A STUDY ON THE DUAL APPLICATION OF BLACK SOLDIER FLY LARVAE (*Hermetia illucens*): PROTEIN-RICH ANIMAL FEED AND BIOLOGICAL POLYHYDROXYBUTYRATE (PHB) EXTRACTION AGENT

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UNIVERSITI SAINS MALAYSIA

2021

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by

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Thesis submitted in fulfilment of the requirements for the degree of Master of Science

November 2021

ACKNOWLEDGEMENTS

In the name of Allah, The Most Gracious, The Most Merciful, Shalawat and Salam for the Holy Prophet Muhammad P.B.U.H.

Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. First and foremost, I would like to express my gratitude to my primary supervisor, Dr. Siti Baidurah Yusoff, for her enthusiasm for this project, for her guidance, patience, understanding, and positive encouragement throughout my years of study and through the process of researching and writing this thesis. It has been a great pleasure and honor to have her as my supervisor. I would also like to extend my sincere thanks to my co-supervisors, Prof. K. Sudesh Kumar and Dr. Hadura Abu Hasan from School of Biological Sciences for their support and knowledge regarding this topic.

Moreover, I would like to extend my heartfelt gratitude to my dear lab mates, Wendy, Sharifah, and Hafis for their motivation and their sincere help during my studies. I am very thankful to all the lab assistants; Mr. Azmaizan, Mrs. Najmah, Mr. Khairul, Mrs. Norita, and Mrs. Noraida for their co-operations, guidance and assistance in machine handling.

My sincere thanks also go to Kuok Foundation MSM Fellowship Award and RUI Grant 1001/PTEKIND/8011022 for the financial support of my master studies.

I would sincerely like to thank all my beloved friends, Wana, Lisa, and Fazari for their kindness and moral support during my study.

Last but not least, nobody has been more important to me in the pursuit of this project than the members of my family. I would like to express my very profound gratitude to my parents and my brother, whose love and continuous encouragement are with me in whatever I pursue. They are the ultimate role models and this accomplishment would not have been possible without them.

Lydia Binti Mohamad

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%	Percentage
ΔH_{c}	Crystallization enthalpy
T_{c}	Crystallization temperature
$T_{\mathrm{decomp.}}$	Decomposition temperature
T_{g}	Glass transition temperature
T_{m}	Melting temperature
X_{c}	Degree of crystallinity
°C	Degree Celsius
A	Weight (g) of crucible + filter paper + dried precipitate
B	Weight (g) of crucible + ash
C	Weight (g) of ashless filter paper
D	Weight (g) of freeze-dried BSFP
F	Weight (g) of round bottom flask + fat residue
M_{n}	Number average molecular weight
M_{w}	Weight average molecular weight
S	Weight (g) of sample (dry and defatted)
T	Weight (g) of empty round bottom flask
V_{B}	Volume (ml) of standardized acid used to titrate reagent blank
V_{S}	Volume (ml) of standardized acid used to titrate a sample
ΔH_0	Melting enthalpy of pure crystalline PHB homopolymer
ΔH_m	Melting enthalpy

LIST OF ABBREVIATIONS

× *g*	Times gravity
µL	Microlitre
µm	Micrometre
3HB	3-hydroxybutyrate
ANFs	Anti-nutritional factors
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Collaboration
BSF	Black soldier fly
BSFL	Black soldier fly larvae
BSFP	Black soldier fly pre-pupae
CDW	Cell dry weight
cm	Centimetre
Da	Dalton
DM	Dry matter
DSC	Differential scanning calorimetry
EAA	Essential amino acid
EM	Effective microorganisms
FAME	Fatty acid methyl ester
FAO	Food and Agriculture Organization of the United Nations
g	Gram
GC	Gas chromatography
GC-FID	Gas chromatography-flame-ionization detection
GC-MS	Gas chromatography-mass spectrometer
GHGs	Greenhouse gases
GPC	Gel permeation chromatography
h	Hour
HPLC	High-performance liquid chromatography
kGy	KiloGray
kPa	Kilopascal
L	Litre

LEAP	Livestock Environmental Assessment and Performance Partnership
LUC	Land-use change
m	Metre
M	Molarity
max	Maximum
ME	Methyl ester
mg	Milligram
Min	Minimum
min	Minute
mL	Millilitre
MM	Mineral medium
mm	Millimetre
MS	Malaysian Standards
NR medium	Nutrient-rich medium
P(HB-*co*-HHx)	Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate)
P(HB-*co*-HV)	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
PDI	Polydispersity index
PEG	Polyethylene glycol
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PKC	Palm kernel cake
Psi	Pounds per Square Inch
rpm	Revolutions per minutes
SCP	Single-cell protein
SDBS	Sodium dodecylbenzenesulfonate
SDS	Sodium dodecyl sulphate
sp.	species
TGA	Thermogravimetric analysis
UNESCO	United Nations Educational, Scientific and Cultural Organization
USM	Universiti Sains Malaysia
v	Volume (unit)
wt	Weight (Unit)

KAJIAN MENGENAI DUA APLIKASI LARVA LALAT ASKAR HITAM (*Hermetia illucens*): POTENSI SEBAGAI MAKANAN HAIWAN KAYA PROTEIN DAN AGEN PENGEKSTRAKAN POLIHIDROKSIBUTIRAT (PHB) SECARA BIOLOGI

