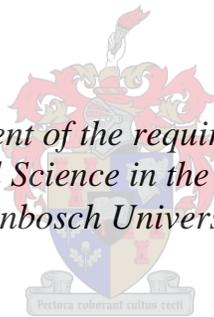


The determination of nutrient requirements and development of artificial diets for the mass rearing of insects of economic importance

by

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Master of Science in Animal Science in the Faculty of AgriScience at
Stellenbosch University*



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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 mass production of various insect species for biocontrol methods and as feed and food is becoming common practice and several different rearing facilities have been established across the world. Although insect mass production facilities have been set in place, the mass production of insects is still in an infantile stage and research is necessary to optimize these systems. As with conventional intensive livestock production the success of these systems, as well as the costs implicated, can largely be contributed to the nutrition of the animals. The false codling moth (FCM) (*Thaumatotibia leucotreta*), black soldier fly (BSF) (*Hermetia illucens*) and yellow mealworm (mealworm) (*Tenebrio Molitor*) are species currently being mass reared and are of economic importance. Three separate experiments were conducted to study the nutritional needs and formulate artificial diets for the mentioned species. It was believed that the current diet used to mass produce the FCM did not meet the requirements of the insects and that it led to nutrient imbalances, and therefore optimal production could not occur. Diets were formulated with novel raw materials and processing methods determined based on various methods. The newly formulated diet led to a ~55% increase in productivity. Current practice for the mass rearing of the BSF involves rearing the insects on chicken layer mash for the first six days of their life cycle, also referred to as the nursery phase. It was once again believed that the nutrient composition of the layer mash in no way resembled the nutrient needs of the larvae and a nursery diet was developed using the comparative slaughter technique. The newly formulated nursery diet led to a ~25% increase in survivability of the neonatal larvae during the nursery period. The protein requirement of mealworms was also studied. Plant (soya bean meal) and animal (ground beef) protein sources at different inclusion levels were tested. The inclusion of ground beef led to a ~50% increase in pupation rate of the mealworm larvae which implied a decrease in production time needed to rear the mealworm and an increase in production efficiency. Overall the results obtained from the different studies were a step in the right direction to understand the nutritional needs of the insect species studied and to solidify the mass rearing of insects for biocontrol methods as well as for feed and food.

Opsomming

Die grootskaalse produksie van verskillende insekspesies vir die doel van biologiese beheer en as voer en voedsel raak algemene praktyk. 'n Verskeidenheid produksie eenhede is al reeds gevestig reg oor die wêreld. Alhoewel grootskaalse eenhede alreeds in plek gestel is, is die grootskaalse produksie van insekte nog in 'n aanvangs fase. Navorsing word benodig sodat hierdie eenhede hul produksie potensiaal kan bereik. Soos in die geval met intensiewe produksie van vee speel voeding 'n groot rol in die sukses sowel as die koste van die produksie van insekte. Die vals kodling mot (VKM) (*Thaumatotibia leucotreta*), venstervlieg (VV) (*Hermetia illucens*) en geel meelwurm (meelwurm) (*Tenebrio Molitor*) is insek spesies waarmee reeds grootskaals geboer word en wat ekonomiese waarde het. Drie verskillende proewe is uitgevoer om die nutrient-behoeftes van die bostaande insekte te bepaal sowel as om voere te formuleer vir die grootskaalse produksie van hierdie spesies. Daar is geglo dat die huidige voer wat gebruik word om die VKM te produseer nie voldoen aan die nutrient-behoeftes van die larwes nie, wat lei tot nutrient wanbalans en afname in produksie. Voere is geformuleer met nuwe rou materiale en die gaarmaak metodes van die nuwe voer bepaal. Die nuwe geformuleerde voere het 'n ~55% toename in die produksie van die VKM tot gevolg gehad. Met die produksie van die VV maak huidige metodes gebruik van lê-hen meel om die larwes groot te maak vir die eerste ses dae van hul lewensiklus. Dit was weereens geglo dat die nutriëntsamestelling van die lê-hen meel nie aan die behoeftes van die larwes voldoen nie en nuwe voere is geformuleer deur gebruik te maak van die vergelykende slag tegniek. Die nuwe voer het 'n ~25% toename in oorleefbaarheid van die VV larwes tot gevolg gehad gedurende hierdie fase. Die proteïen-behoefte van meelwurms was ook ondersoek. 'n Plantproteïen (sojaboon meel) en dierlike proteïen bron (gemaalde beesvleis) teen verskillende insluitingsvlakke is getoets in die dieet van meelwurms. Die gebruik van gemaalde beesvleis as proteïenbron was gemik om die toekomstige moontlikheid van die insluiting van slagpale- afval in die dieet van meelwurms te evalueer. Die insluiting van gemaalde beesvleis het 'n ~50% toename in die tempo van papie wording tot gevolg gehad wat impliseer dat produksie effektiwiteit verdubbel het. Algeheel was die resultate van die proewe 'n stap in die regte rigting vir die grootskaalse produksie van insekte en het dit meer insig geskep oor die nutriëntbehoeftes van die insekte wat nagevors was.

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Notes

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

Abbreviations

ADG	Average daily gain
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BSF	Black soldier fly
CP	Crude protein
CH ₄	Methane
DE	Digestible energy
DM	Dry matter
FCM	False codling moth
FCR	Feed conversion ratio
GHG	Greenhouse gas
GLM	General linear model
kg	Kilogram
km	Kilometre
R	South African Rand
SIT	Sterile insect technique
W	Watt
°C	Degree Celsius

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Chapter 1

Introduction

The world is currently facing a new set of challenges. Current population of 5.1 billion is rapidly growing and is estimated to plateau at some 9 billion by the middle of this century (Godfray *et al.*, 2012). Accompanying growth in human population is increased wealth which leads to greater consumption of processed food, meat, dairy and fish. A 60-70% increase in consumption of animal products is expected by 2050 (Makkar *et al.*, 2014). More protein of animal origin will need to be produced to keep up with the ever-growing demand. Simultaneously food producers are experiencing greater competition for land, water and energy. Detrimental effects of food production on the environment can no longer be ignored and has played a substantial role in climate change (Godfray *et al.*, 2012). Also, it is estimated that 1.3 billion tons of food is wasted each year. This implies that sustainable intensification has to be implemented which entails producing more food from the same area of land while reducing the environmental impacts (Petersen & Snapp, 2015). Insects can provide a natural and legitimate solution to this problem on both economic and environmental levels.

Approximately one million insect species are already known and another ten million more are expected to be discovered. Of the one million known insect species, 1900 species are consumed worldwide of which most are in developing countries (Bukkens, 1997). Depending on species, edible insects have high nutritional value. They contain high levels of protein, fat and minerals (Rumpold & Schlüter, 2013). They are quality food and feed that have high feed conversion efficiencies and emit low levels of greenhouse gasses. When formulating feeds, fish- and soya bean meal have successfully been replaced by insect meal (Barroso *et al.*, 2014). In 2011 global industrial feed production for all livestock species was estimated at 870 million tons and worth approximately US\$400 billion (IFIF, 2015). Due to over exploitation of fish populations the price of fishmeal has increased three fold over the last ten years (Olsen & Hasan, 2012). With competition for land, water and fossil fuel the price of soya has also increased (Asche *et al.*, 2013). Sustainable intensification is an ever growing necessity and this has paved the way for insects to emerge to the forefront as main alternative protein source as food and feed (Makkar *et al.*, 2014). Great interest has been shown in finding an alternative protein source to replace, or supplement,

fish- and soya bean meal. The most promising insects for industrial production are the black soldier fly (*Hermetia illucens*), the common house fly (*Musca domestica*), the yellow meal worm (*Tenebrio molitor*), silkworm (*Bombyx mori*) and various grasshopper (*Orthoptera: Acrididae*) species (Van Huis *et al.*, 2015).

Insects are normally not considered a domesticated resource and only a few species are reared, but it has been proven that certain species are suitable and can successfully be domesticated. The house cricket (*Acheta domesticus*), the black soldier fly, the yellow meal worm and the giant water bug (*Abedus herberti*) (Thailand) are insects that have successfully been domesticated and currently being farmed with for feed and food. Domestication of insects are not only beneficial for feed and food but are of importance for biocontrol of certain insect species. One such species is the false codling moth (*Thaumatotibia leucotreta*). The sterile insect technique (SIT) is a programme developed for biocontrol of mentioned pest species which entails the mass production of sterile moths which in turn are released into citrus orchards to reduce feral male populations (Hofmeyr *et al.*, 2015).

Definite procedures for mass rearing of insects need to be developed. This is a challenge for industries specialized in the mass rearing of insects for biocontrol such as the sterile insect technique (SIT), and for food and feed (Rumpold & Schlüter, 2013). There are a few major issues that need to be addressed when forming a mass rearing system for insects. This includes quality, reliability and cost effectiveness. Mass produced insects should compare favourably to conventional protein sources (Kok *et al.*, 1990).

Great efforts have been made to solidify insect protein as a future commercial protein source. Currently prototype factories for the mass rearing of the black soldier fly and mealworm have been built and are functioning. Considerations that need to be taken into account when designing such large-scale insect production are the intrinsic rate of increase, weight gain per day, feed conversion ratio, invulnerability to disease, the potential to rear insects on organic side streams, suitability for automation and selection of high quality strains (van Huis, 2013). This very youthful industry faces many challenges. One must assure cost-effectiveness and reliable production of an insect biomass of high and consistent quality will be of crucial importance. It also faces numerous other challenges which includes food safety issues (pesticides, contaminants, heavy metals, pathogens,

allergenicity) (FAO, 2013) and processing procedures for converting insects to a protein meal to be used in the animal feed industry or as food source for humans (Klunder *et al.*, 2012). A collaboration of government, industry and academia will be crucial in determining the eventual success of mass producing certain insect species to serve as a sustainable protein source.

The black soldier fly, the yellow mealworm and false codling moth have been identified as insects of economic importance and have a big demand for mass rearing. Nutrition will play a massive role in successfully mass producing these insects and therefore their specific nutrient requirements need to be identified and met. Ensuring this will bring us closer to successfully mass producing these insects.

The purpose of this study was threefold:

- I. To determine nutrient requirements and optimize current commercial diet for the mass rearing of the FCM
- II. Develop a nursery diet for the mass rearing of the BSF
- III. Evaluate protein sources in the diet of mass reared mealworms

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Chapter 2

Literature Review

2.1 Introduction

Many insects have the capability to incorporate organic waste into their diets as principle feed stuff. They have the ability to recycle nutrients and incorporate residual proteins and other nutrients into their biomass. (Melorose *et al.*, 2015). Recent studies have indicated that insects are quality feed and food high in protein (Rumpold & Schlüter, 2013).

There is an ever growing demand for potential mass rearing of certain insect species (Oonicx *et al.*, 2010). The main distinction between mass rearing and laboratory rearing of insects is one of scale and economics. In laboratory rearing, it is important to obtain the maximum yield of insects and costs up to 15-25 cents/insect may not be excessive. On the other hand, mass rearing has the simple objective to rear large numbers of quality insects at the lowest possible cost (Singh, 1976).

Nutrition of mass reared insects plays a crucial role in the eventual success of the production system. It is of utmost importance that insects in mass production systems receive the correct nutrients, in the correct balance, as environmental stress is high. This is crucial to minimize mortalities and maximize production. Unfortunately, most current publications on insect nutrition are based on ingredient composition and not nutrient composition. The field of insect nutrition is under published (and possibly under researched) and the exact nutrient requirements of insects, seen to be of economic importance, needs to be quantified. By knowing the exact nutrient requirements of insects currently being farmed in intensive production systems one can efficiently formulate diets incorporating both conventional raw materials as well as organic waste. Mastering this crucial aspect will solidify insects as sustainable feed and food by successfully mass rearing these animals.

2.2 History and current status of insect nutrition

In the past when insect nutrition was referred to, it was commonly held that entomologists did not respond enthusiastically to this topic (Slansky, 1982). However, the understanding and implementation of insect nutrition is showing an ever growing interest. This could be contributed to the fact that this field does not just entail basic nutritional requirements, but the amount and rates of feed eaten, digested, assimilated and converted to tissue, are all important. Insect nutrition is a more sophisticated field than what it was thought to be. It is seen to be more than just common dietetics and involves many metabolic processes. Research on this topic determines essential nutrients and highlights metabolic pathways, genetic mechanisms, and comparative biochemistry (Lipke & Fraenkel, 1956).

The use of artificial diets for rearing insects has been developed since the 1950's in order to meet the demand for large number of insects required in the fields of physiology, ecology and genetics, and for insect control techniques such as male sterilization, pathogen production, hormone and pheromone manipulations, and biological and integrated control programmes (Singh, 1976). Over the past 40 years much effort has been expended in attempting to combine the 40-50 nutrients common to most foodstuffs into an acceptable feed for insects. This has resulted in papers describing diets for more than 750 species of insect (Singh, 1976). Only recently has the focus turned to identifying and formulating feeds according to nutrient requirements of the different insect species.

The success of entomology over the last century has a large part to do with the ability to rear insects on artificial diets and probably much of the future of entomology depends on this factor (Cohen, 2015). Insects are no longer just seen as pests, but the potential of mass producing certain species for feed and food has been realized. Insects reared on artificial diets are used in many programs. These programmes include biological control and sterile insect techniques (Bloem *et al.*, 2007), feed for other animals (Rumpold & Schlüter, 2013), as bioreactors for the production of pharmaceuticals and other recombinant proteins (Wall, 1999), and as food for people (van Huis, 2013).

As with the nutrition of any other animals, insect nutrition can be broken down into nutrient classes and diet components. These components include amino acids, carbohydrates, lipids, vitamins and

minerals (Chapman, 2012). Most insects have qualitatively similar nutritional requirements because their chemical composition and metabolic capabilities are broadly uniform. It was discovered that certain insect species have specific associations with certain microorganisms and that insects have adapted to particular diets (Cohen, 2015). These factors contribute to variation in their nutritional requirements.

Amino acids are essential for the production of proteins which in turn is used for structural purposes and receptor molecules (Israelewitz *et al.*, 2001). Nitrogen concentration significantly influences the performance of insects. It has a major impact on weight gain and egg production rate (Joern & Behmer, 1997). Dietary protein is the main source of amino acids for most insect species. Protein quality is defined as how well the essential amino acid profile of a protein source matches the requirements of the animal; the digestibility of the protein source and bioavailability of the amino acids also play a role. The nutritional value of proteins depends on the capability to meet the amino acid requirements of the animal in question without over- or undersupplying certain amino acids while considering digestibility, absorbency and mobilizability. It was determined that, when using artificial diets to rear insects, the nutritive effect of proteins on growth of insects after hatching is greatly dependent on the type of amino acids in the diet. When larvae are reared on diets containing proteins with a poor amino acid profile, haemolymph protein is decreased and uric acid secretion is accelerated immensely (Horie & Watanabe, 1983). Supplementation of essential amino acids are crucial in determining the success of artificial diets. Insects are not able to synthesize nine or ten amino acids which are called essential amino acids (Chapman, 2012). If essential amino acids are omitted from the diet the insect will not be able to grow whatever the total diet supplementary amino acids. Although certain non-essential amino acids can be synthesized from other amino acids through the process of transamination, insects are still dependent on precursor molecules derived from the diet. Sustained growth of insects is impaired by gross dietary imbalances and therefore it is of utmost importance that insects consume a balanced mix of dietary amino acids.

Carbohydrates, including simple sugars, starch and other polysaccharides, are important components of the insect diet. Carbohydrates make up roughly between 4% and 27% of the natural diet of insects (Joern & Behmer, 1997). They are the usual respiratory fuel, can be converted to lipids and through transamination can provide the carbon skeleton for the synthesis of various

amino acids (Chapman, 2012). Insects generally do not have the capability to digest and utilize certain carbohydrates such as cellulose. Although certain insect species are not able to digest cellulose, it can be used as a filler in their diet and help to promote intestinal stability (Cohen, 2015). Most mass reared insects fail to perform on diets that contain less than 50% carbohydrates and it is also important to keep in mind that the type of carbohydrate must be fitted to the specific insect species. Insects such as the screw-worm fly, on the other hand, can grow without any carbohydrates and only from live animal tissue (Chapman, 2012). There may be differences in the ability of larvae and adults to utilize carbohydrates (Huffaker, 1999).

