The assessment of black soldier fly (*Hermetia illucens*) pre-pupae, grown on human faecal waste, as a protein source in broiler and layer diets

by

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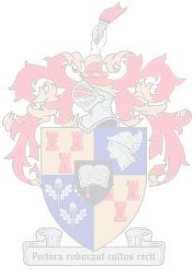
Thesis presented in fulfilment of the requirements for the degree of
Master of Science in Agriculture (Animal Sciences) at
Stellenbosch University



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March 2017

Declaration

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Date: March 2017

Abstract

The aim of this study was to investigate the effect of processing of black soldier fly (*Hermetia illucens)* pre-pupae meal (BSF), grown on human waste, as a protein source in broiler and layer hen diets (10% inclusion level). For the broilers, the diets were iso-nitrogenous and iso-energetic, containing 10% pre-pupae meal. The potential of these (BSF) as a protein source in broiler diets were evaluated, along with the production parameters, carcass quality (physical and chemical), possible toxicities, feed safety and digestibility of the pre-pupae were investigated in broilers. Eight different processing treatment methods were used on the BSF in the broiler trials. The methods included: washed in water at 62°C for 30min (Trt1), 62°C for 60min (Trt2), 72°C for 5min (Trt3), 72°C for 15min (Trt4), 100°C for 2min (Trt5), 100°C for 5min (Trt6), rinsing in 5% propionic acid (Trt7) and rinsed in 5% formic acid (Trt8).The production trial indicated no treatment differences for cumulative feed intake and average daily gain (ADG). Treatment differences were observed between the BSF diets and the control, with the BSF diets achieving better results regarding feed conversion ratio (FCR), protein efficiency ratio (PER), european protein efficiency factor (EPEF) and final live weight. The organ weights and the gut toxicity were measured. The analysis indicated no treatment differences with regards to the gut pH, organ weights and neither with the histomorphology of the duodenum and jejenum. No significant treatment differences were observed regarding the slaughter weight, breast muscle yield and the proximate analysis. However, significant differences were observed in the dressing percentage, with the control diet achieving higher dressing percentages than most of the BSF diets. The breast muscle of broilers receiving BSF diets produced meat that was redder than that of the control diet. Significant differences were observed between Trt2 and Trt8 with regards to pH of the meat (pH of 6.4 and 6.0 respectively). For the total tract digestibility study the following were evaluated: protein, fat, fiber, ash, amino acids and apparent metabolisable energy (AME). There were significant differences among all the treatments with regards to AME and the other nutrients. Trt1, Trt2, Trt3 and Trt5 had the highest coefficient of total tract digestibility (CTTD) over all the nutrients analysed. These treatments had CTTD's over 0.9 for crude protein and the essential amino acids.

The layer trial only investigated four out of the initial eight treatment methods of the pre-pupae (Trt1, Trt3, Trt5 and Trt8), with two housing methods for each treatment (naturally ventilated and free range). The eggs were stored at room temperature at different time intervals before analysis: 1) the same day, 2) one day after collection, 3) one week later, 4) two weeks later and 5) one month later. The data collected were egg weight, shell weight, yolk weight, yolk colour and albumen height. The albumen height was used to determine the Haugh

unit (HU). It was concluded that there were no significant differences between the dietary treatments with regards to shell weight, HU, shell thickness and colour. There were, however, observed differences between dietary treatments for egg weights and yolk weight. Trt3 house (naturally venitlated) differed significantly from the other treatments with regards to egg weight. While Trt3 house (naturally venitlated) and Trt5 house (naturally venitlated) differed significantly from the control group regarding yolk weight. It was observed that with increased storage time the quality of the eggs degraded (egg weight, yolk weight and HU). There were significant differences between treatments with regards to egg weight and yolk weight in storage group 1. There were also significant differences in storage group 2 as pertaining to egg weight, Trt3 house being significantly heavier than the other treatments except for Trt1 house. Trt1-3 free range also differed significantly from the other treatment groups.

It can be concluded that BSF grown on human waste can successfully be used as a protein source in broiler and layer diets. Good production values along with carcasses of acceptable physical and chemical quality can be produced in broilers, with no measurable toxic effects. The BSF are also highly digestible irrespective of treatment. It was also concluded that eggs produced from diets containing BSF grown on human waste were still of good quality with no adverse effects to be found.

Opsomming

Die doel van hierdie studie was om die effek van die verwerking van swart soldaat vlieg (*Hermetia illucens*) pre-papie meel te ondersoek (BSF), gegroei op menslike afval, as 'n proteïenbron in braaikuiken en lêhen dieet (10% insluiting vlak). Vir die braaikuikens, die dieet is ISO-stikstof en ISO-energiek, met 10% pre-papiemeel. Die potensiaal van hierdie (BSF) as 'n proteïenbron in braaikuiken dieet was geëvalueer, saam met die produksie parameters, karkasgehalte (fisiese en chemiese), moontlik toksisiteite, voer veiligheid en verteerbaarheid van die pre-papie is ondersoek in braaikuikens. Agt verskillende verwerkings metodes is gebruik op die BSFin die braaikuiken proewe. Om die voer veiligheid aan te spreek, was die agt behandeling metodes geëvalueer. Die metodes sluit in: gewas in water by 62°C vir 30min (Trt1), 62°C vir 60min (Trt2), 72°C vir 5min (Trt3), 72°C vir 15min (Trt4), 100°C vir 2min (Trt5), 100°C vir 5min (Trt6), spoel in 5% propioonsuur- (Trt7) en afgespoel word 5% mieresuur (Trt8).Die produksie proef het geen behandeling verskille met betrekking tot kumulatiewe voerinname en gemiddelde daaglikse toename (GDT) aangedui nie. Behandeling verskille is waargeneem tussen die BSF dieet en die kontrole, met die BSF dieet wat beter resultate aandui met betrekking tot voer omskakelings verhouding (VOV), proteïen doeltreffendheid verhouding (PER), Europese proteïen doeltreffendheid faktor (EPEF) en finale lewendige gewig. Geen beduidende verskille tussen die BSF groepe is waargeneem in die onderskeie parameters nie. Die orgaan gewigte en die ingewande pH is gemeet. Die ontleding het aangedui geen behandeling verskille ten opsigte van die derm pH, orgaan gewigte en die histomorfologie van die duodenum en jejenum. Geen beduidende behandeling verskille is waargeneem met betrekking tot die slaggewig, bors spier opbrengs en die onmiddellike ontleding. Maar beduidende verskille is waargeneem in die uitslagpersentasie, met die kontrole dieet wat hoër uitslag persentasiebereik as die meerderheid van die BSF dieete. Die bors spiere van die braaikuikens wat BSF ontvang het het vleis produseer wat meer rooi as die kontrole dieet is. Beduidende verskille is waargeneem tussen Trt2 en Trt8 met betrekking tot pH van die vleis (pH van 6.4 en 6.0 onderskeidelik). Vir die totale sisteem verteerbaarheid studie is die volgende geëvalueer: proteïen, vet, vesel, as, aminosure en skynbare metaboliseerbare energie (AME). Daar was beduidende verskille tussen al die behandelings met betrekking tot AME en die ander voedingstowwe. Trt1, Trt2, Trt3 en Trt5 het die hoogste koëffisiënt van totale kanaal verteerbaarheid (CTTD) van al die voedingstowwe ontleed. Hierdie behandelings het 'n CTTD gehad oor 0.9 vir ru-proteïen en die essensiële aminosure.

Die lêhen proef het net vier uit die aanvanklike agt behandeling metodes ondersoek, met pre-papies (Trt1, Trt3, Trt5 en Trt8) en twee behuisings metodes vir elke behandeling (natuurlik geventileerde en vryloop). Die eiers is gestoor by kamertemperatuur en op

verskillende tydsintervalle geanaliseer: 1) op dieselfde dag, 2) 'n dag nadat versameling, 3) 'n week later, 4) twee weke later en 5) 'n maand later. Die data wat ingesamel was was eier gewig, dop gewig, eiergeel gewig, eier kleur en albumen hoogte. Die albumen hoogte is gebruik om die Haugh-eenheid (HU) te bepaal. Dit is tot die gevolgtrekking gekom dat daar geen beduidende verskille tussen die dieet behandelings met betrekking tot gewig, HU, dop dikte en dop kleur was nie. Daar was egter waargeneemde verskille tussen dieet behandelings met eier gewigte en die gewig van die eiergeel.Trt3 huis het beduidend verskil van die ander behandelings met betrekking tot eier gewig. Terwyl Trt3 huis en Trt5 huis beduidend verskil van die kontrole groep met betrekking tot eiergeel gewig. Dit is waargeneem dat met 'n verhoogde bergings tyd die gehalte van die eiers afneem (eier gewig, eiergeel gewig en HU). Daar was beduidende verskille tussen behandelings met betrekking tot eier gewig en eiergeel gewig in die stoor groep 1. Daar was ook beduidende verskille in die stoor groep 2 met betrekking tot eier gewig, Trt3 huis wat aansienlik swaarder as die ander behandelings behalwe vir Trt1 huis. Trt1-3 vryloop is beduidend verskillend van die ander behandeling groepe.

Dit kan afgelei word dat BSF wat gegroei is op menslike afval suksesvol gebruik kan word as 'n proteïenbron in braaikuiken en lêhen dieete. Goeie produksie waardes saam met karkasse van aanvaarbare fisiese en chemiese kwaliteit kan geproduseer word in braaikuikens, met geen meetbare toksiese effekte. Die BSF is ook hoogs verteerbaar ongeag behandeling. Daar is ook bevind dat eiers wat BSF bevat en gegroei is op menslike afval steeds van goeie gehalte met geen newe-effekte geproduseer kan word.

Dedication

I would like to dedicate this thesis to my parents, Anton and Elna van Schoor, who helped me become the person I am today. Due to their sacrifice and commitment I was able to not only get my degree but now my masters. They taught me that through hard work and dedication I can achieve whatever I set my mind to. Thank you for being there for me in the tough times and continuously pushing me to be better.

Acknowledgements

I wish to extend my gratitude towards the following people, without them the completion of this thesis would not have been possible.

Firstly I would like to thank The Lord for giving me the opportunity and ability to persue my studies. With Him by my side I was able to endure the hard times and become a stronger person.

Secondly, I would like to thank both my supervisors Dr. E Pieterse and Prof. L. C. Hoffman for their guidance and support throughout this thesis.

Thirdly, I wish to thank the technical team of the Animal Science department of the Stellenbosch University, who lent me their support, expertise and help whenever I needed it. I would like to especially thank Mr. Danie Bekker who not only lent me his expertise when needed, but was there for me in the good and bad times, thank you for all the laughs that I had.

Fourthly, I extend my grattitude to the team from AgriProtein who helped me when I was doing my trial. Your help will not be forgotten.

Fithly, I say thank you to a Mr. Daniel van der Merwe who gave me friendship and support. I also want to say thank you to Davina Hopley who helped me through my trials, your help is much appreciated and without you I don't see how I would've finished the trial phase.

Finally, but definitely not the least, I say thank you to my family and friends for your support that you gave me. Also thank you so much to my girlfriend, Callan Schafer, for standing by me and supporting me and always believing in me, even when my mood wasn't the greatest.

Notes

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

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Abbreviations

a*	Redness
AA	Amino Acids
ADG	Average daily gain
AgriLasa	Agriculture Laboratory Association of Southern Africa
Al	Aluminium
AME	Apparent metabolisable energy
AMEn	Apparent metabolisable energy nitrogen corrected
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists International
B	Boron
b*	Yellowness
BSF	Black soldier fly
BW	Body weight
C	Celsius
Ca	Calcium
CF	Crude fibre
CH_4	Methane
CO_2	Carbon dioxide
CP	Crude protein
CTTD	Coefficient of total intestinal tract digestibility
Cu	Copper
d	Day
DM	Dry matter
EMB	Eosin methylene blue agar
EPEF	European protein efficacy factor
etc.	Et cetera
FAO	Food & Agriculture Organization
FCR	Feed conversion ratio
Fe	Iron
g	Grams
GE	Gizzard erosion
GHG	Greenhouse gasses
GIT	Gastro intestinal tract
ha	Hectare

hr	Hours
HF	House fly
HU	Haugh unit
K	Potassium
KCl	Potassium chloride
kg	Kilograms
L	Litres
L*	Lightness
Lis	*Listeria* selevtive
M	Molar
Mn	Manganese
ME	Metabolisable energy
Mg	Magnesium
mg	Mili grams
MJ	Mega joules
min	Minutes
ml	Milliliters
N	Nitrogen
NA	Nutrient agar
Na	Sodium
NRC	National Research Council
NSP	Non-starch polysaccharides
O_2	Oxygen
P	Phosphorous
PSE	Pale Soft Exudate
PER	Protein Efficiency Ratio
SSA	*Salmonella/Shigella* agar
T	Ton
TMA	Trimelthylamine
Trt	Treatment
µl	Micro litres
µm	Micro meters
wk	Week
Zn	Zinc

Chapter 1. General Introduction

The human population is growing at a dramatic rate which means that there are more people to feed (Dar & Gowda, 2013). At the same time, the demand for animal products are also increasing (Steinfeld, 2004; Pingali, 2006), especially in Asia, where the increase in wealth leads to an increase in the consumption of animal products (Steinfeld, 2004; Pingali, 2006). However, certain factors are placing stress on the livestock industry to meet these needs. These factors include climate change (Thompson, 2010; Tirado *et al*., 2010; Williams *et al*., 2012), the above mentioned diet shift in Asia (Steinfeld, 2004; Pingali, 2006), competition for plant feed from the biofuel industry (Tyner, 2008) and indirect pressure applied by aquaculture utilizing plant protein for feed (Olsen & Hasan, 2012). Due to the effects of these factors, the agricultural sector needs to adapt to this changing world, while maintaining high production levels.

Climate change is arguably the factor that has the largest influence on the agriculture sector. Due to the change in global surface temperature certain areas will become more susceptible to droughts and floods, while total agricultural production could decrease due to the effect of heat stress (Vergé *et al.*, 2007; Tirado *et al*., 2010). Pests are also expected to increase/become more abundant (Vergé *et al.*, 2007; Tirado *et al*., 2010). The shift in dietary (animal protein) preference will lead to a change in livestock production levels, and although this shift is positive for the animal production sector, it could lead to an increase in greenhouse gas emissions. This is due to an increase in waste production from the feacal matter produced in intensive production systems (Mallin & Cahoon, 2003), as well as from abattoir waste from slaughtering of these animals (Affes *et al*., 2013), post-harvest losses (Affes *et al*., 2013) and increased demand for feed sources which could compete with human food needs (Rosegrant, 2008). Grains used in the production of biofuel are also used in livestock feed and human food (Ajanovic, 2011; Tirado *et al*., 2010; Tyner, 2008). The growth of the biofuel industry has caused an increase in grain prices, especially those used in the production of biofuel (Rosegrant, 2008). This increase in the grain price will directly affect the profitability of the livestock industry (Tyner, 2008; Ajanovic, 2011; Tirado *et al*., 2010).

Similar to the above mentioned, fishmeal is used in both aquaculture and terrestrial livestock production. The usage of fishmeal in both these fields, causes competition between the two (Olsen & Hasan, 2012). It is noteworthy that the use of fishmeal in livestock production (excluding aquaculture) has declined (New & Csavas, 1995; Huntington & Hasan; 2009) due to over fishing, pollution of the oceans and destruction of habitat (Olsen & Hasan, 2012). These factors leads to an increase in fishmeal prices, which affects the profitability of the livestock industry, especially the monogastric industry when using fishmeal. Therefore, instead of using

fishmeal in livestock feeds, a plant protein alternative is frequently used. Soybean meal is the acceptable and most widely used plant protein in the terrestrial livestock industry (Boland *et al*., 2013). However, the sustainability of soya production is of great concern due to the destruction of rain forests for crop plantations (Pimentel & Pimentel, 2008). The world's arable land is mostly farmed, but productivity is also declining due to unwise farming practices (Pimentel & Pimentel, 2008). Therefore, alternative protein sources need to be found to relieve the added pressure and reduce the above-mentioned competition.

The amount of organic waste produced by the agricultural industry, cities and urban areas is increasing. With the increase in human population and the subsequent increase in demand for feed and food, waste production is also increasing (Roberts & de Jager, 2004; Seng *et al*., 2013). These wastes include agricultural waste such as post-harvest losses (Fox, 2011), food factory waste that includes food not eaten or that has gone off (Kantor *et al*., 1997), intensive animal production wastes such as manure, mortalities and feed waste (Mallin & Cahoon, 2003). This organic waste has the potential to be circulated back into the food chain (Cordell *et al*., 2009). Previous studies have indicated that organic waste is a good growth media for insects, which again can be used in livestock feed as a feasible protein source (Newton *et al*., 2005; Ogunji *et al*., 2008; Diener *et al*., 2009; Pretorius, 2011; Uushona, 2015). Insects can break down organic waste (Ocio *et al*, 1979; El boushy, 1991; Sheppard *et al*., 1994; Čičková*et al*., 2012; Zhang *et al*., 2012; Wang *et al*., 2013), whilst the residue produced from the break down process can also be used as a soil amender (Newton *et al*., 2005).Therefore all of the products produced in the breakdown of the organic waste are recyclable. Although, there are many insect species suitable for this function, the BSF (*Hermetia illucens*) larvae are known as ravenous consumers of organic matter and they reduce the dry matter of the waste (Newton *et al*., 2005; Kim *et al*., 2011). The larvae that are grown on the organic waste are high in fat and protein, making them a good potential feed source in animal feeds (Jeon *et al*., 2011). These larvae therefore are potentially a good substitute for not only fishmeal but also soybean meal in livestock feed.

Studies have indicated that BSF larvae can be grown on different types of faecal matter, including human faecal matter (Sheppard *et al*., 1994; Newton *et al*., 2005; Diener, 2009; Banks *et al*., 2011). However, the larvae that were grown on human faecal matter, in the previous studies, were not used as a protein source in poultry diets.

Aim

Therefore, the aim of this study is to evaluate the pre-pupae that have been grown on human faecal matter as a potential protein source in poultry diets.

Objectives

The production parameters for these pre-pupae diets will be determined. Along with this the organ health and carcass characteristics will be evaluated. The total tract digestibility

coefficient values of the pre-pupae will also be determined. This study will also evaluate the pre-pupae meal when added to layer diets in terms of production levels and egg quality.

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Chapter 2. Literature Review

2.1 Factors influencing food security

2.1.1 Growth of the world population

The world population is growing at an alarming rate, currently estimated at 1.2% per annum; it is projected that in the year 2050 the world population will be 11 billion (UN, 2004; Pimentel & Pimentel, 2007). This would lead to greater demand for food and, with the population growth not showing any signs of slowing down, the demand for food is going to increase (Pimentel & Pimentel, 2007). The total grain yield of a country is of great importance, as grains constitute ~80% of the human diet, while animal feeds also utilize large quantities of grains. Therefore, the sustained production of grains is vital (Pimentel & Patzek, 2007). It is therefore important to focus on grain yield when taking the population growth into consideration. Grain yield per hectare is increasing in developing and developed countries, although this increase is slowing down (Pimentel & Pimentel, 2007). As an example, the grain yield of the United States of America (USA) between 1950 and 1980 had a growth of 3% per annum, however, since 1980 the growth has only been 1% per annum (Pimentel & Pimentel, 2007).The annual loss of cropland is also of great concern (Pimentel & Pimentel, 2007). Fertile topsoil is a precious agricultural resource and once lost it takes extremely long to reform (Pimentel & Pimentel, 2007). It is estimated that soil erosion cause at least 50 million hectare (ha) of world cropland to be abandoned and lost to production per annum (Pimentel & Pimentel, 2007). As a result, deforestation is implemented to replace the lost cropland (Pimentel & Pimentel, 2007). It is also important to note that the world population is growing at a faster rate than that of the annual increase of grain production (Pimentel & Pimentel, 2007).

More than 99% of the world's food is produced from the terrestrial environment, while only 1% comes from the ocean (Pimentel & Pimentel, 2007). Considering this, factors that affect the terrestrial food production must be considered because of the effect it would have on the food production potential of a country (Pimentel & Pimentel, 2007). The increase of a country's population causes the expansion of cities and suburbs, into the rural farmlands (Pimentel & Pimentel, 2007). This loss of farmland would then cause a decrease in total agricultural production (Pimentel & Pimentel, 2007). Regarding the state of California, which for many years has been one of the highest producing agricultural states in America, it is observed that over 100 000ha are being lost each year due to urbanization (USCB, 2004). This then places stress on the agriculture sector to not only keep production stable, but also increase production while at the same time using less land. As mentioned above, China's high population growth puts enormous stress on their agricultural sector through urbanization and

increased demand for food (Pimentel & Pimentel, 2007).Therefore, to keep up with the increased demand for food, agricultural production systems must increase production. It is also important to note that due to urbanization agriculture must also become more efficient, through the means of using less land while keeping production high.

One facet of the agricultural sector that will be affected by the growth of the human population is the animal production sector (Pimentel & Pimentel, 2007). It is important to note that due to urbanization, decrease in grain yield and loss of cropland, animal production would be under severe stress to produce affordable products. The growth of the world population would however cause an increase in demand for animal-based products (Steinfeld, 2004). Animal products provide 27% of the food calories in developed countries and 13% in developing countries (FAOSTAT, 2001). Animal products are high in proteins and are energy dense; they also serve as a good source of minerals and vitamins. It is estimated that worldwide 3.7 billion people are malnourished (Pimentel & Pimentel, 2007), and considering their high nutritional quality, animal products are a great food source for adults and children to receive their daily dietary needs from (Pimentel & Pimentel, 2007).

Growth of the world population causes direct and indirect pressure on the agricultural sector of the world (Pimentel & Pimentel, 2007). With other factors also contributing to this pressure, it has become imperative to try to stabilize the population in order to make it liveable for everyone or else, to increase total agricultural production (Pimentel & Pimentel, 2007). Thus agriculture must find ways to become more efficient, therefore, research into more efficient animal production is of great value and necessary for sustainable livestock production.

2.1.2 Effects of climate change

The major issue that the world agricultural industry faces is the effect that global warming has on the industry (Tirado *et al.*, 2010). This increase in temperature can be detrimental to animal and plant production (Thompson, 2010); leading to decreased production levels (Williams *et al.*, 2012). Certain areas would likely benefit from longer growing seasons, but other areas would be affected negatively due to temperature related factors (Vergé *et al.*, 2007). These factors include the increase of droughts and floods and the increase of pest- and disease outbreaks (Vergé *et al.*, 2007; Tirado *et al.*, 2010). Gasses that contribute greatly to the increase of global surface temperatures are known as the greenhouse gasses (GHG). These gasses are carbon dioxide (CO_2), methane (CH_4), ozone and nitrous oxide. Yearly, over 29 billion metric tons of CO_2 is emitted into the atmosphere (Boden *et al.*, 2009), attributing to the rise in atmospheric pressure (Williams *et al.*, 2012). With the world population on the rise, the use of fossil fuels will increase and more GHG will be released into the atmosphere, further contributing to climate change and global warming (Williams *et al.*, 2012).

Livestock accounts for 40% of the world's agricultural production (Vergé *et al.*, 2007). It is also the largest user of agricultural land, be it directly or indirectly through the plantation of crops for animal feed or as grazing land (Vergé *et al.*, 2007). For animals to survive these changes, adaptations are necessary; for instance a change in their distribution patterns, changes in their behaviour and/or animals need to make adjustments in their physiology (Chown *et al.*, 2010). However, the longer the generational interval of the animal, the longer it will take to evolve and adapt to the environment (Williams *et al.*, 2012). With the rise in temperature, for example, dairy cattle are expected to suffer from heat stress even more than they already do (Klinedinst *et al.*, 1993; Williams *et al.*, 2012). This would place extra pressure on the dairy sector and thereby directly affect milk production and profitability (Klinedinst *et al.*, 1993). Taking the global surface temperature into account for the years 1850 to 2000, it was observed that there was a rise in average global surface temperature of 0.8˚C (IPCC Synthesis Report, 2007). Along with this rise in surface temperature, came a rise in average sea level and decrease in snow cover (IPCC Synthesis Report, 2007). Animal production systems use large amounts of land; therefore, it will be greatly affected by the rise in sea level and decrease of land, even if it is at a slow rate (Tirado *et al.*, 2010). It is important to note, that the areas where the snow cover is decreasing is not necessarily arable land, therefore, it does not mean more available farming land. The rise in global temperature would also indirectly affect the food chain, with the rise in food borne diseases and food spoilages (Tirado *et al.*, 2010). A great number of spoilage microbes will benefit from the rise in global temperatures, causing more favourable growing environments (Tirado *et al.*, 2010). Animal diseases would also be affected with a rise in temperature and are expected to increase (Parry *et al.*, 2007). Tick-borne diseases are being affected the most due to having a longer favourable environment for the vector (Parry *et al.*, 2007).

As mentioned above, the GHG are the gasses that greatly affects the rise in global surface temperature. There was an increase in CO_2 and CH_4 emissions in the last couple of years (Loulergue *et al.*, 2008; Luthi *et al.*, 2008). Carbon dioxide is also produced and released into the atmosphere, due to the use of fossil fuels in farming operations (Dyer & Desjardins, 2003). Therefore, the increase of farming operations for the purpose of increase in production, would cause a rise in CO_2 emissions. Manure levels are also rising with the increase in livestock production systems and the intensification thereof (Mallin & Cahoon, 2003), which would lead to an increase in CH_4 emission into the atmosphere. Therefore, better waste management systems must be set in place so that the increase in agricultural production does not compromise the environment (Vergé *et al.*, 2007).

The change in global temperature will affect the agriculture sectors across the world differently (Parry *et al.*, 2007), with some being affected positively and others negatively. However, it is a certainty that the change in climate does have an effect on the world

agricultural sector (Tirado *et al.*, 2010). It is imperative for the sector to evolve so that the effects that are caused do not cripple the industry. If the industry suffers losses, the food chain would be affected and, with the growing population, this would be catastrophic for food security (Vergé *et al.*, 2007).

2.1.3 Westernization of Asian diets

Rapid economic growth has led to a shift in Asian diets away from the traditional towards a diet consisting of higher contents of animal products, fruits and vegetables, and also fats and oils (Mendez *et al.*, 2004; Ma *et al.*, 2004; Pingali, 2006). Traditional Asian diets emphasize carbohydrates, as opposed to the western diets, which consist of higher fat and protein contents (Pingali, 2006). The total amount of calories obtained from animal sources have increased since 1979, whilst a decline in calories obtained from cereals was observed during this period (Pingali, 2006). Two aspects, namely a higher income and, diet globalization and westernization (Pingali, 2006), drive this diet transformation. Income growth is arguably the aspect that has the largest effect on this diet shift (Pingali, 2006). With an increase in income more money is spent on convenience, this includes fast foods, which contains high levels of energy and animal protein (Pingali, 2006). The process of urbanization and integration has also brought about different dietary needs and lifestyle changes (Popkin, 1999; Regmi & Dyck, 2001). If current consumption patterns continue, the total amount of meat consumed in 2030 will be 72% higher than that of 2000, led mostly by an increase in poultry and pork consumption (Fiala, 2008).

