The use of Hermetia illucens and Chrysomya chloropyga larvae and pre-pupae meal in ruminant nutrition

by

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DECLARATION

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Date: March 2016

ABSTRACT

Title: The use of *Hermetia illucens* and *Chrysomya chloropyga* larvae and pre-pupae meal in ruminant nutrition

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Protein resources in ruminant diets such as fish meal are often limited in supply and therefore have become more expensive. Insects such as larvae of different fly species are now more frequently studied and considered as an affordable protein source which could be used as an alternative animal sourced protein in different animal species diets. The objective of this study was to determine the nutrient value and obtain the rumen degradable potential of different fly larvae and pre-pupae treatments. The species used in this trial were the *Hermetia illucens*, known as the black soldier fly (BSF), and the *Chrysomya chloropyga*, better known as the copper-bottom blowfly (CC). The pre-pupae of BSF have a blackish exoskeleton made up of chitin and were therefore named as BSF B whereas the larvae were white and therefore named as BSF W. Furthermore, BSF B and BSF W were included as full fat (ff) treatments and defatted (df) treatments. Thus, there were four treatments for the BSF species, but only one full fat treatment for the CC. The five treatments were as follow:

1. BSF B ff – black soldier fly pre-pupae, full fat.
2. BSF W ff – black soldier fly larvae, full fat.
3. CC ff – *Chrysomya chloropyga*, full fat.
4. BSF B df – black soldier fly pre-pupae, defatted.
5. BSF W df – black soldier fly larvae, defatted.

The different meal treatments were analyzed in the Department of Animal Sciences (Stellenbosch University). Analyses showed that larvae and pre-pupae meal are a good potential source of protein, amino acids and calcium. Two trials were done to determine the

effective degradability (D_{eff}) of the different larvae and pre-pupae meals. The trials were done over a 48 hour incubation period with bag removals at 0, 2, 4, 8, 16, 24 and 48 hours. In the first trial the effective degradability and rumen undegradable protein (RUP) fractions were determined in an *in vitro* digestibility trial. The CC ff had the highest D_{eff} (lowest RUP) value of all treatments. The two defatted treatments (BSF B df and BSF W df) had higher D_{eff} values than the full fat treatments of the same species. It was concluded that the fat content of the larvae and pre-pupae meals were negatively correlated with degradability. The second trial was a repetition of the first trial, except that the protein degradability was determined in an *in sacco* digestibility trial. The degradability values in the *in sacco* trial were higher than those obtained in the *in vitro* trial for all five treatments. As was observed in the *in vitro* trial, CC ff had the highest D_{eff} value. The defatted treatments once again showed higher D_{eff} values than the full fat treatments of the same species. A final conclusion was made that the fat content of the fly larvae and pre-pupae meal has a significant effect on the degradability of the meal. It was suggested that further studies have to be conducted in an attempt to defat larvae and pre-pupae meal more effectively and also to put these meals through different processing methods to attempt to increase the RUP value of the meal. Once the above mentioned objectives is met, larvae and pre-pupae meal can be considered in trials to investigate the meal as an alternative protein source and replacement for fish meal in production animal diets.

UITTREKSEL

Titel: Die gebruik van *Hermetia illucens* en *Chrysomya chloropyga* larwe en pre-papiemeel in herkouer voeding.

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Proteïenbronne wat gebruik word in herkouerdiëte, soos vismeel, is dikwels beperk tot beskikbaarheid wat dan bydrae tot die verhoogde kostes van hierdie produk. Al hoe meer navorsing word gedoen op insekte, soos die larwes van verskillende vliegspesies, in die soeke na alternatiewe proteïenbronne. Vlieglarwes word oorweeg as ’n bekostigbare en alternatiewe bron van proteïen vir die gebruik in diëte van verskillende dierespesies. Die doel van hierdie studie was om vas te stel wat die nutriëntwaarde en rumendegradeerbaarhede van larwe -en pre-papiemeel van twee verskillende vliegspesies is. Die twee spesies wat in hierdie navorsing gebruik is, was *Hermetia illucens*, meer algemeen bekend as die venstervlieg (BSF), en *Chrysomya chloropyga*, wat ook bekend staan as die koperbrommer (CC). Die pre-papie van BSF het ’n swart eksoskelet wat uit kitien bestaan en waarna verwys word as die BSF B. Die larwe, aan die anderkant, het ’n wit kleur gehad en geen eksoskelet nie en daar word dus verwys na die BSF W. Verder was daar vir die BSF B en BSF W volvet (ff) en ontvette (df) behandelings, terwyl daar vir CC net ’n volvetbehandeling (ff) was. Die vyf behandeings is as volg benoem:

1. BSF B ff – venstervlieg pre-papie, volvet.
2. BSF W ff – venstervlieg larwe, volvet.
3. CC ff – *Chrysomya chloropyga*, volvet.
4. BSF B df – venstervlieg pre-papie, ontvet.
5. BSF W df – venstervlieg larwe, ontvet.

Die verskillende meelbehandelings is vir chemise samestelling ontleed in die Departement Veekundige Wetenskappe (Universiteit van Stellenbosch). Die analises het getoon dat larwe-

en pre-papiemeel 'n goeie potensiële bron van proteïen, aminosure en kalsium is. Twee proewe is gedoen om die effektiewe degradeerbaarheid (D_{eff}) van die verskillende larwe-en pre-papiebehandelings te bepaal. Elk van die proewe het 'n 48 uur inkubasieperiode ingesluit en sakkies is op die volgende tye verwyder: 0, 2, 4, 8, 16, 24 en 48 ure. In die eerste proef was die D_{eff} en rumen nie-degradeerbare proteïen (RUP) fraksie bereken deur 'n *in vitro* verteringsproef. Die CC ff het die hoogste D_{eff} (laagste RUP) waardes getoon en die twee onvette (df) behandelings (BSF B df en BSF W df) het hoër D_{eff} waardes getoon as die volvet (ff) behandelings van dieselfde spesie. Die gevolgtrekking is gemaak dat die vetinhoud van die larwe- en pre-papiemeel negatief gekorreleerd was met degradeerbaarheid. Die tweede proef was 'n herhaling van die *in vitro* proef maar hierdie keer is die degradeerbaarheid bepaal deur 'n *in sacco* verteringsproef. Vir al vyf behandelings was die degradeerbaarheidswaardes wat in die *in sacco* proef verkry is, hoër as dié wat in die *in vitro* proef verkry is. Weereens het die CC ff die hoogste D_{eff} waarde getoon. Verder het die onvette behandelings, soos in die geval van die *in vitro* proef, hoër D_{eff} waardes getoon as die volvet behandelings vir elke vliegspesie. Die finale gevolgtrekking is gemaak dat die vetinhoud van vlieg- larwemeel en pre-papiemeel 'n groot invloed het op die degradeerbaarheid van die meel. Dit word voorgestel dat verdere navorsing gedoen behoort te word om larwe- en pre-papiemeel meer doeltreffend te ontvet. Verskeie verwerkingsprosesse kan ook ondersoek word met die doel om die RUP waarde van die onderskeie mele te verhoog. Indien suksesvol toegepas, kan vlieglarwe- en pre-papiemeel oorweeg word in verdere studies as alternatiewe of plaasvervangerproteïenbronne vir vismeel.

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LIST OF ABBREVIATIONS

µL	Microliter
a	Rapidly degradable fraction
AOAC	Association of Official Analytical Chemists
B	Black
b	Potentially degradable fraction
BSF	Soldier fly
c	Rate of digestion
Ca	Calcium
CAF	Central Analytical Facility
CF	Crude fat
CO_2	Carbon dioxide
COOH	Acidic carboxyl
CP	Crude protein
DM	Dry matter
DNA	Deoxyribonucleic acid
EE	Ether extract
FM	Fish meal
g	Gramm
GE	Gross energy
GIT	Gastro intestinal tract
HCl	Hydrochloric acid
Kg	Kilogramme
N	Nitrogen
NDF	Neutral detergent fiber
NFE	Nitrogen free extract
NH_3	Ammonia
NPN	Non-protein nitrogen
P	Phosphorus
RDP	Rumen degradable protein
RNA	Ribonucleic acid
RUP	Rumen undegradable protein
SCP	Single-cell protein
t	Time
VFA	Volatile fatty acid
W	White

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CHAPTER 1

General Introduction

Livestock production has always been an important component of agriculture. The production of livestock for the use of a multitude of purposes is essential to the existence of the human population. Meat, milk and eggs are produced by livestock for human consumption, while wool and feathers are some other products obtained through animal production and used in the clothing and décor industries (McDonald *et al*., 2002). As shown in Table 18.7 of McDonald *et* al (2002) there is an escalated future demand for animal sourced products as the human population grows over time. This places a tremendous amount of pressure on improving animal production and productivity.

A number of factors affect the production, productivity and profitability of livestock. These factors include climate, location, and type of animal. Nutrition is an important factor that makes or breaks the productivity and profitability of any livestock production system. The nutrient requirements of an animal must be satisfied in order for the animal to produce at an optimal level. Even though ruminants have specific requirements for a number of nutrients the two main nutrients looked at in ruminant nutrition are dietary energy and protein. This study focused on the nutrition of ruminant animals with specific reference to the use of protein and the composition of protein fractions in the ruminant rations

Dietary proteins are molecules constructed from a number of diverse amino acids (McDonald *et al*., 2002). Due to differences in amino acid composition, the large protein molecules vary in shape, character, size and solubility. Proteins are incorporated into the structural walls and cellular content of animal and plant tissues where they are used for different purposes (Ferreira & Valadares., 2003). In ruminants, dietary protein enters the rumen where it is exposed to micro-organism populations that cleave the protein particles and modify their structure. The dietary proteins are divided into two fractions, commonly known as rumen degradable protein (RDP) and rumen undegradable protein (RUP) (Van der Merwe & Smith., 1991). Protozoal and bacterial organisms in the rumen incorporate the RDP components into their own structures or biomass to finally form microbial protein (MP) that flows through, together with RUP, to the abomasum and small intestines, where it is further broken down to amino acids and absorbed. These absorbed amino acids are then incorporated into tissue and used for the production of milk, fetus growth and muscle (amongst other, meat). Rumen degradable protein is important to supply nitrogenous components to the microbial populations

in the rumen. The microbes use the nitrogen to grow and repopulate and most importantly, become useful to the host animal by serving as a protein source. Microbial protein makes up for a great amount of the required protein demand in ruminant animals (McDonald *et al*., 2002; Van der Merwe & Smith., 1991). However, the supply of MP alone does not satisfy the protein and/or amino acid requirements of an animal in a highly productive state. This is where the importance of RUP or bypass protein becomes a necessity in the nutrition to provide the animal with additional protein to be broken down and absorbed in the lower gut as amino acids. Rumen degradable protein helps an animal to maintain and in some cases increase its production. The feeding of diets that contained optimal amounts of RUP has shown to have a positive effect on colostrum and milk production in lactating ewes, as well as fetus growth in late gestation ewes (Hoon *et al*., 2000). In early lactating dairy cows the supply of RUP has shown positive effects on milk yields (Mccormick *et al*., 1999; Santos *et al*., 1998). As mentioned, the importance of the RUP is to supply additional amino acids to the small intestine in the high producing animals' requirements (NRC., 2001). Many studies and trials have been conducted to quantify the RDP and RUP portion of different feed ingredients (Orskov & McDonald., 1979).

In vivo digestibility methods are the best way of determining the digestibility of feeds as the feed material is subjected and exposed to the whole digestive tract of the animal (Van der Merwe & Smith., 1991). Nonetheless, this method is expensive and time consuming and additionally requires quite a number of animals as replicates to ensure significant and accurate results (Ferreira & Valadares., 2003). The *in vitro* digestibility method, which means an in-glass simulation of rumen degradability of feed particles, is an alternative method to determine digestibility of feeds (Khan *et al*., 2003). The *in vitro* method offers quick and fairly precise predictions of *in vivo* degradability. It is a simple way of determining digestibility but is also subjected to several variables that may influence the outcome of results (Khan *et al*., 2003). The *in sacco* digestibility method is the preferred method for estimating the rumen degradable portion of feed resources (Alexandrov, 1998). Animals with surgically inserted cannulae are used to conduct such trials. Although *in vitro* methods are easy and convenient (Rossi *et al*., 2003) the *in sacco* method is preferred (Alexandrov, 1998) due to the fact that the treatment particles in the nylon bags are exposed to an actual ruminal environment (Stern *et* al., 1997) with several factors affecting the degradation and digestion of nutrients from different feed sources – unlike with *in vitro* methods.

Conventional feed sources that are rich in proteins are from both animal and plant origin. Protein oilcakes are processed by-products from plant oilseeds such as soybeans, sunflower,

cottonseed and canola. According to Cutrignelli *et al*. (2011) soybean meal is the source of protein most utilized in intensive animal production systems. Nevertheless, not one of these are the perfect source of protein and each product has different short comings. Some contain anti-nutritional factors, others inhibit the uptake of certain amino acids and some are very susceptible to mycotoxins (McDonald *et al*., 2002). The most popular animal-sourced protein is fish meal (FM). Fish meal is an excellent source of RUP, minerals and amino acids such as lysine and methionine. Due to its high RUP content, FM is used in diets of early lactating cattle to promote milk production (Mccormick *et al*., 1999). The problem with FM is that it is unpalatable and therefore its inclusion and use in diets are restricted. Besides palatability problems, FM has become very expensive and often varies in quality (Van der Merwe & Smith., 1991). The high production costs and variable quality of conventional animal protein sources such as FM are two of the main driving forces behind the ongoing search for alternative sources. In this study, a product known as larvae meal, which is the larvae and pre-pupae of different fly species, will be investigated as an alternative source of protein that may be considered to be used in animal nutrition.

The use of dried fly larvae and fly pre-pupae meal was investigated in aquaculture diets by Loyacano Jr. in 1974 (Bondari & Sheppard., 1981). Newton *et al.* (1977) studied the use of dried *H. illucens* (black soldier fly) meal as a supplement in swine diets. Larvae meal has been shown as a successful substitute for FM in aquaculture (Ogunji *et al*., 2008) and poultry diets (Dashefsky *et al*., 1976; Ocio & Vinaras., 1979; Pretorius, 2011). The larvae or pupae meal has been shown d to be a valuable source of essential amino acids and a well-balanced protein source for the use in poultry diets (El Boushy, 1991). In this study, two fly species were used in different trials. The species of interest were the black soldier fly (*Hermetia illucens*) and the copper bottom blow fly (*Chrysomya chloropyga*). No documented information or data could be found on the use of larvae meal in the diets of ruminant animals. The results obtained from the current study will be the first of its kind, therefore more questions could be raised and further investigations would have to be conducted. The objective and goal of this study was to determine the dry matter (DM) and crude protein (CP) degradability values of larvae meal produced from different fly species, as obtained from *in vitro*- and *in sacco* digestibility trials.

A review of compiled literature applicable to this study is presented in Chapter 2. The focus and goal of the literature review was to gather as much information regarding the use of protein in ruminant diets, the factors affecting protein degradation in ruminants and the use of alternative protein resources, such as fly larvae meal in the use of animal diets. In Chapter 3

the treatments were analysed for nutrients such as dry-matter, crude protein, fat, ash, minerals and amino acids. Thereafter (Chapter 4), an *in vitro* digestibility trial was conducted where the dry matter (DM) and crude protein (CP) disappearance values were determined. The DM and CP disappearance results of the *in sacco* digestibility trial are described in Chapter 5. In Chapter 6, a final conclusion was made using the compiled data and results obtained.

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CHAPTER 2

Literature Review

2.1 Introduction

Ruminant animals are unique in their physiological way of feed digestion and processing in the gastro intestinal tract. Ruminants have four distinct stomach compartments; the reticulum, rumen, omasum and abomasum (Church., 1979). The rumen is a large sac or storage space for ingested feed. Feed particles are degraded by microorganisms, such as bacteria and protozoa, which are hosted in the rumen (Van der Merwe & Smith., 1991). The ruminant animal benefits from the presence of these microbes as they assist in the breakdown and processing of feed material to provide essential nutrients, such as amino acids, required by the host animal for growth and production. The microorganisms incorporate free amino acids and peptides from the animals feed, into their own mass which makes up part of the protein used by the animal. This protein fraction is known as microbial protein (MP) and makes up a great amount of the animal's protein requirements (McDonald *et al*., 2002). The MP passes through the rumen to the small intestine where it is further digested. In animals which need to maintain a high production status, MP is often limited in amino acid supply. Ruminants need additional protein that will be digested in the small intestine to meet requirements. The protein needs to withstand or bypass the rumen's fermentation and degradation process and flow through, together with the MP, to the lower gut (McDonald *et al*., 2002; Van der Merwe & Smith., 1991). Researchers refer to this protein as bypass-protein, rumen undegradable protein (RUP) or undegradable digestible protein (RDP). The RDP makes up a part of the total protein content of a feed source. Feed materials differ in RDP fractions and various factors affect the digestibility, some being dietary factors and animal factors (Church, 1979). Researchers put in great amounts of time and expenses to learn more about these factors and feed sources in order to accurately determine degradation and digestion of feed materials at different sites in the animal's gastro intestinal tract (GIT). There are various methods on how to perform different trials. According to literature the Nylon or Dacron bag method is virtually the best and most convenient way of quantifying protein degradation in the rumen (Van der Merwe & Smith., 1991).

Conventional protein sources known for their high protein and, more importantly, high RDP-values are animal-origin proteins such as fish meal, feather meal, bone and blood meal and by-products of plant oilseed known as oilcakes. However, these resources are becoming more

expensive and some limited in availability. Furthermore, due to ethical reasons and by laws, inclusion of blood and bone meal is restricted and in some cases illegal to feed to livestock in South Africa (Act 36/1947). For the above-mentioned reasons, there is an ongoing quest for alternative resources of protein for use in animal diets. A product brought under consideration, and that has already shown promising results in trials with monogastric animals, is fly larvae meal. The use of dried fly larvae and pupae, also known as maggot meal or magmeal, is not a new trend and has been studied and used in aquaculture diets about a decade or two ago. Newton *et al*. (1977) already studied the use of dried *H. illucens* (black soldier fly) meal as a possible supplement in swine diets. The use of this animal product may not be a new trend, but is receiving attention as more and more research is being conducted in search of additional feed resources and insects being used as feedstuffs. The reason for the search of alternative resources is due to high production costs and variable quality of conventional animal protein sources such as fish meal. Larvae meal has been shown to successfully substitute fish meal in aquaculture (Ogunji *et al*., 2008) and poultry diets (Dashefsky *et al*., 1976; Ocio & Vinaras., 1979; Pretorius, 2011). The larvae or pupae meal is a good source of essential amino acids and a well-balanced protein source for the use in poultry diets (El Boushy. A. R., 1991). In this chapter the production of larvae meal and how it ends up as a feedstuff, appreciated for its protein value, will be discussed. There is still limited literature available on the black soldier fly (BSF) and especially the copper bottom blowfly (*Chrysomya chloropyga*) (CC). With regards to this particular study, there has been no research conducted on the CC, but there is a lot of literature available on the *Musca domestica* (housefly). This chapter will discuss the production of the larvae meal and how it ends up a high protein feedstuff as well as a holistic overview of larvae meal with specific reference to *M. domestica* and *H. illuccens*

2.2 Anatomy and physiology of the rumen

The ruminant animal is unique in the anatomy of its digestion tract and its way of digesting different feed particles. Ruminants have the ability to consume unpalatable herbage, such as straw and hay, as well as fibrous material which contain high amounts of cellulose and hemicellulose. The animal utilizes these materials and turns them into usable energy and protein. Unlike monogastric species, the ruminant animal's stomach is made up of four compartments known as the rumen, reticulum, omasum and abomasum (Church, 1979). Ingested feed passes through the stomach in the above-mentioned order. The rumen and reticulum join to form the reticulo-rumen. The reticulo-rumen assures a continuous culture system for anaerobic microorganisms. Products yielded from these combined organs are volatile fatty acids (VFA's), microbial cells, methane and CO_2 (McDonald *et al*., 2002).