The value of lipids, including sterols, oils, fats and phospholipids have been underestimated in insect nutrition. Failing to provide the right amount and type of lipids has been one of the major failings in insect dietetics. In most circumstances lipids have been undersupplied (Cohen, 2015). The absorption and digestion of lipids by insects are similar to those of vertebrates but do differ in some important aspects. Fat metabolism in insects centres around the lipid transport system; diacylglycerol is the major lipid transported in insect metabolism by means of a high-density lipoprotein called lipophorin. Lipophorin is a reusable shuttle that picks up lipid from the gut and delivers it to tissue for storage and utilization without using the endocytic processes common to vertebrate cells (Nichol *et al.*, 2002). All insects require dietary sterols, but because it is difficult to dissolve it is often omitted from their diets (Fraenkel & Blewett, 1946). Sterols are often given in the wrong form. Leaf eating insects are not able to digest sterols from animal origin such as cholesterol, and require plant sterols such as β -sitosterol or campesterol (Cohen, 2015). Lipids function as building-blocks of cell membranes, hormones (fatty acids are converted to juvenile hormone and sterols to moulting hormone), source of energy, nutrient transporters and as structural material for building other materials. Insects cannot produce sterols like vertebrates can and therefore sterols are essential nutrients to include into the diets of insects (Clayton, 1964).

Vitamins are organic compounds required in trace amounts for sustained growth. The general understanding of these organic structures in insects is frustratingly limited and our understanding of vitamin function in insects comes largely from knowledge as applicable to vertebrates. Vitamins can be classed in two distinct categories, water soluble or fat soluble. Water-soluble vitamins are readily excreted from the insect's metabolic pool and therefore they have a relatively short half-life compared to lipid-soluble vitamins which tends to accumulate in lipid stores.

Water-soluble vitamins include B vitamins, vitamin C (ascorbic acid), and some miscellaneous compounds. Vitamin C serves as an antioxidant and is an essential phagostimulant for many phytophagous insects (Vanderzant *et al.*, 1962). Ascorbic acid is most commonly present in its L-ascorbic form, a component that is found in high concentrations in several kinds of fresh fruits and green tissues of plants. For example, in broccoli and fresh green peppers one can expect to find amounts as high as 90 mg/100 g (USDA, 2016). The concentrations of ascorbic acid in plant components that are not green or in fruit is very low or absent. Therefore, when formulating artificial diets using grains and seeds one should include ascorbic acid if the target insect has a requirement for ascorbic acid. Ascorbic acid functions both in the diet and as a factor in the metabolic pathway of the insect that has ingested it (Briggs, 1962). It is also thought that ascorbic acid promotes collagen synthesis in the extracellular matrix of insects (Hunter *et al.*, 1979). The B vitamins function as cofactors in various metabolic pathways. These metabolic pathways include decarboxylation (Vitamin B₁, thiamine), flavoproteins (Vitamin B₂, riboflavin), cytochromes in ATP production (Vitamin B₃, niacin), acyl group transfer reactions (vitamin B₅, pantothenate), amino acid metabolism (vitamin B₆, biotin) and one carbon transfer reactions (vitamin B₉, folic acid). The B₁₂ vitamins (choline, carnitine and cyanocobalamin) and lipoic acid are water-soluble vitamins that are required by insects in very small amounts (Chapman, 2012).

Insects only have requirements for two of the four lipid-soluble vitamins. They have requirements for the vitamin A complex (β -carotene and related carotenoids) and vitamin E (tocopherols), but not for vitamin D (calciferols) and vitamin K (phylloquinone). Vitamin A complex are essential for the formation of eye pigments and other pigments and for normal growth (House & Barlow, 1958). Carotenoids are extremely effective antioxidants and prevent damage to cell membranes and vacuoles (Goodwin, 1986). Vitamin E is important for reproduction of insects, including spermatogenesis and egg maturation (Meikle & Mcfarlane, 1965).

Mineral nutrition is the most poorly understood aspect of insect nutrition. This is due to the difficulty in performing definitive nutritional studies due to the uncertainty that ingredients in a diet are entirely free of the mineral in question (Cohen, 2015). Insects require sodium, potassium, phosphate and chloride to be added to their diets for cellular ionic balance. Insects do not have the same requirements for calcium and iron as vertebrates which utilize these minerals in bone and

haemoglobin formation (Chapman, 2012). Due to this, commercial (for livestock) mineral mixtures cannot be added into the diets of insects.

The supply of the above mentioned nutrients must coincide in the correct balance. This balance is of utmost importance. Therefore, proteins, carbohydrates, lipids, vitamins and minerals must all be present in optimal quantities. The quantitative balance of nutrients is the dominant factor in the success of the diet (Singh, 1976). Although some growth can occur on foods that contain widely differing levels of nutrients, optimal performance will only be achieved if the nutrients are in the correct balance. Insects have the capability to select feedstuff as is required to satisfy their nutrient demand and balance (Raubenheimer, 1992). An unsatisfactory nutrient balance may lead to nutritional diseases affecting growth, development, reproduction and other life processes. Compensation is therefore an important means whereby insects maintain nutritional homeostasis in the face of dietary imbalances or food shortages.

2.3 Methods of developing artificial diets

When reviewing previous literature, the exact logic behind the methods for formulating artificial diets for various insect species is contradictory and rationally unclear. This is unfortunate because it forces all other researchers to start from scratch when developing methods to formulate artificial diets, repeating the same mistakes predecessors have made. Previously artificial diets have been developed using the following strategies: diets developed for insects with similar feeding habits; use of food analysis as a basis for diet development; use of whole carcass analysis in diet development (comparative slaughter); radioisotopes and diet deletion techniques; use of digestive enzymes as aids in diet development; nutrient self-selection and the eclectic approach.

Each species has its own feeding habits. When formulating diets, insects with the same feeding habits are likely to perform on similar diets. For example, leaf-feeding insects such as the cabbage looper is more likely to be suitable to a generalist diet formulated for leaf feeding insects such as that for the armyworm whereas a diet for carnivores or a phloem sap eater or even a specialist on other plant tissues such as an insect that consumes seeds or fruits, would be less suitable (Cohen, 2015). Diets that have previously been found to be suitable can then be used as a starting point for insects with the same feeding habits.

A nutrient analysis of the insect's natural feed could also be used to formulate diets using raw materials other than what would be eaten in their natural environment. The principle being that the artificial diet should mimic as closely as possible the general composition of the natural feed. This was found to play a significant role in determining the eventual success of the diet. In this method, the study of the exact feeding choices of the target species is important. This method could be misleading if the wrong assumptions are made on the natural feeding habits of the target species as well as faulty nutrient compositional analysis. The USDA nutrient data base (USDA, 2016) could be useful when the feeding habits of insects are known to be restricted to foods used by humans and whose nutritional composition are well documented.

Another method uses the whole-carcass analysis (comparative slaughter) to formulate artificial diets. It was thought that the composition of an insect's body reflects its nutritional needs (Rock & King, 1967). This method incorporates using the profile of various nutrients in a target insect's body as template for artificial diets. The comparative slaughter technique is a protocol used to estimate changes in the body composition of animals during an experiment. Although this method has been previously considered by Cohen (2015), entomologists lack basic understanding of mentioned technique and no diets have been published using this method. The comparative slaughter technique has been widely used by animal scientists to determine protein and energy requirements of various domesticated animals (Blaxter, 1967).

In previous studies used to formulate artificial diets, researchers provided a diet that contained all the well-known amino acids except for one. Carbon (^{14}C) or hydrogen (^3H) was also included into the diet either in the form of acetic acid or as a sugar. After the insects had been given a chance to consume and metabolize the diet the carcass was hydrolysed and analysed by conventional chromatography techniques. The separated components were analysed for radioisotopes. If, for example, histidine was not included into the trial diet but was found to be present and labelled with the isotope, it was concluded that the insect was able to produce the amino acid from the precursors provided in the diet (Rock & Hodgson, 1971). It was determined that this method could be used to determine dietary requirements.

Digestive enzymes are used as aids in diet development. When a digestive enzyme is present it indicates that the insect can utilize the substrate that the enzyme hydrolyses. When an insect is

able to hydrolyse a certain substrate it in turn means that the insects may be prepared to use the food material from which the substrate originates in its diet. The application of this basic technique has proven to be useful in the development or improvement of artificial diets for several species of insects (Cohen, 2015). This method aids in selecting raw materials but diets should still be formulated according to the nutrient needs of the target insect.

Nutrient self-selection has also been thought to be an adequate way to develop artificial diets. Insects are born with an innate “knowledge” about what is healthy or nutritional sound feed. If given a proper set of choices, the insect will select food or a combination of foods that completely fills its nutritional needs (Cohen *et al.*, 1987). Previous studies on the *Helicoverpa zea* showed that this species was capable of selecting on optimal mixture of protein and starch when offered diets with these components that were spatially separated (Cohen *et al.*, 1987). This method can only be used for insect species that do not live in their feed substrate.

The most robust approach in the development of artificial diets was found to be the “eclectic” approach. This method is described as using multiple strategies, combining all the previously discussed methods to develop an artificial diet. By combining all these strategies and principles one is most likely to formulate a diet that is most suited to the nutritional requirement of the target species. It will lead to biological parameters that indicate greater fitness. Higher fecundity, fertility and body weight, among many other biological parameters can be expected.

2.4 Insects identified for mass production

2.4.1 False codling moth, *Thaumatotibia (Crytophlebia) leucotreta* (FCM)

2.4.1.1 Life cycle

The false codling moth (FCM), *Thaumatotibia leucotreta*, is indigenous to southern Africa and is a pest of numerous crops, including citrus and deciduous fruits, cotton and maize. They have been reported in South Africa since 1899 (Bloem *et al.*, 2007) and have also been found in sub-Saharan Africa and the Indian Ocean Islands (Malan *et al.*, 2011). The pest was unknown to the Western Cape Province of South Africa, one of the biggest citrus producing provinces, until the end of the 1960s when it was first identified in the Paarl region. By 1980 the pest had spread across the Olifants River Valley, approximately 180 km north of Paarl (Hofmeyr *et al.*, 2015).

The life cycle of FCM is 25-60 days and six to eight non discrete generations per annum have been found in southern Africa (Malan *et al.*, 2011). Females lay between 100 and 250 individual eggs on fruit or foliage (Hofmeyr *et al.*, 2005). Neonatal larvae penetrate the fruit where they complete their development. The last-instar FCM larvae drop with a silken thread to the ground where they then spin tightly woven cocoons in the soil or in the cracks of bark. The prepupa in the cocoon changes into a pupa after a period of approximately 2-3 days. The adult moth emerges after a further 12-16 days at 25 °C with longer intervals at lower temperatures (Malan *et al.*, 2011). It was found that prepupal development takes on average 19 days to complete in artificial rearing facilities and 17 days in nature (Howell, 1970).

2.4.1.2 Economic importance

For the South African economy, citrus represents a huge investment in both foreign exchange earnings and human resources and is a major export based industry. Citrus has been exported from South Africa to all over the world for more than a hundred years. South Africa is the twelfth largest citrus producer worldwide and since 2006 it has been the second largest exporter of this commodity. All provinces of South Africa, excluding the Free State, have a total cultivated citrus area of just over 68 000 ha. The majority of orchards are planted in Limpopo, the Eastern Cape, Mpumalanga and the Western Cape provinces (CGA, 2016).

The presence of the FCM results in crop damage and consequently large economic losses. The South African Department of Agriculture, Forestry and Fisheries listed the FCM as a pest of quarantine concern for exports of citrus and other fruit shipped to the United States of America, China, Korea, Japan, Mexico and Israel (DAFF, 2016). It is estimated by the United States Department of Agriculture that yield losses of up to 20% can be expected in Ugandan cotton, as well as citrus, peach and macadamia crops if FCM are present (USDA, 2010). Their occurrence causes economic loss to farmers through various means. Firstly, the presence of FCM larvae in the host fruit causes infested fruit to drop early and secondly, the fruit that are infested close to harvest may go undetected and is a major concern for destinations of export produce (Boardman *et al.*, 2012).

Currently a combination of chemical, microbial and cultural techniques is used by the South African citrus industry to suppress FCM. Control methods of this pest insect species currently

include the use of biological control, including the *Cryptophlebia leucotreta* granulovirus (CrleGV) (Begemann, 2008), as well as the sterile insect technique (Hofmeyr *et al.*, 2005), orchard sanitation (Moore *et al.*, 2004) and mating disruptions (Hofmeyr *et al.*, 2005). Research has been done on control measures to target the soil-borne stages of the FCM. As the last-instar FCM larvae fall onto the ground and into soil it offers a window of opportunity for the use of nematodes as biological control agents against the FCM. Nematodes can be used as control method in early spring, summer, autumn and after harvest when traditionally no control methods are implemented (Malan *et al.*, 2011). The egg parasitoid *Trichogrammatoidia cryptophlebiae* Nagaraja has been released in citrus orchards against the FCM with fair amount of success in the Transvaal Lowveld of South Africa (Newton, 1988). It was also said that the entomopathogenic fungi *Beauveria bassiana* (Begemann, 2008) and *Aspergillus allicues* (Moore, 2002) could contribute to the natural mortality of this pest insect species.

The most successful biological control method for the FCM has proven to be the sterile insect release (SIT) program. This program is currently being operated in South Africa by XSIT (Pty, Ltd), Citrusdal (Hofmeyr *et al.*, 2005). The SIT was seen to be a long-term solution in the fight against the FCM and this program was initiated in 2002. A mass rearing facility was built capable of producing and rearing 21 million insects per week. Between 2007 and 2008 the pest threat was systematically reduced in the Citrusdal region by releasing commercial sterile insects into 1500 ha of citrus orchards. Between 2008 and 2009 the number of citrus orchards wherein the sterile insects were released increased to 3000 ha and between 2009 and 2010 to 4000 ha. Feral male populations were reduced 3-, 8-, and 10 fold, pre-harvest crop losses decreased by 50%, 80% and 93% and post-harvest export fruit rejections in the SIT area dropped by 13%, 25% and 38%, respectively compared to the non-SIT area (Hofmeyr *et al.*, 2015).

The sterile insect release programme is greatly dependent on the mass rearing of sterile moths. The success of mass rearing the FCM is highly correlated to the diet being fed. Previous artificial diets have been developed (Bot, 1965; Huber, 1981; Guennelon *et al.*, 1981; Moore, 2013) for this insect species. The diet developed by Moore (2013) is currently being used in the mass production facility at XSIT, Citrusdal. It is believed that the current diet can be further improved.

2.4.2 Yellow mealworm, *Tenebrio molitor* (mealworm)

2.4.2.1 Life cycle

The yellow mealworm is the larvae of the darkling beetle (*Tenebrio molitor* Linnaeus, 1758) of the Tenebrionidae family (Makkar *et al.*, 2014). The yellow mealworm is indigenous to Europe but currently distributed world-wide (Ramos-Elorduy *et al.*, 2002). The life cycle of the yellow mealworm spans around two months (45-65 days) depending on environmental conditions. The yellow meal worm goes through four stages to complete a full life cycle. These stages can be described as: egg, larva, pupa and beetle.

An adult female can lay around 160 eggs in her lifetime. After the female has laid her eggs it will take approximately seven days for the mealworm eggs to hatch and the larvae to emerge. In the larval stage a mealworm will moult 10 to 14 times. During the last moult it loses its carapace and changes into a curved pupa. The new pupa is a creamy white colour and changes slowly to brown before emerging as an adult. It will remain in its pupae form from six to 300 days depending on the incubation temperature.

A newly emerged mealworm beetle will sit still as its wings unfold and dry. This beetle is also known as the darkling beetle. It will appear a creamy colour and will brown over a period of 2 to 7 days. Once the beetle has browned they will be sexually mature and begin to look for a mate. Adults typically live 2-4 weeks in captivity.

2.4.2.2 Economic importance

The demand for food of animal origin is rapidly growing on a global scale and is expected to increase between 70-80% between 2016 and 2050 (Pelletier & Tyedmers, 2010). Currently 70% of all agricultural land is used by the livestock sector. The expansion of agricultural land is a major source of greenhouse gas (GHG) emissions and one of the largest contributors to global warming (Pan *et al.*, 2011). The selection of certain diets by people play a role in GHG emissions and other environmental issues. It has been suggested that a mitigation measure is to shift towards protein from lower impact animal species. It is suggested that insects are an environmentally more friendly alternative to conventional livestock (Oonincx & de Boer, 2012). Husbandry contributions to GHG emissions is much lower for insects compared to that of conventional livestock. Insect

husbandry produces 2-122 g/kg mass grain of GHG emissions compared to that of beef cattle (2850 g/kg mass grain), and pigs (80-1130 g/kg mass grain) (Oonincx *et al.*, 2010).

