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

by

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Declaration

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

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Summary

The 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.

Insect protein as a source in poultry feed is slowly gaining popularity in terms of research. Therefore the purpose of this study was to look at unexplored aspects of insect protein, specifically its role in animal feeding. This involved investigating the chemical composition of selected insect species who have yet to gain popularity in this field, possible effects on gizzard erosion in broilers and total tract digestibilities, and also layer production and egg quality characteristics.

In the first study, only eight of the potential nine insect species were selected for proximate analysis. The purpose of this study was to determine and evaluate the chemical composition of some insect species which have yet to be used in livestock feeds and which have the potential for mass rearing. On this basis, the following species were selected: *Tenebrio molitor*, *Zophbas morio*, *Naophoeta cinerea*, *Blaptica dubia*, *Gromphardhina portentosa*, *Periplaneta americana*, *Oxyhaloa duesta* and *Blatta lateralis*. These species were reared at the Department of Animal Sciences of Stellenbosch University. With 60.34%, *N. cinerea* yielded the highest CP value, which is comparable to that of fishmeal. The protein value for the other species were comparable to that of soya oilcake meal, with values ranging from 43.13% to 55.28%. The amino acid profiles for *G. portentosa*, *P. americana* and *B. lateralis* related best to the ideal amino acid profile for broilers.

The purpose of the second study was to evaluate the possible effects of mealworms (the larvae of *T. molitor*) and the larvae- and pre-pupae of the black soldier fly (*Hermetia illucens*) on gizzard erosion in broilers. These two species were selected since they were easy to acquire in the required volumes. Also, the information available on *H. illucens* mainly regards animal broiler production parameters, but not this specific animal factor. Results of the study indicate the following: The mealworms caused significant ($P < 0.05$) gizzard erosion, whereas the others did not. The erosion may have been due to the high histidine content of the mealworms, which may have been transformed into histamine thereby causing erosion. Histidine may have also been transformed into gizzerosine, a potent inducer of gizzard erosion, during the drying process. The erosion observed may have also been due to the presence and form of chitin in the mealworms. Chitin is structurally similar to fibre, which was presented in a coarse form. Coarse fibres have been shown to increase the acidity of gizzard contents, which may lead to gizzard erosion.

In the third study the total tract digestibility for mealworms was evaluated and compared to values from studies on other species as well as that of soya oilcake meal and fishmeal. It was found that the dry matter (DM) digestibility for mealworm meal is similar to that of housefly larvae meal and housefly pupae meal, but lower than that of black soldier fly meal. The CP digestibility was similar to that of black soldier fly meal and soya oilcake meal, but higher than that of housefly larvae meal and

housefly pupae meal. The coefficient of total tract digestibility (CTTD) values for methionine and threonine are similar to that of soya oilcake meal and fishmeal. The CTTD value for lysine, however, was lower than all other protein sources presented. The low digestibility value may be attributed to specific processing conditions, especially overheating.

The effects of the inclusion of mealworm meal and black soldier fly larvae- and pre-pupae meal on layer production performance and egg quality characteristics were evaluated by comparing to a balanced control diet. There were no significant ($P > 0.05$) differences in average daily gain (ADG) between treatments. The feed conversion ratio (FCR) for the black soldier fly pre-pupae meal was significantly ($P < 0.05$) lower than the other treatments. The egg weights for the control diet was significantly ($P < 0.05$) less than the treatment diets. There were however no significant differences between the other treatments. The yolk weights did not differ significantly between the treatments and control. The shell weight for the mealworm diet was significantly higher than that of the control and the black soldier fly larvae- and pre-pupae diets. The albumin weight and albumin height for the mealworm diet differed significantly from the control, although it did not differ significantly ($P < 0.05$) from the rest.

General results are in support of the use of these insects as protein in poultry feeds.

Opsomming

Evaluering van Tenebrio molitor, Zophobas morio, Naophoeta cinerea, Blaptica dubia, Gromphardhina portentosa, Periplaneta americana, Blatta lateralis, Oxyhalao duesta and Hermetia illucens vir gebruik in pluimvee voeding

Insek proteïen in pluimveevoeding raak al hoe meer gewild in terme van navorsing. Die hoofdoel van hierdie proef was om na onverkende aspekte van insek proteïen te kyk, veral die rol wat dit speel in dierevoeding. Dit behels die volgende: evaluering van die chemiese samestelling spesifiek van geselekteerde insek spesies, moontlike newe effekte op spiermaag gesondheid, asook die verteerbaarheid daarvan en laastens die effek op lê-hen produksie en eier kwaliteit eienskappe.

In die eerste studie was agt insek spesies geselekteer vir chemiese ontleding, nl. *Tenebrio molitor*, *Zophobas morio*, *Naophoeta cinerea*, *Blaptica dubia*, *Gronphardhina portentosa*, *Periplaneta americana*, *Oxyhaloa duesta* en *Blatta lateralis*. Tans word hierdie spesies nie in veevoere gebruik nie. Hierdie spesies toon ook die vermoë vir grootskaalse groot maak. Die resultate vir die studie is as volg: Die ru-proteïen (RP) inhoud van *N. cinerea* was die hoogste met 60.34%. Hierdie waarde kan met die van vismeel vergelyk word. Die RP waarde van die ander spesies (43.13 - 55.28%) kon met die van sojaboon meel vergelyk word. Die aminosuur profiel van *G. portentosa*, *P. americana*, en *B. lateralis* kon met die ideale aminosuur profiel vir braaikuikens vergelyk word.

Die doel van die tweede studie was om insek spesies te evalueer vir moontlik newe effekte op spiermaag gesondheid, veral spiermaagerosie. Meelwurms en die larwes- en pre-papies van *Hermetia illucens* is vir hierdie studie gebruik. Die meelwurms het betekenisvolle ($P < 0.05$) spiermaag erosie veroorsaak, terwyl die *H. illucens* larwes en pre-papies geen erosie veroorsaak het nie. Die moontlike oorsaak van erosie is die hoë histidien inhoud van meelwurms. Histidien het ’n neiging om na histamien te verander wat tot erosie in die spiermaag kan lei. Dit is ook moontlik, gedurende die drogingsproses, dat histidien in gizzerosien omgeskakel kon word. Gizzerosien is bekend as die oorsaak van spiermaag erosie in braaikuikens. Die teenwoordigheid en vorm van chitien in meelwurms kon ook ’n bydraende faktor gewees het. Die bouvorm van chitien is soortgelyk aan die van vesel, wat in ’n growwe vorm gevoer is. Dit is moontlik dat growwe vesel die pH van die spiermaag kan laat styg wat tot spiermaag erosie kan lei.

In die volgende studie was die totale spysverteringskanaal verteerbaarheid van meelwurms geëvalueer. Waardes wat gevind was, is vergelyk met die van vorige studies op insekte asook die van vismeel en sojaboonmeel. Resultate toon aan dat die droë materiaal (DM) verteerbaarheid van meelwurms soortgelyk is aan die van huisvlieg larwe- en papie meel, met onderskeidelike waardes van 0.80, 0.81 en 0.8. Die ru-proteïen verteerbaarheid van die meelwurms was soortgelyk aan dit van *H. illucens* pre-papie meel en sojaboonmeel, maar hoër as dit van huisvlieg larwe- en papie meel, met waardes van 0.90, 0.90, 0.85, 0.69 en 0.79, onderskeidelik. Die verteerbaarheid van metionien en treonien is soortgelyk aan die van sojaboon meel en vismeel. Die totale spysverterings verteerbaarheid waarde vir lisien (0.74) was egter laer as al die ander proteïen bronne. Hierdie laer verteerbaarheid kan moontlik toegeskryf word aan spesifieke verwerkingsomstandighede, veral oorverhitting.

In die finale studie, was die effek van meelwurms, *H. illucens* larwe en pre-papie meel as supplimentêre proteïenbron in lê-hen diëte geëvalueer. Deur middel van vergelyking met ŉ kontrole, was daar spesifiek gefokus op lê-hen produksie en eier kwaliteit eienskappe. Daar was geen betekenisvolle ($P < 0.05$) verskille in gemiddelde daaglikse toename tussen behandelings en kontrole nie. Die voeromsettingsverhouding vir die *H. illucens* pre-papie meel was betekenisvolle ($P < 0.05$) laer as die van die ander behandelings en kontrole. Die eier gewigte van die kontrole was betekenisvol ($P < 0.05$) laer as die van die ander behandelings. Daar was ook geen betekenisvolle ($P < 0.05$) verskille in eiergeel gewigte tussen die behandeligs en kontrole. Die dop gewigte van die meelwurms was betekenisvol ($P < 0.05$) hoër as die van die kontrole en ander behandelings. Die albumien gewigte en hoogte vir die meelwurm dieet het betekenisvol ($P < 0.05$) verskil van die kontrole, maar het nie betekenisvol ($P < 0.05$t) van die ander behandelings verskil nie.

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List of abbreviations

ADG – Average daily gain

AME – Apparent metabolisable energy

ANOVA – Analysis of variance

BBSF – Black soldier fly pre-pupae

CP – Crude protein

CTTD – Coefficient of total tract digestibility

DM – Dry matter

FCR – Feed conversion ratio

GE – Gross energy

HLM – Housefly larvae meal

HPM – Housefly pupae meal

MW – Mealworms

N - Nitrogen

TME – True metabolisable energy

WBSF – Black soldier fly larvae

Notes

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

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Chapter 1
General Introduction

Poultry products, including meat and eggs, have always been a major source of animal protein for humans (Stenhouse, 2008). With a reported feed conversion ratio ranging from 1.5 to 1.8 (Sengor *et al*., 2008), poultry production has proven to be a highly efficient industry. This makes chicken one of the most economically viable and important source of meat for human consumption (Khusro *et al*., 2012). The global consumption of chicken has steadily increased over the past few years, a trend which is expected to continue. Furthermore, it is expected that most of the global demand for poultry products will be in developing countries (Ravindran, 2013). The increase in poultry meat consumption may be due to the continuing rise in the human population. Poultry meat is generally cheaper in comparison to other meats, and is acceptable to most religions. Globally, poultry meat is the second most consumed behind pork (Khusro *et al*., 2012). Such growth in the industry has an enormous effect on the demand for raw feed ingredients (Ravindran, 2013).

It is becoming increasingly difficult to meet requirements for the raw materials such as maize, soya oilcake meal, and fishmeal, products that are generally used in poultry feeds. The gap between local supply and demand is expected to widen in the coming decades (Ravindran, 2013). This will be mainly due to the increase in population size and unfavourable climatic conditions (Cribb, 2010). Poultry production is largely based on grain, a product also used for human consumption and therefore a high value commodity, which combined with increasing consumption of chicken globally, has seen the cost of grain increase significantly. The increasing cost of grain has prompted the poultry industry to explore alternative sources for use as raw materials in poultry diets (Khusro *et al*., 2012).

Second to energy supplying raw materials, protein supplementation constitutes the largest component of poultry diets. The major plant protein source is soya, and the major animal protein source is fishmeal (Barrows *et al*., 2008; Ravindran, 2013). The role of fishmeal in poultry production is quite significant (Ijaiya & Eko, 2009). It is a feed with a very high nutritional value and is highly digestible. The protein of fishmeal has a high biological value because it is rich in amino acids (Miles & Jacob, 1997; Karimi, 2006). In most developing countries, fishmeal is an important source of animal protein. However, its production, availability, and cost are major concerns for animal nutritionists. Fishmeal is very scarce and expensive and its inclusion in poultry diets may prove to be unprofitable (Ijaiya & Eko., 2009). Also, further expansion possibilities in the fishmeal industry appear to be limited. Production does not seem to have increased over the last 20 years, and given the pressure on world fisheries, is unlikely to do so in the future. Fishmeal has also been included in the overall animal protein ban in Europe, and there is an underlying concern about possible pollutants (e.g. dioxin) levels in fishmeal (Ravindran, 2013).

Soya oilcake meal is also a commonly used source of protein in poultry diets (Willis, 2003; Ravindran, 2013) due to its excellent amino acid composition and high level of digestibility (Willis, 2003). Although a marked increase in soya production has been witnessed over the past few years (Ravindran, 2013), the availability of soya for use in animal nutrition is limited due to competition with human consumption thereof (Ravindaran & Blair, 1992; Ravindran, 2013). Furthermore, more than 50% of the current global crop is genetically modified (GM), mainly for herbicide tolerance, and there is an ongoing debate and campaign to reject GM ingredients from animal diets. If GM sources are

not accepted in the market place the potential for further nutritional quality enhancement and increased productivity will be limited (Ravindran, 2013). There is thus a need to identify alternate

ve protein sources either for total or partial replacements which meet the dietary requirements and reduce feed costs (Ramos-Elorduy *et al*., 2002; Das *et al*., 2009, Razak *et al*., 2012).

Another concern is the effect the large scale use of soya and fishmeal has on the environment. From a global perspective, the increased production of soya has led to the deforestation of areas with a high biological value (Osava, 1999) and high water consumption (Steinfeld *et al*., 2006). The utilization of pesticides and fertilizers and transgenic varieties (Garcia & Altieri, 2005), may cause significant environmental deterioration (Osava, 1999). Furthermore, the production of fishmeal is dependent on the catch, and is therefore qualitatively and quantitatively variable. In addition, the deterioration of the marine environment and stripping of fisheries have resulted in a decrease in fishmeal production and an increase in the price. This trend to increase price is likely to continue with consequent economic repercussions on animal production. The situation reveals the importance of renewable sources of protein (Manzano-Agugliaro *et al*., 2012).

Among potential protein sources that could replace soya oilcake meal and fishmeal is insect protein (Finke, 2002; Premalatha *et al*., 2011; Razak *et al.,* 2012). In nature, insects form a significant biomass, as can be seen with insect pests (Ramos-Elorduy, 1997). There are approximately one million known species of insects, although it has been estimated that that the global diversity is as high as 80 million (Erwin, 2004). Insects are mainly primary consumers, and with their high reproduction rate, tend to dominate energy sources due to competitive exclusion (Ramos-Elorduy, 1997). In modern society, the true value of insects is overlooked and therefore underestimated. For example, insects can be used in both human and animal nutrition, in medicine, and they also have the ability to recycle organic waste.

Insects have been evaluated as potential feedstuff for use in livestock diets since the 1970s (Finke, 2002). Insects can be used to produce cheaper protein from non-food animals. Insects are part of the natural diets of poultry (Zuidhof *et al*., 2003), and scavenging poultry consume a wide variety, including grasshoppers, crickets, termites, acridids, scale insects, beetles, caterpillars, pupa, fleas, bees, wasps and ants (Ravindran, 2013). Insects have a high nutritive value, not only in proteins, but for fats, minerals and vitamins (Chapman, 1998; Khusro *et al*., 2012). Insect protein is reported to range from 40% to 75% (Khusro *et al*., 2012; Ravindran, 2013). And it is particularly for this reason that they are considered to be a promising animal feed ingredient, together with the fact they have a short life cycle and are easy to produce and handle (Ramos-Elorduy *et al*., 2002).

The purpose of this study was to investigate the value of some insect species with the potential for mass rearing, namely, mealworms (larvae of the beetle *Tenebrio molitor*), superworms (larvae of the beetle *Zophobas morio*), Lobster roach (*Naphoeta cinerea*), Orange spotted roach (*Blaptica dubia*), Madagascar hissing roach (*Gromphardhina portentosa*), Palmetto roach (*Periplaneta americana*), the Cape red roach (*Oxyhaloa duesta*) and the Turkestan roach (*Blatta lateralis*). This study also investigated some aspects of insect use in animal feed that has yet to be explored or of which little information exists. These aspects include the effects of Mealworms and the larvae- and pre-pupae of *Hermetia illucens* on layer production and egg quality, as well as the possible effect thereof on gizzard erosion in broilers. The digestibility of mealworms in broilers were also determined.

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Chapter 2
Literature Review

2.1 Introduction

The combined effects of population increase and increasing standards of living, particularly in developing countries, are expected to create a high demand for animal protein by the year 2050. New initiatives will be required to produce the necessary quantities of high quality protein (Boland *et al*., 2013). This is especially important, since over the past decade we have seen a significant increase in the global consumption of poultry products, particularly poultry meat (Ravindran, 2013). Since it is one of the most readily available and cheaper sources of animal protein for human consumption (Khusro *et al*., 2012), this trend is expected to continue. The demand for poultry products in turn leads to a demand for raw materials used in the manufacture of poultry feeds, a commodity which is becoming more difficult to obtain (Pemalatha *et al*. 2011). Thus, there is a concern that the requirements for traditional ingredients (maize, soya, and fishmeal) used in poultry feeds cannot be met. This concern stems from the fact that products such as maize and soya are also used for human consumption. The competition and demand for these products has led to an increase in costs (Ravindran & Blair, 1992). Therefore, a major problem facing the poultry industry is the provision of feeds that will contain all the necessary components for birds to grow rapidly within a short period of time (Oyegoke *et al*., 2006). Associated with this is a need for supplementing grain based rations with good quality protein (Ijaiya & Eko, 2009). It is therefore important that a wide range of initiatives be explored to close the ever widening gap between supply and demand of these products (DeFoliart, 1975; Boland *et al*., 2013).