The rumen is the largest stomach compartment and can range from 100 – 300 liters (Van der Merwe & Smith., 1991) in volume in mature cows, which makes up for two thirds of the total digestive tract. The rumen consists out of three sacks, the cranial, ventral and dorsal sac. The sacs are separated from one another by strong muscular bands known as pillars (Church., 1979). Ingested feed will spend about 20-48 hours in the rumen which indicates the importance of the rumen's role in the digestion process. The muscular pillars contract and relax, taking 50 to 60 seconds to complete one synchronized cycle. The contractions are crucial as they help to blend the cud (small ball of chewed feed and saliva) with rumen liquor and microorganisms. Finger-like papillae which outline the rumen wall help to increase the surface area available for the absorption of VFA's and ammonia (NH_3), which are two end products produced by fermentation (McDonald *et al*., 2002).

2.3 Digestion dynamics in the rumen

Ingested food particles enter the rumen in different shapes and sizes. The particles are caught up in the liquid segment. Constituents of the particles that are soluble, for example sugars, dissolve rapidly and are quickly available for rumen degradation by microbes. McDonald *et al*. (2002) explains that the insoluble fractions of feed are colonized by microorganisms and degradation takes place at a slower pace. Other components such as lignin, which is found in cell wall structures, are completely undegradable in the rumen. It is imperative that the degraded material spends sufficient time in the rumen to accommodate successful digestion. Depending on the product of digestion, it is removed from the rumen either by passing through to the lower gut, where it is further metabolized, or it is directly absorbed through the rumen wall (McDonald *et al*., 2002).

Large food particles remain in the rumen. They move to the top of the rumen where the particles are reduced to smaller fragments (McDonald *et al*., 2002). Once reduced to smaller pieces they can move down in the liquid and move through to the lower gut as explained above. According to McDonald *et al*. (2002), the rate at which fluids pass through the rumen is quicker with roughage based diets than with diets consisting of more concentrates. The explanation for this occurrence lies in the saliva production. High roughage diets promote more rumination which means more mixture of digesta with saliva. This process adds more salt to the digesta and in turn increases water intake. The latter process speeds up the fluid-flow through the rumen and might be detrimental in fibrous diets as it decreases the time spent in the rumen for digestion by microorganisms. However, a faster rate of passage is

advantageous in diets containing high protein and starch, which are more effectively digested in the organs of the lower gut (McDonald *et al*., 2002).

2.4 Ruminal fermentation and protein degradation

The main process that takes place in the rumen is the fermentation of regularly ingested feed material. A diverse species of microorganisms are present in the rumen and each utilizes different substrates of feed. Fermentation is achieved with the help of anaerobic microorganisms consisting of bacteria (10^9-10^{10} per ml rumen content), protozoa (10^6 per ml rumen content) and fungi (McDonald *et al*., 2002). The composition of a diet has an enormous effect on the quantity and type of microbial species present in the rumen and affects the proportion of the VFA's produced (Church, 1979). For optimal fermentation, the rumen environment should be ideal to sustain microbial life and growth. Under normal conditions the pH in the rumen varies from 5.5 (McDonald *et al*., 2002) to 7.3 (Church, 1979) depending on the diet. The pH falls more to the acidic side which is ideal for the microbes (Kopecny *et al*., 1982). The more acidic rumen pH (<7.0) is normal, however, it is not ideal for the rumen environment to become acid (below 5.5). When the pH drops too low acidosis in the rumen can follow which has a detrimental effect on animal health, dry matter intake and may even cause death in severe cases (Van der Merwe & Smith., 1991). Acidosis is related to high concentrate based diets or low roughage diets. Different pH values affect different microorganism species and thus also the production of VFA (Erfle *et al*., 1982*)*. According to the temperature in the rumen varies between 39 – 40 °C (Church, 1979) but Van der Merwe & Smith (1991) stated that it is between 38 – 42 °C. These temperatures are ideal to sustain microbial life. As mentioned earlier the microbes are anaerobic organisms and the continuance of an anaerobic environment is essential. A constant supply of feed material should be available for the growth and repopulation of microbes. The products of ruminal fermentation is the production of microbial protein, VFA's and NH_3, which is dietary and endogenous. Excessive NH_3 produced in the rumen is directly absorbed through the rumen wall and metabolized in the liver to urea which is recycled through the body in saliva, or lost through urine which is a loss of nitrogen (N) (Bach *et al*., 2005). Reduced ruminal protein degradation or increased efficiency of N use by microorganisms can help to reduce N losses (Tamminga, 1979).

All living cells contain proteins. Proteins are complex, organic compounds that have a high molecular mass. Protein structures are made up of carbon, oxygen, hydrogen, nitrogen and some are also made up of sulphur in the form of amino acids such as cysteine and methionine

(McDonald *et al*., 2002). When proteins undergo hydrolysis (with the help of enzymes, acids and alkalize) it produces amino acids and peptides. Amino acids contain an amino group (-NH_2) which specifies each amino acid and an acidic carboxyl unit (-COOH) (McDonald *et al*., 2002).

Unlike plants and microorganisms, animals are unable to synthesize the amino group, and therefore, amino acids must be supplied through dietary sources for animals to construct body proteins. Through a process known as transamination, some amino acids can be synthesized from other amino acids. Peptides are formed through the linkage of two or more amino acids. The linkage takes place between the α-amino group of one and the α-carboxyl group of another (McDonald *et al*., 2002).

As mentioned, proteins are present in all living cells and are imperative for maintenance of vital functions, growth, production and reproduction in animals. Protein from dietary origin is classed into rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) with the latter also known as by-pass protein (McDonald *et al*., 2002). The RDP contains true protein nitrogen and non-protein nitrogen (NPN). For microbial growth the amino acids, peptides and NH_3 is used by the microbes (Bach *et al*., 2005). RUP is resistant to rumen fermentation and breakdown and flows through to the abomasum and small intestine where it is degraded to amino acids and peptides available for absorption. The RDP is important for the maintenance of a healthy and sustained microbial population within the rumen. This degradable portion is hydrolyzed in the rumen to peptides and amino acids through the work of microbes. Amino acids are broken down to carbon dioxide, organic acids and ammonia. Figure 2.1 presents protein metabolism in ruminant animals.

Brock *et al*., (1982) stated that the breakdown of protein starts when the microorganisms attach to feed particles and cell-bound microbial protease activity follows. Craig *et al*. (1987) stated that 70-80% of the feed particles should be covered by microorganism to initiate the breakdown of protein. Roughly half of those microbes have proteolytic activity (Prins *et al*., 1983) which affects the extent and rate at which degradation of protein will occur. However, the type of protein also affects degradability and will be discussed later on. The function of bacteria is to degrade and ferment the nutrients, protein being one of these. Amino acids and peptides are products of protein degradation. The amino acids are engulfed by microorganisms and form part of microbial protein (Bach *et al*., 2005). The peptides can be further broken down to amino acids and also used by microbes or converted to VFA, NH_3 and carbon dioxide (CO_2) by the process known as deamination (Tamminga, 1979).

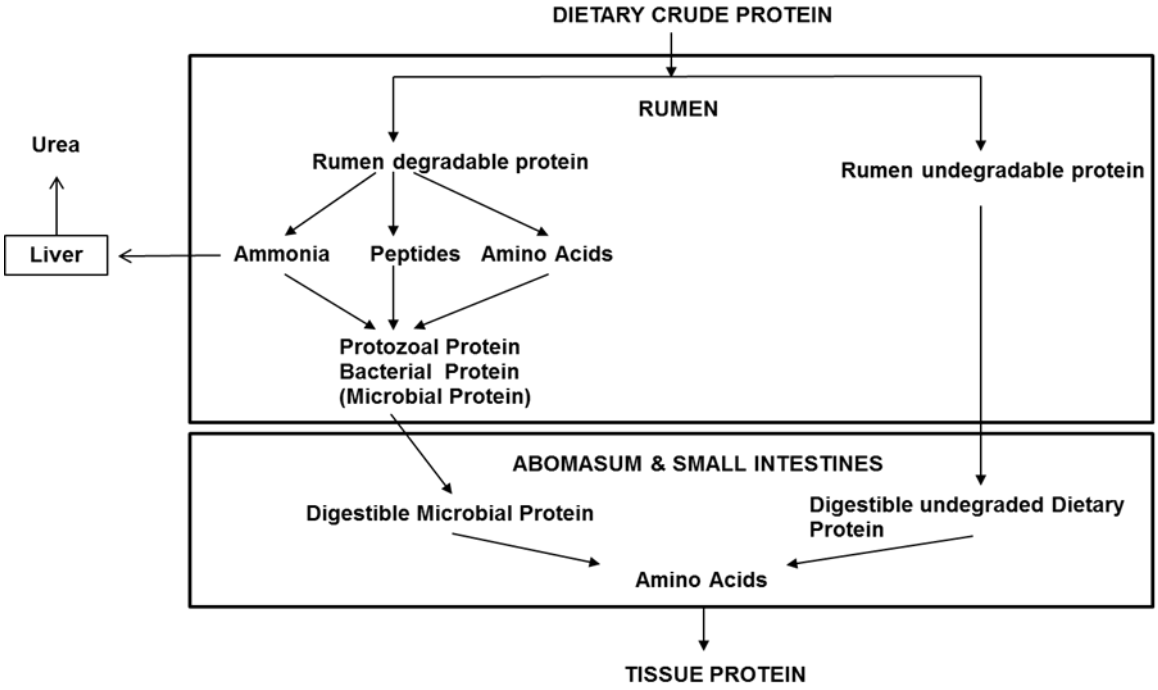

Figure 2.1 Dietary crude protein digestion in the ruminant animal (McDonald *et al*. 2002)

Of all the microbial species in the rumen 30-50% have proteolytic activity (Prins *et al*., 1983). A fraction of the amino acids are further degraded to yield organic acids, NH_3 and CO_2 (Tamminga, 1979). The microbes engulf the amino acids and ammonia which flows through to the other stomachs to be digested in the small intestine as microbial protein. Microbial protein accounts for nearly 80% of the total absorbable protein in the abomasum and small intestine (Storm & Orskov., 1983). For effective production of microbial protein, sufficient amounts of RDP must be available for digestion, as well as enough available energy for microbes for these metabolic processes.

The RUP, also known as “bypass” protein is the portion that resists ruminal degradation and flows through to be digested and absorbed in the abomasum and small intestine, just as the microbial protein (McDonald *et al*., 2002). This part of the protein contributes to production performance, for example an increase in RUP supply in the diet can lead to an increase in milk yield and milk protein and in pregnant animals, a supply of RUP promotes fetal growth. Literature concludes that sources of RUP contributes a great deal to growing or high producing or performing animals (Agbossamey *et al*., 1998; Burke *et al*., 1997). However, RUP feedstuffs are very expensive and availability is sometimes a problem. Nutritionists are continuously searching for and experimenting with alternative resources that might substitute expensive and limited conventional resources.

2.5 Factors affecting ruminal protein degradation

The ruminal degradation of protein is affected by many factors, of which the type of protein is probably the most important. Some other key factors influencing rumen degradation include the interaction of protein with other nutrients in the same feedstuff, which include carbohydrates and microbial protein

2.5.1 Type of protein

The degradability of protein is determined by its solubility which is dependent on the susceptibility to microbial proteases. Several other factors influence the solubility of proteins. Wohlt *et al*. (1976) measured the solubility of casein and soy protein in an autoclaved ruminal fluid and buffer mixture and concluded that solubility can be influenced by factors associated with the solvent. These factors include salt, pH, ionic strength extraction time, degree of agitation and temperature (Wohlt *et al*., 1976).

The structure of the protein has an enormous influence on its susceptibility to microbial proteases. It is one of the most important factors affecting degradability of protein through microbes. Structures containing disulphide bonds (i.e. albumins) are slowly degraded in the rumen (Bach *et al*., 2005), which proves that individual protein degradability is also affected by factors other than solubility (McDonald *et al*., 2002). The bonds in and between the proteins are the key players that determine the degradation of these proteins. Dipeptide bonds in protein structures also affect the degradability (Yang & Russell., 1992). The susceptibility of a protein to microbial proteases is highly influenced by its solubility properties, thus solubility influences degradability. As Romagnolo *et al*., (1994) stated, globulins are highly soluble and highly rumen degradable but glutens and prolamins are the opposite and are consequently degraded at a slower rate.

2.5.2 Rate of passage and ruminal dilution rate

Rate of passage affects microbial synthesis in the rumen. An increase in the rate of passage lowers the maintenance cost requirements of microorganisms due to a limited time spent inter ruminal (Ferreira & Valadares., 2003). According to Orskov (1992), as cited by Ferreira & Valadares (2003), microbial growth will be maximized when the dilution rate is equal to the microbial replication rate, but it is a theoretical point of view. Microbial synthesis is positively related to rate of passage and because rate of passage is a factor of dry matter intake there is also a positive correlation between dry matter intake and microbial synthesis (Verbic, 2002). The passage rate of ingested feed through the rumen is inversely related to protein

degradation (Orskov & McDOnald., 1979). Particle size and density of the particle also play an important role in the rate of passage. In a study by (King & Moore 1957) plastic inert markers were used and it was proven that particles with higher densities and larger size had a lower passage rate thus were more degradable. According the Orskov & McDonald (1979) the rate of passage through the rumen has an inverse effect on protein degradation. The fiber content of a diet also affects rumination. Higher fiber intake will result in more intense and longer periods of rumination. The process will increase the quantity of saliva produced, diluting the rumen contents and increase buffering resulting in an elevated ruminal pH (Valadares *et al* 2003). This all comes down to dry matter intake and the type of ration in which microbial protein production can be increased by insuring an adequate dry matter intake of the animal (Evans, 2003).

2.5.3 Ruminal pH and substrate

The ruminal pH affects ruminal degradation of protein and has an effect on population and species of microorganisms present in the rumen, which in return has its own influence on protein degradation. An interesting observation in the study of Bach *et al.* (2005) was that of a lowered proteolytic activity in the rumen as the pH decreased, but it occurred only in high-forage dairy cattle rations and not in beef-type rations high in concentrates. The ruminal pH, where proteolytic enzyme activity is optimal, falls in the range of 5.5 to 7.0 (Kopecny & Wallace., 1982). Although enzyme activity proceeds well at that pH, the lower pH range does not encourage protein degradation *(Bach et al*., 2005). Cardozo *et al*., (2002) compared rations consisting mostly of forage vs. rations high in concentrates with the pH ranging from 4.9 to 7.0 and demonstrated that for both diets, reduction in protein degradation was observed with decreased pH. Another study showed that when cattle are fed a 100% forage ration the ruminal ammonia concentration reduced when ruminal pH declined from 6.5 to 5.7, but with cattle fed a 90% concentrate the ammonia N concentration was lower regardless of pH (Lana *et al*., 1998). These results imply that the ration type affects the population of microorganisms and that it, together with pH, affects the extent to which proteins are degraded. Erfle *et al*. (1982) concluded through his *in vitro* studies that different microorganisms are affected by pH through the concentrations of VFA's produced in the rumen. Thus, the amount of VFA produced in the rumen can give an indication of the degradation of protein sources. The higher the degradability the more VFA's produced (Erfle *et al*. 1982).

2.5.4 Processing of raw materials

Processing of protein rich feedstuffs are more commonly used in oilseed meals and cakes. Oilseed cakes are the remainder after the oil from the oilseeds are removed. The processing of oilseed cakes begins when the oil is removed. There are two key processes to remove the oil from the seeds. The one involves mechanical applied pressure which forcefully presses out oil and the second process makes use of an organic solvent, hexane. In some cases a combination of the two processes are used (McDonald *et al*., 2002). Processing of feedstuffs is a factor that greatly influences the quality thereof. The more oil extracted from the left over cake the higher the protein content of the end product. Applying heat to the product increases the RDP fraction of the oilcake or meal. In dairy cattle nutrition, a low degradability may be beneficial for protein and amino acid supply to the abomasum and small intestine, therefore production performance. Although, when overheated the quality of the protein drops as it becomes less digestible. The high temperatures of the expellers (cylindrical press) have a tendency to denature protein, resulting in the lower digestibility as mentioned. Substances such as gossypol, which are deleterious, are also controlled or suppressed by the pressures and high temperatures (McDonald *et al*., 2002).

Fish meal goes through a range of processing methods in order to increase RDP and decrease moisture and fat content. The goal is to produce fish meal with a moisture content of 10% and an 8% ether extract. High moisture and fat content promotes oxidation of fats, in which they become rancid, and may even lead to spontaneous combustion of heaped up fish meal (Van der Merwe & Smith., 1991).

2.5.5 Plant and animal protein

Oilcakes are by-products from pressed out oilseeds. Oilcakes are seen as a relatively good “bypass” protein resource. Fish meal of good quality is advantageous when used in diets of young growing animals and high producing animals such as dairy cows fresh in milk or pregnant sows. The amino acid composition of fish meal shows similarity with body tissue protein and therefore the biological value of fish meal is high (Van der Merwe & Smith 1991). Fish meal is rich in amino acids such as lysine, which is the first limiting amino acid, except in poultry, methionine, arginine, glutamic acid and aspartic acid. Fish meal is also a good source of minerals and some vitamins. The essential amino acid profile, compared to lysine, of fish meal compares well with the ideal amino acid profile (Table 2.1) (McDonald et al., 2002).

Table 2.1 The ratio of essential amino acids to lysine in fish meal protein and ideal protein (McDonald et al., 2002)

Amino acid	Fish meal	Ideal protein
Lysine	1.00	1.00
Methionine + cysteine	0.45	0.50
Tryptophan	0.14	0.14
Threonine	0.57	0.60
Luecine	1.01	1.00
Isoluecine	0.62	0.54
Valine	0.73	0.70
Histidine	0.29	0.33
Phenylalanine + tyrosine	1.04	0.96

In ruminants, fish meal is mainly used for its high RDP content. A great part of the protein passes to the lower gut where the amino acids are digested (Van der Merwe & Smith 1991). In one of the studies by Akayezu *et al*. (1997) the substitution of soybean meal with meat and bone meal or fish meal resulted in an increased milk protein content and increased the protein utilization efficiency for milk yield in fresh in lactation cows.

Some feedstuffs are also treated with heat or chemicals to reduce the cleavage or “attack” of the microorganism to the feed particle. The objective is to protect nutrients, such as high quality proteins, from fermentation and digestion in the rumen so that it can be digested post-ruminal (serve more like a by-pass protein). Treatment with a chemical known as formaldehyde is used to adjust protein structures in a manner that prohibits microbial cleavage, however, digestion is still possible through mammalian digestive enzymes (McDonald *et al*., 2002).

2.6 Rapidly degradable fraction (a), potentially degradable fraction (b) and rate of digestion (c) values

With the help of certain methods and trials researchers are able to obtain digestibility results for various feedstuffs and by means of those results able to class raw materials by availability and digestibility of nutrients at different locations in an animal’s gastro-intestinal tract (GIT). Methods used for these trials are *in vivo, in vitro and in sacco* digestibility trials. These methods

will be more thoroughly discussed in chapters 4 and 5. McDonald *et al*. (2002) defines that the digestibility of a raw material is calculated as the difference between the initial N present and the left over N, stated as a proportion of the initial N:

$$\text{Degradability} = [\text{N(initial)} - \text{N(after incubation)}] \div \text{N(initial)}$$

Furthermore, the protein disappearance regressed on time, increases at a dropping rate. The following equation is used to calculate protein disappearance over time:

$$p = a + b(1-e^{-ct})$$

The *p* describes the dry matter disappearance of a nutrient. The *a-value* represents the water-soluble part which is degraded immediately (at zero time). The slower degradable part (*b)* represents the difference between the degradability result from the last removed bag and the *a*-value. The *b*-fraction disappears over time (*t)* at a specific rate (*c)* known as the rate of disappearance (McDonald *et al*., 2002).

2.7 Alternative protein sources

Plant and animal sourced proteins make up quite a big part of a livestock animal's diet to supply sufficient amount of protein and satisfy their amino acid requirements. Protein quality of oilcakes are higher than that of cereals. The oilcakes are generally high in phosphorus which therefore aggravates the lower calcium content thereof. Oilcakes are also generally lower in some essential amino acids including methionine, lysine and cysteine (McDonald *et al*., 2002). However, animal sourced protein like fish meal, blood meal and hydrolyzed feather meal contains high levels of essential amino acids, specifically in methionine, lysine, cysteine and tryptophan. According to McDonald *et al*. (2002) these protein rich sources are usually included in small amounts into an animals' diet just to make up for the shortfall of amino acids and RDP (in ruminant animals) in the diets. They also contain fish meal which has a high mineral content and is a good source of vitamins such as B_{12}, choline and riboflavin. Animal proteins are not very palatable and contribute to limited inclusions in diets (Van der Merwe & Smith., 1991). One of the main reasons fish meal is limited in diets is due to economic reasons. Fish meal is very expensive and sometimes limited in availability.

