Investigations on Black Soldier Fly (*Hermetia illucens* L.) Production and Nutrition: A Sustainable Solution for Poultry Feeding

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Investigations on Black Soldier Fly (*Hermetia illucens*L.) Production and Nutrition: A Sustainable Solution for Poultry Feeding

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Abstract

Black soldier fly (BSF; *Hermetia illucens* L.) is renowned for efficiently converting organic wastes into valuable biomass rich in protein and fat. This makes it a profitable and sustainable method for waste management and a useful feed source for animals like poultry, fish, pigs, and pets. Using BSF reduces the dependence on traditional feed ingredients such as soybean and fish meal, which are often imported and add to the carbon footprint. Despite growing interest and large-scale production of BSF meals, knowledge gaps hinder the widespread adoption of this technology, especially in small and medium-scale operations.

The overall aim of this multidisciplinary research was to better understand the biology and rearing methods of BSF, processing of insect meals, and their utilisation by broiler chickens. The thesis research specifically investigated: (1) methods and techniques that optimise BSF breeding and meal processing relevant to small and medium-sized operations, (2) the impact of substrate type, moisture, and compaction on the non-feeding stages of BSF, (3) disease management in BSF colonies to ensure healthy population dynamics, including the first record of red mites as a parasitic case within BSF colonies, and (4) the nutritional value of the larval and pre-pupal stages of BSF as replacements for soybean meal (SBM) in poultry feeds.

Firstly, the thesis provided a practical guide to establishing and managing a BSF colony, covering the entire process from egg production to larval and pre-pupal meal processing. The procedures were based on the experience of producing 450 kg of BSF larvae (BSFL) and pre-pupae (BSFP) over the course of doctoral research.

Secondly, a study examined the impacts of substrate type, moisture levels, and compaction on BSF pupation success, adult emergence and morality. Among the six substrates evaluated (sand, wood shavings, topsoil, vermiculite, spent wheat middlings, and potting soil), spent wheat middlings presented as the most cost-effective and readily available option. Moisture

level was found to significantly affect substrate performance, with 10% moisture providing the best outcomes for pupation and reducing mortality while avoiding mould growth. Mild compaction did not negatively impact pupation development, suggesting that moderate compaction could enable small farmers to reuse substrate and lower the cost.

Thirdly, an infestation of poultry red mites (*Dermanyssus gallinae*), causing noticeable skin discoloration and anatomical damage to the BSFL and BSFP, was identified. The mites repeatedly bit and fed on the BSF bodily fluids, causing distress. The implications of mite infestation on colony health and viability are discussed, emphasising the need to maintain colony hygiene.

Lastly, the apparent metabolisable energy (AME) and standardised ileal digestibility coefficients (SIDC) of amino acids (AA) of full-fat BSFL and BSFP meals for broiler chickens were determined. The AME and SIDC of BSFL and BSFP were higher than those reported for SBM, the commonly used protein meal in poultry diets. The findings demonstrated that BSFL and BSFP meals are better sources of energy and digestible AA, and are potential substitutes for SBM.

Although the principles behind BSF bioconversion technology may seem straightforward, effective implementation requires deeper understanding of its complex stages. The present thesis provided a thorough overview of BSF technology, including rearing methods, optimising pupation conditions, the identification of a novel ectoparasite affecting BSF colonies, and the potential use of BSFL and BSFP into broiler chicken diets. By addressing these key aspects, the research contributes valuable insights for optimising BSF production and utilisation, particularly for small and medium-scale operations.

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Dedication

Through countless hours of research, study, and staunch dedication, I have experienced significant academic and personal growth. I have confronted challenges with determination, absorbed invaluable lessons, and expanded the horizons of my knowledge. This dedication stands as a testament to the resilience and unwavering resolve that have led me to this point. May it serve as an inspiration to those facing their own struggles, urging them to persist in their journey until they reach the finish line.

Publications

Some studies, completed during the candidature and reported in this thesis have been presented in the following scientific journals:

Mahmoud, A. E., Morel, P. C. H., Potter, M. A., & Ravindran, V. (2023). The apparent metabolisable energy and ileal amino digestibility of black soldier fly (*Hermetia illucens*) larvae meal for broiler chickens. *British Poultry Science*, 64 (3):377-383'.

Mahmoud, A. E., Morel, P. C. H., Potter, M. A., & Ravindran, V. (2023). Poultry red mite (*Dermanyssus gallinae*) poses a risk in the rearing of black soldier fly (*Hermetia illucens*). *Journal of Insects as Food and Feed*, 9(1), 55-63.

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List of Abbreviation

AA	Amino acid
ADF	Acid detergent fibre
AID	Apparent ileal digestibility
AIDC	Apparent ileal digestibility coefficients
Ala	Alanine
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Arg	Arginine
Asp	Aspartic acid
BSF	Black soldier fly
BSFL	Black soldier fly larvae
BSFP	Black soldier fly pre-pupae
Ca	Calcium
ChiSq	Chi-square (χ^2)
CP	Crude protein
Cys	Cysteine
d.f.	Degrees of freedom
DAA	Dispensable amino acids
DDGS	Distillers dried grains with solubles
DM	Dry matter
EAA	Endogenous amino acids
EU	European union
FAO	Food and Agriculture Organisation
Frass	Black soldier fly excrement /residual / spent wheat middlings
g	Gram
GE	Gross energy
GENMOD	Generalised Linear Models
GHG	Greenhouse gases
GIT	Gastrointestinal tract
GLM	General linear model
Glu	Glutamic acid
Gly	Glycine
GT	Grain type
GWP	Global Warming Potential
h/ hrs	Hours
HCl	Hydrochloric acid
His	Histidine
IAA	Indispensable amino acids
Ile	Isoleucine
kg	Kilogram
LCA	Life cycle assessment
LED	light-emitting diode

Leu	Leucine
Lys	Lysine
Met	Methionine
mg	Milligram
mg	Milgram
mg	Milgram
MJ	Mega joule
mL	Millilitre
mm	Millimetre
MUFA	Mono-unsaturated fatty acids
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NFD	Nitrogen free diet
NRC	National research council
P	Phosphorus
Pro	Proline
SBM	Soybean meal
SE	Standard error of mean
Ser	Serine
SFA	Saturated fatty acids
SID	Standardised ileal digestibility
SIDC	Standardised ileal digestibility coefficients
Thr	Threonine
Ti	Titanium
Trp	Tryptophan
US	The United States of America
Val	Valine
WHC	Water Holding Capacity

Chapter 1: General Introduction

1.1 Food loss and waste (FLW) prevalence

The escalating accumulation of organic wastes on a global scale poses a growing threat to the economy, food security, nutrition, biodiversity, and the environment. Among the various contributors to this problem, food loss and waste (FLW) stand out prominently. Over the years, the percentage of FLW in total landfill of municipal solid waste has continued to increase, rising from 13.6% to 24.1% between 1990 and 2020 (Krause *et al*., 2023). It is estimated that approximately 1300 million tonnes of food are lost or wasted every year, equivalent to 33% to 40% of the total food production (Gustavsson *et al*., 2013; Wang *et al*., 2023). Of this staggering amount, 61% is attributed to household waste, equating to an average of 79 kg of food per capita per year (FAO, 2021).

FLW can occur at various stages of the food life cycle, from production to consumption. Both developing and developed countries grapple with managing the continuous increase in FLW, yet in different ways and to varying extents (Figure 1.1) (Jain *et al*., 2018). Typically, food loss is more widespread in developing countries due to lower levels of technology, while food waste is more common in developed countries, driven by high expectations in consumption patterns (Edjabou *et al*., 2016; FAO, 2017; Directive, 2008). For example, the average per capita food waste by consumers in Europe, North America, and Oceania ranges from 95 to 115 kg per year, whereas in Sub-Saharan Africa and, South and South-Eastern Asia, it is only 6 to 11 kg per year (Gustavsson and Cederberg, 2011).

The ramifications of food wastes extend beyond these immediate concerns of wasting food. The apparent adverse effects of FLW are considerable, ranging from food insecurity and nutrient loss to economic loss, and resource wastage. For instance, nearly one third of all agricultural land, totalling 1.4 billion hectares, is utilised for producing food that ultimately goes uneaten and is wasted (FAO, 2013). FLW also contribute to 8% of human-caused greenhouse gas (GHG) emissions (FAO, 2015). The amount of GHG emissions contributed due to global FLW could rank as the third-largest emitter of global GHG emissions, following China (21%) and the United States (13%) (Watch, 2022).

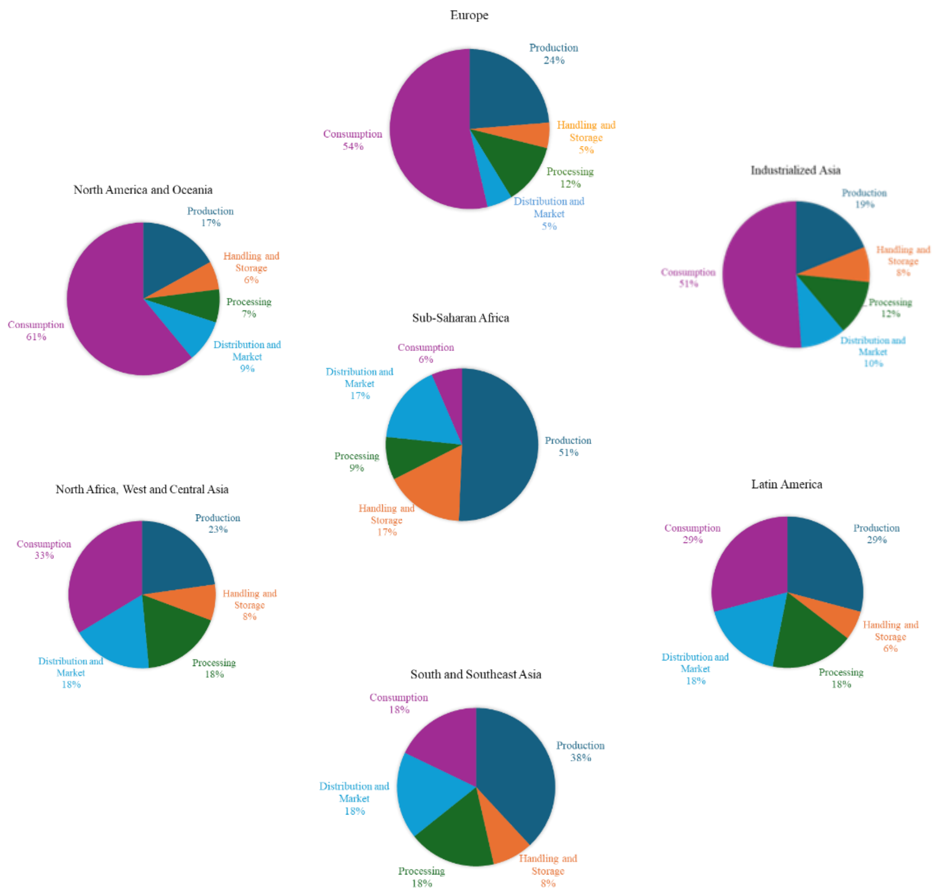

Figure 1.1 Distribution of food loss and waste across different stages of the supply chain in seven regions: North America and Oceania, Europe, Industrialized Asia, West and Central Asia, Sub-Saharan Africa, North Africa, South and Southeast Asia and Latin America. The stages considered include production, handling and storage, processing, distribution and market, and consumption. The food loss often prevalent in developing countries while food waste often prevalent in developed countries. The figure was constructed based on data sourced from Rezaei and Liu (2017) and FAO (2017).

1.2 Current FLW management methods

There is no doubt that preventing FLW in the first place is the best approach. However, achieving this goal can be challenging. Even in the most optimal scenarios where FLW is minimised, there remains a fraction of waste, including inedible plant parts and food preparation leftovers, which often go unused (Ojha *et al.*, 2020). According to the food recovery hierarchy, the next option for FLW is to repurpose it as animal feeds. Unlike other methods (i.e., landfilling and incineration) which incur costs for disposal, the use of FLW as animal feed can generate income (Jain *et al*., 2018). Although feeding animals on waste foods is a common practice due to its valuable nutrients, there are limitations. The variable nature of FLW and its high moisture levels restrict its applications (Jain *et al*., 2018). Additionally, some materials may not be suitable for animal consumption and local regulations may limit this practice to prevent potential disease outbreaks (Shurson *et al*., 2023). As result, the portion of the FLW used for animal feed can be as low as 10 to 15% in countries like the USA and Europe (Shurson *et al*., 2023).

In developing countries, there is a significant portion of FLW ends up in landfills (50%), open dumps (13 to 33%) or disposed into the sea (Hoornweg and Bhada-Tata, 2012). In landfills and open dumps, FLW undergoes anaerobic digestion, resulting in the production of methane (CH_4), carbon dioxide (CO_2), and other GHGs (Melikoglu *et al*., 2013). This process contributes to environmental pollution, loss of the energy content of food wastes, spread of diseases, foul odour, air pollution and, contamination of soil and surface water bodies (Zhang *et al*., 2022). It was reported that CH_4 emissions from landfilled FLW have steadily increased by 295% from 1990 to 2020 (Krause *et al*., 2023). Another an inefficient approach commonly used in developing countries, to manage FLW and organic wastes, is open-burning and uncontrolled incineration. Both methods can cause significant environmental pollution as where smoke and other emissions are released directly into the air include CO_2, CH_4 and nitrous oxide (N_2O). Normally, emissions of CO_2 from waste incineration are more significant than CH_4 and N_2O emissions (Guendehou *et al*., 2006).

A rational and widely accepted method for organic waste disposal is composting, which can reduce waste volume by up to 40%, eliminate pathogens, and produce clean fertilizers (Schaub and Leonard, 1996). This natural aerobic biochemical process involves thermophilic microorganisms breaking down organic materials, ultimately transforming them into a stable soil-like product. However, its effectiveness can significantly vary depending on site-specific conditions, including abiotic factors, pH, carbon-to-nitrogen ratio, particle size, and moisture levels (Schaub and Leonard, 1996). Despite these advantages, composting is time-consuming requiring regular turning and the risk of nutrient loss. Furthermore, it may fall short in enhancing the inherent qualities of the composted material (Ndegwa and Thompson 2001). Composting fruit and vegetable wastes presents a specific challenge due to their high moisture content, necessitating pre-treatment to prevent anaerobic conditions and associated odours (Schaub and Leonard, 1996).

Vermicomposting is a closely related technique to composting. In this process, earthworms feed on organic wastes, passing it through their digestive system to produce well-digested castings that can be used as fertilisers. The advantage of vermicomposting over composting is its low-cost setup. However, vermicomposting does have its limitations. Unlike composting, the temperature required for vermicomposting (below 35°C) may not be sufficient to kill potential pathogens (Ndegwa and Thompson, 2001). Additionally, the process of vermicomposting typically takes at least two months to achieve significant transformation of phytomass into vermicast, and it requires continuous aeration to maintain optimal conditions for the worms (Thakur *et al*., 2022).

1.3 Insect bioconversion of FLW

Insect bioconversion of FLW has emerged as a compelling strategy to address the challenges associated with the existing methods for managing organic wastes. Insects, ranging from detritivores to herbivores, possess diverse dietary preferences and specialized adaptations for consuming various organic substrates (Barry, 2004). These substrates often mirror the food waste generated by agricultural and food processing industries (Fowles and Nansen, 2020). Referred to as bioconversion in the literature, this process involves the controlled decomposition of feedstock or FLW and other organic wastes into insect biomass rich in protein and fat, while residual waste products, also known as frass, can be utilised as soil conditioner (Barry, 2004; Cheng *et al*., 2017). Although bioconversion can utilise various organisms such as yeast, bacteria, and invertebrates, insects from the order Diptera have been among the most prevalent species used in bioconversion (Čičková *et al*., 2013). While many insects are suitable for bioconversion (Table 1.1), because they naturally breed and feed on a wide range of waste materials. Both research and industry have targeted only select species of insects, such as the black soldier fly (BSF; *Hermetia illucens* L. (Stratiomyidae)), and the housefly (*Musca domestica* L. (Muscidae)) (Parry *et al*., 2021). In this thesis, the focus was on BSF production and nutrition as a sustainable solution for poultry feeding.

1.4 The study species

1.4.1 Taxonomy and distribution

The BSF, scientifically known as *Hermetia illucens*, belongs to the Kingdom Animalia, Phylum Arthropoda, Class Insecta, Order Diptera, Suborder Brachycera, Infraorder Tabanomorpha, Superfamily Stratiomyoidea, Family Stratiomyidae, Genus *Hermetia*, and Species *Hermetia illucens* (Leclercq, 1997).

The BSF is a versatile insect that can inhabit various regions from (sub) tropical to temperate areas within the latitudes of 45° N and 40° S (dos Santos, 2016; Dortmans *et al*., 2017; Wang *et al*., 2017). There are three hypotheses about the origin of BSF, but the widely accepted one is that they originated from the Americas and then spread to Europe through trade (Benelli *et al*., 2014). The insect was first discovered in Malta in 1926 and in New Zealand around Auckland in 1956 (May, 1961; Benelli *et al*., 2014). Today, BSF can be found in every zoogeographic region worldwide (Marshall *et al*., 2015). They are synanthropic insects and are generally not considered a nuisance or a mechanical vector of disease (Booth and Sheppard, 1984; Dobermann *et al*., 2019; van Huis, 2020).

Table 1.1 Common insects used for organic waste bioconversion into animal feed and/ or frass fertiliser

Order	Scientific name	Common name	Substrates type	Application	Reference
Diptera	*Hermetia illucens*	Black Soldier Fly	Organic waste (food scraps, manure, agricultural by-products)	Animal feed, soil fertiliser and waste bioconversion	1,2, 3.
	Musca domestica	Housefly Larvae	organic wastes (animal manure, kitchen waste, sludge, and plant residues left after oil extraction)	Animal feed and waste bioconversion	4, 5.
	Sarcophaga dux	Flesh fly	Carrion, meat, and meat by products	Waste bioconversion	1, 6.
	Chrysoma putoria & *Lucilia sericata*	Blow fly	Meat and meat by product, bran flakes and whole eggs	Waste bioconversion	1, 7.
Coleoptera	*Tenebrio molitor*	Yellow mealworm	Wheat bran, mixed-fermented feed and fresh inedible vegetables	Animal feed and waste bioconversion	8.
	Zophobas morio F.	Superworm	Grain-based substrates, fruits, vegetables	Animal feed and waste bioconversion	9, 10.
	Alphitobius diaperinus	Black Beetle Larvae	Grass roots close to the soil and whole plants	Waste bioconversion	11.
	Alphitobius diaperinus	Lesser Mealworm	Several agricultural side-streams (barely, sunflower meal, cotton cake and oat side-stream)	Waste bioconversion	12.
	Oryctes rhinoceros	Coconut rhinoceros beetle	Dead standing coconut palms, coconut stumps and logs and dunk heap.	Animal feed and waste bioconversion	13.
Orthoptera	Various species (e.g., *Acheta domesticus*)	Cricket	Fruits, vegetables, grains, decaying plant matter	Animal feed and waste bioconversion	8.
	Schistocerca gregaria	Desert locust	Agricultural side-streams (*i.e*., tomato leaves)	Waste bioconversion	15.
Haplotaxida	*Eisenia fetida*	Earthworm (tiger worm)	Kitchen scraps, garden waste, manure, paper, cardboard	Waste bioconversion and soil fertiliser	16, 17.
Lepidoptera	*Galleria mellonella*	Waxworm	Creal, bran, honey, cow manure	Waste bioconversion and soil fertiliser	18.
	Gonimbrasia krucki	African golden emperor moth	Leaves of plants	Animal feed and waste bioconversion	19.
Blattodea	*Blattodea*	Cockroach	Organic waste (food scraps, dead plant matter, decaying wood)	Animal feed and waste bioconversion	8.

[1-20] References: [1](Parry *et al*, 2021), [2](Surendra *et al*., 2016), [3](Wang *et al*., 2024), [4](Lu *et al*., 2024), [5](Fan *et al*., 2023), [6](Čičková *et al*., 2015), [7](Cook *et al*., 2024), [8](Siddiqui *et al*., 2024), [9](Jung *et al*., 2023), [10](Kosewska *et al*., 2022), [11](Bell *et al*., 2011), [12](Gourgouta *et at*., 2020), [13](DeFoliart *et al*., 1993), [14](Siddiqui *et al*, 2024), [15](Yakti *et al*., 2023), [16](Sharma *et al*., 2005), [17](Bhat *et al*., 2018), [18](Alwaneen, 2013), [19](Kipkoech *et al*., 2023).

1.4.2 General biology and life cycle

Female BSF lays an average of 400 to 800 eggs, with some laying up to 1,026 eggs per lifetime (Dortmans *et al*., 2017; Gangadhar *et al*., 2018). These eggs are usually deposited in clusters (Figure 1.2A), are elongated-oval, and have a creamy white appearance (Figure 1.2B). They are often cemented together and placed in a dry and sheltered location near decomposed organic matter (May, 1961). This strategic placement helps protect the eggs from predators and desiccation by concealing them in dry crevices (Booth and Sheppard, 1984; Tsagkarakis *et al*., 2015).

Prior to hatching, the chorion becomes translucent, allowing the segmentation, tracheal trunks, head colour, and ocelli (eyespots) to be seen, which are key in distinguishing fertile from non-fertile eggs (Figure 1.2C) (May, 1961). Under optimal conditions, the eggs hatch in 2 to 4 days (Booth and Sheppard, 1984). The newly hatched BSF larvae (BSFL) are white with red eyespots and immediately begin to search for food (Figure 1.2D) and they require proximity to a food source for survival (Dortmans *et al*., 2017). Under optimal feeding conditions, the BSFL grow rapidly, reaching 1.8 mm in length (Figure 1.2E) and 0.4 mm in width, to 2.5 mm in length, 0.5 mm in width, and weighing up to 220 mg in 14 to 21 days (Figure 1.2F) (May, 1961; Dortmans e*t al*., 2017). In adverse conditions, the growth of larvae is much slower, extending their life cycle from 31 days to 4 months (Makkar *et al*., 2014; Oliveira *et al*., 2015).

Due to their negative phototropic behaviour, BSFL require a dark environment to actively feed and achieve optimal growth. BSFL undergo six larval stages that require moulting to progress to the next instar (Tsagkarakis *et al*., 2015). They are omnivorous and can feed on various organic waste materials such as kitchen waste, carcasses, animal manure, and human faeces (Surendra *et al*., 2016). The amount of organic matter that a larva consumes per day can range from 25 to 500 mg, depending on factors such as the larval stage, type and shape of feedstock, temperature, moisture, larval density, and ventilation conditions in the growing unit (Makkar *et al*., 2014; Surendra *et al*., 2016).

The last instar of the BSFL is the sixth stage, also called the pre-pupal stage, which is characterised by a colour change from creamy white to dark grey or brown and eventually to dark brown (Figure 1.2G) (May, 1961; Ushakova *et al*., 2017). In this stage, the pre-pupa stops feeding and migrates from the food site to a dry and sheltered spot to complete the metamorphosis (Diener *et al*., 2011; Spranghers *et al*., 2017).

Pupation is a crucial phase in metamorphosis (Holmes *et al*., 2013) and, influenced by environmental factors such as temperature, moisture, and substrate type (Dzepe *et al*., 2020). Under favourable conditions, pupation lasts 2 to 3 weeks but can last up to five months in unfavourable conditions (Makkar *et al*., 2014; Dortmans *et al*., 2017). During this phase, the pre-pupa's cuticle hardens, marking its transition into the immobile pupal stage (May, 1961; Park, 2016). The pre-pupa and pupa share a similar coloration, but the pupa distinguishes itself by its increased rigidity, with a slight bend visible in the last two segments of the body (Figure 1.2H) (May, 1961).

The pupation stage is pivotal in the life cycle of BSF, akin to many dipteran species. However, research has predominantly focused on the active stages of BSF life. Only two studies have explored the use of different substrates for BSF pupation (Holmes *et al*., 2013; Dzepe *et al*., 2020), with one additional study examining moisture levels in selected substrates (Liu *et al*., 2023). Many aspects concerning optimal pupation conditions for BSF remain less understood, including the interactions between substrate moisture levels, physical properties like particle density, and the suitability of various materials as pupation media.

When the adult is ready to emerge from the pupation stage, the first thoracic segment and the head capsule partially tear off, and a split occurs in the second and third segments for the fly to wiggle out of the exo-skeleton (May, 1961). Within minutes of emergence, the wings of the fly inflate preparing them for flight (Figure 1.1I) (Makkar *et al*., 2014). The adult BSF fly is 13 to 20 mm long, generally lethargic and a weak flier (May, 1961; dos Santos, 2016; Gangadhar *et al*., 2018; Lemke *et al*., 2023). Females are generally bigger than males, and both genders can easily be recognised by the morphology of their genitalia (Figure 1.2J and K) (Tomberlin *et al*., 2002). Their lifespan is about a week, with mating typically occurring two days after emergence. Eggs can be laid two days after mating (Surendra *et al*., 2016). Successful mating requires abundant natural or simulated artificial light and a warm temperature of 25 to 32°C (Dortmans *et al*., 2017). While it is commonly believed that adult BSF flies lack functioning mouthparts and, therefore, do not feed. They have been observed to subsist solely on water and rely on stored fats from their larval stage for sustenance (Makkar *et al*., 2014; Oliveira *et al*., 2015; Tsagkarakis *et al*., 2015). However, some studies have reported that the adult flies can consume liquid-type diets, like water, sugar solutions and milk, which enhance their longevity and egg production, and that they are also capable of consuming non-liquid substances like sugar cubes (Nakamura *et al*., 2016; Bruno *et al*., 2019).

In nature, BSF often inhabit decaying organic materials such as animal manure, carcasses and vegetation (Booth and Sheppard, 1984). They also perceived to outcompetes other species, such as houseflies, for resources due to their larger size and longer developmental period (Miranda *et al*., 2019). The adults possess a non-piercing proboscis, which limits the amount of regurgitated saliva reducing the risk of pathogen transmission, though they themselves can be susceptible to pathogens (Bruno *et al*., 2019; Vogel *et al*., 2022; Lemke *et al*., 2023). Despite their competitive advantages, disease management and maintenance of a healthy BSF colony remain areas of limited knowledge. Müller *et al*. (2017) identified potential risks for BSF colonies include nematodes, microsporidia, and apicomplexa parasites. Additionally, other dipteran species have reported parasites and diseases, such as *Macrocheles subbadius* mites negatively affecting the survival and reproduction of *Drosophila nigrospiracula* flies, and *Macrocheles muscaedomesticae* that feeds on eggs, newly hatched, and small larvae of the house fly Musca domestica L. (Polak, 1996; Abo-Taka *et al*., 2014). However, there has not been any formal report of disease or parasite outbreaks in BSF facilities to date.

Under captive conditions, BSF colony performance can vary. In laboratory settings, BSF exhibited decreases in growth, size, longevity, and caloric content compared to their wild counterparts (Tomberlin *et al*., 2002). However, artificial selective breeding over lab generations has led to significant genetic improvements (Facchini *et al*., 2022). Despite these findings, behavioural performance during mass rearing remains poorly understood. This knowledge gap may be attributed to BSF's inherent phenotypic plasticity, which allows adaptation to various conditions (Nyamukondiwa *et al*., 2010), potentially delaying the need for extensive studies. Furthermore, the mass rearing of BSF is in its early stages, with a focus on creating optimal rearing environments and improving husbandry practices (Hoffmann *et al*., 2021). One study highlighted that the mass rearing of a wild BSF population ultimately collapsed by the fifth generation, most likely due to the adverse effects of inbreeding depression following the fixation of deleterious alleles (Rhode *et al*., 2020). This issue can be mitigated by ensuring the founder colony is collected from diverse populations, maintaining a genetically stable colony (suggested effective population size of 500 individuals: Rhode *et al*., 2020), and carefully augmenting with wild colonies in the early stages of establishment (F0 to F2) (Hoffmann *et al*., 2021).

The issue of inbreeding in BSF colonies mirrors challenges observed in other dipteran species, such as Drosophila, where environmental stress was found to exacerbate the effects of inbreeding depression (Bijlsma *et al*., 2000). Given the current scale of BSF mass rearing, estimated at 200 to 300 billion individuals annually (Rowe, 2020), and the absence of welfare information about BSF in such facilities (Barrett *et al*., 2023), further research is essential to ensure the health and stability of BSF colonies in mass rearing operations.