ABSTRAK

Pertambahan penduduk global mencetuskan permintaan yang lebih besar untuk makanan kaya protein seperti daging unggas dan telah mendorong cabaran dalam industri makanan ternakan. Terdapat batasan dalam makanan haiwan konvensional yang berasaskan pertanian dan oleh itu, penyelesaian alternatif yang menjanjikan perlu dipertimbangkan. Terdapat penerokaan yang lebih mendalam terhadap serangga seperti lalat askar hitam (BSF), *Hermetia illucens*, sebagai alternatif makanan haiwan kaya protein kerana kandungan proteinnya yang tinggi berbanding makanan haiwan komersial. Terdapat beberapa usaha yang telah diambil dalam penggunaan BSF secara meluas dalam industri agro bukan hanya sebagai makanan haiwan sahaja tetapi juga sebagai baja *frass* dalam pertanian organik dan bahan biodiesel. Kebelakangan ini, terdapat pelbagai perspektif mengenai potensi serangga sebagai makanan haiwan dan agen pengekstrakan polimer secara biologi. Polihidroksibutirat (PHB), ialah biopolimer yang disintesis oleh pelbagai jenis bakteria dengan menghadkan sumber nitrogen dan sumber karbon media pertumbuhan yang berlebihan telah mendapat perhatian di seluruh dunia dalam beberapa tahun kebelakangan ini. Walaupun begitu, kos penghasilan PHB yang tinggi berhubung dengan proses pengekstrakan PHB telah membatasi pendekatan hijau untuk berjaya dalam pasaran. Kajian ini menyelidik pengaruh strategi pemberian makanan yang berbeza untuk larva lalat askar hitam (BSFL) terhadap kandungan protein kasar lalat askar hitam prepupa (BSFP) dan mengekstrak polimer secara biologi dengan mengubah suai diet dengan memberi sel bakteria yang mengandungi polimer, PHB. Mod pemberian makanan terdiri daripada dua jenis kawalan pemberian makanan dan empat jenis pemberian makanan yang diubah suai telah diaplikasikan di dalam kajian ini. Kawalan pemberian makanan 2 menunjukkan kandungan protein kasar tertinggi BSFP (81.3 ± 0.21%). Pengubahsuaian pemberian makanan 2 menunjukkan sedikit penurunan kandungan protein kasar BSFP (80.64 ± 1.18%) sekitar 1% berbanding dengan kawalan pemberian makanan 2. Tambahan lagi, penulenan dengan lebih lanjut menggunakan air terhadap polimer yang diekstrak secara biologi diperolehi dari pengubahsuaian pemberian makanan 2 mengandungi kandungan PHB tertinggi dengan 71.00 ± 5.61%. Kajian ini menunjukkan penggunaan BSFL sebagai agen biologi untuk menulenkan sebahagian dari biopolimer dan meningkatkan kandungan protein melalui pemberian makanan dengan sel.

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ABSTRACT

Global population growth resulted in greater demand for protein-rich food such as poultry meat and has driven a challenge in poultry feed industry. The conventional animal feed derived from agriculture-based feed-imposed limitations and therefore, a promising alternative solution need to be considered. There is a considerable interest in exploring insect such as black soldier fly (BSF), *Hermetia illucens*, as an alternative protein-rich feed due to its high protein content as compared to other commercial feed. There are previous efforts on the diverse usage of BSF in agroindustry not only as an animal feed but also as a frass fertilizer in organic farming and biodiesel feedstock. Recently there has been a multi-perspective on the potential role of insects as feed and biological agent of polymer purification. Polyhydroxybutyrate (PHB), a biopolymer synthesized by some bacteria under limiting nitrogen source and excess carbon source growth media has gained worldwide attention in the recent years. Nevertheless, the high production cost of PHB production related to PHB extraction process has limited this green approach to succeed in the market. This study investigated the effect of different feeding strategies of black soldier fly larvae (BSFL) on the crude protein content of black soldier fly pre-pupae (BSFP) and to biologically extract the polymer by modifying the diet with bacterial cells containing polymer, PHB. Feeding mode consisted of two control feedings and four modified feedings were applied in this study. Control

feeding 2 revealed the highest crude protein content of BSFP (81.31 ± 0.21%). Modified feeding 2 showed a slight decrease in BSFP crude protein content (80.64 ± 1.18%) of approximately 1% as compared to the control study 2. Furthermore, further purification with water of the biologically extracted polymer obtained from modified feeding 2 contained the highest content of PHB with 71.00 ± 5.61%. This study demonstrated the utilization of BSFL as a biological agent to partially purify the biopolymer and increase the protein content by feeding with cells.

CHAPTER 1

INTRODUCTION

1.1 Research background

The Food and Agriculture Organization of the United Nations (FAO) projected a remarkably rise in animal protein demand with 40% of the increased global meat consumption accounting for poultry meat (Onsongo et al., 2018). The rising in poultry meat production is unavoidable and is expected to worsen the current challenge of costly conventional feed. Globally, the current poultry production relies heavily on agriculture-derived feed mainly composed of soybean and several other grains such as maize, barley, oat, and wheats. The conflict of agriculture-based products for animal feed will lead to the food security crisis. Furthermore, agriculture production is deemed unsustainable and resulted in many environmental impacts such as the accumulation of all anthropogenic greenhouse gases (GHGs), space competition with human and contribute to higher water footprint. The high cost of agriculture products for animal feed has limiting the poultry sector from expanding to satisfy the market demand. Therefore, a promising alternative solution to conventional sources need an attention to fulfil the poultry production demand.

To mediate this issue, a sustainable diet consists of high-protein sources need to be considered. A considerable amount of literature has been published on the use of insect as a potential source of poultry feed (Allegretti et al., 2017; Onsongo et al., 2018; Rumpold and Schlüter, 2013). Moreover, insects are considered the best alternative as a partial or complete substitution due to their nutritional contents, ease of rearing and high biomass production of larvae or pupae (Tran et al., 2015). A notable example of promising insect in industrial feed sector includes black soldier fly (BSF), common housefly larvae, yellow mealworms and silkworms (Veldkamp et al., 2012; Van Huis et al., 2013; Makkar et al., 2014).

