fan. Once the test tubes were cool, the contents were emptied into an upright separating funnel. The test tubes were then filled with another 10 ml of ethanol and the contents emptied into the sample separating funnel in order to obtain the full sample from the test tube. Once all the samples were placed into their respective separating funnels, 25 ml of diethyl ether was added to each sample. The separating funnels were then shaken for 1 min each, where after 25 ml of petroleum ether was added to each separating funnel and shaken for 1 minute. Once all the samples were shaken, the upper portion of the separating funnel was emptied into a fat cup where after, 15 ml of diethyl ether was added to each sample and shaken for 1 minute. Then, 15 ml of petroleum ether was added to each sample, once again all these samples were shaken for 1 minute. Then, the upper portion of the funnel was again emptied into the fat cup. Lastly, another 15 ml of diethyl ether was added to the sample and shaken for 1 minute followed by the addition of 15 ml of petroleum ether and then shaken for 1 minute. The upper portion was once again emptied into the fat cup. Fat cups were then placed onto a sand bath for approximately 2 hours to allow the ether to evaporate. Once the ether evaporated, the fat cups were placed in a desiccator and allowed to cool for 30 minutes. After the samples have cooled, the samples were then weighed back and the % crude fat calculated using Equation 3.5.

Equation 3.5:

$$\%\text{Crude fat} = 100 \times \frac{(\text{Mass of fat cup plus fat}) - (\text{mass of fat cup})}{\text{Mass of sample}}$$

3.3.3.6 Sample hydrolysis for amino acid determination

The amino acid profile was determined by using the method explained by Cunico *et al*. (1986). Before the amino acid profile could be determined, samples were hydrolysed in acid. A sample weighing 0.1 g was placed in a specialized hydrolysis tube. Six ml hydrochloric acid (HCl) solution and 15% phenol solution was then added to the sample. The tubes were then vacuated and nitrogen (N_2) added under pressure. The tubes were then sealed off and the samples were left to hydrolyse at 110°C for 24 hours. Once hydrolysis was completed, the samples were transferred to Eppendorf tubes and refrigerated. The tubes were then sent to the Central Analytical Facility of Stellenbosch University, where the amino acid profiles were determined as described by Hopley (2015).

3.3.3.7 Determination of mineral composition

Mineral analysis was done by the Western Cape Department of Agriculture located at Elsenburg as described by Hopley (2015).

3.4 Results and discussion

3.4.1 Proximate composition

One of the main reasons why the BSFL have been found to be a beneficial animal feed source is due to their high protein and fat content. The proximate composition of the different processing methods of the BSFL is depicted in Table 3.1. The different processing methods showed variation between the nutritional values of the larvae (on a dry mass basis). Crude protein values were between 36.11% and 48.18%. The full fat meal had the lowest value and the dry rendered meal had the highest value. This value was comparable to the value of protein found in soya oilcake meal (NRC, 1994; Hopley, 2015). These values are also consistent with results from Sheppard *et al.* (1994), Newton *et al.* (2005), Driemeyer (2016) and Haasbroek (2016) which documented protein values ranging from 30%-40%. The fat values ranged from 30.24% to 42.90% between the different processing methods which are higher than fat values of fishmeal and soya bean oilcake meal noted by the NRC (1994) and Hopley (2015). These fat values were consistent with results from Haasbroek (2016). As expected, the full fat meal had the highest value whilst the dry rendered method had the lowest fat composition. Fibre and ash values between the different processing methods were low. Fibre values ranged from 7.76% to 8.51% with the highest fibre value being noted in the extruded larvae meal and the lowest in the dry rendered larvae meal. Fibre values were similar to those documented by Newton *et al.* (2005) which were for larvae reared on pig manure with a fibre value of 7%. Fibre values were slightly higher than those reported by Driemeyer (2016) who noted fibre values of 6.5%. The fibre values were comparable to those found in soya bean oilcake and were higher than the fibre values found in fishmeal (NRC, 1994; & Hopley, 2015). Ash values which are an indication of the amount of inorganic minerals present, were also similar with a range of 10.05% to 12.41%. Fasakin *et al.* (2003) noted that processing had an effect on the mineral content of the larvae meals. They showed that the process of hydrolysis and defatting caused an increase in certain minerals. This could be due to the extraction of oil decreasing the amount of feed product therefore concentrating the specific minerals. The dry rendered processing technique had the lowest ash value while the extruded method had the highest. These ash values were slightly lower than the values documented by Newton *et al.* (2005) and Haasbroek (2016). Values documented Newton *et al.* (2005) had slightly higher ash values 14.6% for larvae fed on poultry manure and 16.6% for that fed on pig manure while Haasbroek (2016) found values of 13.5%.

Table 3.1: Proximate analysis on a dry mass basis of different larvae treatments with comparisons to (Driemeyer (2016) and Haasbroek (2016).

	Crude Protein (%)	**Crude Fibre (%)**	**Fat (%)**	**Ash (%)**	**NFE**
(FF) Larvae Meal	36.11	8.10	42.90	11.90	0.99
(DR) Larvae Meal	48.18	7.76	31.54	10.05	2.46
(EX) Larvae Meal	43.10	8,51	30.24	12.41	5.74
(Driemeyer, 2016)	35.90	6.50	-	7.80	-
(Haasbroek, 2016)	38.05	-	33.87	13.15	-
Fishmeal (1)	61.93	0.54	10.11	-	-
Soya oilcake meal (1)	49.44	7.87	0.45	7.64	-

(FF)-Full Fat larvae meal, (DR)-Dry Rendered larvae meal, (EX)-Extruded larvae meal

(1) NRC, 1994.

3.4.2 Amino acid composition

The amino acid composition of all three processing methods is shown in the Table 3.2. It appears that all three processing methods resulted in good essential amino acid concentrations. However, values between the amino acids and different processing methods showed some variation. The dry rendered meal had the highest concentration of certain amino acids such as arginine, glycine, threonine, methionine, valine, isoleucine, leucine and phenylalanine; as mentioned previously, this may be due to the dilution effect of the oil or due to the high crude protein content in relation to the other processing methods. Similar results were noted by Hopley (2015) who found that the higher crude protein content of *Naophoeta cinerea* relative to the other insect species, led to an expected higher amino acid concentration. Methionine is seen as the first limiting amino acid in poultry (Ravindran & Bryden, 1999; Vieira *et al.*, 2004) whilst lysine is the second limiting amino acid and is not affected by metabolic functions and metabolic conversions, unlike methionine (Lemme *et al.*, 2004). For this reason, lysine is the reference amino acid for describing the ideal amino acid ratio and all essential amino acids are expressed as a ratio to lysine (Han & Baker, 1994). As seen from Table 3.2, the extruded meal seemed to relate best to the ideal amino acid profile even though the valine to lysine ratio may have been too high if compared to the ideal amino acid profiles of Leeson & Summers (2005) and (Bregendahl *et al,* (2008). Neither the Full Fat larvae (FF), Dry Rendered larvae (DR), or Extruded larvae (EX) meals' amino acid composition can be compared to that of fishmeal as the arginine, valine, threonine ratios are too high. The methionine-lysine ratio of the extruded meal compared best with the soya oilcake meal and fishmeal. However, the full fat meal's methionine-lysine ratio compared best with the ideal amino acid profile of Bregendahl *et al.* (2008) and the NRC (1994) and the dry rendered meal's methionine-lysine ratio compared best with the ideal amino acid profile of Leeson & Summers (2005). The arginine-lysine ratios of all three processing methods ranged from 1.01-1.39 (Table 3.3). This shows potential since birds are susceptible to lysine-arginine antagonism in cases where the arginine lysine ratios are less than 1. Lysine-arginine antagonism can cause lysine to compete with arginine in the renal tubules leading to a reduction in arginine retention (Jones *et al*., 1966), high levels of lysine in the diet may cause an increase in the oxidation of arginine (Austic & Nesheim, 1970) and smaller amounts of excess lysine can lead to reduction in the hepatic glycine transaminidase activity in chicks (Jones *et al*., 1966). Lysine content of all insect meals were below 3%. Lysine content greater than 3% may lead to arginine degradation by renal arginase, depression of glycine transamidinase, depression of appetite and arginine loss through urine (Austic & Scott, 1975).

Table 3.2: Comparison of amino acids from the three different processing techniques of black soldier fly larvae meal (g/100g).

	His	**Arg**	**Gly**	**Thr**	**Cys**	**Lys**	**Tyr**	**Met**	**Val**	**Ile**	**Leu**	**Phe**
FF	1.09	1.85	2.24	1.24	0.09	1.37	2.72	0.61	1.78	1.17	2.44	2.32
DR	1.06	2.47	3.05	1.81	0.05	1.77	2.48	0.90	2.36	1.60	3.35	2.62
EX	1.03	2.08	2.36	1.64	0.11	2.05	2.43	0.65	2.13	1.39	2.91	0.00
(Driemeyer, 2016)	1.01	1.93	1.38	1.20	-	1.78	1.75	0.52	1.53	1.25	1.87	1.22
(Haasbroek, 2016)	0.55	0.95	1.73	0.85	0.04	1.17	1.19	0.25	1.31	0.93	1.40	0.77
(St-Hilaire *et al.*, 2007)	1.18	2.65	2.28	1.78	-	2.62	3.08	0.74	2.79	2.03	3.10	2.00

(FF)-Full Fat larvae meal (DR)-Dry Rendered larvae meal, (EX)-Extruded larvae meal

Table 3.3: Calculated amino acids from different processing techniques relative to lysine ratio (%) and the ideal amino acid profile of poultry.