The world food deficit has resulted in an expanded effort to find new sources of protein (DeFoliart, 1975). Efforts include shifting protein sources up the supply chain, use of plant based substitutes or extenders for animal derived protein foods, and use of novel sources for both animal and human nutrition (Boland *et al*., 2013). Currently, insects are being investigated as an alternative source of protein for animal feed (Premalatha *et al*., 2011). This is not surprising, since insects are a natural food source for many animal species (DeFoliart, 1975). Most insects have the ability to efficiently convert low quality plant protein to high quality insect protein resulting in a high protein yield when compared to other breeding animals. The body tissue of edible insects can be expected to contain 44-77% protein, while their plant foods only contain 9-10% protein (Ramos-Elorduy, 1987; Ramos-Elorduy *et al*., 1997; Ramos-Elorduy *et al*., 2002). According to Ueckert *et al*. (1972), insects have a high nutrient value in terms of protein, carbohydrate, fat, vitamins, and minerals. Dufour (1987) suggested that the protein content of ant, termites, and caterpillars are higher than that of dried fish. In a study by Finke *et al*. (1985) it was found that broilers had significantly higher weight gains when soya oilcake meal was replaced with Mormon crickets in the diet (Anand *et al*., 2008). Insects can therefore be used as protein source in livestock diets, and indirectly serve as a protein source for humans (Ramos-Elorduy *et al*., 2002).

2.2 Global utilization of insects

Since insects are the most species' rich taxon in the animal kingdom, they display an immense degree of biodiversity and represent a colossal biomass in nature (Chakravorty *et al.,* 2011). It has been estimated that there could be as much as 80 million insect species globally, although there are only approximately one million known species (Erwin, 2004). Grimaldi (2005) suggested that the

number of insects that have been named and described only constitute 20% of the total. It has also been suggested that insects represent at least 58% of the known global diversity (Footit & Adler, 2009).

2.2.1 Entomophagy

As humans, we have the potential to access a wide variety of food sources from both plant and animal origin. We have, however, limited our food selection base to a restricted number of plant and animal species from which we are to obtain all necessary nutrients. This is especially apparent in developed first world countries. Furthermore, globalization has led to the spread of so-called Western dietary habits to developing countries resulting in loss of food diversity (Paoletti, 2005). Also, globalization has led to changes in both environmental and human systems, which in turn have altered ecosystems throughout the world. Thus, natural resources have acquired high value because they are important to life and the survival of human beings. Among these resources are edible insects (Ramos-Elorduy, 2006).

In previous years, very little attention has been paid to broadening our food resources. This is especially true for food of animal origin. For the layman, the animal kingdom is divided into four broad groups, comprising animals permitted to be eaten, pests, pets and others (Harris, 1998). However, thousands of insects are consumed by humans as food. This fact has been extensively reported in many publications. (Menzel *et al*., 1998; Paoletti, 2005).This practice is referred to as entomophagy (Ekpo *et al*., 2009; Yen, 2009; Chakravorty *et al*., 2011).

Although entomophagy may sound foreign and unfamiliar, it has been practiced for centuries (Sutton, 1988), and as such, has played an important role in the history of nutrition (DeFoliart, 1995). Several species such as grasshoppers and weevils are considered pests; however, many are significant sources of food (Womeni *et al*., 2009). A fact which is especially important in poorer, developing countries where the availability of animal protein is significantly limited (Riggi *et al*., 2013). It is in these countries where entomophagy may in fact be encouraged (Womeni *et al*., 2009). Latham (2003) was of the view that eating insects may appear novel or even repugnant, yet they represent an important high protein food for many rural families in Africa. For example, in Zambia insects serve as a valuable source of animal protein since wild animals are rare (Mwizenge, 1993). Furthermore, in some ethnic groups, insects may provide 5-10% of annual protein as well as fats, energy, vitamins and minerals (McEvilly, 2000). In Nigeria, studies indicate that the practice of entomophagy has significantly alleviated protein deficiencies (Ashiru, 1988; Fasoranti & Ajiboye, 1993). Also, in a study conducted by Dufour (1987) it was shown that in Colombia, insects contributed to 12% of crude protein (CP) of animal origin in men's diets and 26% in women's diets. In many cases insects are consumed to compensate for a decreased availability of fish and game (Dufour, 1987; DeFoliart, 1995; Yen, 2009).

Besides the use of insects as a dietary supplement, the practice of entomophagy also bears cultural significance, especially to people who form part of indigenous populations (Sutton, 1988; DeFoliart, 1990; McEvilly, 2000). For these people, insects are more than just a supplement, but form a regular part of the diet. This is apparent in many Asian, African, Central American and South American cultures (McEvilly, 2000). They therefore place a high value on the insects they consume (DeFoliart, 1989). For example, in Australia, honey ants, witjuti grubs and bardi grubs are important dietary items of aboriginal societies (Yen & Paoletti, 2005). There are also some instances where insects are preferred to meat. For example, the Pedi of South Africa prefer certain insects to beef (DeFoliart, 1989) as do the Yukpa of Colombia and Venezuela (Ruddle, 1973).

An esttimated2086 insect species are consumed by approximately 3071 ethnic groups (Ramos-Elorduy, 2006). DeFoliart (1989, 1990) reported that a wide variety of species of edible insects are prominent items of commerce in the town and village markets of Africa and tropical and semi-tropical regions of the world. According to Ruddle (1973), it has not been possible to get the precise number and identity of edible insects and it is only in a few countries that attention has been given to fully study insect species eaten. But even so, it is apparent that insects may prove to be a valuable food resource. It is thus a shame that all over the world monies worth billions are spent every year to save crops that contain no more than 14% plant protein by killing another food source (insects) that may contain up to 75% of high quality protein (Premalatha *et al.*, 2011).

2.2.1.1 Lepidoptera

The order Lepidoptera consists of butterflies and moths. They are one of the largest orders among insects, and may number up to 100 000 species. They have a wide global distribution and may occur from the extreme arctic to the tropical rainforest and the most arid desert. One of the many benefits of Lepidoptera includes its use as a food source (Klots, 1958). Approximately 80 genera in 20 families are used as food. For most of the species, it is the larvae (caterpillar) that is consumed, although the pupae is used in the species valued as silk producers in the families such as the Bombycidae, Notodontidae, and Saturniidae (DeFoliart, 1995). The use of Lepidopterans as a food source is most common in Africa, where more than 20 species have been reported to be consumed in some countries (Malaisse & Parent, 1980).

A well-known species consumed by many Africans is the caterpillar, *Gonimbrasia belina*. *G. Belina* is a moth species indigenous to Southern Africa. The large edible caterpillar, more commonly referred to as the mopane worm, is an important source of protein to many people native to Southern Africa (DeFoliart, 1995; Glew *et al*., 1999). It has been reported that the Pedi people of South Africa prefer mopane worms to beef and further reports indicate that when mopane worms are in abundance, the sale of beef takes a serious dive.

In some studies, it was found that mopane worms contain, on average, 48.3% protein (Glew *et al*., 1999; Greyling & Potgieter, 2004) and the amino acid profile was adequate enough to compare to that of soya. The amino acid profile also compares well to the Ideal amino acid profile for consumption by humans and broilers as noted in Table 1. Swatson *et al*. (2003) found that the available lysine concentration was, on average, 30.4 g/kg. The study also indicated AME and TME values of 11.34 MJ/kg and 22.12 MJ/kg, respectively. The larvae also appear to be a good source of essential fatty acids, particularly linoleic acid and α-linoleic acid. Furthermore, they also contain several essential minerals, including calcium, magnesium, manganese, and zinc (Glew *et al*., 1999). It is thus evident that the larvae of *G. belina* are an excellent source of many nutrients essential for growth in both humans and animals (Glew *et al*., 1999; Swatson *et al*., 2003).

Table 1 A comparison of the amino acid profile of mopane worms to the ideal amino acid profile for broilers and humans (values calculated as a percentage of lysine)

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

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

A second widely known species of the order Lepidoptera, is *Bombyx mori*, more commonly referred to as the silkworm or mulberry silk moth. This particular species may be the most thoroughly domesticated insect species of the world. It is not surprising, therefore, that it has been used as a food source for both humans and animals, since silkworm pupae is readily available due to silk production, especially in Eastern and South-eastern Asia (DeFoliart, 1995). It has been reported, that in Thailand, silkworm pupae are a popular food and quite expensive in the markets (DeFoliart, 1995). Furthermore, the Nutrition Division of the Thai Ministry of Public Health (1987) include silkworm pupae among the local foods that can be used in supplementary food formulae it developed for malnourished infants and pre-school children. Also, from an animal nutrition perspective, *B. mori* pupae are also fed to fish and chickens in Japan and India. Not only the pupae, but waste material from the reeling factories is used as fish food in pond fish culture in China (Kiuchi & Tamaki, 1990).

2.2.1.2 Coleoptera (beetles, weevils)

Species representing more than 100 genera in 17 families are used as food (Defoliart, 1995). Beetles are believed to be one of the most diverse groups in the animal kingdom. The number of described forms is estimated at around 350 000. The Coleoptera is an order which is rather rich in species which can be found in a wide range of environments, both terrestrial and freshwater. Most beetles are known for their role as pests of crops or stored products. However, there are some that are valuable as predators to suppress a wide range of arthropods (New, 2007). Major families include Cerambycidae, Batocera, Tenebrionidae, Curculionidae, Buprestidae and Dystiscidae (DeFoliart, 1995).

From the Cerambycidae family there are at least 30 genera whose larvae are used as food source. Species of the Batocera are the most widely eaten, being reported from Indonesia, Philippines, Sri-Lanka, and Papua New Guinea (Mercer, 1993). From the Curculionidae Family there are at least a dozen genera used as food, but several species of the Rhynchophorous (palm weevils) family are by far the most important and widely used. Their larvae are greatly esteemed. The major species are *Rhynchophorous palmarum* in the Western hemisphere, *Rhynchophorous phoenicis* in the Africa, and *Rhynchophorous ferrugineus* and *Rhynchophorous bilineatus* in southern Asia, Indonesia and the western Pacific (DeFoliart, 1995).

Another important family is that of Tenebrionidae. A major species from this family is *Tenebrio molitor*, whose larvae, commonly referred to as mealworms, is fed to animals. *T. molitor* is indigenous to Europe, but is currently distributed worldwide (Ramos-Elorduy *et al*., 2002). This species is commonly found in grain and flour stores as pests (Ng *et al*., 2001; Ramos-Elorduy *et al*., 2002). There are however many people who culture their larvae on a large scale for use as food for insectivorous animals used in captivity (Allen & Oftedal, 1989; Ng *et al*., 2001).

2.2.1.3 Hymenoptera

The hymenoptera is one of the largest orders of terrestrial arthropods and comprise the sawflies, wasps, ants, and bees and parasitic wasps (Gauld & Bolton, 1988; Austin & Dowton, 2000). The immature egg stages of Hymenopterans are used as food by indigenous populations in Africa, Asia, Australia, South America and Mexico. They are largely considered as delicacies. In addition to the Apidae and Formicidae, the Vespidae (wasps, hornets) is a family of major food importance (DeFoliart, 1995).

Apidae (Bees)

Larvae and pupae, sometimes called grubs, are widely eaten and often are as highly prized as the honey bee. The Kyapo in the state of Para, Brazil, recognize 56 species of bees, mainly on the basis of ecological niche and behavioural characteristics (Posey, 1983; Posey & Camargo, 1995). Nine species *Apis mellifera* and eight species of stingless bees (subfamily Meliponinae) are semi-domesticated, or at least to some extent manipulated. The larvae and pupae of seven of those species (genera Trigona, Oxytrigona, Scaptotrigona and Tetragonisca) are used as food. In Mexico at least eight species of Meliponae are used (Conconi *et al*., 1984). In Sri-Lanka, the Vedda tribal minority are accustomed to eating bee brood larvae of the giant honey bee, *Apis dorsata* (Nandasena *et al*., 2010).

Formicidae (Ants)

Of all the edible ants, the leaf cuttings, fungus growing ants of the genus Atta are the most fascinating. The genus is restricted to the Western hemisphere. The winged females are collected as they swarm from the nest during the early part of the rainy season. The part eaten is the abdomen. Two species *Atta cepholates* and *Atta sexdens* are the most widely consumed, being relished across the Northern half of South America. They are also preserved and stored for later consumption.

In Southern and Central Africa, the winged sexual stages of *Carebara vidua* are collected as they emerge from their nests after heavy rains. In South Africa, according to Quinn (1959), these ants played an important role in the Pedi diet. In Asia, *Oecophylla smaragadina*, known as the red tree ant, is widely eaten in India, Burma, Thailand and Papua New Guinea. All stages are consumed. In Thailand they are made into salad, fried with eggs or put into bamboo shoot soup (DeFoliart, 1995).

2.2.1.4 Orthoptera

Orthoptera, like many other insects, are highly nutritious and contain large amounts of protein. Various grasshopper, katydid and cricket species are already used for pet and zoo animals (Barker *et al*., 1998; Finke, 2002). Edible species are known in more than 50 genera in seven families. Acrididae (short-horned grasshoppers), Gryllidae (crickets) and Tettigonidae (long-horned grasshoppers) are important families.

Acrididae

Edible insect species are known in about 30 genera. There has been an increased interest by scientists and government in some countries in recent years in harvesting pests as food. In 1983, farmers in Thailand began collecting grasshoppers for sale as an alternative to government sponsored pesticide spraying that was not effective. Also, in Mexico, Chapulines, which are grasshoppers of the Sephanarium genus, and notably *Sephanarium purpurascens*, a pest of alfalfa, are popular edible insects (Cohen *et al*., 2009). In eastern and southern Africa, *Ruspolia differens*, a katydid, is a common food source. In Japan, China, Korea, rice field grasshoppers (including *Oxya yezoensis*, *Oxya velox*, *Oxya sinuosa* and *Acrida Lata*) are harvested for food. In China, the Chinese

grasshopper, *Acrida cineara* was evaluated for possible use in broiler feeds, since they are found in dense concentrations in most areas of China. Also, the grasshoppers were utilized as a human food in central China for centuries. Furthermore, it could be mass reared under controlled conditions (Wang *et al*., 2005).

Locusts are also valuable. Locusts are a group of grasshopper species that become gregarious and migratory when their populations are dense enough. During the swarming phase, locusts destroy or severely damage crops. They are major pest of historical importance in Africa, Australia and the Middle East. A locust swarm can represent a considerable biomass; a single swarm can consist of up to 10 billion insects and weigh up to 30 000 ton (DeFoliart, 1989; Ramos-Elorduy *et al*, 1997). The swarming behaviour makes locusts relatively easy to harvest for food. In Africa, the desert locust (*Schistocera gragaria*), the migratory locust (*Locusta migratoria*), the red locust (*Nomadacris septemfasciata*) and the brown locust (*Locustana pardalina*) are commonly eaten.

Gryllidae

Several species of cricket are used by non-European cultures, notable among which are the Taiwan Giant cricket (*Brachytrupes portentosus)* throughout Asia, and the tobacco cricket (*Brachytrupes membranaceus)* throughout East Africa. Although crickets are not usually thought of as food for humans, the house cricket (*Acheta domesticus*) is one of several insects recommended by Taylor & Carter (1976) for inclusion in their gourmet recipes. The house cricket is a cosmopolitan and easily available insect. Nutritionally, the crickets has been shown to be a protein source of high quality in feeding trials with chicks (Nakagaki & DeFoliart, 1987) and rats (Finke *et al*., 1989).

2.2.2 Entomotherapy

Entomotherapy refers to the medicinal use of insects. It is practiced by many cultures acroos the world (de Figueir, 2015). Insects contain certain substances which may be extracted and used for medicinal purposes (Dossey, 2010). Several authors have studies the medicinal potential of insects. Many observations have been made in cultural practices, but also in the clinical setup (Costa-Neto, 2005). In some studies it was found that termites may be used to treat influenza, asthma, bronchitis and tonsillitis among others (de Figueir, 2015). In Northeastern Brazil, at least 42 insect species have been reported to be used in traditional medicine. In this instance, species of the hymenoptera order were the most prevalent (Dossey, 2010)

2.2.3 Insects in animal feeds

The nutrient requirements of monogastric animals include a high quantity and quality of protein. In animal nutrition, protein sources should have high protein content, a good amino acid composition, exhibit good digestibility and palatability and contain no anti-nutrients (Barrows *et al.,* 2008). In this regard, fishmeal and soybean meal are preferred above other protein sources for use in animal nutrition. However, although there has been a general increase in soya production, competition with human consumption thereof and competition with the biofuel industry, has led to an increase in the cost of soya (Ravindran & Blair, 1992). In addition, the fishmeal industry has suffered a significant loss in production, mainly due the deterioration of the marine environment and stripping of fisheries. This has led to an increase in the cost of fishmeal, a trend which is expected to continue, with consequent repercussions on the animal production industry (International Monetary Fund, 2010). Currently insects are being considered as a new protein source for animal feeds (Premalatha, 2011).

There are many reasons why one should consider using insects in animal feeds. The culturing of insects has both economic and environmental value. Insects have value as recyclers of organic

waste, especially those resulting from the agricultural sector (Pretorius, 2011). They can be raised on products such as manure, abattoir waste and fish offal. Also, termites could be used to break down wood waste while also functioning as a feed (Mitsuhashi, 2010), since they are rich in fat and proteins (Chung, 2010). They have the ability to convert waste into high protein feed sources that could possibly replace the more conventional and expensive protein sources such as fishmeal and soya oilcake meal. Insects can also be reared under varying environmental conditions, which can be adapted to optimise their nutritional value (Sealey *et al*., 2011). Furthermore, the culturing of insects is considered as a sustainable process, since it usually occurs in warehouses where the surface area and water requirements are minimal, especially when compared to crop production. Furthermore, insects are poikilotermic and do not require energy for temperature regulation. As such, they are efficient food converters (Nijdam *et al*., 2012).