Diet diversification has been documented on household level in poor and middle-income countries, in Asia, Africa and Latin America (Behrman & Deolaliker, 1989; Huang & Bouis, 1996; Hoddinot & Yohannes, 2002; Mendez & Popkin, 2004). The evidence indicates that in Asia there is a decline in rice consumption on a per capita basis with growth in income (Huang & David, 1993; Smil, 2005). Urbanization has also exacerbated the negative trend in rice consumption (Huang & David, 1993). Studies have indicated that with this decrease in rice consumption, there is an increase in wheat consumption (Huang& David, 1993) mostly in the form of bread, cakes, pastry and pizza (Pingali, 2006). It is important to note that in western societies, wheat is considered an inferior food and with income growth there is a decline in wheat consumption, unlike what is observed in Asian societies (Pingali & Rosegrant, 1998).

It can be observed from Table 2.1 that there is an increase in consumption of animal based products in Asia over the years 1979 and 2001. This supports the argument that there is an increased demand for animal based products, opposed to the more traditional diets which consist of mainly carbohydrates (Mendez *et al.*, 2004; Ma *et al.*, 2004). Change in diet preference would therefore cause a change in production line and food supply (Pingali, 2006). A rise in livestock production systems would be observed, as well as increased import levels

of animal products (Steinfeld, 2004; Pingali, 2006). Steinfeld (2004) observed how the different livestock commodities have increased over the years 1967 to 1999 and predicted values for the year 2030. However, this trend observed in Table 2.1 is not just isolated in Asia, but is expected to occur in other developing countries across the world.

The livestock production sectors that are predicted to show the highest growth following consumption patterns, would be that of monogastric animals (poultry and pork) (Steinfeld, 2004). This could be due to the fact that these systems require less space for intensive production, and therefore, these animal products are easier to farm and produce, which would then lead to a much more rapid growth as observed (Steinfeld, 2004). However, it is important to note that it is not just the poultry and pork sectors that are exhibiting growth but all livestock sectors (Steineld, 2004). The magnitude of the growth is coupled to land availability and preference and it is predicted that animal production for 2030 is going to be much higher than it is at present (Steinfeld, 2004).

Westernization of Asian diets would lead to agricultural growth, especially the livestock sector (Steinfeld, 2004). This growth of the livestock sector would be positive for the agricultural sector. However, as mentioned above certain factors such as urbanization and climate change would place stress on the sector and would affect the growth (Pimentel & Pimentel, 2007; Thompson, 2010; Tirado *et al.*, 2010). It is important to note that urbanization is coupled to growth of the human population and economic growth of a country (Pimentel & Pimentel, 2007). Ultimately westernization of the diets leads to the increased demand for animal based products across the world, especially in developing countries (Steinfeld, 2004, Pingali, 2006). At the same time, population growth requires energy, directly and indirectly to maintain its growth. As fossil fuels are not only finite, they are also a cause of pollution and alternatives such as green energy sources and biofuels are therefore being sought.

2.1.4 Biofuels

There is an increased demand for bioenergy, especially since the petroleum price continues torise and fluctuate (Tyner, 2008; Ajanovic, 2011; Tirado *et al.*, 2010). Bioenergy has the potential to pose a serious threat on global food prices, especially as pertaining to 1st generation biofuels (Tyner, 2008). This is due to the fact that biofuels are produced from commodities that could have rather been used directly or indirectly as food for humans and animals (Tyner, 2008; Ajanovic, 2011; Tirado *et al.*, 2010). The production of biofuels is expected to affect food prices, seeing as it is produced from agricultural commodities such as corn, wheat, barley, sugarcane, rapeseed, soybean and sunflower (Tyner, 2008; Tirado *et al.*, 2010; Ajanovic, 2011). These ingredients are all major ingredients in animal feeds and even in human food (Tyner, 2008; Ajanovic, 2011; Tirado *et al.*, 2010). However, if the increase of

biofuels in recent years is taken into account, the increase is slow and constant (Ajanovic, 2011).

Table 2.1 Changing consumption patterns of Asian diets between the years 1979 and 2001 (adapted from Pingali, 2006)

		1979-1981	1984-86	1989-1991	1994-1996	1999-2001
Consumption (kg/cap/yr)	Rice	82.3	88.7	89.2	86.0	84.1
	Wheat	54.3	62.5	65.8	69.8	66.7
	Milk	26.1	30.2	32.1	37.5	41.6
	Meat	11.4	14.0	17.1	22.4	26.3
	Beef	2.0	2.3	2.6	3.8	4.1
	Fish and seafood	9.6	11.0	12.6	15.9	17.8
	Animal fat	1.1	1.2	1.3	1.6	1.9
	Vegetable oil	4.6	5.8	6.9	7.8	9.0
	Vegetables	57.1	70.2	76.4	96.3	124.4
	Potatoes	10.6	11.1	12.2	14.7	22.6
	Sweet potatoes	33.3	24.3	19.4	17.6	15.4
	Fruits	27.8	30.1	32.2	40.8	46.0
	Apples	2.8	3.2	3.4	6.0	6.7
	Sugar and sweeteners	14.1	16.0	16.5	16.6	17.2
	Beer	3.1	4.0	5.9	8.3	9.3
Calorie consumption (cal/cap/day)	Total Joules	9565	10305	10669	11033	11213
	% From cereals[a]	65.53	64.68	63.25	59.73	56.27
	Animal source	7.92	8.85	9.96	12.14	13.66

[a]Milk excludes butter; cereals exclude beer; fruits exclude wine

It can be concluded that the biofuel industry is having a smaller effect on global grain prices, especially the 1st generation biofuel grains, than originally anticipated, however, it is not the only driver of commodity price increases (Tyner, 2008; Ajanovic, 2011). Even though

the effect is small at the moment, it could increase in years to come, placing strain on the agricultural sector (Tyner, 2008; Ajanovic, 2011).

Biofuels have been subsidized since 1978 in the United States of America (USA) (U.S Congress, 1978; Tyner, 2008) and since the beginning the industry grew slowly and showed signs of being lucrative (Tyner, 2008). Since 2004, the crude oil price has been increasing and the production of biofuels became even more lucrative (Tyner, 2008). The USA ethanol production grew from 12.9 billion liters in 2004 to 34.0 billion liters in 2008, this increase was greater in these four years than in the previous two decades (Tyner, 2008). The demand for corn for biofuels production has increased since 2008 and in the USA its use has also gone up from 11% to 28% (Tyner, 2008). It is important to note that although corn is in great demand for biofuel production it is not having too great an effect on commodity prices in the USA at present (Ajanovic, 2011).

Due to many other factors playing a role on the pressure being applied to the livestock industry, it is imperative that other sources are found for the production of biofuels. The use of restaurant waste grease as an alternative to 1st generation biofuels, has attracted attention, but it has been observed to produce a lot of solid residual fractions after grease extraction (Zheng *et al.*, 2012). This solid residue poses an environmental concern if not handled properly (Zheng *et al.*, 2012). Due to the ability of insects to easily degrade organic waste, researchers investigated the possibility of using insects to produce or help produce biofuels (Zheng *et al.*, 2012). The ability of BSF larvae to easily breakdown organic matter and produce larvae that are high in fat (Jeon *et al.*, 2011), made them ideal for the studies (Zheng *et al.*, 2012). The larvae were used to breakdown the residual fraction and the results showed great promise, due to the ability of the larvae to breakdown the residue and yield promising levels of oils that are used in the production of biodiesel (Zheng *et al.*, 2012). This indicates that the use of insects in the biodiesel industry, could be quite effective and lucrative (Zheng *et al.*, 2012).

Biofuel production is not having a massive impact on global grain prices, even though grains that are used in food, directly or indirectly, is used to produce biofuel (Tyner, 2008; Ajanovic, 2011; Tirado *et al.*, 2010). However, factors such as climate change, growth of world population and westernization of diets could place great stress on the global grain prices in the future (Tirado *et al.*, 2010). Therefore, using insects in biofuel production as an alternative would help lessen the stress on the agricultural sector and decrease the competition between the uses of grains for biodiesel for food/feed (Zheng *et al.*, 2012). It is not only terrestrial livestock species that require quality grains and animal proteins to maintain their expected growth rates, but so do aquaculture species.

2.1.5 Aquaculture

Fishmeal is a major protein source used in feed for aquaculture species, but has also been used as a protein source in terrestrial livestock (Hardy & Tacon, 2002; Olsen &Hasan, 2012). With the rapid growth of the aquaculture industry, the demand for fishmeal has increased (Figure 2.1), and so has the price thereof (Olsen & Hasan, 2012). Figure 2.1 indicates how the amount of fishmeal used in other livestock sectors (excluding aquaculture) has decreased since 1988. This decrease may be attributed to the rise in fishmeal price, indicated in Figure 2.2 (Olsen& Hasan, 2012). It is important to note that farmed fish and shellfish has a high market value, therefore using high priced fishmeal is justified (Tacon *et al.*, 2006). Due to limited amounts of fishmeal, other alternative protein sources are needed to keep up with the demand of the agricultural industry (St-Hilaire *et al.*, 2007; Olsen & Hasan, 2012).

The global increase of per capita intake of seafood and freshwater fish could be expected to keep on increasing over the years (Ye, 1999; FAO, 2004). This could be driven by factors such as health authorities recommending an increased intake of fish or due to it being advantageous for a person's health (Kris-Etherton *et al.*, 2009). In 2009, 66 million tons (70%) of harvested wild fish were used directly for human consumption, while 23 million tons (30%) were used for non-food purposes (FAO, 2004). These purposes include the making of fishmeal and oil, and it is even used directly in fish and pet foods (FAO, 2004). Over the years (2006 - 2009) there has also been a decrease in the production of fishmeal (FAO, 2004). It was also predicted that there would be a decrease of fishmeal in animal feeds, due to stronger catch restrictions or the increased use of the fish for human consumption (Tacon *et al.*, 2011). This would then mean that less fishmeal is available to be used in animal feeds (terrestrial or aquaculture), therefore, a suitable alternative would be needed (St-Hilaire *et al.*, 2007; Olsen & Hasan; 2012).

The fish species that are mostly used in the production of fishmeal are pelagic fish (Olsen & Hasan, 2012). However, these species deteriorate rapidly post mortem, which leads to the fishmeal quickly becoming of bad quality (Olsen & Hasan, 2012). This would be due to the fact that they mainly feed on zooplankton which contains large amounts of proteolytic enzymes (Felberg *et al.*, 2009). These enzymes leak from the intestines after death and degrade the muscle (Olsen & Hasan, 2012).The quality of fishmeal could vary a lot due to rapid deterioration, therefore more stable products that are easier to produce are needed (Olsen & Hasan, 2012). It can be observed in Figure 2.2, that the price of fishmeal has drastically increased when compared to that of soybean meal (Olsen & Hasan, 2012). The price is also projected to keep on increasing over the years with demand, therefore it is not profitable for livestock industries, other than aquaculture, to use it as their main protein source in feed (Tacon *et al.*, 2006; Olsen & Hasan, 2012). It can be seen in Figure 2.2, that the price

of soybean meal has also slowly increased, but this increase is not as drastic as that of fishmeal (Olsen & Hasan, 2012). This shift that is observed in Figure 2.1 could explain the increase that is seen in Figure 2.2. This increase in price of fishmeal is most likely due to the high demand and low supply thereof (Olsen & Hasan, 2012). The low supply could be attributed to the over exploitation or depletion of the ocean and the use of the caught fish in human food (FAO, 2004; Olsen & Hasan, 2012).

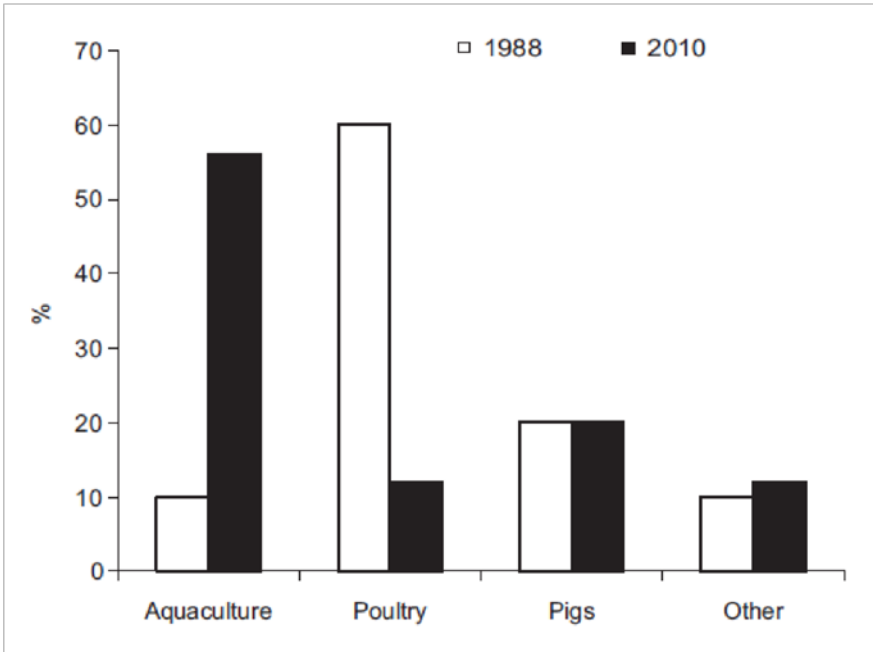

Figure 2.1: Estimated use of available fishmeal in compound feed in different sectors. Data from 1988 from New and Csavas (1995); 2010 from Huntington & Hasan (2009).

Due to the high cost of fishmeal and the low availability, other protein sources need to be found to fill the gap in terrestrial livestock nutrition (Olsen & Hasan, 2012). It is important to note, that the aquaculture industry has made great progress to reduce the amount of fishmeal used in the feed (Tacon *et al.*, 2011). However, finding other sources that can replace fishmeal is still important (St-Hilaire *et al.*, 2007). There have been previous studies that indicate a positive result when using insect larvae meal in aquaculture production (Ogunji *et al.*, 2006; Ogunji *et al.*, 2008a,b; Kroeckel *et al.*, 2012). This opens up good future possibilities for both industries (Aniebo *et al.*, 2009) particularly since a large number of insect larvae can be produced on organic waste.

2.2 Waste products

Organic waste can originate from many different sources and if left untreated this waste can pose potential health risks to the surrounding populace (Roberts & de Jager, 2004). As mentioned above, population growth and urbanization leads to less land available to safely store and dispose of these wastes in the normal fashion (Seng *et al.*, 2013). With an increase

in livestock production, there is also an increase of animal waste, to levels that are difficult to manage and dispose of (Mallin & Cahoon, 2003). Therefore, other methods need to be implemented to dispose of this waste (Wang *et al.*, 2013). The natural environment is a very efficient system at cleaning up after itself, with the help of microbes and insects (Sheppard *et al.*, 1994; Morales & Wolff, 2010). The nutrients still leftover within this waste can be circulated back into the environment and food chain (El boushy, 1991; Li *et al.*, 2011). If the environment uses microbes and insects to take care of the wastes, then it should be possible to use these methods in farming systems.

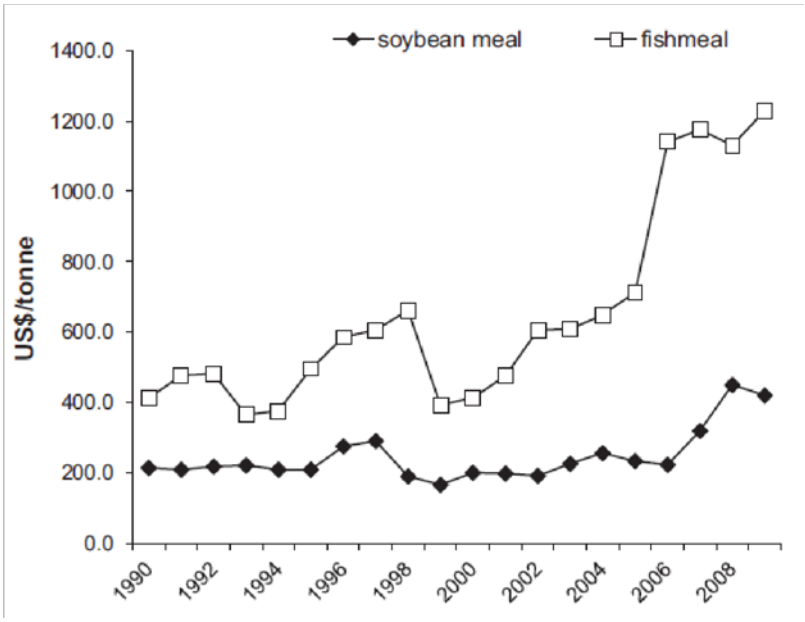

Figure 2.2: Price of soybean meal and fishmeal during the last 20 years (Olsen & Hasan, 2012).

Certain waste products can be a breeding ground for pathogenic microbes, that can cause massive damage to the population and surrounding areas if not managed correctly (Roberts & de Jager, 2004; Mittal, 2006). These wastes are in most cases still high in nutrients, such as protein and energy (Diener *et al.*, 2009). However, due to the nature of the waste the nutrients cannot be used directly in the food chain (Roberts & de Jager, 2004; Mittal, 2006). The organic waste can, however, be circulated into the livestock industry, by using the waste as a feed source for other types of life, such as insects, that can extract the leftover nutrients (El boushy, 1991; Sheppard *et al.*, 1994, Diener *et al.*, 2009; Li *et al.*, 2011). Insects are able to extract the leftover nutrients from waste products and produce condensed forms of nutrients that can be utilised by livestock (Diener *et al.*, 2009; Wang *et al.*, 2013). With the increase in the livestock industry and world population, circulating the waste back into a usable form could be lucrative and beneficial to the environment (Li *et al.*, 2011). Organic waste can be broken down into four different groups namely: agricultural waste, abattoir waste and kitchen waste. Each of these groups are organic based and can be broken down through composting

methods that are aerobic or anaerobic (Banks *et al.*, 2011). This composted material can then be used as animal feed, soil enhancer, soil amender or fertilizer (Banks *et al.*, 2011).

Agricultural waste is derived from many different sectors and can include manure, harvest residue and waste from processing plants (Pretorius, 2011). Manure can typically be used as a fertilizer or soil amender, but it can also be used as a feed source for insects (Diener *et al.*, 2009; Wang *et al.*, 2013). Insects such as the housefly (HF) and Black soldier fly (BSF) have shown promising results where larvae were grown on the waste (Calvert & Martin, 1969; Teotia & Miller, 1974; Ogunji *et al.*, 2006; Adenji, 2007; St.-Hilaire *et al.*, 2007). Research has indicated that chicken manure, where HF larvae were present, has a decreased moisture content, organic matter, and improved texture andodour (Calvert *et al*., 1969; Teoria & MIller, 1974). It is important to note, that growing HF larvae on chicken manure can be dangerous to the surrounding populace, due to the fact that HF carries pathogenic microbes. Chicken manure can, however, differ with regards to nutrient composition due to factors such as age, feed wastage, presence of feathers and storage time (Flegal *et al.*, 1972; El Boushy, 1991). This would then affect the nutrient content of the waste as a possible feed source for insects (El Boushy, 1991).

The waste that is produced from abattoirs includes blood, intestines, intestinal content, bone, carcass trimmings, rejected carcasses, dead on arrivals, heads, hooves, feathers in chicken abattoirs and excess fat (Roberts & de Jager, 2004; Pretorius, 2011). These wastes are often composted to then be used as fertilizers, but an increased amount is being used for biogas production (Abraham *et al.*, 2007). Frequently, the heads and the intestines that are produced are sold as offal or the 5th quarter (Christoe, 2003). The feathers that are produced at a chicken abattoir, can be used in the commercial sector as stuffing for pillows and duvets and it also has uses in animal feed (Dalev, 1994). All the waste products that come from abattoirs are high in protein and fat, which can be a good source of energy and protein if utilised correctly (Adeyemi & Adeyemo, 2007). Blood meal and carcass meal are both produced from abattoir waste, such as the blood and the carcass trimming, rejected carcasses and dead on arrivals (Couillard & Zhu, 1993; Pretorius, 2011). It also poses health risks to the animals and the people who consume the product, due to the possible presence of bovine spongiform encephalopathy. The normal disposing of these abattoir wastes include drainage, oxidation dams, run off into the fields or buried. The condemned carcasses can be incinerated, buried or left in a trench to decompose (Roberts & de Jager, 2004). These methods pose a risk of contamination of the ground water or may cause an outbreak of food borne diseases (Couillard & Zhu, 1993; Mittal, 2006). Abattoir waste typically contains large quantities of fat, which if treated through anaerobic digestion, can produce methane (Affes *et al.*, 2013), which then again can be used to produce electricity (Gonzalez-Gonzalez *et al.*, 2013). Digested

slaughter waste effluent can reclaim significant volumes of water, which can then be used for field irrigation (Gonzalez-Gonzalez *et al.*, 2013).

Certain insect species have been proven to be able to break down and consume abattoir waste (Aniebo *et al.*, 2009; Aniebo & Owen, 2010). These insects then produce a product high in protein, suitable for animal consumption (Aniebo *et al.*, 2009; Aniebo & Owen, 2010). Considering this, these waste products have received attention from researchers for circulation back into the food chain (Aniebo *et al.*, 2009; Aniebo & Owen, 2010). It is important to recognize that even though blood and manure do not really seem like a food source, it is high in certain types of nutrients (Sheppard *et al.*, 1994; Aniebo *et al.*, 2009; Diener *et al.*, 2009; Aniebo & Owen, 2010). Insects are able to consume these nutrients in the given form and grow on it (Sheppard *et al.*, 1994; Diener *et al.*, 2009). Manure is a condensed form of chemical compounds that have been excreted from the body. Some insects are also able to utilise it and convert it into a more usable form (Sheppard *et al.*, 1994; Diener *et al.*, 2009).

Livestock production increases have caused an increase in the amount of manure produced each year (Li *et al.*, 2011; Wang *et al.*, 2013). A dairy cow produces on average 57L of excreta per day (Welsh Ministry of Agriculture, Fisheries & Food, 1991), while it is estimated that 11 million chickens produces 400 000-450 000T of manure per annum (Arkhipchenko *et al.*, 2005). This does not even take into account other livestock sectors, but it does indicate that livestock production is accompanied with high levels of manure accumulation. With the increase in population and the shift in diet that has been seen in Asia, the livestock production sector would increase and with it the amount of manure (Li *et al.*, 2011; Wang *et al.*, 2013).

Manure can differ from species to species but it is mainly affected by the diet of the animal (Kirchmann & Witter, 1992). Manure contains a lot of faecal microbes, such as E. coli and Salmonella, which unlike human waste is not usually treated to get rid of these pathogens (Mallin & Cahoon, 2003). During composting of animal manure, the temperature within the compost rises which is effective at killing most microbes (Mawdsley *et al.*, 1995; Georgacakis *et al.*, 1996; Lalander *et al.*, 2014). Waste slurries on the other hand do not reach these temperatures and therefore do not kill these pathogenic bacteria and it has been reported that E. coli can survive for up to 11 weeks in waste slurry (Mawdsley *et al.*, 1995). Dehydrated poultry excreta used in cattle feed, has a ruminal degradability of 78% and post-ruminal degradability escape of 27% nitrogen (Zinn *et al.*, 1996). The excreta is low in energy (Zinn *et al.*, 1996), but if mixed into feed correctly other raw materials can be added to increase the energy content and become a valuable source of nitrogen in the feed (Chaudhry & Naseer, 2009). Studies have indicated that adding processed broiler litter to the diet of water buffalo steers, has led to an increase in body weight, with the inclusion limit of 26% (Chaudhry & Naseer, 2009).

Kitchen waste can be described as any type of food loss that occur in the production chain (Fox, 2011). This includes losses that occur during the transport from the farm to the retailer, substandard foods, retail losses such as past “sell by” date, as well as consumer and food service losses, which includes rotten or uneaten foods (Kantor *et al.*, 1997). The amount of kitchen waste produced each year is staggering, especially in the developed countries (Fox, 2011). This being due to the fact that people are much more selective when it comes to their food and therefore are more prone to wastage (Fox, 2011). This means that there are a lot of organic waste that has the potential to be used as a feed source when circulating the nutrients back into the food chain (El boushy, 1991; Li *et al.*, 2011). This circulation of organic waste should be quite lucrative and profitable (Diener *et al.*, 2009), particularly if the correct insect species is utilized to enhance this recycling.

2.3 Black soldier fly (*Hermetia illucens*)

The black soldier fly (*Hermetia illucens*) is native to the warm temperate region of south eastern USA (Newton *et al.*, 2005). It has been reported that the BSF can be found all over South America and Asia, but is native to Colombia (Canary & Gonzalez, 2012). The movement of humans across the world has helped the spread of BSF around the world and along with the change in climate, the environment is becoming more favorable for the BSF (Turchetto & Vanin, 2004). Black soldier fly can survive and reproduce in temperatures between 27 and 36^{o}C, but performs optimally at 30^{o}C (Tomberlin *et al.*, 2009).The larvae of the BSF is known to be ravenous consumers of organic decomposing matter whilst the adults are able to live without consuming any feed stuff, living off their stored fat (Figure 2.3; Sheppard *et al.*, 1994; Newton *et al.*, 2005; Kim *et al.*, 2011). Therefore, BSF are more suited to be used in the breakdown of organic waste, because the adults will not be inclined to move to urban areas and thus carry diseases into households (Sheppard *et al.*, 1994; Newton *et al.*, 2005; Kim *et al.*, 2011). The behavior of the BSF larvae, before turning into pupae, is to crawl out of the feedstuff, making them easy to harvest (Sheppard *et al.*, 1994). Due to the high fat and protein content of the BSF larvae, it shows great potential as a feed ingredient (Jeon *et al.*, 2011). Black soldier fly is also a non-pest fly species (Kim *et al.*, 2011); with the female fly only laying one batch of eggs and then dying shortly afterwards (Tomberlin *et al.*, 2009). Another positive effect of adding BSF larvae to organic waste is that they can eradicate the breeding of housefly (*Musca domestica*) larvae and therefore reduce the environmental risk associated with the spreading of disease by the HF (Bradley & Sheppard, 1984). As mentioned, BSF are able to utilize numerous forms of feedstuff such as abbatoir waste, manure and kitchen waste.