	Lys	**Arg**	**Thr**	**Met**	**Val**	**Ile**
FF	100	135	90	44	129	85
DR	100	139	102	50	133	90
EX	100	101	80	31	103	67
Ideal amino acid profile[1]	100	103	80	51	89	79
Ideal amino acid profile[2]	100	-	77	47	93	79
ideal amino acid profile[3]	100	101	68	43	101	94
Fishmeal[4]	100	79	60	27	77	62
Soya oilcake meal[5]	100	117	64	23	77	77

[1] Ideal amino acid profile calculated by Leeson & Summers (2005), [2] ideal amino acid calculated by Bregendahl *et al.* (2008), [3,4,5]NRC (1994), (FF)- Full fat, (DR)-Dry Rendered, (EX)-Extruded

3.4.2 Mineral composition

Minerals are the inorganic components of the feed. They can be classified into two main groups called macro and micro nutrients. Minerals are required for a variety of processes within the body; particular functions can be bone formation, co-factors for enzymes and the control of the osmotic balance (NRC, 1994).

For economical and physiological reasons, the most important mineral for layers would be Ca simply due to egg production (Lukić *et al.*, 2009). Adequate Ca supply will ensure normal physiological functioning as well as production of the egg shell with the desired structural integrity and maintenance of the skeletal structure (Pelicia *et al.*, 2009). The average egg shell contains approximately 2.3 g of Ca (2.0-2.5 g). This is approximately 10% of the total Ca content of the skeleton in the layer, considering the estimated amount of this macro element in skeletons of layers is 20g (Lukić *et al.*, 2009). Calcium homeostasis in the layer is dependent on balancing the ability of Ca absorption in the intestine, renal re-absorption and bone re-absorption of Ca with the needs and requirements of the animal for optimal production (Etches, 1987; Lukić *et al.*, 2009). If Ca is undersupplied, re-absorption of Ca from the bone is increased. This re-absorption of Ca causes a weakening in the bone structure and may lead to the development of osteoporosis in the layer at the end of lay. This metabolic disease is challenging to rectify with very few effective solutions (Whitehead & Fleming, 2000). On the contrary, if Ca is oversupplied, this may interfere with the availability of other minerals such as phosphorous, magnesium, manganese and zinc (NRC, 1994).

Another very important mineral in layer nutrition is P. Phosphorous and Ca have a strong correlation with regard to biological activity, bone structure and egg shell quality (Pelicia *et al.*, 2009). Calcium and P requirements of commercial layers is an on-going challenge for poultry nutritionists and egg producers as the needs for these two minerals seem to constantly change. Phosphorous requirements seem to be decreasing as opposed to Ca. The reasons for these opposite directions are uncertain, but may be related to the fact that high dietary Ca levels reduce the need for bone re-absorption, therefore reducing P needs (Pelicia *et al.*, 2009). Sodium and chloride are essential for all animals and assist with overall growth and performance. However, salt concentrations which are too high may lead to an excessive intake of water. These salts are also important for the regulation of the acid-base balance. Trace elements such as copper, iodine, iron, manganese, selenium, and zinc are needed in small amounts in the diet. Trace elements form part of larger organic minerals. Iron is found in haemoglobin in the blood which is responsible for the transportation of oxygen. Copper, manganese, selenium and zinc are important for the functioning of certain enzymes needed for everyday functioning. Zinc is also found in the structure of the DNA molecule (NRC, 1994).

Mineral comparisons of the BSFL (Table 3.4) were made with those found by Newton *et al.* (2005). However, it needs to be noted that the larvae used in this study were reared on kitchen waste while the larvae reared in Newton *et al.* (2005) were on poultry and pig manure. Therefore the slight variation in minerals could be due to different substrate.

The nutritional role of Ca is closely linked to that of P. More than 70% of animal body ash consists of Ca and P, with about 99% and 80%, respectively, being present in the bones (McDowell, 1992). The metabolic and structural function of these minerals in bone and eggshell formation is essential in poultry production. According to Berne & Levy (1998), Ca is actively absorbed in all intestinal segments, particularly in the duodenum and the jejunum. The speed of Ca absorption is higher than that of any other ion, except for Na. Animal nutritional status affects Ca absorption. Animals fed Ca deficient diets increase Ca absorption levels, whereas high dietary levels of this mineral reduce absorption. Calcium values of the BSFL were higher when reared on the pig manure (Table 3.4). It should be noted that all three processing techniques yielded a Ca concentration higher than the recommended amount for layer hens (3.15 g/day) by the NRC (1994). An excess amount of Ca can cause antagonism of absorption of minerals, causing a reduction in homeostasis of these minerals. Excess P may hamper the release of bone Ca and the adequate mineralization of the eggshell, whereas deficiency of Ca and P might result in poor eggshell quality and reduction in the size and production of eggs (Pastore *et al.*, 2012).

Phosphorus is the second most significant mineral after Ca, participating in metabolic interactions with vitamin D. In layers, requirement for dietary P is mainly due to the need to store Ca in bones prior to egg shell formation. However, P is also essential for metabolism of carbohydrates and fat, and Ca transport in egg formation. The P requirement recommended by the NRC (1994) for laying hen diets is 2.5 g/kg or 250 mg/hen/day, however levels fed in the industry are much higher (Li *et al.*, 2016). Phosphorous values in all treatments comfortably exceeded the recommended daily intake of 250 mg/hen/day or 0.25% (Table 3.4).

Potassium in the layer hen has many important roles including the maintenance of acid-base balances and osmotic relationships, activation of intracellular enzymes, protein and carbohydrate metabolism and ensuring absorption of free neutral amino acids such as glycine (Živkov Baloš *et al.*, 2016). Potassium deficiency is rarely observed since standard poultry feed mixes contain more than 1% K (Puls, 1990) whilst feed with 0.1% K is considered potassium-deficient. Potassium levels found in the larvae (Table 3.4) exceeded the daily requirement of 150 mg advised by the NRC (1994). Potassium toxicity in animals is not very common due to the animal's ability to readily excrete K. Excess K is known as hyperkalemia and could cause reduced renal losses and the redistribution of K (NRC, 2005). Potassium deficiency known as hypokalemia may lead to muscle and limb weakness as well as cardiac and respiratory failure (Živkov Baloš *et al.*, 2016).

Magnesium values found by Newton *et al.,* (2005) were slightly higher than the Mg concentrations found in this trial (Table 3.4). The EX meal concentrations however were very similar to the Mg value found when BSF larvae were reared on poultry manure. Magnesium values in all treatments were far above recommended concentrations for layers (350-355 mg/kg) by the NRC (1994). Excess Mg in the diet however may cause egg shell issues. A study by Atteh & Leeson (1983) suggested that excess Mg will increase the need for Ca and when excess Mg is fed, the Ca content of the diet should be increased to ensure good quality eggshells and improved bone integrity.

Table 3.4: Minerals found in the black soldier fly larvae meal from the different processing methods in comparison to mineral content found by Newton *et al.* (2005) of BSF larvae reared on poultry and pig manure.

	Dry Matter %	**Phosphorus (%) DM**	**Potassium (%) DM**	**Calcium (%) DM**	**Magnesium (%) DM**	**Sodium (mg/kg) DM**	**Iron (mg/kg) DM**	**Copper (mg/kg) DM**	**Zinc (mg/kg) DM**	**Manganese (mg/kg) DM**	**Boron (mg/kg) DM**	**Aluminum (mg/kg) DM**
FF	93.28	0.72	1.17	4.29	0.31	1256.00	609.00	15.86	122.79	99.94	4.34	79.00
DR	92.88	0.76	1.34	3.97	0.24	2061.00	1225.00	15.83	138.14	95.31	4.37	431.00
EX	91.21	0.84	1.53	4.98	0.36	1513.00	1318.00	15.91	212.76	159.57	3.86	833.00
Pm(1)	**-**	1.51	0.69	5.00	0.39	1325.00	1370.00	6.00	108.00	246.00	0.00	97.00
Pigm(1)	**-**	0.88	1.16	5.36	0.44	1260.00	776.00	26.00	271.00	348.00	-	-

(FF)-Full Fat larvae meal, (DR)-Dry Rendered larvae meal, (EX)-Extruded larvae meal, (Pm)-Poultry Manure, (Pigm)-Pig manure, (1) Newton *et al.* (2005)

Sodium is a major cation in extracellular fluid and makes up approximately 93% of the total cation content in blood plasma (Leeson & Summers, 2001). Sodium similarly to K, plays a role in maintenance of the acid-base balance and optimal osmotic relationships. The recommended levels of Na for laying hens ranges from 0.17-0.19% (Živkov Baloš *et al.*, 2016). It needs to be noted that only the DR meal meets this requirement (0.21% Na), both the full fat (0.13% Na) and extruded meal (0.15% Na) are below the recommended amount and will need to be supplemented (Table 3.4). With high Na levels in the diet, the poultry will increase their water intake and remove excess sodium from the body via body excreta. The symptoms of toxicities will not manifest as long as birds have enough drinking water. Deficiency in Na may lead to decreased egg production, impaired growth and even cannibalism. Diets which contain less than 0.012-0.050% are considered Na deficient (Puls, 1990).