In recent years, there has been a surge of interest in insects such as *Hermetia illucens*, commonly known as BSF for poultry industry (Onsongo et al., 2018; Rumpold and Schlüter, 2013; Makkar et al., 2014) due to its rich nutritional value in protein content as compared to conventional animal feed (Tschirner and Simon, 2015; Ewald et al., 2020; Veldkamp et al., 2012; Tran et al., 2015; Spranghers et al., 2017; Liu et al., 2017). Black soldier fly larvae (BSFL) are receiving considerable attention to serve as one of the options to achieve more sustainable production due to their potential feed source, high feed conversion efficiency compared to plant-based feed, a short period of rearing and less space required during breeding (Liu et al., 2017). FAO (2013) has pointed out that agriculture-based feed was the critical factor of anthropogenic-induced climate while insects rearing emits less GHGs (MacLeod et al., 2013). This shows that insect breeding can help to reduce the environmental pressure caused by the agricultural sector.

Furthermore, FAO (2013) reported that BSFL should be considered as animal feed due to its high protein and lipid content which is necessary for a complete diet to support good growth in poultry and aquaculture (Van Huis et al., 2013). Hence, BSFL is regarded as a great potential in the feed sector whereby insect-meal could substitute in part fish-meal and soybean-meal as a protein source in the poultry and aquaculture feed industry (Boccazzi et al., 2017). Due to its nutritional composition, accessibility, easy rearing techniques, and rapid growth rates, BSFL is regarded as an alternative option for a cheap and efficient feed source as well as an excellent opportunity to combat nutritional insecurity (Van Huis et al., 2013; Wang and Shelomi, 2017). Recent interest in this species is deemed as a promising source for bioconversion of organic substance into valuable protein-rich food for the production of animal feed (Gold et al., 2018). The bioconversion of food waste as feeding substrate is an economical way of reducing waste besides producing a cheaper alternative animal feed as the BSFL are voracious feeders of organic material which will then be converted into a protein and fat-rich biomass, ideal as a feed supplement (Diener et al., 2009; Putri, 2019).

While the effect of food waste, fruit and vegetable waste, coffee bean pulp, animal manure, chicken feed, fish offal, and abattoir waste as a rearing substrate on the nutritional value of the insect has already been investigated, the potential of bacterial cells as an additional substrate in BSFL diet has not yet been widely explored (Makkar et al., 2014; Liu et al., 2017; Spranghers et al., 2017; Lalander et al., 2019). This study explores on the possibility of enhancing the protein content of BSFP by incorporating bacterial cells in the diet. In this study, the feeding strategy of BSF at different stages were implemented using various diets consisting a mixture of food waste with different concentration of nutritional value as a control, and bacterial dried cells containing intracellular polyhydroxybutyrate (PHB), as a modified rearing substrate. A non-pathogenic bacterial cell, *Cupriavidus necator* (*C. necator*) wild type H16 (ATCC 17699), formerly known as *Ralstonia eutropha* was utilized in the modified feeding strategy. Non-pathogenic bacterial cells can play an essential role in enhancing the protein content of BSFP due to its properties as a single-cell protein (SCP) (Ong et al., 2018a; Kunasundari et al., 2013). SCP refers to protein derived from cells of microorganism such as bacteria, which are grown on various carbon sources for synthesis. *Cupriavidus necator* can be utilized as a source of SCP for animal feed due to its high protein content that was about 84% protein on dry weight basis (Calloway and Kumar, 1969; Kunasundari et al., 2013; Ong et al., 2018a). Nearly 93% of SCP was found to be digestible by animals (Calloway and Kumar, 1969).

Plastic pollution has been one of the major concerns in environmental issue due to the massive production of single-use plastic driven by consumer demand, hence creating a problem to dispose the plastic waste. Petroleum-based plastics are not biodegradable, and most of the plastic waste were released into the environment or stored in the landfills. The use of biodegradable plastic such as polyhydroxyalkanoate (PHA) can overcome this problem because they can be completely degraded and assimilated by some microorganisms including bacteria, fungi, and algae through aerobic or anaerobic processes into natural compounds such as water, carbon dioxide, methane, nitrogen, and hydrogen (Urbanek et al., 2018; Sankhla et al., 2020). Moreover, it has similar properties to the petroleum-based plastic and can be applied in a wide range of application (Kunasundari and Sudesh, 2011).

Cupriavidus necator is capable of accumulating intracellular granules of PHB under growth media rich with carbon source and limiting nitrogen source (Baidurah et al., 2018). PHB and its copolymers are biodegradable polymers that have similar properties as conventional petrochemical plastics with non-toxic and biocompatibility behaviour which are of particular interest (Murugan et al., 2017; Baidurah et al., 2018). It was reported that PHB granules was found to be non-toxic and tolerated by animals, and this biopolymer was indigestible when consumed by animals, which will eventually be excreted out as a frass without being absorbed by the digestive system (Kunasundari et al., 2013). Although the nutrient aspects of the cells were found promising to be developed as SCP, the PHB granules accumulated by *C. necator* does not possess any nutritive value to the animals (Waslien and Calloway, 1969; Ong et al., 2018a). Hence, by applying these additional cells in the BSF diet, BSF can be utilize as a potential biological agent to partially purify the PHB. This biological extraction method requires less hazardous solvents and strong chemicals to purify the polymer which is environmentally friendly and in due course will partially reduce the production cost of biopolymer with regards to less consumption of chemicals and simpler recovery methods which does not involve with expensive instrumentation.