2.4 Potential manure management of Insects

The ability of the housefly and BSF larvae to break down animal manure have been investigated (Ocio & Vinaras, 1979; Elboushy, 1991; Sheppard & Newton; 1994; Čičková *et al.*, 2012; Zhang *et al.*, 2012; Wang *et al.*, 2013). The use of insects for composting and manure reduction has been shown to reduce manure moisture content which in return reduces the total manure weight (Newton *et al.*, 2005; Diener *et al.*, 2009; Kim *et al.*, 2011; Canary & Gonzalez, 2012; Zhang *et al.*, 2012; Wang *et al.*, 2013). It also reduces the nutrient composition and air pollution, through the quick reduction of the waste leading to less methane being released into the atmosphere (Newton *et al.*, 2005; Diener *et al.*, 2009; Kim *et al.*, 2011; Canary & Gonzalez, 2012; Zhang *et al.*, 2012; Wang *et al.*, 2013).

It can be observed in Table 2.2 that the two insect species, HF and BSF, are efficient at reducing faecal waste. This indicates great future potential, due to the increase of faecal matter caused amongst others, by the growing population (Banks *et al.*, 2014). It is reported that the BSF larvae can consume organic waste quicker than the housefly (Kim *et al.*, 2011), making it a better insect for waste reduction. This can be ascribed to the high concentration of digestive enzymes in the BSF larvae (Jeon *et al.*, 2011). The residue that is left over once the bulk of the manure has been consumed can then be used as a soil amender (Newton *et al.*, 2005; Sheppard *et al.*, 2007). Even if the larvae cannot be used as a feed source, it still holds great potential for other products or just as a waste management tool (Newton *et al.*, 2005; Diener *et al.*, 2009; Li *et al.*, 2011; Čičková *et al.*, 2012; Wang *et al.*, 2013).

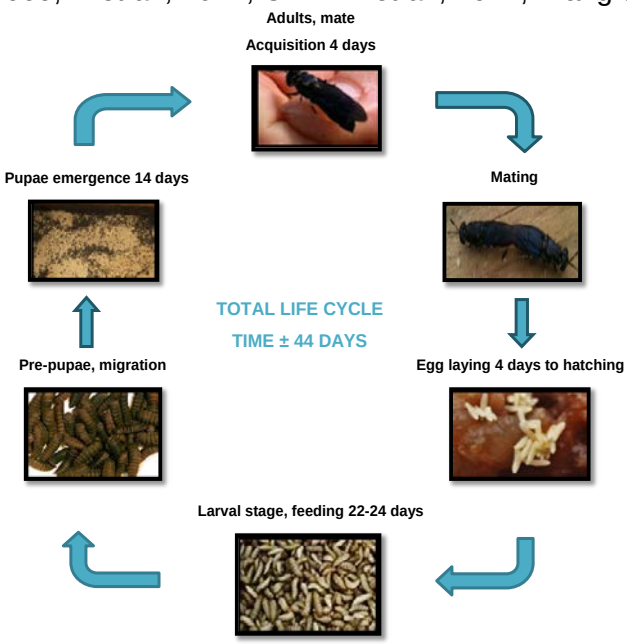

Figure 2.3 Life cycle of the black soldier fly (*Hermetia illucens*) (Alvarez, 2012).

Faecal sludge treated with BSF larvae has been shown to reduce viruses within pig and human faeces to below detection limits within just two weeks (Lalander *et al.*, 2014). Black soldier fly larvae feeding on cow manure reduced E. coli levels (Liu *et al.*, 2008) whilst larvae fed on pig manure also reduced Salmonella spp. levels significantly (Lalander *et al.*, 2014). This is promising for future endeavors with regard to waste, especially faecal waste management (Newton *et al.*, 2005; Diener *et al.*, 2009; Li *et al.*, 2011; Čičková *et al.*, 2012; Wang *et al.*, 2013).

As mentioned above, the amount of manure/faeces is not going to decrease with the increase in world population and shift in Asian diets (Li *et al.*, 2011; Wang *et al.*, 2013). Therefore, the ability of these insects to break down this manure and produce a product that is high in energy and protein is very lucrative and beneficial (Teguia & Beynen, 2005).

Table 2.2 Biomass yield and waste reduction by the housefly (*Musca domestica*) and black soldier fly (*Hermetia illucens*) of different studies

Species	Feed source	Total amount of feed	Residue	Waste Reduction	Yield	FCR	Source
H. illucens*	MOW	151kg	48kg	68%	17.8kg	14.5	Diener (2009)
H. illucens*	Pig manure	68kg	42kg	~39%	~2.7kg	9.6	Newton *et al.* (2005)
H. illucens**	Chicken manure	5240kg	~2,620kg	~50%	196kg	13.4	Sheppard *et al.* (1994)
H. illucens**	Human waste	720.5g	327.1g	55%	131g	3	Banks *et al.* (2014)[a]
M.domestica**	Chicken and cow manure	125kg	95kg	25%	3kg	10	Morgan & Eby (1975)

*Dry weight
**Wet Weight
([a])High density, once fed
FCR - Feed conversion ratio
MOW - Municipal organic waste

2.5 Poultry nutrition

When formulating poultry diets it is important to take into account the different nutritional aspects that need to be met, this includes energy, protein and amino acid requirements, as well as the vitamin and mineral composition. Due to the small size of theintestinal tract of poultry, their diets need to be nutrient dense (Uushona, 2015). Monogastric animals such as chickens get their dietary energy from simple carbohydrates, fat and proteins; as they cannot break down insoluble fiber (Hetland *et al.*, 2004; Ravindran, 2013). However, when diets containing insoluble fiber are fed to poultry in limited quantities, it helps to improve feed

utilisation (Hetland *et al.*, 2004). Anti-nutrients such as non-starch polysaccharides (Rebolé*et al.*, 2010), protease and trypsin inhibitors (Clarke & Wiseman, 2000), mycotoxins (aflatoxins and ochratoxins) (Huff *et al.*, 1980; Awad *et al.*, 2006), adversely affect the feed utilisation when they are present within the diets.

The diets of broilers are formulated in such a way to achieve optimum growth and reach market weight at 30-35 days of age (Butcher & Nilipour, 2002). The feed cost in a broiler production system is the most expensive part; therefore if money can be saved without losing efficiency then it would be very lucrative and advantageous to the livestock agricultural industry and consumer (Teguia & Beynen, 2005). Previous studies have made use of larvae meal in broiler diets to research the production parameters (Teguia *et al.*, 2002; Awoniyi *et al.*, 2003; Adeyemi & Adeyemo, 2007; Pretorius, 2011; Uushona, 2015), as well as the digestibility coefficient of the larvae meal (Zuidhof *et al.*, 2003; Hwangbo *et al.*, 2009; Pretorius, 2011; Uushona, 2015). These studies indicated that diets containing larvae meal achieve good production parameters and digestibility values when compared to traditional diets or protein sources, with soybean being the most widely used protein source (Khusro *et al.*, 2012).

There is little published data about the use of larvae in layer diets, there is however the study by Agunbiade *et al.* (2007) who added BSF larvae into the feed of layers. The effect of replacing fishmeal in a cassava base layer diets was investigated. The results were promising with regard to substituting larvae meal into the diet as a potential protein source (Agunbiade *et al.*, 2007). It is, however, uncommon to add fishmeal to a layer diet, due to trimelthylamine (TMA) that is found in the oxidized form of TMA oxide in fish meal, which leads to a fishy taint in eggs (Pearson *et al.*, 1983). The results obtained indicate that there are positive results when adding larvae meal to layer diets (Agunbiade *et al.*, 2007). The layers also had production levels on par with that of layers fed commercial diets (Agunbiade *et al.*, 2007).

2.6 Dietary protein

One of the main aspects of broiler feed would be the protein content and the quality thereof (Ellinger, 1958). The other aspect being the metabolisable energy (ME) content of the feed (Zaman *et al.*, 2008). The ratio of these two nutritional aspects are important, because the balance can influence the nutrient utilisation (Aletor *et al.*, 2000; Ravindran, 2013). An excess of protein within a diet can cause the metabolism of the chick to catabolise the excess of amino acids (AA), thereby using energy other than for growth; this is similar to the effect of a low protein diet (Kidd *et al.*, 2001). An excess of energy in the diet, however, cause reduced feed intake, which causes decreased growth levels. Therefore, it is important to have a feed that has balanced energy and protein ratio (Aletor *et al.*, 2000; Ravindran, 2013).

Protein is made up of amino acids which are the building blocks of muscle (Boland *et al.*, 2013). The main goal behind the protein of the feed is to provide AA for muscle growth

(Boland *et al.*, 2013). The protein quality is determined by the dietary AA that can be digested and absorbed to facilitate metabolic processes (Boland *et al.*, 2013). The ideal protein and AA composition for humans, pigs, poultry and Nile tilapia are shown in Table 2.3. Synthetic AA are added to the feed to make up the ideal AA content. It has previously been shown that by adding synthetic AA into the feed, can reduce the crude protein inclusion level in the diet by 2% (FAO, 2004). To achieve optimal performance levels, it is important to formulate diets as close to the ideal AA requirement as possible (Boland *et al.*, 2013). Not all the AA needed by the animal for growth can transformed in the body through the process of transaminase and therefore it must be obtained from other sources (Boland *et al.,* 2013).

Table 2.3 Crude protein requirement (% dry matter) and ideal amino acid pattern (g/g lysine) of essential amino acids for growth of different species (adapted from Boland *et al.*, 2013)

Nutrients	Human	Pig	Poultry	Nile tilapia
Crude Protein	10-15.00g	15-29.00	18.-23.00	30
Arginine	-	0.38	1.10	0.82
Histidine	0.33	0.32	0.32	0.34
Isoleucine	0.67	0.54	0.73	0.61
Leucine	1.30	1.00	1.09	0.66
Lysine	1.00	1.00	1.00	1.00
Methionine	0.33	0.27	0.38	0.52
Phenylalanine	0.83	0.60	0.65	0.73
Threonine	0.50	0.64	0.74	0.73
Tryptophan	0.13	0.18	0.18	0.19
Valine	0.87	0.68	0.82	0.55

The digestibility of protein and AA are very important and is determined by the protein source, while also depending on the animal (Boland *et al.*, 2013). The digestibility of a protein and AA differs between animals, each having different digestibility values (Boland *et al.*, 2013). The crude protein levels and the digestibility thereof for different insect meals are depicted in Table 2.4. Insects show good digestibility and high crude protein values, which indicate that it could be a good alternative source of protein. Processing methods also affect the bioavailability of protein in animal feed. For instance, acid and heat can cause denaturation of protein which affects the quality (Boland *et al.*, 2013). Lysineis especially prone to denaturation by extreme heat during processing and is susceptible to the Mailard reaction in the pressence of carbohydrates, reducing the availability for use in the animal (Parsons, 1996).

Table 2.4 Protein content and digestibility values of the protein of different sources within broiler chickens (%).

Protein source	Crude Protein (%)	Apparent digestibility (%)	Reference
Plant products			
Soya bean meal	49.44	85-87.00	Boland *et al*. (2013)
Animal by-products			
Fish meal	60.20	91.30	Pieterse *et al*. (2014)
Insect products			
HF larvae meal	60.38	69.00	Pieterse *et al*. (2014)
HF pupae meal	73.26	79.00	Pieterse *et al*. (2014)
Field cricket	58.30	92.90	Wang *et al*. (2005)
BSF larvae meal	~26.00	86-91.00	Uushona (2015)

HF - Housefly (*Musca domestica*) BSF - Black soldier fly (*Hermetia illucens*)

As mentioned before, the AA content is more important than the crude protein level, due to the possible interactions of AA. Therefore, feeds are formulated based on the availability of the AA in the feed source (Lemme *et al.*, 2004). The AA profile for materials included in the feed must be specific to not have an excess or lack of certain AA (Boland *et al.*, 2013). Considering this, an excess or lack can lead to inhibition of growth especially where methionine, lysine and threonine are concerned. The first limiting amino acid in poultry diet is methionine, the second limiting AA being lysine and threonine being the third limiting AA (Ravindrad, 2013). When formulating diets lysine is used as the reference AA, due to lysine being less affected by metabolic functions and metabolic conversions, unlike methionine (Lemme *et al.*, 2004). It is important to note that an excess of methionine can be toxic, while an excess of lysine may cause anatagonism with other AA. Arginine and Lysine have an antagonistic relationship, but it is prevented when it is fed in a ratio of 1:1 (Austic & Scott, 1975). The interaction of lysine and arginine in animal nutrition is a complex process, but an excess of lysine has three basic consequences. The first being lysine competes with arginine in the renal tubules which cause a reduction in arginine retention (Jones *et al.*, 1966). The second being that the levels of lysine in the diet of poultry causes an increase in renal arginase activity, that causes an increase in the oxidation of arginine (Summers & Leeson, 1997). The third consequence is that smaller amounts of lysine can cause a depression of the hepatic glycine transamidinase activity in chicks (Jones *et al.*, 1966). It is, therefore, due to this antagonistic relationship that the ratio for these two AA should be 1:1 (Chamruspollert *et al.*, 2002). The increase of lysine in the diet can also cause an increase in urea excretion and can slightly increase arginine excretion (Austic & Scott, 1975). It has been observed that when lysine exceeds 3% in the diets the above-mentioned reactions happen (Austic & Scott, 1975). However, these amino acids, particularly the synthetic AA are expensive and the monogastric poultry industry is continuously looking for better, more balanced sources of AA.

2.7 Alternative feed ingredients

The feed cost is the dominating factor associated with broiler production, therefore, reducing the cost by finding different feed ingredients that are less expensive than original, but with same nutrient value would be more profitable (Teguia *et al.*, 2002). Factors earlier mentioned (growth of world population, climate change, shift in Asian diets), have all had a negative effect on the global grain price, which then would affect and place stress on livestock production (Dar & Gowda, 2013). It is important, however, for the alternative ingredient to be easily accessible and not as costly as the original, but the quality must not be lower (Teguia *et al*., 2002). There are many previous studies that have indicated positive results when adding insects into animal feeds as an alternative protein source (Teguia *et al*., 2002; Ogunji *et al*., 2008a, b; Ijaiya & Eko, 2009; Sealey *et al*., 2011). It can be observed in Table 2.5 that insect meals are a goodsource of protein and AA. Previous studies have indicated positive results when adding insects into animal feeds as an alternative protein source (Teguia *et al.*, 2002; Ogunji *et al.*, 2008a, b; Ijaiya & Eko, 2009; Sealey *et al.*, 2011).

Insects are abundant and their numbers surpass that of humans and animals combined (Rumpold & Schlüter, 2013). This could be attributed to their ability to easily adapt and thrive in harsh environments (Rumpold & Schlüter, 2013). It is reported that there are about 2040 edible insect species worldwide (Jongema, 2015). From these edible insects it is reported that 500 species are being used as food for humans in Northern Africa, Asia, Australia and Latin America (Defoliart, 1989).

2.7.1 Housefly (*Musca domestica*) meal

The first evaluation of HF meal in poultry diets was by Calvert *et al.* (1969) and the research indicated that HF meal could partially substitute soybean meal. The use of HF larvae meal and HF pupae meal in broiler diets has also been investigated and it was found that both were highly digestible (Pieterse *et al.*, 2014). Broilers fed HF meal as protein source in a balanced diet was shown to have higher live and carcass weights, with no sensory differences (Pieterse *et al.*, 2014).

Trials done on Oreochromis niloticus (Nile Tilapia) have revealed that HF meal had good AA profile and the results obtained, were closely related to those of fish fed fishmeal (Ogunji *et al.*, 2008a). Another study indicated that with an increase of HF meal into the diets of O. niloticus, that there was a decrease in feed conversion ratio (Ogunji *et al.*, 2008b). Therefore, it is beneficial as less feed is needed to grow the tilapia (Ogunji *et al.*, 2008b). Ogunji *et al.* (2008b) also revealed that a diet containing an inclusion level of 15% can result in acceptable performance ratios. No significant differences in catfish fed HF meal and a commercial feed on the basis of growth parameters and nutrient utilisation were reported (Aniebo *et al.*, 2009). Aniebo *et al.* (2009) also revealed that catfish fed 50% and 100% HF maggot meal had no

significant differences regarding carcass quality and sensory characteristics when compared to a commercial feed. These studies again indicating that cheaper insect feedstuffs can still produce similar or better, production performance in various aquaculture species.

Table 2.5 Comparison of the nutritional content of different insect meals and fish and soya bean meal on dry matter basis.

Parameters	FM[a]	SCM[a]	M[b]	FC[c]	GH[d]	SWC[e]	HFP[f]	BSF[g]
Proximate analysis (%)								
Crude protein	69.13	49.44	55.10	58.30	53.58	50.30	76.23	43.20
Crude fat	10.11	0.90	20.70	10.30	26.52	16.43	14.39	28.00
Crude fibre	0.54	7.87	6.30	8.70	9.21	10.90	15.71	-
Ash	-	5.90	10.40	2.96	4.31	12.03	7.73	16.60
Amino acids (%)								
Lysine	3.57	3.05	2.92	4.79	-	5.02	4.92	2.21
Methionine	1.09	0.70	-	1.93	-	3.02	1.37	0.83
Threonine	1.47	1.95	-	2.75	-	4.50	2.31	1.41
Mineral content (%)								
Ca	1.34	0.33	-	-	-	1.05	0.52	5.36
P	1.77	0.73	-	-	-	2.77	1.72	0.88

Fishmeal (FM); Soya oil cake meal (SCM); Maggot meal (M); Field cricket meal (FC); Grasshopper meal (GH); Silkworm caterpillar (SWC); Housefly pupae meal (HFP); Black soldier fly pre-pupae meal (BSF)
Source: [a]NRC (2004); [b]Awoniyi *et al.*(2009); [c]Wang *et al.*(2005);[d]Hassan *et al.*(2009);[e]Ijaiya& Eko(2009); [f]Pieterse& Pretorius(2014);[g]Newton *et al.* (2005).

Other studies indicated that HF maggot meal fed to broilers with an inclusion level of 5, 10 and 15% had no significant differences with regards to carcass characteristics (Teguia *et al.*, 2002). The Metabolisable energy (ME) level was also higher than that of fishmeal (Teguia *et al.*, 2002). Awoniyi *et al.* (2003) determined that HF larvae meal is a suitable substitute in broiler diets and can replace fishmeal up to 25%, without any negative effects on the growth parameters and carcass characteristics. Diets containing more than 25% HF larvae meal has been shown to cause a reduction in feed intake (Pretorius, 2011) due to the high protein level of the feed. Fishmeal was substituted with 10% HF larvae meal in previous studies and yielded better live and carcass weights (Pieterse *et al.*, 2014). The HF larvae meal also produced heavier breast and thigh muscles than the of the fishmeal diet (Pieterse *et al.*, 2014).
Therefore, HF meal shows great promise as a substitute in broiler feeds and other animal feeds (Pieterse *et al.*, 2014).

2.7.2 Black soldier fly meal

Bondari and Sheppard (1981) evaluated BSF larvae as feed ingredient in tilapia and channel catfish diets and reported no significant differences in fish production/performance between diets. No significant differences were found in the aroma and texture of the meat from

the fish fed diets containing BSF larvae (Bondari & Sheppard, 1981). This indicates that BSF larvae meal is a suitable feed ingredient for livestock (Bondari & Sheppard, 1981; Sheppard *et al.*, 2007; Uushona, 2015). Bondari & Sheppard (1981) concluded that the larvae needs to be crushed in order for the fish to consume it, otherwise feed refusal occurs. Since then studies have been conducted on broiler chicks where BSF larvae meal was added to the feed (Uushona, 2015). Promising results were obtained, with the production parameter values achieving levels on par with that of a commercial diet (Uushona, 2015).

2.7.3 Other insect meals

The field cricket was another insect that has been evaluated in broiler diets to investigate if it can be substituted for fishmeal (Wang *et al.*, 2005). The field cricket was added at a 15% inclusion level and no treatment effects on growth were noted (Wang *et al.*, 2005). It also had better AA digestibility coefficient of 92.9%, compared to the 91.3% of fishmeal (Wang *et al.*, 2005). Grasshopper meal is another suitable feed ingredient, with research indicating positive results when substituting fishmeal (50%) or totally (100%) in broiler starter diets (Hassan *et al.*, 2009). The silkworm (Anaphe infracta) caterpillar was also researched and it was found to be a suitable replacement for fishmeal (Ijaiya & Eko, 2009).

The general conclusion with adding insect meals to animal feed as protein source seems to be positive with regards to growth parameters and carcass characteristics (Wang *et al.*, 2005; Hassan *et al.*, 2009; Ijaiya & Eko, 2009; Pretorius, 2011; Uushona, 2015). In most referenced cases, better results than the commercially used feed ingredient have been reported (Pieterse *et al.*, 2014). To produce insect meal, organic waste is needed and previous studies have indicated that the waste can be reduced up to 50% (Diener, 2009; Sheppard *et al.*, 1994; Banks *et al.*, 2014). This indicates great potential in the waste management sector, as well as animal feed sector (El boushy, 1991; Sheppard *et al.*, 1994, Diener *et al.*, 2009; Li *et al.*, 2011).

2.8 Conclusion

Alternative protein sources have been evaluated for their nutrient value, due to the rise in fishmeal prices and the competition from aquaculture. Currently, fishmeal is the main animal protein source used worldwide, with soybean meal being the major plant based protein source utilised. Both of these are good (well-balanced AA) protein sources, however, both as discussed are experiencing pressure from other users. Scientists are therefore researching the suitability of insects as a potential alternative protein source, but at the same time also their potential use for waste management.

Previous studies have indicated that insects are quite effective at utilising organic wastes of any kind, while producing a product that is high in fat and protein. This product, being the

larvae, can be used in the biofuel industry, livestock feed and aquaculture. This is due to their good nutrient composition and amino acid composition. Studies have also indicated that the digestibility coefficient values rival that of commercial feed sources and in some cases reach higher digestibility values. The nutrient capacity combined with the waste management potential of the larvae, places insect meals in a good light. Studies have indicated that the larvae are able to reduce animal manure and human faecal matter. With these studies it was also observed that the faecal coliform levels were reduced, this being advantageous from a health perspective. The BSF is receiving attention in both animal feed and waste management, as it has proven to be quite effective in both these areas. However, further research is still lacking on the potential of BSF as a suitable replacement in poultry diets to yield a meat product that is safe for human consumption.

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Chapter 3. Comparison of production parameters of broilers fed diets containing black soldier fly (*Hermetia illucens)* pre-pupae grown on human faecal matter, processed with different treatments

Abstract

This study investigated the effect of processing of black soldier fly (*Hermetia illucens)* pre-pupae meal grown on human faecal matter as a protein source in broiler diets (10% inclusion level), with regards to their production parameters. Eight different treatment methods were used on the pre-pupae to assess the potential to remove harmful pathogens and make it safe as a protein source; 62°C for 30min, 62°C for 60min, 72°C for 5min, 72°C for 15min, 100°C for 2min, 100°C for 5min, rinsed in 5% propionic acid and rinsed in 5% formic acid. Seven hundred and twenty, day-old Cobb 500 broiler chicks were randomly allocated to nine treatment groups, the ninth being the control (commercial diet with no pre-pupae larval meal) and each treatment group was replicated eight times. The chicks were raised to slaughter at 29 days of age. There were no treatment differences regarding the cumulative feed intake and average daily gain (ADG). Weekly feed intake only differed significantly around day 22. Treatment differences were observed between the pre-pupae diets and the control, with the pre-pupae diets achieving better results with regards to feed conversion ratio (FCR), protein efficiency ratio (PER), european production efficiency factor (EPEF) and live weight (at day 29, end of trial). There were no significant differences between the pre-pupae groups in these respective parameters. It can be concluded that BSF pre-pupae grown on human waste can successfully be used as a protein source in broiler diets.

Keywords: *ADG, Black soldier fly, Broilers, EPEF, FCR, Human waste, PER, Production, Nutrient recycling*

3.1 Introduction

One of the important aspects to be considered in the formulation of broiler diets is the protein content, but of even more importance is the amino acid (AA) composition (Ellinger, 1958). Considering a broiler diet, a balanced AA profile is important as it regulates the growth regardless of the protein percentage (Deschepper & DeGroote, 1995; Aletor *et al.*, 2000; Rezaei *et al*., 2004; Corzo *et al*., 2005). However, when formulating diets for a specific livestock species, it is necessary to consider the cost of that formulated feed (Téguia *et al*., 2000; Teguia & Beynen, 2005). More than 50% of the costs surrounding broiler production are due to the cost of the feed (Teguia & Beynen, 2005). In recent years, there has been a rise in cost of the protein portion of the feed and therefore it has become important to find good quality alternative protein sources, that are affordable and sustainable (Téguia *et al.*, 2002).

The two protein sources most widely used in broiler diets are soybean meal and fishmeal (Khurso *et al*., 2012). The use of soybean meal in animal nutrition poses a problem, because there can be competition between the use of grains for livestock rearing and human consumption (Teguia & Beynen, 2005; Khusro *et al*., 2012). Soya bean has also been considered for use in biofuel production due to its availability and the ease of fat extraction (Biswas *et al*., 2011). At the same time, global warming also affects the global grain yield and indirectly affects the livestock industry that depends on grain for feed (Dar & Laxmipathi Gowda, 2013). The increase in global temperature is negatively affecting grain yield due to the rise of occurrences of certain factors such as floods, droughts, pests and diseases (Vergé, 2007). It is also noteworthy that due to growth of world population the effect of urbanisation is also affecting grain yield (USCB, 2004; Pimentel & Pimentel, 2007) as less land becomes available for grain plantations (USCB, 2004; Pimentel & Pimentel, 2007). All these factors have caused an increase in the cost of proteins and other feed ingredients (Téguia *et al*., 2002). Fishmeal is the other protein source that is widely used due to its high protein quality and desirable amino acid composition (Olsen & Hasan, 2012). However, there has been an increase in the use of fishmeal in aquaculture and this has caused competition between the livestock industry and aquaculture for fishmeal (Olsen & Hasan, 2012). This “shortage” of fishmeal is exacerbated by the fact that global fish landings have decreased the past few years (Watson *et al*., 2014).

Alternative sources of protein that have shown potential as feedstuffs in livestock feed in recent years are insect meals, due to their high protein levels (36.2% - 76.2%) and desirable amino acid composition (Newton *et al*., 2005; Hassan *et al*., 2009; Ijaiya & Eko, 2009; Barroso *et al*., 2014; Pieterse & Pretorius, 2014). The viability of insect meals as an alternative protein source and a replacement to fishmeal has been researched (Okah & Onwujiariri, 2012; Pieterse & Pretorius, 2014). Studies have indicated that larvae meal made from the common housefly (*Musca domestica*; HF) is a viable protein source for use in broiler diets (Téguia *et al*., 2002; Awoniyi *et al*., 2003; Ogunji *et al*., 2006; Agunbiade *et al*., 2007; Hwangbo *et al*., 2009; Pretorius, 2011). Previous studies using black soldier fly (*Hermetia illucens;* BSF) larvae meal have also indicated increased performance when added to poultry feed, as opposed to diets containing either fishmeal or soybean meal as the protein source (Sealey *et al*., 2011; Okah & Onwujiariri, 2012; Pieterse & Pretorius, 2014).