Previous researchers have conducted trials and experiments in search for alternative protein sources to the conventional sources as these mentioned in the previous paragraph. Some of these alternative sources are already commonly used in animal diets. An example is the use of single-cell proteins (SCP) which are organisms such as bacteria and yeasts (McDonald *et*

al., 2002). These organisms are populated on grains, waste products from food manufactures, plant and wood hydrolysates and unconventional resources like ethanol, alkanes, methanol and organic acids. Table 2.2 shows the chemical composition of some SCP. Inclusion levels up to 15% of SCP in diets for pigs have comparable production levels as those of diets that contained fish meal and soybean meal (McDonald *et al*., 2002).

Table 2.2 Chemical composition (g/kg DM) of single-cell protein grown on different substrates (Schulz & Olsage., (1976), as cited by McDonald et al., (2002))

Substrate	Microorganism	DM	Organic matter	Crude protein	Crude fat	Crude fibre	Ash
Gas oil	*Candida lipolytica*	916	914	678	25	44	86
n-Alkanes	*Picha guillern*	971	941	501	122	76	59
Whey (lactic acids)	*Candida pseudotropicalis*	900	900	640	56	50	100
Methanol	*Pseudomonas methylica*	967	903	819	79	5	97
Molasses	*Saccharomyces cerevisiae*	908	932	515	63	18	68

Other sources of protein are crystalline or pure amino acids which provide specific amino acids to a diet as required by an animal. L-lysine, L-threonine and DL-methionine are the first limiting amino acids often supplemented in diets (McDonald *et al*., 2002). Another source of nitrogen, known as urea, is used in ruminant diets to meet nitrogen requirements of these animals. However, the objective with feeding urea is more to provide nitrogen to the rumen microbes, which they need to digest cellulose, and to improve the microbes ability to hydrolyze dietary protein and in the process incorporate more amino acids into microbial protein (Van der Merwe & Smith, 1991).

In this study research will be done in search of yet another alternative source of protein to be considered in the use of animal nutrition. The product is known as larvae and pre-pupae meal of different fly species. The use of dried fly larvae and pupae meal has previously been studied for its potential use as feed supplement and reported by several authors (Awoniyi et al. 2003; Bondari & Sheppard 1981; Newton et al. 1977). Newton *et al*. (1977) studied the use of dried *H. illucens*, black soldier fly, meal as a supplement in swine diets. More research is conducted on the use of this animal product in search of alternative feed sources and insects used as

feedstuffs. The reason for this search is mainly due to the high production costs, variable quality and scarcity of other conventional animal protein sources such as fish meal (Adeniji, 2007; Awoniyi *et al*., 2003). Larvae meal has been shown to be a successful substituent, with great benefits, for fish meal in aquaculture (Ogunji *et al*., 2008) and poultry diets (Dashefsky *et al*., 1976; Ocio & Vinaras, 1979; Pretorius, 2011). The larvae or pupae meal has attested itself as a valued source of essential amino acids and a well-balanced protein source for the use in poultry diets (El Boushy, 1991; Pretorius. Q., 2011). This chapter will aim to illustrate the net production process of larvae meal and how it ends up as a feedstuff appreciated for its protein value. Limited literature exists on the black soldier fly (*H.illucens*) (BSF) while literature on the copper bottom blow fly (*Chrysomya chloropyga*) (CC) is limited to forensic research. With regards to this particular study, there has been no research conducted on the CC. The most published research on the use of larvae meal for animal feed exists for *M. domestica* (common house fly). This chapter aims to focus on the *H. illucens* and *C. chloropyga* species. The reason for the use of these two particular species was because the species was currently investigated by a company called Agri-protein who was interested in the nutritive value of these species in ruminant diets.

2.8 *Hermetia illucens* (black soldier fly) and *Chrysomya chloropyga* (copper-bottom blowfly)

The *H. illucens*, more commonly known as BSF were first documented in the southern parts of the United States of America (Bondari & Sheppard, 1981). Their current distribution stretch throughout the world and they are to be found in most tropical and subtropical environments (Diener *et al*., 2009; James, 1935; Li *et al*., 2011). BSF is well adapted to a wide range of temperatures (McCallen, 1974). The adult BSF makes use of assorted substances as breeding substrate like rotting fruit and vegetables, soggy grains, decaying organic waste and animal waste (Bondari & Sheppard, 1981).

The life cycle of the BSF consists of five distinct phases (egg, larva, pre-pupae, pupae and adult phase) (Newton *et al*., 2005). The pre-pupae phase, also known as the wondering phase, is a stage at which the mouth part are changed to hooks which will be used for locomotion. They stop feeding and escape the substrate/medium to find an appropriate spot for pupation. It is at this stage when they can be easily harvested / collected. The BSF is not regarded as a pest species (Sheppard *et al*., 1994; Newton *et al*., 2005) since the adult fly do not eat and will therefore not enter buildings in search of food (Sheppard *et al*., 1994). In the adult stage the flies cease to feed, and survives only on the fat reserves stored in their bodies

at earlier larval and pupae stages (Sheppard, 2002). The adult flies mate in flight approximately 2 days after emerging (Sheppard *et al*., 1994) from the pupae with oviposition following two days after that (Tomberlin & Sheppard, 2002). The females lay up to ca. 500 eggs (Sheppard *et al*., 1994) in crevices and cracks near a suitable habitat favorable for larval survival and growth (Newton *et al*., 2004; Furman *et al*., 1959). Li *et al* (2011) stated that the fertilized eggs require 102-105 hours at 24 °C to hatch. Depending on the environmental conditions and food availability, two to four weeks are required for full larval development (Myers *et al*., 2008; Schremmer, 1986). The larvae of BSF are insatiable consumers of various substances. Newton *et al.* (2005) made a statement that manure is a feed source for the larvae of the BSF. Growing through four instars they molt into the fifth instar, they stop eating (Diener *et al*., 2009) because the mouthpart are changed in to hooks (Schremmer. F., 1986) which they use for crawling out of the food an onto a suitable pupation site.

Further benefits associated with the presence and production of BSF is far greater than the common beliefs of the layman or everyday farmer. It is known that the BSF compete for breeding space and habitat with *M. domestica*, the common housefly, and trials conducted by Sheppard, (1983) with BSF resulted in a 94.2% reduction in *M. domestica* numbers. Although BSF's take the place of the house fly they are not a pest species, and do not act as a disease vector. The adult fly does not eat and will therefore not enter houses in search of food (Diener *et al*., 2009; Furman *et al*., 1959). Soldier flies are often found on decomposing materials (Li *et al*., 2011).

As mentioned in previous paragraphs, the larvae of the BSF feed voraciously on mostly all bio waste produced by humans as well as animals. These creatures have the ability to convert waste material into larvae bodies with a high protein and fat content. In the process of bioconversion the mass of waste is reduced, pathogens eliminated (Erickson *et al.,* 2004), reduces excessive nutrients such as nitrogen and phosphorus (Newton *et al*., 2004; Diener *et al*., 2009; Diener *et al*., 2011; Sheppard *et al*., 1994; Sheppard, 1983). Diener *et al.* (2011) claimed a total waste mass reduction of 40% on pig manure and 65-75% on household waste in a trial with BSF. In another study done on cow manure Myers *et al* (2008) obtained results of 43% nitrogen and 67% phosphorus reduction in cow manure. It can be concluded that larvae do in fact hold a great advantage in waste management and reduce pollutions by converting waste of all kinds into a potentially valuable feedstuff.

With reference to *C. chloropyga* (CC), the copper-bottom blowfly, there is very limited research available and none that is applicable to this study. It is known that CC is a carnivorous species

which quickly inhabits carrion and is therefore of forensic importance (Richards *et al*., 2011). Trials done by De Souza Leal *et al* (1982) indicated that larvae grow from a starting weight of 0.5 milligrams to a maximum final weight of 61.5 milligrams in 63 hours which indicated that these larvae are fast growing species. This characteristics is the key to their utilization for the breakdown of abattoir waste.

2.9 Chemical composition of different larvae species

2.9.1 Crude protein content

As mentioned by many researchers in the past 35 years larvae of flies such as the house fly and BSF have the ability to consume and reduce waste products of various kinds and turn them into useful product rich in sought after nutrients, one of which includes protein (Newton *et al*., 2004; Sheppard *et al*., 1994; Li *et al*., 2011). This source of quality protein can be added to animal rations and utilized by farm animals (El Boushy, 1991). Previous studies done on monogastric animals have compared larvae meal as a protein source to other conventional protein sources such as fish meal, soybean meal and groundnut cake. In the study of Awoniyi *et al.* (2003) the protein content of house fly larvae meal was 55.1%. A more recent study done by Pretorius (2011) showed by proximate analysis, that the house fly larvae meal consisted of 60.4% protein. These results are high, but still in range as past studies show protein contents between 39.0% and 70.4% (St-hilaire *et al.,* 2007).

The protein content of BSF is lower than that of the house fly. In a study by St-hilaire *et al*. (2007), protein values of 43.6% for BSF pre-pupae and 70.4% for house fly, were acquired as indicated in the nutrient composition table (Table 2.3). The lower protein content of BSF might be explained by the higher fat content of the BSF larvae and pre-pupae. Different researchers on different research topics found results for protein content of BSF in the range of 31.9% to 47.6% (Diener *et al*., *2009*; Diener *et al*., 2009; Bondari & Sheppard., 1981; Newton *et al*., 1977; Kroeckel *et al*., 2012; St-hilaire *et al*., 2007).

Table 2.3 Nutrient composition of BSF and house fly on dry matter basis (g/kg)

	Black Soldier Fly			House Fly		
Nutrient	Newton *et al*., (1977)	St-hilaire *et al*., (2007)	Kroeckel *et al*., (2012)	Awoniyi *et al*., (2003)	St-hilaire *et al*., (2007)	Pretorius., (2011)-pupae
Moisture, (%)	7.9	8.4	4.4	75	11.9	-
Crude Protein, (g/kg)	421	436	476	551	704	762.3
EE[1]/ Fat (g/kg)	348	331	118	207	161	143.9
Crude Fibre (g/kg)	70	-	-	63	-	157.1
Ash (g/kg)	146	155	159	104	98	77.3
Ca (g/kg)	50	-	65	-	-	5.2
P (g/kg)	15	-	7	-	-	17.2

[1]Ether extract

In terms of fluctuating results of chemical composition within a specific species there are other factors that play a role. Ogunji *et al*. (2008) stated that these differences could be due to different processing and varying methods of drying (Fasakin *et al*., 2003) and storage. The substrate on which the larvae feed also contributes to variation in chemical composition (Newton *et al*., 1977). Other factors such as age at harvest, for instance whether it is harvested at larval phase or at pre-pupae phase, also contributes to the variation in chemical composition (Newton *et al*., 2005; Aniebo & Owen., 2010). According to the proximate analysis results obtained by Pretorius (2011) the housefly pupae meal contained more or less 15% more crude protein (76.2%) than that of the housefly larvae meal (60.4%).

Awoniyi *et al*. (2003) found the larvae meal to be richer in protein when compared to other protein sources such as soybean and groundnut oilcake as illustrated in Table 2.4.

Table 2.4 Proximate analysis (g/100g DM) of the maggot meal compared with other protein sources (Awoniyi *et al*., 2003).

Protein source	Moisture (%)	Crude Protein (%)	Fibre (%)	Fat (%)	Ash (%)
Maggot meal	7.5	55.1	6.3	20.7	10.4
Fish meal	6.3	64.5	1.0	5.3	19.1
Groundnut cake	6.6	45.1	4.1	9.9	7.9

Awoniyi *et al*. (2003) concluded that the use of larva meal as source of protein has the potential to completely substitute fish meal in an iso-nitrogenous basis in the diets of broilers at 4% inclusion with no significant effect on feed consumed, weight gained or efficiency of feed utilization. When considering protein quality, the house fly maggot meal is comparable to that of bone meal and meat meal (Hwangbo *et al*., 2009).

2.9.2 Amino acid profile

Amino acids are a vital component in all living organisms and are building blocks of protein which in turn is the essence of every single organ and its physiological purpose in all living creatures (McDonald *et al*., 2002). The amino acid requirements differ for every animal species and even vary within species due to different physiological stages and needs in specific periods of time (McDonald *et al*., 2002).

According to Spinelli *et al*. (1979), as cited by Ogunji *et al*. (2007), the amino acid composition of larvae meal is equivalent to that of fish meal, including the essential amino acids. El Boushy (1991) stated that pupae or maggot meal is a good source of arginine, lysine and methionine, which are important essential amino acids. Pretorius (2011) also stated that maggot meal could serve as a source of lysine in animal feed mixtures and the pupae meal could also be an arginine source. In Table 2.5 the amino acid profiles of BSF and the house fly are presented. The house fly tends to have higher amino acid contents but this is due to the higher protein content of house fly and lower fat content of BSF. In Table 2.6 the BSF's amino acid profile is put against protein sources consumed by humans. The amino acid content of maggot meal (Table 2.7) is higher compared to that of soybean meal (El Boushy,1991; Hwangbo *et*

al., 2009). When looking at the amino acid profile, maggot meal is suitable as an ingredient for pig diets (Newton *et al*., 1977).

Table 2.5 Amino acid profile of BSF and house fly on a dry matter bases (g/100g.).

	BSF		**House fly**	
	Sealey *et al*., (2011) dairy cow manure	Newton *et al*., (2005) swine manure	St-Hilaire *et al*., (2007) cow manure	Pretorius. Q., (2011)
Methionine	0.77	0.83	1.04	1.37
Lysine	2.05	2.21	3.38	4.92
Leucine	2.66	2.61	3.43	4.14
Isoleucine	1.83	1.51	2.26	2.63
Histidine	0.76	0.96	1.63	0.86
Phenylalanine	1.83	1.49	2.62	3.61
Valine	2.99	2.23	3.26	3.37
l-Arginine	1.78	1.77	3.22	4.5
Threonine	1.58	1.41	2.3	2.31
Tryptophan	-	0.59	-	-
Tyrosine	2.22	2.38	3.22	4.06
Aspartic acid	4.09	3.04	4.55	6.64
Serine	-	1.47	1.88	2.56
Glutamic acid	-	3.99	5.52	9.16
Glycine	1.72	2.07	3.04	3.13
Alanine	2.45	2.55	3.1	3.11
Proline	-	2.12	2.58	-
Cysteine	-	0.31	-	-
Ammonia	-	-	-	-
Glutamine	1.72	-	-	-

Table 2.6 Amino acid profile in different protein resources ([a] (Newton *et al*. 2005);[b] (Uhe *et al*. 1992))

Amino acid	BSF[a]	Chicken[b]	Beef[b]	Fish[b]
Methionine	0.83	1.5	0.8	1.8
Lysine	2.21	4.7	5.4	6.6
Leucine	2.61	5	5.2	5.3
Isoleucine	1.51	2.6	2.9	3
Histidine	0.96	1.1	1.2	1.9
Phenylalanine	1.49	1.8	1.9	2.3
Valine	2.23	2.7	3.2	3.1
l-Arginine	1.77	2.7	3.4	3.9
Threonine	1.41	2.5	3.2	3.4
Tryptophan	0.59	0.3	0.2	0.3
Tyrosine	2.38	1.8	1.7	2.1
Aspartic acid	3.04	-	-	-
Asp + As	-	4.2	5.4	5.9
Serine	1.47	1.7	2.6	2.7
Glu + Gl	-	6.8	8.6	9
Glutamic acid	3.99	-	-	-
Glycine	2.07	2.5	3.2	3.4
Alanine	2.55	3.2	4.5	3.7
Proline	2.12	-	-	-
Cysteine	0.31	-	-	-
Ammonia	-	-	-	-
Glutamine	-	-	-	-

Table 2.7 Amino acid profile of housefly larvae meal and other protein feedstuff (El Boushy. A.R., 1991)

Amino acid	HFLM[1]	Fish meal*	Soya bean meal[2]	Blood meal*
Lysine	6.04	4.55	2.62	5.99
Histidine	3.09	1.36	1.02	3.96
Threonine	2.03	2.6	1.66	3.47
Arginine	5.8	3.99	2.9	3.19
Valine	3.61	3.09	2.06	6.41
Methionine	2.28	1.68	0.52	0.91
Isoleucine	3.06	2.97	2.07	0.9
Leucine	6.35	4.45	3.29	10.1
Phenylalanine	3.96	2.35	2.12	5.47
Tryptophan	-	0.69	0.65	1.02
Cysteine	0.52	0.82	0.74	1.31
Tyrosine	2.91	1.98	1.27	1.73

[1]House fly larvae meal

[2]NRC (1977)

In the study by Wang *et al.* (2013), maggots were produced from swine manure and it was found that 48.5% of the total amino acids came from essential amino acids, these being threonine, valine, leucine, tryptophan and phenylalanine. Furthermore, the maggot meal contained high proportions of histidine, aspartic acid and glutamic acid (Wang *et al*., 2013).

2.9.3 Fatty acid composition

Table 2.8 reveals the results for fatty acid composition of maggot meal from different authors. Pretorius (2011) stated that the essential fatty acid composition of house fly is adequate for the use in broiler diets as broilers require the necessary linoleic acid levels of less than 0.20% of the diets' total amount to attain sufficient growth (Zornig *et al*. 2001, as cited by Pretorius. Q., 2011). The fatty acid contents and composition of the larvae meal are dependent of the fatty acids contained in the diet they feed on. Thus, the final product, the larvae meal can be enriched by feeding waste products high in omega-3 fatty acids. The total lipid content of larvae fed on 50% fish offal and 50% cow manure was 30% compared to a 21% of larvae fed a 100% cow manure diet (St-hilaire *et al*., 2007).

Table 2.8 Fatty acid composition of the housefly larvae and pupae (Pretorius. Q., 2011).

Author	Hwangbo *et al*., 2009	Calvert & Martin, 1969	St-Hilaire *et al*., 2007
Stage of harvest	Larva (age not stated)	Pupae	Pupae
Feed substrate	Milk powder, sugar & layer manure	CSMA**	Cow manure
Fatty acid (%[f])			
Lauric acid	-	-	0.18
Myristic acid	6.83	3.2	2.56
Palmitic acid	26.74	27.6	26.4
Palmitoleic acid	25.92	20.6	13.56
Stearic acid	2.32	2.2	4.77
Oleic acid	21.75	18.3	19.17
Linoleic acid*	16.44	14.9	17.83
Linolenic acid	-	2.1	
α-Linolenic acid	-	-	0.87
Arachidonic acid	-	-	0.07
Eicosapentaenoic acid	-	-	0.05

(*) Essential fatty acids

([f]) % of fatty acids

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

2.9.4 Mineral content

Although minerals are only ingested in small quantities by animals their intake is critical in maintaining physiological functions of an animal. Minerals have an influence on uptake of other nutrients as well as the uptake and availability of other minerals (McDonald *et al*., 2002). They can be antagonistic or synergistic in relation to other nutrients and an oversupply of certain nutrients may even cause toxicity (Van der Merwe & Smith 1991). Although minerals are included as a very small fraction of the total diets of an animal, it is important for sustained health and functionality of an animal. The two minerals predominantly looked at in diets are calcium (Ca) and phosphorus (P), needless to say that all other minerals are equally important.

Kroeckel *et al*. (2012) reported contents of 6.5% Ca and 0.7% P in BSF larvae meal in which the Ca was higher and P lower than results from Newton *et al*. (1977), 5% Ca and 1.5% P. In Table 2.9 the mineral profile of pre-pupae meal fed on poultry and swine manure is tabulated. Variation in results between the authors could be due to age at harvest, methods of processing, change in feed substrates or the adding of premixes (vitamin/mineral) (Pretorius, 2011). Results from the study of Fasakin *et al*. (2003) showed that defatting and hydrolysis of the maggot meal causes elevated levels of manganese (Mn), Ca and magnesium (Mg) (Table 2.10). Fasakin *et al*. (2003) mentioned that the change in mineral levels is due to oil extraction. The volume of the meal decreases but not the content of minerals; therefore it concentrates the meal product which leads to a small increase in every mineral.