The yellow mealworm is considered a pest of grains, grain products and grain by-products although it can also consume meat, feathers and more because of its omnivorous nature (Ramos-Elorduy *et al.*, 2002). Due to these feeding habits of the mealworm they are able to recycle organic waste products. The mealworm represents an inexpensive yet appropriate source of animal protein. When supplemented with methionine the protein quality of the mealworm could be compared to that of casein (Goulet *et al.*, 1978). Mealworms also provide significant amounts of other essential nutrients such as vitamins and minerals (Martin *et al.*, 1976). The whole mealworm is also consumed by humans, therefore the edible portion is seen to be 100% and no waste is produced as with conventional livestock species that, amongst others, also produce abattoir waste. Common production animals vary in edible portion depending on breed, country of production, species and various other factors (Oonincx & de Boer, 2012). The protein and fat content of the yellow mealworm was found to be 76.1% and 6.4% on a dry weight basis, respectively (Li *et al.*, 2013).

Mealworms have a high reproduction rate. A single adult female mealworm can produce 160 eggs in her three-month life cycle. The maturation period is also short and mealworms reach adulthood in 10 weeks. Furthermore, the feed conversion ratio (FCR) for concentrates (kg/kg of fresh weight) for mealworms (2.2) was determined to be similar to that of pigs (2.6) but higher than that of chickens (1.6) and lower than that of beef cattle (4.5-8.8) (Wilkinson, 2011). The large variation in the FCR of beef cattle can be explained by the variation in proportion of roughage relative to concentrates. Also mealworms don't produce any methane (CH₄) (Oonincx & de Boer, 2012). All the above mentioned factors indicate that mealworms are a possible sustainable animal protein source that can be mass produced with little significant negative impact on the environment.

Currently mealworms are used as feed in the captive animal industry but seems to be a promising source of protein for humans with the required fat and essential amino acids. Mealworms are widely available and readily eaten by many insectivorous animal species and therefore they provide a very convenient food source (Martin *et al.*, 1976).

Large scale production units need to be established across the globe for mealworms to become a future sustainable protein source to replace conventional livestock species as food. Currently mass

production units can be found in China (Haocheng), Canada (Ofbug) and France (Ynsects). Haocheng, established in 2002 with 15 breeding farms, is leading the way in terms of mass producing the yellow mealworm. Producing 50 ton of live worms per month as well as 200 ton of dried worms per year for export purposes to The United States of America, Europe, Australia, Southeast Asia and Africa.

Further research into raising the mealworm on a variety of low quality substances such as saw dust, waste paper, corn starch and potato flour were recommended (Ghaly & Alkoaik, 2009). With the availability of land being the most stringent limitation in sustainable feeding of the world's population, mealworms could be considered as a more sustainable alternative to milk, pork, chicken and beef.

2.4.3 Black soldier fly, *Hermetia illucens* (BSF)

2.4.3.1 Life cycle

The black soldier fly (BSF), *Hermetia illucens*, is most commonly found in warmer regions and around the tropics. They have three generations a year and can colonize an extremely wide variety of organic plant and animal waste (Sheppard *et al.*, 2002).

Hermetia illucens mate two days after emergence and oviposition occurs two days after fertilization (Tomberlin *et al.*, 2009). In their natural environment, the BSF will oviposit in dry cracks around and above moist decomposing organic matter (Sheppard *et al.*, 2002). The larvae require 2-4 weeks to develop depending on the environment. Temperature and food availability have large influences on the growth rate of larvae (Myers *et al.*, 2008). The prepupae crawl out of the organic material in search of a dry pupation site. Therefore, the prepupae are self-harvesting and can easily be harvested by constricting their dispersion paths (Tomberlin *et al.*, 2009).

2.4.3.2 Economic importance

Organic waste is the principle food of many insect species and this is especially so for the BSF. Insects have the capability of naturally recycling nutrients. Waste products and residual proteins can be converted into feed and food of high nutritive value. It was estimated that this fly species can reduce nitrogen and phosphorus waste by up to 75% and the mass of manure residue by more than 50% in poultry and swine systems (Melorose *et al.*, 2015). Furthermore, the recycled nutrients

are captured into the biomass of the prepupae, consisting of approximately 40% protein and 30% fat (Newton *et al.*, 1995). Black soldier fly larvae are voracious feeders of organic material and are therefore suitable to be used in simple engineered systems to reduce organic waste and produce high quality protein and unsaturated fat (Diener *et al.*, 2009). While recycling waste, the BSF is also a non pest species that significantly reduce house fly and lesser house fly populations (Sheppard, 1983).

Low- and middle income countries have low waste collection coverage and frequently do not apply the correct treatment of waste. This leads to unfavourable living conditions in townships, villages and in some cases, cities. The inability to effectively dispose of waste has an impact on human health, the environment, and is a major hurdle in economic development (Diener *et al.*, 2009). The recycling of inorganic waste is well under way and in turn creates job opportunities in the informal sector, however the recycling of organic waste is still in the embryonic stage despite its high recovery potential. Organic material can make up more than 50% of the total municipal waste production (Daskalopoulos *et al.*, 1998). There is thus a huge possibility for recycling organic matter and nutrients (Wilson *et al.*, 2006). Therefore, the conversion of organic refuse by the BSF using engineered systems can play a substantial role in reducing and recycling organic waste. The BSF only feed in their larval stage and therefore it does not pose any disease transmission risks.

The development of such waste to feed systems from experimental to full scale waste treatment facilities, using the BSF larvae, offers numerous advantages. Such facilities can be developed and operated at low costs since they are suited to the economic potential of developing countries (Diener *et al.*, 2009). The sale and use of black soldier larvae in the feed industry can strengthen the economic resilience of farmers. Small holder farmers, especially in the poultry and pig industries, are heavily burdened by the excessively high feed cost and can't keep up with commercial farmers as feed cost make up 70-80% of total running costs. BSF larvae offers a solution to the small holder farmer to produce a quality feed stuff and simultaneously reducing farm waste (Melorose *et al.*, 2015).

Recently commercial scale facilities have been developed. In South Africa, Agriprotein (Pty, Ltd) a commercial scale BSF production company, is one of the leading global mass production facilities for the concerned species. Based in Cape Town, South Africa they are in the process of

expanding to Canada and Argentina. Agriprotein (Pty, Ltd) currently produce 7 ton of protein rich larvae meal, extract 3 ton of fat which is turned into a larvae oil high in unsaturated fats, and 22 ton of fertilizer per day. New research is being done on the potential of natural anti-inflammatory properties of larvae to heal livestock, e.g. lame sheep. The high bio-available iron level of the larvae is also being investigated to reduce anaemia in piglets. There are several other BSF mass production systems across the globe; these include The United States of America (Enviroflight LLC & Insect Science resource LLC), Canada (Enterra), Brazil (Entlogics), The Netherlands (Protix), Hawaii (ProtaCulture), China (Haocheng), Malaysia (Entofood), Spain (Entomotech & Bioflytech) and Germany (Hermetia).

The high fat content of the larvae makes it possible to produce biodiesel. A study compared biodiesel produced from BSF larvae to crop-oil such as soybean oil, rapeseed oil and sunflower oil and found that the fuel properties of the larvae biodiesel were comparable to those of rapeseed-based biodiesel and the former also met the European biodiesel standard (Li *et al.*, 2011). The fat content of the larvae can also be manipulated as the lipid content of insects are largely dependent on their diet. It was suggested that BSF prepupae incorporate omega-3 fatty acids (α -linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) when fish offal is added (St-Hilaire *et al.*, 2007). Using fish offal to increase the omega-3 fatty acids of the prepupae can result in the latter being used to replace fish oil (that could be suitable for human consumption) in animal diets.

2.5 Conclusion

Bug husbandry is an ancient practice, but remains largely in its larval stage. The true potential of establishing mass rearing facilities for various insect species, each in their own right, has not been reached. The utilisation of insects for various economic, social and environmental purposes requires mass production. Although mass rearing methods have been developed for some insect species, no other insect cultures have been developed, most likely because of lack of demand. The culture of insects is complicated because insects have strict environmental (temperature and humidity), feeding and population requirements, particularly during reproduction (Sanchez-Muros *et al.*, 2014). Feeding of insects plays a crucial role in determining the eventual success of mass production units. Recent publications indicate that there is no in-depth understanding of insect nutrition and research on this aspect is lacking. This could be due to the fact that entomologist

have in the past focused on pest control and only of late has the trend for mass rearing been developed. Entomologists do not have an in-depth nutrition background and in general, they have no past experience in formulating feeds. Therefore, there is a need for collaboration between human nutritionists, animal nutritionists and entomologists to successfully formulate artificial diets to meet the requirements of specific insect species. Attaining this will be a big leap towards ensuring the successful and efficient mass production of insects of economic and environmental importance. It was therefore seen as important to conduct studies to determine the nutrient requirements and formulate artificial diets for insects that have been identified to have commercial value and are currently being mass reared. These insect species include the BSF, FCM and mealworm.

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Chapter 3

Determination of nutrient requirements and development of diets for the mass rearing of the false codling moth (*Thaumatotibia leucotreta*)

Abstract

Three techniques were used to establish the nutrient composition of novel diets for the mass rearing of the false codling moth (FCM) (*Thaumatotibia leucotreta*). The minimum value for each nutrient from four published diets served as nutrient specifications of the first experimental diet (MS), whilst the comparative slaughter technique based on the composition of the fifth instar larvae served as specifications for the second diet (CS), and lastly the nutrient composition of the natural feed of the insects (oranges) were used for the specification of the third diet (NO). Diets were formulated using current commercial raw materials and production parameters compared. Each diet was replicated 6 times using console jars inoculated with 1000 eggs and 250 g of feed. Larvae were sampled on day 22. The number of larvae harvested on day 22 (yield) was compared to that obtained from the diet currently used at XSIT (Pty, Ltd) (commercial rearing facility). Microwave preparation for 8 minutes, using a 1000 W microwave at 30% moisture, was found to be the optimal cooking time for all treatment diets. Three further diets were formulated based on the same nutrient specifications but including different levels of raw materials and the number of larvae harvested compared. The diet formulated according to the nutrient composition of oranges was not viable for use in a production system as the larvae took too long to develop. The MS diet yielded more larvae (759 ± 29) than the current XSIT diet (652 ± 32) and CS diet (596 ± 46). It was concluded that the nutrient specifications of the MS diet resembled the nutrient needs of the FCM the closest. In order to increase efficiency and decrease costs, a follow up trial with novel raw materials was conducted. Diets (D1 and D2) formulated according to the minimum specifications using novel raw materials produced ($P < 0.001$) more larvae (D1 = 911 ± 40 and D2 = 830 ± 40) than the control (428 ± 40). Formulation costs of the diets were R8.04/kg (D1) and R8.23/kg (D2), respectively compared to R8.55/kg for the control diet. When comparing cost to produce a single larva, the newly formulated diets were ($P < 0.001$) cheaper. XSIT (Pty, Ltd) currently mixes 60

ton of feed and produce roughly 100×10^6 larvae per month. The use of the minimum specification diet using novel raw materials could result in significant annual savings.

***Keywords:** minimum specification, comparative slaughter, natural diet, larvae yield

3.1 Introduction

The false codling moth (FCM) (*Argyroplote leucotreta*; *Cryptophlebia leucotreta*; *Thaumatotibia leucotreta*) is an insect of economic importance for fruit producers in South Africa (Bloem *et al.*, 2007). It is generally found on citrus and deciduous fruits as well as on cotton and maize. Chemical control of these pests has been successful with commonly used organophosphates (Silvie *et al.*, 2001). However, problems associated with chemical control include resistance in the insect as well as the residue of the pesticides remaining in the fruit. Biological methods for the control of the pest include the *Cryptophlebia leucotreta* granulovirus (CrleGV) sprays (Begemann, 2008), orchard sanitation (Moore *et al.*, 2004), mating disruptions (Hofmeyr *et al.*, 2005), as well as the sterile insect technique (Hofmeyr *et al.*, 2005). The growing concern around food safety, as well as the demand for organic produce, is placing immense pressure on the use of pesticides. Pesticides have served mankind for decades, but lowering of the minimum allowable pesticide residue will effectively end the use of these pest control agents in the near future (Begemann, 2008).

Research programmes by numerous institutions are placing more focus on the use of biological control agents in the fight against various pest species affecting crop production. One such biological control method is the sterile insect technique (SIT). The SIT is an integrated, natural pest management system used for the control of various pest species. It includes the mass rearing of the species and the subsequent irradiation of the insects to render them infertile. The mass rearing of the species involves rearing the larvae on artificial diets. As mass rearing factories increase in size the processing of the feed becomes a greater issue of concern. Unlike conventional livestock production, the diet of mass reared FCM cannot be fed raw and the diet needs to be cooked before it can be utilized. Also, feed costs, as well as waste production, are becoming factors requiring assessment. The XSIT production facility in Citrusdal, South Africa is a mass rearing facility specialising in rearing the FCM for the use of biological pest control in citrus orchards in the region. It was established in 2005 when a study on this control method showed good

suppression of the pest and FCM related fruit damage was found to be reduced by 95% (Hofmeyr *et al.*, 2015). Citrusdal is the production region that provides the majority of the citrus that is exported to the USA (CGA, 2014).

XSIT (Pty, Ltd) release sterile moths for a period of ten months stretching from September to June. Moths are released twice per week in orchards on a continuous basis. The sterile animals mate with live feral animals, with no resultant offspring, thereby lowering the total wild population. Currently they rear 100×10^6 sterile moths per month and mix 60 ton of feed per month to feed these insects. Feed cost make out the largest portion of total expenses in the rearing system, somewhat similar to what is observed in intensive poultry and pig production systems.

The diet formulated by Moore *et al.* (2013) is currently in use at the XSIT production system in Citrusdal. This diet was based on artificial diets previously reported for other Lepidoptera. The diet consists of six components (plus distilled water), which included four components of nutritional value and two as anti-microbial agents. It is believed that the current diet does not meet the specific nutrient requirements of the insects and that alternative raw materials to that what was previously used by Moore *et al.* (2013) could be utilized.

The production of these sterile insects represents a large intensive animal production unit consisting of breeders, hatchery and grow out facilities, similar to that observed in intensive poultry and pig production systems. The nutrition of these insects is of utmost importance for economical and efficient production since over and undersupply of nutrients have the same detrimental effect in production as it does in domesticated animals. The undersupply of nutrients could lead to a total break in production while marginal undersupply could lead to immune suppression, decreased efficiency of production or a reduction in fertility and fecundity (Waldbauer, 1968). On the other hand, an oversupply of nutrients could lead to the build-up of primary or secondary metabolites which could be toxic, antagonistic or, nutrient imbalances could lead to metabolic stress (Aryal *et al.*, 2007). In order to minimise any stress associated with the mass rearing of insects it is necessary to formulate feeds with nutrient specifications as close as possible to their requirements. The field of insect nutrition is, unfortunately, under published with most publications supplying ingredient composition and not nutrient composition (Cohen, 2015).

Therefore the objectives of this study were to:

- I. Determine the nutrient requirements of the FCM
- II. Determine an optimal processing method of the diet
- III. Evaluate the use of novel raw materials to formulate FCM diets
- IV. Formulate a more efficient diet for the mass production of the FCM

3.2 Materials and methods

3.2.1 Trial 1: Determining diet preparation procedure

3.2.1.1 Moths and housing

False codling moth eggs were obtained from the XSIT mass production unit (Citrusdal, South Africa). Twelve treatments (Table 3.1) with six replications were randomly allotted to 72, 500mL Consol® glass jars. Feed was subsequently placed into jars. An amount of a 250 ± 1.65 g of feed was placed into each jar. Each Consol® jar containing different treatments was inoculated with a sheet containing 1000 FCM eggs and sealed with a lid containing a brown paper membrane. The egg sheets were surface sterilized by briefly dipping in a 25% formaldehyde solution before placing into the jars. Jars were placed in a controlled environment at the XSIT factory and maintained at 23°C and humidity $50 \pm 10\%$ for the duration of the trial.