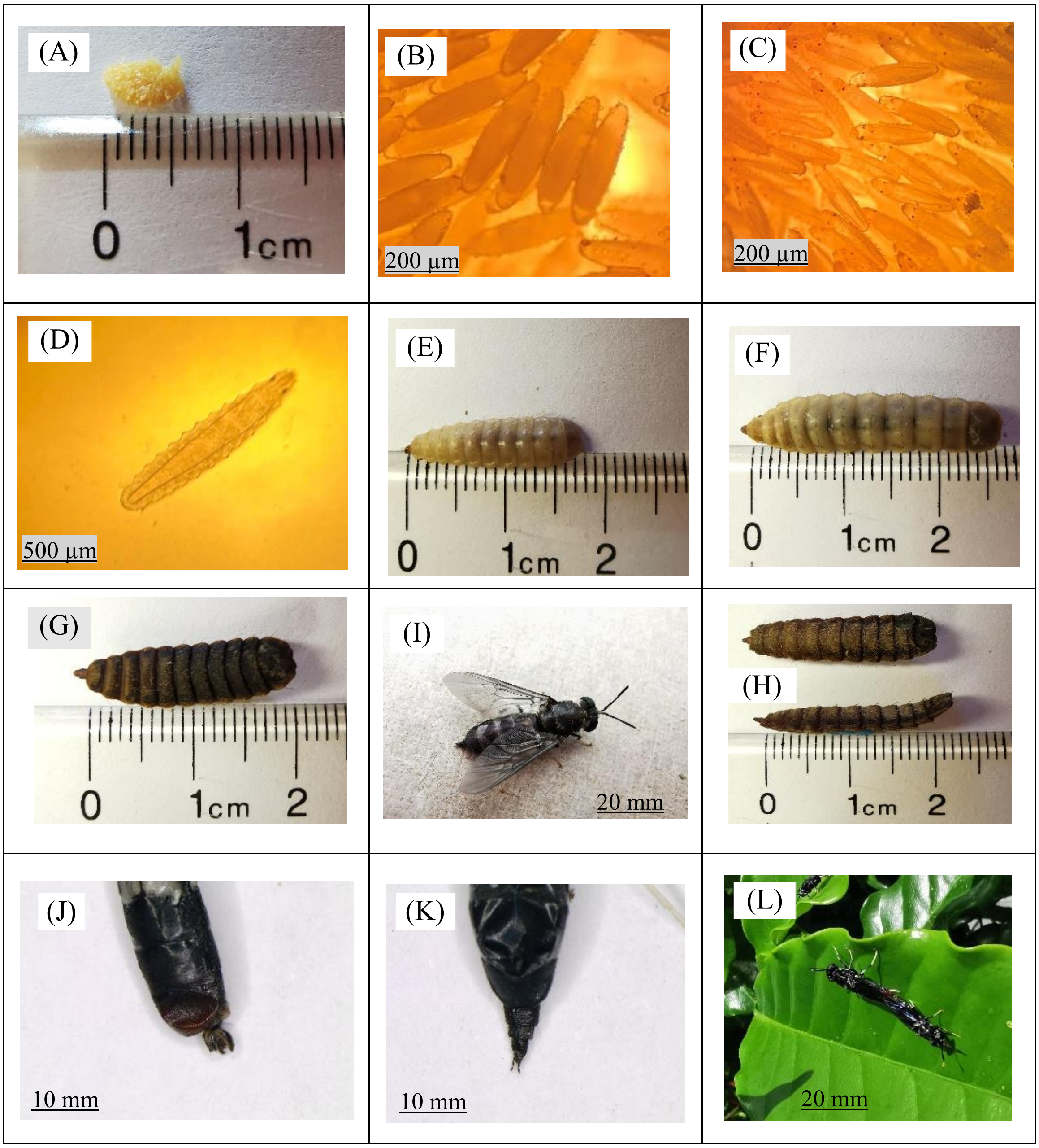

Figure 1.2 Stages of the black soldier fly (*Hermetia illucens* L.; BSF) life cycle: eggs, larvae, pre-pupae, pupae, and adults.
(A) An egg cluster placed together.
(B) Light microscope image of an egg cluster (200 µm).
(C) Light microscope image of fertile eggs showing the eyes, also known as ocelli (200 µm).
(D) Light microscope image of one-hour-old, hatched larva under a light microscope (500 µm).
(E) One-week-old larva, reaching a length of 1.8 cm.
(F) Larvae at the end of the second week, reaching a length of 2.6 cm.
(I) Full-grown adult (20 mm). (J) Close-up image of male genitalia of adult of BSF (10 mm).
(K) Close-up image of female genitalia of BSF (10 mm). (L) Mating position of adult BSF.

1.5 The case of BSF as a sustainable waste management agent

The benefits of using BSFL for waste management are substantial and address several issues associated with current organic waste and FLW management methods. BSFL also present a sustainable source of animal feed, along with several other significant benefits, as outlined below.

1.5.1 Efficiency in waste reduction

BSFL have emerged as highly effective agents for waste conversion due to their substantial feed intake, which can reach up to 200 mg per larva per day (Manurung *et al*., 2016). Their adaptability to a range of organic materials, including food scraps, manure, and agricultural residues, further enhances their utility in waste management (Fowles and Nansen, 2020). With a notably short life cycle of 39 days, BSFL can reduce waste volume by up to 75% within a period of 7 to 14 days in the larval stage (Ibadurrohman *et al*., 2020). This rapid conversion rate not only accelerates waste reduction but also renders BSFL more efficient compared to traditional composting and vermicomposting methods. Additionally, BSFL presence significantly mitigates odour problems associated with organic wastes. Studies have demonstrated that BSFL can reduce odours from poultry, swine, and dairy manures by up to 87%, addressing one of the primary challenges of landfill waste management (Siddiqui *et al*., 2022). This dual functionality of reducing waste volume and mitigating odours makes BSFL a superior choice for waste management in landfills.

The environmental adaptability of BSFL is another crucial factor contributing to their effectiveness in waste management. The optimal temperature for BSFL activity is between 25°C and 32°C (Dortmans *et al*., 2017); yet they can tolerate a broad temperature range from 15°C to 45°C making them versatile in varying environments, including outdoor waste management (Ibadurrohman *et al*., 2020). During their feeding process, BSFL generates heat, which aids in the breaking down of waste materials and enhances microbial activity, thereby accelerating the composting process (Diener *et al*., 2009). This heat generation is particularly beneficial in cooler climates or during colder seasons. Unlike other detritivorous species, such as houseflies and blowflies, which pose concerns of disease spread, BSFL are not considered a pest or a carrier of disease (Dobermann *et al*., 2019; van Huis, 2020). This synanthropic nature of BSFL, combined with their heat-generating capabilities, contributes to their suitability for sustainable waste management practices across diverse climatic conditions.

Furthermore, BSFL thrive in high-moisture environments, requiring a diet with a water content of 60 to 80%, which aligns with the typical profile of food wastes (Ibadurrohman *et al*., 2020). This makes BSFL particularly effective for managing food waste, which is often unsuitable for direct animal feed due to its high moisture content and inconsistency (Jain *et al*., 2018). The ability of BSFL to efficiently process high-moisture wastes means that they can significantly reduce the volume of food waste that would otherwise end up in landfills.

1.5.2 Economic value and market growth of BSF

According to the International Platform of Insects for Food and Feed (IPIFF), European insect companies began commercial operations a few years ago (IPIFF, 2019). Since 2017, the European market has commercialised more than 5,000 tonnes of insects and raised approximately 600 million EUR for investments in insect production as of September 2019 (Franco *et al*., 2021). Currently, European production of insect protein is estimated at approximately 6,000 tonnes per year, with projections suggesting it could reach up to 8 million tonnes by 2030 (Ünver *et al*., 2024). Globally, the insect feed market was valued at USD 1.4 billion in 2024, with forecasts suggesting a future valuation of approximately USD 826.76 million by 2030 (IPIFF, 2021). The primary insects produced include BSF, yellow mealworm (*Tenebrio molitor*), and house cricket (*Acheta domesticus*) (IPIFF, 2021).

The economic benefits of BSF farms extend significantly beyond their contribution to the growing global insect market. BSF technology reduces waste disposal costs for industries and generates revenue streams for BSF farmers (Čičková *et al*., 2015). Additionally, they foster business opportunities in rural areas which contribute to circular economy (Ünver *et al*., 2024).

A cost-benefit analysis conducted in Malawi illustrates the significant income potential of BSF farming, with small-scale operations generating annual revenues of approximately USD 2,535 and large-scale operations reaching up to USD 7,680 (Munthali *et al*., 2023).

Over the past decade, there has been a substantial increase in the number of BSF companies specialised in waste management and animal feed production (Figure 1.3). The BSF technology market is relatively new, and formal distinctions in the scales of operations are still developing. However, three trends namely, large-scale, medium-scale, and small-scale operations, are identified. Large-scale operations can process from several tons to 200 tonnes of waste per day and are found in countries such as South Africa, the USA, Canada, and the Netherlands (Joly and Nikiema, 2019). On the other end, small-scale operations, often household-sized, can process on-site waste and are typically operated by enthusiastic individuals (Joly and Nikiema, 2019). Medium-scale BSF operations are scarce and can process hundreds of kilograms to 10 tonnes of waste daily (Diener *et al*., 2019).

The companies identified in Figure 1.3 primarily fall into the large-scale category. Data on small and local BSF enterprises are absent despite their widespread. According to Raman *et al*. (2022), the BSFL market can accommodate both small and large operations. Large-scale BSFL producers benefit from marketing their products locally and exporting processed meals, leveraging economies of scale and broader distribution networks. Conversely, smallholder farmers can use the BSFL to substitute costly animal or fish feeds with a low-cost, locally produced alternative protein source for poultry and aquaculture. This substitution can significantly reduce dependence on imported feed materials and contribute to local food security and sustainability.

Despite the potential for both large and small-scale BSF operations to thrive, there is a lack of publicly available information about the profitability, operations, and financial performance of these large-scale enterprises. This information remains secretive to maintain the competitive advantage (Joly and Nikiema, 2019). Furthermore, the research is based predominantly on laboratory or bench-scale experiments, with limited practical guides or resources detailing the design and day-to-day activities of medium-scale BSF farms (Caruso *et al*., 2014; Dortmans *et al*., 2017). This creates a critical knowledge gap for small BSF farmers, who struggle to apply laboratory findings to their operations and face difficulties in accessing relevant information from larger-scale operations.

1.5.3 Nutritional value BSF

The nutritional value of harvested BSFL biomass is noteworthy, with the content of protein ranging from 320 to 655 g/kg dry matter (DM), crude fat from 70 to 390 g/kg DM (Schiavone *et al*., 2017), and minerals from 90 to 280 g/kg DM (Barragan-Fonseca *et al*., 2017). This makes BSFL meal a popular component for animal feed, including poultry, fish, and pigs, as demonstrated in number of studies (Table 2.1). These studies have shown the successful partial or full replacement of soybean meal (SBM) or fish meal with BSFL, yet the potential of BSF meals has not been fully explored due to some limitations. Most research has focused on BSFL, with limited studies on other stages such as pre-pupae (BSFP) and adult flies. BSFP has been reported to contain similar protein (320 to 661 g/kg DM) and fat (397 g/kg DM) levels comparable to BSFL, with protein and fat content ranging from 362 to 627 g/kg DM and 180 to 700 g/kg DM, respectively (Heger *et al*., 2016; Caligiani *et al*., 2018). Additionally, BSFP is often mistakenly assumed to contain high levels of chitin. However, Soetemans *et al*. (2020)

reported that the chitin levels in BSFP, BSFL, and adult flies were 31, 36, and 29 g/kg DM, respectively, with only the puparium exhibiting a high chitin concentration of 240 g/kg DM.

Another limitation is that early studies predominantly used BSFL with full-fat content due to the limited use of BSF oil. Recent research, however, has promoted BSF oil as a replacement for SBM oil with favourable results (Kim *et al*., 2020). Furthermore, BSF are responsive to the fatty acid profile in their diet, which can be manipulated to meet the fat level and fatty acid profile required for specific animal species (Barroso *et al*., 2017).

Traditionally, poultry have a natural inclination to consume insects as part of their diet (Kee *et al*., 2023). However, in modern commercial poultry production, the reliance on insects as a primary protein source has been replaced by formulated feeds that provide the necessary nutrients in a balanced manner (Kee *et al*., 2023). Notably, only one study reported that the use of live BSF for broiler chickens improved their welfare (Ipema *et al*., 2020), a practice that is challenging to implement in modern poultry barn setups.

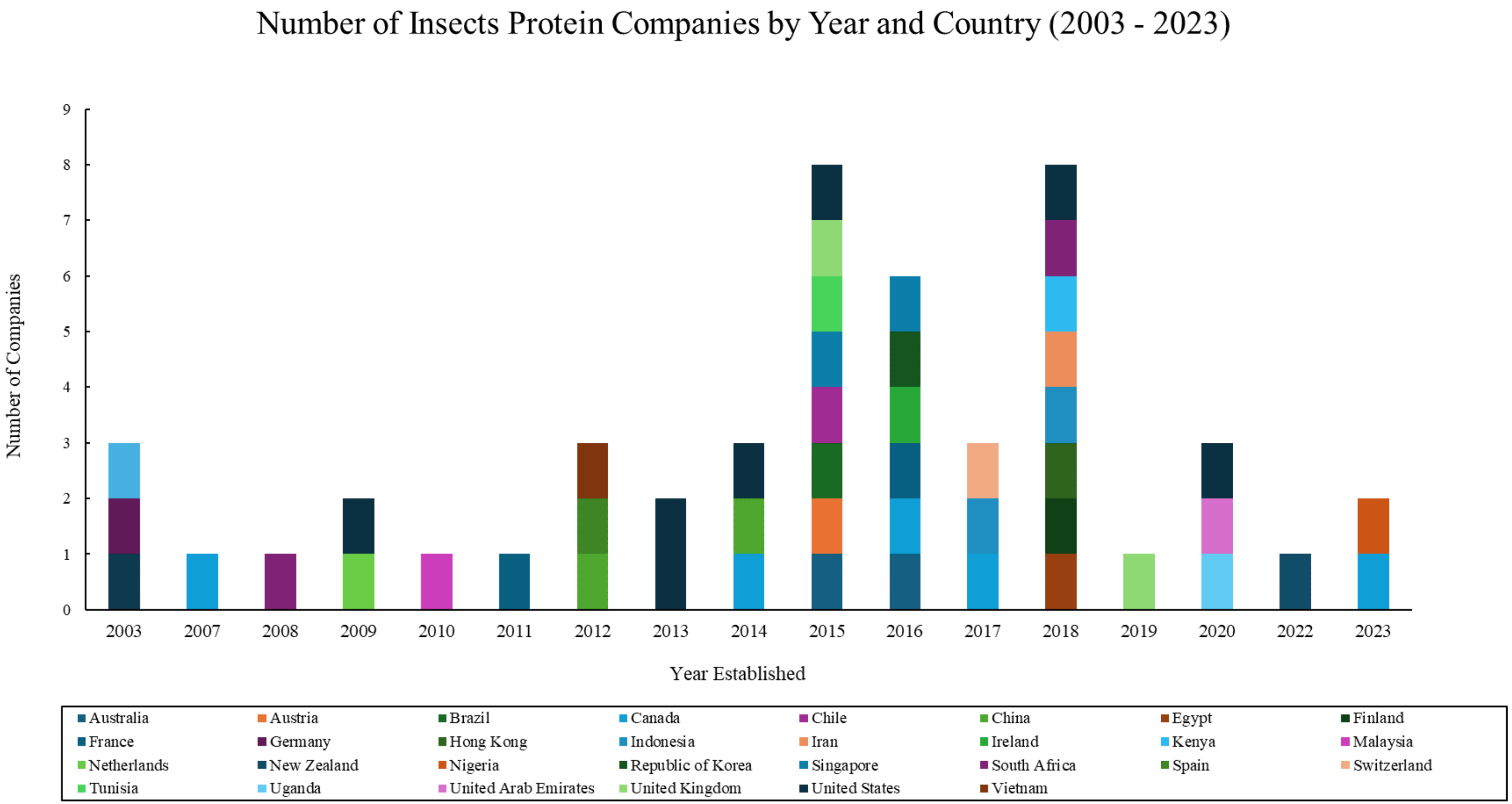

Figure 1.3 The establishment of black soldier fly protein companies across various countries from 2003 to 2023. There is a noticeable increase in the number of companies starting from 2012, with peaks in 2015, 2016, and 2018. Countries like the Netherlands, Canada, and the United States have multiple entries, indicating a significant interest in insect protein production in these regions. Companies included in this graph are: Enterra Feed Corporation, Protix, Ynsect, Agri Protein, Innova feed, Hexa fly, Ento food, Entomo farms, Aspire food, Enviro flight, Beta hatch, Protengo, Nutrinsic, Nextprotein, Entobel, Bio fly tech, Food recycle Ltd, Ento cycle, Fly farm, Soldier fly technologies, Goterra, Buhler insect technology, Nutrition technologies, Better origin, Ento system, Circa biotech, Insectflux, Cyns, Egy Mag, Oberland, Laviran, Symton, F4F, Insectipro, Nutri Industry, JM Green, Hermetia Baruth GmbH, BioflyTech, Guangzhou Unique Biotechnology Co., Ltd, Chapul, Good Grub Agritech, Nambu Group, Manna, Living farms, Magalarva, Biocycle, Akatale on Cloud, ZSI Integrated. (Image by Mahmoud. A. E., 2024; CC BY-ND 4.0).

Table 1.2 The use of black soldier fly larvae (BSFL) and pre-pupae (BSFP) in animal feeds as supplements or as partial or total replacements for soybean meal (SBM), fish meal, and sunflower cake in pigs, ducks, chickens, and quails

Animal Species	Feeding Phase	BSF Inclusion Stage	Alternative Feed Source
Pigs	Weaning	Defatted [1,2,3]	Full replacement of fish meal [1,2]
		Hydrolysed BSFL [1,2]	Partial replacement of enzyme-treated SBM [3]
	Growing	Full fat BSFL [4,5]	Fish meal [4,5]
	Finishing	Full fat BSFL [6,7]	Partial replacement of SBM [6,7]
Duck	Male	Full fat BSFL live [8]	Supplement [8]
	Layer	Full fat BSFL live [9]	Supplement [9]
	Broiler	Partially defatted BSFL [10]	Substitution of corn gluten meal [10]
Chicken	Broiler	Full fat BSFP [11]	Partial replacement of SBM [11,13,15,16]
		Full fat BSFL[13,14,16]	Total replacement of SBM [12,13,15]
		Partially defatted BSFL [12,15]	Partial replacement of fish meal [16]
		Highly defatted BSFL [12]	Supplement [14]
	Layer	Full fat BSFP [17]	Partial replacement of SBM [17,18,19]
		Full fat BSFL[18,19,20]	Total replacement of SBM [17,20,22]
		Partially defatted BSFL[21]	Total replacement of sunflower cake [20]
		Fully defatted BSFL [22]	Supplement [21]
Quail	Broiler	Full fat BSFL [23,25]	Partial replacement of SBM [23,25]
		Fully defatted BSFL [25]	
	Layer	Full fat BSFL [23,24,26]	Partial replacement to SBM [23,24,25]
			Total replacement to SBM [24,26]

[1-26] References: [1](Lee *et al*., 2024), [2](Chang *et al*., 2024), [3](Malla *et al*., 2024), [4](Chia *et al*., 2019), [5](Kar *et al*., 2021), [6](Chia *et al*., 2021), [7](Yu *et al*., 2019), [8](Gunawan *et al*., 2018), [9](Gariglio *et al*., 2018), [10](Gunawan *et al*., 2024), [11](Elangovan *et al*., 2021), [12](Schiavone *et al*., 2017), [13](Murawska *et al*., 2021), [14](Józefiak *et al*., 2018), [15](Dabbou *et al*., 2018), [16](Onsongo *et al*., 2018), [17](Yandigeri *et al*., 2024), [18](Liu *et al*., 2021), [19](Bejaei and Cheng, 2020), [20](Wamai *et al*., 2024), [21](Park *et al*., 2021), [22](Secci *et al*., 2018), [23](Silva *et al*., 2024), [24](Mbhele *et al*., 2019), [25](Cullere *et al*., 2018), [26](Dalle Zotte *et al*., 2019).

1.5.4 Environmental value of BSF

BSF technology provides notable environmental benefits beyond its waste management efficiency and economic advantages. Several studies have examined the environmental impact of BSF rearing, highlighting its advantages using life cycle assessment (LCA) approach (Dalgaard *et al*., 2008; Oonincx and De Boer, 2012; Bosch *et al*., 2019; Ermolaev *et al*., 2019; Boakye-Yiadom *et al*., 2022; Beyers *et al*., 2023; Ferronato *et al*., 2023). Despite variations in the scope and boundaries of these studies, there is a consensus on few key factors: global warming potential (GWP), and the use of energy, land, and water resources.

BSF treatment for organic wastes demonstrates negligible GWP, whether in its fresh form (0.17 kg CO_2-eq.) or dried form (2.1 kg CO_2-eq.), compared to soybean meal (901 kg CO_2-eq.) and even other insects such as meal worm (20 kg CO_2-eq.) (Figure 1.4A). The anaerobic digestion process utilised by BSF is comparable to composting; however, a substantial portion of the energy in the substrate is converted into larval biomass, which has been found to reduce GHGs (Lalander and Vinnerås, 2019) and advantage BSF significantly over traditional composting methods (Perednia *et al*., 2016).

In terms of land use, BSF farming is notably efficient. Smetana *et al*. (2021) found that BSF requires less land compared to conventional livestock farming. Oonincx and De Boer (2012) demonstrated that mealworm production—similar to BSF production—exhibits lower land use compared to beef and chicken production (see Figure 1.4B). When BSF are fed on food waste and organic residues, land use can be minimised to nearly zero. Even when fed traditional substrates like chicken feed and grain by-products, land use remains significantly lower compared to soybean meal (SBM), mealworms, chickens, and beef production (Oonincx and De Boer, 2012).

Another environmental strength of BSF is its efficient use of water. BSFL obtain their water from their diet, leading to significantly lower water usage compared to traditional livestock (Ünver *et al*., 2024). This efficiency helps conserve water resources, making it available for other purposes and benefiting the environment.

The energy use values for BSF technology varied among published data. When considering the energy used to produce fresh BSFL (Boakye-Yiadom *et al*., 2022), it was found to be three to four times higher than the energy used for composting or SBM production (Oonincx and De Boer, 2012) (Figure 1.4C). Inclusion of the drying step in BSF meal production, energy use could reach 99.9 MJ per kg, making BSF meal less efficient than SBM, fish meal, and composting in terms of energy consumption (Dalgaard *et al*., 2008; Oonincx and De Boer, 2012). However, the challenge of high-energy use in BSF operations can be mitigated by employing solar energy (Ferronato *et al*., 2023). Tropical countries, where BSF colonies thrive in outdoor settings, would particularly benefit from this approach as it can be used to process wastes found in dumping sites (da Silva and Hesselberg, 2020). Furthermore, case studies by AgriProtein and Enterra have demonstrated that BSF operations can be profitable even in temperate climate conditions (Joly and Nikiema, 2019). However, achieving profitability in these regions requires significant investment in automation and highly controlled environments to optimise the rearing process (Joly and Nikiema, 2019).

A recent study by Beyers *et al*. (2023) tested the LCA value of BSF when fed different feed sources and agro-wastes. They reported that, regardless of the diets used for BSF, the LCA results showed that BSF production performed worse than conventional SBM and fish meal as a feed source. They suggested that significant improvements would be necessary, such as relocating BSF production to warmer regions or adopting alternative heating sources, to enhance its environmental performance to match or exceed traditional feed sources. However, the use of BSF for managing pig manure and agro-wastes demonstrated positive environmental benefits compared to traditional methods like composting.

It is also worth noting that most studies evaluating the LCA impact of BSF did not include small-scale BSF operations, where food wastes are often sourced locally and the BSFL is marketed locally in fresh form. This setting provides significant advantages for BSF over SBM, which has been reported to travel up to 500 km to mills in Argentina and 150 km in Malaysia (Dalgaard *et al*., 2008).

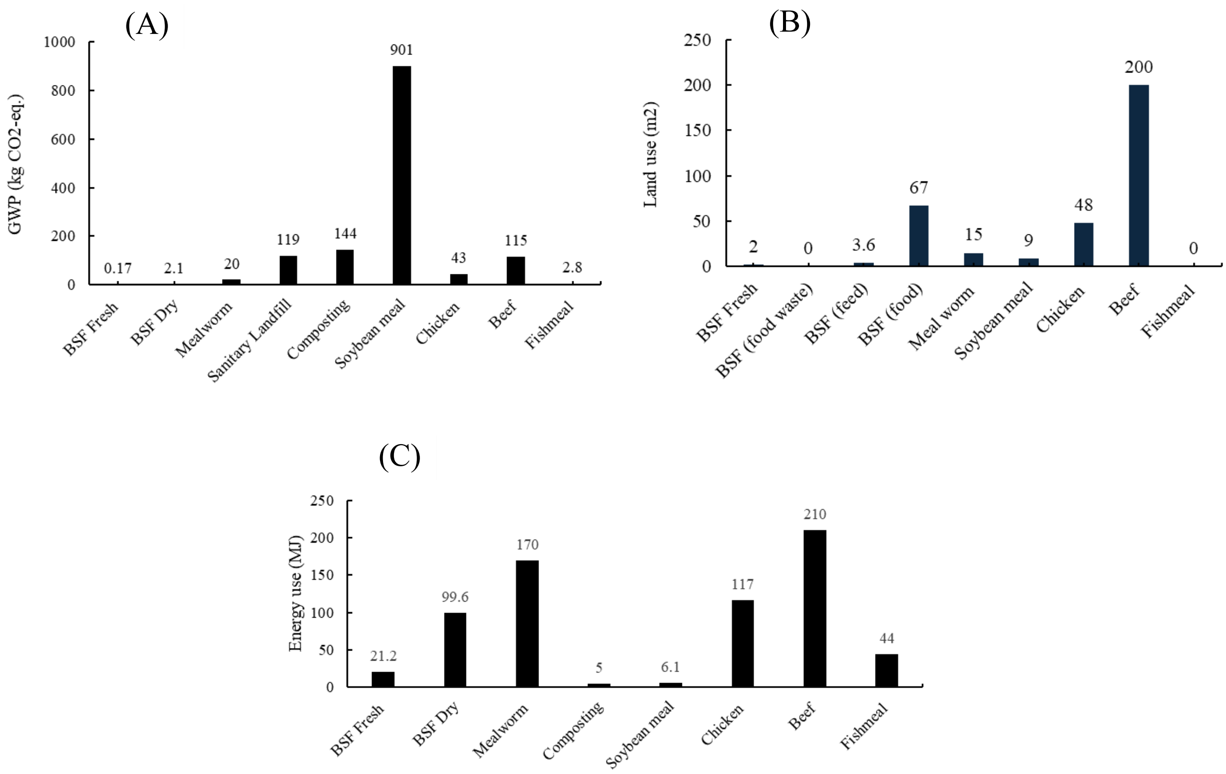

Figure 1.4 Main life cycle assessment (LCA) factors for black soldier fly in comparison to traditional food waste management methods (composting and sanitary landfill), beef, chicken, soybean meal and fish meal production: (A) Global warming potential (GWP) measured in kg CO2-eq. emissions for black soldier fly (BSF) in both fresh[1] and dry[1] forms compared to mealworm[2], sanitary landfill[3], composting[4], soybean meal[5], chicken[2], beef[2], and fishmeal[6]. (B) Land use measured in m^2 for BSF fresh[1], BSF fed on food waste[6] and BSF fed on feeding substrate[6] compared to mealworm[2], soybean meal[5], chicken[2], beef[2], and fishmeal[6]. (C) Energy use measured in MJ for BSF in both fresh[1] and dry[2] forms compared to mealworm[2], composting[4], soybean meal[5], chicken[2], beef[2], and fishmeal[6].

[1-6] References: [1](Boakye-Yiadom *et al*., 2022); [2](Oonincx and De Boer, 2012); [3](Ferronato *et al*., 2023); [4](Ermolaev *et al*., 2019); [5](Dalgaard *et al*., 2008); [6](Bosch *et al*., 2019). Values used for chicken and beef are averaged.

1.5.5 Other applications for BSF

Frass, an important by-product of BSF farms, can be sold to generate income and has been proven to improve soil quality, replacing traditional nitrogen fertilisers (Lopes *et al*., 2022). In recent years, BSF oil has been promoted as a replacement for SBM oil with favourable results (Kim *et al*., 2020). Moreover, BSF oil is endorsed as a non-toxic, biodegradable lubricant (Xiong *et al*., 2020), and can also be used in cosmetic and personal care products (Franco *et al*., 2021).

Other notable applications from BSF farms include the use of dead adults and puparium to produce chitin, which has applications in drug delivery, the food industry, and the medical field (Kaczor *et al*., 2022). Some fractions of BSF protein can be used for bioplastics that can be used in packaging, mulching plastics, and utensils, which do not use toxic additives (Nuvoli *et al*., 2021). Additionally, BSF lipids can be used to produce detergents and biodiesel (Kaczor *et al*., 2022).

BSF also holds potential in the field of entomoremediation, leveraging its remarkable ability to bioaccumulate and degrade pollutants. BSFL can sequester heavy metals such as cadmium (Cd), zinc (Zn), and lead (Pb), and degrade a range of organic pollutants, including mycotoxins, polycyclic aromatic hydrocarbons, insecticides, and antibiotics (Kaczor *et al*., 2022). This versatility positions them as a valuable tool for environmental clean-up. Importantly, this capability does not preclude the use of BSFL for animal feed. Diener *et al*. (2015) reported that BSFL fed on lead-contaminated substrates accumulated the lead in the larval exuviae (the cast-off skins), thereby reducing the lead content in the BSFL themselves. This process makes them safe for use as feed, provided that the skins and cast-offs are separated from the harvested larval mass.

1.6 Thesis aims and layout

The aim of this work was to study the optimal environmental condition for the breeding of BSF and the production of BSFL and BSP meals. It also investigated and compared the nutritional values for BSFL and BSFP poultry feed.