Table 2.9 Mineral content of dried BSF pre-pupae meal reared on swine and poultry manure (dry-matter basis) (Newton *et al*., 2005).

Mineral	Swine	Poultry
Ca%	5.36	5.00
P%	0.88	1.51
Mg%	0.44	0.39
K%	1.16	0.69
Mn (ppm)*	348	246
Fe (ppm)	776	1370
B (ppm)	-	0
Zn (ppm)	271	108
Sr (ppm)	-	53
Na (ppm)	1260	1325
Cu (ppm)	26	6
Al (ppm)	-	97
Ba (ppm)	-	33
Ash%	16.6	14.6

ppm - Parts per million

Table 2.10 Mineral composition of processed maggot meal (Fasakin *et al*., 2003).

Types of maggot meal	Ca (%)	Mg (%)	Na (%)	K (%)	Fe (ppm)*	Zn (ppm)	Cu (ppm)	Mn (ppm)	Pb (ppm)
Hydrolysed/oven-dried	0.31	0.25	0.29	0.50	1317.34	48.87	25.71	47.38	0.71
Hydrolysed/sun-dried	0.36	0.25	0.28	0.51	1369.50	49.61	23.32	46.20	0.72
Hydrolysed/defatted oven-dried	0.32	0.25	0.28	0.43	1280.64	46.03	29.50	45.52	0.50
Hydrolysed/defatted sun-dried	0.37	0.24	0.37	0.36	1225.47	48.71	29.10	46.70	0.61
Defatted oven-dried	0.31	0.14	0.34	0.44	1089.46	46.02	22.59	47.09	0.60
Defatted sun-dried	0.38	0.18	0.24	0.35	1079.89	41.17	26.23	44.75	0.62
Full-fat oven-dried	0.36	0.15	0.33	0.46	1140.47	43.31	29.74	46.95	0.93
Full-fat sun-dried	0.36	0.20	0.38	0.51	1231.21	44.29	25.69	48.73	0.93

*ppm - Parts per million

2.10 Factors influencing the chemical composition of larvae and pre-pupae meal

2.10.1 Source of feed substrate

Various studies have been conducted on maggots produced on different feed substrates with the few most common being municipal wastes, poultry manure, swine, cattle manure and cattle blood with bran combination.

Studies have been done on the yields and nutrient composition of larvae feeding of municipal wastes. Ocio & Vinaras (1979) tested waste-grown larvae as a protein source in poultry diets. It was concluded that municipal wastes can in fact be used as a feed substrate for producing larvae and pre-pupae meal for the purpose of using it as protein source in poultry diets (Ocio & Vinaras., 1979).

Abattoir waste includes blood, intestines, intestinal contents, feathers, hooves or feet, rejected carcasses and fat (Roberts & Jager., 2004). The intestines and also the heads of livestock are sold as the 5th quarter or as offal (Christoe & House., 2003). Feathers are used as a protein source in the feed industry but are also used for duvet and pillow fillings in the domestic sector (Dalev, 1994).

Blood meal is a protein rich source produced from blood waste from abattoirs with a good amino acid profile. Unfortunately blood meal has certain health risks and is therefore not allowed for use in animal feed in certain countries worldwide (Act No 36 of 1947 with adjustment to 2006). The feeding of blood and carcass meal to livestock in South Africa is not prohibited or banned, but the use of some meals from abattoir waste are deemed an unacceptable practice (Act No 36 of 1947 with adjustment to 2006). Feeding these abattoir wastes to house fly larvae can assist with nutrient circulation. This can contribute favorably to the animal feed industry and has a positive effect on the environment (Pretorius, 2011).

Another waste product being used as feed substrate is the waste from products which are already produced. These include waste products from citrus farms which are substandard for the markets, products which are past due dates and rotten and also uneaten products from supermarkets and restaurants (Kantor *et al*., 1997). Estimated by Kantor *et al*. (1997) the retail stores in America produce more or less 2.5 billion kilograms of waste per year. Less than five percent of this amount comes from material which is secure for human consumption (Kantor *et al*., 1997). The amount of waste from consumer and food service origin in the USA is estimated at a figure of 42.3 billion

kilograms with 26% coming from edible matter and fresh fruits together with vegetables accounting for 20% (Kantor *et al*., 1997). These foods are more than suitable for consumption by fly larvae.

Fly larvae have the ability to convert chicken manure into a high-quality protein feedstuff filled with valuable amino acids. Due to different bird species, bird age, different feed rations and amount of feathers present in manure, the chemical composition of the manure varies considerably (El Boushy, 1991). According to Flegal *et al*. (1972), as cited by Pretorius (2011), the time spent in storage has an influence on the loss in crude protein content of chicken manure. Storage time from seven to 98 days, it has been found that the crude protein content decreases from 30.3% to 18.3%. The housefly larvae capture these lost nutrients from the chicken manure serves as a feed substrate. This product can be used as a protein source in animal diets especially poultry diets (El Boushy, 1991). Akpodiete *et al.* (1997) compared three different substrates of poultry droppings, one with fresh droppings, a second with droppings mixed together with groundnut oil and another with droppings mixed together with palm oil. From the results by Akpodiete *et al.* (1997) it was concluded that the yields for the fresh droppings were lower ($P < 0.05$) than the yields of the other two substrates, which statistically did not differ. The poultry droppings mixed with palm oil was considered the best substrate (Akpodiete *et al*., 1997).

Preliminary results from producing BSF maggots on swine manure as a feedstuff have been favorable. It suggested that it could be even more efficient on swine manure than on chicken or hen manure (Newton *et al*., 2005).

2.10.2 Full-fat, defatted and hydrolyzed larvae meal

Fat is an important source of energy and is therefore used in animal diets. Fat has an energy density of 2.5 times more than that of carbohydrates such as starch and has a lower heat increment than carbohydrates (Van der Merwe & Smith., 1991). BSF larvae are rich in fat. Previous results indicated fat contents of larvae meal from 11.8% (Kroeckel *et al*., 2012) to 35% (Newton *et al*., 1977). Fat content has an effect on protein content and dilutes the product and thus leads to reduced total protein content. This effect can be seen in Table 2.11. Ideally the fat would be separated from the protein by a defatting process.

Table 2.11 Proximate composition (mean ± standard error)[1] and gross energy values of processed maggot meal and fish meal used in diet formulation (dry matter basis) (Fasakin *et al.,* 2003).

Type of maggot meal	Moisture (%)	Crude protein (%)	Lipid (%)	Ash (%)	NFE[2] (%)	GE[3] (kcal kg^{-1})
Hydrolysed/oven-dried	8.06±0.05	45.60±0.02	13.28±0.03	13.20±0.02	19.86±0.04	5275
Hydrolysed/sun-dried	8.40±0.01	44.30±0.03	13.65±0.04	13.25±0.01	20.40±0.08	5457
Hydrolysed/defatted oven-dried	7.57±0.02	46.70±0.01	6.28±0.01	13.30±0.01	26.16±0.07	4682
Hydrolysed/defatted sun-dried	8.10±0.01	45.65±0.01	6.30±0.01	12.32±0.02	27.63±0.06	4740
Defatted oven-dried	9.20±0.01	45.75±0.03	7.00±0.02	13.35±0.02	24.30±0.04	4569
Defatted sun-dried	9.65±0.04	45.10±0.05	7.40±0.01	13.45±0.02	24.40±0.03	4872
Full-fat oven-dried	8.25±0.02	43.45±0.03	14.30±0.03	14.35±0.02	19.66±0.02	5089
Full-fat sun-dried	8.55±0.04	43.30±0.01	14.35±0.03	14.65±0.01	19.15±0.02	5219

[1]mean standard error(±SE) of triplicate analysis of samples.

[2]Nitrogen-free extract calculated as: 100 - (moisture content + protein + lipid + ash).

[3]Gross energy

Sheppard *et al.* (2007) stated that the crude protein content of larvae meal could be increased to over 60% when protein and fat is separated. Fasakin *et al.* (2003) compared the results in composition of full-fat and defatted maggot meal together with different drying methods. This was done to evaluate and conclude the nutrient density of the maggot meal and to experience the effects of the different processed meals when included in a diet and partially substituted with fish meal. The outcome of the study was that the processing of maggot meal, such as drying and defatting, definitely had an influence on the density of nutrients of the maggot meal (Fasakin *et al*., 2003).

As seen in Table 2.11, the combination of hydrolysis and defatting resulted in the greatest crude protein value (46.7%) which is due to the decreased lipid or fat content. There was no excessive variation in the protein and lipid value of the different drying methods (Fasakin *et al.,* 2003). According to Castell (1986), as cited by Fasakin *et al.* (2003), hydrolysis and defatting processing increases the crude protein and decreases the lipid value which may lead to total or partial destruction of amino acids such as cysteine, tryptophan and methionine. In the study by Fasakin *et al*. (2003) fish performed better overall on the defatted maggot meals than on full-fat maggot meals. Full-fat larvae meal have successfully been used in the diets of poultry and pigs without any negative effects on the performance of breeding and physiological functions. The decrease in the growth performance of fish that have been fed full-fat maggot meal could be due to factors such as digestibility, palatability and the availability and quality of amino acids available for protein synthesis (Fasakin *et al.,* 2003).

2.10.3 Age at harvest and process of drying

Aniebo & Owen (2010) investigated the effect of age at harvest of the maggots as well as the process of drying, on the proximate composition of the maggot meal. The results indicated that with the increase of days at harvest, or increase in age of maggots, the protein content decreased significantly ($P < 0.05$). The fat content of the maggot meal also increased significantly as the time at harvest increased (Aniebo & Owen., 2010). These results are tabulated in Table 2.12.

Table 2.12 Effect of larvae age and method of drying on the proximate composition of housefly larvae meal (Aniebo & Owen., 2010).

Larvae age days	Drying method	Dry matter (%)	Crude protein (%)	Fat (%)	Crude fibre (%)	Ash (%)
2 days	Oven dried	92.7	55.4	20.8	6.2	6.23
	Sun dried	92.8	51.3	23.4	6.3	6.24
3 days	Oven dried	92.7	50.2	22.2	6.7	6.23
	Sun dried	92.9	47.7	26	6.7	6.23
4 days	Oven dried	92.7	47.1	25.3	7	6.25
	Sun dried	92.9	42.3	29.7	7.1	6.26

Results showed that in housefly maggots the content of fat is inversely related to the protein content. Therefore, the closer the maggot is to the pupae phase, the higher the fat content will be and that will result in a lowered protein content (Aniebo & Owen., 2010). Aniebo & Owen (2010) concluded that the best results were collected from the maggots which were harvested at two days, together with the oven dried method.

2.11 Digestibility and degradability of the house fly larvae meal

The literature on the digestibility of larvae meal as a feedstuff is limited, especially for ruminants. Digestibility is the efficiency of an animal to utilize its feed. This is important for maximum productivity of the animal. A recent study was done by Pretorius (2011) to determine the digestibility of larvae meal in broiler chickens. Pretorius (2011) used acid insoluble markers to determine the total tract apparent digestibility's of three diets, one which included the maggot meal. Hwangbo *et al.* (2009) found the total tract digestibility for crude protein and the amino acids to be 98% and 94.8% respectively for broiler chickens. In the later studies of Pretorius (2011) the values were smaller than that of Hwangbo *et al.* (2009). The results from Pretorius (2011) showed a 69% digestibility for crude protein of housefly larvae meal in broiler chickens and 79% for that of housefly pupae meal. The results from both authors indicate a high nutrient availability for maggot meal and that it can be utilized efficiently by broiler chickens. Findings indicate that the total tract digestibility for amino acids

of housefly maggot meal is superior to that of soya oil cake meal in diets for poultry (Hwangbo *et al*., 2009). Digestibility coefficients of apparent amino digestibility in broiler chickens for the dried maggot meal are presented in Table 2.13.

Table 2.13 Apparent amino acid digestibilities of Domestic House fly maggot meal by chickens (Hwangbo *et al*., 2009).

Essential amino acid	Composition (%)	Digestibility Coefficient
Arginine	3.63±0.12	0.948±0.002
Histidine	1.98±0.35	0.956±0.001
Isoleucine	1.46±0.17	0.937±0.003
Leucine	2.90±0.24	0.922±0.001
Lysine	5.22±0.18	0.947±0.002
Methionine	2.34±0.20	0.976±0.006
Phenylalanine	3.57±0.35	0.956±0.003
Threonine	2.27±0.11	0.968±0.002
Tryptophan	3.17±0.16	0.933±0.001
Valine	2.92±0.25	0.939±0.001

2.12 Toxicities, organ stress and diseases

Some feedstuffs have the tendency to cause some unwanted diseases and toxicities when it is not of good quality or not correctly included and applied in diets. Fish meal for instance has the tendency to cause gizzard erosion in chickens and other poultry species. This causes erosion of the gizzard and presence of ulcers on gizzard musculature (Johnson, 1971). Mycotoxins also play a role in gizzard erosion. In studies done by Pretorius (2011) a yellowish colouration of the inner lining of the gizzard was noted when larvae meal was fed. However, this change in colour was not related to erosion and had no effect on health and performance. Pretorius (2011) also conducted tests on organs of which the resulted data showed no negative effects of symptoms. He concluded that larvae meal is therefore a safe product to incorporate in poultry feed. The available information on any other toxic effects of the house fly larvae meal is limited in the literature published. Awoniyi *et al.* (2003) did not mention any risks regarding toxicity.

2.13 Performance potential in monogastric animals

The potential of BSF as an animal feed ingredient has been tested in trials with varieties of animal species and has proven itself as a worthy product. Newton *et al*. (1977) conducted a trial on pigs and he found that BSF meal is suitable for the use in grower diets. The above mentioned author especially valued BSF meal for its amino acid content as well as for Ca and lipid content. The larvae meal did, however, fall short on threonine, methionine and cysteine which all need to be formulated into a balanced diet (Newton *et al*., 1977). In a trial conducted by (Newton *et al*., 2005), BSF pre-pupae meal replaced 50% of dried plasma in a weaner diet fed to early weaned pigs. Without an amino acid supplementation, this diet had a better production performance (+4% gain and +9% feed efficiency) than the control diet.

In another study where BSF larvae, reared on poultry manure, was minced up and fed to *Ictalurus punctatus (*channel catfish), the minced larvae gave similar results for total length and body weight as the control diet. Even though larvae meal was fed to the fish it had no effect on texture and aroma of the fish and was acceptable to consumers (Bondari & Sheppard., 1981). Similar results were found on blue tilapia (Bondari & Sheppard., 1981). However, when larvae meal was compared to fish meal and substituted, 10% of the fish meal in a channel catfish diet, it was found that the growth rates were slower for caged grown channel catfish in a 15 week period (Bondari & Sheppard., 1987). Newton *et al*. (2005) enlightened that pre-pupae meal will be advantageous up until an inclusion level of 7.5% when substituting menhaden fish meal. In terms of average live weight, weekly feed intakes and cumulative feed intake Pretorius (2011) found that the performance of broiler chickens did not differ significantly when they were fed diets containing house fly larvae meal or fish meal. Pretorius (2011) found that with the inclusion of 10% of the house fly larvae meal in a diet, the average live weight, cumulative feed intake and weekly intake, as well as average daily gain were significantly higher when compared to a commercial broiler diet containing soya oil cake as a major protein source. In a study by Hwangbo (2009) it was found that the weight gain of broiler chickens fed on a maggot meal diet was superior compared to the weight gain when fed on a controlled basal diet. There were no significant differences found in the study by Adenji *et al*. (2007) in weight gain, feed conversion ratio, nutrient retention and feed intake of broiler chickens when groundnut cake was replaced with larvae meal at different levels. It was concluded that larvae meal can replace groundnut cake in the diets of broiler chickens (Adeniji. A., 2007). In the current study, it was decided

to investigate the potential of larvae and pre-pupae meal of two fly species to serve as a protein source, which could be a potential replacement for other protein sources in ruminant diets.

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CHAPTER 3

Nutrient composition of *Hermetia illucens* and *Chrysomya chloropyga* maggot meal.

Abstract

Fly larvae and pre-pupae meal is a protein supplement that has shown to be safe and beneficial to use in different animal species' diets. The crude protein (CP) content of the different larvae and pre-pupae meal treatments range between 31.9% and 47.6%. Larvae meal is rich in fats and oils which makes the product a valuable source of energy. Factors that significantly influence the nutritional value of larvae meal is the variability in age at harvest (larvae or pre-pupae) and the substrate (feed) on which the larvae is reared. The fly species used for this trial were the *Hermetia illucens* or black soldier fly (BSF) and the *Chrysomya chloropyga* or copper-bottom blow fly (CC). For the BSF there were four treatments which consisted of larvae full fat (BSF W ff), larvae defatted (BSF W df), pre-pupae full fat (BSF B ff) and pre-pupae defatted (BSF B df) treatments. Pre-pupae of BSF have a blackish colour due to the chitin exoskeleton so these treatments were called the black (B) treatments and the larvae treatments were the white (W) treatments. For the CC, only a full fat larvae (CC ff) treatment was used. The five different treatments were then analysed for moisture, crude protein, ether extract, ash, neutral detergent fibre (NDF), amino acids and minerals. The crude protein (CP) of the BSF and CC treatments ranged from 34.63% (BSF B ff) to 58.58% (CC ff). BSF B df is a good source of first limiting amino acids such as lysine and methionine. It is evident that with high CP and low CF levels larvae and pre-pupae meal is a good source of Ca.

3.1 Introduction

Fly larvae and pre-pupae meal (maggot meal) are protein sources (Newton *et al*., 2004; Li *et al*., 2011) that have been proved to be safe and beneficial to use in different animal species' diets (El Boushy, 1991). As mentioned in Chapter 2 the crude protein (CP) content falls in a range between 31.9% and 47.6% (Kroeckel *et al*., 2012). Factors that affect the nutritional value of larvae meal are the variability in age at harvest (larvae or pre-pupae; Aniebo *et al*.. 2008; Aniebo & Owen., 2010; Newton *et al*., 2004) and the substrate (feed) on which the larvae was reared (Newton *et al*., 1977). Other factors affecting the nutrient composition were the method of drying and hydrolysis or defatting of larvae and pre-pupae meal (Fasakin *et al*., 2003). As mentioned, maggot meal has a high protein content and good ratio of essential to non-essential amino acids which is quite comparable to that of the amino acid profile of fish meal (FM). As with FM, maggot meal is a great source of the amino

acids lysine and methionine (El Boushy, 1991; Pretorius, 2011). In this chapter the larvae and pre-pupae meal of BSF and CC were analysed for dry matter, ash, CP, ether extract/crude fat, NDF, amino acid and mineral composition.

3.2 Materials and Methods

3.2.1 Rearing of the fly larvae and pre-pupae and defatting process

Brood stock flies were kept in captivity under controlled conditions (temperature, humidity and light) in an insect mass rearing pilot plant. Eggs were collected in the morning from the cages. Eggs were weighed and maintained at constant humidity and temperature for three days. On the fourth day the eggs were placed in a hatchery above feed substrate. At four days of age they were transferred to larger bunkers where they were maintained until harvest at 19 days of age (larvae) or allowed to crawl off (pre-pupae). The harvested larvae or pre-pupae were frozen at -20 ˚C. The larvae or pre-pupae were then dried on sieves in industrial drying ovens at 65 ˚C for 48 hours. The dried product was frozen at -20 ˚C till further used or analysis. For this specific study, a volume of product was also defatted with the help of heating and mechanical presses.

3.2.2 Treatments

The fly species used for this trial were the black soldier fly (BSF) and the copper-bottom blow fly (CC). For the BSF there were four treatments which consisted of larvae full fat, larvae defatted, pre-pupae full fat and pre-pupae defatted treatments. The defatting process was done with a self-constructed grinding machine. Larvae meal was heated and grind into a meal form in attempt to remove some of the fat and oils from the meal. However, as seen in Table 3.1 the process of defatting was not very effective as the defatted treatments still had relatively high fat contents when compared to fish meal and other animal sources protein concentrates. The exoskeleton, consisting of chitin, of the pre-pupae of BSF are black and therefore these treatments were called the black (B) treatments and the larvae treatments were the white (W) treatments. For the CC only a full fat larvae treatment was used. The CC larvae already had a lower fat content than the BSF defatted treatments and this was the reason for not using a defatted CC treatment as well.