2.2.3.1 Poultry

As previously mentioned, one of the major challenges the poultry industry is currently facing, is the acquisition of feeds that comply with all the dietary requirements of the birds to ensure efficient growth (Oyegoke *et al.*, 2006). There is thus a need to supplement predominantly cereal-based diets with animal protein sources that are of superior quality. The enormity of the role of fishmeal in this regard, cannot be overstated (Ijaiya & Eko, 2009). The presence of fishmeal in the diet could substitute some amino acids that are deficient in protein sources of plant origin (Miles & Jacob, 1997).

Insects are the natural prey of wild birds and are consumed in their adult, pupal, and larval forms (Zuidhof *et al*., 2003). The feeding of insects to fowls is therefore not a completely novel idea. However, the extent to which their utilisation occurred was very small. For example, in the eastern Sichuan area, duck breeders collected the larvae of *Musca domestica* for use as feed for ducks. Also, mass rearing of insects is not a popular endeavour. Insects that are reared on a large scale include *Tenebrio molitor*, *Tenebrio obscurus*, *Zophobus morio*, and the diperan house fly larvae which are primarily used for feeding chickens, ducks, and some other animals (Yi *et al*., 2010).

Species of the order Orthopera (Crickets, grasshoppers) are naturally consumed by wild birds and free range poultry. Various studies on species of this order have indicated mostly positive results (Nakagaki & DeFoliart, 1987). Acridids (grasshoppers) have been identified for possible use in poultry feed due to their high protein content. They are also rich in minerals such as Ca, Mg, Zn, Fe, and Cu (Anand *et al*., 2008). In the Phillipines, grasshoppers are fed to chickens raised on pasture. The chicken raised in the manner are said to have a rather delicious flavour and are sold for a much higher price than chickens reared on a commercial feed (Litton, 1993). Wang *et al*. (2005), found that an inclusion level of 15% grasshopper (*Acridia cinerea*) meal in broiler feed did not have any significant effects on growth.

Interest in the use of house crickets as feed ingredients in animal feeds arises from the fact they are conducive to mass rearing under controlled environmental conditions (Nakagaki *et al*., 1985) and can produce from six to seven generations within a year. Also, they are an omnivorous species and preliminary studies indicate that they may have the ability to convert poultry manure into protein rich feedstuff for use in poultry diets on an economically competitive basis. The house cricket is an easily domesticated species (Nakagaki *et al*., 1986; Hardouin & Mahoux, 2003). Razak *et al*. (2012) conducted a study to determine the chemical composition, true metabolisable energy (TME) and crude protein (CP) quality of house cricket meal. They found that house cricket meal had a CP content of 60.4%, which is higher than that of soya oilcake meal, but lower than that of fishmeal. The

house cricket meal had a TME value of 13.03 MJ/kg, which is similar to that of corn at 13.40 MJ/kg, but higher than that of soya oilcake meal at 9.6 MJ/kg. The high value of TME in this study was probably attributed to the high fat content (22.7%). TME value for house cricket was also higher than that of field cricket, 12.39 MJ/kg (Wang *et al*., 2005).

Species from the order Lepidoptera (Butterflies, moths) have also been used in animal feeds. Oyegoke *et al*. (2006) replaced 4% of fishmeal in the diet with the larvae of *C. forda* and found no significant differences in the growth performance between the birds the birds that were fed the larval diet and those fed the conventional diet. In another study by Ijaiya & Eko (2009), where *Anaphe infracta* was used, it was found that there were no significant differences in terms of feed intake, body weight gain, feed conversion efficiency and protein efficiency ratio. It may also be possible to replace dried silkworm pupae (*Bombyx mori*) meal in poultry feeds as they exhibit positive results with regards to growth and egg production (Wijayasinghe & Rajaguru, 1977).

From the order Diptera most studies were on the larvae of *M. domestica* as protein supplement. The house fly is the most frequently occurring of all the Diptera species. It is a worldwide pest and a major carrier of disease, as both the larvae (maggots) and adults feed on manure and decaying organic waste. The ability of housefly maggots to grow on a wide range of substrates can make them useful to turn waste into a utilisable biomass rich in protein and fat. Producing housefly maggot biomass in controlled conditions to feed farm animals have been investigated since the 1960s (Calvert *et al*., 1969; Miller & Shaw., 1969).

Pro *et al.* (1999) ascertained that the protein and energy supplied by dry fly larvae are similar to that supplied by sorghum and soya in terms of production performance. Furthermore, Ocio *et al.* (1979), Awoniyi *et al.* (2003) and Djordjevic *et al*. (2008), found that there were no significant differences in weight gain between birds that were fed diets containing *M. domestica* larvae and those fed diets containing good quality fishmeal. Teéguia *et al.* (2002) established that the weight gain of birds fed diets containing the highest amount of maggots were significantly higher when compared to that of birds fed a conventional fishmeal diet. Also, the lysine and tryptophan content of the breast muscle appears to increase in birds that are fed a diet containing house fly larvae meal. This may be due to the optimal amino acid profile, the high protein content, or high protein digestibility of the larvae (Hwangbo *et al*., 2009). It can therefore be conclude from the above mentioned studies that insects may prove to be a protein source comparable to fishmeal (Oyegoke *et al*., 2006).

Furthermore, dried housefly pupae and dried houseflies grown on chicken manure and subsequently fed to chicks could potentially replace soya oilcake meal as protein source with regard to weight gain and feed intake (Calvert *et al*., 1969). The housefly larvae are also applied to poultry waste because they are able to convert poultry waste into biomass (Calvert *et al*., 1969; El Boushy, 1991; Hwangbo *et al*., 2009). In addition, the black soldier fly can effectively be used to reduce manure waste of laying hens by more than 50% while simultaneously functioning as a feed, thereby reducing cost for fly control and waste removal (Sheppard *et al*., 2007). Other than poultry manure, the black soldier fly has also been reared, with success, on pig and cattle manure, as well as fish waste (St-Hilaire *et al*., 2007; Sealey *et al*., 2011).

Other forms of organic waste can also be used in animal nutrition through insects. Studies indicate that the mealworm, larvae of *T. molitor*, can successfully be reared on waste originating from fruit and vegetable processing plants. The larvae can then be dried and mixed into a diet for poultry. Feeding trials indicate no significant differences in feed uptake, weight gain, and feed efficiency for

feeds containing 0, 5 and 10% mealworm larvae, respectively; making *T. molitor* reared on organic waste a potential supplement for chicken feed (Ramos-Elorduy *et al*., 2002). Information on the use of mealworms in layer diets, on the other hand, are quite scarce, but Giannone (2003) found the larvae of *Tenebrio molitor* and *T. mauritanicus* to be suitable for layer hens. Wang *et al*. (1996) also suggests that ground mealworms may be an adequate substitute for fishmeal.

Nutritionally, these larvae (mealworms) are a rich source of metabolisable energy, protein, phosphorous and many trace nutrients (Martin *et al*., 1976; Klasings *et al*., 2000; Aguilar-Miranda *et al*., 2002). Also, they are easy to breed and feed (Li *et al*., 2012). It is for these reasons that they are used as food for animals in captivity, especially zoo animals (Klasings *et al*., 2000). They are usually fed live, but they are also sold canned, dried, or in powder form (Aguilar-Miranda *et al*., 2002; Hardouin & Mahoux, 2003; Veldkamp, 2012). Their protein quality has been equated to that of soya oilcake meal, although the methionine content might be limiting for poultry (Ramos-Elorduy *et al.,* 2002). The low calcium content may also prove to be a problem in poultry diets. The proximate composition, amino acid profile, vitamin A, vitamin E and selected mineral content have been reported for mealworms (Jones *et al*., 1972; Barker *et al*., 1998). Protein content has been reported to range from 45% to 60% of the dry matter (DM), and a fat content ranging from 30% to 45% DM. Fresh larvae contain approximately 60% water. They have a fairly low ash content and a poor Ca:P ratio (Klasings *et al*., 2000).

2.2.3.2 Pigs

The numbers of studies done on the use of insects in pig feeds are few and far between. There is, however, one study by Newton *et al.* (1977) where the feeding value and palatability of dried black soldier fly larvae (*Hermetia illucens*) were evaluated. The larvae meal was used as a replacement for soya oilcake meal. The digestibility of the larvae meal was evaluated and it was established that the apparent digestibility was significantly lower than that of a conventional soya containing diet. However, the pigs did not discriminate against a diet containing larvae meal.

The use of silkworm pupae in pig feeds has also been studied. There are two experiments which indicate that silkworm pupae meal may be a good replacement for more conventional protein sources. In Brazil, it was possible to partially replace soya oilcake meal in diets for growing and finishing pigs with non-defatted silkworm pupae meal with no significant effects on growth and carcass traits. There was however a negative effect on intake when the substitution rate exceeded 50%, which may have been due to the high energy content or a lower palatability. However, the lower intake was compensated for by a higher feed conversion rate (Coll *et al*., 1992). In India, silkworm meal could fully replace fishmeal in the diet of growing and finishing pigs without affecting carcass and meat quality (Medhi, 2011).

2.2.3.3 Fish

Farmed fish have a high demand for good quality protein supplied in large amounts. Fishmeal is also the major protein source for use in fish diets. Although there are few studies regarding insect protein in fish diets, the interest is increasing. In a study by Alegbeleye *et al.* (2012), growth rate and nutrient utilisation were evaluated. When 25% of fishmeal was replaced with larvae meal of *Zonocerus variegates* there was a significant improvement in both the factors evaluated. Similar results were obtained in African catfish (*Clarias gariepnus*) that were fed slices of mealworm (*T. molitor*). At a 20% replacement rate, both the growth rate and nutritive value of the African catfish were improved (Ng *et al*., 2001). The larvae of *H. illucens* have been evaluated as feed in various species, including channel fish and tilapia (Bondari & Sheppard, 1981).

Silkworm pupae meal is also a valuable protein source in many fish species. When silkworm pupae meal replaced part or all of the fish meal in the diets of the common carp (*Cyprinus carpio*), similar growth performances were observed (Jeyachandran & Raj, 1976; Erencin, 1976; Nandeesha *et al*., 1988; Nandeesha *et al*., 2010). When compared to plant leaf meals, diets based on silkworm meal had better feed conversion rates, digestibility of nutrients, and nutrient retention (Swamy & Devaraj., 1994).

2.3 Assessment of nutritive value

2.3.1 Proximate composition

The determination of the proximate composition of a food source is generally the first step in establishing its value as a reliable feed source. Various studies have been reported on the proximal composition of a variety of insect species. These studies have shown variation in values for crude protein (CP), crude fat and ash across the various species (Table 2).

The percentage of protein is one of the most important criteria to be considered for feed protein sources (McDonald *et al*., 2002; Sànchez-Muros *et al*., 2014). Most studies indicate that insects in general have a high quantity and quality of protein (Ramos-Elorduy *et al*., 1981; de Guevara *et al*., 1995). From the crude protein (CP) values shown in Table 2, a total of nine species are comparable to that of fishmeal (CP = 60% to 80%). The majority of species, however, were comparable or higher than the CP values for soya, which generally ranges from 45% to 50%.

2.3.2 Amino acid composition

Rather than a CP requirement, animals have specific amino acid requirements (Teles *et a*l., 2011). Thus, in order to determine the quality of a protein, one has to look at the specific amino acid composition of the source, especially the balance between the non-essential and essential amino acids (Conconi *et al*., 1984). In other words, how does it compare to the ideal amino acid pofile. Insects have been found to be generally rich in essential amino acids. Vegetable proteins on the other hand are usually deficient in lysine, methionine and leucine (Ockerman, 1992), which in animal nutrition are first limiting amino acids.

Table 3 is a compilation of the amino acid composition of various insect species when compared to that of Fishmeal. As can be seen from Table 3 *Bombyx mori*, *Musca domestica* and *Ephydra hians* are higher in methionine than fishmeal. The leucine values or *B. mori*, *Boopedon flaviventris*, *Sphenarium histrio*, *Callipogn barbatum* and *E. hians* are higher than that of fishmeal, whereas the values for *Cossus redtenbachi*, *Scyphophorous acupunctatus* and *M. domesica* are similar. None of the species evaluated in these studies however, have a higher or similar lysine value than fishmeal.

2.3.3 Mineral composition

In

Table **4** the mineral composition of some insect species are presented. Insects are generally a good source of iron, copper and zinc (Oliveira *et al*., 1976). It can also be seen that the various species have low calcium (Ca) levels. From

Table **4** it is also evident that the fly species *Hermetia illucens* has a much higher Ca content than the rest. The exoskeleton of most insect species is primarily composed of protein and chitin, and

because some insects, including soldier fly larvae, have a mineralized exoskeleton which explains their higher calcium content (Dierenfeld & King, 2008).

Table 2 Proximate compositions of various insect species

	Common name	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	NDF (%)	ADF (%)	Ash (%)	Reference
Lepidoptera								
Achroia grisella (L)	Lesser wax moth	33.97	60.0	NA	19.5	8.19	1.4	Finke, 2002
Bombyx mori (L)	Silkworm	53.75	8.09	NA	6.36	6.36	6.36	Finke, 2002; Frye & Calvert, 1989
Chilecomadia moorei (L)	Butterworm	38.94	73.86	NA	6.53	3.52	2.01	Finke, 2012
Hyalophora cecropia (L)	Cecropia moth	54.7	10.2	14.7	NA	NA	5.9	Landry *et al*., 1986
Collosamia promethean (L)	Promethea silk moth	49.4	10.0	10.8	NA	NA	6.9	Landry *et al*., 1986
Manduca sexta (L)	Tobacco hornworm	58.1	20.7	9.4	NA	NA	7.4	Landry *et al*., 1986
Spodoptera frugiperda (L)	Fall armyworm	57.8	20.2	6.7	NA	NA	5.6	Landry *et al*., 1986
Pseudaletia unipuncta (L)	White speck	54.4	14.9	5.0	NA	NA	6.9	Landry *et al*., 1986
Spodoptera eridania (L)	Southern armyworm	54.7	13.9	7.1	NA	NA	9.8	Landry *et al*., 1986
Samia ricinii (PP)	Eri silkworm	54.2	26.20	3.26	NA	NA	3.80	Longvah *et al*., 2011
Samia racinii (P)	Eri Silkworm	54.6	26.20	3.45	NA	NA	3.80	Longvah *et al*., 2011
Cirina forda	Pallid emperor moth	20.0	12.5	8.7	NA	NA	NA	Osasona & Olaofe, 2010

	Common name	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	NDF (%)	ADF (%)	Ash (%)	Reference
Antheraea pernyi	Chinese tussah moth	71.9	20.1	NA	NA	NA	4.0	Zhou & Han, 2006
Coleoptera								
Z. morio (A)	Superworm	68.05	14.25	NA	50.14	32.06	6.16	Oonincx & Dierenfeld, 2012
Z. morio	Superworm	46.79	42.04	NA	9.26	6.41	2.38	Finke, 2002
T. molitor	Mealworm	49.08	35.17	NA	14.96	6.56	2.36	Finke, 2002
Cotinis ntida	June beetle	51.75	5.41	19.3	NA	NA	12.34	Rakashantong *et al*., 2010
Hymenoptera								
Oecophylla smaragdina	Weaver ant	53.46	13.46	15.38	NA	NA	6.55	Rakashantong *et al*., 2010
Orthoptera								
Acheta domesticus	House cricket	66.56	22.08	NA	22.08	10.39	3.57	Finke, 2002; Bernard *et al*., 1997
Microcentrum rhombifolium (A)	Angel-wing katydids	77.80	9.00	NA	41.14	19.39	9.10	Oonincx & Dierenfeld, 2012
Anurogryllus arboreus	Short-tailed cricket	48.69	20.60	11.61	NA	NA	9.36	Rakashantong *et al*., 2010
Diptera								
H. illucens	Black soldier fly	45.10	36.08	NA	9.79	7.73	9.02	Finke, 2012
Musca domestica (L)	House fly	78.17	7.5	NA	14.29	11.51	6.75	Finke, 2012

	Common name	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	NDF (%)	ADF (%)	Ash (%)	Reference
D. melanogaster (A)	Fruit fly	68.00	19.00	NA	17.66	10.14	7.20	Oonincx & Dierenfeld, 2012; Barker *et al*., 1998
Blattodea								
Blatta Lateralis	Turkestan cockroach	61.5	32.4	NA	9.06	7.12	3.9	Finke, 2012
B. lateralis (S)	Turkestan cockroach	76.05	14.45	NA	11.41	10.87	7.88	Oonincx & Dierenfeld, 2012
B. Lateralis (M)	Turkestan cockroach	62.85	26.50	NA	12.76	12.75	6.89	Oonincx & Dierenfeld, 2012
Eublaberus distanti	Six spotted roach	52.1	43.1	NA	NA	NA	2.98	Oonincx & Dierenfeld, 2012
Gromphadorhina portentosa	Madagascar hissing roach	63.35	20.30	NA	36.54	13.12	8.49	Oonincx & Dierenfeld, 2012
Periplaneta americana	American roach	53.9	28.4	NA	NA	9.4	3.3	Bernard *et al*., 1997