Zinc and Mn, as cofactors of metallo-enzymes are responsible for carbonate and mucopolysaccharide synthesis which play an important role in eggshell formation (Swiatkiewicz & Koreleski, 2008). Mabe et al. (2003) suggested that trace elements Zn, Mn and Cu could affect mechanical properties of the eggshell by effecting calcite crystal formation and modifying the crystallographic structure of the eggshell. The requirements of Mn, Zn for layer hens as mentioned by the NRC (1994) are 20 mg/kg and 40 mg/kg, respectively. Both these requirements are comfortably met by all three insect meals (Table 3.4). In a study by Hess & Britton (1997), excess Mn had a damaging effect on egg production, body weight and egg shell quality. Laying hens can tolerate 1-2 g/kg DM of zinc in their diet, if this however increases up to 4 g/kg DM, this may lead to loss of appetite or retarded growth (Oh *et al.*,1979). Iron values were well over the recommended required amount of (45-55 mg/kg) (NRC, 1994). Mineral composition in general, is probably a function of the food sources of the insect; both the minerals absorbed from the diet as well as those remaining in the gastro-intestinal tract (Oonincx & van der Poel, 2010).

Aluminium values in the DR and EX meal were far higher than those values recorded from BSF larvae reared on poultry manure; however that in the FF meal was lower (Table 3.4). The FF meals sodium concentration was closest to that of the value when BSF larvae were reared on pig manure; however, the DR and EX meals were far higher than the larvae reared on either poultry or pig manure. The Cu value for BSF larvae reared on pig manure was far higher than the BSF larvae reared on kitchen waste in the present study (Table 3.4). However, all three meals in this trial had higher Cu values than the BSF larvae reared on poultry manure. Manganese values were lower in all three meals found in this trial compared to the larvae in Newton *et al.* (2005). The FF meal had the lowest Fe value compared to the other meals; the BSF larvae reared on poultry manure had the highest Fe concentration. Zinc values of BSF larvae in this trial were all higher than values on BSF larvae reared on poultry manure (Table 3.4).

3.5 Conclusion

The results obtained in this study were comparable to previous studies conducted on black soldier fly larvae. The dry rendered meal resulted in the highest crude protein value which could be compared to that of soya oilcake meal. Although none of the processing methods evaluated had amino acid profiles comparable to fishmeal, the extruded meal did relate well to the ideal amino acid profile for layers reported by Leeson & Summers (2005). Mineral concentrations besides sodium, were all above the required amount (NRC, 1994). These results show that black soldier fly processed via the three different methods (FF, DR and EX) could all be used as viable protein alternatives in animal feeds. It is important to note that any mineral that may be deficient in the larvae can be overcome by modification of the feed medium used to rear the larvae. It is well known that chemical composition of insects can be manipulated through the diet; this is definitely an avenue to look into in order to utilize insects in animal feed optimally.

3.6 References

Atteh, J. O., and S. Leeson., 1983. Influence of increasing the calcium and magnesium content of the drinking water on performance and bone and plasma minerals of broiler chickens. Poult. Sci. 62: 869–874.

Austic, R.E. & Nesheim, M.C., 1970. Role of kidney arginase in variations of the arginine requirement of chicks. J.Nut. 100(7): 855–867.

Austic, R.E. & Scott, R.L., 1975. Involvement of food intake in the lysine-arginine antagonism in chicks. J. Nutr. 105(9): 1122–1131.

Berne MR, Levy MN., 1998. Fisiologia. 4th ed. Rio de Janeiro: Guanabara Koogan.

Bregendahl, K., Roberts, S.A., Kerr, B. & Hoehler, D., 2008. Ideal Ratios of Isoleucine, Methionine, Methionine Plus Cystine, Threonine, Tryptophan, and Valine Relative to Lysine for White Leghorn-Type Laying Hens of Twenty-Eight to Thirty-Four Weeks of Age. Poult. Sci. 87(4): 744758.

Cunico, R., Mayer, A.G., Wehr, C.T. & Sheehan, T.L., 1986. High sensitivity amino acid analysis using a novel automated precolumn derivatization system. Biochromatography. 1(1): 14–19.

Driemeyer, H., 2016. Evaluation of black soldier fly (*Hermetia illucens*) larvae as an alternative protein source in pig creep diets in relation to production, blood and manure microbiology parameters. MSc Diss. University of Stellenbosch, Stellenbosch.

Etches, R.J., 1987. Calcium logistics in the laying hen. J. Nutr. 117(3): 619–628.

Fasakin, E. A., Balogun, A. M. & Ajayi, O. O., 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish, *Clarias gariepinus* fingerlings. Aquacult. Res. 34(9): 733–738.

Finke, M. D., 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21(3): 269–285.

Haasbroek, P., 2016. The use of *Hermetia illucens* and *Chrysomya chloropyga* larvae and pre-pupae meal in ruminant nutrition. MSc Diss. University of Stellenbosch, Stellenbosch.

Han, Y. & Baker, D.H., 1994. Digestible Lysine Requirement of Male and Female Broiler Chicks During the Period Three to Six Weeks Posthatching. Poult. Sci. 73(11): 1739–1745.

Hess, J.B. & Britton, W.M., 1997. Effects of dietary magnesium excess in White Leghorn hens. Poult. Sci. 76(5): 703–710.

Hopley, D., 2015. The evaluation of the potential of *Tenebrio molitor*, *Zophobas morio*, *Naophoeta cinerea*, *Blaptica dubia*, *Gromphardhina portentosa*, *Periplaneta americana*, *Blatta lateralis*, *Oxyhalao duesta* and *Hermetia illucens* for use in poultry feeds. MSc Diss. University of Stellenbosch, Stellenbosch.

Jayathilakan, K., Sultana, K., Radhakrishna, K. & Bawa, A.S., 2012. Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. J. Fd Sci. Technol. 49(3): 278–293.

Jones, J.D., Wolters, R. & Burnett, P.C., 1966. Lysine-arginine-electrolyte relationships in the rat. J. Nutr. 89(2): 171-188.

Khusro, M., Andrew, N. R. & Nicholas, A., 2012. Insects as poultry feed: A scoping study for poultry production systems in Australia. Worlds Poult. Sci. J. 68(3): 435–446.

Leeson S., Summers J.D., 2001. Scott's nutrition of the chicken. Chapter 5 – Minerals. 4th edition. Published by University books, Guelph, Ontario, Canada: 341–363.

Leeson, S., & J. D. Summers., 2005. Commercial Poultry Nutrition. 3rd edition. University Books, Guelph, Ontario, Canada.

Lemme, A., Ravindran, V. & Bryden, W., 2004. Ileal digestibility of amino acids in feed ingredients for broilers. Worlds Poult. Sci. J. 60(4): 423–438.

Li, X., Bryden, W.L. & Zhang, D., 2016. Available phosphorus requirement of laying hens. Final Project Report. A report for the Australian Egg Corporation Limited.

Lukić, M., Pavlovski, Z. & Škrbić, Z., 2009. Mineral nutrition of modern poultry genotypes. Biotechnol. Anim. Husb. 25(56): 399–409.

McDowell LR., 1992. Calcium and phosphorus. In: McDowell LR. Books. Vitamins in animal nutrition. London: Academic Press; 1992: 26–77.

Miles, R. D. & Jacob, J. P., 1997. Fishmeal: Understanding why this feed ingredient is so valuable in poultry diets. PhD diss. University of Florida.

National Research Council (NRC)., 1994. Nutrient Requirements of Poultry (NRP). 9th rev. ed. (National Academy Press, Washington D.C.)

National Research Council (NRC)., 2005. Mineral tolerance of animals. 2nd rev. ed. (National Academy Press, Washington D.C.)

Newton, G.L., Booram, C. V., Barker, R.W. & Hale, O.M., 1977. Dried *Hermetia Illucens* Larvae Meal as a Supplement for Pig. J. Anim. Sci. 44(3): 395–400.

Newton, L., Sheppard, C., Watson, D., Burtle, G. & Dove, R., 2005. Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of pig manure. University of Georgia, Tifton, G.A. (available at www.cals.ncsu.edu/ waste_mgt/smithfield_projects/phase2report05/cd,web%20files/A2.pdf)

Oh, S.H., Nakane, H., Deagan, J.T., Whanger, P.D. & Arscott, G.H., 1979. Accumulation and depletion of zinc in chick tissue and metallothioneins. J. Nutr. 109(10): 1720–1729.

Oonincx, D.G.A.B. & van der Poel, A.F.B., 2010. Effects of diet on the chemical composition of migratory locusts (*Locusta migratoria*). Zoo Biol. 30(1): 9–16.

Pastore, S.M., Gomes, P.C., Rostagno, H.S., Albino, L.F.T., Calderano, A.A., Vellasco, C.R., Viana, G. da S. & Almeida, R.L. de., 2012. Calcium levels and calcium: available phosphorus ratios in diets for white egg layers from 42 to 58 weeks of age. Rev. Bras. Zootec. 41(12): 2424–2432.