Organic waste is a good growth medium for rearing insect larvae, as it produces larvae meal that is high in protein and suitable for poultry and fish diets (Ogunji *et al.*, 2008a, b; Pieterse & Pretorius, 2014). Both the common housefly and the BSF larvae have been used to break down other organic wastes, such as faecal matter (Sheppard *et al*., 1994; Erickson *et al*., 2004; Wang *et al*., 2005; Li *et al*., 2011; Čičková *et al*., 2012). The BSF is a non-pest species, unlike the common HF and therefore it can be used safely to break down faecal

matter without any environmental risks (Kim *et al*., 2011). Even though studies reported that BSF larvae can grow and break down human faecal matter (Banks *et al.*, 2014; Lalander *et al*., 2013; Lalander *et al*., 2014), no studies could be found indicating that these larvae were taken further and added to livestock diets. Previous studies have reported that the organic waste consumed by the larvae during growth can affect the nutritional content of the larvae, specifically the fat and fatty acid content (Ramos-Elorduy *et al*., 2002).

This then raises the question of whether pre-pupae grown on human faecal matter could still achieve positive broiler production parameters. Another important aspect that also warrants further study is the preparation of the pre-pupae and/or larvae meal prior to feeding to livestock. Typically, one of the pre-processing steps would be decreasing the moisture content of the larvae and may also sometimes include de-fattening of the meal. By reducing the moisture content, the chances of feed spoilage through microbial growth is reduced. Defattening would also help with better distribution of the pre-pupae meal within the feed during the mixing. In the present investigation the effect of various pre-processing methods of BSF pre-pupae are evaluated by feeding the end products to broilers over a 29-day growthcycle.

3.2 Materials and methods

3.2.1 Pre-pupae treatments

Growth period

The pre-pupae were grown at Mariendahl Experimental farm of Stellenbosch University. Pre-pupae growth was done over a period of 8months in large bins. The growth media of the pre-pupae consisted of a ratio of 60:40 kitchen waste to human faecal matter. Each bin comprised of 80kg growth media, of which 48kg consisted of kitchen waste and 32kg consisted of human faecal matter. The kitchen waste was obtained from the residences of Stellenbosch University and consisted of any leftover or spoiled organic materials, such as cooked food, salad, old fruit and cooked meat. The faecal matter was obtained from Klipheuwel in the Western Cape, an informal settlement close to Durbanville area, where portable sanitation units are used, which facilitated the collection of the faecal matter.

The ratio of BSF eggs to feed media that was added to each bin was 1g of eggs to 10kg of food. However, it is important to note that the eggs weren't added to the bins initially, they were first hatched and at six days of age, the larvae were added to the allotted waste media. The bins were mixed (turned over) at day 8 of growth, to ensure that all the food became exposed to the pre-pupae. The larvae were left in bins for 12 days, after which they were harvested.

After harvesting the pre-pupae were purged (removed from any feedstuff) for 24hr in order to clear the gut. After this purge period they were moved to the treatment area for processing.

Processing treatment

The pre-pupae were grown on a feed media that typically contained high concentrations of faecal coli-forms, which if left untreated would lead to major health problems. The treatment methods of the pre-pupae are meant to remove these faecal coli-forms and make the pre-pupae safe for animal consumption. Each treatment method's effect on the pre-pupae microbiological load was measured as was the effect of the treatments tested on the broilers to determine which treatment method achieves the best results. The treatments differed according to temperature and time of heat application and/or chemical additives. The pre-pupae were divided into eight treatment (TRT) methods:

Trt1 - 62°C for 30min

Trt2 - 62°C for 60min

Trt3 - 72°C for 5min

Trt4 - 72°C for 15min

Trt5 - 100°C for 2min

Trt6 - 100°C for 5min

Trt7 - Rinsed in a 5% Formic acid solution

Trt8 - Rinsed in a 5% Propionic acid solution

Heat based pre-pupae treatments (1 to 6) were done in cooking pots with a volume of 2.5L. The cooking pots were filled with water halfway and then placed on a hot plate. The temperature was measured constantly until the correct temperature was achieved when the pre-pupae were added. The treatment period/time was deemed to start once the correct temperature level was achieved again after the pre-pupae had been added and the temperature was then kept constant for the specific treatment period. The acid treatments (7 and 8) were done in plastic buckets with a volume of 5L. Each bucket contained 3L of tap water and 150ml of the appropriate acid. The pre-pupae were added and kept in the acid for 10 minutes. It was however necessary to stir each bucket 2 times during this period in order to prevent the pre-pupae from crawling out of the bucket. These pupae were then killed by freezing at 16°C for 24hours.

Following the respective treatments, the pre-pupae were dried in a convection oven at 80°C for 48 hours. The dried pre-pupae were removed from the oven, minced and frozen (16°C) until they were mixed into the feed.

3.2.2 Microbial testing

A representative sample of BSF pre-pupae were selected at random for microbiology testing. This was to determine the microbial benchmark of the pre-pupae and if the pre-pupae post-drying was safe for animal consumption. Pre-pupae were selected i) post-harvest, ii) after the processing treatments and iii) after drying. For microbiology testing, individual pre-pupae were placed in a 1ml physiological salt solution in a 1.5ml Eppendorf tube. The pre-pupae were then crushed with an eppi pestle. From the slurry, a dilution series was then prepared (10^{-2} to 10^{-5}) that were plated on four different media plates (Table 3.1) prior to incubating for 48hrs at 30°C.

Table 3.1 Selective growth media utilised for the identification of pathogens found on the black soldier fly pre-pupae

Growth Media:	Supports growth for:
Nutrient agar (NA)	Large number of microbial species
Salmonella/Shigella agar (SSA)	Salmonella and Shigella species
Eosin methylene blue agar (EMB)	Enterobacteriaceae, which include E. coli
Listeria selective (Lis)	*Listeria* species

3.2.3 Chickens and housing system

The trial consisted of 720, day-old Cobb 500 broiler chicks as hatched. The management practices described by Cobb International were followed (Cobb, 2008). The chicks were housed at the poultry section of the Mariendahl Experimental farm of Stellenbosch University. The house was temperature controlled, with ventilation being set to provide a minimum of six changes per hour. The experimental house was equipped with 120 wire cages measuring 0.9m x 0.6m, each of which contained two nipple drinkers and one tube feeder. The chicks had *ad libitium* access to feed and water. The cages were checked twice daily for mortalities and morbidities. The 720 broiler chicks were randomly divided into 72 cages using a random block design, representing nine treatment diets with eight replications per treatment and ten chicks per replication (cage).

Experimental diets

The chicks were randomly assigned to nine different treatment diets, comprising of a starter, grower and finisher phases. The ingredient and the nutrient compositions are shown in Table 3.2. The diets were formulated so that the chicks were maintained on the minimum nutrient specifications as provided by the Cobb 500 International (Cobb-Vantress, 2012). The treatment diets were formulated with a 10% inclusion level of pre-pupae meal in the first

Table 3.2 Ingredients and calculated nutrient composition of the starter, grower and finisher diets which include 10% black soldier fly (*Hermetia illucens*) pre-pupae as fed to the broilers.

		Starter		Grower		Finisher	
	Unit	Diet 1-8 (10% LM)	Diet 9 (Control)	Diet 1-8 (10% LM)	Diet 9 (Control)	Diet 1-8 (10% LM)	Diet 9 (Control)
Ingredients:							
Hermetia illucens pre-pupae	%	10.0	-	10.0	-	10.0	-
Maize	%	41.54	47.76	47.70	48.55	51.21	49.64
Soybean full fat	%	18.83	32.10	20.07	41.37	26.47	37.33
Soybean	%	19.26	7.72	11.68	-	-	-
Maize gluten	%	-	-	2.98	4.80	7.73	7.52
Fish meal	%	3.63	9.25	-	-	-	-
L-lysine	%	0.17	0.06	0.30	0.28	0.25	0.16
DL-methionine	%	0.38	0.36	0.36	0.32	0.18	0.19
L-threonine	%	0.11	0.08	0.11	0.09	0.02	-
Vitamin + mineral premix*	%	0.45	0.45	0.45	0.45	0.45	0.45
Limestone	%	0.30	1.17	0.28	1.50	0.24	1.45
Salt	%	0.11	0.08	0.18	0.25	0.19	0.27
Monocalcium phosphate	%	1.13	0.87	1.24	1.44	1.10	1.32
Sodium bicarbonate	%	0.21	0.11	0.24	0.16	0.22	0.13
Oil - sunflower	%	3.88	-	4.46	0.80	1.94	1.56
Calculatednutritionalcomposition:							
Dry matter	%	89.54	88.73	89.32	88.70	88.93	88.69
AMEn** chick	MJ/kg	12.65	12.65	13.20	13.20	13.40	13.40
Crude protein	%	26.00	26.00	23.00	23.00	23.00	23.00
Lysine	%	1.61	1.63	1.38	1.38	1.22	1.22
Methionine	%	0.78	0.82	0.67	0.68	0.57	0.58
Cysteine	%	0.42	0.40	0.40	0.40	0.41	0.41
Methionine + cystine	%	1.20	1.21	1.07	1.08	0.98	0.99
Threonine	%	1.08	1.09	0.95	0.96	0.86	0.87
Tryptophan	%	0.30	0.29	0.24	0.24	0.22	0.23
Arginine	%	1.68	1.70	1.40	1.43	1.28	1.38
Isoleucine	%	1.13	1.17	0.97	1.02	0.96	1.02
Leucine	%	2.06	2.18	2.03	2.23	2.33	2.41
Histidine	%	0.70	0.70	0.61	0.61	0.60	0.60
Phenylalanine	%	1.15	1.13	1.06	1.09	1.09	1.12
Tyrosine	%	0.99	0.89	0.90	0.85	0.92	0.88
Phenylalanine + tyrosine	%	2.14	2.02	1.96	1.94	2.02	2.00
Valine	%	1.27	1.31	1.10	1.12	1.10	1.14
Ash	%	4.26	5.23	3.41	4.22	3.12	4.03
Crude fibre	%	3.38	3.20	3.25	3.42	3.17	3.26
Crude fat	%	13.89	8.79	14.54	10.43	13.34	10.56
Calcium	%	1.05	1.05	0.90	0.90	0.85	0.85
Phosphorous	%	0.97	0.82	0.90	0.75	0.85	0.72
Available phosphorous	%	0.50	0.50	0.45	0.45	0.42	0.42
Sodium	%	0.16	0.16	0.16	0.16	0.16	0.16
Chloride	%	0.23	0.23	0.23	0.23	0.23	0.23

* Vitamins and minerals are included according to the levels suggested by the National Research Council, 1994.
**AMEn - Apparent metabolisable energy corrected for nitrogen

eight treatment diets and the ninth treatment being the control, which contained no pre-pupae meal.

The treatment diets were representative of diets used commercially. The control diet consisted of a maize/soya diet, an internationally accepted mixture suitable for broiler production. The treatment diets were allocated so that the chicks would receive about 900g of starter, 1200g of grower and 1200g of finisher per bird.

Data collection and analysis

Body weights of broilers were determined at day old and weekly thereafter. Feed was supplied *ad libitum* and weekly intake was determined. Growth and intake data were used for the calculation of FCR, ADG, PER (Boling-Frankenbach *et al*., 2001) and the EPEF. The formulae used are shown in Equation 3.1, Equation 3.2, Equation 3.3 and Equation 3.4.

Statistical analyses were done using STATISTICA, Version 9, by Statsoft Inc. (2009). Where age effects were not a variable, the statistics were done by using one-way analysis of variance (ANOVA) with a Bonferoni least significant difference (LSD) *post hoc* test. Where age and treatments effects were variables, the statistics were done using mixed model repeated measure of ANOVA with a Bonferoni LSD *post hoc* test.

Equation 3.1

$$\text{Feed Conversion Ratio} = \frac{\text{Cumulative Feed Intake (g)}}{\text{Average Live Weight per Chick (g)}}$$

Equation 3.2

$$\text{Average Daily Gain} = \frac{\text{Average Live Weight per Chick (g)}}{\text{Age (days)}}$$

Equation 3.3

$$\text{Protein Efficiency Ratio} = \frac{\text{Weight Gain (g)}}{(\text{Weekly Feed Intake (g)} \times \text{Protein \% feed})/100}$$

Equation 3.4

$$\text{European Production Efficiency Factor} = \frac{\text{Liveability \%} \times \text{Live Weight (g)}}{\text{Age (days)} \times \text{FCR}} \times \frac{100}{1}$$

3.3 Results and discussion

Microbial results

The microbial results from the pre-pupae plating are shown in Table 3.3. It is clear that the benchmark/pre-treatment levels were noticeably high. The benchmark samples had no

prior treatment before sampling and givea good indication of the efficiency of the different treatment methods. The treatments caused a decrease in pathogen counts and there were also differences (P≤0.05) between the treatment methods, directly after the treatment. However, the bacteria numbers were still high in both dilution series (10^{-2} and 10^{-5}). On the other hand, after drying there were no differences (P>0.05) among the treatments. It is important to note that the average count of 250±0.0 indicates that the microbial concentration was too high to count and therefore indicates poor microbial reduction. However, post drying the levels were acceptable and deemed safe due to there being in most cases no more faecal coli-forms on the agar plates. Although microbial growth on the nutrient agar (NA) was noted, there is nocause for concern as this growth could be due to contamination during sampling and asthere wasno growth on the other growth media, this indicates that there was no faecal coli-forms present.As the results obtained indicate that the drying process was the most effective as pertaining to reducing the microbes, the question of whether the treatments were necessary at all for reduction of microbial load needs further research. However, the pre-drying may have had an effect on the nutritional values of the pre-pupae for broilers which needs to be evaluated further in the broiler production study.

Growth trial results

The results obtained during the broiler growth trial are displayed in Table 3.4. The first eight diets consisted of 10% inclusion of the dried pre-pupae meal, while the control diet consisted of maize/soya. There were no differences (P>0.05) between the nine treatments in average live weights at days 8, 15 and 22. There was however, a difference (P≤0.05) between the pre-pupae diets (Trt1-8) and the control diet (Trt9) at day 29, the end of the production period; these differences were only found between the pre-pupae diets and the control diet, but no differences were observed between the pre-pupae diets. It is, however, apparent that there is a trend that indicates that the heat based treatments achieved higher live weights at day 29, than the acid based treatments and it could be argued that if the sample size was bigger or the trial period had been longer, this may have become statistically significant. There were no differences (P>0.05) between the treatments when considering weekly feed intake on days 8, 15 and 29. There were however treatment differences (P≤0.05) between some of the diets at day 22. Treatment 2 (62°C for 60mins) and Trt7 (Formic acid) differed significantly (P≤0.05) between each other but not with the other treatments. The biological difference thatwas seen on day 22 is small and was not observed again at day 29. At days 8, 15, 22 and 29 no differences (P>0.05) between the treatments with regard to cumulative feed intake were observed.

There are no significant differences between the treatment diets with regards to ADG (Table 3.4). The small differences noted may be the reason for the significant differences

Table 3.3 Average (±standard error) microbial countsof the different treatment methods and growth media of dilution 10^{-2} and 10^{-5}.

Beforetreatment	NA	SSA	EMB	Lis
10^{-2}	250.0±00.00	250.0±00.00	250±00.00	250.0±00.00
10^{-5}	196.7±16.864	4.6±04.27	73.3±11.05	49.3±06.43

After treatment	Dilution	Treatment 1	2	3	4	5	6	7	8
NA	10^{-2}	239.2±10.83	243.7±06.33	238.9±11.111	224.4±17.60	238.9±07.64	232.9±54.85	223.3±15.07	250±00.00
SSA	10^{-2}	47.7^{a}±19.54	65^{a}±24.77	32.7^{a}±15.44	43.8^{a}±22.44	44.8^{a}±19.78	64.7^{a}±90.74	236.1^{b}±13.89	222.9^{b}±18.52
EMB	10^{-2}	238.1^{b}±11.89	211.4^{ab}±17.25	162.2^{b}±26.90	201.4^{ab}±22.30	183.2^{ab}±26.18	209.2^{ab}±87.18	236.1^{ab}±13.89	250^{ab}±00.00
Lis	10^{-2}	250.0^{a}±00.00	250.0^{a}±00.00	222.2^{a}±19.06	200.1^{a}±19.46	241.7^{a}±05.96	98.6^{b}±79.12	250^{a}±00.00	250^{a}±00.00
NA	10^{-5}	107.1^{ab}±21.24	90.1^{ab}±23.79	74.6^{ab}±21.27	91.7^{ab}±18.72	80.5^{ab}±26.24	72.3^{b}±17.77	134.3^{ab}±27.78	177.1^{a}±23.57
SSA	10^{-5}	0.0±00.00	0.0±00.00	0.0±00.00	0.4±0.34	3.6±02.98	4.2±4.17	24.9±14.84	21.1±16.57
EMB	10^{-5}	61.7±18.19	80.6±25.94	28.1±19.03	63.6±24.50	64.6±22.19	107.2±27.91	121.7±28.05	116.6±23.63
Lis	10^{-5}	53.5^{b}±21.79	15.8^{ab}±04.32	0.0^{a}±00.00	3.1^{a}±1.23	0.2^{a}±00.12	0.4^{a}±0.39	10^{ab}±1.09	113.6^{c}±21.11
After drying									
NA	10^{-2}	168.7±23.87	199.8±23.24	194.1±24.45	190.7±23.64	161.4±27.38	174.3±26.24	164.4±24.15	193.3±22.45
SSA	10^{-2}	35.7±19.56	2.6±01.24	28.4±19.01	8.1±5.25	15.6±13.89	16.7±11.73	22.7±11.97	15.3±12.56
EMB	10^{-2}	31.3±16.74	18.6±13.98	27.8±19.06	44.1±22.45	42.6±22.51	62.7±25.12	0±0	27.8±19.06
Lis	10^{-2}	0.9±00.45	1.9±01.03	1.9±01.38	1.4±0.64	0.8±00.44	0.4±0.32	0.1±0.06	0.1±0.11
NA	10^{-5}	116.1±25.59	78.2±23.04	79.2±25.74	125.2±27.61	110.9±25.80	88.2±26.05	89.9±25.15	131.4±27.07
SSA	10^{-5}	0.6±00.58	0.0±00.00	1.4±01.44	0.0±00.00	0.3±00.28	0.0±00.00	0.0±00.00	0.0±00.00
EMB	10^{-5}	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00
Lis	10^{-5}	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00	0.0±00.00

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

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

NA - Nutrient agar, SSA - Salmonella/Shigella agar, EMB - Eosin methylene blue agar, Lis - *Listeria* selective.

seen in live weight at the end of the trial (Table 3.4). It is reported that an ADG of 50g per day is required within a normal broiler production unit (Butcher & Nilipour, 2002). In this trial all the treatment diets reached this benchmark (57.5-60.2g), with the control also achieving the minimum ADG, while still being lower than the pre-pupae treatments. The ADG is comparable to results reported by Uushona (2015) with regards to there being no treatment differences.

However, when considering the diet of 10% inclusion, it is observed that the ADG in this current trial is comparable to that of Pretorius (2011). Treatment differences ($P \leq 0.05$) were found between the pre-pupae diets and the control with regards to end live weight (Table 3.4), unlike the study of Uushona (2015). The results with regards to end live weight are comparable to Pieterse and Pretorius (2014), who also achieved higher end live weights when feeding HF based diets than the control diet. Good production efficiency is achieved when an end live weight of 1.5-2kg is reached at 35d of age, with an ADG of 50g and higher (Butcher & Nilipour, 2009), which was achieved in this trial.

As pertaining to the FCR, no differences ($P > 0.05$) were found between the pre-pupae (Trt1-8) treatments, but significant ($P \leq 0.05$) differences were obtained between the pre-pupae treatments and the control (Trt9) diet. The control diet had the highest FCR (1.59) value of the all the treatment diets, however, it did have competitive FCR values early on in the trial. As the trial progressed, the pre-pupae treatments started exhibiting better FCR values and only in the last week did the control exhibit inferior FCR values, with there being significant differences. It is observed from Table 3.4 that the last week of the trial was the period where most of the weight was gained, therefore the lower the FCR value the better. It can then be argued that the pre-pupae treatments are a better protein source in the finisher diet of the broilers.

The results regarding FCR are comparable to previous studies where the pre-pupae diets reached superior FCR values ($FCR \leq 1.5$) than the control (Hwangbo *et al*., 2009; Pretorius, 2011) although Uushona (2015) had an inferior FCR. Butcher and Nilipour (2002) reported that a maximum FCR of 1.85 is necessary for normal broiler production, it is therefore apparent that achieving anything less would indicate positive results. The FCR is an indication of the efficiency with which nutrients are being used by the body of the animal, therefore FCR values as achieved in this trial and previous trials (Hwangbo *et al*., 2009; Pieterse & Pretorius, 2014) show efficient utilisation of the feed.

When considering PER no significant ($P > 0.05$) differences were found between the prepupae (Trt1-8) treatments, but there were differences ($P \leq 0.05$) between the pre-pupae treatments and the control (Trt9) diet. With treatments 1-8 having PER of ≥2.8 and the control 2.3. As with the other production parameters there seems to be a trend where the heat based treatments reached better values than the acid based ones. Similar to theFCR, the control diet under performed in the last week of the trial with regards to PER even though the control diet

did have a competitive PER values early on in the trial. The PER describes the protein utilisation by the animal, in this case the broilers (Johnson & Parsons, 1997). A low PER (≤1.5) is an indication of low protein quality and utilisation (Johnson & Parsons, 1997), with an optimum PER being 3:1 (Wilding *et al.*, 1968). The differences that were found between the pre-pupae diets and the control (Trt9), therefore, can be ascribed to better feed digestion and utilisation, particularly in the final week. Previous studies have indicated that insect meal has a high protein percentage and desirable AA composition (Newton *et al*., 2005; Hassan *et al*., 2009; Barroso *et al*., 2014; Pieterse & Pretorius, 2014) and it is known that a balanced AA profile is required in the feed for growth of a broiler.

There were no differences (P>0.05) between the pre-pupae diets with regard to EPEF, but as with the other parameters, there were significant differences (P≤0.05) between the prepupae treatments and the control (Table 3.4). A flock is regarded to have acceptable growth and liveability parameters when it attains an EPEF >260 (Butcher & Nilipour, 2002). The heat based treatments all had EPEF values >400, while the acid based treatments had EPEF values between 385 and 392 which was still better when compared to the control (327.7). It is apparent that all the diet treatments succeeded in achieving acceptable EPEF values. The trial had a low mortality percentage of less than 1% per treatment, which is within the acceptable range. The EPEF equation takes into account the FCR and the liveability, and due to the good FCR and low mortalities of the treatments, high EPEF values were attained. The EPEF values achieved in this trial are comparable to previous studies by Pretorius (2011) and Uushona (2015), using insect derived protein sources where EPEFs of close to 400 were achieved.

This study suggests substituting a maize/soya diet with pre-pupae meal (inclusion levels at 10%) is a viable alternative source of protein. The results in this study have indicated a positive trend with regards to the production parameters that are associated with broiler production. The results are comparable to the production parameters of previous studies, when regarding the FCR, ADG, PER, EPEF and end live weight (Téguia *et al*., 2002; Okah & Onwujiariri, 2012; Pieterse & Pretorius, 2014). They are, however, not comparable to results reported by Wang *et al*. (2005) and Uushona (2015), where the pre-pupae meal did not perform better than the control with regards to the production parameters. As mentioned above, production efficiency is obtained once the following parameters are reached; an EPEF value of ≥260 units, an ADG of ≥50 g, a FCR ≤1.85 and slaughter live weight of 1.5-2kg at 35d of age (Butcher & Nilipour, 2002). The pre-pupae treatments all reached these benchmarks. A previous study indicated that the BSF pre-pupae have a better digestibility coefficient than that of soybean meal (Uushona, 2015). The digestibility study that shows the chemical composition of the different pre-pupae treatment groups, as well as the digestibility of the different nutrients follows later

Table 3.4 Averages (±standard error) of weekly live weight (g), weekly feed intake (g) and cumulative feed intake (g) and production ratios of broilers receiving pre-pupae meal treated differently in comparison with control (maize/soya).

Treatment diets	1	2	3	4	5	6	7	8	9 (control)
Day 8									
Average live weight	216.9±02.66	211.3±03.37	208.1±03.89	213.1±05.90	210.6±03.46	215.0±00.94	208.1±03.89	206.3±03.50	215.6±04.27
Weekly feed intake	251.3±08.85	238.1±06.81	248.1±08.61	240.0±06.88	226.9±09.01	238.1±13.19	258.1±10.81	244.4±06.84	242.5±08.18
Cumulative feed intake	251.3±08.85	238.1±06.81	248.1±08.61	240.0±06.88	226.9±09.01	238.1±13.19	258.1±10.81	244.4±06.84	242.5±08.18
Day 15									
Average live weight	530.6±11.51	531.9±09.40	523.5±13.03	536.3±16.92	525.0±12.32	537.4±05.74	505.6±11.16	524.0±10.46	523.1±08.01
Weekly feed intake	536.9±09.86	538.1±08.40	541.3±08.00	533.1±10.00	555.6±10.63	544.4±07.87	534.4±08.04	528.1±11.14	540.6±14.47
Cumulative feed intake	788.1±11.42	776.3±11.83	789.4±08.99	773.1±13.26	782.5±15.44	782.5±13.89	792.5±13.53	772.5±08.66	783.1±16.03
Day 22									
Average live weight	1069.4±19.49	1050.6±10.78	1041.5±21.45	1074.2±19.21	1064.4±12.59	1085.2±07.74	1048.8±14.17	1027.6±16.92	1010.6±27.77
Weekly feed intake	679.4^{ab}±14.22	635.6^{b}±08.63	653.8^{ab}±18.17	679.4^{ab}±20.23	670.6^{ab}±39.38	741.9^{a}±20.04	691.3^{ab}±30.10	708.8^{ab}±14.32	663.1^{ab}±15.44
Cumulative feed intake	1467.5±20.75	1411.9±19.20	1443.1±24.69	1452.5±27.27	1453.1±36.68	1524.4±23.57	1483.8±39.22	1481.3±18.02	1446.3±24.62
Day 29									
Average live weight	1740.9^{a}±26.70	1716.3^{a}±20.93	1724.9^{a}±43.83	1712.6^{a}±32.73	1741.9^{a}±20.22	1712.4^{a}±38.36	1659.4$^{a±}$33.15	1662.9^{a}±23.71	1501.9^{b}±21.94
Weekly feed intake	911.9±25.65	940.6±16.62	884.4±23.25	950.6±12.76	910.0±50.21	895.6±29.36	940.0±30.38	915.0±22.58	933.1±33.45
Cumulative feed intake	2379.4±29.57	2352.5±20.53	2327.5±39.28	2403.1±22.79	2363.1±29.98	2420.0±27.56	2423.8±27.75	2396.3±22.36	2379.4±21.64
ADG (g)[1]	60.2±01.01	58.8±00.70	59.4±01.52	59.5±01.09	60.2±00.69	59.6±01.09	57.7±01.05	57.5±00.93	52.5±00.96
FCR[2]	1.4^{a}±00.02	1.4^{a}±00.02	1.4^{a}±00.02	1.4^{a}±00.03	1.4^{a}±00.02	1.4^{a}±00.03	1.5^{a}±00.02	1.5^{a}±00.01	1.6^{b}±00.03
EPEF[3]	428.2^{a}±10.29	432.2^{a}±09.77	429.6^{a}±14.68	411.8^{a}±17.86	443.3^{a}±09.69	407.9^{a}±15.03	392.4^{a}±13.03	387.5^{a}±05.90	327.7^{b}±10.60
PER[4]	3.2^{a}±0.05	3.2^{a}±00.09	3.3^{a}±00.09	2.9^{ab}±00.12	3.3^{a}±00.22	3.0^{a}±00.18	2.8^{ab}±00.13	3.0^{a}±00.06	2.3^{b}±00.06

[1] ADG- Average daily gain, [2] FCR- Feed conversion ratio, [3]EPEF- European production efficiency factor, [4]PER- Protein efficiency ratio.