The five treatments were as follows:

1. BSF B ff – black soldier fly pre-pupae, full fat.
2. BSF W ff – black soldier fly larvae, full fat.
3. CC ff – *Chrysomya chloropyga*, full fat.
4. BSF B df – black soldier fly pre-pupae, defatted.
5. BSF W df – black soldier fly larvae, defatted.

3.2.3 Methodology of analysis

No statistical analyses were done on the results of this chapter. Results were only observed and compared according to numerical value. Before the larvae and pre-pupae meals were analysed, they were milled with a FOSS Knifetec™ 1095 mill into a meal form. The five different treatments were analysed at the laboratory of the Animal Sciences Department of Stellenbosch University. The treatments were analysed for the following:

3.2.4 Proximate analysis

3.2.4.1 Moisture

Moisture determination was done according to the official method of AOAC International (2002), method 934.01 (Loss (moisture) and drying at 95 – 100 °C of feeds). A clean, dry porcelain crucible was taken from an oven (100 °C) and placed in a desiccator to cool down for 30 minutes. The crucible was weighed accurately. The scale was tared and two grams of sample were accurately weighed into the crucible. The crucible and sample were placed in the oven (set at 65 °C to prevent denaturation of protein) for 48 hours. After drying, the crucibles were taken out of the oven and placed into a desiccator to cool down for 30 minutes. The moist free samples were weighed. All the weights were recorded for calculation purposes.

Calculations:

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

- A – Crucible weight
- B – Sample weight
- C – Weight of crucible and sample

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

3.2.4.2 Crude protein

Crude protein determination was done according to the combustion method, AOAC International (2002), method 990.03.With the procedure of the Leco Nitrogen and Protein Analyzer, FP-528 (Leco Corporation, St. Joseph, MI, USA). A small plastic crucible was placed on the scale together with a small piece of aluminium foil. The scale was tared and 0.1000 grams of sample placed in the foil piece. The sample-filled foil was removed from the scale and sealed to form a teardrop. The teardrop shaped foil was placed in the carousel of the LECO. From here the computer analysed the sample and returned a nitrogen (N) value on the computer screen. The N value was recorded for further calculations.

Calculations:

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

3.2.4.3 Fat

Fat content determination was done according to the official method of the AOAC International (2000), method 954.02 which makes use of acid hydrolysis. A clean, dry fat beaker from the oven (100 °C) was placed in a desiccator for 30 minutes to cool down, hereafter the beaker was accurately

weighed. Two g of sample were weighed into test tubes and 2 mL of ethanol added to the tubes to moisten the samples. Hereafter, 10 mL of a HCl solution (38%) was added to the tubes. The tubes were boiled in a water bath for 35 minutes. Tubes were removed from the water bath and left to cool off for 30 minutes to room temperature. The boiled samples were thrown into separator funnel. Then, 25 mL of diethyl ether was added to the separator funnel and shaken for one minute. The top see-through liquid part was carefully thrown into the fat beaker. Next, 15 mL of diethyl ether was added to the funnel and shaken for one minute, followed by the addition of 15 mL of petroleum ether. The funnel was shaken again for one minute. The top, separated layer was carefully transferred to the fat beaker. The step of adding the diethyl ether and petroleum ether was repeated once more. Hereafter the fat beaker was placed in the sand bath to heat the sample and to ensure that all the ether evaporates. The beaker was then placed in a desiccator to cool down for 30 minutes and accurately weighed for calculations.

Calculations:

$$\text{Fat (\%)} = \frac{(\text{fat beaker weight} + \text{Sample weight}) - (\text{fat beaker weight})}{\text{sample weight}} \times 100$$

3.2.4.4 Ash

Ash determination was done according to the official method of AOAC International (2002), method 942.05. The sample was first dried according to the method of determining the moisture content as described before. Thereafter, the sample was transferred to an incinerator at 500 °C for six hours. After six hours, the incinerator was switched off and the sample left to cool for at least two hours before transferring it to a desiccator for 30 minutes and accurately weighed. Weights were recorded for further calculations.

Calculations:

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

- A – Weight of clean crucible
- D – Weight of crucible and ash

3.2.4.5 Neutral detergent Fibre (NDF)

The NDF content of the samples was determined with the aid of an ANKOM$^{200/220}$ Fiber Analyzer (Ankom® Technology Corp. Fairport, NY, USA) according to method suggested by the manufacturers. The filter bags were soaked in acetone, air dried and then oven dried at 100 °C for two hours. Samples of 0.5 g were weighed into individual bags and the bags were heat sealed. Sample weights were adjusted to DM according to their moisture content. The sample bags were then placed on the appropriate bag suspender trays in the ANKOM$^{200/220}$ Fiber Analyzer. The machine was filled with a neutral detergent solution until all the bags were covered (1.9 liters). Heat stable α-amalyse and sodium sulphite were added to the solution. Bags were processed for 75 minutes at 100 °C, after which the solution was drained from the analyser. Samples were then rinsed once with hot water and α-amalyse and thereafter twice with hot water only. After removal, the bags were placed on paper towels and lightly pressed to remove excess water. The bags were then soaked in acetone for three minutes and allowed to air dry before transferring them to 100 °C oven to dry for an additional two hours. The NDF content was calculated using the following equation:

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

- W1 = Bag tare weight (g)
- W2 = Sample weight (g)
- W3 = Weight after extraction process (g)
- C1 = Blank bag correction factor
- DM = Dry matter content of sample

3.2.4.6 Amino acids

Each treatment sample was analysed for amino acid composition. The preparation and hydrolysis of the samples were completed at the Department of Animal Sciences (Stellenbosch University). The hydrolysis (method of AOAC, 1997) involved the weighing of 0.1g of sample into a specialized glass tubes. Thereafter, 6 mL of 6 M hydrochloric acid (HCl) and 15% of a phenol solution were added to the samples. Nitrogen was added to the samples under pressure vacuum. The glass tubes were carefully sealed under a blue flame. The samples were then left to hydrolyze at 110 °C for 24 hours. After hydrolysis a sample was taken from each treatment and transferred to Eppendorf tubes and refrigerated. The prepared samples were taken to the Central Analytical Facility (CAF) at the Department of Biochemistry (Stellenbosch University). The Water API Quattro Micro instrument was used and the scope of analysis was for the total amino acid content of the amino acid hydrolysates. The prepared samples were subjected to the Waters AccQ Tag Ultra Derivitization Kit. Preparations involved 10 microliters (µL) of the undiluted sample added to the Waters AccQ Tag Kit constituents and placed in a heating block at 55 °C for 10 minutes. Amino acids were determined by ion-exchange chromatography.

3.2.4.7 Minerals

The mineral analysis was done at the Western Cape Department of Agriculture's Institute for Plant Production at Elsenburg. Mineral content was determined on 0.5 g of dried and finely ground samples. Each sample was incinerated at 460-480 °C for 6 hours and left to cool down. After cooling, 5 mL of 6 M HCl was added. The sample was then placed in an oven for 30 minutes at 50 °C. Subsequently 35 mL of distilled water was added and the solution was filtered into a brown bottle and made up to a final volume of 50 mL with distilled water (ALASA Method 6.1.1, 2007). Elements were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. Element concentrations were calculated using iTEVA Analyst software. Argon gas flow rate was 2-5 mL/min and instrument settings were as follows: camera temp -27 °C, generator temp 24 °C, optics temp 38 °C, RF power 1150 W, pump rate 50 rpm, aux gas flow 0.5 L/minute, nebulizer 0.7 L/minute, coolant gas 12 L/minute and normal purge gas flow. Wavelengths for the elements were as follows: Al 167.079 nm, B 249.773 nm, Ca 317.933 nm, Cu 324.754 nm, Fe 259.940 nm, K 766.490 nm, Mg 285.213 nm, Mn 257.610 nm, Na 589.592

nm, P 177.495 nm and Zn 213.856 nm. After 11 samples, standards with a high, medium and low range were analysed for quality control.

3.3 Results and discussion

Table 3.1 Proximate analyses (dry-matter base) of the *H. illucens* (BSF) and *C. chloropyga* (CC) larvae (W) and pre-pupae (B) meal

Treatment	Moisture	DM	Crude-Protein	Crude Fat	NDF	Ca	P	Ash
	%	%	%	%	%	%	%	%
1 - BSF B ff	6.93	93.07	34.63	40.10	21.85	3.35	0.68	8.75
2 - BSF W ff	5.27	94.73	35.10	39.13	16.85	2.73	0.58	8.03
3 - CC ff	10.09	89.91	58.58	20.99	23.81	0.34	0.79	4.37
4 - BSF B df	3.42	96.58	47.72	27.42	18.77	6.04	0.67	12.97
5 - BSF W df	2.40	97.60	38.05	33.87	16.13	5.54	0.63	13.15

The comparisons in this chapter's discussion and conclusion section are from numerical comparison and was not statistically analyzed. The moisture contents of the fly larvae and pre-pupae meal treatments are presented in Table 3.1. The moisture in BSF from other literature (Newton *et al*., 1977; St-hilaire *et al*., 2007) came out higher (7.9-8.4%) than the results observed in this study. The moisture content of the CC treatment was, by numerical comparison, the highest (10.1%) whereas the moisture content of the defatted treatments were lower than results observed for the full fat treatments.

The CP content of the BSF and CC treatments ranged from 34.6% (BSF B ff) to 58.6% (CC ff). The CP content of the full-fat (ff) treatments were lower than the defatted (df) treatments, which were closer to results observed by Newton *et al*. (1977), which was reported to be 42.1% and the 43.6% reported by St-hilaire *et al*. (2007). If CP and crude fat (CF) are looked at together, it is assumed that there was a negative correlation between CF and CP content. However, this was not statistically proven, fat content and effective degradability was statistically analyzed for correltation. As the CF fraction of the larvae or pre-pupae meal decreased the CP fraction increased. The BSF B ff with the lowest CP value had the lowest CP:CF ratio (0.86:1) compared to CC ff with a CP value of 58.6% and a much higher CP:CF ratio (2.79:1). With a 12.7% units reduction in fat between the BSF B ff (40.1%) and BSF B df (27.4%) treatments the elevated CP difference was 13.1% (from 34.6% (BSF B ff) to 47.7% (BSF B df)).

The amino acid results of the full-fat BSF and CC treatments, as well as the BSF W df treatments, were lower than the results reported by St.-Hilaire *et al*. (2007), Newton *et al*. (1977), Newton *et al*. (2005) and Kroeckel *et al*. (2012). The source of feed and nutritional content thereof may have a possible effect on the nutritional composition of the larvae meal. In a study by Newton *et al*. (2005) a group of larvae were fed on beef manure and another group on swine manure. The amino acid content of larvae fed on beef manure was higher than that fed on swine manure. Thus, it appears that feed material affect the larvae or pre-pupae meals nutritional content. Results in Table 3.2 suggest that the CF content affects the CP content and therefore amino acid content. The amino acid content increased prominently in BSF B as the CF content decreased. Lysine increased from 1.23 g/100g protein in BSF B ff to 2.96 g/100g protein in BSF B df.

Table 3.2 Amino acid composition H. illucens (BSF) and *C. Chloropyga* (CC) larvae (W) and pre-pupae (B) meal (g/100g of CP).

	BSF B ff	**BSF W ff**	**CC ff**	**BSf B df**	**BSF W df**
His	0.67	0.55	1.07	1.50	0.66
Ser	1.05	0.97	1.33	2.06	1.19
Arg	1.08	0.95	1.24	2.29	1.16
Gly	1.95	1.73	2.14	3.58	1.79
Asp	1.91	1.95	2.60	4.56	2.21
Glu	2.68	2.59	3.50	4.89	2.85
Thr	0.89	0.85	1.23	1.90	1.00
Ala	1.61	1.59	1.77	2.87	2.07
Pro	1.50	1.40	1.33	2.65	1.76
Cys	0.04	0.04	0.03	0.10	0.05
Lys	1.23	1.17	1.96	2.96	1.34
Tyr	1.36	1.19	2.31	2.78	1.43
Met	0.35	0.25	0.62	0.80	0.37
Val	1.43	1.31	1.67	2.72	1.55
Ile	0.89	0.93	1.30	2.09	1.13
Leu	1.58	1.40	1.30	3.34	1.83
Phe	0.88	0.77	1.99	1.87	0.93

The mineral content of the treatments are shown below in Table 3.3. It is evident from the results that BSF larvae and pre-pupae meal is a good source of calcium (Ca), especially the treatments with the lower fat contents (BSF B df and BSF W df). The Ca:P ratio ranged from 4.7 – 9.0, with the defatted treatments having the highest Ca:P ratios. The BSF and CC larvae and pre-pupae

meals are also a good source of trace minerals. The iron (Fe) levels in the larvae and pre-pupae meals were high. The results in Table 3.3 suggest that the content of some minerals increased with decreased fat content but for other minerals its content decreased even though the ash content increased. It would've been expected that the absolute amount would increase, however, results showed that ratios between minerals changed as well. It could be that some minerals are bound to the fat component. Unfortunately there is no evidence or results that support the previous statement as definite.

Table 3.3 Mineral composition of *H. illucens* (BSF) and *C. Chloropyga* (CC) larvae (W) and pre-pupae (B) meal.

		BSF B ff	BSF W ff	CC ff	BSF B df	BSF W df
P	%	0.68	0.58	0.79	0.67	0.63
K	%	1.02	0.88	1.08	0.93	1.06
Ca	%	3.35	2.73	0.34	6.04	5.54
Mg	%	0.30	0.25	0.14	0.35	0.27
Na	mg/Kg	1603.00	1405.00	5513.00	926.00	1392.00
Fe	mg/Kg	459.00	1568.00	184.40	3670.00	1181.20
Cu	mg/Kg	13.82	8.89	21.44	12.52	9.80
Zn	mg/Kg	105.16	83.93	177.80	101.70	66.76
Mn	mg/Kg	124.42	115.80	22.38	184.30	119.56
Bo	mg/Kg	2.91	4.22	1.21	6.19	4.65
Al	mg/Kg	128.00	130.00	18.00	81.00	76.00

3.4 Conclusion

It was concluded that larvae and pre-pupae meal from both BSF and CC are high in crude protein which can be considered for further investigation as a protein rich concentrates or feed ingredients in ruminant nutrition. Furthermore, the different larvae and pre-pupae meals are very oily and high in fat content which makes it a good potential source of energy. Further trials should be conducted on different methods to isolate or drain the oil as an exclusive energy source. With the fat and oil drained, the residual meal would then be concentrated in terms of protein. Moreover, the larvae and pre-pupae meals are good sources of amino acids, especially of lysine, arginine and leucine. Additionally, the meal is high in Ca and trace minerals. Finally, it was observed that the defatted treatments had higher crude protein values than the full fat treatments of the same species.

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CHAPTER 4

In vitro digestibility trial on dry matter and crude protein disappearance of fly-larvae and pre-pupae treatments

Abstract

The objective of this study was to determine the effective degradability of larvae and pre-pupae meal from different fly species. Effective degradability of treatments were obtained by determining the in vitro dry-matter (DM) and crude protein (CP) disappearance of the different meals. The fly species used in the trial were the soldier fly (BSF) and the copper bottom blow fly (CC). For the BSF there were four treatments, which consisted of full fat larvae, defatted larvae, full fat pre-pupae and defatted pre-pupae treatments. The pre-pupae of BSF has a blackish colour due to the chitin exoskeleton, and thus these treatments were called the black (B) treatments while the larvae treatments were classified as the white (W) treatments. For the CC only a full fat larvae treatment was used. Four ruminally cannulated Holstein cows were used as rumen liquor donours. The DM and CP disappearance values were determined at the following incubation times 0; 2; 4; 8; 16; 24 and 48 hours. From the disappearance results the degradability parameters were estimated. Treatment 2-BSF B ff had the lowest effective degradability value of 35.08% with treatment 4-CC ff having the highest effective degradability value of 80.19%. It was concluded that fly larvae meal is a good source of crude protein. However, the fat content of the larvae meal were negatively correlated with the effective degradability of the meal which resulted in untrue rumen undegradable protein (RUP) values. Further studies are necessary for a more accurate evaluation of larvae meal as a source of by-pass protein.

4.1 Introduction

Feed raw materials are evaluated on their nutritive value. The digestibility of feeds or feed ingredients is probably the most significant factor that determines the nutritive value thereof (Khan *et al*., 2003; Ferreira & Valadares, 2003). Digestibility is especially important when looking at protein utilization in ruminants. The best possible way to establish the digestibility of a feed is with *in vivo* digestibility methods (Van der Merwe & Smith, 1991). However, this method is time consuming, expensive and requires quite a number of animals to overcome variation between animals and to ensure accurate results (Ferreira & Valadares, 2003). An alternative method to determine degradability is through *in vitro* digestibility methods which refers to an in-glass simulation of rumen degradability of feed particles (Khan *et al*., 2003). The *in vitro* method offers economical, faster and precise predictions of *in vivo* degradability. This method provides accurate predictions but, Aregheore (2000)

recommended that the results obtained from *in vitro* digestibility trials should only be used and treated as estimates and indications. *In vitro* methods are a simple way of determining digestibility but, are also subjected to a number of variables that may influence the outcome of results (Khan *et al*., 2003).

Many *in vitro* studies have been conducted to predict the digestibility of several feed materials, mainly to establish what percentage of the protein is degradable in the rumen and simultaneously discover what amount of the protein is not subjected to rumen degradation. Protein that is not degraded and digested in the rumen (rumen undegradable protein) flows through to the small intestines where it is broken down to amino acids and peptides that can be absorbed and incorporated into tissue protein (McDonald *et al*., 2002). Rumen undegradable protein (RUP) is an important nutrient in young growing animals and high performing or producing animals to provide adequate protein and essential amino acids to maintain high production levels (McDonald *et al*., 2002). Different sources of protein have different RUP values. Many studies have proved that animal-sourced proteins, such as fish meal, contain high RUP values (Van der Merwe & Smith, 1991) but these values are not constant due to the over and under processing of feed (McDonald *et al*., 2002, p. 602). Oilcakes are by-products of plant oilseeds which are rich in protein and contain quite a high amount of RUP. Even though RUP values of oilcakes are high the values thereof are in most cases lower than that of fish meal (Polan *et al*., 1997). Soya-, cotton- and sunflower oilcakes are used in ruminant rations.

The larvae or pupae meal has attested itself as a valued source protein source in monogastric (Newton *et al*., 1977) and aquaculture (St-hilaire *et al*., 2007) nutrition. No trials, thus far, have been conducted to determine the value of larvae meal in ruminant diets.

Four out of the five treatments used in this particular trial are from the Soldier fly species and the other from the Blowfly species. The Soldier fly treatments consist of larvae and pre-pupae meal of which each has a full fat and low fat or defatted treatment – making up the four treatments. Thus, the trial consisted of five different larvae meal treatments.

The objective of this study was to determine the *in vitro* dry matter and crude protein disappearance of larvae and pre-pupae meal of different fly species and from the results determine the effective degradability.

4.2 Materials and Methods

4.2.1 Animals and diets

Four Holstein cows were used in the trial. They were kept on the Welgevallen experimental farm of Stellenbosch University and managed with the rest of the herd. The four cows were fitted with ruminal cannulae by a veterinarian and ethical clearance for the surgical procedure was obtained from the Stellenbosch University (Reference SU-ACUM13-00029).

The cows were in different production phases (two in lactation and two dry) and therefore received different diets. The two lactating cows received Lucerne hay *ad lib* plus 26 kg of a commercial semi-complete feed provided by Afgri animal Feeds, Malmesbury, Western Cape. The dry cows received mostly oat hay as roughage and some kikuyu from pasture in the paddocks. Feed constituents have an influence on microbial population in the rumen which might contribute to variation in the results of the trials.

4.2.2 Treatments

The fly species used in this trial were the Soldier fly (BSF) and the Copper-bottom Blow Fly (CC). For the BSF the four treatments consisted of full fat larvae, defatted larvae, full fat pre-pupae and defatted pre-pupae treatments. The pre-pupae of BSF have a blackish colour due to the chitin exoskeleton, and thus these treatments were called the black (B) treatments with the larvae treatments being classified as white (W) treatments. For the CC, only a full fat larvae treatment was used.