Pelicia, K., Garcia, E., Faitarone, A., Silva, A., Berto, D., Molino, A. & Vercese, F.,

2009. Calcium and available phosphorus levels for laying hens in second production cycle. Rev. Bras. Ciência Avícola. 11(1): 39–49.

Premalatha, M., Abbasi, T., Abbasi T. & Abbasi, S. A., 2011. Energy-efficient food production to reduce global warming and Eco degradation: The use of edible insects. Renew. Sustain. Energy Rev. 15(9): 4357–4360.

Puls R., 1990: Mineral levels in animal health. Diagnostic data. Published by Sherpa International, Clearbrook, British Columbia, Canada: 112–131.

Ravindran, V., 2013. Poultry Feed Availability and Nutrition in Developing Countries: Main Ingredients used in Poultry Feed Formulation. Poult. Dev. Rev. 2(10): 694–695.

Ravindran, V. & Bryden, W.L., 1999. Amino acid availability in poultry—in vitro and in vivo measurements. Aust. J. Agric. Res. 50(5): 880–889.

Serrano X., 1997. The extrusion-cooking process in animal feeding. Nutritional implications. Zaragoza : CIHEAM. 26: 107–114.

Sheppard, C. D., Newton, L. G., Thompson, S. A. & Savage, S., 1994. A value added manure management system using the black soldier fly. Bioresour. Technol. 50: 275–279.

St-Hilaire, S., Sheppard, C., Tomberlin, J. K., Irving, S., Newton, L., McGuire, M. A., Mosley, E. E., Hardy, R. W. & Sealey, W., 2007. Fly pre-pupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. J. World Aqua. Soc. 38(1): 59–67.

Stamer, A., 2015. Insect proteins — a new source for animal feed.EMBO reports. 16(6): 676–681.

Swiatkiewicz, S. & Koreleski, J., 2008. The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality. Vet. Med. (Praha). 53(10): 555–563.

Veldkamp, T. & Bosch, G., 2015. Insects: a protein-rich feed ingredient in pig and poultry diets. Anim. Front. 5(2): 45–50.

Veldkamp, T., 2012. Insects as a sustainable feed ingredient in pig and poultry diets: A feasibility study. Wageningen UR Livestock Research. 63(October): 1–62.

Vieira, S.L., Lemme, A., Goldenberg, D.B. & Brugalli, I., 2004. Metabolsim and Nutrition Responses of Growing Broilers to Diets with Increased Sulfur Amino Acids to Lysine Ratios at Two Dietary Protein Levels. Poult. Sci. 83: 1307–1313.

Whitehead, C.C. & Fleming, R.H., 2000. Osteoporosis in Cage Layers. Poultry Science. 79: 1033–1041.

Živkov Baloš, M., Jakšić, S., Knežević, S., Kapetanov, M. & Baloš, M.Ž., 2016. electrolytes-sodium,potassium and chlorides in poultry nutrition. Arh. Vet. Med. 9(1): 31–42.

Chapter 4

Effect of black soldier fly larvae (*Hermetia illucens*) fed full fat, dry rendered or extruded on production and egg quality parameters of layer hens

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4.1 Abstract

A study using three different processing techniques of *Hermetia illucens* (Black soldier fly) (BSF) was conducted to evaluate the production parameters of 40 layer hens. The first treatment was a full fat (FF) BSF larvae meal, the second treatment was a dry rendered (DR) larvae meal and the third treatment was an extruded (EX) larvae meal. Treatments were all compared to a control diet with soya meal as the protein source. Birds were fed over a period of 41 days; eggs were collected and marked daily from the six hens per treatment which laid eggs the most consistently throughout the trial. Every five days, feed refusals were collected from all the birds to determine individual consumption. Data were compared to determine the effect of the different treatments on production parameters such as average daily gain (ADG), feed conversion ratio (FCR), egg lay percentage (amount of eggs laid by an individual bird over the duration of the trial), intake (every five days) and cumulative intake. Differences ($P \leq 0.05$) were found with regard to ADG as well as egg lay percentage. With regard to ADG and egg lay percentage the FF, EX and the control groups differed significantly from the DR group. Egg quality parameters which included egg weight, shell weight, shell thickness, albumin weight, yolk weight, yolk height and albumin height were also measured. Data for egg quality parameters were analyzed over three different time periods, namely day 1 to 14, day 15 to 41 and day 1 to 41. This was done as dietary effects were expected after day 14. Egg quality parameters for the duration of the trial (day 1 to 41) had very positive results. A difference ($P \leq 0.05$) was found between treatments with regard to albumin weight. The FF, DR and EX meal all differed significantly with heavier albumin weights compared to the soya based control diet. This chapter concludes that black solider fly larvae, irrespective of the processing method can be used as a protein source for the feeding of layer hens with no adverse effects but rather positive effects on production and egg quality.

Keywords: ADG, FCR, processing techniques, intake, black soldier fly larvae, egg quality

4.2 Introduction

Poultry products, such as meat and eggs, remain the major source of animal protein for humans (Scanes, 2007). This comes as no surprise as eggs offer a moderate calorie source (about 150 kcal/100 g) and a high quality protein at a low economic cost (Carrillo *et al.,* 2012). This places eggs within reach of most of the population. Eggs also provide 18 minerals and vitamins as well as culinary versatility. The balance between these minerals and vitamins found within the egg can be affected or manipulated by the hen diet, age, strain and environmental factors (Samman *et al*., 2009; Fraeye *et al*., 2012). The ability to manipulate these compositions in such ways also makes eggs a desirable source of nutrition. Eggs can also be used in the inclusion of diets for people at different life stages due to its rich fat-soluble compounds. These characteristics of the eggs are highly important for groups of people which may be nutritionally deficient such as pregnant women, children and the elderly (Natoli *et al*., 2007). Eggs are also a highly desirable source of nourishment due to its lack of exclusive restrictions at a religious level*.* Although eggs contain rich amounts of minerals and vitamins, scientific evidence has also reported eggs to contain biologically active compounds with antimicrobial, immunomodulator, antioxidant, anti-cancer and anti-hypertensive properties, which allow them to play a part in the prevention and healing of certain chronic and infectious diseases (Abeyrathne *et al.,* 2013).

The rise in the consumption of poultry products may be due to their high-quality and the relatively low price due to efficiency of production. Such growth in the need for poultry products has placed pressure on the availability and demand for raw feed ingredients. Producing these feed raw materials such as soya, fishmeal, maize (corn) and oilcake meal has become increasingly more challenging due to climatic changes, urbanization and general decrease in arable land. This is an ongoing problem as the gap between local production and demand is seen to become increasingly larger each year (Ravindran., 2013).

The reliance on soya as a protein source in the diets of pigs and poultry raises sustainability issues, both environmentally and economically. Economically, the huge demand for soya causes fluctuations on global markets, making the price of animal feeds and raw materials a challenge for producers (Lee *et al.*, 2016). According to

Scanes (2007), the production of chicken meat and eggs had increased between 1995 and 2005 by 53% and 39%, respectively. This ongoing growth impacts on the demand for raw materials for poultry feed (Ravindran, 2013). Soya oilcake is a popular protein source used in poultry diets due to its high level of digestibility and desirable amino acid composition (Willis, 2003). Although there has been an increase in soya production, soya as a protein source for animals is limited due to competition with human consumption. Fishmeal is a high protein feedstuff used to feed poultry; however, crude protein content can vary from 57% to 77% depending on the species of fish used. Fishmeal is added to poultry diets as a source of high quality animal protein due to its rich concentration of essential amino acids (Miles & Jacob, 1997). However, fishmeal prices are on the rise. For small farmers this means that fishmeal is less accessible (FAO, 2013). Growth in the fishmeal industry appears to be limited and production of fishmeal has not increased over the past 20 years. This trend will continue due to pressure on world fisheries (Ravindran, 2013).

Alternative protein sources to fishmeal and soya bean meal may be in the form of insects for feeding animals (Vrabec *et al.*, 2015). Insects are part of the natural diet for poultry (Zuidhof *et al.*, 2003; Hopley 2015). Insects have a high protein, fat, minerals and vitamins content (Khusro *et al.*, 2012; Hopley 2015). Few studies have been conducted on the use of insect meals for the feeding of layer hens. A study by Agunbiade *et al.* (2007) concluded that a replacement of fishmeal for up to 50% of the dietary protein with maggot meal, had no adverse effects on egg production and shell strength. Another study by Van Schoor (2017) concluded that black soldier fly pre-pupae reared on human waste was a viable alternative protein source for the feeding of layer hens as no pathogens were detected in the eggs. Also, with an inclusion level of 10%, positive effects were noticed on the production parameters of the layers whilst the standard of the eggs were also not affected with the inclusion of the pre-pupae meal. Hopley (2015) compared the production of layer hens fed black soldier fly larvae (BSFL) and pre-pupae meal and mealworms and concluded that these insects could be fed to layer hens with positive effects on the egg quality and production.

However, questions have risen on what the effect of processing of the BSFL could have on the egg production and quality when fed to layer hens as partial replacement for protein. The aim of this study was therefore to determine whether BSFL processed using three different techniques could be used as an alternative protein source for layer hens without any adverse effects on egg quality and production.