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

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

in this thesis (Chapter 5.3). This should shed more light on why certain pre-pupae treatments showed better production parameters than others.

3.4 Conclusion

The aim of this study was to determine whether pre-pupae reared on human waste could be used as a protein source in broiler diets without negatively affecting broiler production parameters. Although different pre-drying processes were used prior to drying the pre-pupae, these processes did not decrease the microbiological load sufficiently nor did they have an influence on the production parameters of the broiler. However, the drying of the pre-pupae prior to incorporating these into the diets resulted in all bacteria (and pathogens) being destroyed. The results of the production trial also showed that the pre-pupae treatment diets did better than the controlin that they all achieved values above the benchmarks (control diet) in ADG, FCR, EPEF, PER and live weight within a normal growth period. A trend was noticed between the heat based treatments and the acid based ones, with the heat based treatments exhibiting better production parameters. More studies need to be done to determine whether heat is therefore a better treatment method than the acid when considering production parameters. It can therefore be concluded that pre-pupae grown on faecal matter are a viable source of protein in broiler feeds. It is assumed that these pre-pupae are much more cost efficient than other types of protein sources and therefore research needs to be done to determine what the cost efficiency would be in using these types of pre-pupae.

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Chapter 4. The effect of feeding broilers black soldier fly (*Hermetia illucens*) pre-pupae meal, grown on human waste, on slaughter and gut health

Abstract

The effect of using different treatment methods on black soldier fly (*Hermetia illucens*) pre-pupae grown on human waste on broiler organ weights and any possible toxicity was investigated. Carcass characteristics, physical and chemical quality of the broiler meat were also investigated. Eight different pre-drying methods of preparing the pre-pupae meal were used in this study. Seven hundred and twenty, day-old Cobb 500 broiler chicks were randomly allocated to nine treatment diets (ninth being the control), each treatment was replicated eight times. The chicks were grown to slaughter weight (at 31 days), with eight chicks per treatment being selected for the organ weighing and toxicity study, along with another eight birds per treatment being selected for carcass quality studies. The organs were removed and weighed, gut pH measured and gut samples taken for histomorphology analysis. The analysis indicated no treatment differences with regards to the gut pH and organ weights. There were no treatment differences with regards to histomorphology of the duodenum and jejenum. Regarding the carcass quality, no significant treatment differences were observed regarding the slaughter weight, breast muscle yield and the proximate analysis. Significant differences were observed in the dressing percentage, with the control diet achieving higher dressing percentages than most of the pre-pupae diets. This study revealed that the breast muscle of broilers receiving the pre-pupae treatment diets produced meat that is redder than that of the control diet. Significant differences were observed between Trt2 (62°C for 60min) and Trt8 (Propionic acid) with regards to pH of the meat; Trt2 had a pH of 6.4 while Trt8 had a pH of 6.0. Overall, these results indicate that black soldier fly pre-pupae grown on human waste can still produce broiler carcasses of acceptable physical and chemical quality. No negative effects on organs and the gastro intestinal tract (GIT) were observed.

Keywords: *Black soldier fly, Broilers, Carcass characteristics, Gut pH, Histomorphology, Human waste, Nutrient recycling, Organ weights, pre-pupae meal*

4.1 Introduction

The intestinal health of broilers is important in a production system as it influences the efficiency of the digestive tract of the animal (Van der Klis & Jansman, 2002). A healthy intestinal barrier with good digestion and absorption rates is a key factor to reach efficient production levels (Sealey *et al*., 2011). Diets could affect the structural integrity of organs depending on the nutrient composition (Boling-Frankenbach *et al*., 2001). The granule size of

the feed has also been indicated to affect certain organs, such as the gizzard, negatively (Engberg *et al*., 2002).

When formulating a diet for broilers, it is important to ensure that the diet contains all the nutrients with no deficiencies (Ensminger, 1992). Deficiencies can cause poor growth in the animal, which again can compromise the health of the animal (Ensminger, 1992). Arginine deficiency, for instance, can cause poor growth in the lymphoid organs of the bird (Uushona, 2015). The bursa, thymus and spleen are examples of organs which help make up the lymphoid system, which forms part of the immune system (Yegani & Korver, 2008). Healthy, well-developed lymphoid organs help maintain production levels, while fighting off infection and harmful pathogens (Butcher & Nilipour, 2002). As mentioned above, the development of these organs is essential for optimal immune response (Kwak *et al*., 1999), which in turn would help production levels and decrease production costs (Collett, 2005).

The intestinal wall is made up of finger-like projections known as villi, which help increase the absorption capacity of the intestine (Silverthorn *et al*., 2009). The length of the villi is an indication of intestinal health, with a reduction in villi length indicating a reduction in nutrient availability (Pluske *et al*., 1996; van Beers-Schreurs *et al*., 1998). The villi are, however, not the only characteristics that gives an indication of intestinal health and absorption. At the base of the villi, crypts extend down into the connective tissue (Silverthorn *et al*., 2009), where absorptive cells known as enterocytes are produced. A positive correlation exists between crypt depth and cell production within the crypts (Hedemann *et al*., 2003). Villi-length-to-crypt-depth ratio is used as an indication of digestive capacity and small intestine health, with a small ratio indicating poor digestion and absorption capacity (Montagne *et al*., 2003). The desired villi-to-crypt ratio in the jejunum would be 3.2:1 (Van der Klis & Jansman, 2002).

Gizzard erosion (GE) is a huge problem that affects the poultry industry worldwide (Johnson & Pinedo, 1971). The gizzard plays a role in the digestion of feed; therefore the health of the organ is important. When the gizzard lining erodes it leads to reduced feed intake (Johnson & Pinedo, 1971). Many factors are associated with GE, but the most common factor would be the excessive secretion of the pariental glands (Itakura *et al*., 1981), which causes an increase in gastric acid (Miyazaki & Umemura, 1987). Other factors that cause GE include mineral composition (Fisher *et al*., 1973) and pelleting of the feed (Ross, 1979), mycotoxin (Hoerr *et al*., 1982; Dorner *et al*., 1983; Diaz & Sugahara, 1995) and fishmeal concentration in the feed (Harry *et al*., 1975; Itakura *et al*., 1981; Itakura *et al*., 1982; Shimasaki *et al*., 2006) and stress on the animal (Grabarević *et al*., 1993; Džaja *et al*., 1996).

With the emerging trend of using fly larvae as feedstuff in broiler diets, questions arise as to the effect that these may have on the gut health of the broilers receiving these. Téguia *et al.* (2002) and Pretorius (2011) both observed no treatment differences in organ weights

with a 10% inclusion level of larvae meal in the diet. Pretorius (2011) also indicated that this larvae inclusion did not induce gizzard erosion. Both of these studies were done on larvae meal made from House fly (HF) larvae. Previous studies that used Black soldier fly (BSF) pre-pupae that were grown on kitchen waste, indicated that the inclusion of BSF showed no significant differences with regards to organ weights, intestinal pH or intestinal morphology (Uushona, 2015).

Two main factors influence consumers' decisions when purchasing meat and meat products: (i) the appearance of the meat, which has the largest initial impact, and (ii) the physical characteristics, which include taste and juiciness. Both of these factors determine the quality of the meat and are established by the consumers (Allen *et al*., 1998; Qiao *et al*., 2001; Fletcher, 2002; Swatland, 2004; Hoffman & Cawthorn, 2012). Although poultry is seen as a white meat, muscle pH still plays an important role in determining the quality thereof, especially as pertaining to the phenomenon known as PSE (pale soft and exudative) which is particularly found in poultry and pork (Swatland, 2004; Fletcher, 2007). Furthermore, the pH also affects the tenderness and water holding capacity, which are important aspects of meat quality (Van Laack *et al*., 2000; Fletcher, 2002; Huff-Lonergan & Longergan, 2005). Previous studies related to larvae meal supplementation in broiler diets have indicated colour differences in the meat (Pieterse *et al*., 2014). It is important to note that the pre-pupae meal produced by Pretorius (2011), was produced from the common HF. Whereas studies using BSF pre-pupae meal did not demonstrate the same findings with regard to colour of the meat produced (Uushona, 2015). Therefore, unlike the common HF it would appear that the BSF pre-pupae meal does not have an effect on the colour of the meat. These studies also indicated no treatment differences with regards to the pH of the meat (Pieterse *et al*., 2014; Uushona, 2015).

Additional characteristics that are taken into account when investigating the quality of broiler carcasses include the total yield and portion yield. Hwangbo *et al*. (2009) reported significant differences with regards to the dressing percentage and breast muscle yield, whereas other studies reported no such differences regarding these two aspects (Teguia *et al*., 2002; Awoniyi, 2003; Uushona, 2015). It is important to note that the above-mentioned articles were all done to observe what the effect of increased inclusion levels of larvae meal in broiler diets are. The breast muscle is of a particularly high market value across the world and it is therefore important to determine whether there might be yield increases in the current study. A study conducted previously using BSF pre-pupae meal indicated no differences with the other portions which include the thigh, wings and drumsticks (Uushona, 2015).

Considering the conflicting results with regards to the carcass characteristics, the efficacy of BSF pre-pupae is still unclear. Furthermore, Uushona (2015) was the only author that used BSF pre-pupae meal, but it is important to note that these pre-pupae were grown on

100% kitchen waste. Therefore, the aim of this study was to determine what the effects of BSF pre-pupae grown on human faecal waste are on the intestinal and organ health and, the effect thereof on the carcass quality characteristics. It will also be interesting to see how these results compares to that of Uushona's (2015) study. No studies could be found regarding BSF prepupae that were grown on human faecal matter in broiler diets.

4.2 Materials and methods

4.2.1 Pre-pupae treatments

Pre-pupae treatments are presented and explained in Chapter 3.2.1.

4.3.2 Chickens and data Collection

Chickens and Experimental Procedure

One hundred and fourty four Cobb 500 broiler chicks were slaughtered at 31days of age after having received the experimental diets (The diets and experimental outlay have been described in detail in Chapter 3.2.3). The chickens received a starter, grower and finisher diet according to one of nine treatments (Table 3.2). Half (72) of the 144 broilers were used to investigate organ stress, while the other half were used for the carcass quality (characteristics, chemical and physical quality) determination. One chick per pen was selected from the mean according to weight, rendering eight chicks per treatment for carcass characteristics testing. Another chick per pen was selected for organ stress and toxicity. Chicks were slaughtered according to acceptable commercial standards by electrical stunning, followed by exsanguination and dressing.

Data collection: Digestive tract measurements

The chicks were weighed before slaughtered and live weight recorded. The heart, liver, spleen and bursa were removed from the fresh carcass and weighed. The gizzard was also removed and cut open longitudinally and rinsed under running water. After rinsing it was scored for gizzard erosion (GE) on an ordinal scale as described in Table 4.1.

Samples of the gut, duodenum, jejunum and ileum were taken within 15 minutes *post mortem.* The pH of the duodenum, jejunum and ileum were measured using a calibrated pH meter (standard buffers pH 4.0 and 7.0) portable Crison pH25 meter (Lasec (Pty) Ltd, South Africa), by inserting the pH electrode into the centre of the area of the digestive tract to be measured. The probe was rinsed with distilled water between readings.

Table 4.1 Gizzard erosion scoring scale (Johnson & Pinedo, 1971).

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

A sample of each part of the small intestine was taken, rinsed with 0.9% saline solution and then fixed in a 10% buffered formalin solution until further analysis was done.

The duodenum and jejunum histology samples were processed according to Presnell *et al.* (1997). The samples were then cut to size and placed into embedding cassettes, processed and saturated with paraffin wax (Histosec, Merck). Thereafter tissue processing was done using an automated tissue processor (TISSUE TEK II, model 4640B, Lab-Tek division, Miles Laboratories Inc, Naperville, IL). Cross-sections of 5 µm were then cut using a rotary microtome (Reichert Jung, Heidelberg, Austria)

The slides were examined with an Olympus IX70 microscope equipped with a digital camera (colour view II) and analysed with Analysis Imaging Software (build 5.1.0.2640) supplied by the Olympus company. The 4X magnification objective lens was used for measuring villi height, width and area, and crypt depth. The villi area and length were measured from the tip of the villi to the villous-crypt junction (in areas with intact villi's), while crypt depth was measuring as the vertical distance from the villous-crypt junction to the lower limit of the crypt. Each parameter was estimated by measuring 10 consecutive measurements and the average was then used further.

Data Collection: Carcass characteristics, chemical and physical quality

Before and after slaughter, broiler live weights and dressed weight were recorded, to determine dressing percentage. The breast and thigh muscles pH were measured using a hand held portable Crison pH 25 pH meter with automatic temperature adjuster 15min *post mortem*. The Crison pH 25 was calibrated before pH measurements were taken with the standard buffers (pH 4.0 and pH 7.0) as provided by the manufacturer. The pH meter probe was placed directly into the left breast and thigh muscles, the instrument was given time to stabilize before the pH reading was taken. Between each measurement the probe was rinsed with distilled water and rested in a 3M KCl electrolytic solution.

The cold carcass (24hr *post mortem*) was cut into portions by first cutting it in half with a portion cutter. By cutting above the thigh, towards the acetabulum and behind the pubic

bone, the thigh and drumsticks were removed and separated. The breast muscles were removed by cutting from the *clavicale furcula* bone alongside the carina (keel) bone. The muscles were cut out and the skins were removed and the muscles left to bloom for an hour in order to measure the surface colour of the breast muscle. The colour measurements were taken, off the surface of the breast muscle, with a CIElab colour meter (BYK-GardnermbH, Gerestried, Germany). The CIElab colour system was used (Commition International de L'Eclairage, 1976) with three measurements; L* (lightness), a* (redness) and b* (yellowness). Positive a* values are a measure of redness and negative a* values are a measure of greenness. Positive b* values are a measure of yellowness and negative b* values indicate blueness. The breast muscle was weighed to obtain portion yield for each bird and treatment. After the breast muscle was removed and weighed, it was frozen until it was homogenised for proximate analysis.

4.2.3 Chemical analysis

All chemical analyses were performed at the Department of Animal Science, Stellenbosch University, except for the mineral composition, which was done at the Western Cape Department of Agriculture, Elsenburg. The analytical laboratory partakes on a Agrilasa profiency test every three months to ensure accuracy.The dry matter (DM), ash, crude fat (Chloroform-Methanol), crude protein (CP) and mineral determination were done according to the Association of Official Analytical Chemists International (AOAC, 2002).

Dry matter determination (Official Method 934.01) was done by weighing two subsamples, each weighing 2g, and then placing them in a crucible drying for 24hr at 100°C, after which they are weighed again. Ash was determined (through Official Method 942.05) by combusting the subsamples in combustion oven for six hours at 500°C, after which they are weighed and ash determined. Crude fat determination (Chloroform-Methanol) was determined through the method described by Lee *et al.* (1996). Crude protein determination was done by measuring total nitrogen content (Official Method 4.2.07, in a LECO FP528) in two subsamples and then read directly from the LECO FP528, then CP calculated through an equation. Mineral determination was determined (method as described by the Agricultural laboratory association of Southern Africahandbook of feeds and plant analysis volume 1, method no. 6.1.1) by combusting 2g of dry sample and then adding 1:1 Hydrochloric acid solution to the sample, made up to 40ml adding distilled water and results were then read from the inductively Coupled Plasma. Minerals P, K, Ca, Mg, Na, Cu, Mn, Fe, Al, Zn and B were determined through this method.

4.2.4 Microbiological testing

Samples of the breasts were taken to determine whether the meat produced from BSF pre-pupae grown on human faecal waste was safe. The sample taken was of the deep muscle and not of the outer portion. This was done so that the results would not be affected by the unintentional transfer of microbes onto the sample during the slaughter and dressing procedures. Samples were taken from all the treatment groups, with eight samples per treatment and one sample per bird. The sample was removed with a sterilised circular puncture knife where after the core portion was removed. The meat sample was then placed in a 1ml physiological salt solution in a 1.5ml Eppendorf tube. The meat sample were crushed with a sterile eppi pestle and from the slurry, dilution series were then prepared (10^{-2} to 10^{-5}) that were plated on 4 different media plates (as described in Table 3.1, Chapter 3). The plates were incubated for 48hr at 30°C.

4.2.5 Statistical analysis

Statistical analysis was done using the general linear models procedure of STATISTICA (data analysis software system), Version 9, by Statsoft Inc. (2009). Before proceeding with analysis the Shapiro-Wilk test was used to test for normality of the data and also homoscedasticity. If significant non-normality values ($P \leq 0.05$) appeared, outliers were identified and residuals greater than three were removed.Where age effects were not a variable the statistics were done by using one-way analysis of variances (ANOVA) with Bonferroni *post hoc* test being applied.

Differences were considered significant if P-values were less than 0.05, thus the 5% significant level was used.

4.3 Results and discussion:

Results obtained from the trial regarding organ weights, organ weight relative to body weight and organ weight ratios are reported in Table 4.2. There were no significant ($P>0.05$) differences between the eight treatment diets where these measurements are concerned.

It is important to take the organ weights of the broilers into account, as amongst others, this indicates oxidative stress, while spleen-to-bursa, spleen-to-body-weight, bursa-to-bodyweight and spleen-to-liver ratios could indicate immune stress (Cooper *et al*., 1966; Collett, 2005). Morales and Wolff (2010) observed that broilers fed larvae meal produced from HF larvae have significantly larger gizzards and smaller hearts, but no significant differences when looking at liver weights. The bursa produces B-cells, which play a role in the immune protection of the birds (Glick, 1991). Therefore, an enlarged bursa would indicate immune stress. The results in this trial are comparable to Schouw *et al*. (2002), Pretorius (2011) and Uushona (2015), who indicated no treatment differences between the pre-pupae diets and the control.

The pH values of the duodenum, jejunum and the ileum of the nine different treatment groups are reported in Table 4.3. There were no differences (P>0.05) between the different treatments where the pH is concerned. Table 4.4 indicates the histological characteristics of the duodenum and the jejunum and it was observed that there were no treatment differences (P>0.05).

It is reported that a healthy gut has a high nutrient absorption capacity and an improved immune status in the animal (Salim *et al*., 2013). In this trial it was observed that the pH values of the duodenum, jejunum and ileum all fall within the normal pH range of 5.5-6.2 for the duodenum, 5.8-6.9 for the jejunum and 6.3-8.0 for the ileum (Van der Klis & Jansman, 2002). These results are comparable to the results obtained by Uushona (2015), who also indicated that the inclusion of BSF pre-pupae meal at 10% does not negatively affect the pH range of the small intestine. The pH of the small intestine is important for microbial population and its survival, therefore the results obtained would indicate favourable pH conditions (Van der Klis & Jansman, 2002).

The digestion and absorption of nutrients occurs mostly in the small intestine, with the jejunum playing the biggest role (Nourmohammadi & Afzali, 2013). The villi size and crypt depth determines the absorption capabilities of the gut (Awad *et al*., 2009). The shortening of villi and deepening of crypts indicates the presence of toxins in the gut (Choct, 2009). As observed in Table 4.4 that there were no differences between the treatment diets and it can be concluded that the toxins are at or below accepted levels. The villi length and size, combined with the crypt depth, indicate the rate of tissue turnover of the epithelial cells, energy requirement and the absorption capabilities in the gastro intestinal tract (Awad *et al*., 2009; Choct, 2009; Zhang *et al*., 2013). It is concluded that the inclusion of these BSF pre-pupae did not negatively affect the absorptive capabilities of the broiler chicks. Due to broilers being bred for fast growth and efficiency, the feed must not negatively affect the gut health.

GE scores for the nine different treatments are depicted in Table 4.5. It is observed that the GE score was widely spread between 1 and 5, with 1 being no erosion and 5 being severe erosion. Trt1 (62°C for 30min), Trt6 (100°C for 5min) and Trt9 (control) did not exhibit GE scores of 5, but they did have GE scores of 3 and 4. Trt9 (control) also did not have desirable GE scores. It is noteworthy that the GE scores of Trt9 (control) were centred around a score of 3 and 4, whereas the other treatments appear to be more widely spread. The macroscopic evaluation of the gizzards indicated GE across all the treatments at unacceptable levels. It can however, be argued that the inclusion of pre-pupae meal is not the reason for the erosion, since the control treatment diet also indicated high levels of erosion. It also important to note that the study conducted by Uushona (2015) also revealed no severe erosion similar to that observed in this trial.

Table 4.2 Averages (± standard error) of liver, heart, spleen and bursa weights, together with organ ratios of broilers receiving different treatment diets (weighed in g).

Organs	Treatment								
	1	2	3	4	5	6	7	8	9
Liver (%BW)	1.8±0.07	1.8±0.05	1.82±0.12	1.7±0.04	1.8±0.03	1.8±0.08	1.8±0.04	1.9±0.06	1.9±0.09
Heart (%BW)	0.7±0.03	0.7±0.03	0.7±0.04	0.6±0.02	0.6±0.02	0.6±0.02	0.6±0.03	0.6±0.02	0.6±0.03
Spleen (%BW)	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01
Bursa (%BW)	0.3±0.02	0.3±0.04	0.3±0.02	0.2±0.01	0.3±0.02	0.2±0.01	0.2±0.03	0.2±0.02	0.2±0.02
Gizzard (%BW)	2.0±0.11	2.1±0.09	1.8±0.11	2.1±0.09	2.1±0.07	2.2±0.17	2.1±0.08	2.1±0.12	2.2±0.11
Spleen: BW	0.001±0	0.0011±0	0.0011±0	0.001±0	0.0009±0	0.0009±0	0.001±0	0.0008±0	0.001±0
Bursa: BW	0.0027±0	0.0029±0	0.0029±0	0.0025±0	0.0028±0	0.0021±0	0.0025±0	0.0024±0	0.0023±0
Spleen: Bursa	0.4±0.02	0.4±0.04	0.4±0.04	0.4±0.04	0.3±0.02	0.4±0.04	0.4±0.07	0.3±0.05	0.5±0.04

*BW: Body weight

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

Table 4.3 Averages (± standard error) of small intestine pH of broilers receiving different treatment diets.

Small intestine	Treatments								
	1	2	3	4	5	6	7	8	9
Duodenum	6.0±0.15	6.2±0.15	6.2±0.09	6.2±0.14	6.0±0.20	5.9±0.28	5.8±0.12	5.8±0.27	6.2±0.10
Jejunum	6.0±0.13	6.2±0.10	6.2±0.11	6.2±0.08	6.2±0.07	6.2±0.11	6.4±0.10	6.2±0.13	6.3±0.09
Ileum	7.1±0.12	6.9±0.23	7.1±0.08	6.8±0.12	7.1±0.13	7.2±0.37	7.1±0.14	7.2±0.12	6.9±0.15

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

The results obtained from the trial regarding the physical carcass characteristics are summarised in Table 4.6. As expected (due to the sampling procedures), no significant (P>0.05) differences were observed for the treatments regarding the live weight and carcass weight. However, differences (P≤0.05) were observed for dressing percentage. Trt8 (propionic acid) and Trt9 (control) differed (P≤0.05) with the other treatments being statistically equal and intermediary between the two treatments. The control achieving the highest dressing percentage of all the treatments with 73.3% and Trt8 (propionic acid) had the lowest with 64.2%. The other treatments had dressing percentages between 66.6% and 71.9%.

No differences (P>0.05) were observed for portion yield, for the breasts, CieLab L* and b* did not differ (P>0.05) between treatments, while a* showed significant differences between Trt3 (72°C for 5min) and Trt6 (100°C for 5min). The a* value is an indication of redness in the meat and it observed that Trt3 (72°C for 5min) and Trt6 (100°C for 5min) differed (P≤0.05) from each other, with Trt3 (72°C for 5min) exhibiting a lower a* value than Trt6 (100°C for 5min). This indicates that the breast meat of Trt6 (100°C for 5min) was much redder when compared to the other treatments. The other treatments, however, did not differ (P>0.05) from one another or with Trt3 (72°C for 5min) and Trt6 (100°C for 5min). Significant (P≤0.05) differences were measured between the treatments regarding the pH of the breast muscle, where Trt2 (62°C for 60min) differed from Trt8 (Propionic acid). As mentioned, pH and colour are correlated (Allen *et al*., 1998; Qiao *et al*., 2001; Swatland, 2004). Meat with low pH is generally paler than at a high pH where the meat is usually redder (Swatland, 2004). This has to do with the light scattering of the meat and thus the absorption of light by the myoglobin, therefore at a higher pH more light is captured and the meat looks redder (Swatland, 2004).
Inclusion of pre-pupae meal into broiler diets has produced broilers that had higher live weights and carcass weights, when compared to the control diet (Hwangbo *et al*., 2009; Pieterse *et al*., 2014) using HF meal. However, Uushona (2015) added BSF pre-pupae meal to broiler diets and found no differences (P>0.05) in the live weight and carcass weight. Neither of these studies had similar findings when regarding the dressing percentage differences that were revealed in this study. The dressing percentage is calculated by dividing the carcass weight with that of the live weight, therefore differences in the dressing percentage could be ascribed to other factors such as feather weight and weight of the intestines; it should be noted that the same techniques for dressing of the carcasses were used between the study of Uushona and this study.