The five treatments were as follows:

1. BSF B ff – black soldier fly pre-pupae, full fat.
2. BSF W ff – black soldier fly larvae, full fat.
3. CC ff – *Chrysomya Chloropyga*, full fat.
4. BSF B df – black soldier fly pre-pupae, defatted.
5. BSF W df – black soldier fly larvae, defatted.

4.2.3 Experimental design

A completely randomized block design was used with cows and runs as blocks. The trial was repeated once (thus two runs) in order to obtain sufficient replicates. Six incubation jars were used in each of the two runs and two jars represented one cow per run counting to be 3 replicates per run. With a total of 12 jars and two jars per replicate, the total replicates or observations were counted to be six. This number of observations was obtained to suit statistical requirements. All five treatment substrates (larvae and pre-pupae meals) were incubated in each jar and for each treatment three bags were inserted per jar (six per two jars) to represent the different incubation periods. The incubation periods were 0, 2, 4, 8, 16, 24 and 48 hours. For 0 hours incubation no bags were incubated, but washed in clean water to obtain the soluble protein values (a-values).

4.2.4 Collection of rumen fluid from Holstein dairy cows

Rumen fluid was required for the *in vitro* digestibility study. The rumen fluid was collected early in the mornings, prior to incubation runs, from the four different cows. The rumen content was mixed by hand inside the rumen to ensure that samples contain enough fluid. The samples of fibrous material extracted from the rumen was placed into two layers of cheesecloth and squeezed so that fluid could be filtered and drained into one liter thermos flasks. The thermos flasks were preheated to 39 °C with water and emptied just prior to collection. The flasks were filled to the brim to exclude air. A fistful of fibrous material was also included into each flask, as the fibrous material contains microbes attached to the fiber particles which are also necessary for digestion of feed material. A volume of 2 L of rumen fluid was taken with each run. The fluid was taken back to the laboratory where the trials were to be conducted. In laboratory the rumen fluid was poured into a heavy duty industrial blender while purged with CO_2 to ensure an anaerobic environment. Furthermore, the rumen fluid was handled and used according to the ANKOM Technology Method 3 for *In vitro* true digestibility using the DAISY^{II} Incubator (ANKOM Technology - 08/05). The above mentioned method will be discussed in the *in vitro* digestibility method section below.

4.2.5 Buffer solution preparation

The ANKOM technology Method 3 was used for the *in vitro* digestibility trial but, the buffer solution described by the latter method was not used and instead, the buffer solution described in Table 4.1 by Goering & Van Soest (1970) was used. This buffer was preferred as it has successfully been

used in similar trials previously done in the Laboratory of Animal Sciences at Stellenbosch University. The reducing agent was only added to the final buffer solution prior to rumen fluid collection.

4.2.6 *In vitro* digestibility

For this *in vitro* digestibility study the ANKOM Technology Corp. (Fairport, NY) Method 3 was used with the use of the DAISY^{II} Incubator. The following materials were required:

- Flask for acetone
- Heat sealer
- Graduated cylinders – one for the buffer solution and one for the rumen inoculum
- Thermos flasks (1 L)
- Cheesecloth
- CO_2 gas and regulator
- Five liter flask

The ANKOM DAISY^{II} Incubator was used for the incubation of treatments in the buffer and rumen inoculum mixture. The incubator stood in a temperature controlled room at 39 °C and so all glassware and apparatus used during preparation for incubation were kept at a constant temperature of 39 °C. The samples were put in filtration bags which allowed microorganisms to pass through to ensure microbial digestion of the feed. The dacron bags were, similar to those used in the *in sacco* digestibility trials. The rumen fluid was collected with two 1 L thermos flasks and was blended with a heavy duty industrial blender to mix the rumen inoculum thoroughly.

Dacron bags were soaked in acetone for four minutes to ensure the removal of the waxy surfactant covering the material and which may inhibit microbial passage through the pores of the bags. The soaked bags were left to air dry for 40 minutes. The bags were each marked with an individual number (e.g. 123) according to the jar, treatment and bag number. The bags were then placed in a 100 °C oven for 24 hours to obtain complete moisture free bags. After drying, the bags were weighed and weights were recorded. The scale was tared and a sample mass of 0.50 g was weighed into each bag. Once more the weight was recorded, the bags sealed using a heat sealer and placed in the jars. Eighteen bags plus one blank bag were placed into each jar, making it a total of nineteen bags per jar.

With completion of bag preparation, the buffer was prepared for incubation. The reducing agent was added to the final buffer solution beforehand and the reduced buffer solution was heated to 39 °C. A volume of 800 mL of buffer solution was added to each of the digestion jars and the jars were purged with CO_2 for 30 seconds and placed into the DAISY^{II} Incubator. The pH of the buffer was taken, which was between 6.9 and 7.0. The agitation and heat switches were turned on and jars were left for 30 to 45 minutes to allow temperature to equilibrate.

While the jars were left to equilibrate, the rumen fluid was collected from the Holstein cows on the Welgevallen Experimental Farm. Rumen fluid was collected from four Holstein dairy cows and drained through two layers of cheesecloth into the preheated thermos flasks. A fistful of fibrous rumen mat was also taken and put into each filled up thermos. The thermoses containing rumen fluid were taken to the laboratory of the Animal Sciences Department where the *in vitro* trials were conducted.

The rumen fluid together with fibrous material was poured into a blender preheated at 39 °C and frequently purged with CO_2 for 30 seconds. The content was blended at high speed for another 30 seconds. The blended contents were filtered through four layers of cheesecloth into a five liter flask that had been preheated at 39 °C. The flask was continuously purged with CO_2 and thereafter rumen fluid was added to each digestion jar, 200 mL of rumen inoculum per jar, and purged with CO_2 gas for 30 seconds. The pH of the inoculum mixture in each jar was recorded before the lids of the jars were tightly screwed on and the jars were placed back into the incubator. Incubation lasted for 48 hours with removal of one bag of each treatment from each jar at 0; 2; 4; 8; 16; 24 and 48hours. As each bag was removed they were rinsed with cold tap water until the water ran clear. Bags were sampled in big plastic bags according to time of removal and stored at -18 °C until analyzed

After completion of the trial, all the bags were dried in a 100 °C oven for 24 hours to obtain moisture free bags. The weights of the bags were recorded. The remaining crude protein content in each bag was determined using the Leco FP-428 Nitrogen and Protein analyser (Leco Corporation, St. Joseph, MI, USA) and the crude protein content was calculated according to the combustion method (Method 990.03, AOAC, 2002). The digestibility or disappearance of dry matter and crude protein could then be calculated with the following equations:

In vitro dry matter digestibility:

$$a = ((b - \left(c \times \frac{d}{e}\right)) / f \times 100)$$

- a = In vitro dry matter digestibility (%)
- b = Dried bag weight (post-incubation) (g)
- c = Original bag tare weight (g)
- d = Blank bag weight (post incubation) (g)
- e = Blank bag tare weight (g)

Disappearance of dry matter or crude protein:

$$\text{Nutrient D} = \left(\frac{a - b}{a}\right) \times 100$$

- Nutrient D = Disappearance of relevant nutrient (%)
- a = Total mass of dry matter before incubation (g)
- b = Total mass of dry matter after incubation (g)

Table 4.1 Buffer solution used for in vitro digestibility trials, described by Goering & Van Soest (1970)

Reagents	Quantity added
Rumen buffer solution	
Deionised water	2.0 L
NH_4HCO_3	8.0 g
$NaHCO_3$	70.0 g
Macro-mineral solution	
Deionised water	2.0 L
NaH_2PO_4 (anhydrous)	11.4 g
KH_2PO_4 (anhydrous)	12.4 g
$MgSO_4 \cdot 7H_2O$	1.17 g
Micro-mineral solution	
Deionised water	100 mL
$CaCl_2 \cdot 2H_2O$	13.2 g
$MnCL_2 \cdot 4H_2O$	10.0 g
$CoCl_2 \cdot 6H_2O$	1.0 g
$FeCl_3 \cdot 6H_2O$	8.0 g
Cysteine sulphide reducing agent	
Deionised water	48 mL
Cysteine hydrochloride	312 mg
1 N NaOH	20 mL
$Na_2S \cdot 9H_2O$	312 mg
Final buffer solution (per liter)	
Deionised water	500 mL
Rumen buffer solution	250 mL
Macro-mineral solution	250 mL
Resazurin (0.2%, w/v)	2 mL
Micro-mineral solution	0.12 mL
Tryptase	1.25 g
Reducing buffer solution	
Final buffer solution	570 mL
Cysteine sulphide reducing agent	30 mL

4.2.7 Chemical analysis

The bags were dried and transferred to a desiccator to cool and weighed back to get the residue weights of the samples. With the initial weights and the residue weights, the dry matter (DM) disappearances (% of the initial sample weights) of the treatments were calculated for each of the bags.

Furthermore, the remaining nitrogen (N) in the samples of each bag was determined using the Leco protein analyser (LECO FP-528, AOAC Official Method 992.15). The result is an N value expressed in percentage. The N value (%) was multiplied by a factor of 6.25 to obtain the crude protein content in the sample. Crude protein disappearance was expressed as a percentage of the CP in the initial sample.

4.2.8 Data analysis

The data was fit to a model described by Orskov & McDonald (1979) with the help of the Solver function in Excel (Windows Office 2013) programme. The following parameters were determined:

$$Y = a + b(1-e^{-ct})$$

- Y = degradation at time t
- a = soluble and rapidly degradable fraction
- b = fraction potentially degraded over time
- c = rate of degradation of fraction b

The effective degradability of the dry matter (DM) crude protein (CP) was calculated with the following formula (Orskov & McDOnald 1979):

$$D(eff) = a + \left(\frac{b \times c}{c + k}\right)$$

- D_{eff} = effective degradability of DM/CP
- a,b and c = degradability parameters (obtain by the model)
- k = passage rate

Rumen undegradable protein (RUP) was then calculated as:

$$RUP = 100 - D(eff)$$

(NRC, 2001)

4.2.9 Statistical analyses

Repeated observations were compared with a Repeated Measures ANOVA for 0, 2, 4, 8, 16, 24 and 48 hours. All variables were tested individually with a one-way ANOVA and tested for homogeneity with Levene's test. Thereafter, a detailed ANOVA was used together with a non-parametric Kruskal-Wallis test for residues (observations per treatment) not normally distributed. Bonferroni test was used to separate the treatments and significance was declared at $P < 0.05$. The Statistica 10 (2011) program was used for these analyses. The correlations between fat content and effective degradability of the treatments were obtained by the Spearman Rank Order Correlations Method.

4.3 Results and discussion

4.3.1 *In vitro* DM disappearance

From the statistical analyses conducted the results showed significant differences between some of the treatments. Table 4.2 presents the non-linear parameters (a, b and c) of the different treatments after 48 hours of incubation.

4.3.1.1 Rapidly degradable fractions (a-values) of the treatments

The highest and lowest a-values were observed for the CC ff (28.5%) and BSF B ff (3.9%) treatments, respectively. The results from the detailed ANOVA proved significant differences between the a-values of the treatments ($P < 0.05$), except for between the BSF B ff and BSF W df treatments. The treatments with a high fat content had a lower disappearance from the bags than the low fat treatments ($P < 0.05$). The explanation for this might be due to the fact that the feed particles of the high fat content treatments were covered with fat and formed clumps. These fat-covered clumps might have prohibited microbial attachment to the feed, making it more challenging to digest the feed. Even though no other literature could be found on rumen degradability studies with larvae and pre-pupae meal to compare to the values of the present study, the results from this study were compared with results from conventional concentrated protein sources. The a-value of

BSF B df (19.2%) was close to the a-value (21.3%) of canola meal presented in an *in situ* study of Maxin *et al* (2013). In the same study of Maxin *et al* (2013) he reported an a-value of 30.1% for soybean meal, which value is close to that of the CC ff (28.5%) treatment.

4.3.1.2 Potentially degradable fractions (b-values) of the treatments

The treatments with the highest and lowest b-values were CC ff (72.7%) and BSF W df (55.4%). The results obtained from the study showed that BSF W df had the lowest DM degradability of all five treatments. The difference between values of BSF B ff and CC ff and BSF B df was significant ($P < 0.05$). The CC ff treatment differs from the species BSF B ff and BSF B df, which both pre-pupae from the Soldier fly species. The BSF B df (69.0%) compared well with the b-value (69.9%) of soybean meal (Maxin *et al*., 2013) whilst the b-values of the other three BSF treatment seemed to be rather similar to that of canola meal (59.4%).

Statistically there were no significant difference between BSF B ff (55.9%), BSF W ff (60.2%) and BSF W df (55.4%) and no significant difference was found between CC ff (72.7%) and BSF B df (69.0%).

4.3.1.3 Rate of disappearance (c-value) of the potentially degradable (b-value) fraction

There was no significant difference between the rate of disappearance of BSF B ff, BSF W ff, CC ff and BSF B df but results illustrated a significant difference ($P < 0.05$) between BSF B ff and BSF W df disappearances at specific incubation periods

For BSF B ff at 16 hours incubation, the disappearance value was 52.84 %. This value did not differ significantly from the BSF B ff value at 48 hours (55.85%) of incubation. It illustrated that at 16 hours most of the DM of the feedstuff was degraded. Similar results were observed for BSF B df between 16 and 48 hour of incubation. Both BSF B ff and BSF B df are treatments of the same species (Soldier fly). The BSF B ff was the full fat treatment and BSF B df the defatted treatment. Both treatments had almost reached their potential DM disappearance at 16 hours of incubation, with BSF B ff (full fat treatment) having a lower rumen degradable value. This proved that fat content of the feedstuff significantly ($P< 0.05$) affected the rumen degradability of that meal.

Table 4.2 Non-linear parameters (a,b and c) for *in vitro* DM disappearance of the different treatments and (including standard *errors).*

Treatment	a-value	±SE	b-value	±SE	c-values	±SE
	0 hrs		48 hrs			
BSF B ff	3.91[a]	±0.001	55.86[b]	±1.644	0.19[c]	±0.009
BSF W ff	16.46	±0.003	60.18[b]	±0.472	0.14[c]	±0.018
CC ff	28.48	±0.003	72.74	±1.402	0.16[c]	±0.107
BSF B df	19.17	±0.030	69.02	±0.772	0.13[c]	±0.007
BSF W df	4.19[a]	±0.003	55.35[b]	±2.446	0.12[c]	±0.012
P-value	0.0036		0.0006		0.3692	

[a,b,c] Treatments with the same superscript did not differ significantly ($P > 0.05$).

Fat content had an effect on the degradability of larvae and pre-pupae meal. The BSF B ff and BSF W ff treatments were the high fat treatments. Although BSF W df was a low fat treatment, its fat content was still relatively high and more in the range of the high fat treatments. The CC ff was one of the high fat treatments but its fat content was more in the range of the low fat treatments. The BSF B ff, BSF W ff and BSF W df treatments had fat contents greater than 30 % and illustrated lower DM degradability than the CC ff and BSF B df treatments which had fat contents below 30 %.

4.3.2 *In vitro* CP disappearance

From the statistical analyses the results showed significant differences between some of the treatments. Table 4.3 presents the values of different treatments at 0 to 48 hour incubation periods and Figure 4.1 represents the CP disappearance of the treatments fitted to a non-linear model.

4.3.2.1 Rapidly degradable fractions (a-values) of the treatments

The highest and lowest a-values were observed for CC ff (27.9%) and BSF B ff (0.0%), respectively. The lowest and the highest values for CP disappearance corresponded with the respective DM disappearance values. The results from the detailed ANOVA proved significant differences between the a-values of the treatments, except for BSF W ff (7.9%) and BSF W df (6.5%) which showed no significant difference for CP disappearance at 0 hours of incubation.

4.3.2.2 Potentially degradable fractions (b-values) of the treatments

The treatments with the highest and lowest values were the CC ff (71.6%) and BSF W ff (46.1%) irrespectively. Although BSF W ff had a slightly lower value (46.1%) than BSF B ff (47.9%) no significant difference was observed between the two treatments. Therefore, according to the results obtained in this study, BSF B ff and BSF W ff were potentially the two treatments with the highest RUP values of the five different larvae and pre-pupae meal treatments.

Although the values between BSF B ff (47.9%) and BSF W df (51.9%) appeared to differ, statistical analyses proved that the difference between these values, and thus the treatments, were not significant. The same was observed for C ff (71.60%) and BSF B df (69%) where no significant differences were obtained ($P < 0.05$).

4.3.2.3 Rate of disappearance (c-value) of potentially degradable (b-value) fraction

There was no significant difference between the rate of disappearance of all five treatments.

4.3.2.4 Disappearance at specific incubation periods

For BSF B ff at 16 hours incubation the disappearance value was 42.6 %. This value did not differ significantly from the BSF B ff value at 48 hours (47.9%) of incubation. It stipulates that at 16 hours most of the rumen degradable CP of the feedstuff was degraded. Both BSF B ff and BSF W ff were treatments of the same species (Soldier fly) but BSF B ff consisted of pre-pupae meal and BSF W ff consisted of larvae meal. The larvae and pre-pupae have physiological and structural dissimilarity and this influences the nutrient composition of the two meals. In this trial both treatments had a fat content that was very similar (40.1% for BSF B ff and 39.1% for BSF W ff). The fat content might be the explanation to the no statistical difference between the CP disappearance at 48 hours incubation of BSF B ff (47.9%) and BSF W ff (46.1%).

Table 4.3 Non-linear parameters (a, b and c) for *in vitro* CP disappearance of the different treatments and effective degradability values (including standard errors).