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

Table 3 Amino acid composition (g/100g) dry matter of some insect species

		Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Ala	Reference
Lepidoptera																				
Samia ricinii	Eri silkoworm	4.4	2.7	1.4	6.6	6.5	2.3	5.2	4.8	NA	5.4	9.9	0.5	13	4.9	6.5	5.3	6.4	6.1	Longvah *et al*. (2011)
Bombyx mori	Silkworm	6.8	2.5	5.7	8.3	6.5	4.6	5.1	5.4	0.9	5.6	11	1.4	15	4.6	4	4.7	5.4	5.5	Rao (1994)
Cossus redtenbachi		6	1.6	5.1	7.9	4.9	2.1	9.3	4.7	0.6	6.1	11	1.3	17	5.5	5.5	5.9	6.2	6.5	Ramos-Elorduy *et al*. (1982)
Coleoptera																				
Scyphophorous acupunctatus	Agave weevil	4.4	1.5	4.8	7.8	5.5	2	4.6	4	0.8	6.2	9.1	2.2	16	6.1	5.4	6.6	6.4	6.5	Ramos-Elorduy *et al*. (1997)
Zophobos morio	Superworm	2.3	1.4	2.2	4.5	2.4	0.5	1.6	1.9	0.4	2.4	3.8	0.35	5.7	2.3	2.6	2.2	3.3	3.4	Finke (2002)
Tenebrio molitor	Mealworm	2.7	1.5	2.5	5.2	2.7	0.6	1.7	2	0.4	2.9	4	0.4	5.5	2.7	3.4	2.5	3.6	4	
Hymenoptera																				
Vespa basalis	Hornet	1.7	1.1	2.6	3.5	1.9	0.9	1.9	1.8	NA	2.6	3.4	ND	7.5	3.6	3.7	1.9	2.5	3.4	

		Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Ala	Reference
Polistes sagittarius	-	1.6	1.1	2	2.8	1.6	0.5	1.8	1.5	NA	2.4	3	ND	6.2	2.5	3.2	1.6	1.8	2.6	Ying *et al*. (2010)
Orthoptera																				
Boopedon flaviventris	Yellow belly boopie	4.3	2.4	4.7	8.8	5.5	1.8	4.1	4.4	0.6	5.7	8.8	2	15	7.5	6.8	4.3	7.4	5.9	Ramos-Elorduy *et al*. (1997)
Gryllus testaceus	Field cricket	3.7	1.9	3.1	5.5	4.8	1.9	2.9	2.8	NA	4.4	6.3	1	9.1	3.6	4.5	3.7	3.9	5.6	Wang *et al.* (2005)
Sphenarium histrio	Chapulin del zacate	6.6	1.1	5.3	8.7	5.7	2	12	4	0.6	5.1	9.3	1.3	4.3	5.3	7.2	5.1	7.3	7.7	Ramos-Elorduy & Pino (1982)
Callipogon barbatum	Longhorn beetle	5.9	2.2	5.8	10	5.7	2	4.7	4	0.7	7	9.1	2	10	9.2	6.2	3.7	4.2	8	Ramos-Elorduy *et al*. (2006)
Diptera																				
Musca domestica	Housefly	5.2	2.9	4.4	7.8	7.3	4.6	13	4.4	0.6	5.1	11.1	2.4	13	5.8	4.8	3.7	7	6.5	Ramos-Elorduy & Pino (1982)
Ephydra hians	Alkali fly	2.7	1	5	8	5.8	3.8	10	4.6	0.4	5.6	11	2.2	16	4.9	6.5	3.8	5.1	12	
Hermetia illucens	Black soldier fly	3.17	1.52	2.0	3.1	3.1	0.87	2.0	1.8	0.77	3.3	4.3	0.25	5.1	2.3	2.6	1.8	3.1	3.1	Finke (2012)
Blattodea																				
Blatta lateralis	Turkestan roach	4.5	1.8	2.5	3.9	4.2	1.1	2.5	2.5	0.55	3.9	4.9	0.45	7.4	3.9	3.6	2.7	4.5	5.5	Finke (2012)

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Asp	Cys	Glu	Gly	Pro	Ser	Tyr	Ala	Reference
Fishmeal	6.14	3.6	4.8	7.8	7.9	2.5	4.1	4.4	1	5.2	9	1	13	6.2	4.5	4	3.2	6.3	Lall & Anderson (2005)

NA- not applicable, ND- not detected

Table 4 Selected mineral content of various insect species

	Common name	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Reference
Lepidoptera									
Galleria mellonella	Honeycomb moth	0. 6	0. 9	12	3.06	77.27	3.28	77.78	Barker *et al*. (1998)
Bombyx mori	Silkworm	1	3	14	20.81	95.38	24.86	177.46	Finke (2002)
Chilecomadia moorei	Butterworm	0.3	0.7	5.7	7.4	35.18	1.78	89.70	Finke (2012)
Coleoptera									
Tenebrio molitor	Mealworm	1.2	2.8	14.2	17.77	39.7	6.79	131.02	Barker *et al*. (1998)
Zophobas morio	Superworm	1.2	1.8	8.3	13.94	50.34	1.54	87.50	
Rhynchophorous phoenicis	African palm weevil	2.1	1.3	6.9	16	158	35	158	Rumpold & Schlüter (2013)
Orthoptera									
Acheta domestica	House Cricket	2.1	0.8	7.8	8.5	112.33	29.65	186.36	Barker *et al*. (1998)
Diptera									
Hermetia illucens	Black soldier fly	24	4.5	9.2	10.39	171.65	159.28	144.85	Finke (2012)
Drosophila melanogaster	Fruitfly	1.7	1.7	13.2	16	400.5	16.5	223.0	Oonincx & Dierenfeld (2011)

	Common name	Ca (g/kg)	Mg (g/kg)	P (g/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Reference
Blattodea									
Blatta Lateralis	Turkestan roach	1.2	0.8	5.7	25.66	47.89	8.54	105.83	Finke (2012)
Gromphadorhina portentosa	Madagascar hissing roach	2.5	2.4	9.3	22.5	153.5	10.0	202.0	Oonincx & Dierenfeld (2011)
Eublaberus distanti	Six spotted roach	0.8	0.8	4.6	12	55	5	124	Oonincx & Dierenfeld (2011)

2.3.4 Digestibility

The digestibility of a protein source is an important indication of the quality of the source (Sànchez-Muros *et al*., 2014). The potential value of a food for supplying a particular nutrient can be determined by chemical analysis, but the actual value of the food to the animal can be arrived at only after making allowances for the inevitable losses that occur during digestion, absorption and metabolism. The component of feed that is not absorbed, but rather excreted represents the first toll on that feed (McDonald *et al*., 2002). The digestibility of a protein source is thus most accurately defined as that protein which is not excreted in the faeces and which is, therefore, assumed to be absorbed by the animal. The digestibility of a specific nutrient is determined by performing a digestibility trial with a target group of animals. This involves measuring feed intake and faecal output for a specified period (Khan *et al*., 2003; Lemme *et al*., 2004).

When determining digestibility, it is important to remember that nutrients may be absorbed from several parts of the digestive tract. A food constituent that is digested at one site may give rise to nutrients differing quite considerably from those resulting from its digestion at another site, and the nutritive value to the animal of that constituent will therefore depend not only on the extent to which it is digested, but also the site of digestion (McDonald *et al*., 2002). There are also various factors that may influence the digestibility of a specific compound (Khan *et al*., 2003). The first is the composition of the specific food component under investigation (Poppi *et al*., 1987; Sarwar *et al*., 1985; Luginbuhl *et al*., 1994). Digestibility of one feed is believed to differ from that of a similar feed because each may contain different contents of certain chemical entities, particular since some of these diminish the opportunity for digestive enzymes to come in contact with their respective substrates (Khan *et al*., 2003). For example, foods such as barley, which varies little in composition from one sample to the next, will show little variation in digestibility. While other foods, particularly fresh or conserved herbages are much less constant in composition and therefore vary more in digestibility. Furthermore, the fibre component of a food may have the greatest influence on its digestibility, and both the amount and chemical composition of the fibre are important (McDonald *et al*., 2002).

A second factor is the composition of the feed in which the substance under investigation is included (McDonald *et al*., 2002). A third factor is that of the animal itself, where genetics plays a major role (McDonald *et al*., 2002; Khan *et a*l., 2003). Plane of nutrition is another important factor and is considered to be one of the primary factors to affect digestibility of feeds (Khan *et al*., 2003). Studies have shown that livestock usually digest a larger portion of the nutrients in their feed when fed restricted than when fed *ad libitum* (Okine & Mathison, 1991). Most data indicate a reduction in apparent digestibility as level of feed intake is increased. This may be due to the more rapid movement of feed through the digestive tract, thus limiting the time at which feed is exposed to digestive enzymes (McDonald *et al*., 2002).

It is important to note, however, that it is the individual amino acid requirement rather than the protein requirement that is essential in animal (particularly monogastric animals) nutrition (McDonald *et al*., 2002). The assessment of amino acid digestibility of feedstuffs is thus essential if poultry are to be fed balanced diets (Short *et al*., 1999). A proportion of dietary amino acids is excreted undigested and individual feed ingredients differ widely in this respect (Lemme *et al*., 2004). Thus, the higher the inclusion levels of feed ingredients with low amino acid digestibility in diets formulated on the basis of total amino acids, the less reliable will be the prediction of performance (Esteve-Garcia *et al*., 1993; Fernandez *et al*., 1995).

The CP figures provide a measure of the nitrogen present in food but give little indication of its value to the animal. Before the food becomes available to the animal it must undergo digestion, during which it is broken down into simpler substances which are absorbed into the body. The digestible protein in food may be determined by digestibility trials in which nitrogen intake is measured along with the nitrogen voided in the faeces. There is very limited data available on the digestibility of insect protein in animals. There are however two studies of note on broilers. The first study was conducted by Pretorius (2011), and tested the total tract digestibility of *M. domestica* pupae and larvae meal. The second study was by Uushona (2014) in which the total tract digestibility of *H. illucens* pupae meal was tested. The coefficient of total tract digestibility (CTTD) results of these studies are provided in Table 5, with digestibility values for soya oilcake meal and fishmeal for comparison. It is clear from the values displayed here that the amino acid digestibility for the fly species are higher than both that of fishmeal and soya oilcake meal.

Table 5 Coefficient of total tract digestibility values for amino acids of some fly species compared to that of fishmeal and soya oilcake meal

	M. domestica **larvae meal[1]**	***M. domestica*** **pupae[1]**	***H. illucens*** **pupae meal[2]**	**Soya oilcake meal[3]**	**Fishmeal[3]**
Threonine	0.93	0.97	0.94	0.79	0.81
Valine	0.91	0.91	0.92	0.79	0.80
Methionine	0.95	0.99	0.97	0.88	0.87
Isoleucine	0.91	0.95	0.94	0.81	0.82
Leucine	0.92	0.96	0.92	0.84	0.85
Phenyalanine	0.91	0.95	0.96	0.85	0.83
Histidine	0.87	0.87	0.95	-	0.80
Lysine	0.95	0.99	0.97	0.86	0.85
Arginine	-	0.93	0.98	0.91	0.86
Aspartate	0.93	1.00	0.95	0.85	0.79
Serine	0.86	1.00	0.91	0.85	0.79
Glutamate	0.91	0.99	0.94	0.87	0.82
Alanine	0.90	0.86	0.92	0.74	0.77
Tyrosine	0.96	0.96	0.95	0.85	0.80

[1]Pretorius (2011); [2]Uushona (2014); [3]Ravindran *et al*. (1999)

2.4 Mass rearing insects

If we are to successfully utilize insect meal in animal nutrition, it would require raising insects on a large scale. This would ensure that there is significant production of insects needed for animal feed production. Methods for mass rearing insects have only been developed for a few species. This may be mainly due to a lack of demand (Sánchez-Muros *et al*., 2014).

Even though the majority of insects consumed today are collected from the wild (Laos, 2010), records indicate that domestic rearing of insects have been practiced for more than 7000 years. This was mostly for the production of silk (sericulture), the production of shellac, the production of honey (apiculture) and also the production of medicinal products. In 1936 the first mass production of screw-worm fly (*Cochliomyia hominivorax*) reared on an artificial diet was accomplished, leading to the development of the sterile insect technique (SIT), a biological method for controlling pests. So far, a lot of progress has been made with regards to the development of artificial diets for insects and mass rearing for use as biological pesticides (Singh & Moore, 1985; Singh, 1982). Today, there are industrial warehouses in Okinawa, Japan, that produce *Bactrocera cucurbitae* (Melon fly) at a rate of 40 million larvae per week. Other cultured species include butterflies and moths, beetles, flies and mosquitoes, crickets and grasshoppers, cockroaches, termites, and fleas (Leppla, 2008). The culture of insects is a complex process and it is rather difficult to establish a colony that can produce continuous generations (Sánchez-Muros *et al*., 2014). In order to make mass rearing of insects both an attractive and competitive industry, it is important to develop rearing, harvest, and post-harvest techniques that will include safety and quality control (Rumpold & Sclüter, 2013). There are some companies in South Africa who have managed this. AgriProtein, a company based in Cape Town, rears black soldier fly larvae for use in animal feeds (Stame, 2015)

Sufficient information about the species habitat, food and behaviour is required. Recreation of soil and aquatic environments, symbiotic relationships, and specialized food can make rearing difficult. Some insects undergo temperature and photoperiod dependent diapause or require host plant cues to terminate multi-year cycles. Feeding by trophallaxis may necessitate maintenance of an entire colony, as in termite, ants, and other social insects (Peters & Barbosa, 1977). Because of these and other peculiar life history characteristics, the easiest insects to rear are relatively small, multivoltine (multiple generations per year), are typically plant feeding terrestrial species with wide host ranges and few unusual environmental requirements. Commonly cultured insects include pests of common crops, stored products or landscape plants. Examples include army worms, loopers, hornworms, mealworms, weevils, mealy bugs, aphids, ants, grasshoppers and lady beetles (Leppla, 2008). In this manner, mass rearing has been developed, particularly for silk production, fishing bait and pet food.

One of the main purposes for mass rearing insects would be the production of protein for use in feed for both humans and animals. Thus, in order to optimise protein yield, suitable insect species should be selected. As with any other livestock, potential insects are selected based on their size, social behaviour, nutritional benefits, potential for storage and marketability (Schabel, 2010). The insect species should display good reproductive traits in terms of egg production rate, high hatchability. The selected species should also display good production traits in terms of duration of larval stage, synchronization of pupation, weight of larvae or pupae, high conversion rate, high increase in biomass per day. Other factors to consider include feed costs, feed composition and quality, vulnerability to disease, and most importantly, the quality of protein (Peters & Barbosa, 1977; Scriber & Slansky, 1981; Sharaby *et al*., 2010)

Caterpillars are one of prime candidates recommended for mass rearing since they are coldblooded, wingless and have the ability to convert plant biomass to animal biomass at a rate that's 10 time more efficient than cattle and on much less land (Schabel, 2010). The rearing of orthoptera (grasshoppers, locusts, crickets) as food or feed has also been suggested. A lab based study on the farming of the grasshopper *Oxya Fusovittata* resulted in a production of 1 kg biomass in 29 to 35 males by 84 females (Haldar *et al*., 1999).

The cricket *A. domestica* has high quality protein (Finke *et al*., 1989), is omnivorous and easily raised. In Laos it is commonly bred on small scale (100-500 kg/year) by local families as a supplementary income. Cylindrical tanks covered with mosquito nets are used as rearing containers and usually chicken feed and vegetable materials are fed (Laos, 2010). Parajulee *et al.* (1993) developed a mass rearing system for *A. domestica* as food and resulted in a production model that provided a harvest of 6000 crickets per day using 32 rearing cages (50x44x20.5 cm^3). At an estimated wet weight of 0.41 g to 0.46 g per cricket (Nakagaki & Defoliart, 1991), a daily production of 2.4-2.7 kg crickets is achieved.

2.6 Conclusion

From the information presented above, it can be concluded that insects are a potential feed source or use in animal feeds. They may present an alternative to soya oilcake and fishmeal as major protein sources in animal feeds. As such, insects have an adequate amino acid profile although the amino acid information is very sparse. In light of this, more studies have to be conducted on the nutritional value of insects (typically available in South Africa) in order to select the species with the best amino acid profile. However, if insect protein is to be introduced as a feed ingredient, additional information regarding its feeding value, inclusion level in diets, and other functional properties, are required.

2.7 References

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Chapter 3
Nutritional Composition of eight selected insect species with the potential for mass rearing

3.1 Abstract

The nutritional composition of eight small scale mass reared insects were compared in terms of proximate composition, amino acid composition and mineral content. These species were *Tenebrio molitor*, *Zophobas morio*, *Naophoeta cinerea*, *Blaptica dubia*, *Gromphardhina portentosa*, *Periplaneta americana*, *Oxyhalao duesta* and *Blatta lateralis*. The roach species, *N. cinerea* yielded the highest crude protein (CP) value, 60.3%; which is comparable to that of fishmeal. The CP values for the other species were comparable to that of soya oilcake meal, with values ranging from 43.1% to 55.3%. The amino acid profiles for *G. portentosa*, *P. americana* and *B. lateralis* compared favourably to the Ideal amino acid profile for broilers. The arginine to lysine ratios for all species ranged from 1.01 to 1.34. These values are promising since birds are susceptible to lysine-arginine antagonism if the ratio is <1. In general, results obtained in this study are in support of the notion that insects may be a valuable protein source for use in animal feeds.

3.2 Introduction

The demand for protein of animal origin is rising globally, and by the year 2050 it would be expected to have increased by 70 to 80% (Steinfeld *et al*., 2006; Pelletier & Tyedmers, 2010). Poultry products, including meat and eggs, have always been a major source of animal protein for humans (Stenhouse, 2008). With a reported feed conversion ratio ranging from 1.5 to 1.8 (Sengor *et al*., 2008), poultry production has proven to be a highly efficient industry. This makes chicken one of the most economically viable and important sources of meat for human consumption (Khusro *et al*., 2012). With the high rate at which the world population is growing, the global consumption of chicken has also increased significantly over the past few years. It is also expected that most of the global demand for poultry products will be evident in developing countries (Rama Rao *et al*., 2004; Ravindran, 2013). However, the increased demand for poultry products has placed the industry under immense pressure, especially in terms of the acquisition of raw materials for use in poultry feeds (Ravindran, 2013). In this regard, protein sources are becoming increasingly difficult to acquire. Thus, it is imperative to identify and develop food resources that can be sourced locally (Conconi *et al*., 1984).