Meat colour plays an important role in the purchase decision of the consumer (Qiao *et al*., 2002; Hoffman & Cawthorn, 2012). The L* value is an indication of the lightness of meat and could indicate inferior meat quality (Chen *et al.,* 2013*)*. Van Laack *et al*. (2000) note that normal broiler meat colour should have a L* value ranging between 50 and 56. Taking this into account it is observed that the broiler meat from the different treatment groups fall within

Table 4.4 Average (± standard error) of duodenum and jejunum histomorphology sections (µm) as influenced by treatment diets

Small intestine	Treatments 1	2	3	4	5	6	7	8	9
Duodenum:									
Area (µm^2)	122949±13483	122488±14228	145776±105825	116175±13199	152056±7748	117015±9255	145868±10944	128743±12344	112384±6882
Villi Length	1089.2±11.67	1058.5±71.27	1326.8±83.26	1096.0±34.35	1134.2±43.10	1052.3±67.18	1176.6±60.71	1098.8±49.71	1063.7±45.74
Crypt Depth	202.9±09.85	212.9±07.42	192.5±09.27	196.3±15.55	190.2±08.96	170.7±10.15	176.2±13.83	185.3±12.19	202.3±10.37
Jejunum:									
Area (µm^2)	105066±23165	93680±8703	104246±8692	88371±10170	93890±5448	111843±4112	114409±9745	109802±10515	125855±16223
Villi Length	1032.6±97.18	966.8±79.39	1037.2±36.33	1053.2±62.58	957.7±41.50	1084.8±37.82	1030.7±50.58	994.0±65.03	1095.5±68.22
Crypt Depth	222.2±22.19	219.0±14.82	223.9±13.29	200.6±11.86	206.1±27.73	172.6±11.81	230.7±13.27	181.9±17.53	219.6±13.67

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

Table 4.5 Number of observations per category of gizzard erosion score recorded for the different treatment groups

GE*-Score	Treatments 1	2	3	4	5	6	7	8	9
1	-	-	1	-	-	-	1	-	-
2	1	-	1	1	4	2	1	1	-
3	1	2	2	3	2	3	3	3	2
4	6	4	1	3	1	2	2	2	6
5	-	1	2	1	1	-	1	2	-

GE* - Gizzard erosion

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

the normal range. Therefore, according to Van Laack *et al*. (2000), based on the L* value the study revealed no pale soft exudative (PSE) or dark-firm-dry meat. A previous study concluded that there is a correlation between pH and meat colour, where darker muscle meat is concerned (Fletcher, 1999). Fletcher (1999) concludes that darker meat had a higher pH value than that of a lighter meat, however these findings do not correlate to this study. In this trial Trt3 (72°C for 5min) did not differ significantly with Trt6 (100°Cfor 5min) where pH is concerned. The reason for the difference in colour ordinate a* (redness of the meat) is then not related to muscle pH. The pH of the breast meat in this study is higher than that which is deemed normal (5.96), as described by Van Laack *et al*. (2000). The range of the muscle pH of the different treatment groups was 6.0 to 6.4. These pH levels may be attributed to *ante mortem* factors that were unique to this group of broilers slaughtered although care was taken to ensure that the birds from the various treatments received similar *ante mortem* treatments. Nonetheless, the results obtained are comparative to previous studies with regards to pH (Pretorius, 2011; Uushona, 2015). Only Pretorius (2011) reported meat that has the same *a value range as that found in this study. This trial therefore produced breast meat with a higher than normal pH and an a* value that is within the normal range (Van Laack *et al*., 2000).

Table 4.7 indicates the results obtained from the proximate analysis of the meat. It was observed that no significant (P>0.05) differences were found between the different treatment diets with regards to the moisture (74.2-75.4%), protein (21.6-23.5%), fat (1.6-2.1%) and ash (1.1-1.2%) content. The mineral composition of the different breast meat treatment diets is summarised in Table 4.8. No significant (P>0.05) differences were observed between the different diets.

There is very little published data concerning the chemical composition of broiler meat where the broiler diets had larvae meal inclusion, with Uushona (2015) reporting the chemical composition; the results in this trial were comparable to those found by Uushona. The meat in this trial had a higher moisture content with lower fat and protein levels compared to Uushona (2015). Pieterse *et al*. (2014) notes that broilers who received larvae meal inclusion do have acceptable chemical composition when compared to those fed soya bean and fishmeal.

The results from the microbial plating of the breast muscle samples revealed only trace amounts of microbial growth. The microbial growth that was observed was attributed to the sampling method and not due to the pre-pupae being contaminated. This being due to the fact, that the concentration of microbial growth still present after drying was very low (refer to Chapter 3). The chances of contamination during sampling was much greater. However, the results still indicate that the meat that was produced was safe for consumption.

Table 4.6 Average (±standard error) broiler carcass measurements as influenced by treatments

	Treatment diets								
	1 (62°, 30min)	2 (62°, 60min)	3 (72°, 5min)	4 (72°, 15min)	5 (100°, 2min)	6 (100°, 5min)	7 (Formic)	8 (Propionic)	9 (Control)
Live weight (g)	1766.7±65.40	1741.7±30.05	1683.3±38.01	1691.7±53.88	1758.3±27.13	1791.7±27.13	1691.7±35.16	1783.3±49.44	1616.7±30.73
Carcassweight (g)	1216.7±40.14	1158.3±15.37	1208.3±30.05	1216.7±51.10	1250.0±22.36	1208.3±20.07	1141.7±35.16	1141.7±20.07	1183.3±21.08
Dressing percentage	69.0 ^{abc} ±1.34	66.6 ^{bc} ±0.97	71.9 ^{ab} ±1.96	71.8 ^{ab} ±0.92	71.1 ^{ab} ±0.72	67.5 ^{abc} ±1.09	67.4 ^{abc} ±1.07	64.2 ^{c} ±1.40	73.3 ^{a} ±1.56
Portion size									
Breast muscle (g)	231.0±8.12	217.5±2.68	224.0±12.24	224.7±14.06	237.2±11.19	240.2±4.05	221.0±9.13	230.0±7.00	216.8±10.64
Colour and pH measurements									
L*	52.0±1.05	50.7±0.86	53.1±1.44	54.2±1.27	53.7±1.42	51.8±1.00	52.9±1.04	54.3±1.09	54.4±0.49
a*	1.6 ^{ab} ±0.43	1.3 ^{ab} ±0.43	0.4 ^{b} ±0.26	1.6 ^{ab} ±0.42	1.8 ^{ab} ±0.45	3.1 ^{a} ±0.36	2.3 ^{ab} ±0.62	1.8 ^{ab} ±0.19	1.2 ^{ab} ±0.25
b*	9.1±0.76	9.6±0.51	8.6±1.10	9.2±0.50	11.1±0.60	10.2±1.02	10.0±0.85	10.1±1.80	10.3±0.43
pH	6.4 ^{ab} ±0.08	6.4 ^{a} ±0.10	6.2 ^{ab} ±0.07	6.3 ^{ab} ±0.07	6.2 ^{ab} ±0.06	6.2 ^{ab} ±0.06	6.2 ^{ab} ±0.09	6.0 ^{b} ±0.07	6.2 ^{ab} ±0.04

L*- lightness, a*- redness, b*- yellowness

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

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

Table 4.7 The averages (±standard error) of the proximate analysis of the broiler breast meat as influenced by treatments

	Treatment diets								
	1	2	3	4	5	6	7	8	9
Moisture	75.1±0.22	74.9±0.36	74.3±0.26	74.8±0.18	74.8±0.22	74.9±0.24	74.5±0.37	75.4±0.35	74.2±0.26
DM	24.9±0.22	25.1±0.36	25.7±0.26	25.2±0.18	25.2±0.22	25.1±0.24	25.5±0.37	24.6±0.35	25.8±0.26
Protein	22.3±0.37	21.6±0.46	23.0±0.35	22.5±0.37	21.8±0.48	22.1±0.56	23.2±0.40	22.4±0.38	23.5±0.51
Fat	2.1±0.22	2.2±0.12	2.0±0.18	1.9±0.16	2.1±0.18	2.0±0.23	1.8±0.11	1.8±0.13	1.6±0.20
Ash	1.2±0.08	1.1±0.01	1.2±0.06	1.1±0.04	1.2±0.08	1.1±0.03	1.2±0.04	1.1±0.04	1.1±0.02

DM: Dry Matter

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid

4.4 Conclusion

The aim of the study was to determine what the effect on organ weight, gizzard erosion and carcass characteristics were when BSF pre-pupae grown on human faecal matter, was included in broiler diets. Taking all the results into account it can be concluded that the meat produced in this study is acceptable and of good quality, with no measurable effects on the organs or the intestinal wall. The pre-pupae treatment diets were comparable to the control with regards to organ weights, gut pH and histomorphology. It is, however, important to note that very high erosion within the gizzard was observed. This erosion was observed across all the treatments and not isolated within the pre-pupae treatment groups. This indicates that the pre-pupae were not the reason for the erosion. It is unfortuanetly not clear as to the reason of the GE findings, but it is suspected to be due to the age of the birds and enviromental factors such as stress. It is important to note that the broilers were at the end of the trial. The carcass characteristics observed in the trial indicated that the pre-pupae diets were also comparable to that of the control in most aspects. Overall, irrespective of the dietary treatment, the breast meat, however, shows higher pH values than what have been previously reported for broilers. As discussed, these differences in pH may be attributed to *ante mortem* factors. There were, however, no treatment differences between the pre-pupae diets and the control with regards to the proximate chemical composition and mineral composition of the breasts. Pre-pupae grown on faecal waste can therefore still produce broiler meat that is comparable chemically and physically to meat that is produced from the commercial standard, soya or maize, diets with no toxic effects. As expected, there was no bacterial contamination from the BSF pre pupae into/onto the broiler meat indicating once again that sufficient processing of the pre-pupae ensures that no potential pathogens survive. This investigation shows that the use of BSF pre-pupae grown on human faecal matter that could be regarded as being inferior produces microbiologically safe quality broiler meat. This then raises the questions and the opportunity for further study to determine whether the public would accept poultry meat produced from feed containing BSF pre-pupae of this sort.

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Table 4.8 The average (±standard error) of the mineral composition of the broiler breast meat as influenced by treatments

	Treatment								
	1	2	3	4	5	6	7	8	9
Phosphorous	0.7±0.02	0.6±0.06	0.7±0.05	0.7±0.03	0.7±0.05	0.7±0.04	0.7±0.02	0.7±0.03	0.7±0.05
Potassium	0.7±0.02	0.7±0.05	0.7±0.03	0.7±0.03	0.7±0.04	0.7±0.03	0.7±0.05	0.7±0.02	0.7±0.03
Calcium	0.03±0.002	0.02±0.003	0.02±0.002	0.03±0.005	0.02±0.002	0.02±0.002	0.03±0.002	0.02±0.002	0.02±0.002
Magnesium	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.00	0.1±0.01	0.1±0.01
Sodium	999.7±41.11	958.8±80.50	1049.8±42.44	916.3±41.62	963.0±76.88	895.5±27.84	1009.2±72.72	933.0±56.66	795.8±150.15
Iron	31.4±3.66	30.5±3.64	25.6±3.97	31.4±4.30	38.0±8.44	45.2±11.84	24.4±3.41	29.3±3.92	33.1±4.50
Copper	0.8±0.34	0.6±0.21	0.5±0.30	0.5±0.16	1.7±0.96	0.9±0.57	0.5±0.30	0.4±0.20	1.0±0.52
Zinc	22.1±2.44	20.3±2.77	20.4±2.16	21.8±1.81	22.6±2.94	21.2±2.22	19.4±1.14	22.7±3.36	20.6±2.42
Manganese	0.8±0.11	0.7±0.07	0.6±0.06	0.8±0.09	0.8±0.13	0.9±0.21	0.6±0.07	0.9±0.21	0.9±0.13
Boron	0.3±0.03	0.3±0.03	0.2±0.03	0.3±0.04	0.3±0.06	0.4±0.10	0.3±0.03	0.3±0.01	0.3±0.04
Aluminium	26.7±6.83	25.2±5.35	17.9±5.34	21.9±4.28	29.6±9.23	29.1±15.33	21.1±6.77	27.3±9.77	22.5±6.48

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid

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Chapter 5. The evaluation of the coefficient of total tract digestibility of differently treated black soldier fly (*Hermetia illucens*) pre-pupae meal grown on human waste in the diets of broiler chicks

Abstract

The total tract digestibility of black soldier fly (*Hermetia illucens*) pre-pupae grown on human waste was investigated in one hundred and forty four broiler chicks. The different dietary/sterilisation treatments were on the pre-pupae and consisted of submerging in water for: 30min at 62°C, 60min at 62°C, 5min at 72°C, 2min at 72°C, 2min at 100°C, 5min at 100°C, rinsed in either 5% propionic acid or in 5% formic acid for 10min. The pre-pupae were then dried, minced and mixed into the poultry diet. The following aspects of the feed and faecaes were then evaluated: protein, fat, fiber, ash, amino acids and apparent metabolisable energy (AME). The chicks were allocated at random to each treatment, with each treatment being repeated eight times. The birds were acclimatised to the feed for three days and the daily intake was determined, after which the trial started and lasted for three days, during which the faecal matter was collected for analysis. There were significant differences among all the treatments with regards to AME and the other nutrients. Treatments 62°C for 30min, 62°C for 60min, 72°C for 5min and 100°C for 2min had the highest coefficient of total tract digestibility (CTTD) over all the nutrients analysed. These treatments had CTTD's over 0.9 for crude protein and the essential amino acids. It can be concluded that these pre-pupae still indicate high digestibility values. These results will help determine with a more accurate inclusion level of black soldier fly pre-pupae into diets.

Keyword: *Amino acids, Black soldier fly, Broilers, CTTD, Human waste, Nutrient recycling, Pre-pupae meal*

5.1 Introduction

Digestibility is an indication of how efficiently an animal utilises a feed substrate (Goodwin, 2009) and this is important from a nutritional perspective to ensure that maximum productivity is achieved. Therefore, to determine how much nutrients within a feed source are digested and utilised, a digestibility trial is typically conducted (Scott & Boldaji, 1997). It has been reported that animals fed highly digestible feed attain superior growth performance, as opposed to those fed poor digestible feeds (Thang *et al*., 2010; Salem *et al*., 2013). Research has indicated that larvae meal produced from the HF and BSF is a promising protein source for use in poultry diets (Pretorius, 2011; Uushona, 2015).

The methods used to determine digestibility differ between species and are dependent on the structure of the digestive tract. Therefore, the method that is employed has to be suited

to the digestive tract of the animal; for instance, ruminant digestion methods cannot be used to determine monogastric digestion and vice versa (Scott *et al*., 1998; Yahya *et al*., 2011; Kroeckel *et al*., 2012; Salem *et al*., 2013). Precision feeding is a preferred method to determine feed digestibility as it is inexpensive, rapid and requires less feed (Parsons *et al*., 2002). It is also the most commonly used method for determining the digestibility of a feed (Parsons *et al*., 2002). It should however be noted that precision feeding is criticised as being less accurate than ileal-digestibility determination (Parsons *et al*., 2002). Therefore, markers (inert and external) are added in excreta assay diets to minimise the error with regards to inaccurate measurements of the feed intake, excreta output and external contamination (Sibbald, 1987). As mentioned, the excreta assay is criticised as being less reliable, but studies have reported that there were no differences between this method and ileal-digestibility regarding sorghum and maize (Ravindran & Bryden, 1999).

Previous studies using larvae meal, made from HF larvae, revealed that the digestibility is high when added to diets of turkey poults (Zuidhof *et al*., 2003) and broiler chicks (Hwanbgo *et al*., 2009). Both these studies reported a crude protein total tract digestibility of 98%, while Hwangbo *et al*. (2009) reported a 94.8% total tract digestibility of the essential amino acids. When comparing a commercially accepted feed source such as soya oil cake with that of larvae meal, it is reported that larvae meal has a higher total tract digestibility of energy, crude protein and amino acids in turkeys (Zuidhof *et al*., 2003). A previous study conducted by Uushona (2015) revealed that, similar to the HF larvae meal, BSF pre-pupae meal is highly digestible. It is reported to have a total tract digestibility for crude protein, fat and amino acid of 86-91%, 100% and over 90%, respectively (Uushona, 2015). BSF larvae therefore show great potential as a feed source within broiler diets (Hale, 1973).

Certain factors are reported to influence the digestibility of a potential feed (Boland *et al*., 2013). These factors include heat and acid treatments, which could lead to denaturation of the protein (Boland *et al*., 2013). Proteins are made up of amino acids, with the amino acid lysine being the most affected by extreme heat processing which reduces its availability (Parsons, 1996). Therefore, in a digestibility study it is important to also determine the bioavailability of the AA within a processed feed source. An optimal and balanced Metabolisable energy (ME) content and protein ratio ensures maximum nutrient and protein utilisation (Ogunji *et al*., 2008; Zaman *et al*., 2008). Imbalances in the ME and protein content influence the performance (growth) of the chicks (Zaman *et al*., 2008).

The aim of this study was to determine the CTTD of BSF pre-pupae grown on faecal matter and how the different heat treatment methods applied to the pre-pupae influence the digestibility. It is also important to quantify how the digestibility of these pre-pupae grown on human faecal matter differ from those of previous studies grown on kitchen waste.

5.2 Materials and methods

5.2.1 Pre-pupae treatments

In depth pre-pupae treatments are presented and explained in Chapter 3.2.1. For clarity the different methods are listed below:

Trt1 – 62°C for 30min

Trt2 – 62°C for 60min

Trt3 – 72°C for 5min

Trt4 – 72°C for 15min

Trt5 – 100°C for 2min

Trt6 – 100°C for 5min

Trt7 – Rinsed in a 5% Formic acid solution

Trt8 – Rinsed in a 5% Propionic acid solution

It is important to note that after the treatments the pre-pupae were placed in a drying over at 80°C for 48hr, after which they were minced and frozen at -16°C until mixing.

5.2.2 Digestibility trial

Chickens

One hundred and forty four, day-old Cobb 500 broiler chicks as hatched were used.

Housing system

During the first twenty days the chicks were kept in a temperature controlled house at the poultry section of the Mariendahl Experimental farm of Stellenbosch University according to the management practices described by die Cobb International (2012). Artificial lighting was provided at a pattern of 16 hours light and 8 hours darkness. Ventilation in the house was set to provide a minimum of six air changes per hour. At day twenty the chicks were moved into their experimental groups and cages. Each cage containing a nipple drinker and feeder. The chicks had *ad libitum* access to feed and water during the duration of the experimental period.

Experimental diets

During the first twenty days the chicks were maintained on a commercial starter diet formulated to produce marketable chickens weighing 1.9kg at 35d according to the nutrient specifications provided by Cobb 500 international (2012). Hereafter the chicks were switched over onto one of the ninetreatment diets which are shown in Table 5.1.

Experimental design and trial procedure

Chicks were randomly allocated to the pens and treatments in the experimental house with two chicks per cage and eight repetitions per treatment. Digestibility of the pre-pupae meal dried at 60°C were done using the Acid Indigestible Assay as described by Scott and Hall (1998).

The broiler chicks were moved into their cages and groups on the 20th day of age. From day 20 to 25 the chickens were left to adapt to their new environment, during this time the chickens were fed a commercial grower diet. From day 25 to 27 the chicks were adapted to the various treatment diets, during this time the individual group *ad libitum* intakes were determined. From day 28 to 31 the actual digestibility trial (data collection period) was conducted.

Table 5.1 Ingredient composition of the commercial starter diet, with the different treatment diets for the digestibility trial (% of the diet).

Ingredients:	Unit	Starter	Diet 1-8 (10% LM)	Diet 9 (Control)
Hermetia illucens pre-pupae	%	-	50.000	50.000
Maize	%	47.755	50.000	50.000
Soybean full fat	%	32.101		
Soybean	%	7.718		
Fish meal	%	9.245		
L-lysine HCl	%	0.062		
DL methionine	%	0.358		
L-threonine	%	0.079		
Vit+min premix *	%	0.450	0.450	0.450
Limestone	%	1.174		
Salt	%	0.077		
Monocalcium phosphate	%	0.873		
Sodium bicarbonate	%	0.107		
Oil - sunflower	%	-		
Acid insoluble ash (CeliteTm)	%		1.000	1.000

* Vitamins and minerals are included according to the levels provided by the National Research Council, 1994.
LM: Larvae meal inclusion

Data collection and analysis

At the onset of the trial a 500g representative sample of each treatment diet was collected and frozen at -20°C until used in the laboratory analysis.

The broiler chicks were weighed on the beginning (20th day) and at the end (31st day) of the digestibility trial. During the period when the chickens were left to adapt to the environment, no measurements were done or data collected. From the 25th to 27th day the daily feed intakes and refusals were measured and the feed offered was adjusted to adapt to the *ad libitum* feed intakes. On the 28th day of the data collection period, faecal trays covered in plastic were placed under the cages and faeces were collected daily and weighed until the 31st day. During the data collection period daily feed intakes and feed refusals were determined. All procedures were conducted at 08:00 in the morning.

5.2.3 Analytical methodologies

Analytical methodologies were performed at the Department of Animal Science, Stellenbosch University, with the exception of the mineral composition, which was done at the Western Cape Department of Agriculture, Elsenburg. The Analytical laboratory partakes in an Agrilasa

proficiency test every three months to ensure accuracy. The dry matter (DM), ash, crude protein (CP) and mineral determination were done as explained in 4.2.3. The gross energy, crude fibre (CF), crude fat (acid hydrolysis), AA hydrolysis and AME determintation was done according to the Association of Official Analytical Chemists International (2002).

The gross energy was determined using a C200 (IKA) oxygen bomb calorimeter as described by the digital data system C200 (IKA) operating manual. Two subsamples of each sample weighing 0.5g respectively were pelleted and placed in the bomb, which was filled with pure oxygen. The bomb was placed into the C200 Calorimeter and the gross energy was directly read from it (MJ/kg). CF was determined (Official Method 962.09) with taking two subsamples of each sample, weighing 1g, and then placing it into a glass crucible and thereafter into the Fibertec extrusion apparatus, cooked with 0.128M H_2SO_4 for 30min. Samples were washed three times with distilled water, thereafter cooked with 0.313M sodium hydroxide for 30min and then washed again. After which the the crude fat, acid hydrolysis, (official method 920.39) determination was done by placing 2g subsamples in a test tube, adding 2ml Ethanol and 10ml HCl (38%) and this was then placed in a water bath for 30min. The tubes were cooled and contents poured into a seperating funnel. Then 25ml diethyl ether was added and shaken (1min), then 25ml petroleum ether was added and shaken. The upper portion of the fat was then poured off into fat beaker, after which 15ml of diethyl ether was added and shaken, thereafter 15ml of petroleum ether was added and shaken. The previous step (adding 15ml of each) was repeated twice more with the fat poured off. The fat beakers were placed on a sand bath for 45min, then cooled and weighed.

The AA profile was determined by (official method 994.12) preparing samples through hydrolysis and then the total AA profile was determined by using a Water API Quattro Micro instrument. During hydrolysis 0.1g of the sample was placed in a hydrolysis tube, along with 6ml Hydrochloric acid and a 15% Phenol solution. Vaccuum was created and nitrogen was added, then tube was sealed. After hydrolysis the samples were removed and placed into Eppendorf tubes.Samples were then subjected to Water AccQ Tag Ultra derivitization kit for cleaning. Amino acid standards were prepared by adding 40µl of standard to 760µl water and 200µl internal standard. Each sample was diluted according to their protein content. After that, 10µl of sample was added to 70µl Borate buffer and 20µl of reconstituted AccQ Tag reagent, they were then removed and cooled. Then placed on a heating block (55°C) for 10 min. Then 1µl of the sample was injected into the apparatus for analysis and different amino acids were determined (g/100g).

Gross energy was determined and then used to determine the AME using the following equation:

Equation 5.1

$$\text{Apparent metabolisable energy} = \text{GE}_{\text{diet}} - [\text{GE}_{\text{excreta}} \times (\frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{excreta}}})]$$

The coefficient of total tract digestibility (CTTD), of each analysed nutrient were calculated by using the following equations:

Equation 5.2

$$\text{Nutrient consumed}\left(\frac{\text{g}}{\text{trial}}\right) = \text{Nutrient}_{\text{excreta}} \times \text{DM}_{\text{intake}}$$

$$\text{Nutrient excreted(g/trial)} = \text{Nutrient}_{\text{excreta}} \times \text{DM}_{\text{excreta}}$$

$$\text{Digested Nutrient(g/trial)} = \text{Nutrient}_{\text{consumed}} - [\text{Nutrient}_{\text{excreta}} \times (\frac{\text{Marker}_{\text{diet}}}{\text{Marker}_{\text{diet}}})]$$

$$\text{CTTD(g/kg)} = \frac{\text{Digested}}{\text{Consumed}}$$

CTTD = coefficient of total tract digestibility

The total tract apparent digestibility obtained for the 100% maize meal diet was used to correct for the digestibility of the pre-pupae-and-maize diets.

5.2.4 Statistical analysis

Statistical analysis was done using the general linear models procedure of STATISTICA (data analysis software system), Version 9, by Statsoft Inc. (2009). Before proceeding with analysis the Shapiro-Wilk test was used to test for normality of the data and also homoscedasticity. If significant non-normality values ($P \leq 0.05$) appeared, outliers were identified and residuals greater than three were removed. Where age effects were not a variable the statistics were done by using one-way analysis of variances (ANOVA) with Bonferroni *post hoc* test being applied.

Differences were considered significant if P-values were less than 0.05, thus the 5% significant level was used.

5.3 Results and discussion

The proximate analysis for the different treatment diets in the digestibility study can be seenin Table 5.2. There were differences ($P \leq 0.05$) among the treatment diets with regards to the nutrient composition. The most apparent difference was Trt7, the Formic acid based treatment had the lowest fibre content as well as the lowest fat and ash content. It was also revealed that Trt4 (72°C for 15min) had the lowest fat and amino acid composition of the heat based treatments. Results obtained from the digestibility study are summarised in Table 5.3.

There were differences (P≤0.05) between the treatment diets regarding AME, with Trt8 (Propionic) having the highest AME and Trt1 (62°C for 30min), 2 (62°C for 60min) and 5 (100°C for 2min) being the closest to Trt8 (Propionic). The CTTD for protein and fat indicates treatment differences (P≤0.05), with Trt1 (62°C for 30min), 2 (62°C for 60min), 3 (72°C for 5min) and 5 (100°C for 2min) exhibiting better digestibility values. It can be observed from Table 5.3 that Trt7 (Formic) showed the best fibre digestibility of the pre-pupae treatment methods. There were differences (P≤0.05) between the treatments with regards to ash digestibility, with the heat based treatments (except Trt4 (72°C for 15min) having higher digestibility values than the acid based treatments.

The crude protein CTTDs reported in previous studies have indicated the digestibility of soya bean to be 80.7% (Sebastian *et al*., 1997), however, Hwangbo *et al*. (2009) reported 98%. This indicates that there is a big variation in the research, but the BSF pre-pupae grown for this trial achieved CTTD values for protein between 80.7% and 98%. However, it is reported that a high amino acid digestibility and complete AA composition is more important (Ellinger, 1958). As with Uushona (2015), the results obtained in this trial indicated high levels of CTTD with regards to crude protein. The CTTD of crude protein was, however, slightly lower than that obtained by Uushona (2015). The fat digestibility in this trial closely resembles that which was observed in a previous study on HF larvae (Pretorius, 2011), but it was lower than that which was reported for BSF pre-pupae meal (Uushona, 2015).