In Chapter 2, I aimed at optimising the rearing and production processes of the BSF for small operations. This research addressed the knowledge gaps in BSF production for smaller operations, which are often overlooked, compared to medium- or large-scale operations capable of processing several hundreds of tonnes of waste daily. The focus was on developing efficient methods for harvesting and processing insect meals on a smaller scale and ensuring year-round egg production. The Chapter also covered the necessary substrates, tools, and equipment for each stage and provided guidance on post-harvest processing of BSFL or BSFP.

Chapter 3 examined the influence of pupation substrate types (sand, wood shavings, topsoil, vermiculite, spent wheat middlings, and potting soil), moisture levels (0, 10, 20, 30, and 40%), and manual compaction on pupation and adult stages, as well as their mortality levels.

In Chapter 4, I report the issues of pathogens and parasites in the mass rearing of BSF, with specific focus on a case of poultry red mite infestation observed during this thesis research.

Chapters 5 and 6 presented digestibility assays on meals from two developmental stages namely BSFL and BSFP meals, for broiler chickens. These chapters reported experiments measuring the apparent metabolizable energy (AME) and ileal amino acid digestibility. The AME and amino acid digestibility of these meals were compared to SBM, the traditional protein source in poultry diets.

Chapter 7 presented a general discussion of the experimental results. This chapter addresses the major findings and draws some conclusions from the data generated. The potential for the upscaling of BSF bioconversion technology for small and medium-scale production, and optimising non-feeding stages (pupal and adult emergence) were discussed. This chapter also acknowledged and discussed limitations inherent in the studies and potential directions for future research.

Chapter 2: General Materials and Methods

(Colony Establishment and Maintenance)

Abstract

This chapter provide a detailed overview of the establishment and maintenance of a black soldier fly (BSF; *Hermetia illucens*) colony as the basis for the experiments carried out in this thesis. The knowledge presented herein was drawn from the production of 450 kg of BSF larvae (BSFL) and pre-pupae (BSFP). The chapter covered technical aspects of each production stage, including factors that influence egg yield, BSFL growth, BSFP and pupal development, and adult colony care to promote longevity. Furthermore, the chapter presented harvest and cleaning methods for BSFL and BSFP. Additionally, it explored waste management within the colony, highlighting novel applications for frass (colony residue) and outlining the daily activities required for optimal production. Post-harvest processing of BSFL and BSFP, including drying techniques and grinding of insect meals, is also discussed.

Keywords: Egg production, larvae and pre-pupae yields, pupation substrates, insect meals.

2.1 Introduction

The production process of Black Soldier Fly (BSF) is sensitive to environmental conditions such as temperature, humidity, and mating. For instance, the amount of organic matter that a larva consumes per day can range from 25 to 500 mg, depending on factors such as the type and shape of substrate, temperature, moisture, larval density, and ventilation conditions in the rearing unit (Makkar *et al*., 2014; Surendra *et al*., 2016). In unfavourable conditions, the larval growth cycle can extend by months (Makkar *et al*., 2014; Oliveira *et al*., 2015).

In large-scale BSF production, optimisation is more readily achieved by controlling environmental conditions with automated processes (Joly and Nikiema, 2019). In contrast, small-scale operations, often household-sized and processing on-site waste, are typically managed by enthusiastic individuals who lack the knowledge required for effective rearing and processing of BSF (Joly and Nikiema, 2019). Previous studies have explored select aspects of laboratory scale BSF rearing (Tomberlin *et al*., 2002; Diener, 2010; Gobbi, 2012; Oonincx, 2015).

This chapter aims to provide comprehensive methods to optimise mass rearing of BSF for small-scale operations based on findings from production of 450 kg of BSF larvae and pre-pupae during the course of the doctoral thesis. It includes year-round rearing methods focusing on egg production in a glasshouse environment, as well as feeding, harvesting, and processing techniques for the larvae and pre-pupae of BSF. Post-harvest processing of BSFL and BSFP, such as drying techniques, grinding, and processing insect meals, is also addressed. Experiments were also conducted to validate the methods presented.

Further details of the production process are provided in the Appendices A to H.

2.2 Origin of the colony

The colony was initially established with 3000 BSFL at two weeks of age, sourced from a laboratory colony at Massey University, Manawatu campus. Prior to being bred in the laboratory, the colony originated from three distinct locations in New Zealand: Thames (37°03′S, 175°52′E), Pukenui (34°82′S, 173°15′E), and Palmerston North (40°36′S, 175°64′E). These larvae were reared to adulthood under controlled conditions, and their eggs were subsequently harvested to maintain the colony cycle within the laboratory setting.

2.3 Adult rearing unit

The adult colony was maintained year-round in a glasshouse in a semi-outdoor environment (Figure 2.1). The glasshouse accommodated five cages, each cage measuring 2.30 × 1.82 × 0.92 m. The cages were made of a wooden frame covered with a polyester mesh (1 mm) (Figure 2.2). The glasshouse environment was controlled to maintain a temperature of 25 to 28 °C during winter and 28 to 32 °C during summer. The humidity was held between 40 to 60% with a humidifier. In the summer months (December to February), the glasshouse microclimate was drier than in winter. For this reason, additional moisture was supplied by spraying the greenhouse floor and adult cages with water mist twice a day. The roof of the glasshouse was also covered with shade cloth to lower the heat.

Figure 2.1 Glasshouse where the adult colony of the black soldier fly (BSF; *Hermetia illucens* L.) was maintained in a semi-outdoor environment.

Adults need quality sunlight for successful mating (Tomberlin *et al.*, 2002; Zhang *et al.*, 2010). The BSF adult colony was kept and bred in this facility using natural light supplemented with artificial light. The sun was the primary light source for adult breeding, with two JM GREEN breeding LED lights (150 watts each) to supplement natural light on cloudy days (Figure 2.3). The breeding light was controlled by a timer and light sensor consistent with the natural light cycle.

Figure 2.2 The adult colony of the Black Soldier Fly (BSF; *Hermetia illucens* L.) was housed in wooden-framed cages covered with 1 mm mesh. These cages were subjected to natural light, supplemented by two artificial breeding lights (JM GREEN breeding LED light), during the winter months. The cages were locally constructed.

Figure 2.3 JM GREEN Breeding LED Light (150 watts) utilised to ensure optimal lighting conditions for the adult colony of the Black Soldier Fly (BSF; *Hermetia illucens* L.) during the winter months.

The survivability of adult flies is known to vary based on the rearing condition (i.e., temperature, moisture content) and the food source provided (Bertinetti *et al*., 2019; Salam *et al*., 2022).

In this facility, adult male and female BSF were exposed to three different food treatments: a control (no added substances), water with sugar cubes, and milk, at 3-day intervals under specific temperature and humidity conditions. The results revealed that milk markedly extended the survival of the flies, followed by the water with sugar cubes, compared to the control group (Figure 2.4A and B). However, as the BSF adults aged, they became increasingly susceptible to mortality regardless of the treatment. The survival of BSF was higher with the milk treatment, particularly at advancing age. Due to the cost and tendency to ferment, milk was not chosen as the preferred food source. Instead, each breeding cage was equipped with at least five water bowls, each covered with a lid containing a small incision through which tissue paper or cloth was inserted to allow the flies to drink without the risk of drowning (Figure 2.5). The water containers were refilled and replaced every week, and several sugar cubes were placed in each bowl.

As the adults aged, both genders became more vulnerable to mortality regardless of the treatment. However, the survival of BSF was notably higher with the milk treatment, particularly as the flies aged, but milk was not chosen as the preferred food source due to its cost and tendency to ferment. Instead, it was decided to proceed with sugar cubes and water,

Each breeding cage was equipped with at least five water bowls, each covered with a lid containing a small incision through which tissue paper or cloth was inserted to allow the flies to drink without the risk of drowning (Figure 2.5). The water bowls were refilled and replaced every week; several sugar cubes were placed in each bowl.

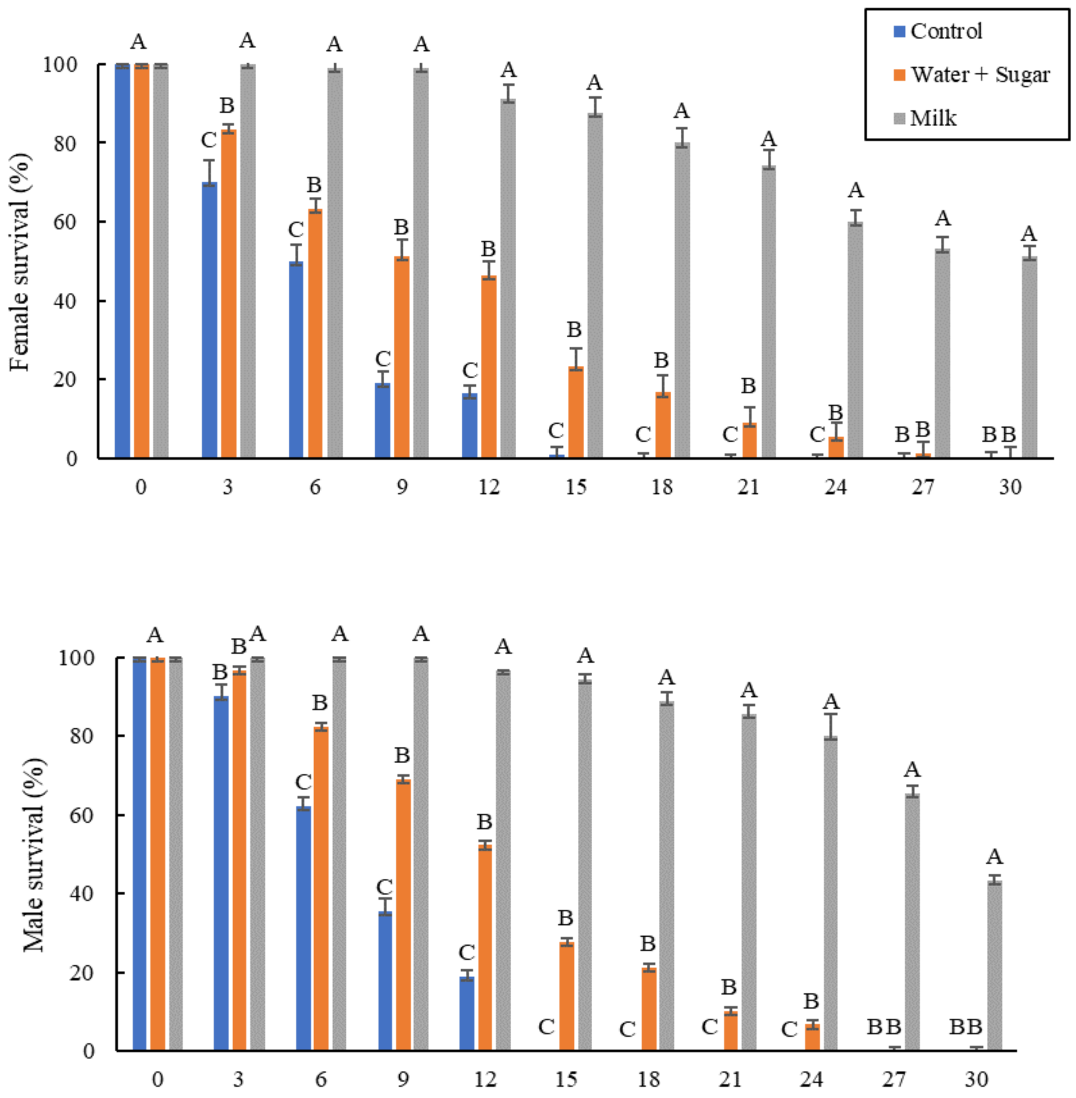

Figure 2.4. Survival rates for female and male of adult black soldier fly (BSF; *Hermetia illucens* L.) under varied food regimes. Values represent the mean of percentages, with error bars indicating the standard error (SE) based on a sample size of n = 30. Different letters (A, B and C) indicate significant differences between treatments ($P < 0.05$).

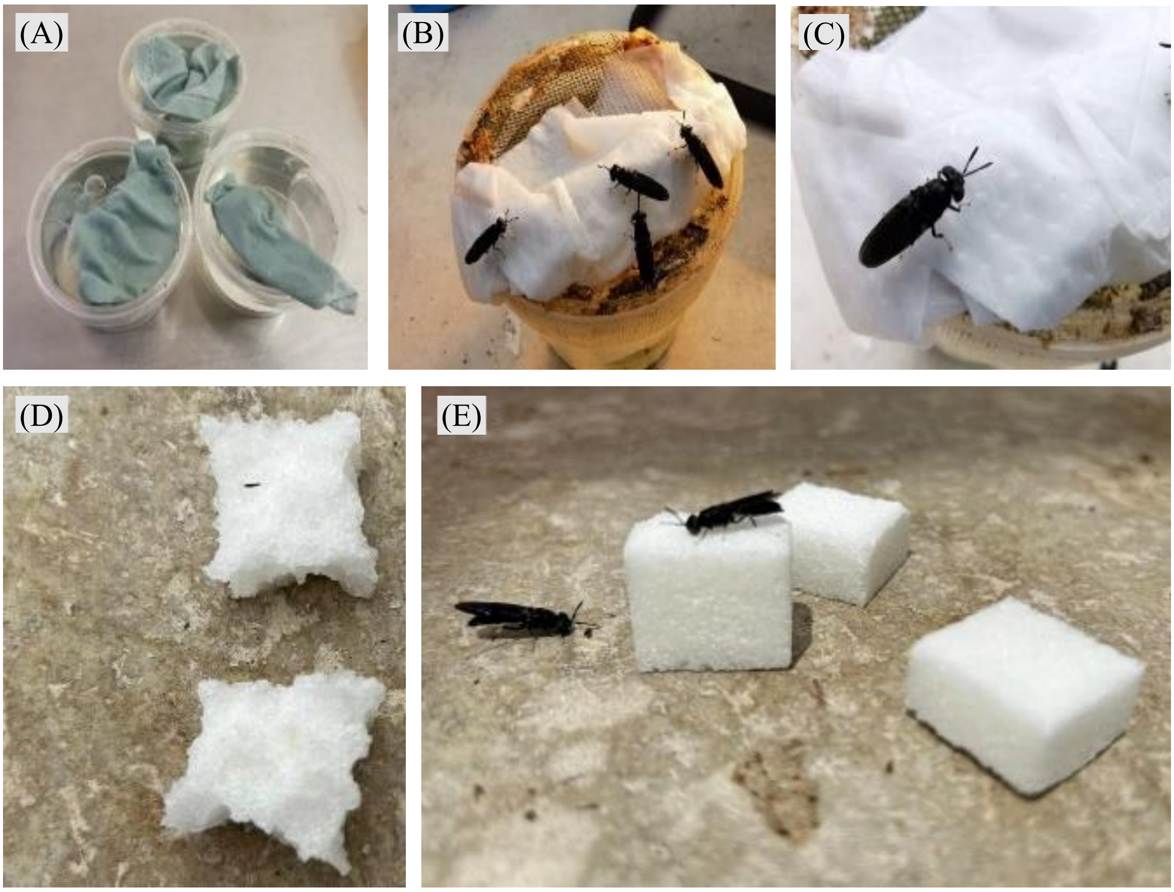

Figure 2.5 (A, B and C) Water bowls with a lid containing an incision fitted with tissue paper or a piece of cloth for adults of the black soldier fly (BSF; *Hermetia illucens* L.) to drink. (D) an image of consumed sugar cubs by the flies. (E) fresh sugar cubes are also placed inside the breeding cages to give the adults extra energy for mating and extended longevity.

2.3.1 Adult emergence and mating

According to Dortmans *et al*. (2017), two methods exist to establish breeding cages. One is to release all the flies simultaneously during the cage setup, while the other involves releasing flies on multiple occasions within the same cage. For this study, the second method was employed, wherein 800 pupae close to the emergence of flies were initially placed in each cage. Weekly additions of adult flies were made to maintain a density of 800 to 1000 individuals per cage and compensate for natural adult mortality. This method allowed for sustainable egg harvests lasting 2 to 4 months, reducing the need for multiple breeding cages and extra labour (Figure 2.6).

When adults are ready to emerge, the puparia split along the interior dorsal aspect allowing emergence. The emerging adults do not fly right away, and it takes them 2 to 3 minutes for the wings to inflate in preparation for flight (Figure 2.7).

The sex of the BSF fly can usually be identified once they become reproductive adults (Figure 2.8). The posterior end of the female body is split like a scissor or fork shape (Figure 2.8A), and the male has a rounded body end with a claw shape (Figure 2.8B). The genitalia of the adults are tubular and expand during mating and for females when they deposit their eggs (Figure 2.8E and F). In this facility, the ratio of males and females was not controlled. However, it was observed that males generally emerged in the first 1 to 2 days, followed by females. Also, the weight of males was lighter, and the length was shorter than those of females (Table 2.1).

Two to three days following emergence, adults ready. The mating starts with lekking behaviour by the males (Hoffmann *et al*., 2021). The lekking behaviour is observed when several males (4 to 12) group together in a mating display by bending their abdomen onto one another while fanning their wings (Figure 2.7A). They can also fly in this aggregate position (Giunti *et al*., 2018). Sometimes, the aggregation bundle includes males only or may contain females. The successful male and female mate initially and couple descend to a resting spot to complete the mating process (Figure 2.9).

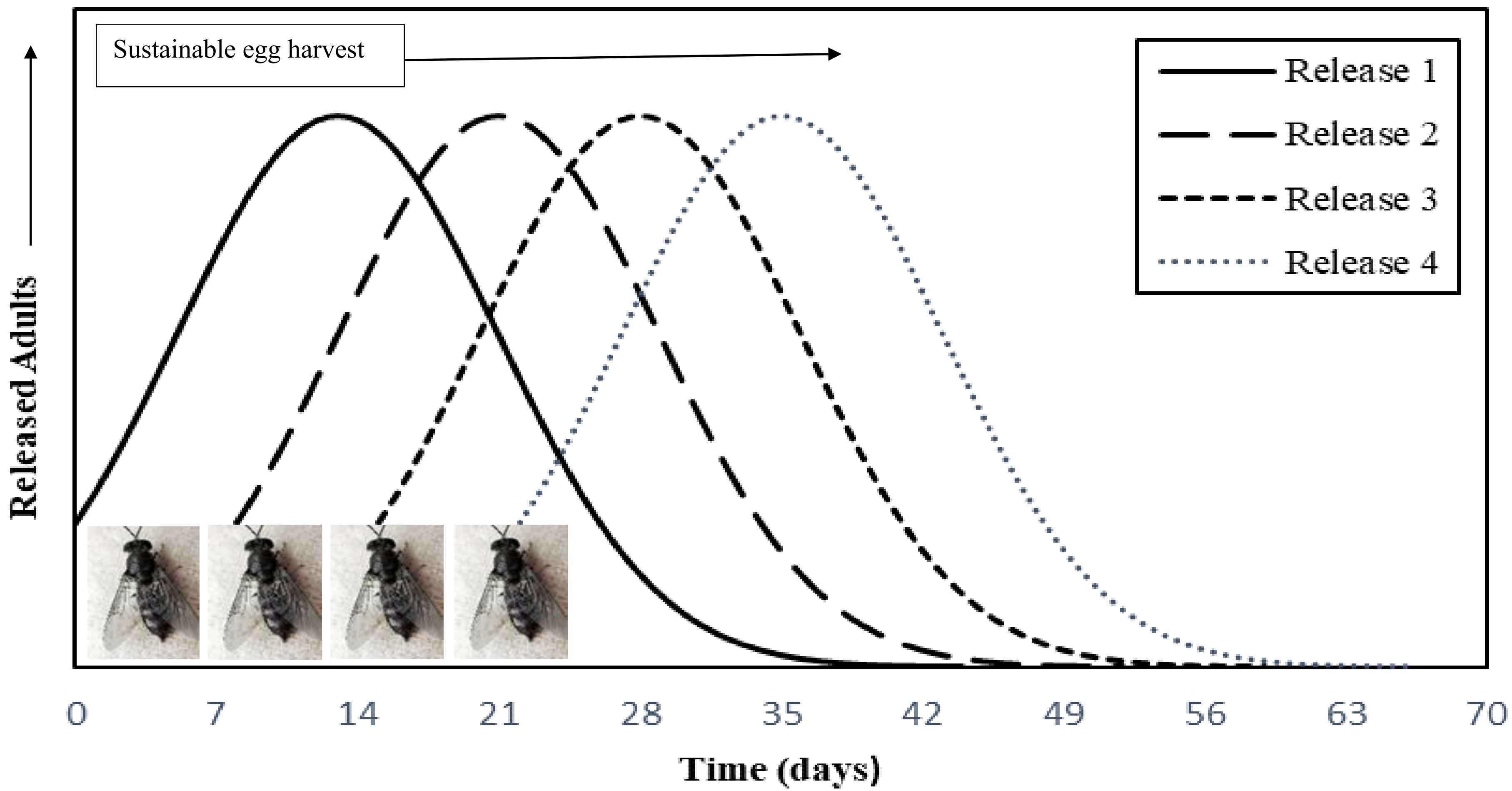

Figure 2.6 Following the initial setup of a breeding cage, adults of the black soldier fly (BSF; *Hermetia illucens*L.) were released on a weekly basis to ensure a consistent and sustainable egg harvest, extending for a duration of 2 to 4 months.

Figure 2.7 (A) The black soldier fly (BSF; *Hermetia illucens* L.) puparium splits open in the mid-dorsal aspect creating a T-shape spilt. (B)The head of the puparium falls off, allowing the new adult to emerge. (C) A pre-mature adult image shows the head started to form. (D) A newly hatched female adult. (E) Adult wings inflated a few minutes after emergence.

Table 2.1 Characteristics of adult male and female black soldier fly (BSF; *Hermetia illucens* L.)

Adult characteristics	Mean ± SD*
Male weight (mg)	42.8 ± 0.01
Female weight (mg)	80.1 ± 0.11
Male length (mm)	13.0 ± 1.5
Female length (mm)	15.4 ± 1.4

*Values are mean ± standard deviation, n = 21.

After successful mating, fertile females search for sites near a food source that is dry, shady, and safer to deposit eggs (Figure 2.10). The females are attracted to the smell of 'attractants' such as decaying organic substrates. A range of substrates can be used as attractants, including slurry of spent wheat middlings (wheat milling by-products, locally known as broll) from the larval nursery, dead adult flies, kitchen wastes, and BSFL frass (cuticle casting or excreta). These substrates must be fermented (5 to 10 days) to produce a strong odour to attract the fertile females to lay. In the present setup, two attractants, namely kitchen wastes and spent wheat middlings with dead flies, were tested. The experiment was conducted by placing each cage in an environment where two different attractant boxes were positioned at opposite ends. The flies were allowed to naturally interact with these attractants within the cage. The second attractant (spent wheat middlings with dead flies) showed a higher egg yield (2.10 ± 1.91 g; n = 10) and a greater number of egg clusters (4.60 ± 1.17 g; n = 10) (Table 2.2). Hence, it was used for the rest of the production and made by mixing dead adult flies (~50 per container) with spent wheat middling and tap water at a ratio of 1:6 by volume. Each cage was provided with one attractant box (40 ×25 ×33 cm) covered with mesh to prevent contamination and keep the eggs clean above the attractant box. Above the attractant box, egg traps were placed close to the fermented substrate boxes (Figure 2.11). The attractant box was monitored weekly to ensure microbial activity produced a strong smell to attract fertile females. When necessary, the boxes were replaced or topped up with a fresh attractant. The presence of bubbles accompanied by a strong unpleasant smell indicated fermentation and an active attractant.

Furthermore, another experiment was conducted with adult BSF to examine the influence of lighting on egg production. Some cages in a glasshouse were exposed only to sunlight, while others were supplemented with artificial light on cloudy days. Light sensors were activated on cloudy days to ensure the cages received adequate illumination. The use of artificial lighting on cloudy days resulted in a 492% improvement in egg yields. In contrast, during sunny days, natural sunlight led to a 2052% increase in egg yields compared to artificial lighting (Table 3.2).

Table 3.2 Egg characteristics and egg yields of black soldier fly (BSF; *Hermetia illucens* L.) under different conditions of attractants and breeding light

Egg yield with different attractants	Mean ± SD	*P* Value
Fermented kitchen waste (g)	0.18 ± 0.16^{B}	0.005
Fermented consumed wheat middlings + dead flies (g)	2.10 ± 1.91^{A}	
No. of egg clusters from the fermented kitchen waste	2.40 ± 0.97	
No. of egg clusters from consumed wheat middlings + dead flies	4.60 ± 1.17	
Sunlight only (during cloudy days)	0.18 ± 0.22^{B}	0.005
Sunlight + LED light (150 watt each)	1.07 ± 0.66^{A}	
Sunlight only (during sunny days)	4.02 ± 1.49	0.005
Sunlight + LED light (150 watt each)	0.19 ± 0.23	

^{AB} means in a column with different superscripts are significantly different ($P < 0.05$). Values are mean ± standard deviation; n = 21.

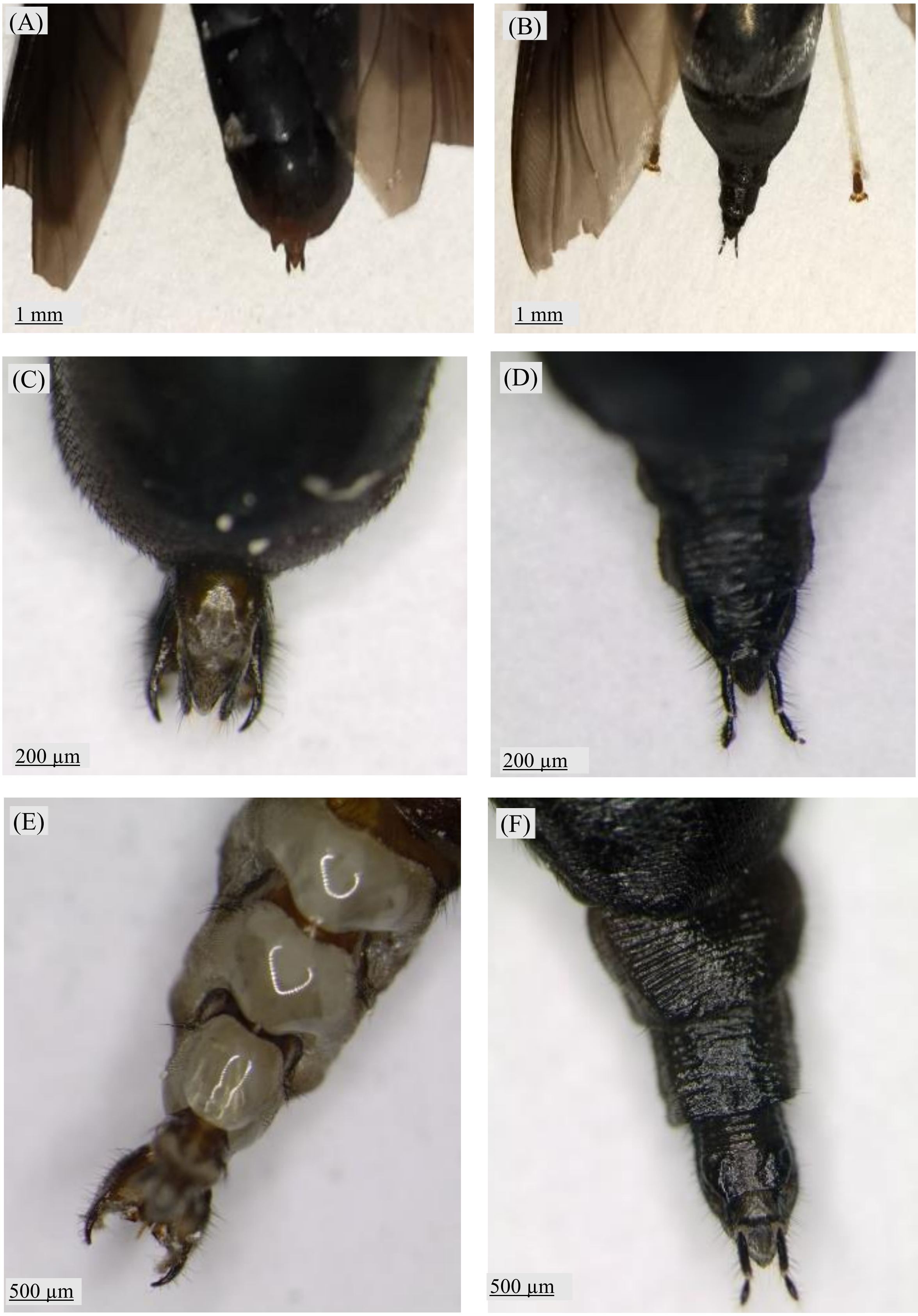

Figure 2.8 Images of the black soldier fly (BSF; *Hermetia illucens* L.) show the difference between males and females. The adult male genitalia (A) show rounded end and claw-shaped genitalia, and a female adult (B) shows a pointed, fork-shaped end. C. Microscopic image of male genitalia. D. Microscopic image of female genitalia. E. Microscopic image of tubular male genitalia in an expanded position. F. Microscopic image of tubular female genitalia in an expanded position (porrect female ovipositor).