One resource that has come under consideration is insects (Finke, 2002; Premalatha *et al*., 2011; Razak *et al*., 2012). In nature, insects form a significant biomass, as can be seen with pests (Ramos-Elorduy *et al*., 1997). There are approximately one million known species of insects, although it has been estimated that the global diversity is as high as 80 million (Erwin, 2004). Insects should not only be considered due to their abundance in nature, but also their nutritional value (Conconi *et al*., 1984). Insects have been shown to have a high CP content and insect protein have been reported to be a good source of essential amino acids, equivalent or even superior to soya protein (Finke *et al*., 1989). In general, insect protein tend to be low in sulphur containing amino acids but high in lysine and threonine, one or both of which may be deficient in cereals. Insects are generally a rich source of fat, as well as a good source of vitamins and minerals (Oliviera *et al*., 1976; Malaisse & Parent, 1980; Banjo *et al*., 2006).

However, the utilization of insect meal in animal feeds requires mass production of insects, ensuring a significant production of insects that are necessary for animal production. Virtually any insect can be collected as an egg, larvae or nymph and maintained through stages in metamorphosis until it becomes an adult. However, it is considerably difficult to establish a colony that produces continuous generations (Leppla, 2008). For a maximum protein yield, a suitable insect species to be raised has to be selected. Candidate insects are selected based on their size, social behaviour, safety, epidemic tendencies, reproductive and survival potential, and nutritional benefits (Schabel, 2010). The selected species should also display good production traits in terms of duration of larval stage, synchronization of pupation, weight of larvae or pupae, high conversion rate, high increase in biomass per day protein (Peters & Barbosa, 1977; Scriber & Slansky, 1981; Sharaby *et al*., 2010). Due to these and other peculiar life history characteristics, the easiest insects to rear are relatively small, multivoltine (multiple generations per year); plant feeding terrestrial species with wide host ranges and few unusual environmental requirements (Leppla, 2008). Insects that fit the description ae generally feeder insects, i.e. insects used to feed insectivorous animals living in captivity (Finke, 2002). The mass production of insects for use in animal feeds is a relatively new undertaking in South Africa and we need to establish which species will be suitable for this purpose.

Therefore the aim of this study is to determine the chemical composition of insect species that have the potential to be mass reared, with the aim of using them as a protein source in animal feeds. A total of eight insect species were selected for mass rearing, namely, Mealworms (larvae of the beetle *Tenebrio molitor*), Superworms (larvae of the beetle *Zophobas morio*), Lobster roach (*Naophoeta cinerea*), Orange spotted roach (*Blaptica dubia*), Madagascar hissing roach (*Gromphardhina portentosa*), Palmetto roach (*Periplaneta americana*), the Cape red roach (*Oxyhaloa duesta*) and the Turkestan roach (*Blatta lateralis*). These insects showed potential for mass rearing as they are small, produce multiple generations per year and have very few environmental requirements. Furthermore, these insects are generally reared on a small scale and used as feed for reptilian pets and are referred to as feeder insects (Finke, 2012).

3.3 Materials and Methods

3.3.1 Insect rearing and drying

Mealworms and superworms were reared on a diet of wheat bran and water was supplied in the form of carrageenan gel (98% moisture) and carrots. Mealworms were collected by placing egg cartons in the holding container. The mealworms then crawled onto egg cartons just before they were ready to pupate. Egg cartons were collected and mealworms shaken off. Superworms were collected by sifting them out of the bran. Roaches were reared in plastic containers and were maintained on a diet consisting of cooked layer mash with moisture supplied in the form of carrageenan gel (98% moisture) and some carrots. Empty toilet paper rolls were placed in rearing containers. The roaches would crawl into the toilet rolls. The toilet rolls were then collected and the roaches shaken off into a sealable bag. All insects were killed by freezing at -20 °C for 24 hours. Insects were then removed from the freezer and allowed to thaw after which they were dried in a ventilated oven at 65 °C for 72 hours. After drying, insects were ground using a homogeniser.

3.3.2 Methods for chemical analysis

3.3.2.1 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. Two samples, weighing 2 g, of each species were placed in a porcelain crucible and dried at 100 °C for 24 hours. Thereafter, the dried samples were weighed and the DM content calculated using Equation 1.

Equation 1:

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

$$\%\text{DM} = 100 - \%\text{Moisture}$$

Where:

A = weight of empty crucible

B=weight of air dried sample

C = weight of crucible and dry sample.

3.3.2.2 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. Samples were combusted in a combustion oven at 500 °C for six hours. Thereafter the combusted samples were weighed and the ash content calculated using Equation 2.

Equation 2:

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

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

Where:

A = weight of empty and dry crucible

D= weight of crucible and ash

3.3.2.3 Determination of CP

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 a (LECO) FP528 apparatus. Two samples of each species 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.

Equation 3:

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

3.3.2.4 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. Approximately 1 g of sample was weighed into a weighed ANKOM filter bag and heat sealed. In order to extract fat from the samples, bags were soaked in petroleum ether for 10 minutes, after which the bags were air dried. Bags were then placed in the ANKOM fibre analyser and agitated at 100 ºC in 1.9 L of the aforementioned 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 ºC in 1.9 L of the 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 4. One blank containing no sample was also included in the run in order to determine the blank bag correction factor.

Equation 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).

3.3.2.5 Determination of gross energy

The gross energy values for all samples were determined using the (IKA) calorimetric system C200. All samples were formed into tablets which were then placed into the decombustion vessel. The decomposition vessel was then filled with oxyen until a pressure of 3000 kPa was reached, after which it was placed in the IKA C200 calorimeter for combustion. The subsequent reading in MJ/kg was then taken as the gross energy value. It should also be noted that the IKA C200 calorimeter was first calibrated. This was done by combusting certified benzoic tablets with a known calorific value.

3.3.2.6 Sample hydrolysis for amino acid determination

The amino acid profile was determined using methods described by Cunico *et al*. (1986). Before the amino acid profile could be determined, samples had to be hydrolysed in acid. During this process, a sample weighing 0.1 g was placed in a specialized hydrolysis tube. Six millilitres hydrochloric acid (HCl) solution and 15% phenol solution was then added to the sample. The tubes were then vacuated by using a vacuum pump and nitrogen (N) added under pressure. The tubes were subsequently sealed off with a blue flame and the samples were left to hydrolyse at 110 ˚C for 24 hours. After hydrolysis the samples were transferred to Eppendorf tubes and refrigerated till it could be sent to the Central Analytical Facility of Stellenbosch University, where the amino acid profiles were determined by subjection to the Waters AccQ Tag Ultra Derivitization kit.

3.3.2.7 Determination of mineral composition

Samples were sent to the Western Cape Department of Agriculture located at Elsenburg, for mineral analysis.

3.4 Results and Discussion

3.4.1 Proximate composition

The proximate composition of the evaluated insects species are presented in Table 6. Results from the study indicate some variation in crude fat content of the species evaluated with values ranging from 24.2% to 38.3%. This may likely be due to undocumented differences in the reproductive state (Myers & Pedigo, 1977; Redford & Dorea, 1984; Mason *et al*., 1990; Pennino *et al*., 1990). These values are considerably higher than that of fishmeal and soya oilcake meal. These results, however, are not unexpected since in many studies on the chemical composition of invertebrates, high fat values were obtained (Raksakantong *et al*., 2010; Longvah *et al*., 2011; Melo *et al*., 2011; Oonincx & Dierenfeld, 2011; Finke 2012). The crude fat values for *N. cinerea, B. dubia, and G. portentosa* were similar, with values of 24.7%, 24.2% and 24.5%, respectively. *B. lateralis* had the highest crude fat content with a value of 38.33%. This value however is higher when compared to studies by Finke (2012) and Oonincx & Dierenfeld (2011) who noted values of 32.4% and 26.5%, respectively. The crude fat value for *Z. morio*, however, was slightly lower than those found in studies by Finke (2002) and Barker *et al*. (1998). They found values of 42.04% and 40.80%, respectively. The crude fat value for *T. molitor*, was similar to values found by Finke (2002), Barker *et al*. (1998) and Jones *et al*. (1972), but higher than values noted by Frye & Calvert (1989). They found values of 35.2%, 35.4%, 35.4% and 20.8%, respectively. These differences may be due to possible differences in rearing methods and diets since some studies have shown diet to be a major factor that may influence chemical composition (Simpson & Raubenheimer, 2001; Ramos-Elorduy *et al*., 2002; Oonincx & van der Poel, 2010). In a specific study by Li *et al*. (2012) it was found that *T. molitor* larvae fed plant waste had a lower fat content when compared to those fed the conventional bran diet. The value for *P. Americana* is higher than values for American roaches (28.7%) found by Bernard *et al*. (1997). This may possibly be due to the method by which fat content was determined.

Table 6 Proximate composition of selected insect species

Insect species	Common name	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Gross energy (MJ/kg)
T. molitor	Mealworm	50.16	31.09	5.77	3.70	25.32
Z. morio	Superworm	43.13	38.21	4.22	2.68	27.95
N. cinerea	Lobster roach	60.34	24.76	7.70	4.97	23.20
B. dubia	Orange spotted roach	46.25	24.17	8.50	4.06	21.64
G. portentosa	Madagascar hissing roach	55.28	24.46	8.12	4.56	23.43
P. americana	Palmetto roach	49.03	37.27	5.87	3.42	26.09
O. duesta	Cape red roach	52.59	28.85	6.72	3.78	24.99
B. lateralis	Turkestan roach	45.94	38.33	7.92	3.36	25.68
Fishmeal[1]		61.93	10.11	0.54	-	18.57
Soya oilcake meal[1]		49.44	0.45	7.87	7.64	18.92

([1]) NRC, 2004

Naophoeta cinerea yielded the highest CP value for this study with a value of 60.34%. The value differed markedly from the other roach species. This is also the only species whose CP content is comparable to that of fishmeal, whereas the rest is comparable to that of soya oilcake meal as indicated (Table 6). Superworms had significantly less CP than mealworms. According to Studier *et al*. (1991) larger larvae may have a lower nitrogen content due to a decrease in the surface area to mass ratio accompanying growth, since significant amounts of nitrogen are contained within the exoskeleton. Superworms are larger than the mealworms, which may account for their lower nitrogen level. The CP value for mealworms is similar to those found by Finke (2002) and Jones *et al*. (1972) but quite lower than those found by Frye & Calvert (1989). They found values of 49.1%, 52.8% and 64.7%, respectively. It was also lower than the value found by Li *et al*. (2012) who had a value of 76.1%. Mealworms in this study however, were fed plant waste materials which may explain the large difference. The CP value for *B. lateralis* was also lower than those found by Finke (2012), with a value of 61.5% and Oonincx & Dierenfeld (2011), with a value of 62.9%. The CP value for superworms were however similar to those found by Finke (2002), with a value of 46.8% and Barker *et al*. (1998), with a value of 43.1%. Crude protein for *P. americana* is comparable to values for American roaches (54.0%) found by Bernard *et al*. (1997). Insects were previously documented as an excellent source of dietary nitrogen (Martin *et al*., 1976; DeFoliart *et al*., 1982; Landry *et al*., 1986; Frye & Calvert, 1989; Zhou & Han, 2006; Elemo *et al*., 2011; Longvah *et al*., 2011; Melo *et al*., 2011). The CP content of all species investigated are comparable to those of soya oilcake meal (NRC, 1994), which has an estimated protein value of 49.44%, whereas the CP value for *N. cinerea* is comparable to that of fishmeal (68.84%) (de Koning, 2005). Estimates of protein content may however be misleading. Some of the nitrogen is contained within the N-acetylglucosamine subunit of the chitin polymer and may be unavailable to insectivores (Finke *et al*., 1989). The overall protein values for this study were however similar to those in a study conducted by Raksakantong *et al*. (2010).

Ash values, a measure of inorganic (mineral) content, were generally low (<10%), as previously reported (Martin *et al*., 1976; Redford & Dorea, 1984; Pennino *et a*l., 1991). Values ranged from 2.7% to 5.0% with *N. cinerea* having the highest value. The ash value found for *G. portentosa* is lower than those found by Oonincx & Dierenfeld (2011), who reported a value of 8.5%. However, in this study, all the roach species had similar ash values and the value for *B. lateralis* is comparable to the 3.9% found by Finke (2012). The ash values for all species, however, are lower than both that of fish meal and soya oilcake meal (NRC, 1994; de Koning, 2005).

Estimates of fibre content were also relatively low with values ranging from 4.2% to 8.5%, while the highest value was recorded for *B. dubia*. The mealworm and the super worm had the lowest values. This was expected, since they were in a larval state and contained no exoskeleton, and therefore also no chitin (Chitin may present itself as fiber). The crude fibre value for *N. cinerea, B. dubia*, *G. portentosa*, *O. duesta*, *B. lateralis* is comparable to the of soya oilcake meal which, according to the NRC (1994) has a value of 7.9.

There did not appear to be any marked differences in gross energy content for the species evaluated in this study. Values ranged from 21.6 MJ/kg to 28.0 MJ/kg, with the highest value recorded for *Z. morio.* The higher gross energy value for *Z. morio* may be related to its higher fat content. Fats have higher calorie content (37.7 MJ/kg) than proteins (16.7 MJ/kg) or carbohydrates (16.7 MJ/kg), thus providing a more concentrated energy source. It should also be noted that these values are higher than that of Fishmeal and soya oilcake meal.

3.4.2 Amino acid composition

The amino acid composition for the selected species is shown in Table 7. All species appear to be a good source of all the essential amino acids. The values for *N. cinerea* appear to be the highest. This may be related to its high protein content in relation to the other species evaluated.

Table 8 illustrates how the amino acid compositions of the various insect species compare to the ideal amino acid profile for broilers. Since lysine is the first limiting amino acid for broilers, the ideal amino acid profile for broilers was determined by expressing the essential amino acids as a percentage of lysine (NRC, 1994; Schutte & de Jong, 2004).

It can be seen from Table 8 that the amino acid profiles for *G. portentosa*, *P. americana*, and *B. lateralis* relate best to the ideal amino acid profile even though the valine to lysine ratio for these species were relatively high. None of the amino acid profiles for any of the insect species can be compared to that of fishmeal; the arginine, valine and isoleucine ratios are too high. The methionine to lysine ratio for *Z. morio* related best to that of soya oilcake meal and fishmeal, but the methionine to lysine ratio for *O. duesta* was close to that of the ideal amino acid profile for broilers. It is also evident form Table 8 that soya oilcake meal had the best amino acid profile. It should however be noted that the phytate present in soya oilcake meal may lead to a decrease in the bio-availability of all amino acids (Thompson & Serraino, 1986). Thompson & Serraino (1986) found that a complex that is formed between the proteolytic enzymes, phytate and the proteins within the animal's stomach, may lead to a decrease in amino acid and protein digestibilities.

The arginine to lysine ratio for all species ranged from 1.0 to 1.3. This is promising since birds are susceptible to lysine-arginine antagonism in cases where the arginine lysine ratios are <1. Consequences of lysine-arginine antagonism include the following: lysine competes 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; Leeson & Summers, 1997) and smaller amounts of excess lysine can lead to reduction in the hepatic glycine transaminidase activity in chicks (Jones *et al*., 1966). It should also be noted that the lysine content for all species ranged from 0.7% to 0.99%. This is promising since if the lysine content were to be greater than 3% it may lead to arginine degradation by renal arginase, depression of glycine transamidinase, depression of appetite and arginine loss through urine (Austic & Scott, 1975).

The isoleucine to leucine ratio for *N. cinerea*, *B. dubia*, *G. portentosa*, *P. americana*, and *O. duesta* were calculated to be 0.57 and that of *B. lateralis* to be 0.59. These values are below optimal especially considering that the isoleucine requirement for broilers is 0.9% (NRC, 1994). If the concentration of leucine is too high, it may lead to a reduction in the utilization of isoleucine (Leeson & Summer, 1997). Burnham *et al.* (1992) found that with a severe decrease in the isoleucine to leucine ratio in fed diets, there was a reduction in feed intake and consequently also weight gain. Ratios for *T. molitor* and *Z. morio* were however better, but not by much, with calculated values of 0.63 and 0.66, respectively. If these species were to be used in a complete diet, isoleucine would have to be supplemented in order to overcome the oversupply of leucine (Burnham & Gous, 1992).