Each treatment of 6, 8 or 10 minutes cooking using a 1000 W microwave were replicated six times for each of the three newly formulated diets. The diet formulated by Moore (2013) currently in use at XSIT production facility, cooked for eight minutes, was used as the control. Eggs hatched on day two and hatching was confirmed using digital microscopy (Keyence VX-5000). On the 20th day after hatching jars were opened and the larvae were killed instantly by dropping them one by one onto a sieve above rapidly boiling water. Thereafter they were immediately removed from the steam and counted.

3.2.1.2 Treatments and experimental diets

Nutrient compositions of published diets (Bot, 1965; Huber, 1981; Guennelon *et al.*, 1981; Moore *et al.*, 2013) were calculated as ingredient composition as no nutrient composition were provided. The minimum value for each nutrient from the four published diets served as nutrient specifications of the first experimental diet as it is thought that the current diet oversupplies nutrients compared to the demand of the insects. The comparative slaughter technique based on

the composition of the fifth instar larvae served as specifications for the second diet, and lastly the nutrient composition of the natural feed of the insects (oranges) were used for the specification of the third diet. The diet formulated by Moore *et al.* (2013) that is currently in use at XSIT, cooked for eight minutes using a 1000 W microwave, was used as the control. The ingredient composition of the diets is shown in Table 3.2.

Diets were formulated using the current XSIT raw materials (milk powder, canola oil, maize meal, brewer's yeast, wheat germ and nipagin), except for the diet based on their natural feed which included limestone and sugar. The diets were mixed and the moisture content of the feed brought to 20% through the addition of distilled water.

Each diet was cooked in a 1000 W microwave oven for treatment periods of 6, 8, and 10 minutes. Subsamples were taken from the microwave feeds and moisture content determined after cooking. From this value the moisture content of the feed was corrected to $48 \pm 1\%$ by the addition of distilled water. Formalin 0.2% was added to the distilled water at a concentration of 1 mL/L to ensure an aseptic diet and reduce contamination.

Table 3.1 A description of dietary treatments used throughout Trial 1

Treatments	Description
XSIT (CON)	Current XSIT diet cooked for 8 minutes
MS6*	Diet formulated according to minimum nutrient specifications of current published diets, cooked for 6 minutes
MS8	Diet formulated according to minimum nutrient specifications of current published diets, cooked for 8 minutes
MS10	Diet formulated according to minimum nutrient specifications of current published diets, cooked for 10 minutes
CS6	Diet formulated using the comparative slaughter technique, cooked for 6 minutes
CS8	Diet formulated using the comparative slaughter technique, cooked for 8 minutes
CS10	Diet formulated using the comparative slaughter technique, cooked for 10 minutes
NO6	Diet formulated according to the nutrient specifications of natural diet (oranges) cooked for 6 minutes
NO8	Diet formulated according to the nutrient specifications of natural diet (oranges) cooked for 8 minutes
NO10	Diet formulated according to the nutrient specifications of natural diet i.e. oranges, cooked for 10 minutes

* The number after the dietary treatment indicates the time period of cooking (mins)

Table 3.2 Ingredient and calculated nutrient composition of the diets used to determine optimal cooking time

	XSIT(CON)	MS	CS	NO
Ingredients (% of diet)				
Full cream milk powder	1.5	1.1	1.7	1.5
Wheat germ meal	8.4	31.6	20.1	1.4
Brewer's yeast	4.2	10.4	76.2	--
Ascorbic acid	--	--	--	0.2
Benzoic acid	--	0.8	1.2	0.2
Nipagen	0.9	0.4	0.6	--
Sorbic acid	0.4	0.2	--	--
Canola oil	0.3	--	--	--
Maize meal	84.3	55.2	--	81.9
Sugar	--	--	--	13.1
Limestone	--	--	--	1.6
Calculated nutritional value (%)				
Dry matter	87.2	87.3	89.4	88.5
Moisture	12.8	12.7	10.6	11.5
Crude protein	13.7	12.1	23.9	7.7
Crude fibre	2.7	2.3	2.8	1.8
Calcium	1.2	0.2	0.8	0.6
Available phosphorous	0.5	0.1	0.2	0.1
Sodium	0.1	0.1	0.4	--
Potassium	1.2	1.5	4.9	1.8
DE ¹ (Pig)	14.8	14.2	13.9	14.6
Lysine	0.4	0.5	1.2	0.3
Methionine	0.2	0.2	0.4	0.2
Sulphur amino acids	0.5	0.5	0.7	0.4
Tryptophan	0.1	0.1	0.2	0.1
Isoleucine	0.4	0.4	0.8	0.3
Leucine	1.2	1.2	1.6	1.0
Threonine	0.4	0.5	0.9	0.3

XSIT(CON): Current XSIT diet

MS: Diet formulated according to minimum nutrient specifications of current published diets

CS: Diet formulated using the comparative slaughter technique

NO: Diet formulated according to the nutrient specifications of natural diet

¹DE: Digestible energy (MJ/kg)

-- No value available

3.2.2 Trial 2: Formulation of diets with current raw materials at different inclusion levels and nutrient specification evaluation

3.2.2.1 Moths and housing

Four treatments (Table 3.3) with six replications were randomly allotted to 24, 500 mL console glass jars. Feed was subsequently placed into jars at a rate of $250 \text{ g} \pm 1.65 \text{ g}$ per jar. All diets were cooked for 8 minutes using a 1000 W microwave as this was determined to be optimal in Trial 1.

The trial was conducted and production parameters compared as described in Materials and Methods for Trial 1.

3.2.2.2 Treatments and experimental diets

The inclusion levels of current XSIT raw materials used to formulate Trial 1 diets were changed (Table 3.4) to study the effect that different raw material inclusion levels would have on larval production and determine if it would be viable to use different raw materials compared to the current status.

Table 3.3 A description of dietary treatments used throughout Trial 2

Treatments	Description
XSIT (CON)	Current XSIT diet
MS	Diet formulated according to minimum nutrient specifications of published diets with current raw materials at different inclusion levels
CS	Diet formulated using the comparative slaughter technique with current raw materials at different inclusion levels
NO	Diet formulated according to the nutrient specifications of natural diet (oranges) with current raw materials at different inclusion levels

Table 3.4 Ingredient and calculated nutrient composition of diets formulated with current raw materials at different inclusion levels

	XSIT(CON)	MS	CS	NO
Ingredients (% of diet)				
Full cream milk powder	1.5	1.0	1.0	1.5
Wheat germ meal	8.4	11.6	45.4	--
Brewer's yeast	4.2	4.3	17.4	--
Ascorbic acid	--	--	--	0.3
Benzoic acid	-	0.7	0.7	0.3
Nipagen	0.9	0.9	0.9	1.3
Sorbic acid	0.4	0.2	--	--
Canola oil	0.3	0.3	0.3	0.5
Maize meal	84.3	81.1	34.4	81.8
Sugar	--	--	--	12.9
Limestone	--	--	--	1.6
Calculated nutritional value (%)				
Dry matter	87.2	87.3	89.1	88.1
Moisture	12.8	12.7	10.9	11.9
Crude protein	13.7	12.1	23.1	7.3
Crude fibre	2.7	2.3	2.9	1.4
Calcium	1.2	0.2	0.8	0.6
Available phosphorous	0.5	0.1	0.2	0.1
Sodium	0.1	0.1	0.4	--
Potassium	1.2	1.5	4.9	1.8
DE ¹ (Pig)	14.8	14.2	13.6	14.9
Lysine	0.4	0.5	1.2	0.3
Methionine	0.2	0.2	0.4	0.2
Sulphur amino acids	0.5	0.5	0.7	0.4
Tryptophan	0.1	0.1	0.2	0.1
Isoleucine	0.4	0.4	0.8	0.3
Leucine	1.2	1.2	1.6	1.0
Threonine	0.4	0.5	0.9	0.3

XSIT(CON): Current XSIT diet

MS: Diet formulated according to minimum nutrient specifications of published diets with current raw materials at different inclusion levels

CS: Diet formulated using the comparative slaughter technique with current raw materials at different inclusion levels

NO: Diet formulated according to the nutrient specifications of natural diet with current raw materials at different inclusion levels

¹DE: Digestible energy (MJ/kg)

-- No value available value

3.2.3 Trial 3: Formulation of diets based on minimum specifications principle using novel raw materials

3.2.3.1 Moths and housing

Three treatments (Table 3.5) with eight replications were randomly allotted to 24, 500 mL Consol® glass jars

The trial was conducted and production parameters compared as described in the Materials and Methods for Trial 1.

3.2.3.2 Treatments and experimental diets

In order to increase efficiency and decrease the cost, a follow up trial with novel cheaper raw materials was conducted. Diets (D1 & D2) were formulated according to the minimum nutrient specifications (Table 3.6) as it was determined in Trial 2 that this best resembled the nutrient requirements of the insects. Therefore, the nutrient specifications of the MS diet were taken as the nutrient requirements of the moths.

Table 3.5 A description of dietary treatments used throughout Trial 3

Treatments	Description
XSIT (CON)	Current XSIT diet
D1	Diet formulated according to minimum nutrient specifications of published diets with alternative raw materials
D2	Diet formulated according to minimum nutrient specifications of published diets with alternative raw materials at a different inclusion level

Table 3.6 Ingredient composition of diets with novel raw materials based on the minimum nutrient specification technique

	XSIT(CON)	D1	D2
Ingredients (% of diet)			
Full cream milk powder	1.5	1.0	1.0
Wheat germ meal	8.4	13.6	8.4
Brewer's yeast	4.2	--	4.2
Nipagin	0.9	0.9	0.9
Sorbic acid	0.4	0.4	0.4
Canola oil	0.3	0.3	0.3
Maize meal	84.3	79.9	81.5
Wheat bran	--	1.1	3.1
Soya bean meal (46% CP) ¹	--	3.0	0.6
Limestone	--	0.2	--
Calculated nutritional value (%)			
Dry matter	87.2	87.2	87.2
Moisture	12.8	12.8	12.8
Crude protein	13.7	12.1	12.1
Crude fibre	2.7	2.5	2.5
Calcium	1.2	0.2	0.2
Available phosphorous	0.5	0.1	0.1
Sodium	0.1	0.1	0.1
Potassium	1.2	1.8	2.0
DE ² (Pig)	14.8	14.6	14.2
Lysine	0.4	0.5	0.5
Methionine	0.2	0.2	0.2
Sulphur amino acids	0.5	0.5	0.5
Tryptophan	0.1	0.1	0.1
Isoleucine	0.4	0.4	0.4
Leucine	1.2	1.2	1.2
Threonine	0.4	0.4	0.4

XSIT(CON): Current XSIT diet

D1: Diet formulated according to minimum nutrient specifications of published diets with alternative raw materials

D2: Diet formulated according to minimum nutrient specifications of published diets with alternative raw materials at different inclusion level

¹Crude protein²DE: Digestible energy (MJ/kg)

-- No value available

3.2.3 Statistical analysis

For all three trials, similar statistical analyses were conducted. Analysis of variance was performed on larvae yield data using the ANOVA procedures of SAS (2009) with treatment as the main effect. All the parameters were tested for normality and homoscedasticity before analysis. Significance was declared at $P \leq 0.05$. Means was separated with Bonferroni *post hoc* test (SAS, 2009).

3.3 Results and Discussion

Trial 1: Determining diet preparation procedure

The results for diet preparation time are expressed as the total larvae yield/number after 20 days (Table 3.7), as well as the physical appearance of the diet (Table 3.9). The larvae reared on the diet formulated according to the nutrient specifications of the natural diet (N0) of the FCM was not counted as it performed poorly for all time treatments producing low numbers of larvae.

Table 3.7 Mean (\pm standard deviation) of total larvae yield (number), at 20 days after hatching, of different diets at different time treatments

Treatment	Larvae yield (number)
XSIT (CON)	681 \pm 23 ^b
MS6	488 \pm 38 ^c
MS8	863 \pm 29 ^a
MS10	503 \pm 32 ^c
CS6	462 \pm 19 ^c
CS8	753 \pm 15 ^b
CS10	428 \pm 13 ^{cd}
NO6, 8, 10	--

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

XSIT(CON): Current XSIT diet cooked for eight minutes

MS6: Diet formulated according to minimum nutrient specifications of published diets, cooked for six minutes

MS8: Diet formulated according to minimum nutrient specifications of published diets, cooked for eight minutes

MS10: Diet formulated according to minimum nutrient specifications of published diets, cooked for ten minutes

CS6: Diet formulated using the comparative slaughter technique, cooked for six minutes

CS8: Diet formulated using the comparative slaughter technique, cooked for eight minutes

CS10: Diet formulated using the comparative slaughter technique, cooked for ten minutes

NO6,8,10: Diet formulated according to the nutrient specifications of natural diet (oranges) cooked for six, eight and ten minutes

--No value available

For both the MS and CS diets, a cooking time of 8 minutes using a 1000 W microwave at 20% moisture was found to be optimal producing the highest number of viable larvae. A cooking time of 8 minutes increased ($P < 0.05$) larvae production for all dietary treatment groups compared to 6 and 10 minute treatments (Table 3.7). There were no differences between the number of larvae produced on the CS8 and XSIT(CON) treatments. MS8 produced more larvae ($P < 0.05$) than CS8 and XSIT(CON).

The consistency of the diets was evaluated using the matrix described in Table 3.8 with a score of 2 being optimal for commercial use. The texture and consistency of the MS8 and CS8 diets, after cooking and correcting the moisture content to $58 \pm 1\%$, visually compared favourably to that of the XSIT diet with all scoring 2. The appearance of MS8 and CS8 was suitable for use on a commercial scale within the XSIT mass product unit. On appearance, the diets cooked for 10 minutes appeared to be heat damaged with dark areas and dry crusts forming in the diet with both MS10 and CS10 scoring 3. Diets MS6 and CS6 appeared dense and undercooked scoring 1 (Table 3.9).

Table 3.8 Matrix used to evaluate appearance of Trial 1 diets after being cooked for different treatment times

Description	Score
Dense and undercooked	1
Light and well cooked	2
Dry, dark colour with burnt crusts	3

Table 3.9 Matrix scores to evaluate appearance of diets after being cooked for different treatment times

Treatment	Score
XSIT (CON)	2
MS6	1
MS8	2
MS10	3
CS6	1
CS8	2
CS10	3
NO6, 8, 10	--

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

XSIT(CON): Current XSIT diet cooked for eight minutes

MS6: Diet formulated according to minimum nutrient specifications of published diets, cooked for six minutes

MS8: Diet formulated according to minimum nutrient specifications of published diets, cooked for eight minutes

MS10: Diet formulated according to minimum nutrient specifications of published diets, cooked for ten minutes

CS6: Diet formulated using the comparative slaughter technique, cooked for six minutes

CS8: Diet formulated using the comparative slaughter technique, cooked for eight minutes

CS10: Diet formulated using the comparative slaughter technique, cooked for ten minutes

NO6,8,10: Diet formulated according to the nutrient specifications of natural diet (oranges) cooked for six, eight and ten minutes

-- No value available

Trial 2: Formulation of diets with current raw materials at different inclusion levels and nutrient specification evaluation

The results for the formulation of the diets with the raw materials currently used at different inclusion levels are expressed as the total larvae yield/number after 20 days, for each treatment (Table 3.10). The diet based on the natural food (oranges) of the insects was once again not counted due to the fact that the larvae grew too slow and too few numbers were produced; thus this diet would not be viable for commercial use

Table 3.10 Mean (\pm standard deviation) of total larvae yield, at 20 days after hatching, of different treatment diets

Treatment	Larvae yield (numbers)
XSIT (CON)	652 \pm 32 ^b
MS	759 \pm 29 ^a
CS	593 \pm 46 ^b
NO	--

^{a,b} Means within columns with different superscripts differ significantly ($P < 0.05$)

XSIT(CON): Current XSIT diet

MS: Diet formulated according to minimum nutrient specifications of current published diets

CS: Diet formulated using the comparative slaughter technique

NO: Diet formulated according to the nutrient specifications of natural diet (oranges)

-- No value available

The MS diet produced more larvae ($P < 0.05$) than that of the CS and XSIT(CON) diets. The XSIT(CON) diet performed intermediately with the CS diet producing the least number of larvae ($P < 0.05$). Therefore, it could be assumed that the nutrient specifications of the MS diet better resemble the nutrient requirements of the moths.