Treatment	a-value (0 hrs)	±SE	b-value (48 hrs)	±SE	c-value	±SE	Eff. Deg. (k = 5%)	±SE
BSF B ff	0.00	±0.000	47.88[b]	±1.090	0.14[c]	±0.011	35.08[d]	±0.799
BSF W ff	7.90[a]	±1.644	46.12[b]	±1.043	0.10[c]	±0.011	38.59[d,e]	±1.416
CC ff	27.90	±0.393	71.60[c]	±1.338	0.13[c]	±0.016	80.19	±1.179
BSF B df	16.17	±0.251	68.97[c]	±0.592	0.12[c]	±0.006	65.33	±0.422
BSF W df	6.45[a]	±0.360	51.93[b]	±1.618	0.11[c]	±0.012	41.76[e]	±1.070
P-value	0.0000		0.1318		0.1008		0.0262	

[a,b,c,d,e] Treatments with the same superscript did not differ significantly ($P > 0.05$).
Eff. Deg. – Effective degradability

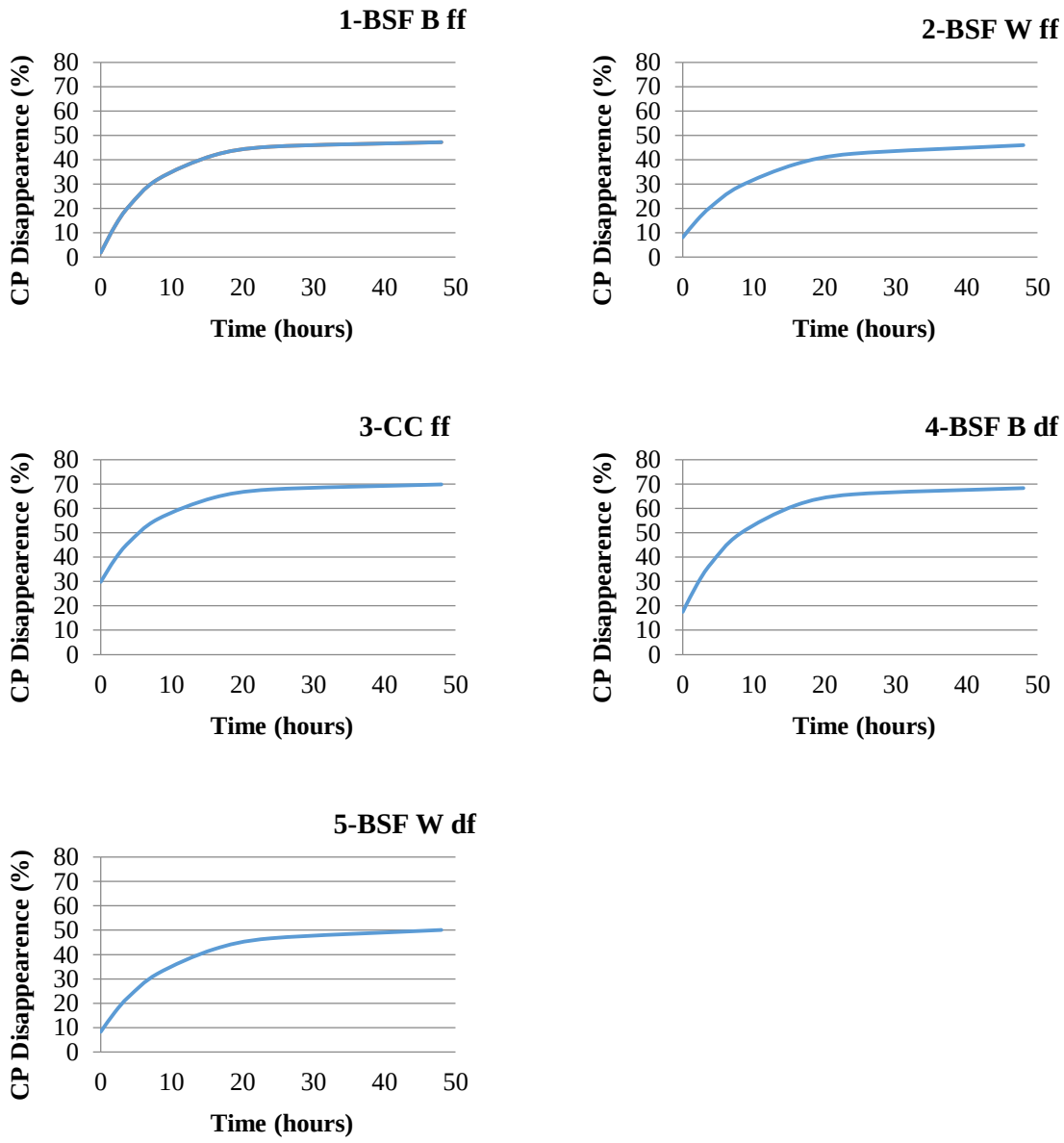

Figure 4.1 Crude protein disappearance (%) over 48 hours of incubation fitted to non-linear model

4.3.2.5 Effective degradability of crude protein (CP)

The effective degradability values for all the treatments can be observed in Table 4.3. The treatments with the lowest and highest effective degradability values for crude protein were BSF B ff (35.1%) and CC ff (80.2%), respectively. There was no significant difference between effective CP degradability of BSF B ff (35.1%) and BSF W ff (38.6%). The BSF B ff and BSF W ff were both from the same species but BSF B ff was harvested at pre-pupae stage and BSF W ff harvested at larvae stage. The pre-pupae have an exoskeleton made up of chitin that, contains nitrogen. Chitin is not degradable and therefore might explain the lower value for effective degradability of BSF B ff – harvested at pre-pupae stage. The larvae do not have the exoskeleton covering and therefore it might have a slightly higher degradability value than that of BSF B ff. No significant difference was found between BSF W ff (38.6%) and BSF W df (41.8%). Both these treatments were from the same species and the slight difference in degradability might be explained by the difference in fat content. Although the BSF W df is the defatted treatment of BSF W ff the difference in fat content to the BSF W ff might not be significant and therefore explains that there is no significant difference between the degradability of the two BSF W treatments. From Figure 4.2 it can be observed that there was a definite negative correlation (Spearman R = -0.939) between fat content and effective degradability of the treatments. It illustrates that the low fat content treatments were more degradable that those with high fat contents.

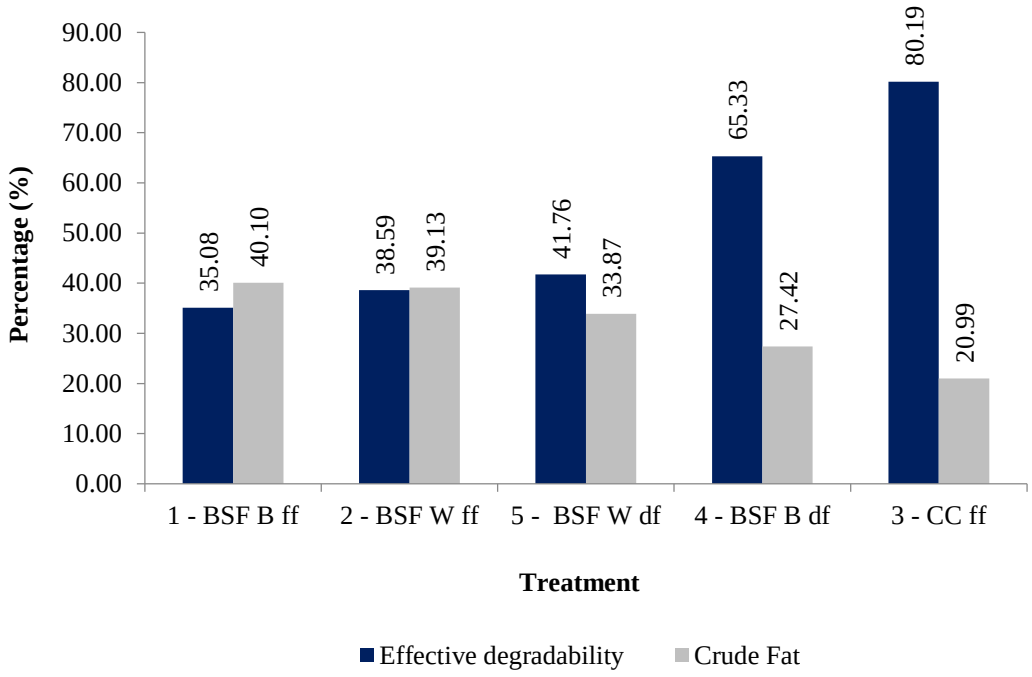

Figure 4.2 Correlation between fat content (%) and effective degradability

4.3.2.6 Rumen undegradable protein (RUP) fraction

The rumen undegradable protein fraction was determined as a percentage value from 100 – RDP (%) as described by the (NRC., 2001) and presented in Table 4.4.

Table 4.4 Effective rumen degradable protein (RDP) and rumen undegradable protein (RUP) values for the different treatments.

Treatment	BSF B ff	BSF W ff	CC ff	BSF B df	BSF W df	*P*-value
Deff[a]/ RDP[b] (%)	35.08	38.59	80.19	65.33	41.76	0.0262
RUP[c] (%)	64.92	61.41	19.81	34.67	58.24	

[a] – Effective degradability (K_p = 5%/ hour)
[b] – Rumen degradable protein
[c] – Rumen undegradable protein (RUP = 100 – RDP)

The treatment with the highest RUP value was BSF B ff with a value of 64.92%. The RUP value of BSF W ff was 61.41% and was lower than that of BSF B ff, however, BSF W ff did not differ significantly from BSF B ff. These results were higher than the RUP results for fish meal (58.1%), meat and bone meal (48.6%) and solvent extracted soybean meal (26.0%) obtained from an *in vitro* digestibility study using the inhibitor *in vitro* method (England *et al*., 1997)The CC ff treatment had the lowest RUP value (19.81%) and differed significantly from the other four treatments. Also, CC ff had the lowest fat content (20.99%) of all the maggot treatments. As mentioned in previous discussions the fat content has a definite influence on the RUP content of the meal. The two defatted treatments, BSF B df and BSF W df, showed lower RUP fraction than the full fat treatments of the same species and stage of growth (larvae or pre-pupae). These results support the fact that fat content of larvae or pre-pupae meal was negatively correlated with CP degradability and suppressed solubility of the larvae meal. Figure 4.3 illustrates the RDP- and RUP fraction as a percentage of the total crude protein of the different treatments and is ranged in a decreasing order of RUP.

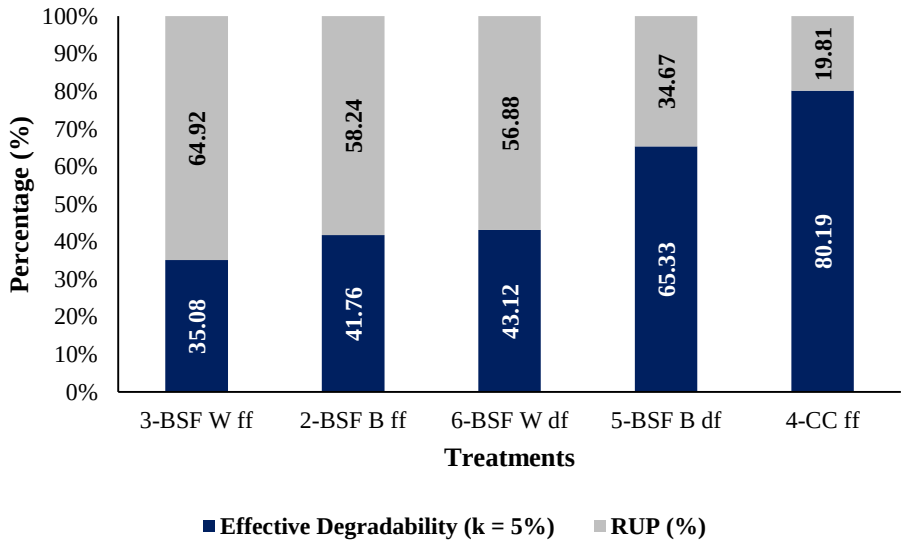

Figure 4.3 Effective rumen degradable protein (RDP) and rumen undegradable protein (RUP) values of the different treatments

4.4 Conclusion

It was concluded that larvae and pre-pupae meal is a good potential source of protein and amino acids. However, it was observed that the fat content of the meals had a definite negative effect on the effective degradability of the meals. The degradability of the meal was negatively correlated with the fat content thereof, which gave a false impression that the meals high in fat had a higher RUP value than that of the defatted meal. What was observed was that the fat formed a layer over meal particles and formed clumps which influenced the solubility of dry matter and crude protein of the meal and possibly gave rise to an apparent and untrue rumen undegradable protein (RUP) value. To get past this issue the larvae and pre-pupae meal should undergo a successful defatting process to achieve results of less than 10% fat. Furthermore, research should be done and trials conducted where larvae and pre-pupae meal undergoes different processing treatments (as with fish meal) to alter the digestibility of the meal. Once this has been successfully achieved larvae and pre-pupae meal can be considered, with more confidence, as a RUP source to compete with animal sourced proteins such as fish meal.

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CHAPTER 5

In sacco digestibility trial on dry matter and crude protein disappearance of fly-larvae and pre-pupae treatments

Abstract

The objective of this study was to determine the in sacco dry matter and crude protein disappearance of five different fly larvae and pre-pupae meal treatments as well as the rumen degradable protein (RDP)- and rumen undegradable protein (RUP) fractions. Four of the five treatments were from the H. illucens or soldier fly (BSF) species and the other one from the C. chloropyga or copper bottom blow fly (CC) species. The BSF treatments consisted of larvae and pre-pupae meals, of which each had a full fat and low fat or defatted treatment. Four ruminally cannulated Holstein cows were used in an *in sacco* digestibility trial. The incubation periods were 0; 2; 4; 8; 16; 24 and 48 hours. Degradability parameters, RDP- and RUP-values were estimated using the disappearance values of dry matter and crude protein. In this trial, BSF W ff had the lowest effective degradability value of 37.9% with CC ff having the highest value of 88.8%. Fat content played a significant ($P < 0.05$) role in the DM and CP disappearance of the larvae meal. It was concluded that fly larvae meal is a good source of crude protein however, further studies are necessary to more accurately evaluate larvae meal as a source of by-pass protein.

5.1 Introduction

Researchers and nutritionists in the animal feed industry are curious and interested in the rumen undegradable protein (RUP) portion of protein sources. Rumen undegradable protein sources are a high cost ingredient used in animal rations (Dunlap *et al*., 2000) and therefore many research projects focus on the search for alternative or less expensive feedstuffs to use in animal rations. Rations with adequate RUP levels are required by animals that are in a physiological state that demands a high nutrient flow, for example in pregnant or lactating ewes (Mikolayunas-Sandrock *et al*., 2009), young growing animals or early lactation dairy cows (Santos *et al*., 1998; McDonald *et al*., 2002). These animals have high energy and protein requirements and RUP assists in meeting the animals' amino acid requirements needed for optimal performance (NRC., 2001).

Fish meal and oilcakes are two major sources of RUP in animal nutrition. The use of these raw materials in diets has shown positive effects on milk production and milk protein in lactating cows of different parity (Burke *et al*., 1997; Allison & Garnsworthy, 2002) as well as increased average daily gains in growing animals (Agbossamey *et al*., 1998).

The purpose of this study was to evaluate fly larvae and pre-pupae meal as a potential source of RUP in ruminant diets. Fly larvae and pupae meal has not yet, to date, been used in digestibility studies in ruminants. It has been successfully used in pig (Newton *et al*., 1977) and poultry (Awoniyi *et al*., 2003; Pretorius, 2011; Adeniji, 2007; Hwangbo *et al*., 2009) trials as a replacement or alternative protein source to fish meal. Four of the five treatments were from the Soldier fly species and the other from the Blowfly species. The Soldier fly treatments consisted of larvae and pre-pupae treatments, of which each had a full fat and low fat or defatted treatment, making up the total of four treatments.

In vivo methods are the preferred option to estimate the digestibility of feeds and *in sacco* trials are an acceptable method for estimating the rumen degradable portion of feed resources (Alexandrov, 1998). Animals with surgically inserted cannulae are needed to conduct such studies. Even though *in vitro* methods can be done in the laboratory making them quick and convenient, the *in sacco* method is preferred due to the fact that the treatment particles in the nylon bags are exposed to an actual ruminal environment with several factors affecting the degradation and digestion of feed. Thus, reasonably accurate estimates can be derived from *in sacco* digestibility trials done with cannulated animals.

The objective of this study was to determine the *in sacco* dry matter and crude protein disappearance values of larvae and pre-pupae meals from different fly species.

5.2 Materials and Methods

5.2.1 Animals and diets

The same four cannulated Holstein cows that were used for the *in vitro* digestibility trial (rumen fluid collection) were used for the *in sacco* digestibility trial. As mentioned in the previous chapter, the cows were kept on the Welgevallen Experimental Farm of Stellenbosch University. The feeding and management of the cows were discussed n the previous chapter

5.2.2 Treatments

The fly species used for this trial were the *Hermetia illucens* (Soldier fly) and the *Chrysomya chloropyga* (copper-bottom blow fly). For the Soldier fly (BSF) there were four treatments which consisted of larvae full fat, larvae defatted, pre-pupae full fat and pre-pupae defatted treatments. The

pre-pupae of BSF have a blackish colour due to the chitin exoskeleton and thus these treatments were titled as the black (B) treatments, with the larvae treatments being titled as the white (W) treatments. For the *C. chloropyga* (CC) only a full fat larvae treatment was used.

The five treatments were as follows:

1. BSF B ff – black soldier fly pre-pupae, full fat.
2. BSF W ff – black soldier fly larvae, full fat.
3. CC ff – *Chrysomya Chloropyga*, full fat.
4. BSF B df – black soldier fly pre-pupae, defatted.
5. BSF W df – black soldier fly larvae, defatted.

5.2.3 Experimental design

A completely randomized block design was used with all five treatments incubated in each cow at the same time. Cows were thus regarded as blocks. Two cows were used for a second time bringing the total amount of replicates to six. For each treatment six bags were inserted to represent the different incubation periods. The incubation periods were 0, 2, 4, 8, 16, 24 and 48 hours. For the zero hours incubation, bags were not incubated in the rumen, but washed in clean water to obtain the soluble protein fraction.

5.2.4 *In sacco* technique

The *in sacco* technique enables the researcher to study ruminal digestion directly in the rumen without the necessity of simulating the rumen environment, as with *in vitro* methods (Vanzant *et al*., 1998). According to Erasmus *et al*. (1988) the best available method is the nylon bag technique.

The Dacron bags of Bar Diamond (Parma, ID, USA) were used in this study. The bags are 100 x 200 mm in size and have a designated mean pore size of 53 µm. Prior to drying the bags, they were marked with a permanent marker pen according to cow number and treatment. Bags were dried in a 60 °C oven for 24 hours. After drying, the bags were weighed and bag weights were recorded. An 8 g sample was accurately weighed into each bag prior to folding the bag and sealing it with a small

cable tie. The total weight of the bag was also recorded as the weight of the cable tie had to be accounted for.

The method described by Cruywagen (2006), using women's opaque stockings as incubation vessels, was used to retrieve the nylon bags from the rumen. This method involves the placement of a large marble in the toe part of the stocking to serve as a weight that pulls the stocking down into the rumen content. Six filled Dacron bags were inserted into each stocking and each was individually sealed off with a cable tie. A total of six stockings were inserted into each cow's rumen. Each stocking was tied to a shorter stocking called the "catcher" that was again tied to the cannula plug for easy retrieval. Four blank bags were included to correct for microbial contamination.

The incubations started at 07h30 on the morning of each run. The incubation period was, as recommended by the NRC (2001), 0; 2; 4; 8; 16; 24 and 48 hours. Blank bags were removed at incubation periods 4, 16, 24 and 48. After each removal of bags the bags were soaked in clean cold water and excess water squeezed out. The bags were placed in plastic zip lock bags and stored at -18 °C until further analyzed.

Dry matter and crude protein degradability values were calculated with the following equation:

Disappearance of dry matter (DM) or crude protein (CP):

$$D = (\frac{a-b}{a}) \times 100$$

- Where D = Disappearance of relevant nutrient (%)
- a = Total mass of dry matter before incubation (g)
- b = Total mass of dry matter after incubation (g)

5.2.5 Chemical analyses

The bags were dried and transferred a desiccator to cool and weighed back to obtain sample residue weights. With the initial weights and the residue weights, the DM disappearance (% of the initial sample weights) of the treatments were calculated for each of the bags.

Furthermore, the residual nitrogen (N) in the sample of each bag was determined using the Leco protein analyser (LECO FP-528, AOAC Official Method 992.15). The N value (%) was multiplied by a factor of 6.25 to obtain the CP content of the sample. The CP disappearance was expressed as a percentage of the CP in the initial sample.

5.2.6 Data analysis

The data was fitted to a model described by Orskov & McDOnald (1979) with the help of the Solver function in the Excel programme. The following parameters were determined:

$$Y = a + b(1-e^{-ct})$$

- Y = degradation at time t
- a = soluble and rapidly degradable fraction
- b = fraction potentially degraded over time
- c = rate of degradation of fraction b
- t = time incubated in hours

The effective degradability of the dry matter (DM) crude protein (CP) was calculated with the following formula:

$$D(eff) = a + \left(\frac{b \times c}{c + k}\right)$$

- D_{eff} = effective degradability of DM/CP
- a,b and c = degradability parameters (obtain by the model of Ørskov and McDonald., 1979)
- k = passage rate (5% per hour) – as recommended by the NRC (2001) page 59.

Rumen undegradable protein (RUP) was then calculated as:

$$RUP=100-D(eff)$$

(NRC., 2001)

5.2.7 Statistical analyses

The non-linear parameters a, b and c were analyzed with a main effects ANOVA with treatment and block as main effect. A Bonferroni test was used to separate the treatments and significance was declared at $P < 0.05$. The Statistica 10 (2011) program was used for these analyses.

5.3 Results and discussion

5.3.1 *In sacco* DM disappearance

From the statistical analyses the results showed significant differences between some of the treatments. Table 5.1 presents the values of the different treatments at 0 and 48 hours of incubation.

5.3.1.1 Rapidly degradable fractions (a-values) of the treatments

The values at 0 hours (a-values) of incubation or a-values can be seen in Table 5.1. These samples were not incubated in the rumen, but were only washed with water as described in the materials and methods section of this chapter.

The BSF B ff had the lowest a-value (3.7%) with CC ff having the highest a-value (19.3%). The results showed that BSF B ff, BSF W ff and BSF W df differed significantly ($P < 0.05$) from CC ff and BSF B df and that there was no significant difference between BSF B ff, BSF W ff and BSF W df.