Table 7
Amino acid composition (g/100g) dry matter for selected insect species

	Insect Species							
	T. molitor	***Z. morio***	***N. cinerea***	***B. dubia***	***G. portentosa***	***P. americana***	***O. deusta***	***B. lateralis***
Common name	Mealworm	Superworm	Lobster roach	Orange spotted roach	Madagascar hissing roach	Palmetto roach	Cape red roach	Turkestan roach
His	0.332	0.137	0.422	0.170	0.182	0.209	0.215	0.221
Ser	1.862	1.650	2.208	1.431	1.542	1.483	1.547	1.558
Arg	1.992	1.838	3.122	1.754	1.853	2.214	1.942	2.336
Gly	2.255	1.950	3.538	2.245	2.488	2.122	2.069	2.284
Asp	2.962	3.040	4.409	2.663	3.052	2.955	3.202	3.067
Glu	4.522	4.874	5.819	3.635	4.210	4.218	4.319	4.519
Thr	1.497	1.348	1.913	1.208	1.068	1.214	1.061	1.413
Ala	3.213	2.900	4.193	2.909	3.536	2.487	2.517	2.896
Pro	2.637	2.177	2.918	1.952	2.126	1.944	1.841	1.932
Cys	0.048	0.067	0.147	0.079	0.121	0.064	0.107	0.050
Lys	1.971	1.762	2.528	1.547	1.629	1.941	1.448	2.097
Tyr	2.517	2.579	4.144	2.902	2.999	2.934	2.694	2.727
Met	0.579	0.472	0.903	0.531	0.533	0.578	0.594	0.630

Table 7
Amino acid composition (g/100g) dry matter for selected insect species

	Insect Species							
	T. molitor	***Z. morio***	***N. cinerea***	***B. dubia***	***G. portentosa***	***P. americana***	***O. deusta***	***B. lateralis***
Common name	Mealworm	Superworm	Lobster roach	Orange spotted roach	Madagascar hissing roach	Palmetto roach	Cape red roach	Turkestan roach
Val	2.869	2.455	3.560	2.318	2.490	2.375	2.268	2.363
ILe	1.966	1.730	2.092	1.339	1.374	1.427	1.400	1.518
Leu	3.132	2.609	3.652	2.336	2.417	2.483	2.416	2.651
Phe	1.650	1.348	2.216	1.273	1.397	1.462	1.549	1.517

Table 8 Calculated amino acid to lysine ratios (%) in comparison to the ideal amino acid profile for broiler chicks.

		Amino acids					
		Lysine	**Threonine**	**Arginine**	**Valine**	**Isoleucine**	**Methionine**
T. molitor	Mealworm	100	76	101	146	100	29
Z. morio	Superworm	100	77	104	139	99	27
N. cinerea	Lobster roach	100	76	123	141	83	36
B. dubia	Orange spotted roach	100	78	113	150	87	34
G. portentosa	Madagascar hissing roach	100	66	114	153	84	33
P. americana	Palmetto roach	100	63	114	122	74	30
O. duesta	Cape red roach	100	73	134	157	97	41
B. lateralis	Turkestan roach	100	67	111	113	72	30
Fishmeal	-	100	60	79	77	62	27
Soya oilcake meal	-	100	64	117	77	73	23
Ideal amino acid profile[1]	-	100	65	110	80	70	38

([1]) Ideal amino acid profile as determined for broilers as determined by Schutte & de Jong (2004)

3.4.3 Mineral Composition

Table 9 illustrates the mineral composition of the selected insect species. The low calcium levels obtained here are consistent with values found in previous studies on a variety of insects (Frye & Calvert, 1989; Barker *et al*., 1998; Finke, 2002; Oonincx & Dierenfeld, 2011). The Ca value for *B. lateralis* is similar to that found by Oonincx & Dierenfeld (2011) but much higher than the value found by Finke (2012). The values obtained in these studies were 2.4 g/kg and 1.1 g/kg. This may likely be due to the difference in age of the roaches evaluated. The value for *G. portentosa* was also similar to that found by Oonincx & Dierenfeld (2011), who obtained a value of 2.5 g/kg. The values for *T. molitor and Z. morio* were similar to those found by Oonincx & Dierenfeld (2011) and Finke (2002), but lower than values obtained by Barker *et al*., (1998). These differences could possibly be due to the method used to determine mineral composition. The calcium content for the species evaluated here are also lower than some fly species (Finke, 2012). The exoskeleton of most insect species consists mainly of protein and chitin. Some fly species however, such as the soldier fly, have a mineralized exoskeleton that explains their higher calcium content. A very important observation from the data obtained here though, is that the Ca content for all species, with the exception of *B. dubia*, do not meet the minimum Ca requirement for poultry (NRC, 1994). Many of the species contain an inverse Ca:P ratio which, without supplementation, may lead to clinical Ca deficiency. This specific imbalance has been previously reported for other insect species (Pennino *et al*., 1991; Bernard *et al*., 1997; Barker *et al*., 1998; Finke, 2002).

Table 9 Mineral composition of selected insect species

		Species							
		T. molitor	***Z. morio***	***N. cinerea***	***B. dubia***	***G. portentosa***	***P. americana***	***O. duesta***	***B. lateralis***
		Mealworm	Superworm	Lobster roach	Orange spotted roach	Madagascar hissing roach	Palmetto roach	Cape red roach	Turkestan roach
Phosphorous	g/kg	7.9	4.9	7.6	5.9	7.7	6.0	7.0	6.8
Potassium	g/kg	8.6	7.6	10.5	8.4	11.3	8.3	9.6	9.9
Calcium	g/kg	0.4	0.6	3.4	5.8	2.9	2.9	2.5	2.3
Magnesium	g/kg	3.2	1.1	1.3	1.3	1.3	0.9	1.3	1.1
Sodium	g/kg	1.1	1.0	4.1	3.9	4.1	2.5	3.0	3.2
Iron	mg/kg	39.94	52.84	68.57	61.11	65.57	57.47	59.40	55.77
Copper	mg/kg	2.12	1.65	5.97	5.17	7.41	2.15	6.53	6.16
Zinc	mg/kg	101.70	63.50	251.60	239.20	245.50	87.91	307.00	107.30
Manganese	mg/kg	17.54	13.10	13.37	20.07	14.00	14.47	15.09	14.51
Boron	mg/kg	1.97	2.82	4.73	3.19	4.70	5.16	4.51	5.09
Aluminium	mg/kg	13.00	22.00	15.00	15.09	15.00	12.00	12.00	11.00

The phosphorous values obtained for *B. lateralis* are similar to values found by Finke (2012), but much lower than values found by Oonincx & Dierenfeld (2011). The value found in these studies were 5.7 g/kg and 9.5 g/kg, respectively. The value obtained for *G. portentosa* is however higher than the value of 5.7 g/kg obtained by Oonincx & Dierenfeld (2011). The values for *T. molitor* and *Z. morio* are similar to those found by Finke (2002) and Oonincx & Dierenfeld (2011), but lower than values found by Barker *et al*. (1998). The phosphorous content was much higher than calcium content for all species evaluated excluding *B. dubia*, who had a Ca value of 5.8 g/kg and a P value of 5.9 g/kg. Values reported in this study are however similar to those reported for other feeder insects (Frye & Calvert, 1989; Barker *et al*., 1998; Finke, 2002). It should also be noted that unlike plant-based phytate-phosphorous, the phosphorous in insects is more readily available as is shown for face fly (*Musca autumnalis*) pupae (Dashefsky *et al*., 1976).

Magnesium values for *T. molitor* are similar to those found by Barker *et al.* (1998), but higher than those found by Finke (2002) and Oonincx & Dierenfeld (2011). Values obtained in these studies were 0.28%, 0.21% and 0.19%, respectively. The values for *Z. morio* are similar to those found by Barker *et al*. (1998), Finke (2002) and Oonincx & Dierenfeld (2011). They found values of 0.18%, 0.12% and 0.15%, respectively.

Levels of the trace minerals were variable but similar to those previously reported for feeder insects (Martin *et al*., 1976; Barker *et al*., 1998; Finke, 2002; Oonincx & van der Poel, 2010; Oonincx & Dierenfeld, 2011). 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 (Finke, 2003; Oonincx & van der Poel, 2010). Studies on wild insects show both seasonal variation as well as variation between different populations of the same species living in the same general area (Finke, 1984; Studier *et al*., 1991).

3.5 Conclusion

The results obtained in this study are comparable to many of the previous studies conducted on a wide range of insect species. The roach species, *N. cinerea,* yielded the highest CP value, which turned out to be comparable to that of fishmeal. Most of the other species, however, were comparable to that soya oilcake meal. Although none of the species evaluated amino acid profiles could be compared to that of fishmeal, *G. portentosa*, *P.americana*, and *B. lateralis* did relate well to the ideal amino acid profile for broilers. These results are in support of the notion that insects may in future become a valuable protein source for use in animal feeds. It is therefore important that the role of insect protein in other aspects of animal feeding be investigated. The only factor that may depreciate the value of insects as protein source is their low calcium content. Perhaps in future, it would be possible to manipulate the diet in order to optimise the chemical composition of insects and by extension, optimising its use in animal feeds. It is definitely an avenue worth investigating.

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Chapter 4
Comparison of mealworm meal (*Tenebrio molitor*), black soldier fly (*Hermetia illucens*) pre-pupae and larvae meal in terms of gizzard erosion and total tract digestibilities

4.1 Abstract

A study using two insect species was conducted in which the possible effects on gizzard erosion was investigated and coefficient of total tract digestibility (CTTD) determined. Four treatments, namely *Tenebrio molitor* larvae (mealworms), *Hermetia illucens* pre-pupae, *H. illucens* larvae and control (maize meal) were fed. Mealworm meal caused significant ($P < 0.05$) gizzard erosion in broilers. Causative effects were possibly related to processing conditions, particularly excessive heat exposure during drying. The CTTD value for mealworms was 0.90, a value which is comparable to that of black soldier fly meal and soya oilcake meal. The CTTD value for lysine, 0.74, was however lower when compared to other protein sources, including fishmeal and soya oilcake meal. As with gizzard erosion, this low value may be attributed to unfavourable processing conditions. The *H. illucens* pre-pupae and larvae did not have any negative effects and results are in favour of their possible use in animal feeds. It is however recommended that further research be done regarding processing conditions.

4.2 Gizzard erosion study

Gizzard erosion in poultry is characterized by defects and inflammation in the gizzard mucosa. The condition as a problem in broilers and has been associated with many and diverse factors that have been assumed to play a causative, predisposing or preventative role (Kaldhusdal *et al*., 2012).These factors may include stress (Grabaravić *et al*., 1993; Dżaja *et al*., 1996), adenoviral infections (Abe *et al*., 2001; Ono *et al*., 2003), the ingestion of mycotoxins (Diaz & Sugahara, 1995; Ono *et al*., 2003) and also the presence of histamine and gizzerosine in the diet (Harry *et al*., 1975; Okazaki *et al*., 1983; Sugahara *et al*., 1988; Ono *et al*., 2003). The disease is characterized by a crop distended with black ingesta, chocolate coloured faeces and dark blood tinged vomit (Montes *et al*., 1980). The lining of the proventriculus and gizzard would show signs of erosion as well as signs of ulceration of the gizzard musculature (Johnson *et al*., 1971).

There is very limited published literature available on the toxic effects of insect meal. In a study by Pretorius (2011) it was found that the inclusion level of *M. domestica* larvae and pupae meal did not cause gizzard erosion in broilers. This appears to be the only insect species on which such a study has been conducted. It is therefore important that further studies on other species with a similar potential for use in animal feeds be conducted. Thus, the aim of this study was to determine if the inclusion of mealworm meal, black soldier fly pre-pupae and larvae meal could cause gizzard erosion in broilers.

4.2.1 Materials and Methods

Ethical clearance was obtained from the ethical committee of Stellenbosch (SU-ACUM14-00034; SU-ACUM14-00031).The experimental trial was performed at the poultry section of the Mariendahl experimental farm of Stellenbosch University. A total of forty day-old Cobb broiler chicks, as hatched,

were used. Chicks were kept in a temperature controlled house according to the management practices described by Cobb International (2008), until the end of the study.

Chicks were maintained on a commercial starter diet for the first seven days before they were switched over to the treatment diets. There were four treatment diets namely, the control (maize meal), MW (mealworm meal), BBSF (black soldier fly pre-pupae meal) and WBSF (black soldier fly larvae meal). Ten birds were randomly allocated to each treatment and birds were allowed *ad lib* access to their respective treatment diet for seven days. Table 10 presents the composition of the different treatment diets.

The black soldier fly pre-pupae- and larvae were obtained from AgriProtein ™. In preparation for inclusion in diets, they were killed in hot water at 62 ºC and subsequently dried at 60 ºC for 24 hrs. They were then milled finely. The mealworms were reared at the Department of Animal Sciences, Stellenbosch University. After collection, the mealworms were killed by exposure to boiling water (100 ºC) for four minutes. They were then dried in an oven at 100 ºC for two hrs. Once dried they were crushed and included in diet.

For the determination of differences between treatments, a categorical data analysis was done using SAS Enterprise Guide 5.1

Table 10 Composition (%) of treatment diets on as is basis

Inclusion levels of specific raw materials for each diet (%)					
Ingredient	**Starter**	**Control**	**MW[1]**	**BBSF[2]**	**WBSF[3]**
MW meal	-		50		
BBSF meal	-			50	
WBSF meal	-				50
Maize	47.75	100	50	50	50
Soybean full fat	32.10				
Soybean 46	7.72				
Fishmeal 65	9.25				
L-lysine HCl	0.06				
DL- methionine	0.36				
L-threonine	0.08				
Vit+min premix	0.45				
Limestone	1.17				
Salt	0.08				
Monocalcium phosphate	0.87				
Sodium bicarbonate	0.11				

([1]) MW- Mealworm, ([2]) BBSF- Black soldier fly pre-pupae, ([2]) WBSF- Black soldier fly larvae

On day fourteen, birds were culled by cervical dislocation and their gizzards removed. Gizzards were weighed and a gizzard score was given to each according to the following:

	Gizzard Erosion Scoring Description
1	No erosion
2	Light erosion (roughness of epithelia)
3	Modest erosion (roughness and gaps)
4	Severe erosion (roughness, gaps ad ulcers on stomach and showing light hemorrhaging
5	Extreme erosion (roughness, gaps and hemorrhagic ulcers on stomach wall and separation of epithelia from stomach wall)

The amino acid composition of the mealworms were determined by the Central Analytical Facility of Stellenbosch University as indicated in Chapter 3 (3.3.2.6 Sample hydrolysis for amino acid determination).

4.2.2 Results and Discussion

Table 11 illustrates the different gizzard erosion scores obtained after evaluation of individual gizzards. Statistical analysis indicate that there were no differences ($P > 0.05$) between the control, BBSF and WBSF treatments. The MW treatment did however differ significantly ($P < 0.05$) from the rest. From Table 11 it is evident that the frequency for a gizzard erosion score of 4 was higher for the MW treatment than for the rest. This is indicative of prominent erosion. The level of erosion observed for this treatment may be due to the high histidine content in mealworms. Several bacteria can transform histidine into histamine. Histamine is a biogenic amine which stimulates receptors in the proventricular glands, increasing hydrochloric acid secretion and thus causing superficial gizzard erosion (Contreras & Zaviezo, 2007). The cause of the erosion observed may also have been caused by gizzerosine. Gizzerosine is a bioactive amine, generally associated with overheated fishmeal (Kaldhusdal *et al*., 2012), and is a potent inducer of gizzard erosion in chicks (Mori *et al*., 1983). Histidine in fishmeal becomes gizzerosine due to excess heating during processing (Okazaki *et al*., 1983; Masumura & Sugahara, 1985; Sugahara *et al*., 1987; Kaldhusdal *et al*., 2012; Gjevre *et al*., 2014). There may have been a similar occurrence in the mealworms, since histidine content of the mealworms was found to be 0.332 g/100g. This value is higher than the value for fishmeal: 0.237 g/100g. The mealworms were also dried at 100 ºC for 2 hours. During heating, the ε-amino group of lysine reacts with the imidazolylethyl group of histidine to form gizzerosine (Okazaki *et al*., 1983; Masumura & Sugahara, 1985; Sugahara *et al*., 1988). Gizzerosine acts on the H_2-receptor of histamine, thereby stimulating gastric acid secretion.

Table 11 Number of observations per category of gizzard erosion scores recorded for the different treatment groups

Score	Control	MW	BBSF	WBSF
1	4	0	9	8
2	4	0	1	2
3	1	2	0	0
4	1	7	0	0
5	0	1	0	0

The erosion may also be explained by the presence of chitin in the mealworms. Chitin is a polysaccharide consisting of a β(1→4) polymer joined by a β(1→4) glycosidic bond, which is a crude fibre (Lindsay *et al*., 1984). Since the mealworms were included whole, the fibre in the form of chitin

was presented as very coarse. The ingestion of coarse fibres has been shown to increase gastric acid secretion, thereby lowering the pH of gizzard contents and causing gizzard erosion (Jiminéz-Moreno *et al*., 2009). The inclusion of coarse fibres has also been shown to stimulate gizzard development (Svihus, 2011). This may explain why the gizzard weights for the birds receiving the mealworm diet was significantly ($P < 0.05$) higher than those receiving the control, BBSF and WBSF diets. Also, the crude fibre content or BSF (9.13%) is higher than that of mealworms, thus further supporting the argument regarding the form of the fibre.

4.3 Digestibility study

The digestibility of a protein source is an important indication of the quality of the source (Sanchez-Muros *et al*., 2014). The potential value of a feed ingredient for supplying a particular nutrient can be determined by chemical analysis, but the actual value of the food to the animal can be arrived at only after making allowances for the inevitable losses that occur during digestion, absorption and metabolism. The first tax imposed on a food is that represented by the part of it which is not absorbed and excreted in the faeces (McDonald *et al*., 2002). It is important to note, however, that it is the individual amino acid requirement rather than the protein requirement that is essential in animal nutrition (McDonald *et al*., 2002). The assessment if amino acid digestibility of feedstuffs is thus essential if poultry are to be fed balanced diets (Short *et al*., 1999). A proportion of the dietary amino acids is excreted undigested and the specific amount differs among feeds (Lemme *et al*., 2004).