In general, this present study focused on the dual application of BSF as a protein-rich animal feed and biological PHB extraction agent. In the latter feeding strategies by incorporating cells in the diet, two products were obtained: (1) the BSFP as a promising alternative protein-rich feed, and (2) the extracted PHB through a biological extraction method, by utilizing the intestine of BSFL. The obtained BSFP was further subjected to the proximate analysis to determine its nutritional value consisting of crude protein, crude fat, crude fiber, moisture, and dry matter, all of which are critical in evaluating poultry feed parameters. The proximate data were compared with currently available animal feed products and with previous studies. The polymer characterization using gas chromatography (GC), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and gel permeation chromatography (GPC) was also carried out on the biologically recovered PHB excreted along with the frass.

1.2 Problem statements

Increasing in the global population growth and urbanization will drive a significant demand for protein-rich food such as poultry and aquaculture (FAO, 2016). Makkar et al. (2014) pointed out a remarkable shift in people's dietary preferences with increased consumption of an animal product and this is probably to continue in the coming decades. Rising demand in protein has prompted an agricultural production of poultry feed industry sector to scale up rapidly. However, concerns have been raised following animal feed due to insufficient availability of natural resources, negative impact on the environment, and competition between human food and animal feed (Makkar et al., 2014). FAO (2013) argued that the poultry industry is among the rapidly growing animal food-producing sector, but the limitation faced by the farmers such as critical availability of low-priced and high-quality feeds are expected to affect the growth of poultry production.

Plant-derived feed such as cereals (maize) and oil seed meal (palm kernel meal) are low in protein content (Iji et al., 2017; Samtiya et al., 2020). In addition, plant protein sources, are generally deficient in essential amino acids (with the exception of soybean meal) and nutritionally unbalanced, causing the manufacturer to add an accompanying source of amino acids or other protein sources such as animal protein (Ravindran, 2014; Beski et al., 2015). Furthermore, most of the plant protein ingredients have limitation on their usage since it contains anti-nutritional factors (ANFs). This has caught an attention in feed industries because ANFs will combine with nutrients and reduce the nutrient bioavailability (Samtiya et al., 2020). Other factors such as trypsin inhibitors and phytates, found primarily in cereals, may hinder the digestibility of proteins and mineral absorption, which eventually will cause micronutrient malnutrition and mineral deficiencies (Samtiya et al., 2020).

Therefore, it is essential to exploit a sustainable protein-rich poultry feed. The use of insect protein in poultry diets has received a considerable interest as a sustainable raw material for animal feed source (Józefiak and Engberg, 2015; Sogari et al., 2019). Other findings reported on the positive outcomes on utilizing insect as a partial or total replacement of animal feed relating to growth rate, body weight, animal health and performance, gut health, and product quality (Makkar et al., 2014; Sogari et al., 2019). Rearing of insect such as BSF as an alternative animal feed source is found to be promising in this twenty-first century. The incorporation of *C. necator* which act as SCP in the BSF diet will enhance the nutritional value of this animal feed protein. Furthermore, the PHB accumulated in *C. necator* will be excreted by the larvae as a whitish frass which contain the PHB granules. The properties of the biopolymer excreted through biological means is comparable to conventional extracted polymer using solvents.

1.3 Research scope and objectives

This study aims to experimentally investigate the effect of bacterial cells containing intracellular polyhydroxybutyrate (PHB) in BSFL diet to further increase the protein content for the applications of animal feed and promising alternative protein-rich feed, and simultaneously to biologically extract the polymer by utilizing the digestive system of BSFL. The specific objectives of the study are as below:

(1) To identify the effects of black soldier fly larvae feeding strategy on the protein profile.

(2) To elucidate the possibility of the black soldier fly pre-pupae-derived protein to complement the current conventional animal feed.

(3) To characterize PHB extracted from BSF through biological extraction method.

CHAPTER 2

LITERATURE REVIEW

2.1 Current environmental issue: high protein demand and plastic pollution

Increase in food protein production, such as poultry sector will also increase the feed production to satisfy the global demand. Conventional feeds available commercially such as cereal grains and oilseed meals have commonly been used in poultry production. Nevertheless, these conventional feeds have created extensive drawbacks in feed industry: (1) nutritional unbalanced especially protein content and deficiency in amino acids; (2) high cost of feeds; (3) creating environmental pollution; (4) competition with human food source. These issues have urged the researchers to find an alternative protein-rich feed for poultry (Iji et al., 2017; Cappellozza et al., 2019; Motte et al., 2019; Parolini et al., 2020).

Recent interest in insects farming as a source of feedstuff have a great potential for a numerous reason such as their ability to consume a wide range of organic wastes and convert into protein-rich biomass, rich in nutrient contents (energy, crude fat, vitamins, and minerals) necessary for poultry development and growth, easy rearing, low space requirement, and low environmental impact (Makkar et al., 2014; Surendra et al., 2016; Zotte et al., 2019).

Furthermore, in this modern lifestyle, the use of petroleum-based plastic is undeniably crucial in the daily life of humans. It has been reported that two million tonnes of plastics were produced in 1950, and the annual production in 2015 had increased almost 200-fold, reaching 381 million tonnes (Ritchie, 2018). Plastics are semi-synthetic organic polymer composed of long, chain-like molecules of high average molecular weight (Zainab et al., 2019). Plastics are cheap, durable, resistance to degradation, and highly versatile, making it suitable to be used in a wide range of consumer and industrial application. Despite of the advantages possessed by petroleum-derived polymers, plastics have attracted serious concern as a large-scale pollutant (Moore, 2020).

Ethylene and propylene are the common monomers used to make plastics, and none of the petroleum-derived plastics are biodegradable (Ritchie, 2018) because they are intractable to microbial degradation (Geyer et al., 2017; Zainab et al., 2019). Consequently, rather than decompose, these synthetic plastics tend to accumulate in the landfills, oceans, and the natural environment. Replacement of petroleum-derived plastics with biopolymer such as PHA has attracted much attention due to its beneficial properties. PHA is a bio-based polymer synthesized by the bacterial cells such as *C. necator* under specific growth conditions as carbon and energy storage (Ong et al., 2018a).