These last three mentioned treatments had crude fat values higher than 30% which may have contributed to the lower disappearance or lower a-values that had been observed. The treatments which had crude fat values below 30% had much higher 0 h disappearance values than the high fat treatments. The 0 hour values of the five treatments in the present study were markedly lower than the 0 hours results for solvent extracted soybean meal (29.6%) and raw full fat soybean (30.3%) (Canbolat *et al*., 2005).

5.3.1.2 Potentially degradable fractions (b-values) of the treatments

The potentially degradable fraction or b-value is the DM fraction that has disappeared after 48 hours (in this study) of incubation. The BSF W ff treatment had the lowest b-value (71.8%) and CC ff had the highest b-value (84.0%). There were no significant differences between the different meal treatments, except for BSF W ff (71.8%) and CC ff (84.0%). The CC ff (84.0%) and BSF W df (79.5%) seemed to be similar to that of solvent extracted soybean meal (81.5%) and raw full fat soybeans (81.9%) (Canbolat *et al*., 2005). The fact that the maggot treatments had very similar values after 48 hours of incubation, suggests that the effect of fat decreased as time of incubation increased.

5.3.1.3 Rate of disappearance (c-value) of potentially degradable (b-value) fraction

The BSF B ff treatment had a c-value of 0.51% per hour which was similar to the 0.59% per hour of CC ff. The two defatted treatments, (BSF B df and BSF W df) had much higher c-values than the other three treatments. The c-value for BSF W ff (a full fat treatment) was significantly lower than BSF B ff which was also a full fat treatment.

Table 5.1 *In sacco* DM disappearance at zero hours, 48 hours and rate of disappearance of treatments (including standard errors).

Treatment	a-value 0 h	±SE	b-value 48 h	±SE	c-values	±SE
BSF B ff	3.67[a]	±0.523	77.19	±2.061	0.51[c]	±0.116
BSF W ff	5.75[a]	±0.397	71.78[b]	±1.625	0.12	±0.024
CC ff	19.30	±3.257	84.02[b]	±2.754	0.59[c]	±0.067
BSF B df	12.94	±1.172	77.68	±2.660	0.87[d]	±0.069
BSF W df	4.22[a]	±0.273	79.51	±1.659	0.99[d]	±0.094
P-value	0.0000		0.2516		0.2648	

[a,c,d] Treatments with the same superscripts did not differ significantly ($P > 0.05$)

[b] Treatments with the same superscripts differed significantly ($P < 0.05$)

5.3.2 *In sacco* CP disappearance

From the statistical analyses the results showed significant differences between some of the treatments. Table 5.2 presents the values of different treatments at 0 and 48 hours of incubation and Figure 5.1 illustrates the CP disappearances fitted to a non-linear model.

5.3.2.1 Rapidly degradable fractions (a-values) of the treatments

The larvae meal treatment with the highest fat content (BSF B ff = 40.1%) had the lowest soluble CP value (0.64%) and the treatment with the lowest fat content (CC ff = 21%) had the highest soluble CP value (22.4%). The a-values for CP degradability of all the treatments obtained in this study were noticeably lower than that of solvent extracted soybean meal (33.1%) and the 30.8% of raw full fat soybeans Canbolat *et al*., 2005). Also, the a-values presented in this study was lower than that of fish meal (41.2%), meat and bone meal (39.6%) and blood meal (13.2%), except for the CC ff (22.4%) treatment which was higher than that of blood meal (England *et al*., 1997).The BSF B ff and BSF W ff did not differ significantly and this may be the reason for the close similarity regarding soluble CP values. CC ff had a much lower fat content than the other treatments and also a much higher a-value than the other four treatments. The two defatted treatments had higher a-values. The BSF B df treatment had a soluble CP value of 8.8% and the BSF W df treatment a value of 2.4%. These two treatments differed ($P < 0.05$) from one another. One could again make an assumption that the fat content of the meal played a role in the solubility of the product. Due to the high fat and

oil content the meal formed small clumps which possibly resulted in less material disappearing through the nylon bag pours.

5.3.2.2 Potentially degradable fractions (b-values) of the treatments

The BSF W ff treatment had a potential CP degradability of 58.9%. The two BSF full fat treatments had very similar fat contents and a-values, but the BSF W ff treatment had an apparent lower b-value (59.9%) than the BSF B ff treatment (64.1%). However, it was found that these two treatments did not differ significantly which was unexpected as the BSF B ff is a pre-pupae treatment. Pre-pupae has an exoskeleton consisting of chitin which is insoluble and one would expect that the pre-pupae meal would have a lower degradability than the BSF W or larvae meal. The same result was observed with the BSF defatted treatments, although as with the full fat treatments, the two defatted treatments did not differ significantly from one another. There was no significant difference between BSF B ff and the BSF W df treatments but, BSF W ff differed significantly from the two defatted (BSF W df and BSF B df) treatments. The BSF W ff (58.9%) treatment had a b-value which seemed to be similar to the 58.5% of raw full fat soybeans presented by Canbolat *et al* (2005). The b-values the five larvae and pre-pupae meal treatments seemed to be significantly higher than the b-values for meat and bone meal (43.9%) and blood meal (18.1%) as presented by England *et al* (1997).

5.3.2.3 Rate of disappearance (c-value) of potentially degradable (b-value) fraction

The c-value is the rate at which the potentially degradable fraction (b-value) disappears and is measured as percentage per hour. The BSF W ff treatment had a c-value of 0.11%, which was much lower than the other meal treatments and could be explained by the high fat content of this treatment. However, the BSF W df treatment had a much higher fat content than the CC ff treatment but showed higher c-values than that of the CC ff. This result contradicts the statement that fat content was the reason for the lower rate of DM disappearance in treatments. There were numerical differences between the treatments for rate of disappearance, but no significant difference between the three full fat treatments (BSF B ff, BSF W ff and CC ff) were observed. The BSF W df differed significantly from the full fat treatments but BSF B df differed only from the BSF W ff treatment. Looking at these results, the conclusion can be made that there was little difference in the rate of disappearance (c-value) between the five larvae meal treatments and it can also be concluded that fat content did not affect the c-value as much as it affected the a- and b-values of the larvae meal treatments.

Table 5.2 *In sacco* CP disappearance (%) at zero hours, 48 hours and rate of disappearance (%) of treatments (including standard errors).

Treatment	a-value 0	±SE	b-value 48	±SE	c-values	±SE	Eff. Degr. k= 5%	± SE
BSF B ff	0.64[a]	±0.451	64.10	±3.459	0.37	±0.062	56.58[b]	±3.526
BSF W ff	0.89[a]	±0.591	58.92	±2.565	0.11	±0.030	37.88	±2.494
CC ff	22.43	±2.589	83.38	±3.001	0.34	±0.090	88.78[c]	±3.887
BSF B df	8.76	±1.023	78.18	±3.660	0.64	±0.094	80.37[c]	±3.197
BSF W df	2.36[a]	±0.542	72.84	±3.136	0.75	±0.053	70.52[b]	±2.624
P-value	0.0000		0.7382		0.1771		0.8296	

[a,b,c] Treatments with the same superscripts did not differ significantly ($P > 0.05$)

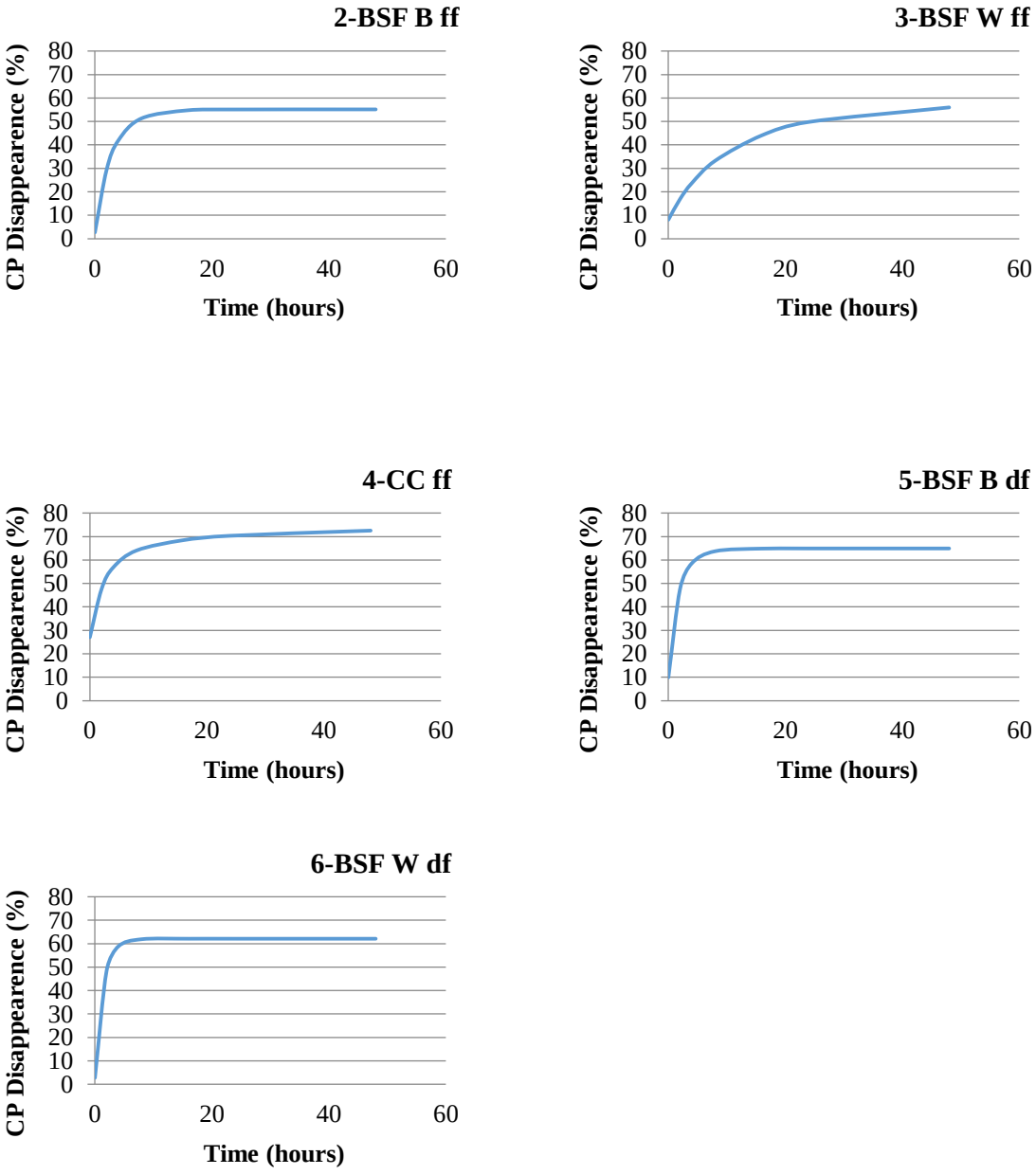

Figure 5.1 Crude protein disappearance (%) over time for the five treatments

5.3.2.4 Effective degradability of crude protein (CP)

The effective degradability was calculated using the non-linear parameters a, b and c (or K_d) obtained from the model used by Orskov & McDonald (1979). The K_p- value was estimated as 5% per hour. The CC ff treatment had the highest D_{eff} value (88.8%) of all the treatments and seemed to be similar to that of soybean meal (88.3%) in a study of Maxin *et al* (2013). The BSF W ff treatment had the lowest D_{eff} value (37.9%) and differed significantly (P <0.05) from BSF B ff with the value of 56.6%. The three treatments with the highest D_{eff} values were CC ff, BSF B df and BSF W df, but, the CC ff treatment also had a relatively lower fat value compared to the full fat and the defatted BSF treatments. Looking at Figure 5.2 it appears that there was a negative correlation between the crude fat content and the D_{eff} values of the treatments. The Spearman rank order correlation indeed showed a negative correlation with an (R value of -0.812.

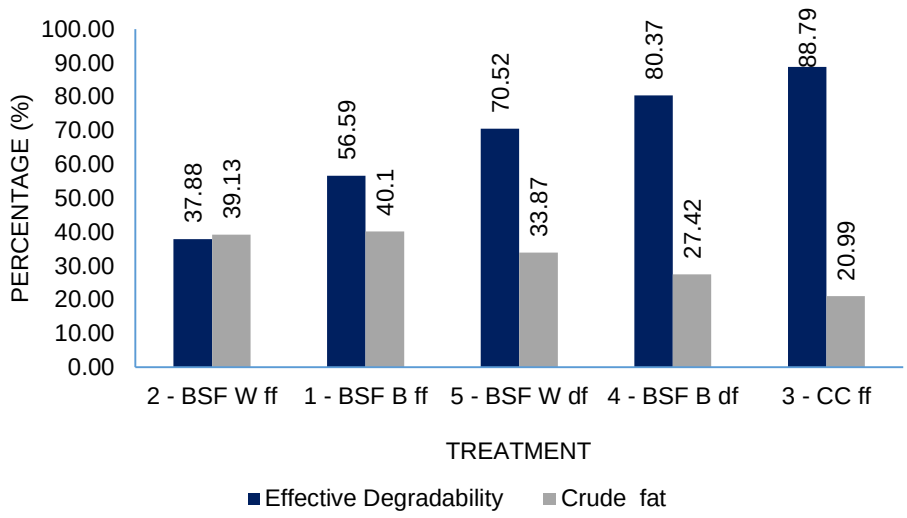

Figure 5.2 Effective degradability (%) and crude fat (%) values for different treatments.

5.3.2.5 Rumen undegradable protein (RUP) fraction

The RUP fraction was determined as a percentage value from 100 – RDP (%) as described by the NRC (2001) and is showed in Table 5.3.

Table 5.3 Effective rumen degradable protein (RDP) and rumen undegradable protein (RUP) values for the different treatments.

Treatment	BSF B ff	BSF W ff	CC ff	BSF B df	BSF W df	*P*-value
Deff[a]/ RDP[b] (%)	56.59	37.88	88.79	80.37	70.52	0.8296
RUP[c] (%)	43.41	62.12	11.21	19.63	29.48	

[a] – Effective degradability (K_p = 5%/ hour)
[b] – Rumen degradable protein
[c] – Rumen undegradable protein (RUP = 100 – RDP)

The treatment with the highest RUP value was the BSF W ff treatment with a value of 62.1%. The BSF B ff had a value of 43.4% and differed significantly from BSF W ff. The BSF W df treatment was from the same species (BSF) and same stage (larvae) of harvest as the BSF W ff, but was subjected to a defatting process. The outcome from BSF W df (defatted treatment) was a much higher RDP value resulting in a significantly lower RUP value (29.5%) compared to the RUP value of BSF W ff (62.12%). Figure 5.3 illustrates the RDP- and RUP fraction as a percentage of the total crude protein of the different treatments and is ranged in a decreasing order of RUP. These results confirmed that the fat content of the larvae and pre-pupae meals had a negative influence on degradability and therefore the high fat treatment, BSF W ff, showed the highest RUP value and the low fat treatment, CC ff, had the lowest RUP value.

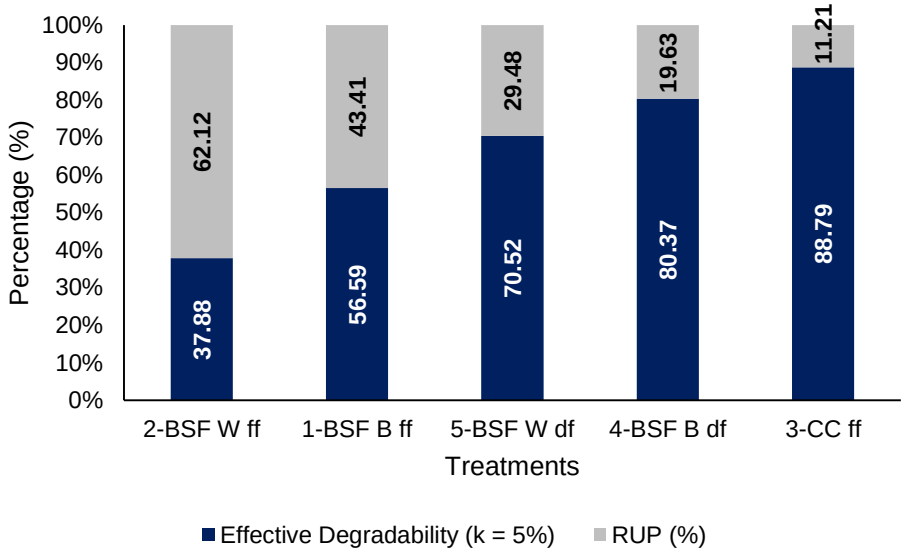

Figure 5.3 Effective rumen degradable protein (RDP) and rumen undegradable protein (RUP) values for the different treatments.

5.4 Conclusion

From all the results observed in this chapter it was very clear that the *in sacco* disappearance or solubility (degradability) of the treatments were significantly affected by the crude fat content of the treatments. Statistical analysis showed a strong negative correlation between fat content and effective degradability. Without undergoing any processing, other than the defatting of the meal, the larvae meal treatments had high RDP fractions, especially the defatted treatments. Larvae and pre-pupae meal would contribute to the N-requirements of the microbes in the rumen and would be incorporated into the microbial protein portion. Further studies would have to be conducted where larvae meal would be successfully defatted and exposed to processing methods similar to that of fish meal (FM) to increase the RUP value of the larvae or pre-pupae meal. These studies will give a more accurate and true reflection of the degradability of larvae meal and could then be compared to that of FM and other protein sources. One can conclude that larvae meal has potential as a protein source and may have a similar or even superior RUP content than fish meal in terms of RUP when exposed to processing methods.

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CHAPTER 6

General Conclusion

The livestock industry is faced with many challenges. For livestock farmers a great challenge lies in producing products (meat, milk, wool etc.) at a more cost-effective and profitable way. For animal feed manufacturing companies the challenge lies in the formulation and production of high quality rations at the cheapest possible cost (least-cost formulation). Raw material costs and availability are the most determining factors that affect ration costs. Even though maize (an energy source) greatly affects ration costs its cost per ton is much less than that of the sources included as protein supplements (oilcakes and animal proteins). The purpose of this study was to investigate the use of different fly species' maggot meal as a potential source of protein in ruminant diets.

A significant amount of time and money are spent on research to find ways to improve the quality of conventional protein sources as well as finding alternative sources to supplement or substitute conventional resources. Fish meal, a source of animal protein is probably one of the most used and high quality animal protein sources used in ruminant diets to increase the UDP fraction (bypass protein). However, fish meal is becoming unaffordable and unprofitable to use in diets as the availability thereof is often limited. This study was conducted with larvae and pre-pupae meal of *Hermetia illucens* and *Chrysomya chloropyga* fly species to look at its nutritional value and whether it could be used as an additional source of animal protein.

The nutrient analysis from Chapter 3 suggests that larvae ad pre-pupae meal are both high in protein and also in fat. The larvae and pre-pupae meal treatments with the highest and lowest crude protein value were CC ff (58.6%) and BSF B ff (34.6%). The fat content of the meal significantly affected the protein content – the higher the fat content the lower the protein content. Furthermore, the meal proved to be a good source of amino acids, calcium and other trace minerals.

In the *in vitro* digestibility trial treatment BSF W ff showed the lowest effective degradability and therefore had the highest RUP-value of all treatments. The same results were observed in an *in sacco* digestibility trial and treatment BSF W ff again had the highest RUP-value of all the treatments. However, in the *in sacco* digestibility trial all the treatments had higher effective degradability values than in those obtained in the *in vitro* trial due to a better environment in the rumen where microbial populations are higher and other rumen environmental factor has an effect on degradability. It was

concluded that as the fat content of larvae and pre-pupae meal decreased so did the RUP-value. Furthermore, fly larvae and pre-pupae meal are good sources of protein but their potential to serve as a RUP or UDP sources in ruminant diets is still very vague and questionable.

Further trials and studies should be conducted on methods of processing to successfully and significantly reduce the fat content of the meal to overcome problems with rancidity during storage and the major effect that fat content has on the degradability of dry matter and protein content of the maggot meal. Only once these challenges are overcome an accurate conclusion, with the help of repetitive trials, can be made on the quality of fly maggot meal and its potential to be used as a RUP/UDP supplement in ruminant diets.