4.3 Materials and methods

Ethical clearance was obtained from the ethical committee of Stellenbosch University for conducting this experimental trial (SU-ACUD16-00107).

The study took place on Mariendahl experimental farm, Stellenbosch University. A total of 40 Hy-line Silver hens which were 35 weeks of age, were used in this experiment. All mortalities were subjected to a full *post-mortem* analysis. For the duration of the trial, the hens were kept in a naturally ventilated layer house equipped with “A” type layer cages. The hens were fed 150 g of feed per day and had *ad lib* access to water throughout the duration of the trial.

The hens were assigned to four different treatment diets which included a control diet. Treatment diets are shown in Table 4.1. The diets were formulated so that the hens were maintained on the minimum nutrient requirements. The nutritional compositions of the BSFL were as described in Chapter 3. The control diet for this trial was soya based since it is an internationally accepted mixture suitable for poultry production. The hens were fed an adaptation diet for two weeks. After two weeks, the hens were fed their allocated diets respectively. The hens were fed their allocated diets for 41 days.

The 40 layer hens were each placed in individual cages. The layout was a completely randomized design with ten replicates per treatment and one hen per replicate.

The body weight of each hen was determined at the start and end of the trial (Equation 4.1). Feed was supplied (150 g/day) daily and refusals weighed back for determination of feed intake every five days. From this data, the food conversion ratio (FCR) was calculated as per Equation 4.2.

Equation 4.1:

Weight gain/loss = Weight of hen at start of trial - Weight of hen at end of trial

Equation 4.2:

$$\textbf{FCR} = \frac{\text{kg of feed consumed}}{\text{kg of egg produced}}$$

Each day of the trial, eggs were collected from the layer hens at around 1pm. Hen number, date of lay and treatment diet was recorded on the egg. Marking of the egg was done using an Hb graphite pencil. The collected eggs were weighed and then placed in egg trays according to their allocated treatment diet. The eggs were stored in a laboratory at room temperature (15 to 18°C). Broken or cracked eggs were discarded and noted. Dirty eggs (contaminated by excess faeces or dirt) were discarded and noted. If a certain layer hen did not lay an egg a note was also made. Eggs were selected from hens which followed the average mean egg production per treatment. The eggs were all analyzed within a month of lay in order to preserve freshness. Eggs were analyzed in order of date of lay. The data collected was used to determine the feed conversion ratio (FCR) for egg production, weight gain over the duration of the trial and egg quality parameters.

4.3.1 Measuring egg quality parameters

The layer hen eggs post-trial were first analyzed for any external abnormalities such as cracks, pinholes, dirt or abnormal pigmentation, indicating problems within the reproductive tract or a nutritive imbalance. Once external abnormalities had been analyzed, the eggs were cracked open gently onto a large piece of transparent glass, ensuring egg yolks and whites remained intact on the glass. The cracked egg shell was weighed and shell thickness was recorded using a digital caliper (measurements were done in millimetres to 2 decimal places). A Haugh meter was used to measure the height of the yolk and albumin determining the quality of the egg (measurements were done in millimetres to 2 decimal places). A careful observation was then conducted to ensure no meat or blood spots were present on the yolk or the albumin. These meat and blood spot abnormalities, if present, were then recorded. Once yolk and albumin heights were recorded, the yolk was separated from the albumin using a glass rubber wiper. Upon separation, both the yolk and albumin were placed in separate petri dishes and the weight of each component was recorded. A BYK-Gardiner Colour Guide (Catalogue no:6805; BYK-Gardner, USA) colour colorimeter was then placed onto the yolk in order to retrieve the colour readings which consisted of: “L*” a measurement of lightness, “a*” which denotes the red/green spectra and “b*” which denotes the yellow/blue spectra. Once the colour readings had been recorded, the yolk was discarded and the next egg could be measured accordingly. All weights were recorded using a laboratory scale measuring in grams to 4 decimal points.

The weight of the eggs determined their classification according to the South African standards as follows [Minimum Mass (g) per egg].

Jumbo	≥ 66g
Extra Large	≥ 59g
Large	≥ 51g
Medium	≥ 43g
Small	≥ 33g

Table 4.1: Ingredient and calculated nutrient composition of treatment diets (as is basis).

Ingredients	**Unit**	**Full fat**	**Dry rendered**	**Extruded**	**Control**
BSF[1] (FF[2])	%	15.00	-	-	-
BSF (EX[3])	%	-	-	15.000	-
BSF (DR[4])	%	-	15.00	-	-
Maize	%	59.685	65.821	64.463	58.603
Soybean full fat	%	15.158	9.235	10.476	26.506
L-lysine HCl	%	0	0.054	0.042	0
DL methionine	%	0.032	0.018	0.021	0.169
L-threonine	%	0.481	0	0	1.433
Vit + Min premix	%	0.250	0.250	0.250	0.250
Limestone	%	7.195	7.229	7.253	8.010
Salt	%	0.150	0.136	0.073	0.175
Monocalcium phosphate	%	1.250	1.257	1.424	1.696
Sodium bicarbonate	%	0.355	0.379	0.349	0.357
Oil – sunflower	%	0.442	0.621	0.649	2.800
Calculated nutritional value	**Unit**	**Full fat**	**Dry rendered**	**Extruded**	**Control**
AME[5]n_adult	MJ/kg	13.000	13.000	13.000	13.000
Dry matter	%	89.090	89.633	88.525	89.140
Crude protein	%	16.000	16.000	16.000	16.000
Lysine	%	0.780	0.780	0.780	0.780
Methionine	%	0.320	0.320	0.320	0.406
Methionine+cystine	%	0.739	0.781	0.773	0.692
Threonine	%	1.116	0.640	0.640	2.000
Tryptophan	%	0.196	0.198	0.198	0.162
Arginine	%	1.047	1.031	1.035	0.966
Isoleucine	%	0.680	0.665	0.669	0.647
Leucine	%	1.468	1.480	1.478	1.423
Histidine	%	0.442	0.441	0.441	0.420
Phenylalanine	%	0.701	0.683	0.687	0.694
Tyrosine	%	0.568	0.566	0.566	0.531
Phenyl+tyrosine	%	1.269	1.250	1.253	1.225
Valine	%	0.870	0.891	0.886	0.751
Ash	%	9.728	10.129	9.608	9.879
Crude fibre	%	3.546	3.355	3.749	2.747
Crude fat	%	11.257	9.552	7.826	9.903
Calcium	%	3.250	3.250	3.250	3.250
Phosphorous	%	0.691	0.675	0.708	0.742
Avail. phosphorous	%	0.500	0.500	0.500	0.500
Sodium	%	0.180	0.180	0.180	0.180
Chloride	%	0.150	0.150	0.150	0.150
Potassium	%	0.555	0.482	0.523	0.629
Linoleic acid	%	2.892	2.489	2.586	4.436

(1)BSF-Black Soldier Fly, (2)-Full fat, (3) DR-Dry rendered, (4) EX-Extruded (5) AME-Apparent metabolisable energy.

4.3.2 Statistical analyses

Statistical analysis was done using STATISTICA, version 9 by Statsoft inc. (2009). Where age effects were not a variable, statistics were done using a one way analysis of variance (ANOVA). Significant differences were found if $P \leq 0.05$. Diet was the main effect evaluated.

The egg laying periods were divided into three different time periods, namely day 1 to 14, day 15 to 41, and day 1 to 41, in order to observe how the egg characteristics improved, became worse or remained constant over time. The data was divided into these different time periods as expected dietary effects would only become evident after day 14. Data for the full trial (day 1 to 41) was also analyzed to observe the overall trend in quality of the eggs.

4.4 Results and discussion

The experimental data was compared to determine the effects of the different treatments on production parameters such as average daily gain (ADG), feed conversion efficiency (FCR), egg lay percentage (number of eggs laid during the two time period and over the whole duration of trial), intake (every five days) and cumulative intake (Table 4.2). With regard to ADG, the full fat (FF), extruded (EX) and Control diet all differed ($P \leq 0.05$) from the dry rendered (DR) diet (Table 4.2). A difference ($P \leq 0.05$) was found between treatments with regard to egg lay percentage. The FF, EX and Control diet all differed ($P \leq 0.05$) from the DR diet (Table 4.2) with all three having larger egg lay percentages. This could be due to over processing of the dry rendered product which could have caused destruction of amino acids having a negative effect on egg production.

In general there was a slight decrease in body weights between all the treatments, besides the EX meal. This general decrease may be due to the age of the birds since at the onset of the trial they were already 36 weeks old; feed intake and body weight gains for laying hens tend to follow a sigmoidal curve. According to Bordas & Minieville (1999), after 20 weeks of age, laying hens reach a plateau and the values for these two parameters remain relatively constant. The lower performance for the DR treatment with regard to egg lay percentage and ADG may be due to the processing methodology. The rendering process uses intense heat (see Chapter 3 for details) which can negatively influence the nutritive value through the production of hydrolyzates (Hendriks *et al.*, 2000, 2006; Piva *et al.*, 2001; Bellagamba *et al.*, 2015). The exposure of proteins to certain treatments causes the formation of cross-linked amino acids (Liardon & Hurrell, 1983; Man & Bada, 1987; Friedman, 1999). This denaturation in protein structure may lead to the reduction in protein digestibility (2 to 7%) especially the digestibility of cysteine (16 to 26%) and aspartic acid (7 to 11% reduction) (Miller, 2004; Bellagamba *et al.*, 2015). These factors may be the reason for both the reduced feed intake and egg production with layer hens being fed the DR larvae meal.