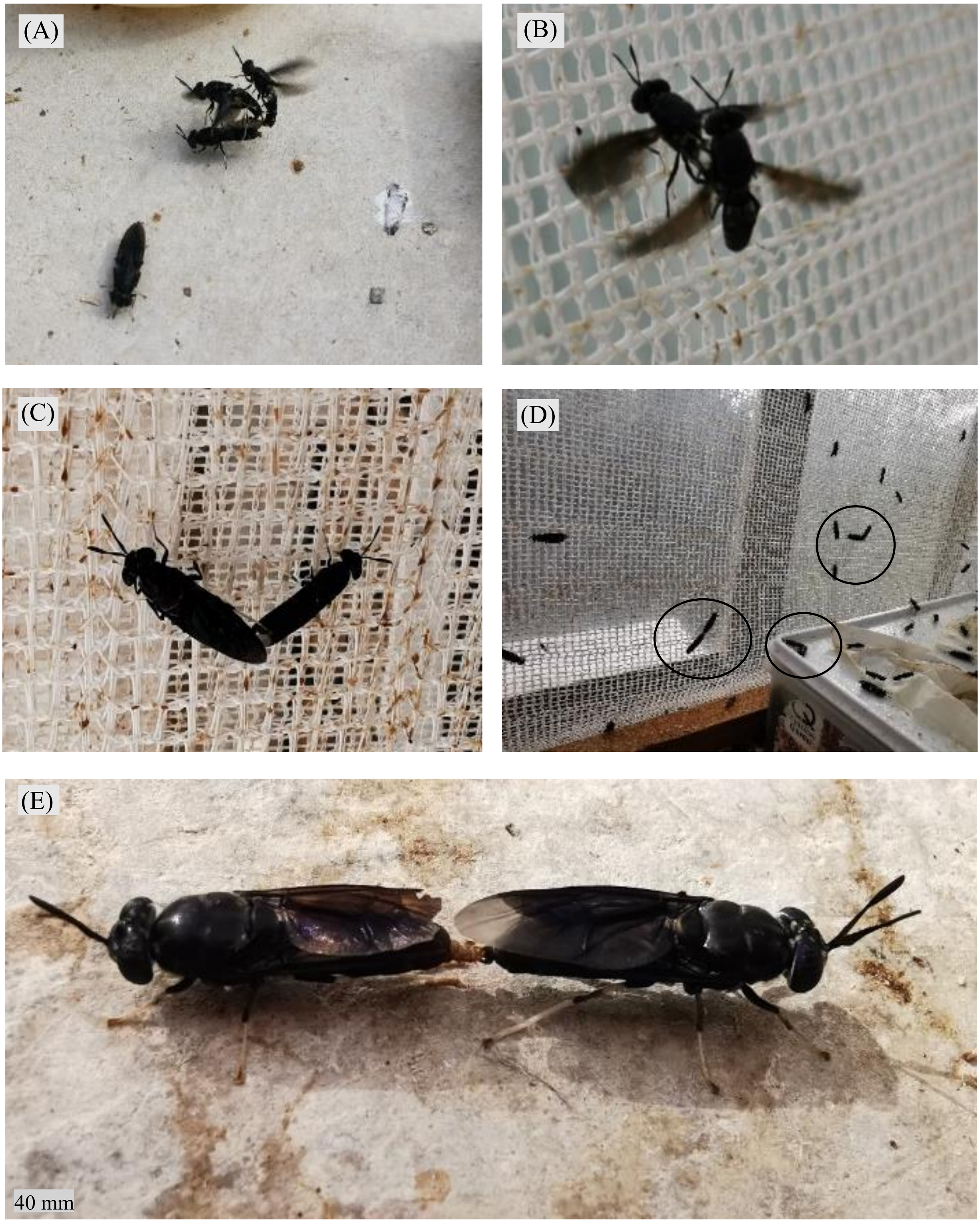

Figure 2.9 Mating in the Black Soldier Fly (BSF; *Hermetia illucens* L.) transpired two to three days following emergence. (A) Male individuals vied for a single female partner, while (B) successful mating ensued in mid-air. (C) and (D) depict varying mating positions. (E) The copulated pair descended to a resting location to finalise the mating process.

The egg traps can be made from corrugated cardboard, wood slices, or similar shapes, providing small gaps (cracks) where females can lay eggs. Several egg traps were used in the current setup, including coarse wood slices, corrugated cardboard, smooth wood (medium-density fibreboard), and plastic balls. The gaps in all traps were 1 to 2 mm (Figure 2.12).

An experiment was conducted to test the effectiveness of three different types of egg-laying traps for BSF: coarse wood slices, corrugated cardboard, and smooth wood. The traps were designed to attract female BSF for egg-laying and were placed directly above an attractant box to draw the insects to the traps. The setup involved placing each type of trap above the attractant box, which contained BSF attract. Eggs were collected from each trap daily over a four-week period in total 28 time. The results showed that the coarse wood slices were the most effective, yielding the highest average egg mass (0.91 ± 0.69 g; n = 28). This success is likely due to the coarse wood's ability to retain the odour and symbiotic microbes from the BSF eggs, which encouraged females to return and continue laying eggs in the same location. Additionally, the rough texture of the wood may have provided a more accessible surface for the females to glue their eggs, enhancing the trap's effectiveness (Table 2.3).

Therefore, the wooden traps used in this facility. It was made with four coarse wood slices (2 ×4 ×1cm) separated with pushpins. The pushpins created a small gap of about 1 to 2 mm between the wooden sheets, allowing space for egg clusters. The wood slabs were held together by a rubber band at each end of the bundle.

Figure 2.10 An image of a female of the black soldier fly (BSF; *Hermetia illucens* L.) depositing eggs into a wooden trap.

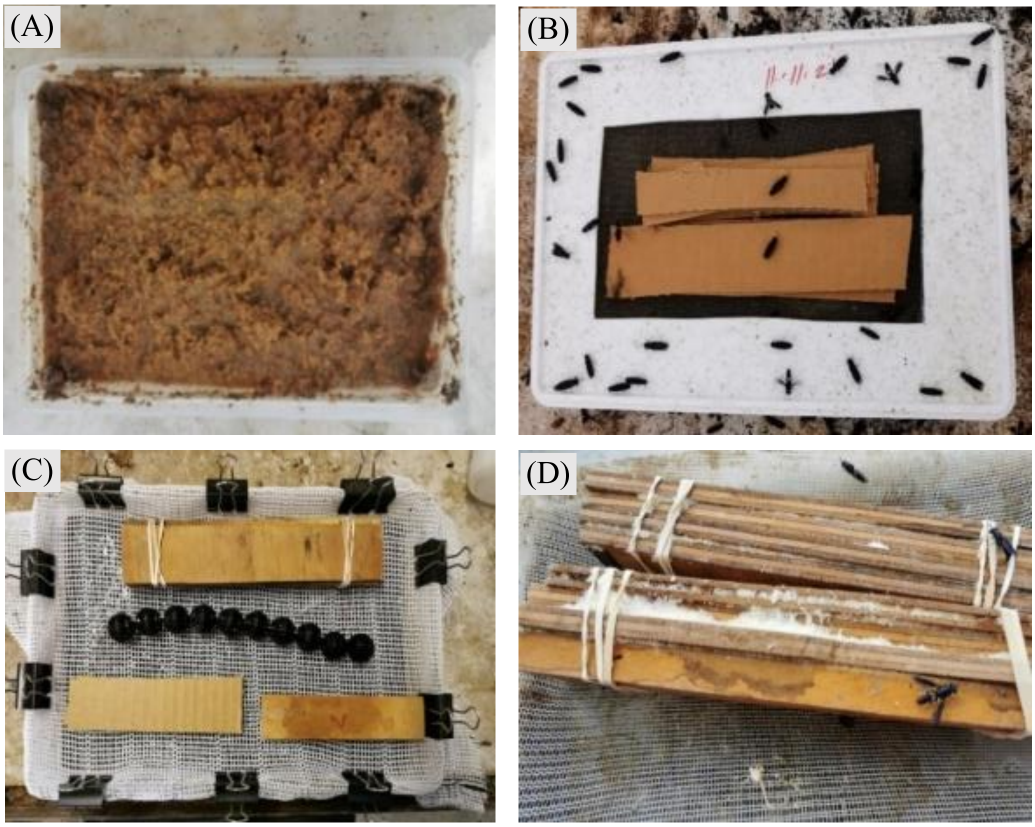

Figure 2.11 (A) Attractant box housing fermented larvae frass for female Black Soldier Fly (BSF; *Hermetia illucens* L.). (B), (C), (D) Different egg traps constructed from corrugated cardboard, wood slices, and plastic balls.

Table 2.3. Egg yield (g) of the black soldier fly (BSF; *Hermetia illucens* L.) with different egg trap materials*

	Mean ± SD	*P* Value
Wooden slices	0.91 ± 0.69^{A}	<.0001
Corrugated cardboard	0.15 ± 0.22^{B}	
Smooth wood (Medium-density fibreboard)	0.32 ± 0.36^{B}	
Plastic balls	0.04 ± 0.11^{B}	

^{AB} means in a column with different superscripts are significantly different ($P < 0.05$). Values are mean ± standard deviation; n = 29.

Egg traps must be emptied periodically, especially during summer when egg production is at its highest because females tend to lay elsewhere when the traps are full. For instance, it was observed that females laid their eggs in cage corners, under the attractant box or outside the cage on the netting (Figure 2.13). This behaviour can cause eggs to be lost, dried or contaminated.

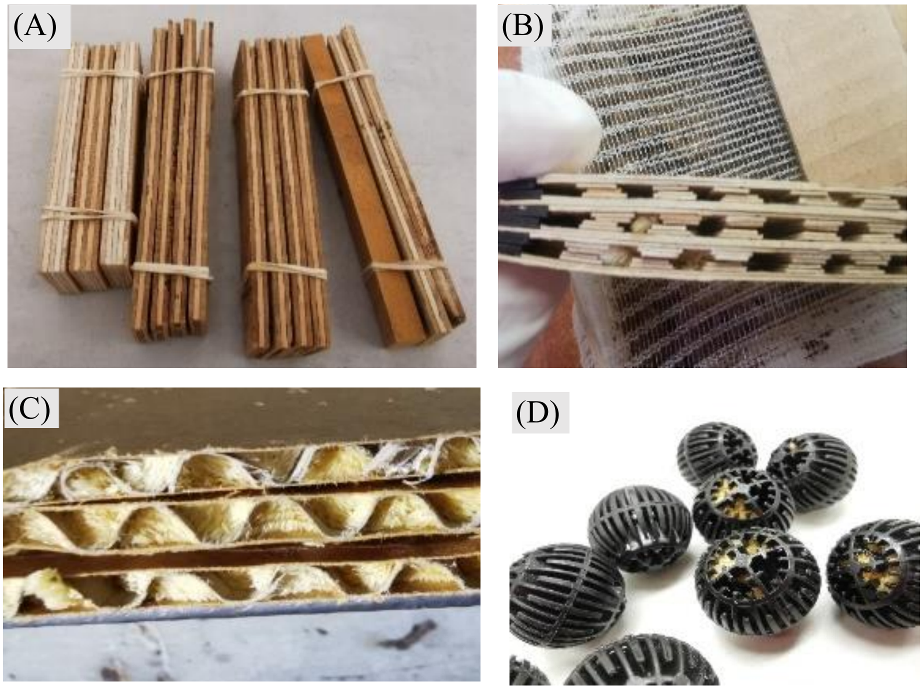

Figure 2.12 Different egg traps for female black soldier fly (BSF; *Hermetia illucens* L.) to deposit eggs. (A) Wood slices, (B) cardboard, (C) corrugated cardboard, and (D) grooved plastic balls.

Figure 2.13 A fertile female black soldier fly (BSF; *Hermetia illucens* L.) is laying her eggs outside the egg trap (nylon netting of the cage).

2.3.2 Egg deposition and harvest

Fertile females laid eggs in clusters (15.1 ± 4.2 mg; n = 21) inside the egg traps, with the eggs glued to each other. The number of eggs in each cluster varied (686 ± 191 eggs; n = 21). For three clusters, the number of eggs was counted separately, and the average egg weight was calculated by dividing the total weight by the number of eggs (Table 2.4). The average egg weight based on these three clusters was 0.022 ± 0.01 mg.

Table 2.4 Characteristics of black soldier fly (BSF; *Hermetia illucens* L.) eggs.

Parameter	Mean ± SD	n
Individual egg weight (mg)[1]	0.02 ± 0.01	3
Egg cluster weight (mg)	15.1 ± 4.20	21
Number of eggs per cluster	686 ± 191	21
Fertility (%)[2]	91.2 ± 10.1	15

[1]Egg individual weight was calculated by dividing the total weight by the egg number. [2] Egg fertility percentage was calculated by dividing the number of fertile eggs by the number of whole eggs × 100; SD: standard deviation.

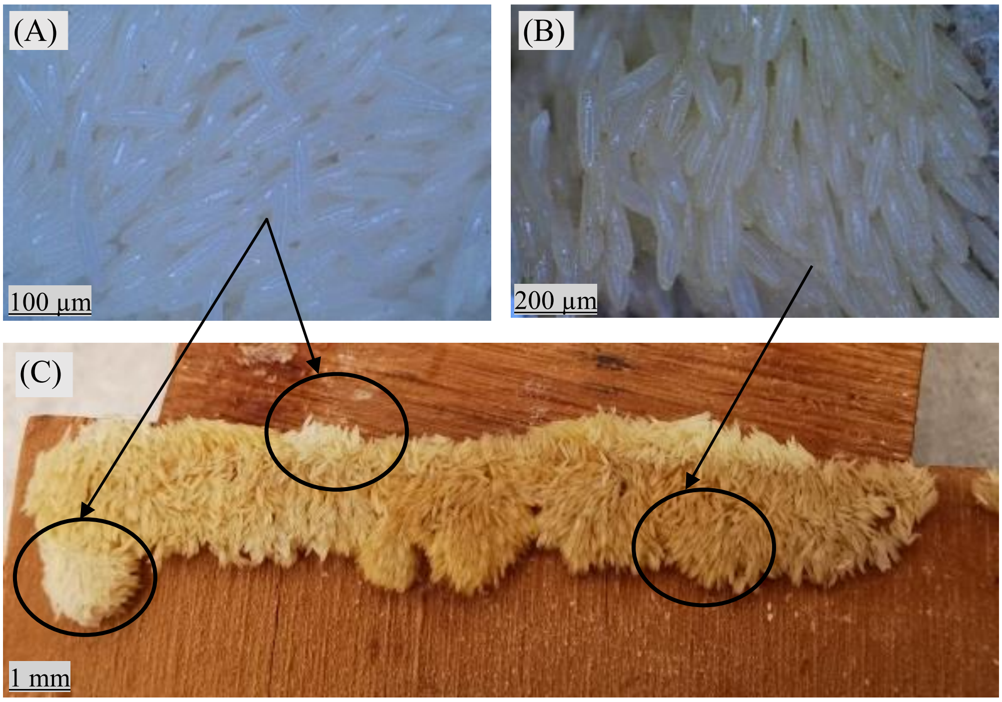

Figure 2.14 The colour of the black soldier fly (BSF; *Hermetia illucens* L.) eggs progressed from pale white (A) when freshly laid to golden yellow (B) two days after laying, with the colour changing to light yellow upon hatching.

In this facility, eggs were handled differently based on the type of trap used. In the case of wooden traps, eggs were carefully removed with a sterile blade and placed on clean filter paper in clean Petri dishes. On the other hand, the eggs laid in corrugated cardboard and plastic balls were not emptied to reduce the handling of the eggs and the risk of causing damage. Instead, they were incubated intact. All harvested eggs were then incubated in an enclosed box (15 ×26 × 21 cm) in a controlled temperature (25°C) room environment with moist tissues or a layer of water (~2 mm) for 2 to 4 days to allow the embryo to develop. The layer of water was inside the box, but the eggs were kept dry inside the Petri dish (Figure 2.15). During this time, the eggs develop two dots known as "eyes" and the embryo can be identified with a light microscope (Figure 2.16). The eggs must be kept warm (28 to 30°C) and at high moisture (70 to 90% RH) for this development to occur.

As soon as fertile eggs were identified under a light microscope, the eggs were moved to a rearing container containing wheat middlings and water at a 1:2 ratio (Figure 2.17) for a week.

The boxes remain covered from day one to day seven to maintain the moisture content of the diet that facilitates the hatching process.

It is worth mentioning that elevating the eggs above the hatching substrates is recommended to prevent the mould and improve hatching. Furthermore, a dry layer of diet was spread on top of the moist diet to control mould growth. In addition, this dry layer keeps the neonates from moving away from the diet during the first few days. The hatched eggs can be identified from their colour and the shells left behind (Figure 2.18).

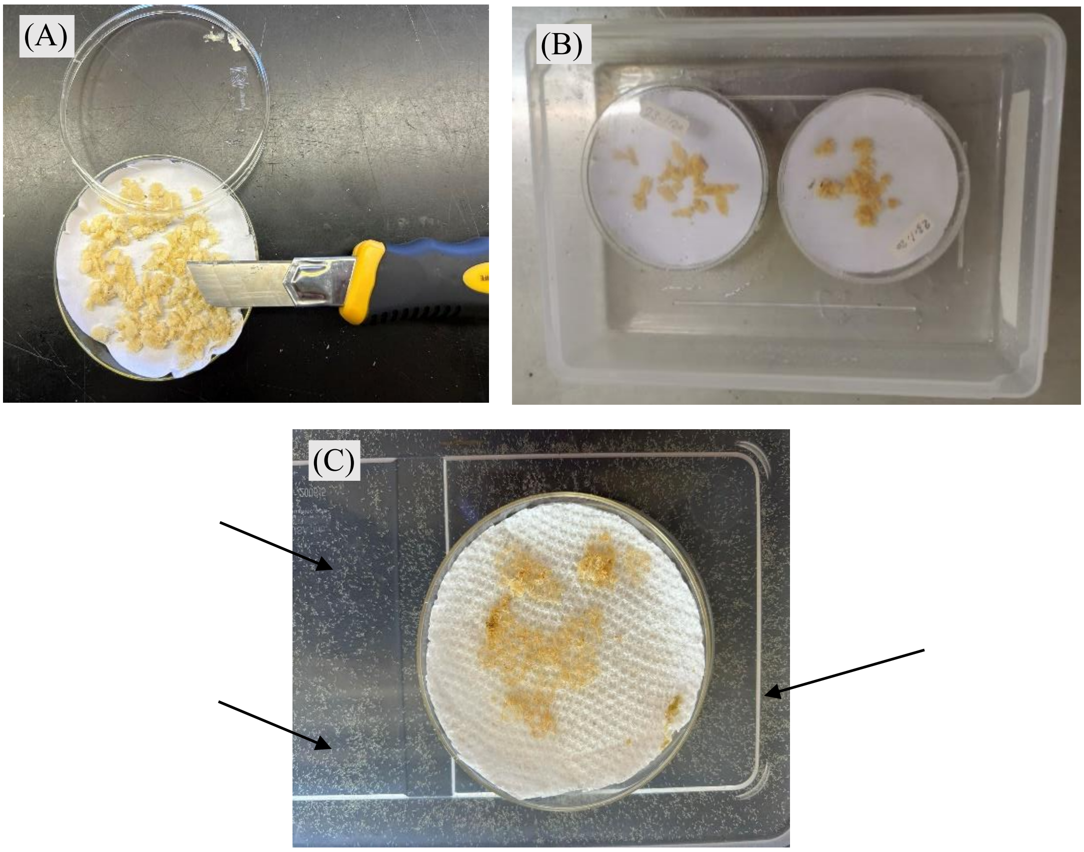

Figure 2.15 (A) Tool used to arrange the eggs on clean filter paper inside a Petri dish. (B) Petri dish containing black soldier fly *(BSF; Hermetia illucens L.)* eggs are placed inside a tray. A 2 mm layer of water was added to the tray to create a microclimate with high moisture (80%) and a warm temperature (25°C), essential for the development of the fertile eggs. The eggs remain dry inside the Petri dish. (C) This setup provides the optimal conditions for the eggs to develop into neonates, indicate newly hatched neonates seen in the water.

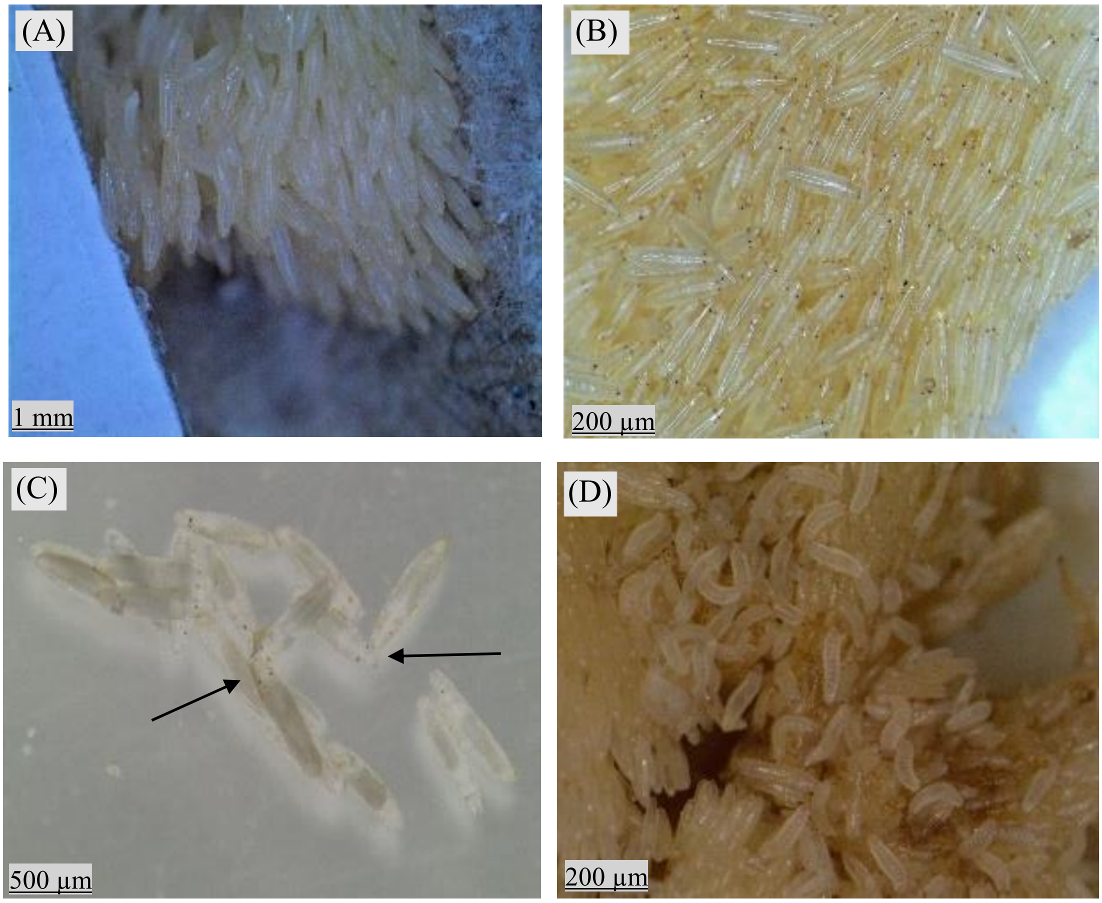

Figure 2.16 Egg development of Black Soldier Fly (BSF; *Hermetia illucens* L.); (A) A non-fertile egg cluster. (B) A fertile egg cluster. (C) Microscopic view of fertile eggs under a light microscope; arrows point to the ocellus (eyes). (D) Newly hatched larvae observed under a light microscope.

Figure 2.17 Black soldier fly (BSF; *Hermetia illucens* L.) eggs were extracted from wooden traps and subsequently incubated in Petri dishes, alongside corrugated cardboard traps. These setups were positioned above the diet, with a layer of dry feed atop to deter the growth of mould.

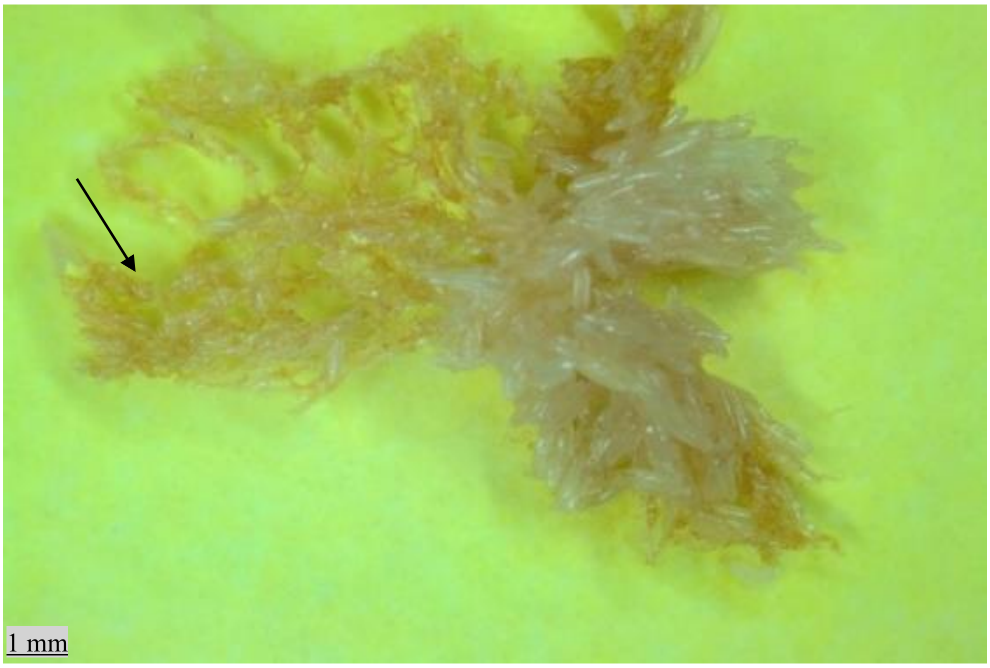

Figure 2.18 The image shows a cluster of black soldier fly (BSF; *Hermetia illucens*L.) eggs. Hatched eggs are empty and translucent, while non-hatched eggs are opaque and intact. Arrows point the remaining eggshells from hatched eggs.

2.4 BSFL and BSFP rearing units

The BSFL and BSFP were reared in a controlled environment. During first week post-hatch, the BSFL were placed in small trays (40 × 25 × 33 cm). After the first week, the content of each tray was transferred to a larger plastic tray (80 × 60 × 25 cm) under the same temperature of 28 ± 2°C and RH 60 ± 10%. Finally, the trays were stacked uncovered on shelves (Figure 2.19). The rearing unit had two humidifiers and air conditioning to maintain the required temperature and relative humidity.

2.5 Feeding substrates and feeding regime

2.5.1 Larval stage

The BSFL were initially fed wheat middlings during week 1, followed by minced food waste *ad libitum*. The food waste supplied to the BSFL throughout this period came from the Massey University staff and student cafeterias. The food waste included mostly cooked food such as rice, pasta, bread, meat, vegetables, and coffee grounds. The waste contained some uncooked organic wastes such as fruit peels, raw vegetables, and damaged fruits. The food waste was mixed in a Kenwood 1440w mincer using a coarse screen (8 mm) before feeding and the moisture content of the waste was between 80 to 90%. No additional water was added (Figure 2.20).

Figure 2.19 Indoor rearing facility for larvae and pre-pupae of black soldier fly (BSF; *Hermetia illucens* L.).

Whenever there was a shortage of restaurant wastes, the colony was fed on wheat-middlings and wastes such as distiller's dried grains with solubles (DDGs), chicken feed, and chicken feed ingredients (e.g., soybean meal, wheat, corn). Detailed information regarding the various diets utilised throughout the study, as well as their chemical composition, can be found in Appendix D.

The week 1 intake was standardised at 0.027 to 0.033g of dry feed per larva per day. Each tray started with 0.1g of fertile eggs (~300 to 3,700) and 700g of wheat middlings mixed with 1400g of water, and the diet was offered once daily.

After week 1, the BSFL were fed twice a week. The feeding rate was determined by using two methods:

1. Calculating the feed offered using the total number of the BSFL and individual BSFL feed intake as follows:

$$\text{Diet offered (g)} = \text{larva daily feeding rate DM (g)} \times \text{Larvae number} \times \text{Time (1day)}$$

Larval daily feeding rate was fixed at 100 mg on a dry matter basis for grains and 200mg for restaurant and kitchen wastes.

The daily feeding rate can vary significantly based on the diet type (i.e., vegetables, meat, grains), processing condition (i.e., cooked, raw, fermented), and particle size (i.e., minced, large particles). Previous studies reported that the daily BSFL intake varied from 100 to 200 mg of feed per larva daily, depending on the diet type (Diener *et al*., 2009; Parra Paz *et al*., 2015; Permana and Putra, 2018). Diener *et al*. (2009) reported that 100 mg chicken feed per larva daily was optimum for growth and provided a nutrient-rich pre-pupa in a short time. On the other hand, Permana and Putra (2018) reported 200 mg spent coffee grounds resulted in the fastest development rate for the larva and the highest pre-pupa biomass.