Insect meals contain chitin (makes up the exoskeleton of insect body) and thus is expected that the digestibility values would be lower (Diener *et al*., 2009). It has been revealed that an increase of chitin in the diet causes a decrease in nutrient digestibility (Razdan & Pettersson, 1994; Kroeckel *et al*., 2012). BSF are known to be high in chitin (Newton *et al*., 2005), but pre-pupae meal has lower chitin values than that of pupae meal (Pretorius, 2011). The milling of materials before feeding it to the animals has been revealed to increase the digestion surface (McDonald, 2002). All the pre-pupae treatments were milled and, therefore, even though differences in the fibre content of the pre-pupae treatments are observed, chitin might not have played that large a role in the digestibility. It is noteworthy that to determine chitin content, the methods used for determining fibre were used, as with previous studies (Uushona, 2015). The fibre level, therefore, can indicate the difference in level of chitin of the insect diets. Therefore, from here on when referring to fibre, it is a referral to chitin. It is observed from Table 5.2 that the fibre content of Trt7 (Formic acid) was the lowest. However, if the CTTD of nutrients for Trt7 (Formic acid) is taken into consideration, it can be observed that Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min) and 5 (100°C for 2min) exhibited higher CTTD's than Trt7 (Formic acid). Previous studies have indicated that fibre

Table 5.2 The analysed nutritional composition of black soldier fly (*Hermetia illucens*) pre-pupae grown on human faecal matter of the different treatment methods.

		Treatment								
	Unit	1	2	3	4	5	6	7	8	9
Gross energy	MJ/kg	20.91	20.77	19.59	19.77	20.55	20.19	20.62	22.39	16.85
Crude Protein	%	21.31	24.34	19.84	20.78	23.34	19.03	19.63	21.31	8.53
Ash	%	7.49	9.12	10.64	10.23	9.35	9.72	5.75	7.20	2.06
Fat	%	22.61	21.32	18.92	18.58	20.32	21.21	16.59	24.56	3.72
Crude protein	%	21.31	24.34	19.84	20.78	23.34	19.03	19.63	21.31	8.53
Crude fibre	%	4.07	5.00	3.99	3.97	4.73	4.47	3.58	4.09	2.19
Histidine*	g/100g	0.44	0.43	0.31	0.18	0.36	0.29	0.28	0.30	0.13
Serine	g/100g	0.82	0.79	0.64	0.35	0.73	0.64	0.57	0.66	0.30
Arginine*	g/100g	0.96	0.95	0.77	0.40	0.86	0.69	0.67	0.73	0.29
Glycine	g/100g	1.01	0.93	0.70	0.40	0.84	0.71	0.65	0.80	0.25
Aspartic acid	g/100g	1.45	1.48	1.12	0.61	1.44	1.11	1.16	1.23	0.41
Glutamic acid	g/100g	2.69	2.22	1.99	1.13	2.39	2.01	1.98	2.21	1.23
Threonine*	g/100g	0.70	0.71	0.55	0.30	0.61	0.53	0.50	0.57	0.21
Alanine	g/100g	1.19	1.20	0.98	0.49	1.04	0.94	1.04	1.22	0.44
Proline	g/100g	1.22	1.15	0.93	0.50	0.99	0.95	0.89	0.99	0.48
Cysteine	g/100g	0.04	0.04	0.04	0.02	0.03	0.04	0.03	0.02	0.02
Lysine*	g/100g	0.90	0.97	0.77	0.39	0.90	0.74	0.71	0.82	0.19
Tyrosine	g/100g	1.01	0.91	0.60	0.41	0.70	0.57	0.58	0.64	0.18
Methionine*	g/100g	0.25	0.22	0.20	0.13	0.25	0.17	0.18	0.24	0.07
Valine*	g/100g	1.06	1.07	0.80	0.46	0.96	0.78	0.77	0.91	0.30
Isoleucine*	g/100g	0.74	0.75	0.56	0.31	0.65	0.52	0.55	0.62	0.21
Leucine*	g/100g	1.53	1.43	1.10	0.62	1.29	1.10	1.09	1.13	0.72
Phenylalanine*	g/100g	0.80	0.73	0.53	0.30	0.64	0.48	0.49	0.53	0.25
Phosphorous	%	0.45	0.49	0.57	0.53	0.45	0.50	0.42	0.31	0.24
Calcium	%	2.60	3.02	3.73	3.74	4.06	3.56	2.14	1.29	0.12

*Essential amino acids

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

influences the passage rate of feed in the digestive tract, as it causes a reduction in digestion in the upper digestive tract and increases digestion in the lower digestive tract of almost all nutrients (Wenk, 2001). This could then explain why Trt7 (Formic acid) did not exhibit the same level of CTTD as those treatments.

There were differences (P≤0.05) between the treatment diets regarding the AA digestibility (Table 5.3). Where the essential AA's are concerned, Trt1 (62°C for 30min), 2 (62°C for 60min), 3 (72°C for 5min) and 5 (100°C for 2min) had the best digestibility values. There were, however, also treatment differences (P≤0.05) regarding the other AA's. It can be observed that there was a trend, not just limited to AA, with Trt1 (62°C for 30min), 2 (62°C for 60min), 3 (72°C for 5min) and 5 (100°C for 2min) indicating better digestibility values compared to the other treatment diets.

Over-processing, especially overheating, is considered to be a primary cause of a reduced AA bioavailability in broiler diets (Parsons, 1996). Lysine is the AA that is mostly affected by over-exposure to heat due to its susceptibility to the Mailard reaction (Parsons, 1996). As observed from Table 5.2, extending processing time, such as 62°C at 30 mins (Trt1) and 62°C at 60mins (Trt2) caused a decrease in AA content. This trend was observed between Trt1 (62°C for 30min) and Trt2 (62°C for 60min), Trt3 (72°C for 5min) and Trt4 (72°C for 15min), Trt5 (100°C for 2min) and Trt6 (100°C for 5min). Thus it can be said that with shorter heat treatment time, the higher the AA content. The CTTD for the essential amino acids was higher than that reported for soya bean (Sebastian *et al*., 1997) and comparable to those found in previous studies for larvae meal, with regards to the AA CTTD (Zuidhof *et al*., 2003; Hwangbo *et al*., 2009; Pretorius, 2011; Uushona, 2015). Methionine is the first limiting amino acid, with lysine second and threonine third, therefore, a high CTTD of these AA is of great importance in broiler diets. Sebastian *et al*. (1997) and Hwangbo *et al*. (2009), respectively, reported that soya bean meal had a digestibility of methionine at 91.6% and 93%. Lysine is used as a reference AA in feed formulation and is important for muscle growth (Lemme *et al*., 2004). L-Threonine and lysine in poultry diets optimises the use of body protein deposition and weight gain, it is also vital in the immune response of the bird (Taghinejad-Roudbaneh *et al*., 2013). It can be observed in Table 5.3 that the CTTD of the first three limiting AA were above 0.9 for Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min) and Trt5 (100°C for 2min).

The interaction of lysine and arginine in animal nutrition is a complex process, but an excess of lysine has three basic consequences. The first being lysine competes with arginine in the renal tubules which cause a reduction in arginine retention (Jones *et al*., 1966). The second being that the levels of lysine in the diet of poultry causes an increase in renal arginase

Table 5.3 Averages (±standard error) of coefficient of total tract digestibility (CTTD) of black soldier fly (*Hermetia illucens*) pre-pupae grown on human faecal matter of the respective treatment diets and the apparent metabolisable energy (AME) for broilers.

	Treatment							
	1	2	3	4	5	6	7	8
AME (MJ/kg)	18.34^{b} ±0.070	18.27^{b} ±0.068	17.26^{d} ±0.048	17.43^{cd} ±0.081	18.03^{b} ±0.036	17.64^{c} ±0.084	18.23^{b} ±0.072	19.70^{a} ±0.099
Ash (%)	0.81^{ab} ±0.004	0.80^{bc} ±0.003	0.83^{a} ±0.005	0.79^{c} ±0.003	0.82^{ab} ±0.004	0.79^{c} ±0.003	0.77^{d} ±0.002	0.76^{d} ±0.007
Protein (%)	0.93^{a} ±0.004	0.94^{a} ±0.004	0.92^{a} ±0.004	0.89^{b} ±0.007	0.93^{a} ±0.004	0.87^{b} ±0.003	0.84^{c} ±0.006	0.83^{c} ±0.007
Fat (%)	0.98^{a} ±0.000	0.99^{a} ±0.002	0.96^{bc} ±0.001	0.95^{d} ±0.001	0.99^{a} ±0.002	0.96^{bcd} ±0.003	0.97^{b} ±0.004	0.95^{cd} ±0.003
Fiber (%)	0.72^{cd} ±0.005	0.75^{b} ±0.003	0.74^{bc} ±0.004	0.71^{de} ±0.003	0.73^{cd} ±0.003	0.70^{e} ±0.002	0.80^{a} ±0.003	0.72^{cde} ±0.005
Histidine* (g/100g)	0.92^{ab} ±0.001	0.90^{b} ±0.008	0.91^{ab} ±0.002	0.88^{c} ±0.001	0.93^{a} ±0.003	0.87^{c} ±0.002	0.83^{e} ±0.004	0.85^{d} ±0.003
Serine (g/100g)	0.89^{a} ±0.004	0.88^{ab} ±0.005	0.86^{b} ±0.004	0.83^{c} ±0.002	0.87^{b} ±0.004	0.81^{c} ±0.004	0.77^{d} ±0.003	0.78^{d} ±0.005
Arginine* (g/100g)	0.97^{a} ±0.003	0.97^{a} ±0.005	0.93^{b} ±0.004	0.89^{c} ±0.004	0.96^{a} ±0.004	0.87^{c} ±0.003	0.81^{d} ±0.003	0.84^{e} ±0.007
Glycine (g/100g)	0.84^{b} ±0.003	0.87^{a} ±0.007	0.80^{c} ±0.008	0.77^{d} ±0.003	0.82^{a} ±0.005	0.73^{e} ±0.005	0.70^{e} ±0.005	0.72^{e} ±0.004
Aspartic acid (g/100g)	0.93^{ab} ±0.001	0.93^{b} ±0.002	0.88^{c} ±0.003	0.83^{d} ±0.002	0.95^{a} ±0.004	0.81^{e} ±0.004	0.75^{g} ±0.004	0.78^{f} ±0.004
Glutamic acid (g/100g)	0.92^{a} ±0.001	0.91^{a} ±0.001	0.89^{b} ±0.003	0.85^{c} ±0.003	0.92^{a} ±0.002	0.84^{d} ±0.003	0.78^{f} ±0.004	0.80^{e} ±0.005
Threonine* (g/100g)	0.91^{a} ±0.002	0.91^{a} ±0.002	0.89^{b} ±0.003	0.84^{c} ±0.001	0.92^{a} ±0.003	0.83^{cd} ±0.002	0.79^{e} ±0.005	0.82^{d} ±0.003
Alanine(g/100g)	0.91^{ab} ±0.002	0.92^{a} ±0.003	0.86^{c} ±0.002	0.80^{d} ±0.002	0.90^{b} ±0.002	0.81^{d} ±0.003	0.75^{f} ±0.004	0.78^{e} ±0.004
Proline (g/100g)	0.93^{a} ±0.001	0.92^{a} ±0.002	0.87^{c} ±0.004	0.83^{d} ±0.001	0.89^{b} ±0.005	0.82^{d} ±0.002	0.76^{e} ±0.003	0.77^{e} ±0.004
Cysteine (g/100g)	0.84^{a} ±0.004	0.84^{a} ±0.004	0.83^{a} ±0.005	0.80^{b} ±0.003	0.85^{a} ±0.004	0.78^{b} ±0.003	0.71^{d} ±0.003	0.74^{c} ±0.006
Lysine* (g/100g)	0.95^{b} ±0.002	0.96^{b} ±0.003	0.90^{c} ±0.001	0.88^{d} ±0.001	0.97^{a} ±0.002	0.86^{e} ±0.003	0.80^{g} ±0.001	0.83^{f} ±0.005
Tyrosine (g/100g)	0.94^{a} ±0.002	0.93^{a} ±0.001	0.87^{c} ±0.005	0.81^{d} ±0.004	0.90^{b} ±0.001	0.78^{e} ±0.005	0.74^{f} ±0.002	0.77^{e} ±0.003
Methionine* (g/100g)	0.95^{b} ±0.002	0.96^{ab} ±0.002	0.90^{c} ±0.003	0.87^{d} ±0.004	0.97^{a} ±0.005	0.86^{d} ±0.004	0.81^{f} ±0.002	0.84^{e} ±0.006
Valine* (g/100g)	0.91^{a} ±0.002	0.91^{a} ±0.002	0.87^{b} ±0.001	0.85^{c} ±0.003	0.90^{a} ±0.002	0.81^{d} ±0.003	0.77^{e} ±0.003	0.81^{d} ±0.003
Ileucine* (g/100g)	0.92^{a} ±0.002	0.92^{ab} ±0.002	0.85^{d} ±0.001	0.87^{c} ±0.003	0.9 ^{0b} ±0.005	0.82^{e} ±0.003	0.76^{f} ±0.003	0.81^{e} ±0.004
Leucine* (g/100g)	0.94^{a} ±0.002	0.93^{a} ±0.004	0.89^{b} ±0.002	0.84^{c} ±0.002	0.93^{a} ±0.003	0.80^{d} ±0.002	0.75^{f} ±0.004	0.78^{e} ±0.005
Phenylalanine* (g/100g)	0.94^{a} ±0.001	0.93^{a} ±0.002	0.87^{c} ±0.004	0.84^{d} ±0.001	0.90^{b} ±0.002	0.80^{e} ±0.003	0.78^{f} ±0.004	0.80^{e} ±0.002
Phosphorous (%)	0.81^{ab} ±0.005	0.83^{a} ±0.018	0.79^{bc} ±0.003	0.75^{d} ±0.006	0.76^{cd} ±0.005	0.79^{abc} ±0.007	0.80^{abc} ±0.006	0.79^{bc} ±0.007
Calcium (%)	0.85^{bcd} ±0.002	0.84^{cd} ±0.003	0.81^{e} ±0.002	0.86^{bc} ±0.002	0.84^{d} ±0.002	0.81^{e} ±0.002	0.87^{a} ±0.003	0.86^{b} ±0.003

*Essential amino acids

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

Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min), Trt4 (72°C for 15min), Trt5 (100°C for 2min), Trt6 (100°C for 5min), Trt7 (rinsed in 5% propionic acid), Trt8 (rinsed in 5% formic acid)

activity, that causes an increase in the oxidation of arginine (Nesheim & Austicandm, 1970; Summers & Leeson, 1997). The third consequence is that smaller amounts of lysine can cause a depression of the hepatic glycine transamidinase activity in chicks (Jones *et al*., 1966). It is, therefore, due to this antagonistic relationship that the ratio for these two AA should be 1:1 (Chamruspollert *et al*., 2002). The increase of lysine in the diet can also cause an increase in urea excretion and can slightly increase arginine excretion (Austic & Scott, 1975). It has been observed that when lysine exceeds 3% in the diets the above-mentioned reactions happen (Austic & Scott, 1975). All the treatment diets had a ratio that is very close to 1:1 (Table 5.2)

Glutamic acid is the AA that is of interest regarding broiler growth, improving live weight and carcass weight (Moran & Stilborn, 1996). The CTTD of glutamic acid was the highest in Trt1 (62°C for 30min), 2 (62°C for 60min), 3 (72°C for 5min) and 5 (100°C for 2min), which was above or close to 0.9 (Table 5.3). This would then indicate that the bio-availability of glutamic acid was high in these treatments. The isoleucine requirements for broilers are reported to be 0.89% (NRC, 1994). Considering this, it can be observed in Table 5.2, that Trt1 (62°C for 30min), 2 (62°C for 60min) were the closest to this value (0.74 and 0.75, respectively). However, when formulating a diet, other raw materials are added together which would make up for the lack of any nutrients in one of the feed sources.

Calcium (Ca) and phosphorous (P) also differed ($P \leq 0.05$) between the treatment diets (Table 5.2). Phosphorous content would appear to be quite similar between the treatment diets, except for Trt8 (Propionic) which had lower phosphorous levels (0.24 compared to the ≥0.31 of the other treatments). Previous studies have reported that BSF pre-pupae meal is high in Ca and P (Newton *et al*., 2005). Broilers are selected for their fast growth and this increases the probability of there being bone deficiencies. The above mentioned, can be due to the fact that the weight gain occurs at a faster tempo than bone development, which causes the bones to become porous and fragile (Hocking *et al*., 2009; Garcia *et al*., 2013). Therefore, it is important to determine the bioavailability of the Ca and P within the diet. This study and a previous study (Uushona, 2015) revealed that the CTTD of calcium and phosphorous in BSF pre-pupae, was above or around 80%. This trial reported values of over 0.8 for Ca and over 0.75 for P.

It is reported that CTTD of nutrients above 70% is viewed as acceptable (Emami *et al*., 2013; Thiamhirunsopit *et al*., 2014). It can be observed that the CTTD of all nutrients in this trial were above this level. The most apparent trend that was observed was where Trt1 (62°C for 30min), Trt2 (62°C for 60min), Trt3 (72°C for 5min) and Trt5 (100°C for 2min) exhibited superior CTTD values. This would explain the similar trend that was observed in the production trial between these specific diets (Chapter 4). Trt 7 (Formic), as an acid base treatment did exhibit good chemical composition for certain nutrients and it was indicated to produce a good digestibility

for fibre. However, Trt4 (72°C for 15min) produced results that were not on the same level as those of the other heat based treatments and this would explain why certain production parameters were so low (Chapter 4). The AA content for Trt4 (72°C for 15min) was also observed to be quite low compared to the other heat based treatments.

5.4 Conclusion

The aim of the study was to determine what the effect of each treatment method was on the digestibility values of the pre-pupae. The treatment methods determined how heat coupled with time and acid effected the pre-pupae composition. The secondary aim was to determine how these pre-pupae compared with previous studies on larvae as that from this investigation were grown on human faecal matter and not kitchen waste. It can be concluded that the digestibility values of the pre-pupae treatments are excellent. The trends observed earlier in the production trials can be explained by the results reported in this study. This trend being that the heat based treatments achieved better production values than the acid based treatments. The bio-availability of the amino acids of these pre-pupae treatments were high and can easily be utilised by the animals efficiently. The heat based treatments methods showed better digestibility results when compared to those of the acid based treatments. The pre-pupae in this trial were comparable to other protein sources and, due to the feed stuff of the pre-pupae, shows great promise. It is important to note that the BSF pre-pupae in this trial were grown on human faecal matter, a waste source that is inexpensive and abundant. It can be concluded that the pre-pupae can easily convert a perceived low quality waste to a high quality feed source that is comparable to commercially accepted protein sources, such as soybean meal. The results obtained in this trial would be of great importance to animal nutritionists.

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Chapter 6. Evaluation of egg quality of battery and free range layer hens fed different processed black soldier fly (*Hermetia illucens*) pre-pupae meal grown on human waste

Abstract

This trial investigated the effect of four different treatment methods of black soldier fly (*Hermetia illucens*) pre-pupae grown on human waste on egg quality of layer hens. The four treatments were: submerging in water for 62°C for 30min, 72°C for 5min, 100°C for 2min and rinsing in 5% propionic acid. One hundred and fifty, 46 weeks old hyline silver hens were used. A hundred of these hens were housed in four free range cages, with 25 hens per treatment. The other 50 were housed in individual battery pens in a naturally ventilated house, with ten hens per treatment (fifth treatment being the control). Hens were adapted on the feed for 14 days. After which eggs were collected daily for 21 days, with five eggs per treatment per day selected. The eggs closest to the average egg weight of each cage, was randomly selected and included in the study. The eggs were stored in the dark, at room temperature at different time intervals before analysis: 1) the same day, 2) one day after collection, 3) one week later, 4) two weeks later and 5) one month later. Data collected included egg weight, shell weight, shell thickness, albumen height (this being used to determine the Haugh unit), yolk weight, and yolk colour. There were no significant differences between the dietary treatments with regards to shell weight, shell thickness, Haugh unit (HU) and colour. Egg weight and yolk weight differed between treatments: Trt2 (72°C for 5min) house differed significantly from the other treatments with regards to a heavier egg weight. While Trt2 (72°C for 5min) house and Trt3 (100°C for 2min) house differed significantly with the control group regarding yolk weight, by having a heavier yolk. With increased storage time, the quality of the eggs decreased (egg weight, yolk weight and HU). It was observed that there were significant differences between treatments with regards to egg weight and yolk weight in storage group 1. There were also significant differences in storage group 2 when regarding egg weight; With Trt2 (72°C for 5min) house being significantly different to all the other treatments except Trt1 (62°C for 30min) house; Trt1 to 3 (62°C for 30min, 72°C for 5min, 100°C for 2min) free range also differs significantly with the other treatment groups, being lighter than Trt2 and Trt3 house. It can be concluded that even though there were significant differences with regard to certain aspects, overall the pre-pupae diets performed as well if not better than the control diet. This then indicates pre-pupae produced from human waste can be used as a good protein source in layer diets.

Keywords: *Black soldier fly, Egg quality, Haugh unit, Human waste, Nutrient recycling, Storage time*

6.1 Introduction

The diet of layers is very important as it plays a major role in producing eggs of good quality (Roberts, 2004). Layer hens need a balanced diet from an early age (before lay) so that they produce eggs that are strong and do not break when laid (Roland & Bryant, 1994). This diet must consist of enough Ca and P in the right ratio (Roberts, 2004); lack of phosphorous in the diet can cause demineralisation of the hen's skeleton (Bar *et al*., 2002; Sohail & Roland, 2002). In addition to the weather (a hot environment can cause reduced intake, which again would affect egg shell quality), the housing may also effect egg shell quality, especially in free range systems (Fraser & Bain, 1994), but this could be explained by the inability to ensure a balanced diet of the hens in such a system. The cage density may also effect the egg shell quality (Mench *et al*., 1986), however, other studies have shown no consistent effects (Lee & Moss, 1995).

Anti-nutrients within the feed also play a role in the quality of the eggs that are produced (Roberts, 2004). Mycotoxins in the feed have an effect on the shell quality and production of the layers, but this can be ascribed to the reduced feed intake of the layers (Suksupath *et al*., 1989). Other anti-nutritional factors within feed, such as non-starch polysaccharides (NSPs), increase the gut viscosity to hold larger volumes of water, causing watery and sticky droppings (Roberts, 2004). This can also negatively affect the eggs by causing dark stripes on the exterior, which not only reduces the quality of the egg, but makes it unsellable. A possible way to deal with NSP's in the diet is to add certain enzymes that degrade NSPs, especially those found in wheat and barley based diets, to improve egg shell quality (Roberts *et al*., 1999). It is, therefore, important to determine whether the chitin, which is found in insect exoskeletons, negatively affect the egg shell quality by acting similar to NSP's. Very little research has been done to determine/quantify this.

The quality of the yolk is determined by two factors, the colour and the strength of the periviteline membrane (Roberts, 2004). The colour of the yolk is associated with healthiness, where a more yellow egg is perceived as being more healthy, there is however no research to support this. The colour is mainly affected by the carotenoids that are found within the feed (Scott & Silversides, 2000). Previous studies have indicated that low levels of vitamin A can cause an increase of blood spots in the egg, which lowers the internal quality of the egg (Pingel & Jeroch, 1997). The strength of the periviteline membrane determines the ease with which the yolk breaks; the weaker it is (older eggs for instance) the easier it breaks (Kirunda & McKee, 2000). A thick albumen is an indication of freshness (Silversides & Scott, 2000). The albumen makes up the largest part of the egg weight (about 66%) and it affects the stability and strength of the yolk, therefore albumen quality is important.

The albumen quality is measured 1cm from the edge of the yolk by measuring the height of the albumen, the height is then converted into HU. Factors that affect HU include: storage time,

storage temperature, hen age, strain of the bird, nutrition (dietary protein and amino acid content for example lysine, methionine, feed enzymes, grain type or protein source), disease, supplements, artificial exposure to ammonia, induced moult and medication (Roberts & Ball, 2004). The main factor that was examined in this trial was storage time. Previous studies have revealed that HU decrease with an increase in storage time (Silversides & Scott, 2001; Samli *et al*., 2005). Albumen breaks down and becomes more fluid as the egg gets older. The changes in the albumen height are related to the changes occurring in the ovomucin, particularly the thick albumen (Kato *et al*., 1971; Kato *et al*., 1981; Toussant & Latshaw, 1999). Some studies have indicated that nutritional factors can affect the albumen quality, although Williams (1992) has concluded that the quality is not greatly affected by nutrition. An increase of albumen quality has been observed with the increase of dietary lysine concentration, supplementation of ascorbic acid and vitamin E (Balnave *et al*., 2000; Kirunda *et al*., 2001; Franchini *et al*., 2002). A decrease of albumen quality has been ascribed to an increase in dietary protein (Hammershøj & Kjaer, 1999).

Although numerous research projects have reported on egg quality in the past, only one study could be found that relates to larvae meal inclusion in the diet on the egg quality. Agunbiade *et al*. (2007) concluded that BSF larvae can be included in layer diets to replace fishmeal up to 50% without having any adverse effects on the egg quality. However, it is noteworthy that the use of fishmeal within layer diets is uncommon (Agunbiade *et al*., 2007). It is also important to note that the BSF larvae that was used by Agunbiade *et al*. (2007) was not grown as was in this trial, but collected from chicken manure. Due to the feed source of the pre-pupae in this investigation being human faecal matter, it is important to determine whether it is still an acceptable protein source and if these pre-pupae can produce eggs of good quality. Also, in this study, the age of the hens and the storage temperature of the eggs are all the same, therefore the quality of the albumen can be determined through Haugh units.

6.2 Materials and methods

6.2.1 Pre-pupae treatments

The pre-pupae in this trial were grown as described in Chapter 3.2.1. After harvesting and purging the larvae were all subjected to pre-treatment aimed at reducing microbial risk associated with feed substrate. Pre-treatment methods used in this trial consisted of subjecting the pre-pupae to different water temperatures and time intervals and with one acid treatment. The four different treatment methods are described in the experimental procedure part of this chapter. It is important to note that the time interval related to the treatments started once the temperature of the water reached the specific temperature/degrees after the larvae were added.

6.2.2 Animals and data collection

Animals and Experimental Procedure

The trial consisted of 150, 46-week-old hyline silver hens. These hens were raised in outside free-range cages up until the start of the trial (from 17weeks of age to 40weeks). They received a standard commercial feed. One half of the free-range cages were open to the outdoors and the other half under roof and sheltered from the environment. The layers, therefore, received shelter from environment and also time in the environment for scratching and basking in the sun. Artificial light was also provided to ensure the layers received 16hr days. The layers also received new layer boxes before they came into lay, so they could learn to lay within the boxes. The boxes were placed under the roof, this was done so that the environment does not affect the eggs. During the trial period, 100hens were housed outdoors in the free-range cages, while fifty were housed in a layer house with natural ventilation. The free-range hens were divided into four groups, 25 hens per group, each group having two hanging feeders and two hanging water bowls. These hens also had layer boxes to lay their eggs in. In the layer house, the 50 hens were divided into five treatment groups, 10 per treatment. They were placed into individual cages, with a slightly slanted floor so that the eggs could roll out. The cages had individual nipple drinkers and feeding trough. Although, the treatments of the pre-pupae, prior to inclusion into the diets, are described in detail in Chapter 3.2.1, not all of these treatments were included in the layer trial. These four treatments were used as a good representative of the treatment groups. The four treatments that were included in the experimental diets are:

Trt1: 62^{o}C for 30mins

Trt2: 72^{o}C for 5mins

Trt3: 100^{o}C for 2min

Trt4: Rinsed with Propionic acid

Treatment 5/Control: Soybean meal diet

Table 6.1 describes the composition (iso-nitrogenous and iso-energetic) of the diets of each treatment group, with treatment groups 1 to 4 having a 10% inclusion level of pre-pupae meal and treatment 5 being the control group formulated using only soybean mealas protein source.