The digestible energy (DE) of the MS diet (14.2 MJ/kg) was intermediate compared to that of the CS (13.9 MJ/kg) diet and XSIT(CON) diet (14.8 MJ/kg) (Table 3.4). The crude protein content of the CS diet (23.1%) was almost double that of the MS diet (12.1%) and regarded as being too high. The diet formulated according to the natural composition of the of the FCM (NO) had a crude protein content of 7.7% which fell short of the needs of the insect in question. Therefore, the crude protein needs for mass reared FCM was concluded to be higher than that of their wild counterparts but lower than that of what is currently supplied by the diet of Moore *et al.*, (2014).

The amino acid composition of the CS diet would therefore also oversupply all the amino acids when compared to that of the MS diet that yielded the highest number of larvae. It should be remembered though that like most other insect larvae, FCM larvae are rich in essential amino acids. Therefore, it is postulated that earlier researchers when formulating a diet based on the composition of the larvae, would include high amounts of the essential amino acids. However, as shown in the case of the FCM, they do not need such high dietary amounts of amino acids. The absolute lysine value of the CS diet (1.2%) was much higher than that of the MS (0.5%) and XSIT(CON) (0.4%) diets. The same implied for methionine, tryptophan, isoleucine, leucine and

threonine. The oversupply of essential amino acids had a negative effect on the larvae numbers. The amino acid composition of the MS and XSIT(CON) diets were similar with the exception of lysine and threonine that were slightly higher for that of the MS diet (which yielded more larvae). This could indicate that the need for these two amino acids are higher than what are currently supplied by the XSIT(CON) diet.

The calcium (Ca) content of the XSIT(CON) diet (1.2%) is exceptionally high compared to that of the MS diet (0.2%). This is unnecessary as insects do not have the same Ca requirements as vertebrates which utilize these minerals in bone formation (Chapman, 2012). The sodium and potassium levels were similar in the MS and XSIT(CON) diets but significantly higher in the CS diet.

It can be concluded that the current diet, XSIT(CON), used to produce moths at the XSIT mass production unit as well as the CS diet oversupplied nutrients and this could possibly have led to metabolic stresses which inhibited the performance of the insects. An oversupply of nutrients could lead to the build-up of primary or secondary metabolites which could be toxic or antagonistic or alternatively, imbalances could lead to metabolic stress (Aryal *et al.*, 2007). The nutrient specifications of the MS diet seems to be the best as it produced most FCM larvae.

Trial 3: Formulation of diets based on minimum specifications principle using novel raw materials

The results for formulation of diets based on the minimum specifications principle using novel raw materials are expressed as the total larvae numbers after 20 days as well as the cost to produce a single larva, for each treatment (Table 3.11).

Table 3.11 Mean (\pm standard deviation) of total larvae yield, at 20 days after hatching, of different treatment diets

Treatment	Larvae yield (numbers)
XSIT (CON)	428 \pm 57 ^a
D1	911 \pm 59 ^b
D2	830 \pm 61 ^b

^{a,b} Means within columns with different superscripts differ significantly ($P < 0.05$)

XSIT(CON): Current XSIT diet

D1: Diet one formulated according to minimum nutrient specifications of current published diets using novel raw materials

D2: Diet formulated according to minimum nutrient specifications of current published diets with alternative raw materials at a different inclusion level

Diets D1 and D2 formulated according to the minimum specifications using novel raw materials produced more ($P < 0.001$) (D1: 911 \pm 59 and D2: 830 \pm 61) larvae than the control (428 \pm 57). No differences ($P > 0.05$) were observed between D1 and D2.

The results for Trial 3 confirmed, as reported in Results and Discussion for Trial 2, that a reduced nutrient specification diet proved to meet the nutrient requirement of the moths more closely than the XSIT(CON) diet. More larvae were produced when using bran and soya as novel feedstuffs. It was found that the use of soya bean meal as protein source favourably influenced production and could partially replace conventional protein sources (milk powder and brewer's yeast) currently used to formulate the ration of the moths. Soya bean meal is higher in protein and essential amino acids such as lysine, methionine and tryptophan compared to milk powder. On the other hand, soya bean meal contains antinutritional factors such as the soya bean Kunitz trypsin inhibitor which affects digestion and protein hydrolyses. In this trial soya bean meal only partially substituted milk powder and brewer's yeast as protein source with a maximum inclusion level of 3%. The effect of higher inclusion levels could be studied but it was determined that the inclusion of soya bean up to a 3% level had no negative effect on larvae yield of mass reared FCM. The amino acid profile of soya could likely meet the amino acid requirements of the insects in question more closely and be utilized more efficiently. Wheat bran meal is a cheaper alternative to the wheat germ meal currently being used to formulate the XSIT(CON) diet. Both function as a filler in the diet and have similar nutritional compositions. The use of alternative raw materials made it possible, not only to maximize larval yield, but to reduce input costs for formulating the diets of the animals (Table 3.12).

Pristavko *et al.* (1979) previously reported that one required 2-4 g of diet to rear one insect when individually fed and 8-12 g per insect when fed in groups. FCM production for treatment diets D1 and D2 were on average 0.3 g of feed per insect, which is significantly lower to that reported by Pristavko *et al.* (1979). It is also lower than that achieved by the diet of Moore *et al.* (2014) which required 0.4 g of diet to produce a single moth. In this study it was found that the diet formulated by Moore *et al.* (2014) (XSIT) needed 0.6 g of feed to produce a single moth which was contradictory and higher than what was previously reported. It was also noticeable that the larvae reared on diets D1 and D2 appeared to have greater synchrony of development compared to that of the XSIT(CON) diet. This implies that less size variance between different instars will occur which in turn will increase the production efficiency of the mass production system.

As noted in Table 3.12, formulation cost of the diets was R8.04/kg (D1) and R8.23/kg (D2) respectively, compared to the R8.55/kg for the control diet (calculated as per price of raw materials on 07/10/2016). The correlation between larvae yield and total cost of the respective treatment diets was used to estimate the production cost to produce a single larva. When comparing cost to produce single larvae (Table 3.13) the newly formulated diets were cheaper ($P < 0.001$) (D1: 0.22 cents/larvae and D2: 0.25 cents/larvae) than XSIT(CON) (0.55 cents/larvae). XSIT (Pty, Ltd) rear 100×10^6 sterile moths per month and mix 60 ton of feed per month thus the use of the minimum specification diet using novel raw materials could result in great reduction in feed costs.

Table 3.12 Raw material and total dietary costs of different treatment (XSIT(CON), D & D2) diets

Treatment	Ingredient	% of diet	Price/kg (R)*	Ingredient price/kg diet (R)
XSIT(CON)	Milk powder	1.50	60.00	0.90
	Wheat germ meal	8.43	7.50	0.63
	Brewer's yeast	4.20	7.50	0.57
	Nipagen	0.85	119.00	1.00
	Canola oil	0.31	17.45	0.05
	Maize meal	84.16	6.00	5.04
	Sorbic acid	0.36	92.00	0.35
				8.55 (R/kg diet)
D1	Milk powder	1.00	60.00	0.60
	Wheat germ meal	13.62	7.50	1.02
	Nipagin	0.85	119.00	1.01
	Sorbic acid	0.36	92.00	0.33
	Canola oil	0.31	17.45	0.05
	Maize meal	79.87	6.00	4.79
	Wheat bran	1.06	3.17	0.03
	Soya (46% CP) ¹	3.04	6.40	0.19
	Limestone	0.22	1.20	0.02
				8.04 (R/kg diet)
D2	Milk powder	1.00	60.00	0.60
	Wheat germ meal	8.44	7.50	0.63
	Nipagen	0.85	119.00	1.00
	Sorbic acid	0.36	92.00	0.33
	Canola oil	0.31	17.45	0.05
	Maize meal	81.51	6.00	4.90
	Wheat bran	3.05	3.17	0.10
	Soya (46% CP) ¹	0.61	6.40	0.04
	Brewer's yeast	4.20	7.50	0.57
				8.23 (R/kg diet)

XSIT(CON): Current XSIT diet

D1: Diet one formulated according to minimum nutrient specifications of current published diets using novel raw materials

D2: Diet formulated according to minimum nutrient specifications of current published diets with alternative raw materials at a different inclusion level

¹CP: Crude protein

*Prices obtained on 07/10/2016

Table 3.13 Mean (\pm standard deviation) of total cost in cents to produce a single larva fed different treatment diets

Treatment	Cost per larvae (cent)
XSIT (CON)	0.51 \pm 0.04 ^a
D1	0.22 \pm 0.02 ^b
D2	0.25 \pm 0.02 ^b

XSIT(CON): Current XSIT diet

D1: Diet one formulated according to minimum nutrient specifications of current published diets using novel raw materials

D2: Diet formulated according to minimum nutrient specifications of current published diets with alternative raw materials at a different inclusion level

3.4 Conclusion

It would seem as if the previously published feeds (and that utilised by the industry) malnourished the insect. This research indicates that the costs of feed could be decreased through reformulating the diets fed to the larvae; this would also increase production efficiency. As the nutrient specifications of the FCM have been more accurately determined in this investigation, this provides a crucial nutrient matrix and identifies alternative raw materials that could be utilized to formulate cost effective diets according to the price of the specific raw materials. FCM larvae live within the substrate and therefore it is of utmost importance that the physical properties of the diet meet the needs of the larvae but simultaneously must be suitable to be used within a mass production system. Therefore the physical properties of the diet and bioconversion of the feed provided warrants further research. The success of the newly formulated diets demonstrates that it is not necessary for a diet to be elaborate and expensive to consistently produce high numbers of FCM for biological control purposes.

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Chapter 4

Development of nursery diets for the mass rearing of the black soldier fly (*Hermetia illucens*)

Abstract

A study was conducted to determine an optimal nursery diet for the mass rearing of the black soldier fly (BSF) (*Hermetia illucens*). Various mass production facilities rear neonatal BSF larvae on chicken layer mash. The rearing of these animals for the first six days of their life cycle, also known as the nursery phase, was found to be suboptimal when reared on layer mash. Mortalities ranged between 20-60%. A nursery diet was formulated based on the amino acid composition of the neonatal larvae using the comparative slaughter technique. Newly formulated diets also tested blood meal as alternative raw material. The effect of a sterol source on the growth and development of neonatal BSF larvae was also evaluated. A total of 5600 freshly hatched neonates were allotted to 56 pill pots. Seven treatments with eight replications were allocated to the pill pots in a randomized design. Treatments were: 1) diet formulated according to general nutrient specifications of layer 120 (control); 2) diet formulated according to general nutrient specifications of layer 120 including blood meal; 3) diet formulated to resemble amino acid composition of BSF larvae; 4) diet formulated to resemble amino acid composition of BSF larvae including blood meal; 5) diet one with the addition of a sterol source; 6) diet three with the addition of a sterol source; 7) commercial layer 120 mash currently in use by factory. Diet 3 significantly increased survivability by 25% compared to Diet 1 and Diet 7. The inclusion of bloodmeal in the two treatment diets also caused a significant increase in survivability. The treatments (Diets 5 and 6) that included a sterol source yielded the lowest number of larvae but had a significantly higher average larval weight compared to all other treatments. A strong negative correlation was obtained between survivability and average larval weight. Certain key factors were observed in terms of physical properties of the diets that need to be addressed in future studies. Addressing these factors will ensure a more productive nursery diet for rearing BSF neonates.

***keywords:** nutrient requirements, survivability, larval weight, blood meal

4.1 Introduction

The black soldier fly (BSF) (*Hermetia illucens*) is an insect of economic importance for numerous commercial industries. These include the global food and feed industry, the organic waste sector and the cosmetics industry (van Huis, 2013). It is a non-pest fly that originates from the USA and has spread throughout the tropics and subtropics. The larvae of the BSF are omnivores that feed on organic waste. The adult fly does not take up any food and only survives on reserves and therefore it does not pose any disease transmission risks. The chitin corrected crude protein content of the BSF prepupae was determined to be 42% (Diener *et al.*, 2009), the fat content 35% (Sheppard *et al.*, 2002) and 44% dry matter. Variation in these numbers can be found depending on the feed provided. The protein and fat components include essential amino and fatty acids. Based on the high crude protein content of the prepupae it is deemed to be a good nutritional source for various domesticated animals. Previous studies have determined that BSF larvae can successfully be used to formulate diets for poultry (Hopley, 2015), swine (Veldkamp & Bosch, 2015) and various fish species (Talamuk, 2016).

A growing population, scarce water and land resources, as well as declining natural fish stocks has left the world in urgent need of a sustainable source of protein. The Agriprotein (Pty, Ltd) production facility in Phillipi, South Africa is a mass rearing facility specialising in the rearing of BSF to address the problem in question. Agriprotein (Pty, Ltd) has been developing insect based protein feed, extruded oil and fertilisers since 2007. The first factory was set up in 2014 and since 2015 it has produced 7 ton of protein rich meal, 3 ton of oil and 22 ton of fertiliser per day. The BSF larvae are reared on abundant waste sources and therefore recycle large amounts of waste due to the voracious feeding nature of the larvae. Current procedures at Agriprotein (Pty, Ltd) recycle 110 ton of waste per day.

The waste received by Agriprotein (Pty, Ltd) to rear the larvae include kitchen waste from surrounding areas. It was found that this waste varies greatly in nutrient composition due to the large variation in the composition of the kitchen waste. Hence larvae are typically reared on soaked poultry layer mash for the first six days after hatching (Diener *et al.*, 2009). The hardier six day old larvae are transferred to the kitchen waste where they then feed for the following 12 days before they are harvested at day 18. The first six days after hatching is referred to as the nursery

phase. Mortality of larvae during this period ranges between 20% and 60% and this is thought to be due to an imbalance in nutrients provided by the layer mash per specific nutrient needs of the neonatal larvae; the nutrient needs of BSF larvae does not resemble that of layers.

The nutrition of these insects is of utmost importance for economical and efficient production since over- and undersupply of nutrients have the same detrimental effect in production as it does in domesticated animals. The undersupply of nutrients could lead to a total break in production while marginal undersupply could lead to immune suppression, decreased efficiency of production or a reduction in fertility and fecundity (Waldbauer, 1968). On the other hand, an oversupply of nutrients could lead to the build-up of primary or secondary metabolites which could be toxic, antagonistic or result in imbalances that could lead to metabolic stress (Aryal *et al.*, 2007). To minimize any stress associated with the mass rearing of insects it is necessary to formulate feeds with nutrient specifications as close as possible to their requirements.

The only proven nutritional difference between insects and most other animals is their dietary need for sterols (Hobson, 1935). Insects do not have the ability to synthesize sterols (Clarck & Bloch, 1959) and therefore it is a necessity that they obtain it from a dietary source. Sterols are required in lipid biostructures, as precursors to important steroid hormones and as regulators of developmental processes (Behmer & Nes, 2003). It was found that cholesterol plays a crucial role in governing larval growth and development by functioning in a metabolic role. Many species of insects can modify the sterols of their diet and presumably such modifications serve to provide a structure that is more appropriate to one or all the insect's requirements. Nevertheless, it is becoming clear that sterols play a role in insect's physiology that seems remarkably similar to that of cholesterol in the mammal (Clayton, 1964). Current status of the neonatal diet lacks in a distinct sterol source and could be a factor contributing to the current high mortality rates.

As the production of BSF larvae as a protein source is a sustainable solution to the growing protein crisis, the development of a nursery diet should follow the same theme. Alternative raw materials that are produced from abattoir waste, such as blood meal and poultry by-product meal, could be utilized to formulate a nursery diet that conforms to the current sustainable agriculture needs. Poultry by-product meal has a crude protein content of 60% (Dong *et al.*, 1993) and bloodmeal 94% (El-Sayed, 1998) which could be effectively utilized by the BSF larvae. Previous heat

processing methods of blood meal such as rotoplate-, steam-tube- and ring-dried methods severely reduced the digestibility of the protein, as well as the lysine and methionine content of the blood (Waibel *et al.*, 1977). Current spray dried blood products such as whole blood, blood cells and blood plasma have increased apparent digestibility coefficients and are therefore more efficiently utilized (Bureau *et al.*, 1999).