PHA has a great potential as an alternative to petroleum-derived plastics due do its properties which are similar to conventional polymers and additionally, possess other important benefits such as biodegradability, biocompatibility, and non-toxic behaviour (Baidurah et al., 2018; Yong Sen et al., 2019). PHB is the most common and simplest form of PHA produced by bacteria in the natural environment (Mostafa et al., 2020; Muneer et al., 2020). These unique properties of PHB gave rise to diverse applications in industries ranging from biodegradable packaging materials (Baidurah et al., 2018), to biocompatible medical devices and tissue engineering (Chaijamrus and Udpuay, 2008; Bhagowati et al., 2015).

2.2 Protein demand for animal feed

Livestock is one of the fastest-growing sectors in agriculture, offers huge potential for economic growth, poverty reduction, improved food security and nutrition (Robinson and Pozzi, 2011). The rising population coupled with increasing income growth and urbanization will result in a greater demand for food, and the preferences will be more on poultry protein products, such as meat and eggs. This is supported by estimation done by FAO (Robinson and Pozzi, 2011), whereby the growth of demand for poultry meat in Southeast and South Asia, is shown to increase by 750% by 2030 (Kim et al., 2019). Based on data retrieved from FAOSTAT, in 2018, 37% of the global meat production was accounted for poultry sector which is the highest among the other livestock. Furthermore, 12% of global meat production was attributed to poultry meat production in 1961 and its share has approaching tripled in year 2018.

According to Kim et al. (2019), the projected growth of animal production, driven by an increasing population, high living standards and preferences towards animal-derived foods, generates a global shortage of feed protein supply. A rapidly growing population and rising incomes in developing countries have resulted in estimated increase in global demand for meat and milk by 57% and 48% respectively between 2005 and 2050 (Alexandratos and Bruinsma, 2012; Kim et al., 2019). Subsequently, the livestock sector is projected to expand by 21% between 2010 and 2025 (Mottet et al., 2017). If feed production continues at the same rate, the global feed supply is anticipated to grow from 6.0 to 7.3 billion tons of dry matter (Kim et al., 2019).

The animal feed protein is gaining an attention worldwide due to its ability to increase the nutritional value of the product derived from animals, thereby increasing the market share of animal protein meals ingredients. Moreover, animal feed protein provides crucial amino acids which can enhance the immunity of the livestock. These feeds aid amino acid synthesis which helps in improving the quality and productivity of animals (Ploegmakers, 2019).

Ritchie and Roser (2020) highlighted the percentage of cereal crops accounted directly for human, animal feed and other uses in year 2013 are 48%, 41%, and 11% respectively. This data shows that there is a strong competition of cereal crop production between human food and animal feed with only difference of 7%. Feed grains has exploited the grains used for human consumption and it is expected to become worsen if the feed productivity remains the same. Furthermore, feed grains have created a competition in terms of land usage with grains planted for human consumption.

Kim et al. (2019) argued that feed protein supplements are one of the costliest and limiting feed supplements. As highlighted by Mottet et al. (2017), poultry meat and eggs are among the most common food source of animals consumed worldwide, across a wide range of cultures, beliefs and religions, thus making them essential to food security and nutrition. FAO projected a remarkably rise in animal protein demand with 40% of the increased global meat consumption accounting for poultry meat (Onsongo et al., 2018). Hence, rising in poultry meat production is unavoidable and is expected to worsen the prevailing challenge to costly conventional feed.

Table 2.1 shows the survey done by Alltech (2020) on the feed production estimations of layer chicken and broiler by region in year 2019. The highest growth of feed production of chicken layer and broiler was seen in Asia-Pacific region with 73.14% and 115.2% respectively. This indicates a growing needs and continual interest in this feed protein source.

Table 2.1 Feed production estimations of layer chicken and broiler by region in year 2019 (Alltech, 2020).

Region	**Poultry**	
	Layer	**Broiler**
Asia-Pacific	73.14	115.2
Europe	33.49	56.3
Latin America	24.03	61.8
North America	15.54	51.8
Africa	5.72	10.0
Middle East	4.86	8.4
Oceania	0.93	3.8
Total	157.7	307.3

* All numbers are in million metric tons

Majority compound feeds rations for mono-gastric livestock such as poultry are based on maize or soybean meal mixed with other grains which most of the ingredients are imported. There are also locally produced raw materials incorporated in the feedstuff, however, the quantity is not sufficient to meet the demands of the local feed industry where it only represents 30% of the total feed ingredients in Malaysia (Loh, 2004). The major issue of the future poultry feed industry is its heavy dependence on imported feedstuff as discussed above.

2.3 Poultry feedstock

2.3.1 Conventional animal feed for poultry industry

Animal feeds are vital in providing all the essential nutrients to keep their body performs properly and their wellbeing remains in excellent condition. It does not only protect them away from pathogens causing disease but also improves the quality of the products derived from them. The traditionally poultry feeds ingredients available in the market includes cereal grains, protein meals, and a mixture of grains with insect-based meals.

Cereal grains are widely used as animal feed especially in poultry sector. Cereal grains are primarily used to satisfy poultry's energy demand. The commonly used cereals in feed are maize, wheat, barley, oat, and rice. The most desirable grain compound feeds for poultry are maize, mixed with other numerous additives to provide necessary amino acids, vitamins, and minerals (Loh, 2004). Maize has high energy value with low fiber content and about 8-11% of protein content (Maner, 1987). Qi et al. (2002) reported that around 70-80% of maize production is used as feed ingredient in the world. In Malaysia, the source of the maize for feed ingredients is mostly imported from Thailand, China, Argentina and USA, although to some extent, locally produced maize is also included (Loh, 2004).