Data Collection

The hens were randomly allocated to the nine (four free-range and five cages) different groups and randomly allocated to their cages. Each hen was weighed when placed in their pen/cage, and weighed again at the end of the trial. In the layer house the feed was weighed weekly and the average intake per group was determined. No data on feed intake was collected

from the free range hens due to unavoidable wastages occurring. Before data collection started, the hens were fed their treatment diets for a two-week adaptation period.

Eggs were collected daily from the free range pens and from the cages in the layer house over a period of 21 days for analysis. Broken or cracked eggs were discarded and noted. Also "dirty" eggs (contaminated with large amount of faeces or dirt) were noted and discarded. From the eggs that were collected, five eggs per pen/cage per treatment were selected and placed into five different groups for analysis. Each one of these five groups were assigned time of storage before analysis as follows:

Group 1: same day
Group 2: next day
Group 3: week later
Group 4: two weeks later
Group 5: one month later

These eggs were selected closest to the average egg weight of the treatment group. The eggs were then stored at room temperature (15-18°C) until egg analyses were done. Analysis of each egg consisted of i) egg weight, ii) weight of the empty shell, iii) weight of the yolk, iv) shell thickness, v) yolk - and albumen height and vi) the colour of the yolk.

The weight of the eggs, yolk and shell was measured with a laboratory scale (Measuring in grams to 4 decimal units). The thickness was measured with a digital caliper (Measuring in millimeters to 2 decimal units). The height of the yolk and albumen was measured with a Haugh measuring tripod.

From the albumen height, the HU can be calculated which is an indication of the egg's freshness,as follows:

Equation 6.1:

$$HU = 100\log(h - 1.7w^{0.37} + 7.6)$$

HU = Haugh units

h = albumin height

w = weight of whole egg in grams

The Haugh unit ranges from 0 - 130 and were ranked as:

AA: ≥72
A: 71-60
B: 59-31
C: ≤30

The size of the eggs determined their classification according to South African standards as follows (Minimum Mass (g) per egg):

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

The colour measurements were taken with a BYK-Gardiner Colour Guide (Catelogue no: 6805; BYK-Gardner, USA) and for the purpose of this study, the CIElab colour system was used (Commission International de L'Eclairage, 1976) with three measurements; L*(lightness), a*(redness) and b*(yellowness). Positive a* values are a measure of redness and negative a* values are a measure of greenness. Positive b* values are a measure of yellowness and negative b* values indicate blueness.

6.2.3 Statistical analyses

Statistical analysis were done with STATISTICA (data analysis software system), Version 9 (Statsoft Inc., 2009). Where age effects (on the egg storage section of the investigation) were not a variable, statistics were done by using one-way analysis of variances (ANOVA) with the Bonferroni least significant different (LSD) *post hoc* test. Where age and treatments effects were variables, the statistics were done using the mixed model repeated measure of ANOVA with the Bonferroni LSD *post hoc* test.

6.2.4 Food safety analysis

Detection of possible pathogens

Due to the growth media (human faeces) of the BSF pre-pupae that were added to the feed, the eggs were tested internally for any possible harmful pathogens. The pathogens that were tested for included *Salmonella*, *Listeria* and *E.coli*. One egg per treatment were randomly selected on day 0, 4, 8, 12, 16 and 20. The surface of the egg shell was sterilised by rolling the egg in 70% ethanol. Contents of the eggs were homogenised in a sterile environment and a dilution series of 10^{-1} – 10^{-5} was prepared. The plating methods are described in detail in Chapter 3.2.3**Error! Reference source not found.**.

6.3 Results and discussion

There were no differences (P>0.05) between the diets with regards todaily feed intake, change in layer weight and egg production percentage for the hens in the layer cages (Table 6.2). The three above-mentioned characteristics were only measured in the layer house and did not

Table 6.1 Ingredient, and calculated nutrient, composition of treatment diets.

	Unit	Diet 1-4 (10% LM)	Diet 5 (Control)
Ingredients:			
Hermetia illucens pre-pupae	%	13.171	-
Maize	%	65.427	58.823
Soybean	%	10.427	4.159
Soybean full fat	%	-	22.643
L-lysine	%	0.128	-
DL methionine	%	0.120	0.131
Vit + min premix *	%	0.250	0.250
Limestone	%	7.214	8.576
Salt	%	0.170	0.285
Monocalcium phosphate	%	1.208	1.494
Sodium bicarbonate	%	0.223	0.090
Oil - sunflower	%	1.662	3.549
Calculated nutritional compositionValue:			
Dry matter	%	89.039	89.080
AMEn adult	MJ/kg	12.908	12.920
Crude protein	%	15.420	15.420
Lysine	%	0.800	0.808
Methionine	%	0.370	0.376
Cysteine	%	-	-
Methionine + cysteine	%	0.680	0.670
Threonine	%	0.551	0.593
Tryptophan	%	0.150	0.170
Arginine	%	0.876	1.004
Isoleucine	%	0.592	0.673
Leucine	%	1.356	1.462
Histidine	%	0.431	0.433
Phenylalanine	%	0.680	0.718
Tyrosine	%	0.628	0.560
Phenylalanine + tyrosine	%	1.308	1.278
Valine	%	0.742	0.779
Ash	%	9.537	10.492
Crude fibre	%	2.580	2.747
Crude fat	%	10.000	10.000
Calcium	%	3.500	3.420
Phosphorous	%	0.882	0.697
Available phosphorous	%	0.450	0.450
Sodium	%	0.150	0.150
Chloride	%	0.220	0.220

*Vitamins and minerals are included according to the levels provided by the National Research Council, 1994.
**AMEn - Apparent metabolisable energy corrected for nitrogen

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include the free-range cages. The average daily feed intake per hen varied between 113 to 130g. The average change in weight of the hens varied between 110 to 170g. The production percentage is calculated by dividing the number of eggs produced by the number of hens per

day per treatment. The treatment groups had a 78-80% production per hen per day indicating that per day, 78-80% of the hens laid an egg.

The results depicted in Table 6.2 are not comparable to the study of Agunbiade *et al*. (2007) with regards to change in live weight and production percentage, however, it is comparable to daily feed intake. Agunbiade *et al*. (2007) noted a decrease in hen weight whereas it was observed that there was an increase in hen weight during this trial. This increase can be ascribed to the housing of the birds before the trial. The hens were raised outside in a free range system and then moved into a hen house with one hen per cage where the hens no longer experienced any competition for food and water. They also no longer contended with environment factors and no longer walked around, which would leave more energy available for production and building of reserves. The production % that was achieved in this trial was higher than that of Agunbiade *et al*. (2007); however, the hens used by Agunbiade *et al*. (2007) were older than the hens used in this trial and as there is a decrease in production with an increase in hen age (Joyner *et al.*, 1987), this difference was expected. It is important to note that as hens get older their production increases and reaches a plateau whereafter the production declines. The layers used by Agunbiade *et al.* (2007) already were past this plateau point and was on the decline. The layers in this trial was also past the plateau and was starting to decline, but it was not as far past as the previous trial.

As pertaining to egg quality characteristics with both diet and housing system as main effects, diet had no effect (P>0.05) on shell weight, yolk height, albumen height, HU, shell thickness and colour (Table 6.3). There were, however, differences (P≤0.05) for egg weight and yolk weight. The significant (P≤0.05) differences that are observed with egg weight, indicated thatTrt2 (72°C for 5min) house differed (P≤0.05) from the other treatment groups; producing eggs that are heavier than the other treatments. The yolk weight of the control group produced lighter eggs (P≤0.05) than Trt2 (72°C for 5min) house and Trt3 (100°C for 2min) house.

There were no differences (P>0.05) between the treatment diets with regards to shell weight and shell thickness, unlike that reported by Agunbiade *et al*., (2007). It is, however, noteworthy that the diet formulated for the layers in this trial is more complex compared to that of Agunbiade *et al.* (2007) with regards to the nutrient aspects. The diet for this trial was specifically formulated for the nutritional requirments of the layers in the trial. This trial diet also consisted of maize and soybean, where Agunbiade *et al*. (2007) used a cassava-based diet.

Table 6.2 Averages (±standard error) of daily feed intake (g), change in weight (g) and production percentage of the layer subjected to the different treatment groups in the layer house.

Parameter	Treatment				
	1	2	3	4	C
Average daily feed intake (g)	118.86±6.49	115.14±3.30	130.29±7.16	113±2.66	115.43±1.21
Average change in weight (g)	130±29.06	170±15.28	125±46.10	110±19.44	130±23.80
Production (%)	80.56±1.89	79.44±1.89	82.78±2.26	78.33±1.67	80.56±2.21

[a,b] Means with different superscripts within the same row differ significantly (P≤0.05)
Trt1 (62°C for 30min), Trt2 (72°C for 5min), Trt3 (100°C for 2min), Trt4 (rinsed in 5% propionic acid), C (control)

The differences in egg weight that were observed between the storage periods can be attributed to the change in albumen weight and therefore correlates to the lowering of the HU Table 6.4. The results in this trial indicated that with prolonged storage there were no significant differences in shell weight and thickness, as opposed to HU (Table 6.4). Previous studies have indicated similar results (Scott & Silversides, 2000; Silversides & Scott, 2001; Monira *et al*., 2003; Jones & Musgrove, 2005) whilst Samli *et al*. (2005) revealed that the shell strength does not decrease with prolonged storage time. There were no significant differences between the storage periods where yolk weight was concerned. Previous studies also indicated that there was no change in yolk weight over storage time (Scott & Silversides, 2000; Silversides & Scott, 2001; Monira *et al*., 2003; Samli *et al*., 2005). In addition, there were no differences (P>0.05) between the diets of the different storage periods with regards to HU and shell thickness. Egg weight differed (P≤0.05) between the treatment diets in the storage periods 1 and 2. Trt2 (72°C for 5min) differed significantly with the other treatments in storage period 1, with being heavier than the rest. In storage period 2 there were also significant differences; with Trt2 (72°C for 5min) house being significantly different from all the other treatments except Trt1 (62°C for 30min) house; Trt1 to Trt3 (62°C for 30min, 72°C for 5min, 100°C for 2min) free range also differed significantly from the other treatment groups, with yolks being lighter than rest. There were significant differences in storage period 1 with regards to yolk weight. Where TrtC (Control) were lighter than Trt2 (72°C for 5min) house, Trt3 (100°C for 2min) house and Trt2 (72°C for 5min) free range.There were also differences between the storage periods with regards to HU, where periods 1 and 2 differed (P≤0.05) from the rest. The egg weight from storage period 1 also differed (P≤0.05) from that of period 5.

The HU is a measure of the freshness of an egg and is measured through the height of the albumen: HU decreases with extended storage time (Scott & Silversides, 2000; Silversides &

Scott, 2001; Monira *et al*., 2003; Jones & Musgrove, 2005; Samli *et al*., 2005). The HU reported by Agunbiade *et al*. (2007) differed from the HU that were obtained in this trial when the eggs were analysed the same day (therefore the HU from storage period 1). Agunbiade *et al*. (2007) did not report the storage time before analysis. In fact,the HU in Agunbiade *et al*. (2007) is more comparable to the HU score of storage period 3 in this trial than that of period 1. Therefore, the differences observed between the two trials can be due to differences in storage time. It is important to note that the hen strain also differed between the two studies, which could also affect the HU. The temperature at which eggs are stored is also known to affect the rate at which the albumen degrades (Jones & Musgrove, 2005; Samli *et al*., 2005). The eggs in this trial were stored at room temperature and when compared to Samli *et al*. (2005), it can be observed that the degradation of HU is on par with that of eggs stored at 20^{o}C. It is has been showed that HU degradation slows down when eggs are stored at 4^{o}C (Jones & Musgrove, 2005; Samli *et al*., 2005). Therefore, the degradation observed in this trial could be reduced by storing the eggs at lower temperatures. All the eggs in this trial were graded as Extra large A-grade eggs, except Trt3 (100°C for 2min) house. Trt3 (100°C for 2min) house was graded as Large A-grade eggs, but the egg weight was very close to 59g (58.43g). The eggs in this trial would also be graded as AA with regards to their HU score and would then be regarded as fresh. However, storage period 5 (stored for a month before analysis) was graded as B - C, but this is a result of storage time and temperature as discussed.

From this trial, no eggs were produced that had any brown or black stripes on the exterior of the shell which would imply that the chitin in the feed did not act as NSP's. This correlates to previous studies (Agunbiade *et al*., 2007) where no eggs with these characteristics were reported. To determine whether the same results can still be obtained with the use of larvae meal high in chitin, more studies are required. Agunbiade *et al*. (2007) showed that larvae meal produced from BSF larvae that has higher levels of chitin shows great promise when added to layer diets.

The eggs analysed for *Salmonella, E. coli* and *Listeria* revealed that there was no growth on any of the plating's, this includes the free range eggs. This indicates that no pathogens were within the eggs or that the levels were too low to detect in the dilution series. As shown in Chapter 3 (Table 3.3), the treatments along with the drying of the pre-pupae were sufficient to destroy any pathogens present in the pre-pupae meal thereby making this nutrient source fit for feeding to layers and the eggs safe for human consumption.

Table 6.3 Averages (±standard error) of egg weight(g), shell weight(g), yolk weight(g), yolk height, albumen height, H.U, shell thickness, colour (L, a, b) of the different dietary and production groups

Characteristic	Treatment								
	House					Free Range			
	1	2	3	4	C	1	2	3	4
Egg Weight	61.09 [b] ±0.63	64.01 [a] ±0.71	58.43 [b] ±0.86	60.96 [b] ±0.9	59.52 [b] ±0.59	59.2 [b] ±0.27	60.69 [b] ±0.31	59.13 [b] ±0.28	59.64 [b] ±0.26
Shell Weight	7.78±0.14	8.14±0.16	7.58±0.16	8.3±0.29	7.93±0.12	7.6±0.22	7.84±0.14	7.56±0.22	7.52±0.21
Yolk Weight	16.71 [ab] ±0.27	17.41 [a] ±0.42	17.24 [a] ±0.31	16.49 [ab] ±0.3	15.53 [b] ±0.41	16.7 [ab] ±0.27	17.19 [a] ±0.30	16.52 [ab] ±0.28	16.33 [ab] ±0.38
Yolk height	18.42±0.18	18.57±0.29	18.36±0.28	18.51±0.28	17.76±0.25	18.15±0.23	18.41±0.36	18.25±0.22	17.71±0.18
Albumin Height	7.06±0.39	7.44±0.37	7.19±0.47	7.53±0.46	6.56±0.59	6.57±0.25	7.34±0.45	6.86±0.45	7.11±0.43
HU	82.34±2.81	84.15±2.51	83.7±2.95	84.83±3.33	76.83±6.09	80.54±1.80	84.05±2.94	81.12±3.54	83.06±2.80
Shell Thickness	0.33±0.01	0.34±0.01	0.34±0.01	0.35±0.01	0.37±0.01	0.36±0.01	0.36±0.01	0.36±0.01	0.34±0.01
Colour:									
L	63.11±1.09	62.95±0.93	61.67±0.98	62.47±1.06	61.31±1.11	64.34±0.88	63.04±1.17	63.04±1.06	65.63±0.56
a	7.54±0.73	8.02±0.72	9.87±0.50	8.33±0.55	10.3±0.67	7.49±0.71	9.39±0.69	9.6±0.65	7.56±0.60
b	53.75±1.84	54.16±2.19	55.91±1.9	54.97±1.82	52.46±2.34	54.47±1.93	57.44±1.53	55.13±2.42	51.98±1.95

[a,b] Means with different superscripts within the same row differ significantly (P≤0.05)
Trt1 (62°C for 30min), Trt2 (72°C for 5min), Trt3 (100°C for 2min), Trt4 (rinsed in 5% propionic acid), C (control)

Table 6.4 Averages (±standard error) of haugh unit (H.U), egg weight (g), shell thickness, yolk weight (g) of the different treatment groups and their different storage groups/time.

	Treatment								
	House					Free Range			
	1	2	3	4	C	1	2	3	4
HU period									
1	82.34±2.81	84.15±2.51	83.7±2.95	84.83±3.33	76.83±6.09	80.54±1.8	84.05±2.94	81.12±3.54	83.06±2.80
2	76.37±3.35	84.21±2.40	86.28±2.06	81.72±2.85	73.06±3.97	78.61±3.33	82.05±2.21	77.87±2.71	81.86±1.70
3	61.3±1.91	66.53±3.48	71.29±1.50	66.39±2.78	62.88±2.58	64.08±2.79	61.31±3.13	61.23±2.50	68.87±2.43
4	47.38±3.37	49.58±2.95	55.47±4.51	56.73±2.36	46.24±3.66	48.13±3.63	45.88±4.09	46.72±3.91	54.83±3.83
5	37.81±3.21	33.6±2.02	35.93±2.80	33.22±2.92	38.71±4.16	34.03±1.89	38.05±3.39	41.03±2.38	37.66±2.63
Egg Weight period									
1	61.09^{b} ±0.63	64.01^{a} ±0.71	58.43^{b} ±0.86	60.96^{b} ±0.90	59.52^{b} ±0.59	59.2^{b} ±0.27	60.69^{b} ±0.31	59.13^{b} ±0.28	59.64^{b} ±0.26
2	62.18^{ab} ±0.54	63.45^{a} ±0.77	59.76^{bc} ±0.67	60.21^{bc} ±0.84	60.13^{bc} ±1.3	59.22^{c} ±0.30	60.57^{c} ±0.37	59.85^{c} ±0.39	59.84^{bc} ±0.24
3	60.26±0.58	59.86±1.09	59.21±0.83	59.23±0.81	59.1±1.05	58.73±0.29	60.19±0.42	58.71±0.27	59.42±0.26
4	59.69±0.47	58.12±0.97	57.79±0.67	58.19±1.05	59.31±1.1	57.17±0.38	59.01±0.37	57.95±0.45	58.95±0.36
5	54.07±0.89	56.09±0.82	55.9±1.01	55.31±0.98	52.99±0.91	55.09±0.61	54.21±0.62	54.67±0.62	55.77±0.22
Shell Thickness period									
1	0.33±0.01	0.34±0.01	0.34±0.01	0.35±0.01	0.37±0.01	0.36±0.01	0.36±0.01	0.36±0.01	0.34±0.01
2	0.35±0.01	0.34±0.01	0.35±0.01	0.35±0.01	0.34±0.01	0.34±0.01	0.33±0.01	0.34±0.01	0.35±0.01
3	0.33±0.01	0.31±0.01	0.32±0.01	0.31±0.01	0.34±0.01	0.32±0.01	0.33±0.01	0.34±0.01	0.34±0.01
4	0.33±0.01	0.32±0.01	0.32±0.01	0.33±0.01	0.32±0.01	0.34±0.01	0.32±0.01	0.33±0.01	0.34±0.01
5	0.33±0.01	0.32±0.01	0.31±0.01	0.32±0.01	0.3±0.02	0.33±0.01	0.3±0.02	0.33±0.01	0.32±0.02
Yolk Weight Group									
1	16.71^{ab}±0.27	17.41^{a} ±0.42	17.24^{a} ±0.31	16.49^{ab}±0.30	15.53^{b} ±0.41	16.7^{ab}±0.27	17.19^{a} ±0.30	16.52^{ab}±0.28	16.33^{ab} ±0.38
2	16.77±0.25	16.53±0.34	17.19±0.14	16.95±0.35	18.15±0.59	16.71±0.41	17.19±0.26	16.81±0.26	17.68±0.34
3	17.29±0.23	17.31±0.42	18.5±0.21	17.15±0.36	16.8±0.54	17.82±0.32	18.09±0.32	17.33±0.26	17.88±0.29
4	17.42±0.80	16.24±0.85	17.48±0.89	16.48±0.87	17.5±0.44	17.19±0.84	16.51±0.84	16.61±0.87	16.41±1.03
5	17.83±0.32	18.75±0.40	19.55±0.47	18.22±0.35	236.53±218.21	19.14±0.28	18.37±0.46	18.37±0.65	18.95±0.49

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

Trt1 (62°C for 30min), Trt2 (72°C for 5min), Trt3 (100°C for 2min), Trt4 (rinsed in 5% propionic acid), C (control

6.4 Conclusion

It can be concluded that diets containing BSF pre-pupae that were grown on human faecal waste are an acceptable safe alternative protein source in the diets of layer hens as no pathogens were detected in the eggs. When added at an inclusion level of 10% there were no adverse effects on any of the layer production parameters and there were also no significant differences between the treatment diets containing pre-pupae meal and the commercial control diet. The eggs that were produced were also of good quality and the standard did not decrease when pre-pupae meal was added.It can be concluded, that the different dietary treatments did not affect the rate of degradation of most of the egg quality parameters during storage at room temperature; the degradation of the albumen was due to storage time and temperature. Therefore, due to the positive results in this trial it can be concluded that BSF prepupae is a good protein source in layer diets.

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Chapter 7. General conclusion

This study aimed to determine whether BSF pre-pupae grown on human waste could be used as a partial protein source in broiler and layer diets. Four trials were conducted to determine the effect of a 10% inclusion level on broiler production, carcass quality and organ weights, broiler total tract digestibility and table egg quality. These trials play a fundamental role in determining whether there is any value to be had from utilizing pre-pupae grown on human waste. Care has to be taken when supplementing diets with pre-pupae of this nature (care must be taken to assure that the pre-pupae was treated properly), due to the high microbial risks involved.

It was determined (in Chapter 3) that although different processes (62°C for 30min, 62°C for 60min, 72°C for 5min, 72°C for 15min, 100°C for 2min, 100°C for 5min, rinsed in 5% propionic acid and rinsed in 5% formic acid) were used prior to drying the pre-pupae, these pre-drying processes did not decrease the microbiological load sufficiently. However, the drying (80°C for 48 hours in a convection oven) of the pre-pupae prior to incorporating these into the diets resulted in all tested bacteria (*E.coli*, *Salmonella* and *Listeria*) being destroyed. This then raises the question whether any pre-treatments are necessary and whether drying alone would not suffice? It is, however, important to note that the treatments played a vital role in killing the prepupae (not microbes) before they were placed in the oven. Future trials can therefore investigate more efficient means in killing the larvae before drying and incorporating the meal into the diets. It is important to kill the larvae before drying, because if they are alive when placed into the oven enzymatic deterioration can take place. Which then can cause inefficiency of pathogen reduction in those clumps.

The pre-pupae drying treatments all performed superior to the control diet as pertaining to all broiler production parameters (as can be observed in Chapter 3); the control diet is a commercially accepted maize/soya diet. This is therefore very positive results for the poultry industry. To be more specific the pre-pupae diets achieved values superior to the control in all the production parameters: ADG, FCR, EPEF, PER and live weight within a normal growth period/cycle. This indicates that good alternative protein sources are available to replace expensive and scarce fishmeal in broiler diets.

The meat that was produced in the follow up trial (Chapter 4) was of acceptable and good quality with no differences ($P>0.05$) being found between the pre-pupae diets and that of the control. The proximate chemical composition and mineral composition of the breast meat revealed no differences between the pre-pupae diets and the control. It is, however, important to note that irrespective of the diet, the meat that was produced had a higher pH value than what was previously reported for breast meat,

this phenomenon is attributed to pre- and post mortem environmental factors rather than dietary treatments. As expected, there was no bacterial contamination from the BSF pre pupae into/onto the broiler meat indicating once again that sufficient processing of the pre-pupae ensures that no potential pathogens survive. Also no measurable toxic effects could be observed on the organs or intestinal wall of the broilers receiving the pre-pupae diets. The pre-pupae treatment diets were comparable to the control with regards to organ weights, gut pH and histomorphology. It is, however, important to note that very high erosion within the gizzard was observed. This erosion was observed across all the treatments and not isolated within the pre-pupae treatment groups. This indicates that the pre-pupae were not the reason for the erosion. The reason for the erosion is unknown but it can be speculated to be due to enviromental issues (long term stress).

With the total tract digestibility trial (Chapter 5) it was concluded that the digestibility values of the pre-pupae wereof an excellent quality. The digestibility values for these pre-pupae were on par with those of raw materials that are commercially used. There was a trend that was observed in the production trial that was also seen agin in the digestibility trial, it would seem as if the heat based treatments (not including the 72°C for 15min) could be a better treatment method than the acid base treatment, due to better (although not significant) production values. This trend was seen once again when the heat based treatments had better (this time significant) digestibility values than the acid based treatments. More research should, therefore, be done to determine whether heat is the best treatment source for pre-pupae processing. Irrespective of the treatment method, it was determined that the digestibility values for the pre-pupae were within acceptable ranges. In addition, the BSF larvae were able to convert organic material with a low economical value (human faeces) into a highly digestible protein source.

It can also be concluded that diets containing BSF pre-pupae that were grown on human faecal waste are an acceptable safe alternative protein source in the diets of layer hens as no pathogens were detected in the eggs (Chapter 6). When added at an inclusion level of 10% there were no adverse effects on any of the layer production parameters and there were also no significant differences between the treatment diets containing pre-pupae meal and the commercial control diet. The eggs that were produced were also of good quality and the standard did not decrease when pre-pupae meal was added. It can also be concluded that the eggs of the different dietary treatment did not affect the rate of degradation of most of the egg quality parameters during storage at room temperature; the degradation of the albumen was due to storage time and temperature and not different dietary treatment methods.

Taking all of these results into consideration, it can be concluded that pre-pupae grown on faecal matter is a viable source of protein in poultry diets. It is important to remember that the BSF pre-pupae in this trial were grown on human faecal matter, a waste source that is inexpensive and abundant. It can also be concluded that BSF prepupae can easily convert a perceived low quality waste to a high quality feed source that is comparable to commercially accepted protein sources, such as soya bean meal. It can, therefore, be assumed that these pre-pupae are more cost efficient than other types of protein sources and therefore research needs to be done to determine what the cost efficiency would be in using these pre-pupae. More studies are required to evaluate the economic viability of a pre-pupae plant growing larvae similar to that used in this investigation. More trials are necessaryto determine the quality of pre-pupae when they are grown on human waste when the waste has already been treated to some extent as the human faecal matter used in this investigation was raw human faeces that had undergone no treatment.