Egg quality parameters including egg weight, shell weight, yolk weight, albumin weight, yolk height, albumin height, shell thickness and colour L, a, b are depicted in Table 4.3. Data were analyzed statistically over three different time periods, namely day 1 to 14, day 15 to 41 and day 1 to 41. This was done as dietary effects were expected after day 14. However, egg quality parameters for the duration of the trial (day 1 to 41) had positive results. A difference ($P \leq 0.05$) was found between treatments with regard to albumin weight; the FF, DR and EX diets all differed with heavier albumin weights compared to the soya based control diet.

No differences ($P > 0.05$) were found between yolk height and albumin height throughout the trial between the different treatments (Table 4.3). Egg weight, shell weight, yolk weight showed no difference ($P > 0.05$) if all the data from day 1-41 was tested between the different treatments. However, for day 1-14, a difference ($P \leq 0.05$) was found between egg weight, shell weight, yolk weight and shell thickness between the different treatments. With regard to egg weight, the full fat diet (56.60 g) differed from the control (53.50 g). The extruded meal (7.16 g) showed a significant difference from the control meal (7.46 g) with regard to shell weight. With regard to yolk weight, the full fat diet (16.86 g) differed ($P \leq 0.05$) from the DR (16.10 g), EX (16.59 g) and control diet (16.43 g). The higher yolk weight found from the FF diet can be caused by the higher fat content which results in stimulation of oviduct protein synthesis causing heavier yolk weights (Whitehead *et al.*, 1991). However this became negligible once the data from the whole period of day 1-41 was analyzed. A difference ($P \leq 0.05$) between the EX meal (0.44 mm) and control (0.46 mm) was found with regard to shell thickness. The period from day 15-41 also showed differences ($P \leq 0.05$) between egg weight, shell weight, yolk weight and shell thickness between the treatments. With regard to egg weight, the FF (57.40 g), DR (55.76 g) and EX (57.84 g) meal differed from the control (54.70 g). The FF diet (6.96 g) differed ($P \leq 0.05$) from the DR (7.49 g), EX (7.56 g) and control (7.59 g) with regard to shell weight. However, the DR diet (16.49 g) differed ($P \leq 0.05$) from the FF (17.89 g), EX (17.75 g) and control diet (17.26 g) with regard to yolk weight. With regard to shell thickness, the FF diet (0.39 mm) differed ($P \leq 0.05$) from the DR (0.43 mm), EX (0.43 mm) and control (0.44 mm) diets. For the period day 1-41, the control (0.45 mm) diet differed ($P \leq 0.05$) from the FF (0.42 mm), EX (0.43 mm) and DR (0.43 mm) diets.

With regard to colour (Table 4.3), no differences ($P > 0.05$) were found between all the treatments when the data for the whole period (day 1-41) was analyzed. During the period of day 1-14, the FF diet differed ($P \leq 0.05$) from the DR, EX and control diet for the L* colour measurement. During day 15-41, the EX diet differed ($P \leq 0.05$) from the FF, DR and control for the L* colour measurement. With regard to the a* colour measurement, during the periods of day 1-14 and 15-41, the control differed ($P \leq 0.05$) from the DR, EX and FF diets. No significant differences were found between the treatments during the different time periods for the b* colour measurements. No differences between colour over the duration of the trial (day 1-41) was expected as yolk colour is determined primarily by the pigmenting carotenoids found in yellow maize. All treatments had the same yellow maize at similar levels resulting in no differences with regard to colour.

The data for meat spots, blood spots, thin albumin spread, thick albumin spread and the colour fan (used to measure shade of yellow of yolk: higher number denotes darker shade of yolk) were converted to categorical data for better interpretation of the effect of the treatment on the BSFL (Table 4.4).

Table 4.2: Averages of weekly live weight (kg) (±standard error), weekly feed intake (g), and cumulative feed intake (g) of layers receiving different diets.

	Full fat[1]	**Dry rendered[2]**	**Extruded[3]**	**Control**
Day 5				
Average live weight (day 1)	1.94 ± 0.020	1.95 ± 0.026	1.98 ± 0.025	1.97 ± 0.025
Average feed intake	608.40	596.63	638.38	532.80
Cumulative feed intake	608.40	596.63	638.38	532.80
Day 10				
Average feed intake	488.15	524.62	530.42	434.45
Cumulative feed intake	1096.55	1121.25	1168.8	967.25
Day 15				
Average feed intake	493.53	516.67	550.77	470.27
Cumulative feed intake	1590.08	1637.92	1719.57	1437.52
Day 20				
Average feed intake	561.92	506.72	543.35	527.65
Cumulative feed intake	2152.00	2144.64	2262.92	1965.17
Day 25				
Average feed intake	572.13	475.33	557.84	561.34
Cumulative feed intake	2724.13	2619.97	2820.76	2526.51
Day 30				
Average feed intake	558.37	468.96	572.64	545.59
Cumulative feed intake	3282.50	3088.93	3393.40	3072.10
Day 35				
Average feed intake	514.14	487.57	586.63	521.60
Cumulative feed intake	3796.64	3576.50	3980.03	3593.70
Day 40				
Average feed intake	554.14	432.70	584.13	550.00
Cumulative feed intake	4350.78	4009.20	4564.16	4143.70
Average live weight (day 41)	1.92^{a} ± 0.049	1.78^{b} ± 0.031	1.98^{a} ± 0.021	1.95^{a} ± 0.034
ADG[4] (kg)	-0.02^{a} ± 0.042	-0.17^{b} ± 0.036	0.000^{a} ± 0.018	-0.02^{a} ± 0.021
Egg lay %	98.78^{a} ± 0.545	92.68^{b} ± 1.889	98.78^{a} ± 0.833	94.31^{b}± 1.626
FCR[5] (per kg egg mass)	1.99 ± 0.054	1.99 ± 0.050	2.06 ± 0.039	2.1 ± 0.070

(1) FF-Full fat, (2) DR-Dry rendered, (3) EX-Extruded, (4) ADG-Average daily gain, (5) FCR-Feed conversion ratio

(^{a,b,c})Values with different superscripts differ significantly within rows.

Table 4.3: Average (± standard error) egg quality measurements as influenced by diets.