The development of a sustainable, effective nursery diet that meets the specific nutrient needs of the juvenile BSF larvae is crucial to solidify the prospect of BSF larvae replacing conventional protein sources such as fish and soya bean meal as a sustainable option. A diet meeting the nutritional needs of the animals in question will decrease mortality and effectively optimize production.

Therefore the objectives of this study were to:

- I. Determine the amino acid requirements of neonatal BSF larvae
- II. Formulate a commercial nursery diet for the mass production of BSF
- III. Evaluate the use of alternative, more sustainable raw materials in the formulation of BSF nursery diets
- IV. Evaluate the use of a sterol source in the diets of neonatal BSF larvae

4.2 Materials and Methods

4.2.1 Flies and housing

Freshly hatched *Hermetia illucens* neonatal larvae were obtained from Agriprotein (Pty, Ltd) (Phillipi, Cape Town). Seven treatments with eight replications were randomly allotted to 56 politop 100 pill pots with a volume of 100 mL. Neonates were counted under a magnifying glass and randomly allotted to one of seven treatment groups, with 100 neonates assigned to each of the 56 pill pots. A fine net was placed over the top of the pill pot and secured using an elastic band. Pill pots were kept in a controlled-environment with a constant temperature of $27 \pm 1^\circ\text{C}$ and relative of humidity of $65 \pm 5\%$.

Based on standard practise at Agriprotein (Pty, Ltd) which provide 0.04 g of feed per larvae per day, 24 g of feed was placed in each pill pot. The temperature of the feed was brought to 25°C by

leaving the feed in the controlled-environment overnight, before placing the neonates on the feed. This was done to ensure that larvae were not placed onto cold feed which would have slowed their metabolic rate (Čičková *et al.*, 2015). Neonates were placed on tissue paper on top of the feed and not directly onto the feed to prevent them from drowning.

Pill pots were monitored daily to ensure that the moisture content of the feed did not drop to such an extent that the neonatal larvae were not able to consume the feed. On days three and five, 3 mL of water was added to each pill pot using a spray bottle as the feed had started to dry. Moisture content of the feed was not allowed to drop below 50% as it was determined that larvae are not able to feed when the moisture content drops below this point (Beard *et al.*, 1973; Sheppard *et al.*, 2002).

Neonatal larvae were harvested six days after being provided the feed. The larvae and remaining feed were separated where after the larvae were counted. Survivability and average larval weight were determined.

4.2.2 Treatments and experimental diets

The neonatal larvae were assigned to seven different treatment diets. The seven experimental treatments are described in Table 4.1. The first two treatment diets were formulated based on the nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day. The first formulated feed served as control (L120) and the second included 10% blood meal (L120B) to the control.

A comparative slaughter was done on six day old larvae which were reared on a commercial layer 120 mash. The amino acid profile was determined using methods described by Cunico *et al.* (1986). Before the amino acid profile could be determined, samples were hydrolysed in acid. During this process, a sample weighing 0.1 g was placed in a specialized hydrolysis tube. Six mL hydrochloric acid (HCl) solution and 15% phenol solution was then added to the sample. The tubes were then vacuated 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 sent to the Central Analytical Facility of Stellenbosch University, where the amino acid profiles were determined by means of the Waters AccQ Tag Ultra Derivatization method.

The amino acid profile of the six day old larvae are described in Table 4.2. Diets B0.69 and B0.69B were formulated to resemble the amino acid profile of the six day old larvae. The diet was formulated to resemble 69% of the lysine content of the larvae, similar to that of the layer mash. A diet formulated to resemble 100% of the lysine composition of the larvae, according to the comparative slaughter, was not feasible as the lysine content of such a diet would not be achievable using commercial raw materials. Diet three was formulated using current raw materials (B0.69) and diet four included 10% spray dried blood meal (B0.69B).

Diets five and six were achieved by including a sterol source (pork brains) to diets L120 (L120+S) and B0.69 (B0.69+S). Pork brains were obtained from Winelands Pork (Stikland, Cape Town), and homogenised. This was included into the mentioned diets at a rate of 3%.

The ingredient composition for each treatment diet is indicated in Table 4.3. For commercial purposes the production potential of a commercial layer 120 mash, currently used in mass production facilities, was compared to that of the treatment diets. The general nutrient specifications of the commercial diet were as follows: protein – 13%, fat – 25%, fibre – 7%, moisture – 20%, calcium – 4%, phosphorus – 0.5% and total lysine – 0.5%.

Diets were mixed at Mariendahl experimental farm (Stellenbosch). The moisture content of the diet was brought to 60% by the addition of warm water. Feed was left to absorb the water for 12 hours before being fed to the larvae.

4.2.3 Statistical Analysis

Analysis of variance was performed on larvae yield data using the ANOVA procedures of SAS (2009) with treatment as the main effect. All the parameters were tested for normality and homoscedasticity before analysis. Significance was declared at $P \leq 0.05$. Means were separated with Bonferroni *post hoc* test (SAS, 2009). The results were used to compare the yield of larvae between different treatments.

Table 4.1 A description of BSF nursery diet treatments used throughout the trial

Treatment	Description
L120	Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day (control)
L120B	Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day with the inclusion of 10% blood meal
B0.69	Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120)
B0.69B	Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the inclusion of 10% blood meal
L120+S	Diet formulated according to nutrient specifications of the post lay hyline brown with feed intake of 120 g per day with the addition of 3% pork brains
B0.69+S	Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the addition of 3% pork brains
F	Commercial store bought layer feed presently used in Agriprotein (Pty, Ltd) factory

Table 4.2 Amino acid profiles of six day old BSF larvae and treatment diets with lysine concentration as 100 and other amino acid concentrations expressed as a percentage of lysine.

Amino Acid	Larvae	L120	L120B	B0.69	B0.69B
Arginine	95	130	129	70	73
Glycine	83	49	50	37	40
Threonine	65	79	78	65	63
Tyrosine	57	81	80	50	51
Methionine	28	44	70	31	46
Valine	79	108	106	116	110
Phenylalanine	52	96	95	88	85
Isoleucine	61	89	88	34	37
Leucine	99	210	204	204	189
Histidine	64	59	57	75	70
Lysine	100	100	100	100	100
Cysteine	98	42	42	30	29
Tryptophan	25	22	22	16	16

L120: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day (control)

L120B: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day with the inclusion of 10% blood meal

B0.69: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120)

B0.69B: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the inclusion of 10% blood meal

L120+S: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day with the addition of 3% pork brains

B0.69+S: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the addition of 3% pork brains

F: Commercial store bought layer feed presently used in Agriprotein (Pty, Ltd) factory

Table 4.3 Ingredient and calculated nutrient composition of BSF nursery diets used in the trial

	L120	L120B	B0.69	B0.69B
Ingredients (% of diet)				
Maize	77.18	85.63	75.47	79.62
Soya bean meal (46% CP ¹)	18.62	0.22	20.12	4.43
Spray dried blood meal	--	10.00	--	10.00
DL - Methionine	0.07	0.07	0.29	0.27
Vit+min premix ²	0.15	0.15	0.15	0.15
Limestone	1.82	1.87	1.81	3.13
Salt	0.25	0.21	0.25	0.21
Monocalcium phosphate	1.67	1.64	1.67	1.64
Sodium bicarbonate	0.24	0.21	0.24	0.21
Calculated nutritional Value (%)				
Dry matter	87.57	87.26	87.68	87.58
Moisture	12.43	12.74	12.32	12.42
Crude protein	15.19	15.95	15.95	17.61
Crude fibre	2.63	1.90	2.67	1.98
Calcium	1.00	1.00	1.00	1.46
Available phosphorous	0.50	0.50	0.50	0.50
Sodium	0.18	0.18	0.18	0.18
Potassium	0.64	0.31	0.67	0.38
DE ³ (Pig)	14.06	14.04	14.04	13.85
Lysine	0.73	0.77	1.02	1.14
Methionine	0.32	0.54	0.32	0.53
Sulphur amino acids	0.63	0.86	0.63	0.86
Tryptophan	0.16	0.17	0.16	0.18
Isoleucine	0.65	0.68	0.35	0.42
Leucine	1.53	1.57	2.08	2.16
Threonine	0.58	0.60	0.66	0.72

¹CP: Crude protein

²Vit+min premix: Commercial layer vitamin and mineral premix

³DE: Digestible energy (MJ/kg)

L120: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day (control)

L120B: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day with the inclusion of 10% blood meal

B0.69: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120)

B0.69B: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the inclusion of 10% blood meal

L120+S: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day with the addition of 3% pork brains

B0.69+S: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the addition of 3% pork brains

F: Commercial store bought layer feed presently used in Agriprotein (Pty, Ltd) factory

-- No value available

4.3 Results and discussion

4.3.1 Survivability and larval weight

The effects that diets (L120, L120B, B0.69, B0.69B, L120+S & B0.69+S) had on the survivability of the neonatal larvae are presented in Table 4.4. Differences ($P < 0.05$) in survivability at six days of age were detected between most treatment diets. Survivability was significantly higher for larvae in the two blood meal treatments (L120B & B0.69B) and the diet formulated to resemble the amino acid composition of BSF larvae (B0.69) compared to that of the control (L120) and factory (F) diets. Treatment groups containing the pork brains (L120+S & B0.69+S) had the lowest survivability. The layer 120 mash treatment (L120) formulated to serve as control was statistically comparable to that of the current factory diet (F) in use at the Agriprotein (Pty, Ltd) factory. No differences in survivability were detected between the two treatment diets. These two treatments were intermediary as pertaining to the number of neonatal larvae surviving compared to other treatment diets. No differences were observed between the two blood meal treatments (L120B & B0.69B) and the diet formulated to resemble the amino acid composition of BSF larvae treatment groups (B0.69). These treatment diets had on average a 25% increase in survivability compared to the control treatments. Both diets formulated to resemble the amino acid composition of the larvae (B0.69 & B0.69B) had higher survivability ($P < 0.05$) than current diets formulated based on layer 120 nutrient specifications. The success of these diets was thought to be attributed to the fact that the amino acid profile of the experimentally formulated feeds more closely resembled that of the larvae. When comparing the amino acid profile of these diets to that of the larvae threonine, tyrosine, methionine, histidine and isoleucine had similar ratios compared to that of the larvae. Also, the crude protein content of diets B0.69 (16.95%) and B0.69B (17.61%) were higher than that of the layer specifications diets, L120 (15.19%) and L120B (15.95%). The crude protein requirements for the first six days of development of the larvae may be higher than what is currently being provided.

The inclusion of 10% blood meal as additional raw material proved to increase survivability of the larvae. When comparing L120 and L120B there was a 23.5% increase in survivability caused by the addition of blood meal. When blood meal is fed to conventional livestock it does not have a very high palatability and cannot be included at high rates. However, it was determined that a 10% inclusion rate in the diets of neonatal BSF larvae had a desired effect on production and seemed

more palatable to the larvae compared to that of conventional livestock. The success of bloodmeal in the diet of neonatal BSF larvae has paved the way for the possible use of fresh blood if legislation is rewritten in the future regarding this topic.

The two treatments including a sterol source (L120+S and B0.69+S) had the lowest survivability and on appearance had a sticky texture. When assessing the crude fibre content of these treatment diets, both diets contain less than 3% fibre. Fibre in the diets of insects acts as bulking agents that can be used to modify the texture of the diet. Raw materials such as wheat germ or wheat bran that are high in fibre act as carriers for lipids and lipoproteins (Cohen, 2015). Fibre can thus be used to carry lipids into the diet matrix and contribute to the desirable texture of diets. The fibre content of these diets were determined to be suboptimal and the pork brain provided as sterol source was not readily available to be utilized by the insects and this could explain the low survivability of these two treatment diets. However, this aspect warrants further research where the same diet containing brains with different levels of fibre is evaluated. Alternative sources of sterols should be considered and researched. It was observed that the inclusion of a sterol source also had an influence on the larval weight.

The effects that diets had on the larval weight are presented in Table 4.4. No differences ($P > 0.05$) in live weight were observed between treatments L120, L120(B), B0.69, B0.69(B) and F. The only differences observed were between the two sterol treatments (L120+S & B0.69+S) that produced heavier ($P < 0.05$) larvae than other treatment diets. This could be contributed to fact that BSF larvae do not have the ability to synthesize sterols (Clarck & Bloch, 1959) and therefore it is a necessity that they obtain it from a dietary source. It was found that cholesterol plays a crucial role in governing larval growth and development (Behmer & Nes, 2003). It was found that there was a strong negative correlation (-0.82) between the number of larvae produced and the average larval weight for each dietary treatment. These results suggest that the increase in larval weight observed for treatments L120+S and B0.69+S can not only be contributed to the inclusion of a sterol source, but the correlation factor between larval density and larval weight needs to be considered. Even more so when conducting this experiment in pill pots due to the extremely confined environment that led to greater competition. The increase in larval weight for treatments L120+S and B0.69+S could be explained by either of these two factors or a combination of the two and thus the direct reason for increased larval weight could not be accurately pinpointed by this study.

Severe microbial growth was observed during the last three days of the trial. Treatment diets were not rendered to be aseptic and the influence of these cultures on the survivability and growth performance of the larvae were unknown. Most other artificial diets for mass producing insects, as the one formulated by Moore *et al.* (2014) for the production of the false codling moth (*Thaumitotibia leucotreta*), make use of aseptic diets. The inclusion of nipagin and/or formalin into the diets could have a desired effect on production and also warrants further research.

Table 4.4 Mean (\pm standard error) survivability (%) and larval weight (g) of neonatal larvae grown from hatch to six days of age receiving different treatment diets.

Treatment	Survivability (%)	Larval weight (g)
L120	69.8 \pm 3.98 ^b	0.1013 \pm 0.00739 ^b
L120B	91.3 \pm 4.26 ^a	0.1086 \pm 0.00739 ^b
B0.69	92.0 \pm 3.98 ^a	0.0779 \pm 0.00739 ^b
B0.69B	93.9 \pm 3.98 ^a	0.0807 \pm 0.00739 ^b
L120+S	34.8 \pm 3.98 ^c	0.1715 \pm 0.00739 ^a
B0.69+S	11.1 \pm 4.26 ^d	0.1869 \pm 0.00739 ^a
F	68.2 \pm 3.98 ^b	0.0986 \pm 0.00739 ^b

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

L120: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day (control)

L120B: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day with the inclusion of 10% blood meal

B0.69: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120)

B0.69B: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the inclusion of 10% blood meal

L120+S: Diet formulated according to nutrient specifications of the post lay hyline brown hen with feed intake of 120 g per day with the addition of 3% pork brains

B0.69+S: Diet formulated to resemble amino acid composition of BSF larvae at a lysine level comparable to that of the post lay hyline brown hen specifications (L120) with the addition of 3% pork brains

F: Commercial store bought layer feed presently used in Agriprotein (Pty, Ltd) factory

4.4 Conclusion

The nutrient requirements of neonatal BSF larvae were more accurately met when diets formulated according to their amino acid composition, compared to that of the layer mash currently being used in the industry, were fed. This led to a 25% increase in survivability of neonatal larvae compared to that of the survivability determined for current Agriprotein (Pty, Ltd) factory operations. The nutrient specifications determined for neonatal BSF larvae could be used as matrix for further studies. Also, the inclusion of blood meal as additional raw material had a desired effect on production. Unprocessed blood could possibly be provided to BSF larvae as a cheaper alternative, although the weight of the liquid could be an impediment. For safety reasons blood must be heat treated to be used in conventional livestock feeds in order to destroy potential pathogens. The ability of BSF larvae to detoxify and metabolize these pathogens should be studied to evaluate the safety of incorporating unprocessed blood into their diets. The use of fresh blood could help reduce environmental impact as modern drying techniques require high amounts of energy and including blood meal into the diets of BSF larvae removes potentially contaminating slaughter wastes from the environment. Certain key observations were made through the duration of the trail that may affect the survivability of the larvae and should be considered in future studies. The moisture content of the feed seemed to be a major factor determining feed utilization by the larvae. Larvae live within their feed and therefore their direct environment is determined by the feed provided. The fibre content of the newly formulated diets was found to be too low and an optimal inclusion level needs to be determined. Fibre can be used to carry lipids into the diet matrix and contribute to the desirable texture of diet. The texture, water holding capacity and density of the feed play a crucial role in determining the success of the treatment diets and in future studies, physical properties of the diets need to be considered.