There have been a few digestibility studies conducted on selected insect species; Zuidhof *et al*. (2003) investigated the digestibility of housefly larvae meal when included in the diets of turkey poults. Results from this study indicate that the housefly larvae meal had significantly higher total tract digestibilities for energy, crude protein (CP) and all the amino acids, excluding cysteine, when compared to soya based commercial diet. Hwangbo *et al*. (2009) conducted a similar study on broilers and yielded a total tract digestibility for CP and essential amino acids of 98% and 94.8%, respectively. No work has been conducted on the digestibility of meal worms.

4.3.1 Materials and Methods

4.3.1.1 Digestibility trial

Ethical clearance (SU-ACUM14-00034; SU-ACUM14-00031) was obtained from the ethical committee of Stellenbosch University. The experimental trial was conducted at the poultry section of Mariendahl Experimental farm of Stellenbosch University. A total of 16 day-old Cobb 500 broiler chicks as hatched were used. Chicks were kept in a temperature controlled house according to the management practices described by Cobb International (2008) until the end of the study. Up until day twenty, chicks were housed in 0.9x0.6 m metabolic cages in groups of eight. After day 20, they were individually housed.

For the duration of the study, chicks were housed in the experimental house which comprises of a temperature controlled room equipped with metabolic wire cages measuring 0.45x0.6 m each containing one tube feeder and one nipple drinker. Artificial lighting was provided at a pattern of 18hrs of light altering with 6 hours dark. Ventilation in the house was set to provide a maximum of six air changes per hour. The chicks had *ad libitum* access to feed and water for the duration of the experimental period.

During the first 24 days the chicks were maintained on a commercial starter diet formulated to produce marketable chickens weighing 1.9 kg at 35 days according to the nutrient specifications

provided by Cobb International (2008). Hereafter the chicks were switched over to the treatment diets, the composition of which is illustrated in Table 12 below.

The mealworms included in the treatment diet were reared at the Department of Animal Sciences, Stellenbosch University. After collection, the mealworms were killed by exposure to boiling water (100 ºC) for four minutes. They were then dried in an oven at 100 ºC for two hrs. Once dried they were crushed and included in diet.

Table 12 Ingredient composition (%) of the commercial starter diet and the different treatment diets

	Commercial Starter	**Treatment 1 (Control)**	**Treatment 2 (MW[1])**
MW[1]	-	50	-
Maize	47.75	50	100
Soybean full fat	32.10	-	-
Soybean 46	7.72	-	-
Fishmeal 65	9.25	-	-
L-lysine HCl	0.06	-	-
DL - methionine	0.36	-	-
L- Threonine	0.08	-	-
Vitamin and mineral premix	0.45	0.45	0.45
Acid insoluble ash (Celite™)	-	1	1
Limestone	1.17	-	-
Salt	0.08	-	-
Monocalcium phosphate	0.87	-	-
Sodium bicarbonate	0.11	-	-

([1]) MW-Mealworm

Chicks were randomly allocated to pens and treatments in the experimental house with eight cages per treatment and one bird per cage. From day 20 to 24 chicks were allowed to adapt to the change in environment. From day 25 to 26 chicks were allowed to adapt to the treatment diets. During this time the individual group *ad libitum* intakes were determined. From day 27 to day 30 the digestibility trial was conducted.

During the time the chicks were left to adapt to the environment, no measurements were done or data collected (so as to minimise stress). From day 25 to 26 daily feed intakes and refusals were measured and the feed offered was adjusted to adapt to the *ad libitum* feed intakes. Faecal collection took place from day 27 to 30. Faecal collection trays were placed beneath the metabolic cages and faeces was collected and weighed. During the data collection period daily feed intakes and refusals were measured.

4.3.1.2 Analytical Methodologies

Chemical analysis for collected faecal and feed samples for this study were all conducted at the department of Animal Sciences, Stellenbosch University except for the determination of amino acid composition. This was done at the Central Analytical Facility, Stellenbosch University.

Determination of dry matter (DM), ash, CP, crude fat, and crude fibre content were done according to analytical methods described in Chapter 3. In preparation for the determination of amino acid

composition, all samples were hydrolysed according to the methods described in Chapter 3 (3.2.2.5 Sample hydrolysis for amino acid determination).

Gross Energy Determination

The gross energy (GE) was determined according to methods described in Chapter 3. This value was then used to determine the apparent metabolisable energy (AME) for each treatment diet using Equation 5 as described by Scott & Boldaji (1997):

Equation 5

$$\text{Apparent Metabolisable Energy} = GE_{diet} - \left[GE_{excreta} \times \left(\frac{Marker_{diet}}{Marker_{excreta}}\right)\right]$$

Coefficient of total tract digestibility

The coefficient of total tract digestibility (CTTD), of each analysed nutrient was calculated using **Equation 6** as described below.

Equation 6

$$\text{Nutrient consumed (g/trial)} = Nutrient_{analysed\ in\ feed} \times Dry\ matter_{intake}(g/trial)$$

$$\text{Nutrient excreted (g/trial)} = Nutrient_{analysed\ in\ excreta} \times Dry\ matter_{excreta}(g/trial)$$

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

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

4.3.2 Results and Discussion

Table 13 summarises the nutrient composition of the treatment diets as determined by various laboratory analyses.

Table 13 Analysed nutrient composition of treatment diets

	Units	Mealworm meal[1]	Housefly larvae meal[2]	Housefly pupae meal[3]	Black soldier fly meal[4]	Soya oilcake meal[5]	Fishmeal[6]
Gross Energy	MJ/kg	20.86	19.45	19.76	17.40	16.8	-
Ash	%	3.52	8.97	7.25	7.17	7.43	-
Crude protein	%	30.76	31.50	37.19	26.19	22.07	68.87
Crude fat	%	17.51	7.88	7.06	19.46	5.17	-
Crude fiber	%	6.65	5.69	8.99	6.51	-	-
Alanine	g/100g	0.87	1.51	1.57	7.4 7	1.05	4.36
Threonine	g/100g	0.60	0.88	1.38	4.73	0.74	3.02
Serine	g/100g	0.67	0.91	1.85	4.43	1.08	3.80
Glutamic acid	g/100g	2.64	0.00	1.40	14.34	3.89	9.27
Valine	g/100g	0.93	2.15	5.02	7.24	1.21	3.27
Histidine	g/100g	0.32	1.21	1.33	3.77	0.62	1.50
Aspartic acid	g/100g	1.03	0.64	0.74	9.44	2.26	6.15
Arginine	g/100g	0.46	2.35	4.16	6.47	1.49	4.80
Lysine	g/100g	0.53	1.68	2.28	5.55	1.27	4.43
Proline	g/100g	0.81	1.56	1.63	7.80	1.27	-
Methionine	g/100g	0.30	0.47	0.55	1.87	0.19	0.20
Tyrosine	g/100g	0.59	1.27	1.80	8.00	0.74	2.14
Cysteine	g/100g	0.00	0.08	0.12	0.20	0.38	-
Isoleucine	g/100g	0.53	1.06	1.15	5.07	0.97	2.63
Phenylalanine	g/100g	0.71	1.32	1.64	5.54	1.09	2.76
Leucine	g/100g	0.93	2.18	2.61	9.61	1.81	5.09
Glycine	g/100g	1.60	1.03	1.19	7.01	1.21	-

([1]) MW- Mealworm meal ([2]) HL- Housefly larvae meal, ([3]) Housefly pupae meal, ([4]) Black soldier fly meal

([5]) Soybean meal as determined by Valencia *et al*. (2011)

([6]) Fishmeal as determined by Ravindran *et al*. (1999)

Table 14 Average Coefficient of total tract digestibility (CTTD) values (±standard errors) for various insect species

	Mealworm meal	Housefly larvae meal[1]	Housefly pupae meal[1]	Black soldier fly meal[2]	Soya oilcake meal[3]	Fishmeal[4]
AME[5]	15.12 ± 0.021	14.23 ± 20.94	15.95 ± 19.29	17.40 ± 0.200	-	-
DM[6]	0.80 ± 0.04	0.81 ± 0.005	0.83 ± 0.005	0.94 ± 0.004	0.85 ± 0.004	-
Ash	0.92 ± 0.02	0.83 ± 0.004	0.85 ± 0.005	0.85 ± 0.024	-	-
Crude protein	0.90 ± 0.02	0.69 ± 0.009	0.79 ± 0.007	0.91 ± 0.015	0.86 ±0.007	-
Crude fat	0.86 ± 0.03	0.94 ± 0.004	0.98 ± 0.003	1.02 ± 0.001	-	-
Crude fiber	0.84 ± 0.04	0.62 ± 0.012	0.58 ± 0.013	0.74 ± 0.015	-	-
Alanine	0.77 ± 0.050	0.90 ± 0.009	0.86 ± 0.008	0.92 ± 0.008	0.87 ± 0.005	0.77 ± 0.012
Threonine*	0.81 ± 0.041	0.93 ± 0.010	0.97 ± 0.005	0.94 ± 0.004	0.80 ± 0.008	0.81 ± 0.001
Serine	0.80 ± 0.043	0.86 ± 0.027	1.00 ± 0.015	0.91 ± 0.006	0.82 ± 0.010	0.79 ± 0.001
Glutamic acid	0.85 ± 0.032	0.91 ± 0.006	0.99 ± 0.003	0.94 ± 0.005	0.83 ± 0.014	0.82 ± 0.011
Valine*	0.80 ± 0.043	0.91 ± 0.006	0.91 ± 0.005	0.92 ± 0.005	0.87 ± 0.007	0.80 ± 0.003
Histidine*	0.74 ± 0.056	0.87 ± 0.005	0.87 ± 0.004	0.95 ± 0.006	0.87 ± 0.009	0.80 ± 0.008
Aspartic acid	0.78 ± 0.047	0.93 ± 0.006	1.00 ± 0.004	0.95 ± 0.005	0.81 ± 0.012	0.79 ± 0.007
Arginine*	0.62 ± 0.081	-	0.93 ± 0.012	0.98 ± 0.002	0.88 ± 0.010	0.86 ± 0.001
Lysine*	0.74 ± 0.055	0.95 ± 0.005	0.99 ± 0.004	0.97 ± 0.004	0.88 ± 0.013	0.85 ± 0.028
Proline	0.75 ± 0.052	0.91 ± 0.005	0.91 ± 0.005	0.92 ± 0.004	0.87 ± 0.009	-
Methionine*	0.86 ± 0.029	0.95 ± 0.004	0.99 ± 0.003	0.97 ± 0.008	0.88 ± 0.010	0.87 ± 0.007
Tyrosine	0.68 ± 0.069	0.96 ± 0.005	0.96 ± 0.004	0.95 ± 0.005	0.85 ± 0.016	0.80 ± 0.001
Cysteine	-	0.92 ±0.010	0.96 ± 0.009	0.86 ± 0.024	0.81 ± 0.013	-
Isoleucine*	0.79 ± 0.046	0.91 ± 0.005	0.95 ± 0.004	0.94 ± 0.004	0.85 ± 0.013	0.82 ± 0.001
Phenylalanine*	0.82 ± 0.039	0.91 ± 0.005	0.95 ± 0.003	0.96 ± 0.003	0.86 ± 0.010	0.83 ± 0.003
Leucine*	0.75 ± 0.054	0.92 ± 0.005	0.96 ± 0.003	0.92 ± 0.010	0.90 ± 0.011	0.85 ± 0.001
Glycine	0.89 ± 0.022	0.83 ± 0.009	0.89 ± 0.010	0.88 ± 0.011	0.84 ± 0.015	

([1]) CTTD values as determined by Pretorius (2011), ([2]) CTTD values as determined by Uushona (2014)

([3]) CTTD values as determined by Valencia *et al*. (2011), ([4]) CTTD values as determined by Ravindran *et al*. (1999)

([5]) AME- Apparent metabolisable energy, ([6]) Dry matter

The CTTD values for mealworm meal is presented in Table 14. These values are further compared to values from previous studies as well as that of soy oilcake meal and fishmeal. It was found that the DM digestibility for mealworm meal is similar to that of Housefly larvae meal (HLM) and House fly pupae meal (HPM), but lower than the black soldier fly meal (BSFM). The CP digestibility was similar to that of BSFM and soya oilcake meal (SBM) and higher than that of HLM and HPM.

Animals have an individual amino acid requirement, rather than a complete protein requirement (McDonald *et al*., 2002). It is therefore more important to look at amino acid digestibility (Short *et al*., 1999). Digestibility hereof is also an important indicator of amino acid availability. The essential amino acids for poultry are methionine, lysine, threonine, tryptophan and valine (Kidd *et al*., 2000; Baker, 2009; Corrent & Bartelt, 2011), with methionine being the first limiting amino acid (Kidd *et al*., 2000; Corrent & Bartelt, 2011; Dozier *et al*., 2011). The mealworm CTTD values for methionine and threonine are similar to that of Soya oilcake meal and fishmeal, but lower than that HLM, PM and BSFM. The CTTD value for lysine, however, is lower than all other protein sources presented. The low digestibility value may be attributed to specific processing conditions, especially overheating (Parsons *et al*., 1992; Anderson-Hafermann *et al*., 1993; Newkirk *et al*., 2003) it has been found that overheating is one of the primary causes of reduced amino acid availability (Parsons *et al*., 1992). The amino acid most affected is usually lysine (Hancock *et al*., 1990) due to its susceptibility to Maillard browning reactions (Parsons, 1996). Parsons *et al*. (1992) found that increased exposure to heat reduced the concentrations and digestibility of lysine, cysteine and arginine. A similar event may have occurred here, since during the drying process the mealworms were exposed to a temperature of 100°C for 2hrs. Furthermore, the CTTD value for arginine is also much lower than the rest, thus further supporting the argument.

The lower digestibility may also be attributed to the form of the fibre or form of the mealworms in which they were included in the diet. The mealworms were included whole and were slightly crisp and thus slightly hard. According to Carré *et al*. (2002) there is a negative relationship between hardness and digestibility. The effect of hardness may be due to larger particulate size reducing the surface area and thus also exposure to digestive enzymes (Carré *et al*., 2005). Furthermore, coarse grinding of some grains has been shown to lower the digestibility coefficients of nutrients (Carré, 2004). In these cases it was also considered that the low digestibility may have been due to the inability of enzymes to access feed particles (Hesselman & Åman, 1985). Access problems related to coarse particles, however, could not be clearly demonstrated as there were many factors contesting the notion (Carré *et al*., 2002).

4.4 Conclusion

This study indicated that mealworms may cause prominent erosion in broiler chickens. The hypothesised causative effects were mainly related to the high histidine content of mealworms processing and conditions. Similarly with the digestibility study, the lower digestibility value of some nutrients may have been related to unfavourable processing conditions. It is then advisable that further research be done relating to different processing methodologies. Despite this, the promising CP digestibility value for mealworms is indicative of its potential as protein source in animal feeding.

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Chapter 5
Comparison of the production and egg quality parameters of laying hens maintained on diets containing mealworm (*Tenebrio molitor*) meal, black soldier fly (*Hermetia illucens*) larvae and pre-pupae meal

5.1 Abstract

The effect of mealworm meal (MW), black soldier fly pre-pupae- (BBSF) and larvae (WBSF) on layer production performance and egg quality parameters were investigated by comparison to a control (Soya oil cake). A total of 32 Hy-line Silver hens were used. Hens were assigned to four different treatment diets (MW, BBSF, WBSF and control) with eight hens per treatment in a completely randomised design. Parameters investigated were average daily gain (ADG), feed conversion ratio (FCR), egg weight, egg shell weight, yolk weight, albumin weight, yolk height, albumin height, shell thickness and colour. There were no significant differences ($P > 0.05$) in ADG between treatments. The BBSF had a significantly lower ($P < 0.05$) FCR. The egg weights for the control treatment was significantly lower ($P < 0.05$) than that of the other treatments. The shell weight for the mealworm treatment was significantly higher than the other treatments. Results obtained in this study are in favour of the use of insect meal in layer diets.

5.2 Introduction

Poultry products, including meat and eggs have always been a major source of animal protein for humans (Stenhouse, 2008). Globally, production of these products has been rising rapidly. This is a clear reflection of consumer preference for these products and the relatively low price because of efficiency of production. According to Scanes (2007), production of chicken meat and eggs had increased between 1995 and 2005 by 53% and 39%, respectively. By 2007 global egg production, expressed as a weight percentage of meat production, was at 78%. It is clear that egg production is growing rapidly, and the importance of research in this area becoming ever greater (Scanes, 2007). It is important to note, however, that such rapid growth in the industry as indicated above may have an enormous impact on the demand for raw materials for use in poultry feeds (Ravindran, 2013). It is becoming especially difficult to acquire suitable protein sources for use in poultry feeds (Khusro *et al*., 2012; Ravindran, 2013).

The major plant protein source is soya and the major animal protein source is fishmeal (Barrows *et al*., 2008; Ijaya & Eko, 2009). The protein of fishmeal has a high biological value, since it has a protein content that ranges from 500-750g/kg and it is rich in the essential amino acids (Miles & Jacob, 1997; Karimi, 2006). In most developing countries, fishmeal is an important source of animal protein. However, its production, availability, and cost are major concerns for animal nutritionists. Fishmeal is very scarce and expensive and its inclusion in poultry diets may prove to become unprofitable (Ijaiya & Eko, 2009). Also, further expansion possibilities in the fishmeal industry appear to be limited. Production does not seem to have increased over the last 20 years, and given the pressure on world fisheries, is unlikely to do so in the future (Ravindran, 2013).