For protein meals, it can be divided into two which are from vegetable and animal protein sources, such as oilseed meals and fish processing by-products. Mainly, after the oil is extracted from the oilseed crops, the remaining protein-rich by-product meal or cake will be used as feed ingredient (Ravindran and Blair, 1992; Loh, 2004). The examples of oilseed crops include soybean, palm kernel, rapeseed, and sunflower. Oilseed meals often make up between 20-30% of a poultry diet and provide over half of the total protein (Dale, 1996; FAO, 2004). Soybean meal is one of the most preferred high quality vegetable protein for animal feed manufacture due to its high crude protein content ranging from 44-50% and also have a balanced amino acid profile (Dale, 1996; FAO, 2004). Furthermore, fishmeal is also a great source of protein for poultry. Fishmeal does not only provide high levels of essential amino acids such as methionine and lysine, but it also contains a good balance of unsaturated fatty acids, minerals and vitamins (Jacob, 2013).

Insects are becoming a popular choice as an ingredient in poultry feed due to its sustainability and nutritious value of the biomass that are comparable with traditional agriculture-based feed. There are numerous feed industries in Malaysia utilizing insect such as BSF in their product, for example LARVIE Chicken Feed Pro-115(S) from Betsol Sdn. Bhd., BIO Protein manufactured by Unique Biotech Sdn. Bhd., and *Hi.*Protein® from Nutrition Technologies Sdn. Bhd. These commercially available products contained a mixture of grains and BSFL together with a trade secret of the company, in order to enhance the nutritional value of the feed.

2.3.2 Limitations of conventional poultry feedstock

The global demand for poultry industry is projected to increase the poultry feedstock demand. According to Makkar (2017), almost 800 million tonnes of cereal which accounted for about one-third of total cereal production are used in animal feed and this trend is estimated to rise over 1.1 billion tonnes by 2050. Although conventional poultry feedstock is readily available in the global market and easily accessible throughout the year, there are some limitations of these traditional feedstock.

Table 2.2 shows the nutritional value of different types of conventional poultry feed. Among this nutritional value, protein is the most important nutrient required to build the tissues and organs, hence becoming a major growth promoting factor in all animals. However, the crude protein content in the cereal grains (maize and polished rice) and oilseed meal (soybean and sunflower meal) are relatively low. Although grain has high level of starch, FAO (2004) suggested that grain is typically low in protein quality due to its unbalanced protein. Plant protein sources are lack in certain essential amino acid and nutritionally unbalanced, hence reducing their biological value as they may not supply the necessary limiting amino acids needed by birds for the development of egg and meat (Beski et al., 2015).

Table 2.2 Proximate data on dry matter basis in various poultry feed (Anjum et al., 2014).

No.	**Nutrients (%)**	**Types of poultry feed**			
		Maize	**Polished rice**	**Soybean meal**	**Sunflower meal**
1.	Crude protein	9.77	11.50	42.48	27.70
2.	Crude fat	10.15	15.12	4.14	4.10
3.	Crude fiber	4.20	14.80	6.80	17.50
4.	Ash content	2.50	14.00	7.25	5.75

2.3.3 Environmental issues arising from conventional poultry feedstock production

In poultry system, feed production significantly contributes to the environmental footprint globally. The demand for feedstuff will also increase with the rising of poultry production, generating greater burden on natural resources (LEAP, 2014). This is especially alarming as the livestock sector is already a big consumer of land and water, with current total cropland usage of 35% and 20% of water consumption for feed production (Opio et al., 2013; LEAP, 2014). FAO (2018) stated that, to sustain feed crops irrigation, more than 90% of the water use in the livestock sector are withdrawn from river, underground water, and lake which is not sustainable (Wada, 2012). The example of environmental impacts related to poultry feed production includes climate change, carbon footprint, greenhouse gas (GHG) emissions, water pollution, land exploitation, eutrophication, and acidification (LEAP, 2014; Wilfart et al., 2016).

Climate change occurred owing to the carbon footprint and GHG emissions is the biggest environmental pressure facing by worldwide. In detail, feed production accounts for about 50-85% of climate change impact (Wilfart et al., 2016). Altogether, feed-related emissions of the livestock industry (including land-use change (LUC)) represent around 3.3 gigatonnes of carbon dioxide (CO_2)-equivalent, or nearly half of total emissions from livestock supply chains (Gerber et al., 2013). Furthermore, there are three major GHGs emission associated with animal feed chains that are methane (CH_4), nitrous oxide (N_2O), and CO_2 as shown in Table 2.3 (MacLeod et al., 2013). According to Livestock Environmental Assessment and Performance (LEAP) Partnership (2014), the global GHG emissions associated to the production, refining, and transportation of feed is responsible for approximately 45% of total sector emissions (Gerber et al., 2013).

Table 2.3 Categories of GHG emissions (MacLeod et al., 2013).

Category		Description
Feed N_2O		Direct and indirect N_2O emissions from organic and synthetic N applied to feed crops and crops residues.
Feed CO_2	Feed: non-crop	CO_2 arising from the production of fishmeal and synthetic feed additives (and lime for chickens).
	Feed: fertilizer production	CO_2 from energy use during the manufacture of urea and ammonium nitrate (and small amounts of N_2O).
	Feed: processing and transport	CO_2 from energy use during crop processing (e.g. oil extraction) and transportation by land and (in some cases) sea.
	Feed: field operations	CO_2 arising from the use of energy for field operations (tillage, fertilizer application). Includes emissions arising during both fuel production and use.
Feed LUC CO_2		CO_2 from LUC associated with soybean cultivation.
Feed rice CH_4		CH_4 arising from the anaerobic decomposition of organic matter during rice cultivation.