4.5 References

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Chapter 5

Evaluation of protein sources in the diets of the yellow meal worm (*Tenebrio molitor*)

Abstract

The effect of a dietary protein source on the production performance of the yellow mealworm (mealworm) (*Tenebrio molitor*) was investigated. Mealworms are commonly reared on grain by-products and thus lack a distinct protein source in their diet. The use of either ground beef (70% lean) or soya bean meal 46 were included into the diets of juvenile mealworms at 10, 20 and 30% levels on a weight to weight basis whilst wheat bran alone served as control diet. The mealworms were allowed to grow for 35 days. Treatments were: 100% wheat bran (CON); 10% ground beef / 90% wheat bran (M10); 20% ground beef / 80% wheat bran (M20); 30% ground beef / 70% wheat bran (M30); 10% soya / 90% wheat bran (S10); 20% soya / 80% wheat bran (S20); 30% soya / 70% wheat bran (S30). The inclusion of ground beef (70% lean) and soya bean meal 46 at all inclusion levels increased ($P < 0.05$) the cumulative live weight for the duration of this period as well as the growth rate. The treatment groups containing 20 and 30% ground beef yielded heaviest larvae with the fastest growth rates while the performance of the soya groups were intermediate. The 20% ground beef treatment group also had the fastest pupation rate and yielded the most pupae over this period. The inclusion of a protein source in the diets of mealworms increased their productivity. However, cheaper alternative protein sources need to be investigated to ensure that the inclusion of a protein source into the diets of mass reared mealworms is economically feasible. Nonetheless, the inclusion of a protein source into the diets of mass reared mealworms is vital for enhanced production.

***keywords:** larval weight, growth curve, pupation rate

5.1 Introduction

With the global population estimated to increase to 9 billion people in 2050 and current food insecurity situation prevailing in many developing countries, insects as food has received wide attention (FAO, 2013). Due to the economic crisis affecting developing countries in general and Africa in particular, it becomes more advisable to encourage the utilisation of non-conventional sources of protein that are widely available (Téguia *et al.*, 2002). Insects have served as protein source for many centuries and are currently being consumed by humans in many parts of Asia, Latin America and Africa (Bukkens, 1997). Currently it is estimated that insects supplement the diets of approximately 2 billion people (DeFoliart, 1992).

The yellow mealworm (mealworm) (*Tenebrio molitor*) is a pest of grain, flour and food stores, but often of little importance since their populations are quite small (Ramos-Elorduy, 1997). They have a valuable amino acid profile high in essential amino acids and are easy to breed and feed. For these reasons, they can be produced in intensive production systems. They are produced commercially as feed for pets and zoo animals, including birds, small mammals, reptiles, amphibians and fish. Mealworms are usually fed live to these animals, but are also sold canned, dried or in powder form. Although insects have been eaten for millennia worldwide, the consumption of raw yellow mealworms by humans is not well accepted in the so called western countries because of their appearance. However, maize flour supplemented with *T. molitor* processed meal is a growing commodity (Aguilar-Miranda *et al.*, 2002). In the East, insects are considered as delicacies, however in the Western world they are considered the last option of food, but due to their accessibility, nutritional value and flavour, insects are taking on an important role in the human diet (Andersen *et al.*, 1995).

Mealworms have the ability to recycle plant and animal waste materials of low quality into high-quality feed rich in energy, protein and fat in a relatively short period. They contain high amounts of crude protein (47-60%) and fat (31-43%) with a favourable amino- and fatty acid composition. Fresh larvae contain about 60% water. Mealworm larvae are low in ash (<5% DM) and are low in calcium (Makkar *et al.*, 2014). The composition of mealworm larvae varies depending on their diet which can be manipulated to supplement for nutrients that are lacking in the larvae (Klasing *et al.*, 2000). Mealworms also possess the ability to detoxify zearalenone by partly metabolizing it

to alpha-zearalenol. There was no risk of zearalenone accumulating in the mealworm larvae at concentrations where they could affect the animals that ate them (Makkar *et al.*, 2014).

Mealworms are omnivorous and can eat different kinds of plant and animal material. They are able to utilize animal and plant by-products such as feathers and saw dust and incorporate it into their biomass as a suitable protein source for human consumption (Ramos-Elorduy *et al.*, 2002). They are typically fed on cereal bran or flour (wheat, oats, maize) and occasionally supplemented with protein sources such as soybean meal, skimmed milk powder or yeast. Fresh fruits and vegetables (carrots, apples, lettuce) are also provided to serve as water source. Mealworms are able to utilize small amounts of water contained in dry feeds but this is not favourable for production. The productivity of water deprived yellow mealworms drop to one generation per year compared to that of six generations per year under normal circumstances; water deprivation can also lead to cannibalism. Relative humidity is positively correlated to fertility, but at high relative humidity it is important to monitor fresh foods as they may turn mouldy (Makkar *et al.*, 2014).

Previous studies indicate that the diet of the yellow mealworm should be balanced out with 20% protein on a dry matter basis (Ramos-Elorduy *et al.*, 2002). It was also found that larvae of the yellow mealworm require a dietary source containing the same 10 amino acids essential for growth in rats, other vertebrates, and some protozoa. Studies also suggest that serine, tyrosine, glutamic acid, and possibly glycine can be excluded from their diet without negatively influencing growth (Davis, 1975), ideal amino acid profiles for these animal have however, not been published.

Therefore, the objectives of this study were to:

- I. Establish the importance of protein source in the diet of mealworms
- II. Evaluate the effect of plant versus animal protein source in the diet of mealworms
- III. Determine optimal inclusion level of protein in the diet of mealworms
- IV. Increase mass production efficiency of the mealworm

5.2 Materials and Methods

5.2.1 Animals and housing

A total of $\pm 42\,000$ juvenile yellow meal worms were obtained from Reptilia, a commercial breeder of reptiles and various insect species (Vanderbijlpark, Gauteng, South Africa). They were transported for a distance of 1400 km via air freight to the Department of Animal Sciences, Stellenbosch University (Stellenbosch, Western Cape, South Africa). Seven treatments with six replications per treatment were randomly allotted to 42 plastic containers (34.0 x 23.5 x 6.5 cm). The density of the worms within the residue that they were transported was determined by thoroughly mixing the residue and worms to create one homogenous mixture. Twenty samples were taken from the mixture and each sample weighed and the number of worms therein, counted. An average ratio was determined between sample weight and number of worms and used to allocate 1000 worms to each treatment. The containers were placed in a controlled environment where temperature ($26 \pm 1^\circ\text{C}$) and humidity ($60 \pm 5\%$) were kept constant for the entire duration of the trial.

Each container contained 350 g of wheat bran and sufficient water bound by carrageenan gel to maintain a solid state at the temperature the trial was conducted. Water was checked daily and supplied *ad libitum*. Different protein sources, at different inclusion levels, were provided daily at 08h00 and protein consumption was recorded. Feed was provided daily at a rate of 1, 2 and 3 g for the S10, S20 and S30 treatments respectively. The same principle applied for the ground beef treatments. It was found that all protein feedstuff provided was utilized daily and no wastage occurred. The trial was conducted for a period of 35 days.

Body weight of 1000 worms were measured at placement and an average weight used to represent the initial weight of all worms. From day 21 onwards, ten worms were randomly selected from each treatment to be weighed every third day. Worms started pupating around day 21 and pupae were collected by means of placing egg boxes on top of the feed that the worms climbed onto prior to commencing pupation. Pupae were counted daily until the end of the trial. At the end of the trail the remaining bran, frass (excrement of larvae) and worms were separated. The residual bran (g) as well as the number of mealworms that had yet to pupate were all recorded.

5.2.2 Treatments and experimental diets

The worms were assigned to seven different treatment diets. The seven experimental treatments are described in Table 5.1. The main difference between the treatments were the type of protein source as well as the inclusion levels of the different protein sources. Lean ground beef (70% lean) was used as animal protein source and soya bean meal (soya oil cake 46) as plant protein source. Three different inclusion levels i.e. 10, 20 and 30% were used for both protein feedstuffs. A 100% wheat bran diet was used as the control. Moisture content of the soya and ground beef were determined and the moisture content of the soya corrected to that of the ground beef (72%) by the addition of water. Amino acid compositions and profiles of protein sources, bran and mealworms are described in Table 5.2.

5.2.3 Statistical Analysis

Analysis of variance was performed on container means for weight, pupation rate and pupal weight data using the general linear models (GLM) and ANOVA procedures of SAS (2009) with treatment as the main effect. All the parameters were tested for normality and homoscedasticity before analysis. Significance was declared at $P \leq 0.05$. Means were separated with Bonferroni *post hoc* test (SAS, 2009). Average daily gain was determined by means of fitting a simple linear regression of weight over time. The slope of the resulting regression function is ADG and was used to compare animals between treatments.

Table 5.1 A description of the dietary treatments used throughout the trial

Treatment	Description
CON	100% bran
S10	10% soybean meal / 90% bran
S20	20% soybean meal / 80% bran
S30	30% soybean meal / 70% bran
M10	10% ground beef / 90% bran
M20	20% ground beef / 80% bran
M30	30% ground beef / 70% bran

Table 5.2 Amino acid composition (g/100 g) dry matter and amino acid profiles of feedstuffs used in treatment diets as well as for *Tenebrio molitor*

Amino Acid	<i>*Tenebrio molitor</i>		Ground beef		Bran		Soya bean meal	
	AV ¹	% of Lys ²	AV ¹	% of Lys ²	AV ¹	% of Lys ²	AV ¹	% of Lys
Alanine	4.0	1.5	0.9	0.5	0.7	1.3	--	--
Arginine	2.7	1.0	0.9	0.5	1.1	1.8	3.5	1.2
Aspartate	4.0	1.5	1.3	0.7	1.1	1.9	--	--
Cysteine	0.4	0.1	0.2	0.1	0.4	0.6	0.7	0.2
Glutamine	5.5	2.0	2.2	1.1	2.9	4.8	--	--
Glycine	2.7	1.0	1.0	0.5	0.9	1.5	1.9	0.7
Histidine	1.5	0.6	0.5	0.3	0.4	0.7	1.2	0.4
Isoleucine	2.5	0.9	0.6	0.3	0.5	0.8	2.3	0.8
Leucine	5.2	1.9	1.1	0.6	0.9	1.6	3.5	1.2
Lysine	2.7	1.0	1.9	1.0	0.6	1.0	2.8	1.0
Methionine	0.6	0.2	0.4	0.2	0.2	0.4	0.6	0.2
Phenylalanine	1.7	0.6	0.6	0.3	0.6	1.0	2.2	0.7
Proline	3.4	1.3	0.7	0.4	0.9	1.5	--	--
Serine	2.5	0.9	0.6	0.3	--	--	--	--
Threonine	2.0	0.7	0.6	0.3	0.5	0.8	1.8	0.6
Tryptophan	0.4	0.1	0.1	0.1	0.3	0.5	0.6	0.2
Tyrosine	3.6	1.3	0.4	0.2	0.4	0.7	1.9	0.6
Valine	2.9	1.1	0.7	0.4	0.7	1.2	2.4	0.8

¹ Absolute value² Lysine

-- No value available

*Hopley (2015)

5.3 Results and discussion

5.3.1 Live weight and weight gain

The effect that different treatments (CON, S10, S20, S30, M10, M20 and M30) had on growth are presented in Table 5.3. No differences ($P > 0.05$) in live weight were detected at the start of the performance trial.

On day 21, mealworms in the M30 treatment group had a 31.9% heavier live weight compared to mealworms in the CON treatment group. Animals in the ground beef treatment groups (M10, M20 and M30) had the heaviest live weights and animals in the CON treatment the lowest. A lack of difference ($P > 0.05$) between the ground beef treatment groups (M10, M20 and M30) and the maximum soya inclusion treatment (S30) existed.

The same trend in live weights were obtained for days 24, 27, 30 and 33 where differences ($P < 0.0001$) were observed between treatment groups. In general, over this growth period, the ground beef treatment groups (M10, M20 and M30) had the heaviest live weights and were similar ($P > 0.05$). For each of these days (24, 27, 30 and 33), the CON treatment group had the lowest live weight. All treatment groups performed ($P < 0.05$) better than that of the CON treatment. The soya bean meal treatments (S10, S20 and S30) performed intermediate and were similar to each other. On days 24, 27 and 30, the average live weights of the S30 treatment group were similar to that of the ground beef treatments. Over the mentioned period, it was observed that mealworms in the M30 treatment had a 23.9% increase in live weight compared to that of the CON treatment that only had a 16.7% increase in live weight. For all treatments, the most growth took place between day 21 and 27 where after the growth rate started to plateau from day 30 onwards.

Results for live weight gain on day 36 showed less difference ($P < 0.025$) compared to days 21, 24, 27, 30 and 33 ($P < 0.0001$). By day 36, the CON treatment did not differ from the soya bean meal treatments (S10, S20 and S30) and only the ground beef treatment groups (M10, M20 and M30) and S30 differed from the CON treatment.

The lack of significant difference and the intermediate growth performance of the mealworms in soya bean meal treatment groups (S10, S20 and S30) could be partially attributed to and correlated

to natural inhibitors of proteolysis known to be present in soya seeds (Powning *et al.*, 1951; Applebaum *et al.*, 1961). Two of these inhibitors are the trypsin inhibitors, while another has been shown to inhibit *Tribolium* proteolytic activity (Applebaum *et al.*, 1964).

Davis (1975) indicated that mealworm larvae require a dietary source of the same 10 amino acids essential for growth in rats and other vertebrates. These amino acids include arginine, histidine, isoleucine, leucine, threonine, lysine, methionine, phenylalanine, tryptophan, and valine. Ground beef contains higher levels of essential amino acids such as lysine and methionine and has a more favourable amino acid composition compared to that of wheat bran (Table 5.2). Therefore, larvae in the ground beef treatment groups (M10, M20 and M30) had more essential amino acids available for protein synthesis and growth. This was also the case for the soya bean meal treatments (S10, S20 and S30) but it is suggested that certain antinutrients such as trypsin inhibitor, caused these treatment groups to perform poorer than the ground beef (M10, M20 and M30) treatment groups.

Fraenkel *et al.* (1950) using casein as protein feedstuff found that optimal growth was still achieved at a 10% casein level compared to that of the normally used 20% level. Growth rate was only affected when the inclusion level of casein was reduced to 5, 2 and 1% levels leading to a progressive reduction in growth. This was contradictory to the findings of the use of ground beef as protein feedstuff as there was a significant difference ($P < 0.05$) in cumulative live weights between treatment groups M10 and M20. On the other hand the findings of Fraenkel *et al.* (1950) for a 10% casein inclusion level were comparable to that of the S10 and S20 treatments as no difference in cumulative live weight was observed between these treatment groups.