2. Visual analysis approach (also known as *ad libitum*) in which the colour and smell of consumed diet are used as an indicator to determine the feeding time (Figure 2.20). When the BSFL are offered wheat middlings, the colour of the residual diet was often dark brown, and a smell of ammonia can be recognised. In the case of food waste, the ammonia smell was not present, but the remaining diet was often dark brown. This method is helpful in medium-scale production when the number of BSFL on each tray is substantial and difficult to count.

In this thesis work, the first approach was used for larval feeding experiments, while the second approach was used for producing BSFL in large quantities for insect meals. For example, each tray (80 × 60 × 25 cm) containing 3 to 4 cm BSFL depth was served with 200 to 400 g minced food waste or a mixture of water and wheat middlings. Also, the maximum feed bed height was maintained at 2 to 3 cm above the BSFL. Therefore, before adding the fresh diet, the remaining uneaten feed was removed by sifting or scraping the upper surface. It was observed that the higher the feed bed, the higher the risk of compaction, which hindered the BSFL growth and reduced airflow within the feed, causing mould to grow and inevitably causing larval mortality.

The BSFL are dense, creamy white with a reddish or light brown head. They undergo several moulting processes during their lifetime through six instars (Figure 2.21). The first four instars are similar in appearance but differ in size. BSFL at the 5th instar are light grey to yellowish. The BSFL can reach 27 mm length and 6 mm width (Diclaro and Kaufman, 2009).

The quality and quantity of larval diets significantly affected their weight and development time (Figure 2.22). For example, BSFL fed chicken feed were heavier in weight (208 ± 0.002 mg; n = 80) in the third week, while BSFL fed on spent coffee grounds were lighter (104 ± 0.07 mg; n = 80). However, the weight on the standard diet (wheat middlings) on day one was 0.0014 to 0.0018 mg, and they developed to a weight range between 74 to 111 mg at 7 days old. Between days 18 to 21, the average harvested size ranged from 180 to 226 mg. Detailed information regarding BSFL weight corresponding to different diet groups can be found in Appendix E. BSFL ability of reducing waste and conversion to insect biomass can be found in Appendix F.

2.5.2 Pre-pupal stage

Sixth instar of the BSF, also known as pre-pupae (BSFP), appears very dark with reduced mouthparts. BSFL and BSFP are relatively similar in size but different in colour (Figure 2.23). The BSFP harvest was typically done at 6 to 8 weeks post-hatch, with BSFP weighing between 83 and 150 mg (117.5± 15.7 mg; n = 80).

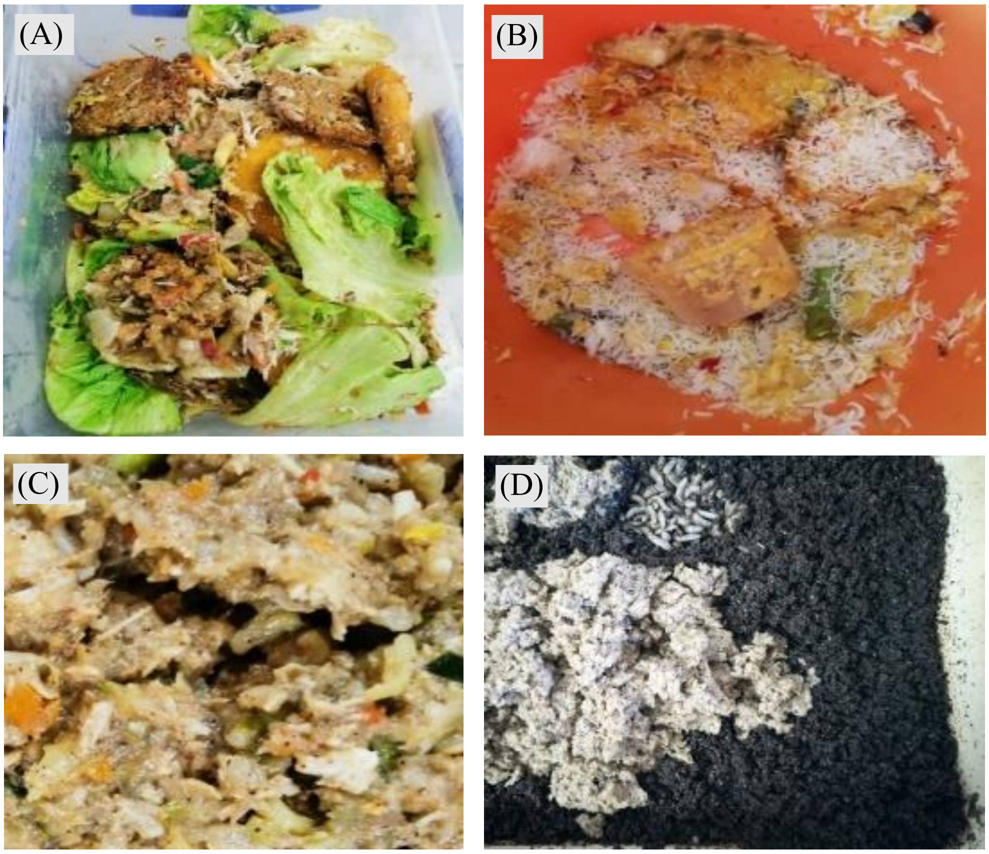

Figure 2.20 Food waste collected from restaurants (A and B) is minced to 8 mm size (C) to feed the larvae of the black soldier fly (BSF; *Hermetia illucens* L.). (D). Shows that excrement is dark in colour, and the fresh feed is light.

Figure 2.21 Different images of larvae of the black soldier fly (BSF; *Hermetia illucens* L.) undergo several moulting from the first instar to the sixth instar.

Figure 2.22 Black soldier fly (BSF; *Hermetia illucens* L.) larvae show different sizes and stages at a similar age.

2.5.3 Pupal stage

The pupal stage begins at the end of the 6th instar, when the final moulting phase for BSFP occurs (Chia *et al*., 2018). Like BSFP, the dark colour identifies the pupae due to dark pigmentation in the skeleton. However, the pupa shows stiffness in the last two segments (Figure 3.24). In addition, other characteristics can identify the pupae, such as their lack of mobility. The pupae are often seen on the surface and remain immobile in preparation for hatching into flies, usually occurring within 7 to 14 days of the pupation period. Unlike the BSFP, the pupae do not seem sensitive to light (Figure 2.24).

2.5.3.1 Pupation substrate preparation

Several materials can be used as pupation substrates for BSF, such as topsoil, potting soil, sawdust, wood shavings, and sand. The current facility used three pupation substrates namely, vermiculite, wood shavings and consumed wheat middlings (also referred to as spent or residual). Each substrate was prepared and used separately.

At the time of collection, exfoliated vermiculite contained 3% moisture. Additional preparation was required for vermiculite by soaking in water for two days to facilitate expansion. Later, it was squeezed and drained for another day to remove excess water. The vermiculite was then oven-dried for several days at 100 ºC to reach ~1% moisture content and adjusted to the desired moisture for pupation at ~10% RH, by adding water directly and mixing it well (Figure 2.25).

After removing the BSFL, residual wheat middlings were sterilised by heating at 150 ºC for 150 minutes to avoid pathogenic microbes (Mubarak *et al*., 2019). Then, the dried residual was placed in plastic bags, sealed, and kept at – 20 ºC for future use as a pupation substrate. Later, the pupation substrate was defrosted, and the moisture content was adjusted to ~10%.

Each pupation box (40 × 25 × 33 cm) contained 700 g of moist vermiculite and 400 g of pupae and was covered with mesh to prevent adults from escaping when they emerged. This ratio of pupation substrate to pupae ensures a minimum depth of 5 cm for the pupae, as recommended for dipteran species (Capinera, 2020). The pupation boxes were stored in the dark at a temperature of 25 to 28°C and 40 to 50% RH for 7 to 14 days. Twelve pupation boxes with vermiculite were monitored to evaluate the duration of pupation. It was found to within the normal pupation for BSF (9.08 ± 0.9 days). When flies emerged in a box, they were either transferred to breeding boxes or released weekly. Additional information on the life cycle and development time of BSF is available in Appendix G.

Figure 2.23 Black soldier fly (BSF; *Hermetia illucens* L.) pre-pupae (PP) are dark brown, while larvae (L) are creamy.

Figure 2.24 Observed in the black soldier fly (BSF; *Hermetia illucens L.*) pupa (P), the last two segments are stiff, while in pre-pupa (PP), they are flexible. Another unique characteristic of pupae is that they translocate to the surface of the substrate.

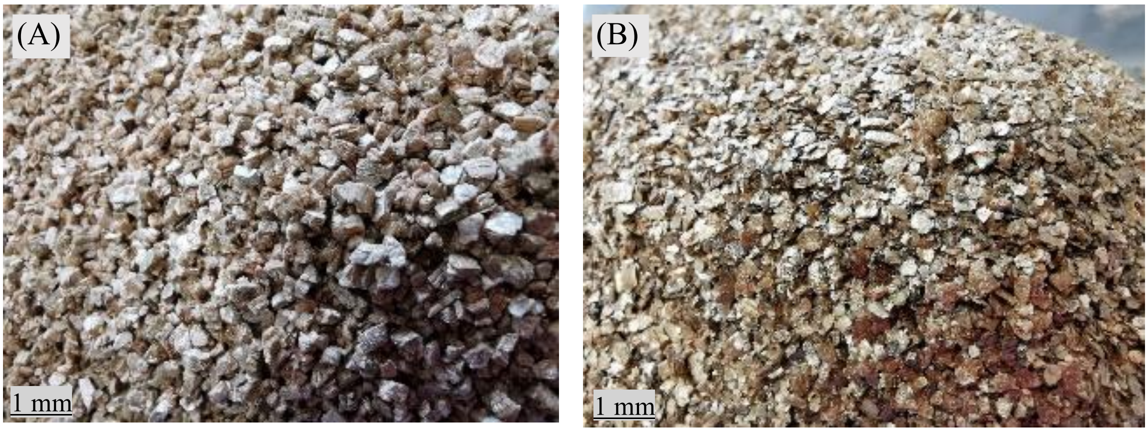

Figure 2.25 (A) Exfoliated vermiculite before soaking and (B) soaked and dried vermiculite to use as pupation substrate black soldier fly (BSF; *Hermetia illucens* L.).

2.6 Frass and residue

In a BSF colony, frass consisting primarily of excrement is obtained after the harvesting of BSFL, while the residue also include frass, uneaten feed, and skin shedding. These two terms, however, are often used interchangeably. Due to the short life cycle of BSFL and BSFP (14 to 28 days), this residue is typically not fully composted. However, after proper composting, such as using earthworms, the residue can be transformed into a valuable soil amendment (see Figure 2.26). Managing this residue can be challenging, particularly in larger BSF rearing facilities.

In this facility, a portion (~2/3) of the residue was used for purposes besides composting.

2 Attractant substrate for adults for oviposition; a portion of residual was used as an attractant after fermentation. The residual was kept in covered buckets with enough water for natural fermentation (one part frass to 4 to 6 parts of water). The buckets were placed in a glasshouse in winter or outdoors in summer and left to ferment naturally, which often takes 7 to 10 days, depending on the temperature.

3 Pupation substrate after sterilisation, as explained above.

Figure 2.26 Compositing site for black soldier fly (BSF; *Hermetia illucens* L.) colony residual before use as a soil amendment.

2.7 Insect biomass harvest

2.7.1 BSFL harvest

In this study, BSFL harvesting time was determined based on three criteria: age (15 to 21 days), size (4 to 6 mm), and/or weight (150 to 220 mg). Several methods were employed to harvest the BSFL and remove them from the feeding site:

1. Manual sieving for BSFL harvest:

   BSFL were separated from their diet using a 1 to 2 mm sieve, chosen according to larval size (Figure 3.27B). A substantial amount (~200 to 300 g) of BSFL and residual were placed in the sieve and manually shaken to pass the frass and residue, while retaining the BSFL. It was observed that withholding feed from the BSFL for one day before harvest resulted in drier residues and greatly improved the efficiency of BSFL separation by sieving.
2. Water bath method for BSFL harvest:

Like manual sieving, BSFL and residue were placed in a sieve and gently shaken in a water bath. The shaking process was repeated until all the residue passed through the sieve, leaving the larvae separated.

3. Food attraction method for larvae harvest:

   The larvae were collected using their natural attraction to fresh food. Fresh food was placed in the middle of the rearing tray and after 30 minutes, the larvae gathered around the food. Subsequently, the larvae were scooped out (Figure 2.27A).

2.7.2 BSFP harvest

In this facility, the harvesting of BSFP used two methods as follows:

1. Self-harvest system: This is the most common harvest system for BSFP. The BSFP usually leave the feeding site and look for dry shelter to eventually enter the pupal stage, also known as the self-harvest stage. Hence, the larvae were reared in trays at a 45-degree angle to help the crawling BSFP self-harvest (Figure 2.28). It was observed that increasing the moisture (~80 %) in the diet at this stage facilitated the emigration from the feeding site. The water gave the BSFP a better grip when climbing over the edge of the tray to leave the feeding site.
2. Water harvest system: This method was proposed for separating the BSFP from larvae or residue using water. This method enables the selective harvest of BSFP and pupae, leaving behind larvae. A tray was harvested as normal by sieving and washing the BSFL and BSFP together. Later, the tray was filled with water and allowed to sit for 2 to 3 minutes. Separation of tray contents happens due to differences in the densities of BSFL, BSFP and residual. BSFP are buoyant, unlike the BSFL, causing them to float and allow them to be scooped out (Figure 2.29).

Figure 2.27 (A) Larvae of black soldier fly (BSF; *Hermetia illucens*L.), roll together near a fresh food source, facilitating the harvest process. B. Sieving the larvae using a 1 to 2 mm sieve.

Figure 2.28 The pre-pupae of black soldier flies (BSF; *Hermetia illucens L.*), leave the feeding area in a self-harvest system.

2.8 Post-harvest processing

The harvested BSFL and BSFP can be euthanised by an array of methods (e.g. freezing, blanching, desiccation, steaming, and hydrostatic pressure). In this facility, the insects were washed with running tap water and euthanised by placing them at -20 °C for at least 12 h. The killing method is a crucial step as it influences the colour, quality, composition, and microbial load of the final product (Larouche *et al.*, 2019). Subsequently, the BSFL were defrosted and placed in metal trays with a mesh base (50 cm×12 cm× 6 cm) to a depth of 2 cm. The trays were then placed into a Unitherm oven at 65 °C until the BSFL were completely desiccated and firm. After the first eight hours, the BSFL were stirred within the trays for even drying. Every eight hrs, the trays were moved around inside the oven to ensure even drying. The dry yield was between 250 to 350 g/kg of the fresh weight. Finally, the dried material was ground in a hammer mill to pass through a screen size of 5.0 mm and stored at 4 ºC for further use (Figure 2.30).

Figure 2.29 The pre-pupae of the black soldier fly (BSF; *Hermetia illucens* L.) are buoyant and can be harvested by scooping them out of a water container.

2.9 General cleaning and high hygiene in the colony

Maintaining optimal rearing conditions for breeding units of BSF require high moisture content and warm temperatures, but these conditions can also invite infestations (e.g., house flies, fruit flies, mites) and lead to mould growth on BSFL feed. Therefore, regular cleaning is crucial to maintain a healthy colony and to prevent these issues.

All surfaces in the rearing rooms were wiped down with a disinfectant solution weekly, while the floors were cleaned using water and chlorine. Monthly, the rooms were emptied and treated with insecticide bombs to eliminate any remaining pests (Appendix H). The rearing rooms were then aired out for two days before resuming production.

In the glasshouse, general cleaning was carried out monthly, including cleaning the cages' floors and removing all dead flies. Sugar containers were checked for any ants and replaced with fresh sugar, while water bowls were emptied and cleaned for any signs of mould growth.

Figure 2.30 (A) Larvae of the black soldier fly (BSF; *Hermetia illucens* L.) were spread on metal trays in preparation for oven drying at 65°C for 24hrs. (B) Larvae in trays after drying. (C) a close view of the dried larvae. (D) Larvae meal after ground using a hammer mill to 5.0 mm size.

Chapter 3: Influence of Substrate Type, Moisture and Density on the Pupation and Adult Emergence of Black Soldier Fly (*Hermetia illucens*)

Abstract

Pupation is a critical phase in the life cycle of dipteran species, including black soldier fly (BSF; *Hermetia illucens* L.), involving complex morphological and physiological transformations. In mass-rearing operations, optimising pupation substrates is essential for successful pupation, adult emergence and a steady egg supply, while managing costs effectively. This study investigated the effects of substrate type, moisture level, and compaction on pupation success, adult emergence and morality. Six substrates namely, sand, wood shavings, topsoil, vermiculite, spent wheat middlings, and potting soil, were evaluated across three experiments. While several substrates supported successful pupation, the use of spent wheat middlings (also referred to as colony residues) is recommended due to its economic viability and abundant availability as a waste product from BSF operations.

Moisture levels significantly influenced substrate performance, with moist substrates generally outperforming the dry ones. Optimal moisture level varied based on substrate characteristics such as dry matter (DM), bulk density, and water-holding capacity (WHC). Generally, 10% moisture achieved not only high percentages of pupation and adults and reduced morality but also avoid the risk of mould growth. Furthermore, mild compaction did not adversely affect BSF development, suggesting that moderate compaction might facilitate substrate reuse. This approach could potentially lower costs in mass-rearing operations while maintaining effective pupation conditions.

Keywords: BSF, pupation substrates, adult emergence, pupae, adult mortality

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3.1 Introduction

In recent years, the utilisation of insects as poultry feed has garnered increasing interest due to their high protein and fat contents (Holmes *et al*., 2013; Makkar *et al*., 2014; Abd El-Hack *et al*., 2020). Among these, the Black Soldier Fly (BSF; *Hermetia illucens* L.) is particularly promising, with protein and fat contents reported between 39 to 63% and 7 to 39%, respectively. These variabilities are notably influenced by the substrate used. Furthermore, BSF possesses a relatively short lifecycle of approximately 41 days under optimal conditions, although this can extend to as much as 131 days under less favourable conditions (Abduh *et al*., 2022). Notably, BSF larvae can only convert organic wastes during their larval stage, yet every life cycle stage is vital for successful BSF production. For instance, adverse conditions during the pre-pupal stage can significantly impede the pupation process, resulting in failed adult emergence and subsequent disruptions in egg supply (Holmes *et al*., 2013; Dzepe *et al*., 2020). Optimising mass-rearing of BSF thus requires maintaining low adult mortality rates and ensuring a continuous egg supply. A crucial aspect of achieving this is providing suitable pupation substrates and maintaining optimal conditions for pre-pupae.

Pupation is a critical phase in the life cycle of all dipteran species, including BSF, involving complex morphological and physiological transformations. Wen *et al*. (2016) highlighted that the pupal stage is vulnerable as the pupae lack mobility and are defenceless, rendering them susceptible to predation and other environmental challenges. Research has highlighted the importance of the substrate in facilitating metamorphosis, shortening pupation time, and enhancing adult fitness and longevity (Weston and Desurmont, 2008; Holmes *et al*., 2013; Chen and Shelton, 2014; Aceituno-Medina *et al*., 2017; Parry *et al*., 2017). Various substrates such as sand, wood shavings, different types of soil, coconut husk, wheat bran, volcanic ash, sawdust, and corn cob grits have been utilised in insect mass-rearing facilities for other insects like sawfly, tephritid fruit fly, and *Heortia vitessoides*, a type of moth (Pietrantuono *et al*., 2015; Wen *et al*., 2016; Chantawee and Soonwera, 2018; Pascacio-Villafán, 2021). Additionally, studies on dipteran species have identified several factors that can influence the success of pupation and pupation depth, such as substrate type, moisture, and compaction levels. For example, the absence of substrate generally reduced the pupation rate and adult survivorship in tephritid fruit flies (Pascacio-Villafán, 2021). Furthermore, substrate moisture saturation was found to negatively impact pupation, increase mortality, and drive pupation to the surface in corn silk flies (Allan, 2023) while a minimum depth of 5 cm for dipteran pupation is recommended (Amaral *et al*., 2021; Capinera, 2020). The compaction of substrate has been shown to affect the depth of pupation for the green bottle fly (*Lucilia sericata*), by reducing pore space and air circulation, as it requires more energy and prolongs the time to pupation (Cammack *et al*., 2010; Bubnov *et al*., 2015; Shi *et al*., 2021).

While insights from earlier studies on other dipteran species contribute to the understanding of BSF pupation, there remains a lack of research specifically tailored towards the BSF. Two studies by Dzepe *et al*. (2020) and Holmes *et al*. (2013) examined various pupation substrates and their impact on pupation time and adult emergence rate, comparing these against conditions with no substrates. Liu *et al*. (2022) investigated BSF pupation in two substrates (vermiculite and wood chips) across a range of moisture levels from 0 to 150%. Collectively, these studies concur that absence of pupation substrates showed negative affect the pupation process. While Dzepe *et al*. (2020) did not report the moisture levels in their substrates, they agreed with the other two studies (Holmes *et al*., 2013; Liu *et al*. 2022) that moist substrates perform better than the dry ones for pupation. However, excessively high moisture levels are reported to hinder pupation progress (Holmes *et al*., 2013; Chen and Shelton, 2014; Wang *et al*., 2017).

The present study investigated the effects of substrate type and moisture content on BSF pupation rate, adult emergence rate, and mortality respectively. The substrates tested included spent wheat middlings, vermiculite, topsoil, wood shavings, potting soil, and sand, selected for their availability and/or affordability on BSF farms. Moisture levels were maintained below 40% to avoid the negative impacts of high moisture levels, as reported in previous studies (Holmes *et al*., 2013; Chen and Shelton, 2014; Wang *et al*., 2017). Since the substrate physical properties can vary, an additional test hypothesis was that the optimum moisture level for BSF pupation will differ depending on substrate type. The study also examined the effect of compacted substrate on pupation and adult emergence, an aspect not previously explored.

3.2 Materials and Methods

3.2.1 Insect collection and pre-pupae harvest

The methods used in insect collection and BSF pre-pupae (BSFP) harvest were the same as previously described in Chapter 2, section 2.4 (BSFL and BSFP rearing units) and section 2.7.2 (BSFP harvest).

3.2.2 Pupation substrate preparation

Six substrates were chosen for the experiments based on their local availability, cost, and previous use with other dipteran species (Figure 3.1). The substrates included topsoil, potting soil, sand, wood shavings, spent wheat middlings, and exfoliated vermiculite.

To ensure uniform moisture content, the substrates were prepared accordingly. Topsoil, potting soil, sand, and wood shavings were oven-dried at 100°C for two days to achieve a moisture content of 0 to 1%. Spent wheat middlings, a residue from BSFL rearing obtained after two-weeks of feeding from an existing BSF colony, were also oven-dried to the same moisture content.

Exfoliated vermiculite, which initially had a moisture content of only 3%, required additional preparation. It was soaked in tap water for two days to allow for expansion and to increase the bulk density. Excess water was removed by squeezing and draining for an additional day, and the material was then oven-dried for several days at 100°C to reach a moisture content of 0 to 1%.

Moistened treatments of substrates were prepared by adding water directly to the substrate and thorough mixing to achieve the desired moisture content. The amount of water added was based on the volume of each substrate. Each substrate was filled to a depth of 7 cm in a 400 ml cardboard cup and filled with distilled water, and left for 24 hours. The saturation level of each substrate was calculated by measuring the weights before and after saturation. Dry treatments with 0% moisture content did not require any water. The saturation level of each substrate was determined using the equation (1), considering the different proportions of water ranging from 0% to 40%. All dry substrates were weighed, stored in airtight plastic bags, and sealed for future use.

Equation 1 (Chen and Shelton, 2007):

$$\text{Saturation level (\%))} = \frac{\text{Weight of distilled water added}}{\text{weight of saturated substrate} - \text{weight of dry substrate}} \times 100$$

3.2.2.1 Substrate water holding capacity

The water holding capacity (WHC) was determined using 2.5 g pupation substrate that was weighed into pre-weighed 30 ml plastic centrifuge tubes. Ten millilitres of distilled water were added to each tube and mixed well. Samples were kept at room temperature (22 ± 2°C) for 30 min and then centrifuged at 1200 × g (3709 rpm) for 30 min (Model 3K 15, rotor 11133, Sigma, Schönwalde-Glien, Germany). The supernatant was carefully decanted and the tube was re-weighed (Traynham *et al*., 2007).

The WHC was calculated using Equation 2 (Traynham *et al*., 2007).

Equation 2:

$$\text{WHC (g/g)} = \frac{[(\text{tube and wet sample weight} - \text{tube and dry sample weight}) - \text{total weight of substrate}]}{\text{dry sample weight}}$$

3.2.3 Experimental design

Three experiments were conducted to investigate the influence of substrate type, moisture level, and substrate compaction on BSF pupation, adult emergence and adult morality. Each experiment was repeated four times.

Experiment 1: Substrate effect

A total of 4,800 BSFP were collected from a self-harvest system on day 1, randomly placed in 48 cardboard cups (100 pre-pupae per cup)) with each cup being allocated to a different substrate treatment. The 12 treatments included six substrates (sand, wood shavings, topsoil, vermiculite, spent wheat middlings, and potting soil) in dry (0%) and moist (10%) conditions.

Experiment 2: Moisture level effect

A total of 2,000 day-old BSFP were collected from a self-harvest system and randomly assigned to 20 treatment combinations (100 BSFP per treatment). The 20 treatments involved four substrates (sand, topsoil, potting soil, and spent wheat middlings) at five saturation levels (0, 10, 20, 30 and 40%).

Experiment 3: Substrate compaction

A total of 1600 day-old BSFP were collected from a self-harvest system and randomly assigned to four treatment combinations and four replicates. The treatments were examined two substrates (vermiculite and spent wheat middlings) under manual compaction and loose state on pupation and adult emergence percentages. Substrate preparation began with adjusting the moisture content to 10%, ensuring uniformity thorough mixing. Compaction was achieved by manually reducing the substrate height from an initial 7 cm to 5 cm. A wooden tamp (square block, 0.5 cm thick) placed inside the pupation cup above the substrate and manual pounding using a rubber hammer to achieve the desired depth (5 cm), as described by Cammack *et al.*

(2010). The experiment was performed in 400 mL cups, with the substrate filled to 7 cm for non-compacted treatments and 5 cm for compacted ones.

All treatments were then placed in a dark growth chamber with an average temperature of 28 ± 2°C and 50 ± 10% RH. Each sample cup was covered with mesh and secured with a rubber band to prevent BSFP from escaping.

In all experiments, BSFP were introduced at the surface of each cup. After a week of incubation, pre-pupae that had not developed into pupae and those found dead on the surface of the substrate were considered deceased, as suggested by Wang *et al*. (2017). Conversely, any pupae either found on the surface or penetrated under the substrate were considered to have survived. The pupae were identified based on the descriptions reported by May (1961) and Dzepe *et al*. (2020), where pupae become completely immobile with a rigid puparium without elasticity and the last abdominal segment bent in a ventral position.

At the end of the 3-week incubation period, each cup was individually sieved, and the counts for dead pupae, successful pupae, dead adults, and emerged adults were recorded. The experiment was extended by one week beyond the average pupation time for BSF, which is approximately 14 days from pupation to emergence (Barragan-Fonseca *et al*., 2017), to ensure that all potential emergences were accounted for.

Table 3.1 Different moisture percentages and the corresponding amount of water added to each of the pupation substrate (sand, wood shavings, topsoil, vermiculite, spent wheat middlings and potting soil). These substrates were later used for the pupation of black soldier fly (BSF; *Hermetia illucens*). Water was added on a volume to volume (v/v) basis.

Substrate [a]	Substrate weight (g) in 7 cm depth	Water added (mL) at each saturation (%) level [b]				
		0	10	20	30	40
Sand	370	0	4.30	6.80	12.9	17.2
Wood shavings	24.0	0	6.30	12.7	19.0	25.3
Topsoil	129	0	6.00	12.1	18.1	24.1
Vermiculite	66.8	0	7.00	14.0	21.1	28.1
Spent wheat middlings	95.0	0	6.90	13.7	20.6	27.4
Potting soil	87.8	0	6.30	12.5	18.8	25.0

[a] Substrate weight varied among substrates, so volume was standardised to 7cm in a 400 mL cup [b] Each substrate was weighed to 7 cm depth, saturated with water, and soaked for 24h. The total value of the water absorbed overnight was recorded as 100%.

3.2.4 Statistical analysis

Data on pupal, adult emergence, and mortality percentages were categorical. To handle this, nature logit transformations were applied to convert the probabilities of categorical outcomes into a linear relationship with predictor variables. The PROC GENMOD procedure in SAS (SAS, 2018) was used, employing a generalised linear model (GLM) with a binomial distribution and logit link function for categorical outcomes (event/ trial) as described by Agresti (2002).

For each experiment, the analysis was conducted in two steps. Firstly, for the pupation and mortality analysis, binomial data were analysed with the logit link function modelling the probability of successful pupation versus mortality. Secondly, adult emergence and mortality were analysed as a separate binomial set, applying the logit link function to model these outcomes.

GLM was employed to assess the impact of various factors in each experiment. In the first experiment, we examined how substrate type, substrate state (dry vs. moist), and their interaction influenced pupation. The second experiment focused on the effects of substrate type and varying moisture levels (0, 10, 20, 30, and 40%) on pupation. The third experiment analysed the influence of substrate type and compaction on pupation. The null hypothesis in each case postulated no effect of substrate type, state (dry and moist), moisture levels, compaction, or their interactions on pupation, adult emergence, and mortality.