Besides contribution to climate change from carbon footprint and GHG emissions, approximately one-quarter of the associated feed emissions (less than 10 percent of sector emissions) are related to LUC (Gerber et al., 2013; LEAP, 2014). Land-use change may be accompanied by distinct or extreme changes in land quality, such as declines in biodiversity, nutrient depletion, increased soil compaction, and impacts on water supply and quality (LEAP, 2014). Such environmental reductions reflect the ecological harm caused by land-use change.

2.4 Insects as a high-protein feedstock for poultry industry

The potential of insect protein to be used in poultry diet has received much interest in tackling the sustainability and supply concerns relating to agriculture-based feed. Besides high protein content of the biomass, insects have adequate nutritional value including fat, energy, vitamins and minerals (Khan, 2018). The inclusion of insect proteins as a raw material for commercial feed manufacturing and the development of intensive agricultural systems has been explored (Józefiak and Engberg, 2015). Insect species such as BSF, the common housefly, yellow mealworm, silkworm, grasshoppers, and crickets are among the desired species with rich nutritional value suitable to be applied in the poultry feed (Veldkamp et al., 2012; Van Huis et al., 2013; Makkar et al., 2014; Józefiak and Engberg, 2015; Khan, 2018). Focusing on BSF, this insect can be fully utilized by implementing dual application, first as a high protein biomass, and second to biologically extract the polymer. Due to its nature of being a voracious feeder, BSF can ultimately improve the food waste management concern.

2.4.1 Lifecycle of the black soldier fly (BSF), *Hermetia illucens* L.

The BSF, *H. illucens* (Linnaeus 1758) (Diptera: Stratiomyidae), is an endemic fly species from the tropical, subtropical and warm temperate areas of America (Hoc et al., 2019) and can be found worldwide through human-mediated dispersal and natural dispersal (Marshall et al., 2015). Like most flies, the BSF does not have a stinger and even though they produce loud buzzing when flying, adult soldier fly poses no danger (Diclaro and Kaufman, 2009). Studies showed that this species is one of the most useful flies in existence and are considered to be non-pest in nature (Sheppard et al., 2002; Diclaro and Kaufman, 2009; Oliveira et al., 2016). This is because the adult BSF does not possess functional mouthparts and do not feed waste, therefore they are not associated in disease transmission. On the other hand, Shumo et al. (2019) described that an actively feeding BSFL excrete an infochemical that keeps other fly species away, thus repelling potential insect pest and disease vector such as the common housefly, *Musca domestica*.

Hermetia illucens L. encounters a complete life cycle (Figure 2.1) consisting of five life stages: egg, larva, pre-pupae, pupa, and adult. The female adult lays a cluster of about 500 to 900 eggs (De Smet et al., 2018) into a cracks and crevices near or in the decomposing organic waste such as food waste, rotting fruit or vegetables, municipal waste and manure (Diclaro and Kaufman, 2009; Spranghers et al., 2017). Studies showed that shortly after oviposition, the female dies (Tomberlin et al., 2009; Dortmans et al., 2017). The eggs will take about four days to hatch into larvae (Diclaro and Kaufman, 2009; Dortmans et al., 2017) and the first instar larvae will crawl or fall into their food source nearby to start feeding.

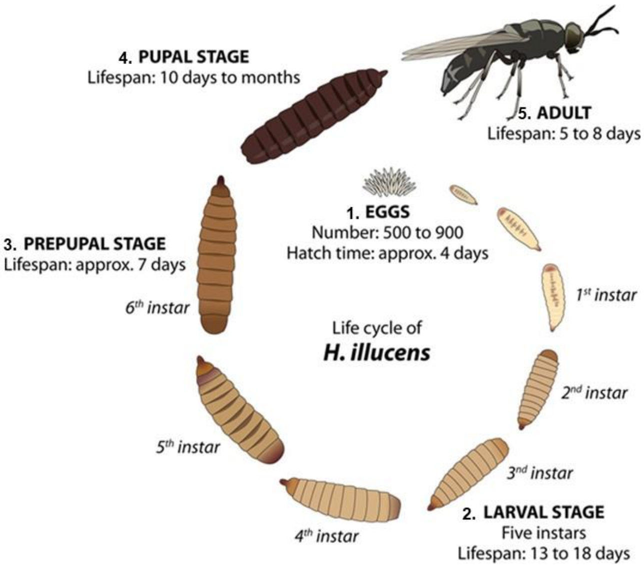

Figure 2.1 The life cycle of *H. illucens*. The various stages of the development of *H. illucens* are shown, as well as the average length of the duration of these different stages (De Smet et al., 2018).

The larvae's development will take approximately 13-18 days under optimal conditions of relative humidity and temperature as well as ideal food quality and quantity (De Smet et al., 2018). Larvae stage is the only phase which the BSF feeds throughout the entire life cycle and therefore the larvae must reserve enough fat and protein before turning into adults. Larvae stage could be extended to a maximum of four months in the event of food shortage (Ortiz et al., 2016).

After completing the five larval stages, the larvae enter the sixth and final stage that is pre-pupal stage, during which time the larvae stop eating. Throughout the transition process from larvae to pre-pupae, the larvae replace its mouthpart with a hook-shaped structure and turns from dark brown to charcoal grey in colour (Dortmans et al., 2017). They will use the hook to easily migrate from the food source and searching for a dark, dry, and protected environment for hiding prior to pupation.