Soya oilcake meal is a commonly used source of protein in poultry diets (Willis, 2003; Ravindran, 2013), due to its high level of digestibility and excellent amino acid composition (Willis, 2003), although the concentrations of cysteine and methionine may be suboptimal (McDonald *et al*., 2002). Furthermore, soya oilcake meal contains a number of toxic substances including allergenic,

goitrogenic and anti-coagulant factors. Of particular importance are the protease inhibitors, the Kunitz anti-trypsin factor and the Bowman-Birk chymotrypsin factor. Although a marked increase in soya production has been witnessed over the past few years (Ravindran, 2013), the availability of soya oilcake meal for use in animal nutrition is limited due to competition with human consumption thereof (Ravindaran & Blair, 1992; Ravindran, 2013). There is thus a need to identify alternative protein sources either for total or partial replacement which meet the dietary requirements of poultry and reduce feed costs (Ramos-Elorduy *et al*., 2002; Das *et al*., 2009, Razak *et al*., 2012).

Among potential protein sources that could replace soya oilcake meal and fishmeal is insect protein (Finke, 2002; Premalatha *et al*., 2011 Razak *et al*., 2012). In nature, insects form a significant biomass, as can be seen with insect pests (Ramos-Elorduy, 1997). Insects can be used to produce cheaper protein from non-food animals. Insects are part of the natural diets of poultry (Zuidhof *et al*., 2003), and scavenging poultry consume a wide variety, including grasshoppers, crickets, termites, acridids, scale insects, beetles, caterpillars, pupa, fleas, bees, wasps and ants (Ravindran, 2013). Insects have a high nutritive value, not only in proteins, but also in fats, minerals and vitamins (Chapman, 1998; Khusro *et al*., 2012). Protein levels in insectis are reported to range from 40 to 75% (Khusro *et al*., 2012; Ravindran, 2013). It is particularly for this reason that they are considered to be a promising animal feed ingredient, together with the fact they have a short life cycle and are easy to produce and handle (Ramos-Elorduy *et al*., 2002).

Very few studies have been done on the use of insect protein in layer diets. There are however some studies on the use of the larvae of *Musca domestica* (maggot meal) as protein source in layer diets. Agunbiade *et al*. (2007) concluded that maggot meal could replace 50% of the dietary animal protein supplied by fishmeal without adverse effects on egg production and shell strength. In another study Akpodiete *et al*. (1998) found that partial substitution of fishmeal with maggot meal had no significant impact on feed intake, hen-day egg production, egg weight, feed efficiency and liveability. It was also found that the albumen height was significantly higher in birds fed with a diet which contained equal levels of maggot meal and fish meal.

The aim of this study was therefore to evaluate the effects of mealworm meal (*T. molitor*), black soldier fly (*H. illucens*) pre-pupae meal and black soldier fly larvae meal on layer production performance and egg quality parameters.

5.3 Materials and Methods

Ethical clearance (SU-ACUM14-00034; SU-ACUM14-00031) was obtained from the ethical committee of Stellenbosch University. The study was conducted on Mariendahl experimental farm, Stellenbosch University. A total of 32 Hy-line Silver hens, 32 weeks of age, were used for this experimental trial. The hens were kept in a naturally ventilated layer house equipped with A-type layer cages for the duration of the trial. The hens had *ad lib* access to both feed and water at all times.

The hens were assigned to four different treatment diets (including the control diet). Treatment diets are shown in Table 15. The diets were formulated so that the hens were maintained on the minimum nutrient requirements. However, the diet containing the mealworm meal had a slight oversupply of protein. The treatment diets were allocated so that each hen received approximately 150 g feed per day. The control diet for this trial was soya based since it is an internationally accepted mixture suitable for egg production. The 32 layers were divided into four different cages with eight hens per

cage. The layout was a completely randomised design with eight replicates per treatment and one hen per replicate.

The body weights of each hen was determined on day 0 of the experimental trial and weekly thereafter. Feed was supplied on an *ad libitum* basis and weekly feed intake was determined. The data collected was used to determine the feed conversion ratio (FCR) for egg production (Equation 7) and average daily weight gains (ADG) (Equation 8).

Equation 7

$$\text{FCR (per kg egg mass)} = \frac{\text{kg of feed consumed}}{\text{kg of egg produced}}$$

Equation 8

$$\text{ADG} = \frac{\text{Average live weight per hen}}{\text{days}}$$

Table 15 Ingredient and calculated nutrient composition of treatment diets (as is basis)

		Treatment diets			
	Unit	**Diet 1 (Control)**	**Diet 2 (10% MW[3])**	**Diet 3 (10% WBSF[4])**	**Diet 4 (10% BBSF[5])**
Ingredients					
WBSF[4]	%	-	-	10	-
BBSF[5]	%	-	-	-	10
MW	%	-	10	-	-
Maize	%	58.82	64.35	64.19	64.13
Soybean full fat	%	22.64	-	-	-
Soybean 46	%	4.16	13.17	13.24	13.44
L-lysine HCl	%	-	-	0.10	0.01
DL methionine	%	0.13	0.1	0.12	0.10
Vit+min premix	%	0.25	0.25	0.25	0.25
Limestone	%	8.58	8.66	7.38	7.38
Salt	%	0.29	0.08	0.19	0.21
Monocalcium phoshate	%	1.49	1.37	0.28	1.28
Sodium bicarbonate	%	0.09	0.36	0.20	0.17
Oil	%	3.55	1.67	3.06	3.03
Calculated nutritional value					
DM[1]	%	89.08	89.08	89.04	88.66
AME[2]	MJ/kg	12.92	12.92	12.91	12.92
Crude Protein	%	15.42	15.42	15.42	15.42
Ash	%	10.49	10.49	9.54	10.01
Crude Fibre	%	2.75	2.74	2.58	3.35
Crude fat	%	10.00	10.00	10.00	7.65
Calcium	%	3.42	3.42	3.50	3.50
Phosphorous	%	0.69	0.70	0.88	0.90

Available phosphorous	%	0.45	0.45	0.45	0.45
Chloride	%	0.22	0.22	0.22	0.22
Potassium	%	0.65	0.61	0.53	0.53
Lysine	%	0.81	0.81	0.80	0.80
Methionine	%	0.38	0.37	0.37	0.37
Threonine	%	0.59	0.59	0.59	0.55
Tryptophan	%	0.17	0.17	0.15	0.15
Arginine	%	1.01	1.00	0.88	0.94
Isoleucine	%	0.67	0.67	0.59	0.64
Leucine	%	1.46	1.46	1.36	1.44
Histidine	%	0.43	0.43	0.43	0.47
Phenylalanine	%	0.71	0.72	0.68	0.68
Tyrosine	%	0.56	0.56	0.62	0.71
Valine	%	0.78	0.77	0.74	0.82

([1]) DM- Dry matter, ([2]) AME- Apparent metabolisable energy, ([3]) MW- Mealworm meal, ([4]) WBSF- Black soldier fly larvae meal, ([5]) BBSF- Black soldier fly pre-pupae meal

To determine if there were any significant differences between treatments, ANOVAs were done on all data using SAS Enterprise Guide 5.1. The correlation coefficient were also determined for some parameters.

For the first two weeks of the experimental trail, hens were allowed to adapt to the feed. From the onset of week three, eggs from each hen was collected and weighed on a daily basis for a total of 21 days.

Eqq quality parameters measured included egg weight, shell weight, yolk weight, shell thickness, yolk height, albumin height and yolk colour. The yolk colour color was determined using a (CIElab) colorimeter. Colour parameters measured were L* (denoting lightness), a* (denoting red/green value) and b* (denoting yellow/blue value).

5.4 Results and discussion

Table 16 summarises all production parameters for the layers. There were no significant ($P > 0.05$) differences in average daily gain. The FCR for birds receiving the BBSF treatment diet, however, was significantly lower than the rest. From Table 16 it can be seen that there is no real change over the five weeks in live weight and feed intake as is indicted by the low ADG values for all birds. This 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 & Minvielle (1999), after 20 weeks of age, laying hens reach a growth plateau and the values for these two parameters remain relatively constant. The same is witnessed for the birds in this trial.

Table 16 Averages of weekly live weight (kg) (±standard error), weekly feed intake (kg), and cumulative feed intake (kg) of layers receiving different treatment diets.

	Diet 1 (Control)	Diet 2 (10% MW[1])	Diet 3 (10% WBSF[2])	Diet 4 (10% BBSF[3])
Day 7				
Average live weight	1.76 ± 0.14	1.79 ± 0.17	1.80 ± 0.14	1.82 ± 0.09
Weekly feed intake	0.840	0.763	0.763	0.762
Cumulative feed intake	0.840	0.763	0.763	0.762
Day 14				
Average live weight	1.81 ± 0.17	1.80 ± 0.16	1.79 ± 0.14	1.81 ± 0.08
Weekly feed intake	0.800	0.775	0.756	0.769
Cumulative feed intake	1.640	1.538	1.519	1.531
Day 21				
Average live weight	1.84 ± 0.15	1.88 ± 0.17	1.88 ± 0.11	1.89 ± 0.08
Weekly feed intake	0.795	0.800	0.831	0.806
Cumulative feed intake	2.435	2.338	2.350	2.337
Day 28				
Average live weight	1.86 ± 0.17	1.89 ± 0.23	1.91 ± 0.13	1.89 ± 0.08
Weekly feed intake	0.812	0.786	0.769	0.75
Cumulative feed intake	3.247	3.124	3.119	3.087
Day 35				
Average live weight	1.86 ± 0.14	1.89 ± 0.19	1.91 ± 0.15	1.89 ± 0.09
Weekly feed intake	0.612	0.571	0.794	0.338
Cumulative feed intake	3.859	3.695	3.913	3.425
ADG (kg)	0.053 ± 0.004	0.054 ± 0.006	0.054 ± 0.004	0.0054 ± 0.002
FCR (per kg egg mass)	3.15 ± 0.09	3.19 ± 0.24	3.43 ± 0.29	2.78 ± 0.12

([1]) MW- mealworm meal, ([2]) WBSF- Black soldier fly larvae meal, ([3]) BBSF- Black soldier fly pre-pupae meal,
([4]) ADG- average daily gain, ([5]) FCR- feed conversion ratio

Egg quality parameters including egg weight, shell weight, yolk weight, albumin weight, yolk height, albumin height as well as colour characteristics are given in Table 17. The egg weights for the control diet was significantly lower ($P < 0.05$) than that of the rest. Egg weights for diets containing MW,

WBSF and BBSF did not differ significantly (P > 0.05) from each other. When one considers egg size classification, then it should be noted that the eggs from the control group fell under the large classification (51 g to 59 g), whereas the rest were extra-large (59 g to 66 g). Yolk weight did however not differ significantly (P > 0.05) between the treatments. Since yolk weight and egg weight are positively correlated, one would expect the same result as for the egg weights. However, it should be noted that the egg weights for all treatments were very close, even though they differed statistically.

Table 17 Average (± standard error) egg quality measurements as influenced by treatments

	Diet 1 (control)	**Diet 2 (MW[1])**	**Diet 3 (WBSF[2])**	**Diet 4 (BBSF[3])**
Egg Weight (g)	58.39 ± 3.19^{b}	60.39 ± 3.08^{a}	59.42 ± 4.83^{b}	59.89 ± 3.58^{b}
Shell weight (g)	7.38 ± 0.64^{a}	7.65 ± 0.96^{b}	7.34 ± 0.81^{a}	7.51 ± 0.60^{a}
Yolk weight (g)	17.28 ± 1.58^{a}	17.48 ± 1.43^{a}	17.29 ± 1.56^{a}	17.79 ± 1.69^{a}
Albumin weight (g)	34.14 ± 2.78^{b}	35.22 ± 2.44^{a}	35.21 ± 2.44^{a}	35.01 ± 2.16^{a}
Yolk height (mm)	17.41 ± 0.90^{c}	18.68 ± 0.96^{a}	18.01 ± 0.99^{b}	18.22 ± 1.08^{b}
Albumin height (mm)	8.07 ± 0.71^{b}	8.41 ± 1.15^{a}	8.69 ± 1.10^{a}	8.48 ± 1.24^{a}
Shell thickness (mm)	0.33 ± 0.04^{a}	0.34 ± 0.04^{a}	0.34 ± 0.04^{a}	0.35 ± 0.04^{a}
Colour				
L*	58.98 ± 0.58^{b}	61.34 ± 0.41^{a}	59.74 ± 0.42^{ab}	59.71 ± 0.47^{ab}
a*	9.99 ± 0.20^{b}	9.55 ± 0.21^{b}	10.94 ± 0.17^{a}	11.58 ± 0.22^{a}
b*	60.89 ± 0.89^{a}	59.20 ± 0.89^{a}	62.32 ± 0.85^{a}	61.98 ± 0.89^{a}

([1]) MW- Mealworm meal, ([2]) WBSF- Black soldier fly larvae meal, ([3]) BBSF- Black soldier fly pre-pupae

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

Shell weight for the MW diet appeared to be significantly higher (P < 0.05) than that of the control, WBSF and BBSF diets. It should be noted however, that these differences are rather small and may be ignored, especially when one considers the fact that there were no significant differences (P > 0.05) in shell thickness between the treatments. This is an important observation, since there may be a positive correlation between shell weight and shell thickness (Perek & Snapir, 1970).

The albumen weight and albumen height for the MW diet differed significantly (P < 0.05) from the control, although it did not differ significantly (P > 0.05) from the WBSF and BBSF treatments. These results are the same as that for the egg weights, since there may be a positive correlation between albumen weight and egg weight, as well as between albumen height and egg weight (Silversides & Budgell, 2004). The lower albumen height associated with the control, may be attributed to proteolysis of ovomucin, cleavage of disulphide bonds, interactions with lysozyme, and changes in the interactions between α and β ovomucins (Stevens, 1996).

When looking at colour, MW differed significantly (P < 0.05) from the control for the L* parameter, which is a measurement of lightness. For the a* parameter, which denotes the red/green value, MW and control did not differ significantly (P > 0.05), but their values were significantly lower (P < 0.05)

than that of the WBSF and BBSF treatments. There were no significant differences (P > 0.05) for the b* parameter, which denotes the yellow/blue value. These results may be explained by the presence of specific carotenoids in the diet. According to Finke (2013), black soldier fly larvae contain the carotenoids beta-carotene (<0.20 mg/kg), lutein (0.6 mg/kg) and zeaxanthin (1.3 mg/kg). These carotenoids are generally responsible for the yellow pigmentation in feedstuffs. This may explain the higher values for the a* parameter in the WBSF and BBSF diets. Mealworms on the other hand contain only a small amount of lutein (0.2 mg/kg) and no zeaxanthin (Oonincx & Dierenfeld, 2011). This may explain why there were no difference in the a* parameter between MW and the control, since the primary source for the yellow pigment would be the yellow carotenoids found in maize.

5.5 Conclusion

For production traits, the insect meals performed favourably when compared to the control, with only the BBSF diet showing a lower FCR. In terms of egg quality characteristics, the insect meals were either comparible with or better than the control; a diet representative of that which is fed commercially. The MW diet had the highest values for egg weight, shell weight, yolk weight, and albumin weight and yolk height. The control diet on the other hand, generally had the lowest values for most egg quality parameters. Results obtained in this study is a clear indicator that insect meals may be used in layer diets without adverse effects. It is recommended that further research be done to corroborate these findings in order to further cement the standing of insects as potential protein source in animal feeds.

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Chapter 6
General conclusion

The various insect species investigated here showed promise as a potential protein source for use in animal feeds. The first important indicator was the crude protein (CP) and amino acid content of the selected species. The roach species, *Naophoeta cinerea*, yielded a protein value of 60.34%, which is comparable to that of fish meal. The protein value of other species investigated were comparable to that of soya oilcake meal, with values ranging from 43% - 55%. These species were also good sources of all the essential amino acids and the amino acid profile for *Gromphardhina portentosa*, *Periplaneta americana* and *Blatta lateralis* related well to the Ideal amino acid profile for broilers. The only potentially limiting factor with regards to chemical composition, is the general low calcium content for all species. It is therefore recommended since, according to literature, the diet of insects has an influence on its chemical composition that further studies be done on manipulating the diet of the insects. By doing so it may be possible to optimise the chemical composition of the insects to better meet nutrient requirements of poultry.

Although mealworms caused significant gizzard erosion in broilers, the larvae and pre-pupae of *Hermetia illucens* did not. The CP digestibility of mealworms was comparable to that of black soldier fly meal and soya oilcake meal. The digestibility of lysine, the second limiting amino acid for broilers, was however lower than that of other proteins sources including fishmeal and soya oilcake meal. The causative effects for both the gizzard erosion and the poor digestibility of mealworm lysine were most likely related to processing conditions, particularly overexposure to heat and particle size. Further research should therefore be done on drying methods that do not require exposure to temperatures that are too high.

The layer production study yielded promising results. There were no significant differences ($P > 0.05$) in average daily gain between treatments. Layers fed the *H. illucens* pre-pupae meal had a significantly lower ($P < 0.05$) feed conversion ratio. The egg weights for the control treatment was significantly lower ($P < 0.05$) than that of the other treatments. The shell weight for the mealworm treatment was significantly higher than that of the other treatments. Results obtained in this study are in favour of the use of insect meal in layer diets. Further studies should however be done to corroborate these findings and also perhaps on other animal factors that influence production performance.

A stage will soon be reached where the use of insect protein in animal feeds will no longer be a novel idea. Research in this area is progressing rapidly, but there are still some areas that require further investigation. Insect protein is more frequently researched in poultry and fish nutrition. There are however other animals that may also be able to utilise insect protein. More research would further validate the use of insect protein. This is important, because all the research done so far needs to be applied commercially. Global warming may inevitably lead to feed shortages, necessitating the utilization of insects.