Statistical significance was determined using chi-square tests with a significance level set at $P < 0.05$. When a treatment effect was statistically significant, Post hoc analyses were performed using least squares means (LS means) with pairwise comparisons to identify significant differences between the different treatments. The data were back-transformed for visual presentation to provide an intuitive understanding of the results.

3.3 Results

3.3.1 Characteristics of pupation substrates

The physical characteristics of the substrates used in the study varied greatly, as shown in Table 3.2 and Figure 3.1. The sand had the highest bulk density and the lowest WHC. In contrast, wood shavings had the lowest bulk density and the highest in WHC, followed in both values by spent wheat middlings.

Table 3.2 Physical characteristics of the pupation substrates for black solider fly (BSF; *Hermetia illucens* L.) tested

Substrate	Type	Dry matter (g/kg)	Volume (cm^3)	Bulk density (g/cm^3)	WHC (g/g) [a]
Sand	Coarse sand	978	4	1.50	0.35
Wood shavings	Pine shavings	888	66	0.09	4.77
Topsoil	Coarse soil	709	11	0.55	0.90
Vermiculite [b]	Golden, silicate mineral	950	13	0.46	1.73
Vermiculite compacted [c]	Golden, silicate mineral	950	5	1.38	1.73
Spent wheat middlings	A by-product of wheat milling	835	25	0.24	3.02
Spent wheat middlings compacted [c]	A by-product of wheat milling	835	5	1.2	3.02
Potting soil	Coarse soil	892	22	0.27	1.66

[a] WHC, water holding capacity calculated based on an equation by Traynham *et al.*, (2007) WHC (g/g) = [(tube and wet sample weight - tube and dry sample weight) – total weight of substrate] / dry sample weight [b] Vermiculite was soaked with water for two days and then oven-dried at 100^{o}C, while the other substrates were oven-dried at 100^{o}C as sourced [C]. Compaction was achieved manually by reducing the substrate height from 7 cm to 5 cm using a small hammer and a wooden tamp fitted inside the pupation cup, as described by Cammack *et al.* (2010).

Figure 3.1 A total of six different pupation substrates were used for the black soldier fly (BSF; *Hermetia illucens*). (A) Difference between wheat middlings. (B) Exfoliated vermiculite before soaking in water, while (C) Vermiculite after soaking for 48 hours and then drying. (D) Topsoil, (E) pine wood shavings, (F) potting soil, and (G) washed coarse sand.

3.3.2 Substrate effect

During the pupation stage, the type of substrate (GLM: $\chi^2 = 60.4$, df = 5, $P < 0.0001$), moisture content (GLM: $\chi^2 = 130$, df = 1, $P < 0.0001$) and the interaction between them (GLM: $\chi^2 = 13.4$, df = 5, $P = 0.0200$) significantly influenced the pupation percentage. Generally, moist substrates performed better than dry ones. The pupation percentages of moist forms of spent wheat middlings, topsoil, vermiculite, potting soil, and wood shavings, with pupation percentages of 97.3, 96.5, 95.5, 95.3, and 94.3%, respectively (Figure 3.2A). Among the dry substrates, potting soil and dry sand exhibited the lowest ($P < 0.05$) percentages, at 79 and 77%, respectively (Figure 3.2A).

The Adult emergence percentages were significantly impacted by substrate type (GLM: $\chi^2 = 128$, df = 5, $P < 0.0001$) and moisture content (GLM: $\chi^2 = 191$, df = 1, $P < 0.0001$) as did the interaction of substrate type and moisture content (GLM: $\chi^2 = 21.6$, df = 5, $P = 0.0006$). Similar to the pupation stage, the moist substrates performed better than the dry ones. Among the substrates, moist vermiculite supported the highest adult emergence (92%), followed by moist spent wheat middlings (90%), moist topsoil (87.2%), and moist wood shavings (82%), respectively (Figure 3.2B). Dry sand had the lowest value (44.4%) and those of dry topsoil and potting soil were also lower (65 and 63%, respectively) compared to dry treatments.

The mortality of pupae was significantly influenced by substrate type (GLM: $\chi^2 = 18.4$, df = 5, $P < 0.0025$) and moisture (GLM: $\chi^2 = 39.7$, df = 1, $P < 0.0001$). However, no significant interaction between these factors was detected (GLM: $\chi^2 = 6.29$, df = 5, $P = 0.2793$). Moist substrates generally resulted in lower or negligible mortality compared to their dry counterparts, with dry sand exhibiting the significantly the highest mortality percentages at 4% (Figure 3.2C) while all moist substrates showed practically no mortality.

In the adult stage, mortality was higher than during the pupation stage. The substrate type (GLM: $\chi^2 = 20.9$, df = 5, $P < 0.0009$) and moisture (GLM: $\chi^2 = 45.7$, df = 1, $P < 0.0001$) significantly affected adult mortality, but no significant interaction was observed between these factors (GLM: $\chi^2 = 6.29$, df = 5, $P = 0.2875$). Similar to pupation, dry sand showed the highest adult mortality (42%), while vermiculite and spent wheat middlings were associated with the lowest adult mortality, at 7.8% and 10%, respectively (Figure 3.2D).

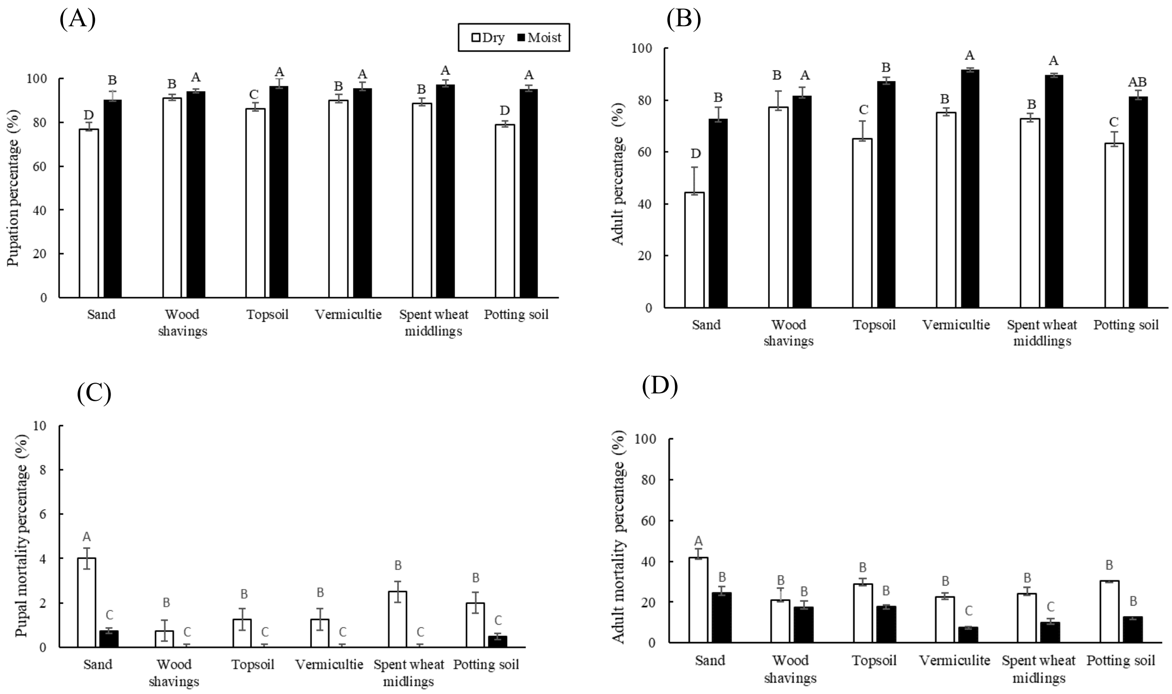

Figure 3.2: Effects of dry (0%) and moist (10% water) substrates on the pupation, adult emergence, and mortality percentages of black soldier fly (BSF; *Hermetia illucens* L) across developmental stages. Values represent the mean of percentages, with error bars indicating the standard error (SE) based on a sample size of n = 4. Different letters (A, B, C and D) indicate significant differences between treatments ($P < 0.05$).

3.3.3 Moisture level effects

In the pupation stage, significant effects were observed for substrate type (GLM: $\chi^2 = 37.3$, df = 3, $P < 0.0001$), moisture levels (GLM: $\chi^2 = 183$, df = 4, $P < 0.0001$), and their interaction (GLM: $\chi^2 = 133$, df = 12, $P < 0.0001$). Significant differences among substrates were noted, with pupation rates ranging from 60 to 95%. The highest pupation percentages across all substrates were generally observed at 10% moisture level (Figure 3.3A). However, more than one moisture level proved optimal for topsoil and spent what middlings and potting soil. It was also observed that mould growth increased when moisture levels exceeded 10% in spent wheat middlings. Furthermore, for all substrates at higher moisture levels (> 10%), most of the pupae remained in the upper, drier layer of the substrate, measured to be 2 to 3 cm from the surface, indicating an avoidance of excessively moist conditions.

Similar to the pupation stage, significant effects were observed at the adult stage for substrate type (GLM: $\chi^2 = 33.5$, df = 3, $P < 0.0007$), moisture levels (GLM: $\chi^2 = 117$, df = 4, $P < 0.0001$), and their interaction (GLM: $\chi^2 = 27.3$, df = 12, $P = 0.003$). Significant differences were also observed among substrates. 40% moisture level consistently yielded the highest adult emergence percentages across all substrates and there were no considerable differences among the five tested moisture levels (Figure 3.3B).

The mortality percentages in pupal stage were significant effected by substrate type (GLM: $\chi^2 = 17.03$, df = 3, $P < 0.0007$), moisture levels (GLM: $\chi^2 = 93.5$, df = 4, $P < 0.0001$), and their interaction (GLM: $\chi^2 = 29.8$, df = 12, $P = 0.003$). Significant differences were also observed among substrates. However, the mortality percentages did not follow a consistent pattern across all substrates (Figure 3.3C). For example, the highest mortality was observed at 0, and 20% moisture in sand. Conversely, for topsoil and potting soil, higher mortality rates were noted when moisture levels exceeded 10%. No significant differences were observed for spent wheat middlings across the tested moisture levels.

Mortality in adult emergence were significantly influenced by substrate type (GLM: $\chi^2 = 33.5$, df = 3, $P < 0.0001$), moisture levels (GLM: $\chi^2 = 117$, df = 4, $P < 0.0001$), and their interaction (GLM: $\chi^2 = 27.3$, df = 12, $P = 0.0069$). Significant differences among substrates were also noted. However, substrates such as sand and spent wheat middlings did not exhibit significant differences across moisture levels tested here (Figure 3.3D).

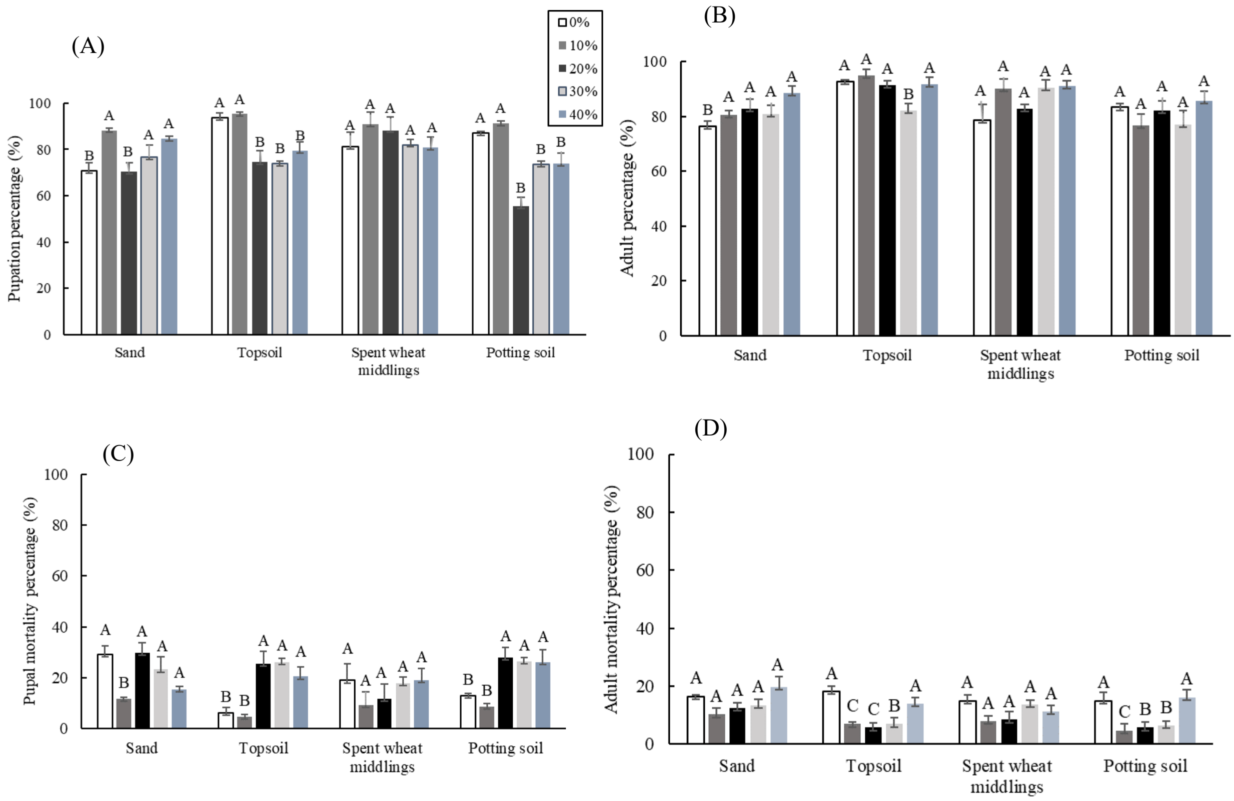

Figure 3.3 Effects of using substrate type (sand, topsoil, spent wheat middlings and potting soil) and levels of moisture (0, 10, 20, 30 and 40%) on black soldier fly (BSF; *Hermetia illucens*) pupation, adult emergence, and mortality percentages in both stages (pupal and adults). Values represent the mean of percentages, with error bars indicating the standard error (SE) based on a sample size of n = 4. Different letters (A, B and C) indicate significant differences between treatments ($P < 0.05$).

3.3.4 Substrate compaction

Pupation percentages were significantly influenced by substrate type (GLM: $\chi^2 = 28.5$, df = 1, $P < 0.0001$), compaction (GLM: $\chi^2 = 9.36$, df = 1, $P < 0.0001$), and their interaction (GLM: $\chi^2 = 6.27$, df = 1, $P = 0.0007$). Loose spent wheat middlings achieved the highest pupation percentage at 93%, which was significantly different compared to other substrates, which ranged from 81 to 88% (Figure 3.4A).

In the adult stage, substrate type (GLM: $\chi^2 = 73$, df = 1, $P < 0.0001$) and compaction (GLM: $\chi^2 = 5.3$, df = 1, $P = 0.018$) significantly affected the adult emergence percentages. However, their interaction did not significantly impact this outcome (GLM: $\chi^2 = 1$, df = 1, $P = 0.28$). Consistent with the pupation results, only loose spent wheat middlings showed a significantly higher adult emergence percentage at 92.5% (Figure 3.4B).

Mortality during the pupation stage was significantly influenced by substrate type (GLM: $\chi^2 = 130$, df = 1, $P < 0.0001$) and compaction (GLM: $\chi^2 = 4.38$, df = 1, $P = 0.01$). However, the interaction between these factors was not significant (GLM: $\chi^2 = 1$, df = 5, $P = 0.28$) (Figure 3.4A). Although compacted substrates generally exhibited higher mortality percentages than loose substrates, no significant differences were observed between compacted and loose substrates. However, spent wheat middlings (both loose and compacted) had significantly lower mortality percentages (7.5 and 9.5%, respectively) compared to vermiculite (18.3 and 16.3%, respectively).

Similarly, mortality in adult stage was significantly affected by substrate type (GLM: $\chi^2 = 73$, df = 1, $P < 0.0001$), compaction, and density (GLM: $\chi^2 = 5.3$, df = 1, $P = 0.0004$). However, their interactions were significant (GLM: $\chi^2 = 1$, df = 1, $P = 0.05$). Among the substrates, only loose spent wheat middlings showed a significantly lower mortality percentage at 2.5% (Figure 3.4B).

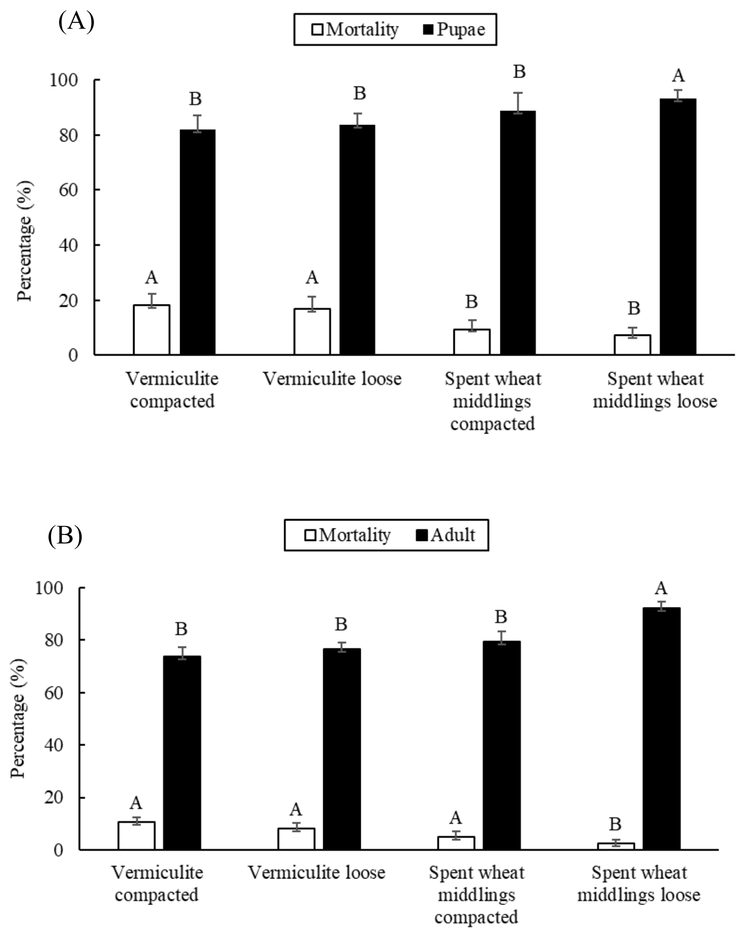

Figure 3.3 Effects of using substrate type (spent wheat middlings and vermiculite) and compaction (loose and compacted on black soldier fly (BSF; *Hermetia illucens*) pupation, adult emergence, and mortality percentages in both stages (pupal and adults). Values represent the mean of percentages, with error bars indicating the standard error (SE) based on a sample size of n = 4. Different letters (A and B) indicate significant differences between treatments ($P < 0.05$).

3.4 Discussion

3.4.1 Substrate type

Moist substrates consistently improved pupation and adult emergence percentages, and reduced mortality at both stages. The absence of moisture had a pronounced effect on mortality, particularly in adult than pupation stage. This observation aligns with the understanding that insects typically experience water loss through the integument during the pupal stage (Vargas *et al*., 1987). Conversely, desiccation from dry substrate conditions can delay BSF pupation and increase mortality (Holmes *et al*., 2012). Higher mortality in dry substrates, such as sand

and potting soil, can be attributed to excessive heat and energy losses because of increased contact among pupae, causing delays in pupation and death from exhaustion. This phenomenon has been documented in studies examining the absence of suitable substrates (Holmes *et al*., 2013; Dzepe *et al*., 2020).

Sand led to significant water losses, as previously reported by Vargas *et al*. (1987), consistent with the present findings. Dry sand resulted in the highest failure rates during both pupation (4% mortality) and adult emergence (42% mortality). The poor performance of dry sand could also be due to its high bulk density and low WHC, which hindered pre-pupal penetration and led to desiccation-related mortality (Holmes *et al*., 2013; Dzepe *et al*., 2020; Shi *et al*., 2021). In dry sand treatments, BSFP tended to settle on the surface and struggled to penetrate the substrate. This exposure of the BSFP could have a similar impact to the absence of substrate, as previously reported by Holmes *et al*. (2012).

Potting soil, despite having a higher WHC (1.66 g/g) compared to sand (0.35 g/g), exhibited the second-highest mortality and lowest adult emergence percentages. This may be attributed to its firm texture, which fails to provide adequate thigmotactic stimuli necessary for BSF pupae. Thigmotactic stimuli, which involve tactile pressure, are crucial for triggering the burrowing behaviour and helping pupae locate suitable microenvironments for pupation (Holmes *et al*., 2013).

On the other hand, vermiculite (WHC, 1.73 g/g) and spent wheat middlings (WHC, 3.02 g/g) caused the highest percentages of pupation and adult emergence with the lowest mortality. The findings suggest that these substrates provided additional advantages for optimal pupation, due to their texture and ability to offer suitable thigmotactic stimuli. Although BSF are not known to respond to thigmotaxis (movement towards tactile stimuli), lack of a suitable substrate can adversely affect their development, as noted by Holmes *et al*. (2013). Their study demonstrated that when pre-pupae come into direct contact or rub against each other due to inadequate substrate, pupation is delayed. Similar results have been reported for other insects, such as *Tribolium freemani* (Kotaki and Fujii, 1995), indicating that both mechanical and chemical stimuli from conspecifics can delay pupation. Therefore, appropriate substrate textures are crucial, as observed in the differences between the rigid structures of potting soil and sand versus the fine and penetrable particles of vermiculite and spent wheat middlings.

3.4.2 Moisture levels

All substrates evaluated performed well when maintained at 10% moisture, resulting in the lowest mortality rates for both the pupae and adults. Some substrates also showed good responses at other moisture levels. For instance, spent wheat middlings performed well at 10, 30, and 40% moisture levels for both pupation and adult emergence. During the experiments, mould growth was observed when moisture levels exceeded 10%, indicating that higher moisture contents created favourable conditions for mould development. Therefore, it is recommended to use 10% moisture level in substrates with high WHC, such as spent wheat middlings, to minimise mould growth. Previous studies reported that higher the moisture level during pupation, the higher is the pupation percentage and lower is the mortality (Vargas *et al*., 1987; Liu *et al*., 2022). However, the present results demonstrate that the desired moisture level of each pupation substrate vary depending on their physical characteristics, such as DM, bulk density, and WHC.

Higher moisture levels improved the adult emergence only in some substrates, such as sand and potting soil. This may be explained because both substrates have low WHC values causing them to lose their moisture faster due to the warm ambient temperature in the pupation chamber. Interestingly, spent wheat middlings and topsoil showed similar levels of adult emergence at 10 and 40%, with mould growth being observed at 30 and 40% of moisture in spent wheat middlings. Also, pupation was observed to occur in the upper drier section of the substrate, where less water seems to be present and more oxygen available. This observation aligns with Shi *et al*. (2021), who noted that substrates with extremely high moisture content tend to have their pore spaces filled with water, creating oxygen deficient conditions negative to insect development. Although a minimum pupation depth of 5 cm is generally recommended for dipteran species (Capinera, 2020), a depth of 3 cm appeared sufficient when moisture levels exceeded 10%. This observation could influence the amount and volume of substrate used in mass-rearing facilities based on the moisture levels maintained.

3.4.3 Substrate compaction

Substrate compaction significantly impacted pupation, adult emergence, and mortality at both stages. However, the effects were mainly observed in spent wheat middlings and not in vermiculite. Previous research has demonstrated that compaction, particularly in soils, can reduce oxygen penetration, leading to hypoxia and failed pupation (Bubnov *et al*., 2015; Shi *et al*., 2021). In this study, the mild and manual application of compaction may have somewhat mitigated these effects. For instance, Roach and Campbell (1983) found that post-pupae compaction in their study on Bollworm (Lepidoptera: Noctuidae) moth emergence could be detrimental. Their experiment involved saturating and smoothing the soil before introducing pupae. It was concluded that compaction increased resistance to penetration, delaying development and requiring greater energy expenditure. However, in the present study, mild compaction with only 10% moisture level had no negative impact on pupation, adult emergence, or mortality at either stage. This finding suggests that moderate compaction may not be detrimental to BSF pupation and could potentially allow for the reuse of substrates. Given the cost associated with purchasing new substrate materials (such as vermiculite), this finding could save cost in mass-rearing facilities. Further research is encouraged to explore the impact of mechanical compaction on BSF pupation and development to better understand its implications and optimise substrate reuse practices.

3.5 Conclusions

In a mass-rearing facility for Black Soldier Flies (BSF), achieving high pupation and adult emergence rates while minimising mortality and controlling costs is crucial. The present study is the first to examine how three key rearing conditions namely, substrate type, moisture level, and compaction, affect BSF pupation, adult emergence, and mortality. Among the substrates tested, several proved optimal for BSF pupation and adult emergence. Notably, spent wheat middlings offer both economic and environmental advantages.

Moisture levels significantly influenced substrate performance, with moist substrates outperforming dry ones. Optimal moisture levels varied based on substrate characteristics such as DM, bulk density, and WHC. Higher percentages of pupation were observed at 10% moisture, while higher adult emergence percentages were noted at 40%. However, to avoid mould growth, which was observed in some substrates at moisture levels above 10%, a moisture level of 10% is recommended.

Mild compaction had no impact BSF development, suggesting that moderate compaction could allow for substrate reuse. This approach could help reduce costs in mass-rearing operations while maintaining effective pupation conditions.

Chapter 4: The Poultry Red Mite (*Dermanyssus gallinae*) Poses a Risk in Rearing Black Soldier Fly (*Hermetia illucens*)[1]

[1] This chapter was published in: 'Mahmoud, A. E., Morel, P. C. H., Potter, M. A., & Ravindran, V. (2022). Poultry red mite (*Dermanyssus gallinae*) poses a risk in the rearing of black soldier fly (*Hermetia illucens*). *Journal of Insects as Food and Feed*, 9 (1), 55-63'.

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Amira El sayed Abdalla Mahmoud
Name and title of main supervisor:	Professor Peter Tozer
In which chapter is the manuscript/published work?	Chapter 7

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Amira contributed to experimental design, data collection, data analysis, initial and subsequent draft writing, and addressing feedback from supervisors. This involvement extended to preparing the final manuscript, responding to journal feedback.
Prof. Murray Potter contributed to data analysis, manuscript writing and responding to the reviewers.
Prof. Ravi Ravindran provided inputs to the writing, final manuscript and responding to the reviewers.

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Doctoral Research Committee May 2023

Abstract

The larvae of *Hermetia illucens* L. (Insecta: Diptera: Stratiomyidae), also known as black soldier fly (BSF), has the ability to recycle nutrients from organic wastes. The BSF larvae (BSFL) biomass is a cheap and sustainable protein source that can be used in the feeding of fish, chickens, pigs, and pets. The BSF is a non-pest species, but its mass rearing conditions can attract pathogens and parasites due to the high temperature and moisture requirements. The published literature on the BSF cover aspects such as larval production, conversion efficiency during the rearing stage, adult longevity, and mating. However, no published data are available on any parasitic or disease outbreaks in BSF colonies. The present paper reports the first case of an ectoparasite in a BSF colony. The poultry red mite (*Dermanyssus gallinae*) is an invasive predator of the BSFL and BSFP (pre-pupae), quickly deteriorating the production and health of BSF colony.

Keywords: Pathogen, insect protein.

4.1 Introduction

Insect protein has received increasing attention recently as a sustainable alternative source for food and animal feed worldwide. In 2018, the insect protein industry's investment exceeded $300 million (Chauve, 1998; Joosten *et al*., 2020). Among the insect species, black soldier fly (BSF; *Hermetia illucens* L.) (Diptera: Stratiomyidae) has attracted the most attention due to its significant advantages. BSF can consume and transform a wide range of organic wastes into nutritional materials in two to three weeks (Müller *et al*., 2017; Xiao *et al*., 2018; Scala *et al*., 2020). At the end of the growth cycle, the harvested BSF larvae and pre-pupae (BSFL and BSFP) are used as animal feed (Wang and Shelomi, 2017) for their richness in protein, fat, and minerals (Newton *et al*., 2005; Barragan-Fonseca *et al*., 2017; Franco *et al*., 2021).

Although BSF are known for their resilience to diseases, concerns are increasing about mass rearing and conditions in production facilities. The ideal rearing environment for BSF requires warm temperature (25 to 28 ºC) and humidity (50 to 70%), which create opportunities for multiplication of pests and pathogens not normally found in natural settings (Müller *et al*., 2017). While there have been no reports of parasites attacking BSF, it is not uncommon for dipteran species to host parasites, such as *Macrocheles subbadius* mites impacting the survival and reproduction of *Drosophila nigrospiracula* flies (Polak, 1996). Another example is *Macrocheles muscaedomesticae* (Scopoli), one of several mites that feed on eggs, newly hatched, and small larvae of the house fly *Musca domestica* L (Abo-Taka *et al*., 2014).

In this study, we report the first record of the ectoparasite *Dermanyssus gallinae* infecting a BSF colony and feeding on both BSFL and BSFP. This highlights the importance of addressing parasitic challenges to ensure sustainable insect protein production.