	Full fat	**Dry Rendered**	**Extruded**	**Control**	**P-value**
Egg weight (g) day 1- 14	56.60^{bd} ± 0.255	55.22^{cde} ± 0.226	55.82^{cde} ± 0.271	53.50^{e} ± 0.420	P=0.0027
Egg weight (g) day 15- 41	57.40^{ac} ± 0.246	55.76^{abcde} ± 0.239	57.84^{ab} ± 0.178	54.70^{d} ± 0.291	P=0.0027
Egg weight (g) day 1- 41	57.13 ± 0.185	55.57 ± 0.174	57.15 ± 0.161	54.30 ± 0.242	P=0.0755
Shell weight (g) day 1- 14	7.30^{ab} ± 0.046	7.19^{ab} ± 0.062	7.16^{b} ± 0.052	7.46^{a} ± 0.070	P=0.0000
Shell weight (g) day 15- 41	6.96^{d} ± 0.044	7.49^{c} ± 0.056	7.56^{c} ± 0.039	7.59^{bc} ± 0.051	P=0.0000
Shell weight (g) day 1- 41	7.08 ± 0.034	7.38 ± 0.040	7.42 ± 0.033	7.55 ± 0.041	P=0.2641
Yolk weight (g) day 1- 14	16.86^{bcde} ± 0.122	16.10^{e} ± 0.124	16.59^{cde}± 0.140	16.43^{de} ± 0.146	P=0.0076
Yolk weight (g) day 15- 41	17.89^{a} ± 0.106	16.49^{cd} ± 0.109	17.75^{ab} ± 0.110	17.26^{abc} ± 0.123	P=0.0076
Yolk weight (g) day 1- 41	17.54 ± 0.087	16.35 ± 0.084	17.38 ± 0.093	16.98 ± 0.098	P=0.1416
Albumin weight (g) day 1- 14	32.53 ± 0.185	31.93 ± 0.179	32.07 ± 0.177	29.61 ± 0.286	P=0.2767
Albumin weight (g) day 15- 41	32.56 ± 0.177	31.78 ± 0.181	32.54 ± 0.138	29.84 ± 0.184	P=0.2767
Albumin weight (g) Day 1- 41	32.55^{a} ± 0.132	31.83^{a} ± 0.133	32.38^{a} ± 0.110	29.76^{b} ± 0.155	P=0.0049
Yolk height (mm) day 1- 14	15.36 ± 0.233	14.59 ± 0.219	15.18 ± 0.179	14.80 ± 0.177	P=0.7067
Yolk height (mm) day 15- 41	15.07 ± 0.138	14.25 ± 0.125	15.00 ± 0.146	14.80 ± 0.095	P=0.7067
Yolk height (mm) day 1- 41	15.17 ± 0.121	14.37 ± 0.112	15.06 ± 0.114	14.80 ± 0.086	P=0.0718
Albumin height (mm) day 1- 14	3,03 ± 0.075	2,84 ± 0.066	2,61 ± 0.05	2,72 ± 0.064	P=0.0699
Albumin height (mm) day 15- 41	3,07 ± 0.067	2,78 ± 0.060	2,70 ± 0.053	2,52 ± 0.047	P=0.0699
Albumin height (mm) day 1- 41	3.06 ± 0.051	2.80 ± 0.045	2.67 ± 0.038	2.58 ± 0.039	P=0.0750
Shell thickness (mm) day 1-14	0.45 ± 0.005^{ab}	0.45 ± 0.004^{ab}	0.44 ± 0.004^{b}	0.46 ± 0.006^{a}	P=0.0000
Shell thickness (mm) day 15- 41	0.39^{d}± 0.004	0.43^{c} ± 0.003	0.43^{c} ± 0.004	0.44^{bc} ± 0.003	P=0.0000
Shell thickness (mm) day 1- 41	0.42^{c}± 0.004	0.43^{ab} ± 0.003	0.43^{bc} ± 0.003	0.45^{a} ± 0.003	P=0.0054
Colour					
L* day 1- 14	64.64^{abcd} ± 0.296	63.00^{d} ± 0.279	64.08^{bcd} ± 0.270	63.251^{c} ± 0.308	P=0.0383
L* day 15- 41	64.56^{abcd} ± 0.317	64.38^{abc} ± 0.255	65.273^{a} ± 0.315	64.61^{abd} ± 0.288	P=0.0383
L* day 1- 41	64.59 ± 0.232	63.89 ± 0.196	64.86 ± 0.229	64.15 ± 0.221	P=0.5769
a* day 1- 14	9.93^{a} ± 0.177	9.77^{ab} ± 0.221	9.95^{a} ± 0.179	8.77^{bc} ± 0.238	P=0.0441
a* day 15- 41	7.83^{cd} ± 0.178	8.459^{cd} ± 0.170	8.50^{cd} ± 0.141	7.56^{d} ± 0.133	P=0.0441
a* day 1- 41	8.54 ± 0.146	8.93 ± 0.141	8.99 ± 0.120	7.97 ± 0.125	P=0.1595
b* day 1- 14	68.91 ± 0.735	69.64 ± 0.800	69.16 ± 0.879	68.30 ± 0.827	P=0.8386
b* day 15- 41	61.35 ± 0.520	63.23 ± 0.552	61.76 ± 0.568	60.97 ± 0.533	P=0.8386
b* day 1- 41	63.92 ± 0.483	65.52 ± 0.500	64.32 ± 0.530	63.43 ± 0.504	P=0.5184

^{a,b,c} Values with different superscripts differ significantly within rows (P- values as indicated in the Table)

L*- lightness, **a***- green/red, **b***- blue/yellow

Table 4.4: Categorical data recorded between the different treatments.

	Full Fat	**Dry Rendered**	**Extruded**	**Control**	**P-value**
meat spots	44/242	62/227	59/243	74/229	P=0.31
blood spots	11/242	28/227	23/243	25/229	P=0.79
Thin albumin spread (**1**,**2**,**3**)	**1**=48 **2**=87 **3**=107	**1**=48 **2**=78 **3**=101	**1**=62 **2**=91 **3**=90	**1**=30 **2**=96 **3**=103	P=0.25
Thick albumin spread (**1**,**2**,**3**)	**1**=33 **2**=63 **3**=146	**1**=25 **2**=82 **3**=120	**1**=34 **2**=73 **3**=136	**1**=17 **2**=90 **3**=122	P=0.31
Colour fan (**7**,**8**)	**8**=175 **7**=64	**8**=180 **7**=47	**8**=174 **7**=69	**8**=158 **7**= 71	P=0.24
Yolk membrane	201=good 41=poor	193=good 34=poor	195=good 48=poor	205=good 24=poor	P=0.41

*Small spread=3 Medium spread=2 Large spread=1

Categorical data which consisted of meat spots, blood spots, thin albumin spread, thick albumin spread and colour fan was also tested between the different processing methods. No differences (P>0.05) could be seen between any of the treatments. Blood spots in eggs are formed during the ovulation process. Blood vessels that cross the stigma line rapture as the follicle releases the ova causing blood spots. Blood spots are also confined to the yolk mass. Meat spots are seen as tissue rich deposits which are the result of oviduct breakdown and are confined to the albumin (Solomon, 2002). Stressful and frightening situations are possible causes of blood spots. Therefore it is understandable that no differences between these parameters would be noted between the treatments as all birds were in the same environment and care had been taken to keep the stress to a minimal as is normal in good production practices and as monitored by the animal ethical committee. Blood spots may also have a genetic component with some hens being more prone to blood or meat spots than others. Albumin quality is mostly affected by storage time and temperature (Samli *et al.*, 2005). Increased periods of storage and temperature cause increased albumin spreads (Omana *et al.*, 2011) resulting in a poor quality egg. Excess loss of water from the egg through evaporation due to temperature and relative humidity during the long-term storage conditions has been reported to be detrimental to table and hatching egg quality (Walsh *et al.*, 1995; Scott & Silversides, 2000). It has also been recorded by Silversides & Villeneuve (1994) that pH is a good tool for describing changes in albumin quality and storage time. Albumin pH increases over time due to a loss of CO_2 from the egg. This increase in pH will cause a decrease in viscosity of the albumin causing larger spreading. All eggs analysed in this trial were stored in a room at room temperature and analysed at similar time periods after being laid. Therefore time after lay or freshness and temperature were consistent across all three treatments leading to no differences (P>0.05) between diets in terms of albumin spreads. Factors influencing yolk/vitelline membrane strength are the same factors influencing albumen quality (Fromm & Lipstein, 1964) and therefore differences would also not be expected. Colour plays a major role in our perception of food quality. Surveys performed over 10 years in a number of European countries indicate that consumers are interested in certain characteristics of the egg such as shell strength, albumin consistency and yolk colour (Hernandez *et al*., 2005). It is true that consumers around most parts of the world prefer a deeply hued yolk (Beardsworth *et al*., 2004). Yolk colour in laying hens is primarily determined by the content and profile of pigmenting carotenoids present in their feed and can easily be adapted via feed ingredients (Hernandez *et al*., 2005). Yellow maize is a raw material which contains carotenoids and xanthophyll (Muzhingi *et al.*, 2008). Xanthophyll such as lutein and zeaxanthin have the greatest influence on the yolk colour. Beta-carotene as a representative of carotenoids is present only in small amounts. Each pigment has unique properties, e.g. colour hue and deposition efficiency. Real colour of the yolk (varying from pale yellow to dark orange) is determined by the proportion of the dietary intake of carotenoids that are absorbed and deposited in the egg yolk (Beardsworth *et al*., 2004). All diets in this investigation contained yellow maize in similar concentrations and therefore variation in colour fan readings between treatments would not have been expected.

4.5 Conclusion

For production parameters, egg lay percentage and ADG was found to be the lowest for the DR and the highest for FF and EX treatments. Feed conversion ratio was lowest for FF and highest for the control. With regard to the egg quality parameters, no statistical 14 day treatment effect was experienced; therefore data conclusions were made from the duration of the whole trial (day 1-41). The insect diets were either comparable or outperformed the control diet. A significant difference was only documented for the albumin weights.

Results obtained in this study are a clear indicator that BSFL meals processed using different techniques may be used in layer diets without adverse effects on the production performance and egg quality parameters. It is recommended that further research be done to corroborate these findings in order to further cement the standing of insects as a potential protein source in animal feed.

4.6 References

Abeyrathne, E.D.N.S., Lee, H.Y. & Ahn, D.U., 2013. Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents – a review. Poult. Sci. 92: 3292–3299.

Agunbiade, J.A., Adeyemi, O.A., Ashiru, O.M., Awojobi, H.A., Taiwo, A.A., Oke, D.B. & Adekunmisi, A.A., 2007. Replacement of fish meal with maggot meal in cassava-based layers' diets. J. Poult. Sci. 44: 278–282.

Beardsworth P.M. & Hernandes J.M., 2004: Yolk colour-an important egg quality attribute. Int. Poult. Prod. 12(5): 17–18.

Bellagamba, F., Caprino, F., Mentasti, T., Vasconi, M. & Moretti, V.M., 2015. The Impact of Processing on Amino Acid Racemization and Protein Quality in Processed Animal Proteins of Poultry Origin. Ital. J. Anim. Sci. 14(2): 3760–3770.

Bordas, A. & Minvielle, F., 1999. Patterns of growth and feed intake in divergent lines of laying domestic fowl selected for residual feed consumption. Poult. Sci. 78(3): 317–323.

Carrillo, S., Ríos, V.H., Calvo, C., Carranco, M.E., Casas, M. & Pérez-Gil, F., 2012. n-3 Fatty acid content in eggs laid by hens fed with marine algae and sardine oil and stored at different times and temperatures. J. Appl. Phycol. 24(3): 593–599.