Table 5.3 Mean (\pm standard error) live weights and cumulative live weights (g) of juvenile mealworms reared for 36 days on different treatments

Treatment	Day 0	Day 21	Day 24	Day 27
CON	0.0293 ^a \pm 0.00155	0.0638 ^c \pm 0.00436	0.0727 ^d \pm 0.00449	0.0753 ^c \pm 0.00433
S10	0.0293 ^a \pm 0.00155	0.0688 ^{bc} \pm 0.00436	0.0923 ^c \pm 0.00449	0.1003 ^b \pm 0.00433
S20	0.0293 ^a \pm 0.00155	0.0636 ^c \pm 0.00436	0.0945 ^{bc} \pm 0.00449	0.1144 ^{ab} \pm 0.00433
S30	0.0293 ^a \pm 0.00155	0.0839 ^{ab} \pm 0.00436	0.1069 ^{abc} \pm 0.00449	0.1063 ^{ab} \pm 0.00433
M10	0.0293 ^a \pm 0.00155	0.0815 ^{abc} \pm 0.00436	0.1121 ^{ab} \pm 0.00449	0.1215 ^a \pm 0.00433
M20	0.0293 ^a \pm 0.00155	0.0895 ^a \pm 0.00436	0.1171 ^a \pm 0.00449	0.1214 ^a \pm 0.00433
M30	0.0293 ^a \pm 0.00155	0.0937 ^a \pm 0.00436	0.1172 ^a \pm 0.00449	0.1183 ^{ab} \pm 0.00433
P Value	0.774	0.0001	0.0001	0.0001

Treatment	Day 30	Day 33	Day 36	Day 24-36
CON	0.0758 ^d \pm 0.00417	0.0766 ^d \pm 0.00443	0.0906 ^b \pm 0.00469	0.0746 ^c \pm 0.00190
S10	0.0985 ^c \pm 0.00417	0.1014 ^c \pm 0.00443	0.1039 ^b \pm 0.00469	0.0942 ^d \pm 0.00190
S20	0.1039 ^c \pm 0.00417	0.1044 ^c \pm 0.00443	0.1007 ^b \pm 0.00469	0.0966 ^d \pm 0.00190
S30	0.1117 ^{abc} \pm 0.00417	0.1111 ^{bc} \pm 0.00443	0.1099 ^{ab} \pm 0.00469	0.1019 ^{cd} \pm 0.00190
M10	0.1189 ^{ab} \pm 0.00417	0.1198 ^{abc} \pm 0.00443	0.1093 ^{ab} \pm 0.00469	0.1064 ^{bc} \pm 0.00190
M20	0.1213 ^{ab} \pm 0.00417	0.1226 ^a \pm 0.00443	0.1198 ^a \pm 0.00469	0.1123 ^{ab} \pm 0.00190
M30	0.1231 ^a \pm 0.00417	0.1211 ^{ab} \pm 0.00443	0.1168 ^a \pm 0.00469	0.1157 ^a \pm 0.00190
P Value	0.0001	0.0001	0.0025	0.0001

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

CON: 100% bran

S10: 10% soybean meal / 90% bran

S20: 20% soybean meal / 80% bran

S30: 30% soybean meal / 70% bran

M10: 10% beef mince / 90% bran

M20: 20% beef mince / 80% bran

M30: 30% beef mince / 70% bran

5.3.2 Pupation rate

The effects that different treatments (CON, S10, S20, S30, M10, M20 and M30) had on pupation rate are presented in Table 5.4. Larvae of all treatment groups started pupating 21 days after the start of receiving the different protein treatments. Differences ($P < 0.0001$) were detected between different treatment groups. On day 21 the M20 treatment group yielded the most pupae and CON, S10 and S20 the least number of pupae with the M20 treatment group yielding 86.7% more pupae than that of the CON treatment group. No differences ($P > 0.05$) were observed between the ground beef treatment groups (M10, M20 and M30). The S30 treatment performed intermediate to the ground beef and CON treatment groups.

This trend followed for most of the duration between days 22 and 29 with the ground beef treatment groups (M10, M20 and M30) producing more ($P < 0.05$) pupae compared to that of the CON and soya bean meal treatments groups (S10, S20 and S30). At day 30 no difference was observed between treatment groups ($P > 0.05$) and from this point onwards until day 35, the different treatment groups started yielding a similar number of pupae. This occurrence could be explained due to the fact that a finite number of larvae were used for each treatment and the number of larvae left to pupate started to decrease. The CON treatment group carried on producing the least number of larvae over this period but no differences were observed between soya bean meal (S10, S20 and S30) and ground beef (M10, M20 and M30) treatment groups.

The observed difference ($P < 0.0001$) in cumulative pupae yield of the mealworms between treatment groups (CON, S10, S20, S30, M10, M20 and M30) for days 24-35 was expected taking into consideration the daily yields during this period. The CON and S10 treatment groups performed the poorest compared to the ground beef treatments (M10, M20 and M30) that yielded the most pupae for this period. The soya bean meal treatment groups (S10, S20 and S30) performed intermediate with S10 and S20 being statistically similar to that of the CON treatment and S30 to that of M10. Mealworms in the M20 treatment produced 50.5% more pupae over this period compared to that of the CON treatment group.

It was suggested that the increase in pupation rate over the trial period for the ground beef treatment groups (M10, M20 and M30) could be contributed to fact that the ground beef contains a sterol source. According to the USDA (2016) food composition database ground beef (70% lean)

contains 78 mg/100 g cholesterol. Insects do not have the ability to synthesize sterols (Clarck & Bloch, 1959) and therefore it is a necessity that they obtain it from a dietary source. It was found that cholesterol plays a crucial role in governing larval growth and development (Behmer & Nes, 2003). Lipids are of vital importance to many insects for the production of juvenile hormone which in turn is responsible for embryogenesis and metamorphosis (Gilbert, 1967). Fraenkel *et al.* (1950) found the growth of the mealworm larvae to be exceptionally slow in the absence of cholesterol. Fraenkel *et al.* (1950) further reported that in the absence of cholesterol half the larvae died before reaching the age of 13 weeks and all the larvae were dead by the 20th week.

Table 5.4 Mean (\pm standard error) daily and cumulative pupae yield (g) of mealworms reared for 36 days on of different protein treatments

Treatment	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
CON	7.6 ^c \pm 4.71	5.5 ^b \pm 2.43	10 ^b \pm 2.18	19.7 \pm 2.89	4.7 ^d \pm 1.57	12.7 ^{abc} \pm 2.55	5.2 ^c \pm 2.66	9.0 ^d \pm 3.02
S10	6.5 ^c \pm 4.71	3.7 ^b \pm 2.43	9.7 ^b \pm 2.18	21.3 \pm 2.89	6.2 ^d \pm 1.57	7.3 ^c \pm 2.55	3.8 ^c \pm 2.66	11.0 ^{cd} \pm 3.02
S20	12.3 ^c \pm 4.71	8.7 ^{ab} \pm 2.43	17.7 ^{ab} \pm 2.18	18.8 \pm 2.89	9.7 ^{cd} \pm 1.57	10.7 ^c \pm 2.55	7.7 ^{bc} \pm 2.66	14.3 ^{bcd} \pm 3.02
S30	34.8 ^b \pm 4.71	12.5 ^{ab} \pm 2.43	21.8 ^a \pm 2.18	20.5 \pm 2.89	14.0 ^{bc} \pm 1.57	19.5 ^{ab} \pm 2.55	18.3 ^{ab} \pm 2.66	24.7 ^{abc} \pm 3.02
M10	49.7 ^{ab} \pm 4.71	18 ^a \pm 2.43	15.5 ^a \pm 2.18	15.7 \pm 2.89	19.7 ^{ab} \pm 1.57	20.8 ^{ab} \pm 2.55	24.0 ^a \pm 2.66	20.2 ^{abcd} \pm 3.02
M20	57.0 ^a \pm 4.71	19.5 ^a \pm 2.43	17.5 ^{ab} \pm 2.18	24.0 \pm 2.89	24.5 ^a \pm 1.57	20.0 ^{ab} \pm 2.55	29.3 ^a \pm 2.66	25.0 ^{ab} \pm 3.02
M30	41.2 ^{ab} \pm 4.71	19.0 ^a \pm 2.43	21.7 ^a \pm 2.18	19.0 \pm 2.89	22.0 ^a \pm 1.57	23.3 ^a \pm 2.55	23.5 ^a \pm 2.66	30.0 ^a \pm 3.02
P Value	0.0001	0.0001	0.0007	0.5900	0.0001	0.0004	0.0001	0.0001

Treatment	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 24-35
CON	12.5 ^c \pm 3.93	23.2 ^a \pm 3.32	24.5 ^b \pm 3.27	28.0 \pm 2.93	14.3 ^d \pm 2.43	38.2 ^{ab} \pm 3.98	20.0 ^b \pm 2.85	235.0 ^d \pm 17.03
S10	22.7 ^{cb} \pm 3.93	23.7 ^a \pm 3.32	37.5 ^{ab} \pm 3.27	34.5 \pm 2.93	16.3 ^{dc} \pm 2.43	31.0 ^{ab} \pm 3.98	18.7 ^b \pm 2.85	253.8 ^d \pm 17.03
S20	32.7 ^{ab} \pm 3.93	22.7 ^a \pm 3.32	35.2 ^{ab} \pm 3.27	32.8 \pm 2.93	26.2 ^{abc} \pm 2.43	29.5 ^b \pm 3.98	26.8 ^{ab} \pm 2.85	305.7 ^{cd} \pm 17.03
S30	40.2 ^{ab} \pm 3.93	24.0 ^a \pm 3.32	36.0 ^{ab} \pm 3.27	27.7 \pm 2.93	21.8 ^{abcd} \pm 2.43	41.2 ^{ab} \pm 3.98	26.7 ^{ab} \pm 2.85	383.7 ^{bc} \pm 17.03
M10	36.5 ^{ab} \pm 3.93	23.5 ^a \pm 3.32	30.2 ^{ab} \pm 3.27	26.8 \pm 2.93	20.0 ^{bcd} \pm 2.43	36.0 ^{ab} \pm 3.98	15.0 ^b \pm 2.85	399.6 ^{ab} \pm 18.66
M20	49.0 ^a \pm 3.93	30.5 ^a \pm 3.32	42.2 ^a \pm 3.27	35.7 \pm 2.93	29.3 ^{ab} \pm 2.43	49.0 ^a \pm 3.98	22.2 ^{ab} \pm 2.85	474.7 ^a \pm 17.03
M30	37.3 ^{ab} \pm 3.93	29.5 ^a \pm 3.32	37.8 ^a \pm 3.27	35.5 \pm 2.93	32.5 ^a \pm 2.43	47.8 ^{ab} \pm 3.98	34.7 ^a \pm 2.85	454.8 ^{ab} \pm 17.03
P Value	0.0001	0.4628	0.0151	0.1278	0.0001	0.0065	0.0006	0.0001

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

CON: 100% bran

S10: 10% soybean meal / 90% bran

S20: 20% soybean meal / 80% bran

S30: 30% soybean meal / 70% bran

M10: 10% beef mince / 90% bran

M20: 20% beef mince / 80% bran

M30: 30% beef mince / 70% bran

5.3.3 Pupal weight

The effects that different treatments (CON, S10, S20, S30, M10, M20 and M30) had on pupal weight are presented in Table 5.5. The S10 treatment group had the heaviest pupal weight and S30 the lowest. No clear trend was observed between pupal weight and different treatment groups. No explainable connotation could be made to the inclusion level or protein source and pupal weight. The observed differences may be due to external factors that were not accounted for. Literature suggests that for most arthropods an increased weight and/or size results in increased fecundity (Leather, 1988) due to larger fat body, however this was not found to be the case for the mealworms in this study. Therefore, this topic could be further researched as no clear connotation between pupal weight and pupation rate was observed in this trial.

Table 5.5 Mean (\pm standard error) pupae weight of mealworms reared for 36 days on (g) of different protein treatments

Treatment	Day 24-35
CON	0.1207 ^{ab} \pm 0.00062
S10	0.1225 ^a \pm 0.00061
S20	0.1205 ^{ab} \pm 0.00062
S30	0.1154 ^d \pm 0.00062
M10	0.1187 ^{bc} \pm 0.00061
M20	0.1161 ^{cd} \pm 0.00061
M30	0.1183 ^{bc} \pm 0.00061
P Value	0.0001

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

CON: 100% bran

S10: 10% soybean meal / 90% bran

S20: 20% soybean meal / 80% bran

S30: 30% soybean meal / 70% bran

M10: 10% beef mince / 90 % bran

M20: 20% beef mince / 80 % bran

M30: 30% beef mince / 70% bran

5.4 Conclusion

An inclusion level of 20% ground beef to a bran base was found to yield the heaviest larvae with the fastest pupation rate. Although the inclusion of soya bean meal increased the growth rate compared to the standard control diet used in the industry, it was not on a par with those diets containing ground beef. It is thought that the soy bean Kunitz inhibitor may also affect the growth performance of the mealworm. If this is so, then for soya bean meal to be used as protein feedstuff in the diet of the mealworm it would need to be heat treated for optimal utilization. More research around this theory is required. It was clearly noted that the inclusion of a protein feedstuff in the diets of mealworms improves production. Alternative cheaper forms of protein should be investigated so that the inclusion of a protein source into the diets of mass reared mealworms makes economic sense. It is also suggested that a cheaper plant protein source at inclusion levels of 10, 15 and 20% be investigated with the addition of one percent cholesterol. It was concluded that the ground beef used as animal protein source more closely met the protein requirements of the mealworms compared to that of the soya bean meal used as plant protein source. The success of the animal protein treatment has paved the way for the inclusion of abattoir waste into the diets of mealworms, if legislation around this topic is rewritten. The inclusion of abattoir waste into the diet of mealworms could serve as a sustainable protein source for the mass production of these insects.

5.5 References

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Chapter 6

General conclusion

The primary objective of this study was to determine the nutrient requirements and formulate artificial diets to optimize mass production of the false codling moth (FCM) (*Thaumatotibia leucotreta*), the black soldier fly (BSF) (*Hermetia illucens*) and the yellow mealworm (mealworm) (*Tenebrio molitor*) which have been determined to be of economic importance utilizing acceptable animal nutrition principles. The following parameter measurements were used as response criteria: live weight gain, survivability and pupation rate. Positive results were obtained for all mentioned species and when meeting certain nutrient requirements, led to an increase in productivity. The results obtained were a step in the right direction to efficiently produce these insects on an industrial level.

The formulation of diets more suitable to the nutritional needs of the FCM led to an approximate 55% increase in production efficiency. Similar findings were observed for the BSF diets. When the nutrient needs of neonatal larvae were met more closely, an approximate 25% increase in survivability was observed. This implies that the nursery phase of the production facility can be optimized with about 25% higher yields expected right through the production chain leading to more final product being marketed. The inclusion of a protein feedstuff into the diets of mealworms led to approximately 50% increase in pupation rate. Therefore, the production time of the mealworms were cut in half and production efficiency doubled. However, more work still needs to be done on the nutritional needs of these species with the potential of even greater margins being gained.

Certain key observations were made concerning the physical properties of the FCM and BSF diets. The larvae of these insects live within their feed and the latter therefore determine their direct environment. Variables such as water holding capacity, density and viscosity of the diets need to be considered when formulating further diets for the mass rearing of the mentioned species as this plays a crucial role in determining the success of the diets. When formulating diets, and taking these factors into consideration, better feed conversion could be expected with nutrients more readily available and more efficient production can be achieved within the mass rearing facilities.

These studies focused on the nutrient requirements of the insects in question but some nutritionally inert components can be deliberately added to their diets as bulking agents to modify texture or as carriers for other nutrients. The fibre content of the newly formulated diets of the BSF larvae was found to be too low. Although fibre is seen as a nutritionally inert component it can be used to carry lipids into the diet matrix and contribute to the desirable texture of diet. This aspect warrants further research.

It was demonstrated that diets do not have to be elaborate and expensive to consistently produce high numbers of larvae. Cheaper alternative raw materials to the conventional base raw materials were successfully used to formulate the diet of the FCM. The costs of the newly formulated diets were significantly reduced by the inclusion of soya bean meal and wheat bran as novel raw materials. The mass production of insects is a sustainable solution to the growing protein crisis and insect pest management and the development of commercial mass production diets should follow the same theme. The inclusion of blood meal into the diets of BSF larvae led to an increase in the survivability of the larvae. Possibly unprocessed blood could be included into their diets as an efficient way of recycling this abattoir by-product. The success of the inclusion of ground beef in the diet of the mealworm has also paved the way for incorporating abattoir waste into their diet as means of recycling protein unfit for consumption whilst producing a valuable food and feed source.

The commercial production of these insects represents a large intensive animal production unit consisting of breeders, hatchery and grow out facilities similar to that observed in intensive poultry and pig production systems. Insects can be seen as “micro livestock” or “six-legged livestock” but the farming of these insects is still in an infant stage. As with conventional livestock farming the nutrition of these animals is the biggest and most expensive factor influencing the success of the production systems. Focus has long been placed on the elimination of certain insect species and only recently has the focus moved to mass producing insects for feed and food and biocontrol. Therefore, a collaboration between entomologist and animal scientists is necessary to design and optimize intensive production units to solidify insects as food and feed and for their use in biocontrol methods.

The mass production of the mentioned species has a huge role to play in ensuring food security and the biocontrol of pest species. With the global population estimated to increase, mostly in developing countries by one third of the total population within the next 14 years, insects as food and feed are a more than plausible solution to address food and feed security. The mass production of insects can address numerous other problems such as waste management, the over exploitation of antibiotics in animal production systems, deforestation and reduce the excessive use of water by agricultural industries.

This investigation has shown that animal nutritionists can play an important role in the design of insect feeds utilizing their knowledge of the principles of animal nutrition. Also, this research has shown that there are numerous questions and new directions that the research foci can follow. However, care should be taken that the research does not focus on using feedstuffs that are presently being used in human and animal diets, but rather should focus on the unique ability of insects to recirculate biological wastes.