4.2 Materials and methods

4.2.1 BSF colony maintenance and diet

The methods used colony maintenance are the same as previously described in Chapter 3, section 3.2.1.

4.2.2 Description of Poultry Red Mite

Poultry red mite, *Dermanyssus gallinae* (also known as red mite), is a cosmopolitan species (Lesna *et al*., 2012). They are known to be hematophagous ectoparasites of poultry, and, often, nymphs and females feed on the host's blood for 0.5 to 1.5 hrs, while males are thought to do so only intermittently (Chauve, 1998; Sparagano *et al*., 2014). In adulthood, red mites are oval-shaped (Figure 4.1), usually less than 2 mm long with eight legs, and the front pair are used as sensory organs (Chauve, 1998). They typically appear colourless or grey to brown/red, depending on their feeding status. The colour can turn darker after meal/blood ingestion.

The life cycle of red mites suggests that they live most of their life away from the host (Wood, 1917; Chauve, 1998; Sparagano *et al*., 2014). They are usually associated with wild bird nests, domestic chickens, turkeys, ducks, pigeons, rabbits, and rats (Azad, 1986). Red mites are often found in the cracks and crevices in walls and beneath poultry nest boxes where the hygiene is marginal (Chauve, 1998). The red mites are a reservoir for bacteria, including zoonotic bacteria, easily transmitted between animals and humans (Kaoud, 2010; Sparagano *et al*., 2014; Flochlay *et al*., 2017). They usually attack resting birds in darkness to feed on their blood (Chauve, 1998; Fiddes *et al*., 2005). Infested birds become restless and exhausted, and, in extreme cases, the host can die from exsanguination (Azad, 1986). Red mites may also attack humans, causing mild discomfort, skin disorders, pruritic dermatitis, and potential vectors of viruses (Azad, 1986; Kaoud, 2010).

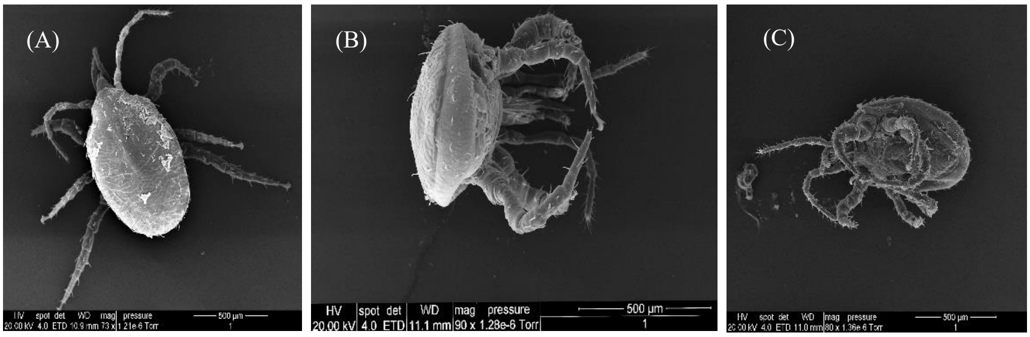

Figure 4.1 Scanning electronic microscopic images (SEM) of (A) dorsal view of poultry red mites, (B) side view for poultry red mites, and (C) under view of poultry red mites (*Dermanyssus gallinae*).

4.3 Experiment

4.3.1 Part A: behaviour and survival of larvae under mite infestation

The first observation of poultry red mite infestation in the BSF colony was in a managed facility located at Massey University. The colony was established successfully for over a year before the first infestation was observed. We randomly selected 64 BSFL with red mite infestation and monitored them for four days. These BSFL were divided into two groups of four replicates (8 BSFL per replicate) each:

- Group 1: BSFL were monitored for survival rate under the red mite infestation in the presence of wheat middlings.
- Group 2: BSFL were monitored for survival rate under the red mite infestation without wheat-middlings feed.

On day 1, the BSFL were transferred to plastic jars (8 cm in diameter, 10 cm depth). The jars were then sealed with a single mesh layer that was screwed in place using a lid ring. All jars were kept in a controlled environment (28 ± 3°C and 60 ± 10% RH). The second group of the BSFL was provided with one serving of fresh feed at 100 mg larva/ day. All jars were checked daily at the same time, and the dead BSFL were counted and removed.

4.3.2 Part B: Scanning electron microscopic analysis

Several samples of mites and damaged BSFL were examined using FEI Quanta 200 Environmental Scanning Electron Microscope (SEM) and energy-dispersive x-ray spectroscopy (EDAX) module (Manawatu Microscopy and Imaging Centre, Massey University, Palmerston North, New Zealand). The samples were prepared firstly in primary fixation with aldehydes, followed by secondary fixation with osmium tetroxide. Dehydration was in a graded ethanol series (50, 60, 70, 80, 90, 96, and 100%; 5 min each) followed, and the samples were then stored in 100% ethanol at 4°C. The gradual hydration allowed the removal of moisture without causing specimen shrinkage. Dried mites and damaged larval samples were sputtered with gold using the Sputtering Device (Balzers Union, Witten, Germany), and several images were taken for examination under the SEM.

4.4 Calculation and statistics

Survival data was analysed using PROC GENMOD with the logit transformation in SAS (SAS, 2016). The procedure was utilised with a binomial distribution and a logit link function, which is appropriate for modelling proportions or binary outcomes. The model evaluated the main effects of feed and day, as well as their interaction, to understand how these factors affect survival outcomes. Post hoc analyses were performed using least squares means with pairwise comparisons to identify significant differences between the levels of feed and day.

Statistical significance was determined using chi-square tests with a significance level set at $P < 0.05$. When a treatment effect was statistically significant, Post hoc analyses were performed using least squares means (LS means) with pairwise comparisons to identify significant differences between the different treatments. The data were back-transformed for visual presentation to provide an intuitive understanding of the results.

4.5 Results and discussion

4.5.1 The survival rate of black soldier fly BSFL under infestation of red mites

In general, red mites attached and fed on BSFL caused the BSFL to be restless. In addition, the BSFL became distressed due to the repeated bites of red mites, which often attacked the host in large groups. One larva of BSF was observed to carry as many as 30 to 40 mites (Figure 4.2).

Figure 4.2 Light micrograph of many red mites (*Dermanyssus gallinae*) feeding on a larva of black soldier fly (BSF; *Hermetia illucens*). The red mites body coloured red when they are fully engorged with food.

The survival data are presented in Figure 4.3. Both the effect of feeding and day were significant (GLM: $\chi^2 = 281$, df = 3, $p < 0.001$), but the interaction was not (GLM: $\chi^2 = 281$, df = 3, $p = 0.54$).

In the first group, where feed was provided, the BSFL showed a higher survival rate. On days 1 and 2, the majority of the BSFL survived the red mite bites. While on day 3, the survival rate dropped markedly from 96.8 to 21.9%. The BSFL survival was 71.8% in the second group on the first two days and dropped dramatically to 3.1%.

Ostensibly the moisture and nutrients in the feed were able to sustain the infested BSFL for more days. Still, eventually, they succumbed to the severity of the injuries caused by the bites. The extreme reduction in survival rate was irreversible, regardless of the feed supply, and caused heavy mortality in the colony within a short period (3 to 5 days).

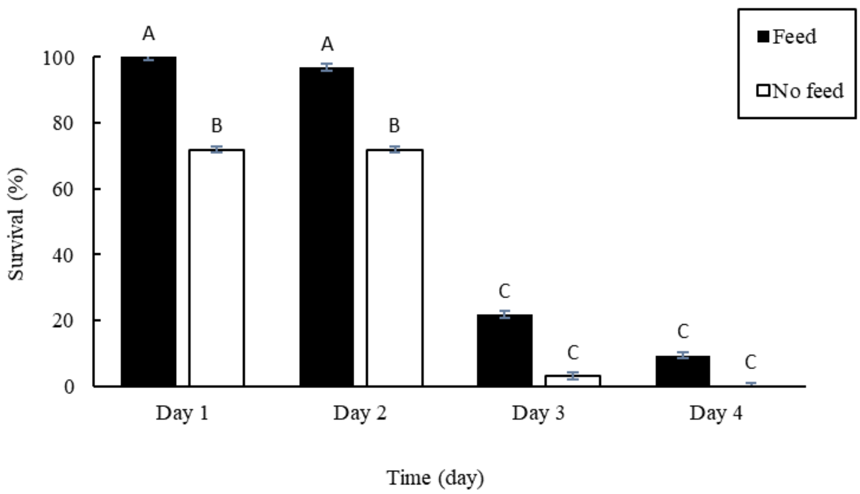

Figure 4.3 The survival percentage of black soldier fly larvae (BSFL; *Hermetia illucens*) posts red mite infestation over the period of four days without and with feed. The black colour represents the larvae survival rate under mite infestation in the presence of food. The white colour represents the larvae survival rate under mite infestation in the absence of food. Both the effect of feeding and day were significant ($\chi^2 = 281$, df = 3, $P < 0.001$), but the interaction was not ($\chi^2 = 281$, df = 3, $P = 0.54$). Values represent the mean of percentages, with error bars indicating the standard error (SE) based on a sample size of n = 4. Different letters (A, B and C) indicate significant differences between treatments ($P < 0.05$).

4.5.2 Parasitic behaviour of red mites in the colony

Red mites were observed to attack the BFS colony during both larval and pre-pupal stages (Figure 4.4). In the absence of BSFL, red mites targeted BSFP as a secondary preference host. The pre-pupa, being more chitinous (Wang *et al.*, 2020), and consuming less than the larva, was found to be less succulent, potentially making BSFP a less preferable meal for the mites. The dry matter content (%; mean ± SD) of BSFL and BSFP were determined to be 37.6 ± 2.1 (n = 5) and 53.4 ± 11.5 (n = 5), respectively.

There was no evidence of red mites attacking pupae or eggs. The eggs were stored in a separate facility (incubator) and often kept in airtight containers until hatching, thus shielding them from any infestation, including red mites. The substantial presence of chitin in the pupae skeleton, which can reach up to 14.1% (Wang *et al*., 2020), may contribute to the protection against red mites.

The feeding behaviour of red mites on the BSFL of BSF is similar to that on poultry (Chauve, 1998). The feeding attack was found to happen in darkness, with the BSFL being infested by groups of red mites ranging from 5 to 40. Red mites often parasitise the BSFL by attaching themselves to the posterior end of the dorsal side of BSFL or BSFP (Figure 4.4). It was observed that when the host (BSFL or BSFP) is exhausted and has stopped movement, red mites seem to spread from the dorsal end to the ventral side, forming a half-ring shape (Figure 4. 5). The BSFL attacked by mites appeared at first restless in an attempt to shake off the red mites of its body. Eventually, the BSFL surrender and show signs of exhaustion, such as decreased feed intake and slow movement.

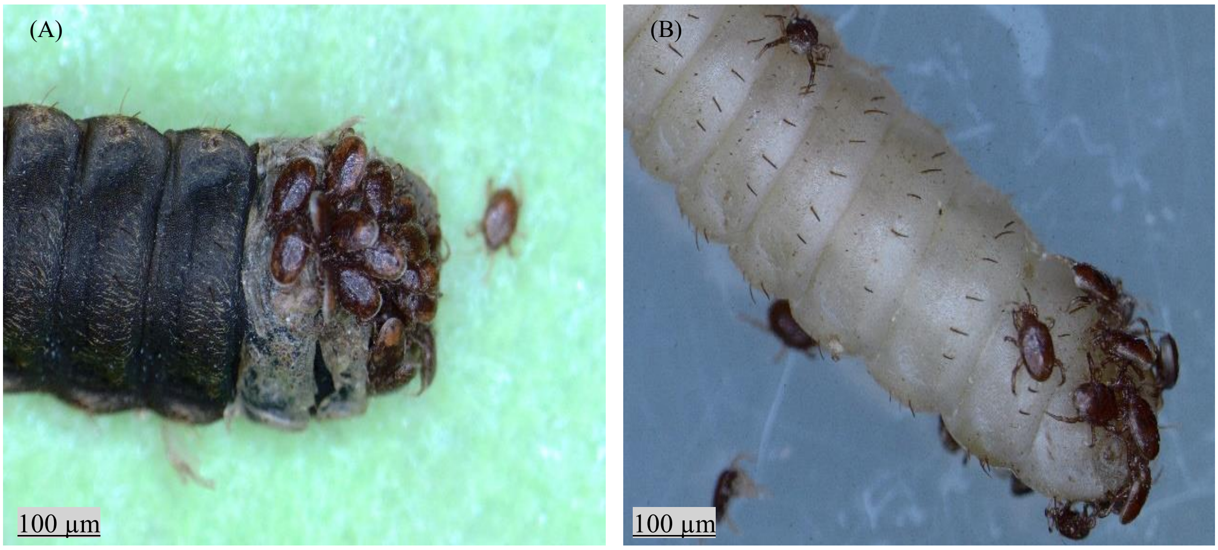

Figure 4.4 Light micrographic views for red mites (*Dermanyssus gallinae*) while feeding on, (A) pre-pupa and (B) larva of black soldier fly (BF; *Hermetia illucens*).

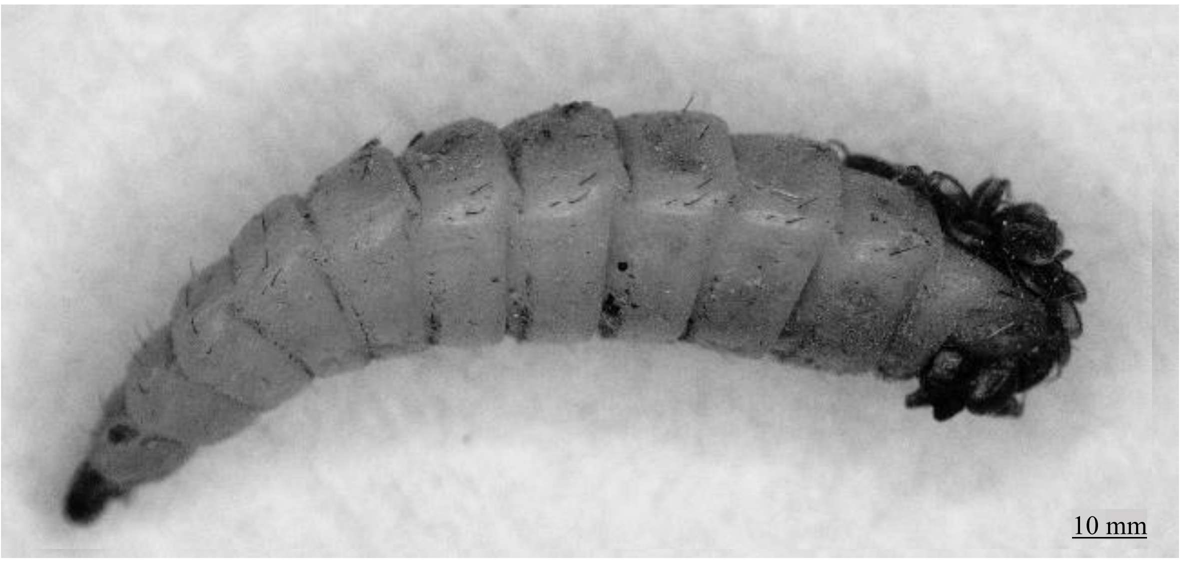

Figure 4.5 Several scanning electron microscope (SEM) images with different magnification, show cuticle damage on larvae of black soldier fly (BSF; *Hermetia illucens*) caused by poultry red mite infestation (*Dermanyssus gallinae*).

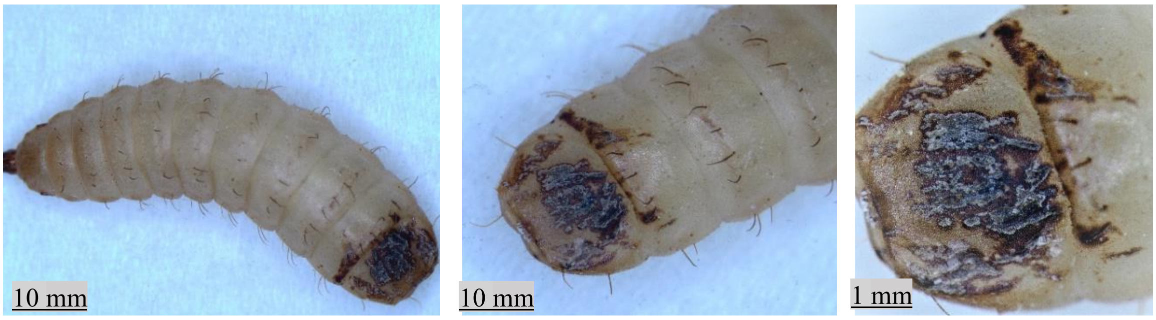

Figure 4.6 Poultry red mites attached in half a ring shape feeding on exhausted and motionless larva of black soldier fly (BSF; *Hermetia illucens*).

4.5.3 Damage caused by red mites to the BSF colony

Parasites are common in nature and can harm their hosts in various ways. They can affect different levels of biological organisation, from the genetics of local host populations to entire ecological communities (Polak, 1996; Abo-Taka *et al*., 2014). Parasites consume nutrients that would otherwise be available to the host and, also interfere with the host's ability to feed and absorb nutrients efficiently (Polak, 1996). This leads to disruptions in the host's physiological functions, weight loss, and increased risk of death (Barnard and Behnke, 1990). Some parasites may reduce the host's ability to produce eggs by altering metabolism or disrupting hormonal controls (Polak, 1996; Benoit *et al*., 2020). In this study, red mite infestation affected BSFL production in several ways:

4.5.3.1 Anatomical damage

The parasitic behaviour of mites on BSFL or BSFP leaves marks on the skin, which was visible to the naked eye. The damage discoloured the larval skin and altered the colour from light cream to dark brown (Figure 4.6). In addition, SEM images clearly showed cuticle damage (Figure 4.7). The mites also caused distress to the BSFL due to repeated bites as they appeared to feed on their blood.

4.5.3.2 Human health concerns

The risk of the presence of red mites in the colony may also extend to the transfer of viruses to the colony and researchers. In the personal experience of the researcher, red mites crawled on her hands and caused skin irritation.

4.5.3.3 Economic loss

The economic loss from red mite infestation of BSF colonies has the potential to be severe for the insect protein industry. It is almost impossible to save the colony once infested, and the infected BSF colony is wholly destroyed. This would be costly in commercial operations, with additional costs incurred for re-establishing colonies, premise disinfection, and sterilising tools and facilities. The risk of transferring red mites from the BSF colony to poultry may limit the application of live larvae as poultry feed.

4.5.4 Control and prevention methods

The control of red mite infestation is a problematic task for several reasons. (i) Red mites are resilient to desiccation and starvation (Chauve, 1998; Flochlay *et al*., 2017). In extreme cases, they can live up to 8 months away from the host (Chauve, 1998; Sparagano *et al*., 2014). (ii) They spend most of their life cycle sheltered in cracks close to their host (Chauve, 1998; Kilpinen, 2001). (iii) They multiply rapidly and the life cycle can be as short as seven days (Chauve, 1998; Sparagano *et al*., 2014). (vi). Only a few pesticides are registered to control or eradicate red mites (Kilpinen, 2001). (v) Red mites feed on a range of alternative hosts, including poultry, wild birds, horses, rodents, and humans (Sparagano *et al*., 2014). Traditionally, red mites in poultry houses are controlled by chemical methods, silica dust, biological control (e.g. Bacillus thuringiensis) and insect growth regulators (Chauve, 1998). Nevertheless, most of these approaches are not suitable at the BSF facilities. Alternative methods to control red mites in poultry are outlined below and shed light on their suitability in the BSF premises.

4.5.4.1 Pesticides

Previous studies reported the toxicity of spinosad, also known as Elector, to red mites in vitro and in vivo (George *et al*., 2010a; Liebisch *et al*., 2011). One dose of spinosad had a 97% successful rate and lasted at least 28 days after the application (Sparagano *et al*., 2014). Since 2010, Elctore has been approved to be used in laying hens' houses in several EU countries (Sparagano *et al*., 2014). Although Elector application may help control red mite infestation in BSF colonies, the application must be in empty premises or limited to walls where red mites often rest and away from the BSF colony. Another successful biopesticide is Bacillus thuringiensis (Bt), with proven toxicity to *O.sylviarum*, a type of mite (Sparagano *et al*., 2014). But it is proven toxicity to insects, and other vertebrates (Sparagano *et al*., 2014) will limit its use in the BSF facilities.

In general, spraying or dusting insecticides near the BSF colony can be problematic. It can cause death for adults, and BSFL may ingest harmful chemical substances to their wellbeing. In addition, if the BSFL store toxic chemicals in their bodies, that could limit their use for animal feed. Therefore, the safest option is to treat the premises with insecticides before establishing the BSF colony, and the periodic spry should be done in the absence of the colony.

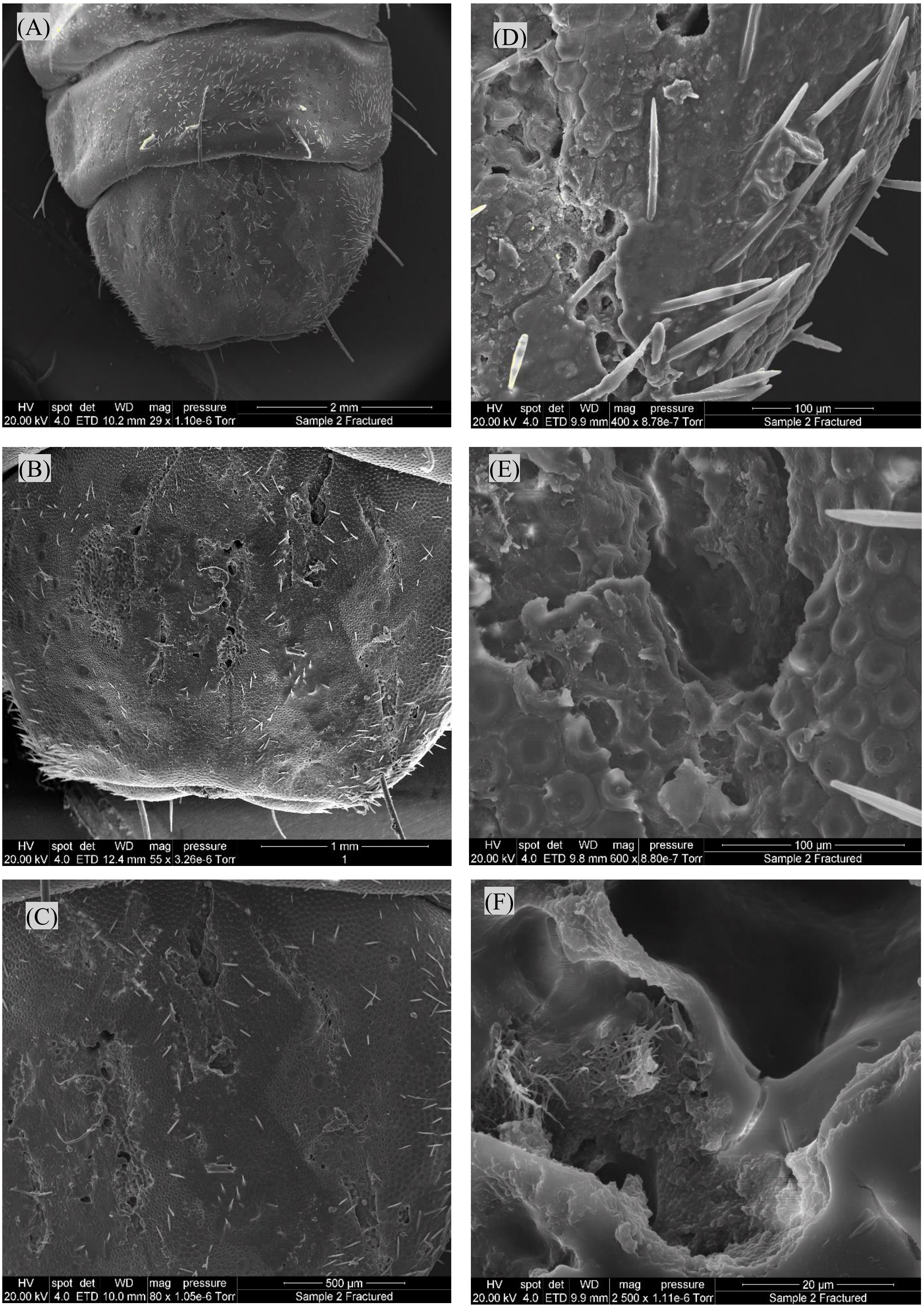

Figure 4.7 Several micrographic views for the larva of black soldier fly (BSF; *Hermetia illucens*) showed skin damage caused by poultry red mite bites (*Dermanyssus gallinae*).

4.5.4.2 Plant-derived products

Plant-based pesticides are used to fight red mite infestations (Maurer *et al*., 2009; George *et al*., 2010b; Sparagano *et al*., 2014). For example, garlic-based acaricide is commonly used against red mites. Similarly, commercial neem-based products are shown acaricidal activity against red mites (Sparagano *et al*., 2014). Another approach is adding essential oil such as thyme (Thymus spp.), burdock (Arctium spp.), and tansy (Tanacetum vulgare) infusion in poultry water to reduce the presence of the red mites (George *et al*., 2008). Although these are seemingly natural products, a topical use to clean premises may be safe for BSF colonies. However, further research will need to evaluate their suitable application near BSFL. Similarly, the infusion of essential oil in the BSF diet might not be favourable for other use of BSF as animal feed.

4.5.4.3 Inert substrates

Inert substrates, such as silica, kaolin, and diatomaceous earth (DE), have been demonstrated to effectively control red mites within poultry houses (Sparagano *et al*., 2014). These substrates offer several advantages, including their non-toxicity to both birds and humans and their low likelihood of resistance development (Mul *et al*., 2009). The mechanism of action of these substrates involves absorbing lipids from the mites, ultimately leading to their death through dehydration (Mul *et al*., 2009; Sparagano *et al*., 2014). This method has been proven effective in controlling mite populations in various agricultural settings.

It is noteworthy that mites and BSF belong to different orders within the Arthropoda phylum, with BSF being classified in the dipteran order. Given this distinction, applying inert substrates to areas where mites typically hide, such as cracks and crevices, may allow for the mitigation of mite infestations without causing significant harm to BSF populations.

4.5.4.4 Temperature

All red mites can be expected not to survive at temperatures below -20°C and higher than 55°C (Sparagano *et al*., 2014). As a result, manipulating the temperature of poultry houses between flocks has been a method to control red mite spread. In Netherland and Norway, heating hen houses to 45°C is a common practice to control red mites (Mul *et al*., 2009; Sparagano *et al*., 2014). For instance, in Norway, the heating is applied with a chemical treatment called phoxime before introducing the new birds (Mul *et al*., 2009; Sparagano *et al*., 2014). While in Netherland, the heat application without chemicals was not adequate, and the red mites appeared after six months from the treatment (Mul *et al*., 2009). Therefore, this heating method can be safe to adopt without chemicals in the BSF premises. However, in general, the heating method has several disadvantages, such as the cost of heating, the damage that may cause to the internal structure (Mul *et al*., 2009; Sparagano *et al*., 2014). Furthermore, some mites can survive within cracks where the temperature is sublethal. Alternatively, the feed of the BSF colony can be frozen at -20°C for seven to ten days to reduce the chances of transferred mites into the BSF colony.

4.5.4.5 Lighting regimen

Red mites feeding behaviour on poultry (Chauve, 1998; Fiddes *et al*., 2005) and BSF colonies happen in long darkness periods. They look for hosts one hour after dark, although the feeding often took place 5 to 11 h in the dark (Sparagano *et al*., 2014). Previous studies reported that intermittent lighting reduced the presence of red mites in poultry houses. For example, a continuous cycle of repeated 15 minutes of light and 45 minutes of dark reduced red mites presence compared to16 cycles of 15 minutes' light and 45minutes' darkness followed by eight hours of darkness every 24 h (Mul *et al*., 2009; Stafford *et al*., 2006). Another lighting regime reported success in commercial poultry farms was three hours' light and three hours' darkness

(Stafford *et al*., 2006). This approach can be worth exploring within the BSF colony as it does not involve harmful chemicals. However, the downside of this approach might be the extra expenses of lighting. In addition, BSFL are negatively phototropic, and the continuous lighting may reduce their feed intake in the long term.

4.5.4.6 Design of premises and hygiene

A simple change in the design of insect breading rooms can reduce the risk of red mites spreading. However, it may not be realistic to remove all red mite refugia areas but reduce them, which could be effective (Mul *et al*., 2009; Sparagano *et al*., 2014). Furthermore, proper hygiene and adequate cleaning are underestimated in preventing red mite spread. Studies showed that periodic cleaning with high pressured water and sanitary clearance could effectively prevent but not enough to eradicate infestations (Nordenfors and Hoglund, 2000; Sparagano *et al*., 2014).

4.6 Conclusions

Black soldier fly is known for its efficient bioconversion of organic wastes while producing high nutritional food and feed components within a relatively short life cycle. In recent years, BSF has gained considerable interest as a high-potential insect in the insect protein industry. Although the BSF is a non-pest species, rearing conditions may make it prone to diseases and predators. Due to a lack of knowledge and research on BSF diseases in the literature, BSF has been mistakenly reported to have high resistance to pathogens. The current findings highlight poultry red mites as an ectoparasite of BSF. Red mites can cause high mortality and economic loss to the BSF colony and jeopardise commercial insect protein production.