Food and Agriculture Organization (FAO)., 2013. Edible insects Future prospects for food and feed security:1–201.

Fraeye, I., Bruneel, C., Lemahieu, C., Buyse, J., Muylaert, K. & Foubert, I., 2012. Dietary enrichment of eggs with omega-3 fatty acids: A review. Fd. Res. Int. 48(2): 961–969.

Friedman, M., 1999. Chemistry, Nutrition, and Microbiology of d-Amino Acids. J. Agric. Fd. Chem. 47(9): 3457–3479.

Fromm, D., & R. Lipstein., 1964. Strength, distribution, weight, and some histological aspects of the vitelline membrane of the hens egg yolk. Poult. Sci. 43: 1240−1244.

Hendriks, W.H., Butts, C., Thomas, D.V. & Ravindran, V., 2000. Protein quality of New Zealand meat and bone meals. Asian-Australasian J. Anim. Sci. 13(1): 1507–1523.

Hendriks, W.H., Cottam, Y.H. & Thomas, D.V., 2006. The effect of storage on the nutritional quality of meat and bone meal. Anim. Feed Sci. Technol. 127(1): 151–160.

Hernandes J.M., Beardsworth P.M. & Weber G., 2005: Egg quality – meeting consumer expectations. International Poultry Production. 13(3): 20–23.

Hopley, D., 2015. The evaluation of the potential of *Tenebrio molitor*, *Zophobas morio*, *Naophoeta cinerea*, *Blaptica dubia*, *Gromphardhina portentosa*, *Periplaneta americana*, *Blatta lateralis*, *Oxyhalao duesta* and *Hermetia illucens* for use in poultry feeds. MSc Diss. University of Stellenbosch, Stellenbosch.

Khusro, M., Andrew, N. R. & Nicholas, A., 2012. Insects as poultry feed: A scoping study for poultry production systems in Australia. Worlds Poult. Sci. J. 68(3): 435–446.

Lee, M.R.F., Parkinson, S., Fleming, H.R., Theobald, V.J., Leemans, D.K. & Burgess, T., 2016. The potential of blue lupins as a protein source, in the diets of laying hens. Vet. Anim. Sci. (1–2): 29–35.

Liardon, R. & Hurrell, R.F., 1983. Amino acid racemization in heated and alkali-treated proteins. J. Agric. Fd. Chem. 31(2): 432–437.

Man, E.H. & Bada, J.L., 1987. Dietary D-Amino Acids. Annu. Rev. Nutr. 7(1): 209–225.

Miles, R. D. & Jacob, J. P., 1997. Fishmeal: Understanding why this feed ingredient is so valuable in poultry diets. PhD diss. University of Florida,Florida.

Miller, E.L., 2004. Protein nutrition requirements of farmed livestock and dietary supply. In FAO Animal Production and Health Proceedings. University of Cambridge UK.29–47.

Muzhingi, T., Yeum, KJ., Russell, RM., Johnson, EJ., Qin, J. & Tang, G., 2008. Determination of Carotenoids in Yellow Maize, the Effects of Saponification and Food

Preparations. Int. J. Vitam. Nutr. Res. 78(3): 112–120.

Natoli, S., Markovic, T., Lim, D., Noakes, M. & Kostner, K., 2007. Unscrambling the research: Eggs, serum cholesterol and coronary heart disease. Nutr. Diet. 64(2): 105–111.

Omana, D.A., Liang, Y., Kav, N.N. V. & Wu, J., 2011. Proteomic analysis of egg white proteins during storage. Proteomics. 11(1): 144–153.

Piva, G., Moschini, M., Fiorentini, L. & Masoero, F., 2001. Effect of temperature, pressure and alkaline treatments on meat meal quality. Anim. Feed Sci. Technol. 89(1): 59–68.

Ravindran, V., 2013. Poultry Feed Availability and Nutrition in Developing Countries: Main Ingredients used in Poultry Feed Formulation. Poult. Dev. Rev. 2(10): 694–695.

Samli, H.E., Agma, A. & Senkoylu, N., 2005. Effects of Storage Time and Temperature on Egg Quality in Old Laying Hens. J. Appl. Poult. Res. 14(3): 548–553.

Samman, S., Kung, F.P., Carter, L.M., Foster, M.J., Ahmad, Z.I., Phuyal, J.L. & Petocz, P., 2009. Fatty acid composition of certified organic, conventional and omega-3 eggs. Food Chem. 116(4): 911–914.

Scanes, C.G., 2007. The Global Importance of Poultry. Poult. Sci. 86(6): 1057–1058.

Scott, T.A. & Silversides, F.G., 2000. The effect of storage and strain of hen on egg quality. Poult. Sci. 79(12): 1725–1729.

Silversides,F.G. & Villeneuve,P., 1994. Is the Haugh Unit Correction for Egg Weight Valid for Eggs Stored at Room Temperature? Poult. Sci. 73(1): 50–55.

Solomon, S.E., 2002. The oviduct in chaos. Worlds. Poult. Sci. J. 58(1): 41–48.

Van Schoor, A., 2017. The assessment of black soldier fly (*Hermetia illucens*) pre-pupae , grown on human faecal waste , as a protein source in broiler and layer diets. MSc Diss. University of Stellenbosch, Stellenbosch.

Vrabec, V., Kulma, M. & Cocan, D., 2015. Bulletin of University of Agricultural Sciences and Veterinary Medicine Animal science and biotechnologies. University of Agricultural Sciences and Veterinary Medicine. 72(2): 116–126.

Walsh, T. J., R. E. Rizk, & J. Brake., 1995. Effects of temperature and carbon dioxide on albumen characteristics, weight loss, and early embryonic mortality of long stored hatching eggs. Poult. Sci. 74(9): 1403–1410.

Whitehead, C.C., Bowman, A.S. & Griffin, H.D., 1991. The effects of dietary fat and bird age on the weights of eggs and egg components in the laying hen. Br. Poult. Sci. 32(3): 565–574.

Willis, S., 2003. The use of soybean meal and full fat soybean meal by the animal feed industry. 12th Australian soybean conference: 1–8.

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

Chapter 5

General conclusion

The aim of this study was to determine whether black soldier fly larvae (*Hermetia Illucens*) processed using three different techniques, namely a full fat, or a dry rendered or as an extruded meal may be used as an alternative supplementary protein source for layer hens without any health implications for the layer or any adverse effects on overall egg production.

There is minimal literature available about the use of different insect species as a viable protein source to layer hens. Available data which has been recorded using the black soldier fly larvae produced desirable outcomes. Not only are these insect species attractive as a nutritive source, but also due to their ability to be reared on a variety of waste streams. As mentioned previously, soya meal and fishmeal are the most commonly used protein sources in poultry diets. It is also known that due to competition with human consumption for soya and dwindling fish stocks for fishmeal, these sources will need to be replaced by an alternative protein source in animal feeds.

In order to determine if the processed BSFL using the three different methods could be used, incorporation into the layer hens' diet was done after the nutritional profile of the BSFL was determined. Each treatment was included into the ration at a level of 15%. The treatment diets were all compared to a control diet which was predominantly soya based.

The dry rendered (DR) meal had the highest crude protein value of 48.18%; this value was comparable to that of soya oilcake. The highest fat value (42.90%) was found in the full fat (FF) larvae. The amino acid profiles for all three processing techniques were favourable. The FF and DR diets had the lowest feed conversion ratios. The FF and extruded (EX) meal had the highest egg lay percentage while the DR meal had the lowest. This result may be due to the processing method where a large amount of heat is used during the EX processing. This may have an effect on nutritional components of the larvae which would affect production performance of the layer.

The layer trial produced positive results in terms of production and egg quality parameters. The treatment diets were comparable to the control diet in all the egg quality parameters. The treatment diets' egg weights were all larger than the control diet. The FF larvae produced the heaviest egg weight. The shell thicknesses for the treatments were all marginally lower than the control. However, this might not be a fair reflection on the performance of the insect meal. If tools with higher accuracy and less variation of position of measurement (where the egg shell thickness was measured per egg) and a larger amount of replications were done, this result may have been different. All the insect diets proved they could be used as viable commercial rations with little variation between them in terms of production and egg quality parameters.

Results in this thesis favour the use of the BSFL using different processing techniques as an alternative protein source. With critical analysis of this trial, minor adjustments could have been made to increase overall accuracy if it were to be repeated. The use of a larger flock would add to the accuracy of the data collected. Temperatures were often high in the bird house; sufficient ventilation could have had positive effects on the results.

More trials should be conducted using the different processing techniques in order to verify their effectiveness in layer hen feeds. These processing methods should also be done on different insect species to study the effect that they may have when used in animal feeds of different animal species. Another area of research which could be investigated is the nutritional concentration of certain insect minerals and vitamins so as to increase these nutritional components found within the egg. As mentioned previously, insects are a good source of minerals, vitamins and amino acids. The concentrations of these compounds are also dependent on the medium on which they are reared. With these properties, the feeding of insects which have been reared on a nutrient rich medium to layer hens could allow for the production of "designer eggs" which will be beneficial for human consumption. Overall the trial proved that the use of BSFL processed using different methods can be incorporated into the layer hen diet without any adverse effects and could be used as an alternative protein source to conventional protein sources.