Pupation is a process where a pupa transforms into fly. The pre-pupae will find a suitable place and it will enter a sleeping state, then becomes immobile and stiff for at least two weeks, during which time the pre-pupae grows further within its exoskeletal casing (Evans, 2018). Moreover, the pupa is formed inside the last pre-pupa skin, a puparium (Oliveira et al., 2015). The casing breaks down at the tip when it is fully developed to release an adult fly in a process called emergence (Sheppard et al., 2002). Adult males generally emerged earlier than females (Tomberlin et al., 2002) and they have a lifespan of 5-12 days (Diclaro and Kaufman, 2009; Dortmans et al., 2017). Females typically will mate two days after emergence (Ortiz et al. 2016) and oviposition will occur two days after mating (Tomberlin et al., 2002).

2.4.2 Morphological and behavioural description of the BSF

It is reported that members of the soldier fly family can range in colour from black, yellow, green or blue, with some having a metallic appearance (Diclaro and

Kaufman, 2009). BSF adults ranging in size from 13 to 20 mm have a wasp-like appearance (Tomberlin et al., 2002) and are black or blue in colour (Diclaro and Kaufman, 2009). However, these two insects can be distinguished by the number of wings they possess. Wasp has four wings whereas BSF has only a pair of smoky wings (Figure 2.2).

Figure 2.2 Adult black soldier fly (Heiman, 2008).

Freshly laid eggs (Figure 2.3) of BSF are oval in shape and measure up to approximately 1 mm long in length (Caruso et al., 2013). The eggs are white in colour but over the time, the colour gradually changing into creamy white or pale yellow. A newly hatched first instar larvae are tiny and white coloured with a length of 0.66 mm long (Caruso et al., 2013), with a small projecting head containing chewing mouthpart (Oliveira et al., 2015).

Figure 2.3 Black soldier fly eggs (Dinh, 2019).

The larva has been described as “torpedo-shaped” (Figure 2.4) and slightly flattened with exoskeleton that is firm, tough, and leathery. During larval development, they are voracious feeders and grow from a few millimeters size to around 25 mm length and 5 mm width (Dortmans et al., 2017). In addition to the size increments, the colour of the larva gradually changes as the instar develop that is from white colour to beige, yellowish, blackish grey and finally brown.

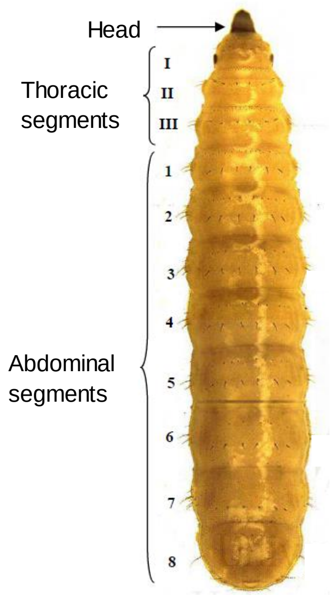

Figure 2.4 General appearance of the *H. illucens* larvae showing the division of the thoracic, abdominal, and cephalic capsules (Gobbi, 2012).

The morphology of BSF larva is divided into three major sections that is head, thoracic and abdominal segments as shown in Figure 2.4. The thorax covered the first three segments after the head. The abdomen is composed of eight segments that are formed by plates covered by numerous small setas (hairs) (Oliveira et al., 2015). Segments one to seven are categorized by spiracles (breathing pores) on both sides (Figure 2.4) while the eight abdominal segment is the last segment (anal) with a rounded shape (Oliveira et al., 2016).

The midgut of BSF can be divided into three main regions that are anterior, middle, and posterior (Figure 2.5). These three regions play an important role in the successive function of the gut such as ingestion of food, digestion and absorption of nutrients, and excretion of frass (Bonelli et al., 2019). The midgut represents the longest part of the digestive system, where the production and secretion of digestive enzymes, as well as the absorption of nutrient occurs in this region (Bonelli et al., 2019). Kim et al., 2011 reported the gut extracts of the BSF are high in amylase, lipase, protease, and trypsin-like protease activity. BSFL are efficient decomposer of food waste and when collecting the rearing substrates from garbage, the unwanted compounds such as plastics that were mixed with food waste might affect the larval growth. However, from the previous study on the effect of microplastic on food waste processing by BSFL, it showed that larval growth of BSF was not inhibited by plastics (Cho et al., 2020). This shows that the gut of BSFL may not digest the polymer from the waste substrates.

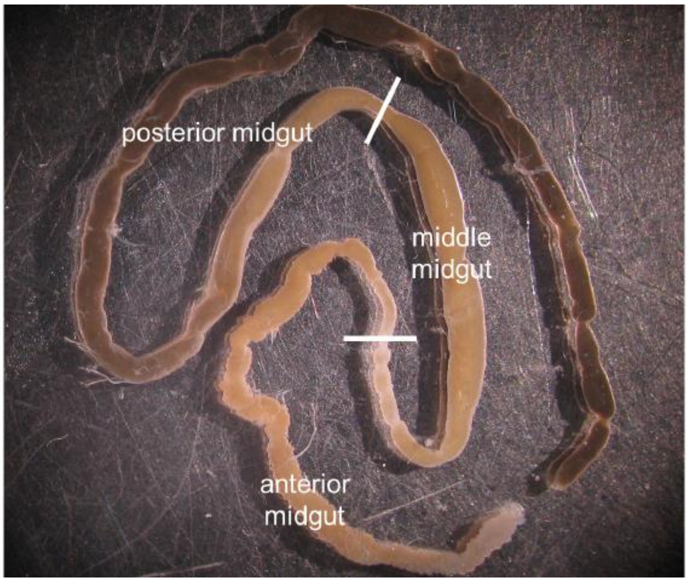

Figure 2.5 Anatomy of the midgut of *H. illucens* larvae. The midgut of *H. illucens* larvae can be subdivided into three main regions: anterior, middle, and posterior (Bonelli et al., 2019).