The work presented herein represents an essential contribution to the knowledge of pathogens for BSFL and BSFP. To our knowledge, this is the first study to document a parasitises outbreak in a BSF colony. More research is warranted to explore this finding further, especially ways of preventing red mite infestation.

The red mite infestation may result in the total loss of the BSF colony, requiring rebuilding the BFS colony in a mite-free environment. Several methods to control red mites include chemicals, natural oils, inert substrates, and physical control such as temperature and lighting. However, the best approach is to prevent the infestation from happening in the first place through careful hygienic procedures, including the examination of the feeds used, especially grains. In addition, it is recommended to keep the BSF colony away from wild birds or poultry farms. Finally, safe, periodic inert dusting or natural oil sparing can help control mite infestation.

Chapter 5: The Apparent Metabolisable Energy and Ileal Amino Digestibility of Black Soldier Fly (*Hermetia Illucens*) Larvae Meal for Broiler Chickens[1]

[1]This chapter was published as 'Mahmoud, A. E., Morel, P. C. H., Potter, M. A., & Ravindran, V. (2023). The apparent metabolisable energy and ileal amino digestibility of black soldier fly (*Hermetia illucens*) larvae meal for broiler chickens. *British Poultry Science,* 64 (3):377-383'.

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STATEMENT OF CONTRIBUTION
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Student name:	Amira El Sayed Abdalla Mahmoud
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In which chapter is the manuscript/published work?	Chapter 8

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Amira contributed to data collection, data analysis, initial and subsequent draft writing, and addressing feedback from supervisors. This involvement extended to preparing the final manuscript, responding to journal feedback.
Prof. Ravi Ravindran guided in experimental design, data analysis, manuscript writing and responding to reviewers, leading to acceptance.
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Abstract

Two experiments were conducted to determine the apparent metabolisable energy (AME) and standardised amino acid digestibility coefficients (SIDC) of black soldier fly larvae (BSFL) for broiler chickens. The BSFL contained, on a g/kg dry matter basis: crude protein, 486; crude fat, 320; ash, 58.5; neutral detergent fibre, 181; calcium, 6.8 and phosphorus, 9.1.In Experiment 1, an AME assay was performed wherein broilers were fed two experimental diets (a maise-soy basal diet and a test diet containing 250 g/kg BSFL) for 4 d from d 18 post hatch. The AME of BSFL was calculated based on the difference between the AME values of basal and test diets. The AME and nitrogen-corrected AME were determined to be 19.1 and 18.0 MJ/kg of dry matter, respectively. In Experiment 2, the ileal amino acid (AA) digestibility of BSFL was determined using 22-dayold broilers by the direct method and the digesta was collected on d 25. The ratios between the AA and titanium in the diet and digesta were used to calculate the apparent digestibility and then standardised using previously published endogenous losses to estimate the SIDC of AA. The SIDC of Lys, Met, Thr, Val and average SIDC of AA in our BSFL sample were 0.85, 0.90, 0.91, 0.87 and 0.84, respectively. The findings showed that the BSFL meal is a good source of available energy and digestible AAand could be a potential substitute for soybean meal in broiler diets.

Keywords: Amino acid digestibility; black soldier fly larvae; broilers; metabolisable energy

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5.1 Introduction

Globally poultry meat is the most popular animal protein consumed. It is projected that the global demand for poultry meat will continue in the future and that such a growth will have a profound effect on the demand for poultry feed and raw materials. This will be the greatest constraint to the future expansion of the poultry industry. In particular, sourcing conventional ingredients will become a major challenge and the obvious strategy is to search for and evaluate potential alternative ingredients. Among the various possibilities, insects represent a promising alternative protein source due to their nutrient composition, low environmental footprint (van Huis, 2013), favourable biomass conversion, and efficient land and water use. Furthermore, insects do not compete with humans for resources and can be reared on a wide range of organic wastes (Diener *et al*., 2011; Makkar *et al*., 2014; Nascimento Filho *et al*., 2021).

Insects have been a part of human diets since ancient times and many communities worldwide still consume insects. Inclusion of insects in poultry diets is, however, more acceptable to consumers rather than the direct consumption avoiding the aversion associated with insects in Western societies. Furthermore, insects are natural foods for free-range poultry (Khan, 2018; Nascimento Filho *et al*., 2021). The Food and Agriculture Organisation of the United Nations is promoting insects as a human food to alleviate protein malnutrition in developing countries (Kryger *et al*., 2010) and this has resulted in increased interest in evaluating the insects in animal feeding.

Insects produce high-value feed ingredients containing 370 to 630 g/kg of crude protein and 70 to 390 g/kg of fat on a dry matter basis (Makkar *et al*., 2014). Among the many insect species, the black soldier fly (BSF; *Hermetia illucens*) larvae (BSFL) are well suited to mass production because of their short life cycle, ease of rearing, and productivity (van Huis, 2013).

Given the growing interest in using BSFL in poultry feeding, the need for knowledge on its nutritional value is self-evident. In particular, information on the apparent metabolisable energy (AME) and amino acid (AA) digestibility of BSFL are needed for precise feed formulations, but published data on these aspects are scant. Despite the large volume of research on the production aspects, gross nutritional composition and feeding value of BSFL (Makkar *et al*., 2014; Abd El-Hack *et al*., 2020), only one published report is available on their AME and ileal AA digestibility of full-fat BSFL for broilers (De Marco *et al*., 2015). The specific objective of the present study was to determine the AME and, apparent ileal digestibility coefficients (AIDC) and standardised ileal digestibility coefficients (SIDC) of AA of full-fat BSFL for broiler chickens.

5.2 Materials and methods

The nutritional characterisation of BSFL meal was carried out in three phases, namely laboratory evaluation, AME assay, and ileal AA digestibility assay. The experimental procedures for animal trials were approved by the Massey University Animal Ethics Committee (protocol number: MU19/123) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

5.2.1 Preparation of BSFL meal

The methods used in rearing the BSFL was the same as previously described in Chapter 4, section 4.2.1. The mature BSFL were removed, washed with water and stored at -20°C. The BSFL were then dehydrated at 65°C in an Unitherm Drier for 24 h until the BSFL were dry, firm, and shrank in size. The average yield was 314 g/kg of the fresh weight (standard deviation, 34.1; n = 21). The dried material was ground in a hammer mill to pass through a screen size of 5.0 mm and stored at 4°C.

5.2.2 Birds and housing

Day-old male broiler (Ross 308) chicks, obtained from a commercial hatchery, were raised in a floor pen until 14 day and fed a commercial broiler starter diet (230 g/kg crude protein and 12.56 MJ/kg AME). The pens were located in an environmentally controlled house and, the temperature was maintained at 31°C for the first 7 day and then gradually reduced to 22°C by day 14. The birds received 20 h of fluorescent illumination per day. Central ceiling extraction fans and wall inlet channels controlled the ventilation. Birds had free access to water, and diets were offered *ad libitum*.

5.2.2.1 Experiment 1. Apparent metabolisable energy assay

The difference method was used to measure the AME of BSFL meal (Nalle *et al*., 2010). In this method, two diets were formulated: a basal diet (Table 8.1) and a test diet containing a 750 g/kg basal diet and 250 g/kg BSFL meal. The assay was based on the classical total excreta collection. On day 15 post hatch, chicks were individually weighed and assigned to cages (6 birds/cage; 6 cages/assay diet) on the basis of body weight so that each cage contained birds of equal average body weight (1600 ± 100 g; n = 12) in a completely randomised design. Diets, in pelleted form, were fed for seven days from day 15, with the first three days serving as an acclimation period. During the last four d (18 to 22 day), feed intake was monitored, and the excreta were collected quantitatively daily, weighed, and pooled within a cage. Pooled excreta were mixed well in a blender, and representative samples were obtained and freeze-dried. Dried excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4ºC until analysed for chemical composition.

5.2.2.2 Experiment 2. Ileal amino acid digestibility assay

On day 22, the birds used in the AME assay were fasted overnight, randomised, and used in the AA digestibility assay. The ileal AA digestibility of BSFL was determined by the direct method (Ravindran *et al*., 2005). A diet based on dextrose and BSFL, as the only source of protein, was formulated to supply 180 g/kg of dietary protein (Table 8.2). The diet contained titanium dioxide as an indigestible marker to determine the digestibility.

The assay diet, in mash form, was offered *ad libitum* for four days to six cages (6 birds/cage) of male broilers. On day 25, all birds were euthanised by an intracardial injection of diluted sodium pentobarbitone solution and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers (Ravindran *et al*., 2005). The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocaecal junction. The digesta from birds within a cage were pooled, frozen immediately after collection, and subsequently freeze-dried.

The diet and dried digesta samples were ground to pass through a 0.5 mm sieve and stored in airtight containers at 4°C until analyses for DM, titanium (Ti), N, and AA.

Table 5.1 Ingredient composition and analysis of the basal and test diets used in the apparent metabolisable energy assay (g/kg; as received basis), Experiment 1.

	Basal diet	Test diet[a]
Maise	611.4	-
Soybean meal	351.8	-
Dicalcium phosphate	21.7	-
Limestone	7.8	-
Salt	2.0	-
Sodium bicarbonate	2.3	-
Trace mineral premix[b]	2.5	-
Vitamin premix[b]	0.5	-
Analysed composition		
Dry matter	885	900
Gross energy	16.2	18.4
Nitrogen (N)	36	42
Crude protein (N×6.25)	225	265
Crude fat	28	103
Crude fibre	2	6
Ash	53	58
Neutral detergent fibre	80	118
Acid detergent fibre	24	48
Indispensable amino acids[c]		
Arg	14.2	14.6
His	5.5	7.0
Ile	9.0	10.8
Leu	17.5	18.9
Lys	12.8	15.0
Met	3.7	4.3
Phe	10.7	11.3
Thr	8.6	10.1
Val	10.7	14.3
Dispensable amino acids[c]		
Ala	10.5	16.0
Asp	24.6	25.9
Cys[c]	3.0	2.8
Gly[c]	9.6	12.6
Glu	40.1	38.0
Pro	12.8	15.3
Ser	10.6	12.3
Tyr	7.9	11.3

[a] Test diet was formulated by substituting 250g/kg (w/w) of the basal diet with BSFL meal.
[b] Provided per kilogram of diet: Co, 0.3 mg; Cu, 5 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Zn, 60 mg; choline chloride, 638 mg; *trans*-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamine, 3.0 mg; riboflavin, 12 mg; niacin, 35 mg; calcium pantothenate, 12.8 mg; pyridoxine, 10 mg; cyanocobalalamin, 0.017 mg; folic acid 5.2 mg; biotin, 0.2 mg; antioxidant, 100 mg; molybdenum, 0.5 mg; selenium, 200 µg.
[c] Semi-indispensable amino acids for poultry.

Table 5.2 Ingredient composition and analysis of the diet used in the ileal amino acid digestibility assay (g/kg, as received basis), Experiment 2.

Item	**value**
Black soldier fly larvae meal	450
Dextrose	491
Soybean oil	20
Titanium dioxide	3.0
Sodium bicarbonate	2.0
Dicalcium phosphate	19.0
Limestone	10
Trace mineral premix[a]	2.5
Vitamin premix[a]	0.5
Salt	2.0
Analysed composition	
Dry matter	897
Gross energy	192
Nitrogen (N)	30
Crude protein (N×6.25)	187
Crude fat	140
Crude fibre	32.2
Ash	59.9
Neutral detergent fibre	126
Acid detergent fibre	71.8
Indispensable amino acids	
Arg	9.02
His	5.53
Iso	7.97
Leu	12.77
Lys	10.73
Met	3.88
Phe	7.47
Thr	7.34
Val	12.11
Dispensable amino acids	
Ala	14.96
Asp	15.83
Cys[b]	1.39
Gly[b]	10.46
Glu	19.34
Pro	12.54
Ser	8.23
Tyr	12.06

[a] Provided per kilogram of diet: Co, 0.3 mg; Cu, 5 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Zn, 60 mg; choline chloride, 638 mg; trans-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamine, 3.0 mg; riboflavin, 12 mg; niacin, 35 mg; calcium pantothenate, 12.8 mg; pyridoxine, 10 mg; cyanocobalalamin, 0.017 mg; folic acid 5.2 mg; biotin, 0.2 mg; antioxidant, 100 mg; molybdenum, 0.5 mg; selenium, 200 µg.

[b] Semi-indispensible amino acids for poultry.

5.2.3 Chemical analysis

A representative sample of BSFL meal was analysed, in duplicate, for dry matter (DM), gross energy (GE), nitrogen (N), crude fat, crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), ash, minerals and AA.

Dry matter was determined after oven-drying the samples at 105ºC according to method 930.16 of the Association of Official Analytical Chemists (AOAC 2016). Ash was determined by complete combustion at 550°C in an electric furnace for 16 h (method 942.05; AOAC 2016). Organic matter was calculated as the difference between dry matter and ash contents. Nitrogen was determined (method 968.06; AOAC 2016) by combustion using a carbon nanosphere-200 carbon, N and sulfur autoanalyser (rapid MAX N exceed, Elementar, Donaustraze, Hanau, Germany). The crude protein (CP) content was calculated as N × 6.25. The crude fat was determined by Soxtec extraction procedure (Method 2003.06; AOAC 2016) using (Soxtec System HT 1043 Extraction Unit, Höganäs, Sweden). The diet samples were analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), and crude fibre using Tecator Fibertec™ (FOSS Analytical AB, Höganäs, Sweden) (method 2002.04; AOAC 2016). The gross energy was determined using a bomb calorimeter (Gallenkamp Autobomb, Weiss Gallenkamp Ltd, Loughborough, UK) standardised with benzoic acid.

For mineral analysis, the samples were wet acid digested with a nitric and perchloric acid mixture, and concentrations of phosphorus (P), potassium, calcium (Ca), magnesium, sodium and iron were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Thermo Jarrell Ash IRIS instrument (Thermo Jarrell Ash Corporation, Franklin, MA).The concentrations of copper, manganese and zinc were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) using a Perkin Elmer Elan 6000 instrument (Melbourne, VIC, Australia).

Amino acids were determined by hydrolysing the samples with HCl (containing phenol) for 24 h at 110 ± 2ºC in glass tubes sealed under vacuum. Amino acids were detected on a Waters ion exchange HPLC system, and the chromatograms were integrated using dedicated software (Millenium 32, Waters, Millipore, Milford, MA) with the AA identified and quantified using a standard AA solution (Pierce, Rockford, IL). Cysteine and methionine were analysed as cysteic acid and methionine sulphone by oxidation with performic acid for 16 hrs at 0ºC and neutralisation with hydrobromic acid prior to hydrolysis. Tryptophan was not determined.

5.2.4 Calculations

The AME of basal and test diets were calculated using the following formulas:

$$\text{AME diet(MJ/kg)} = \frac{\big((\text{feed intake (kg) X GEdiet(MJ)}\big) - (\text{excreta output (kg) X GE excreta (MJ)})}{\text{Total feed intake (kg)}}$$

Total tract N retention, as a percentage of intake, was determined as follows:

$$\text{N retention (\%)} = 100 \times \frac{((\text{FI} \times \text{Ndiet}) - (\text{Excreta output} \times \text{Nexcreta}))}{(\text{FI} \times \text{Ndiet})}$$

The AMEn was calculated by correction for zero N retention by assuming 36.54 KJ per g N retained in the body as described by Hill and Anderson (1958).

The AME or AMEn of BSFL was then calculated as follows.

$$\text{AME or AMEn (MJ/kg)} = \frac{\text{AME test diet} - (\text{AME basal diet X PC basal diet })}{\text{PC test diet}}$$

Where PC basal diet = Proportional contribution of GE of the basal diet in the test diet and PC BSFL diet = Proportional contribution of the energy of BSFL in the test diet.

Apparent total tract retention (ATTR) coefficients of dietary components in the BSFL were calculated as follows.

$$\text{Nutrient content X BSFL} = \frac{[\text{X BSFL diet} - \text{X basal diet} \times (\text{PCx basal diet})]}{\text{PCx BSFL diet}}$$

Where X represents DM, OM, ash, N or GE and PCx represents the proportional contribution of DM, OM, ash, N or GE.

$$\text{ATTR Coefficient} = \frac{\text{Digestible nutrient content}}{\text{Gross content}}$$

The apparent ileal digestibility coefficients (AIDC) and N and AA were calculated from dietary ratios of N and AA to Ti relative to corresponding ratios in the ileal digesta, as shown below.

$$\text{Apparent ileal Amino acid digestibility coefficient (AIDC)} = \frac{(\text{AA/Ti})\text{diet} - (\text{AA/Ti})\text{ileal}}{(\text{AA/Ti})\text{diet}}$$

where, (AA / Ti) diet = ratio of amino acid to titanium in diet

and (AA / Ti) ileal = ratio of amino acid to titanium in ileal digesta.

Apparent digestibility data for N and AA were then standardised using the average basal endogenous N and AA estimates (g per kg DM intake [DMI]) reported by Ravindran *et al.* (2021), as shown below.

$$\text{Standardised ileal digestibility coefficients (SIDC)} = \text{AIDC} + \frac{\text{Basal EAA (g/kg DMI)}}{\text{Ing. AA (g/kg DM)}}$$

Where, AIDC = apparent ileal digestibility coefficient of the amino acid;

Basal EAA = basal endogenous loss of the amino acid

Ing. AA = concentration of the amino acid in the ingredient.

5.3 Results

The data are presented on a dry matter basis, unless indicated otherwise.

The proximate, carbohydrate, mineral, and AA composition of BSFL are summarised in Table 8.3. Crude protein (CP) was the main component in the BSFL followed by crude fat. The BSFL was analysed to contain (g/kg DM basis) CP, 486; crude fat, 320; neutral detergent fibre, 181, calcium (Ca), 6.8 and phosphorus (P), 9.3. Among the indispensable AA, the contents of branched-chain AA, Leu and Val, were highest, followed by Lys, whereas the lowest content was determined for Met. Glu and Asp were the major dispensable AA, and Cys had the lowest content.

Table 5.3 Proximate, carbohydrate, mineral, and amino acid composition of black soldier fly larvae (BSFL; *Hermetia illucens*L.) (dry matter basis)[a]

Proximate and carbohydrate composition (g/kg)		**Indispensable amino acids (g/kg)**	
Gross energy (kj/g)	26.6	Arg	22.0
Nitrogen (N)	77.8	His	13.4
Crude protein (N×6.25)	486.3	Ile	19.3
Crude fat	320.0	Leu	30.0
Crude fibre	62.3	Lys	27.7
Ash	58.5	Met	7.6
Neutral detergent fibre	181.0	Phe	17.1
Acid detergent fibre	92.5	Thr	18.4
Minerals		Val	29.4
Calcium, g/kg	6.8	**Dispensable amino acids**	
Magnesium, g/kg	3.3	Ala	36.9
Potassium, g/kg	13.4	Asp	42.5
Sodium, g/kg	1.2	Cys[b]	3.3
Phosphorus, g/kg	9.3	Gly[b]	26.0
Iron, g/kg	1.6	Glu	48.9
Aluminium, mg/kg	25.5	Pro	27.3
Copper, mg/kg	10.3	Ser	20.3
Manganese, mg/kg	287	Tyr	26.5
Zinc, mg/kg	154.0		
Chloride, g/kg	3.4		
Heavy metals			
Arsenic, mg/kg	<0.10		
Cadmium, mg/kg	0.17		
Lead, mg/kg	0.35		

[a] Dry matter content of black soldier fly larvae (BSFL) meal, 941 g/kg.
[b]Semi-indispensable amino acids for poultry.

The AME and AMEn of BSFL were determined to be 19.1 and 18.0 MJ/kg DM, respectively (Table 8.4). The ATTRC of DM, N, GE, organic matter and ash were 0.70, 0.49, 0.72, 0.76, 0.64 respectively (Table 8.4).

Table 5.4 Apparent metabolisable energy (AME) and apparent total tract retention coefficients (ATTRC) of dry matter, organic matter, ash, nitrogen, and gross energy of black soldier fly larvae (BSFL; *Hermetia illucens* L.), Experiment 1[a,b]

Item	
Metabolisable energy, MJ/kg dry matter	
AME basal diet	15.4
Nitrogen-corrected AME basal diet	14.6
AME test diet	16.3
Nitrogen-corrected AME test diet	15.5
AME of BSFL	19.1
Nitrogen-corrected AME of BSFL	18.0
ATTR coefficient	
Dry matter	0.70
Organic matter	0.76
Ash	0.64
Nitrogen	0.49
Gross energy	0.72

[a] Each value represents the mean of six replicates (six birds per replicate).
[b] Measured by total excreta collection between day 18 and 22 post hatch.

The AA in the BSFL were well digested, with an average AIDC of 0.82 and SIDC of 0.84 (Table 8.5). The AIDC of indispensable AA ranged between 0.77 (Thr) and 0.88 (Met). The SIDC of indispensable AA ranged from 0.82 for His to 0.91 (Thr). Among the dispensable AA, Cys had the lowest AIDC and SIDC of 0.55 and 0.61, respectively.

Table 5.5 Apparent and standardised ileal amino acid digestibility coefficients andcontents (g/kg dry matter) of black soldier fly larvae (BSFL; *Hermetia illucens* L.) for broiler chickens, Experiment 2[a,b]

	Digestibility coefficient		**Digestible amino acid content (g/kg dry matter)**	
	Apparent	**Standardised[c]**	**Apparent**	**Standardised[c]**
Crude protein (Nitrogen × 6.25)	0.74 ± 0.03	-	356 ± 2.71	-
Indispensable amino acids				
Arg	0.87 ± 0.02	0.89 ± 0.02	19.5 ± 0.34	19.8 ± 0.34
His	0.80 ± 0.03	0.82 ± 0.03	10.7 ± 0.36	11.0 ± 0.36
Ile	0.86 ± 0.02	0.87 ± 0.02	16.5 ± 0.35	16.9 ± 0.35
Leu	0.87 ± 0.02	0.88 ± 0.02	26.0 ± 0.50	26.5 ± 0.50
Lys	0.84 ± 0.02	0.85 ± 0.02	23.3 ± 0.63	23.6 ± 0.63
Met	0.88 ± 0.01	0.90 ± 0.01	6.71 ± 0.11	6.84 ± 0.11
Phe	0.87 ± 0.02	0.89 ± 0.02	14.9 ± 0.29	15.2 ± 0.29
Thr	0.77 ± 0.03	0.91 ± 0.01	14.2 ± 0.57	14.8 ± 0.57
Val	0.85 ± 0.02	0.87 ± 0.02	25.0 ± 0.59	25.4 ± 0.59
Dispensable amino acids				
Ala	0.86 ± 0.02	0.87 ± 0.02	31.0 ± 0.69	31.4 ± 0.69
Asp	0.82 ± 0.03	0.84 ± 0.03	34.9 ± 1.11	35.6 ± 1.11
Cys	0.55 ± 0.07	0.61 ± 0.07	1.83 ± 0.22	2.03 ± 0.22
Gly	0.76 ± 0.03	0.78 ± 0.03	19.8 ± 0.86	20.3 ± 0.86
Glu	0.82 ± 0.03	0.85 ± 0.03	40.2 ± 1.34	41.6 ± 1.34
Pro	0.85 ± 0.02	0.88 ± 0.02	23.3 ± 0.61	24.1 ± 0.61
Ser	0.80 ± 0.03	0.83 ± 0.03	16.2 ± 0.54	16.8 ± 0.54
Tyr	0.90 ± 0.01	0.91 ± 0.01	23.8 ± 0.39	24.0 ± 0.39
Overall mean[d]	0.82 ± 0.03	0.84 ± 0.02	-	-
Sum of all amino acids[e]	-	-	348 ± 9.5	356 ± 9.50

[a]Each value represents the mean of six replicates (six birds per replicate) ± standard deviation. [b]Measured on d 25 posthatch.
[c]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg dry matter intake): Arg, 0.29; His, 0.15; Ile, 0.30; Leu, 0.43; Lys, 0.29; Met, 0.09; Phe, 0.31; Thr, 0.50; Val, 0.37; Ala, 0.30; Asp, 0.58; Cys, 0.18; Pro, 0.43; Gly, 0.36; Glu, 0.77; Ser, 0.45; and Tyr, 0.23 (Ravindran 2021).
[d]Average of 17 amino acids. [e]Sum of 17 amino acids.

5.4 Discussion

Soybean meal (SBM) is the most widely used protein source in poultry diets worldwide. Its popularity is due to favourable attributes such as relatively high CP content, an excellent AA profile and high AA digestibility. The demand for SBM is on the increase due to the continuing expansion of the global poultry industry and cannot be met by current soybean production. Thus, there is an urgent need to search for and evaluate alternatives for SBM. In this context, BSFL is emerging as a potential feed ingredient. The present findings show that the protein content and, the ileal digestibility coefficients and digestible AA contents in BSFL compare closely with those reported for SBM (Ravindran *et al*., 2014a). The SIDC of Thr is higher in BSFL (0.91) than in the SBM (0.74 to 0.78), while that of Cys (0.61) was within the range found in SBM (0.59 to 0.66) (Ravindran *et al*., 2014a). These findings suggest that BSFL can completely replace SBM as a protein source in broiler diets. It is also noteworthy that, similar to SBM, the contents of Leu, Val, Ile and Lys were high and those of sulphur-containing AA (Met and Cys) were deficient in the BSFL.

The protein and AA contents of BSFL are lower than those of animal protein sources, such as fish meal and, meat and bone meal (Bryden *et al*., 2009; NRC, 2012; Heger *et al*., 2016). On the other hand, these nutritional attributes in the BSFL are superior to plant protein sources, like canola meal, sunflower meal and grain legumes, that are considered as SBM alternatives in poultry diets (van der Poel *et al*., 2013; Heger *et al*., 2016; Wiltafsky *et al*., 2016; Iji *et al*., 2017). In addition, most plant-based SBM alternatives contain relatively high levels of fibre and/or anti-nutritional compounds limiting their inclusion rates and the replacement value (Ravindran and Blair, 1991).

The analysed composition of BSFL meal was within the range reported in the literature (St-Hilaire *et al*., 2007; Sánchez-Muros *et al*., 2014; De Marco *et al*., 2015; Cullere *et al*.,2016; Spranghers *et al*., 2017; Marono *et al*., 2017; Mwaniki *et al*., 2018a). However, the wide variability in the nutrient composition, especially in fat and protein contents, of BSFL is also recognised, depending on larval age and the rearing substrate (Makkar *et al*., 2014; Abd El-Hack *et al*., 2020; Do *et al*., 2020).

A notable feature of most insect meals is their high fat contents. In the current work, the crude fat content of BSFL was determined to be 320 g/kg; this is substantially higher than those reported for full-fat soybeans (177 to 192 g/kg; (Ravindran *et al*., 2014a) and SBM (10.9 to 20.5 g/kg; (Ravindran *et al*., 2014b). However, much lower fat contents (153 to 207 g/kg) for have been reported in some studies (Matin *et al*., 2021b) and contents of up to 408 g/kg in other studies (Makkar *et al*., 2014; Wang and Shelomi, 2017). These findings suggest that the fat content varies widely depending on the source of BSFL. Although the efficiency of fat utilisation was not determined in the current work, some evidence suggest that the fat in BSFL to be almost completely digested, with an ATTRC of 0.98 (Schiavone *et al*., 2017). Full-fat BSFL, therefore, provides an additional advantage in supplying energy in high-energy broiler diets.

The BSFL was found to contain 62.3 g/kg of crude fibre. According to Lovell *et al*., (1968), the crude fibre provides a rough estimate of the chitin present in insects. Chitin, a nitrogen-containing carbohydrate (N-acylated glucosamine polysaccharide) is the main component of the exoskeleton of insects. Chitin is insoluble in both water and acid, inert and nutritionally unavailable to poultry (Razdan and Pettersson, 1994). Chitin is reported to have no adverse effects on poultry performance (Kobayashi and Itoh, 1991).

To the author's knowledge, no published data are available on the complete mineral profile of the BSFL. In the present evaluation, among major minerals, potassium (13.4 g/kg), Ca (6.8 g/kg), and P (9.3 g/kg) were determined in the greatest concentrations. Of the minerals, the contents of Ca and P are crucial owing to their roles in skeletal health. Matin *et al*., (2021a) reported higher Ca (13.4 and 22.8 g/kg) and P (10.3 and 11.1 g/kg) contents of full-fat BSFL samples. Even higher Ca content (44.3 g/kg) has been reported by Moula *et al*., (2018) in BSFL reared on horse manure. No information was provided on the Ca content in the manure, and it is difficult to ascertain whether the substrate Ca contributed to the observed anomaly. Among the trace minerals, manganese and zinc were found in the greatest concentrations and it is possible that BSFL may be accumulating these two minerals. The Ca and P contents determined for BSFL in the current work were higher than those observed for SBM in the literature (NRC, 2012; Ravindran *et al*., 2014b).

Accurate information on the AME of feed ingredients is required because energy is the costliest component in poultry diets. In the current study, the AME and AMEn of full-fat BSFL were estimated to be 19.1 and 18.0 MJ/kg DM, respectively. These values were found to be higher than the values of 17.38 and 16.60 MJ/kg DM, respectively, reported by De Marco *et al*., (2015). Schiavone *et al*., (2017) determined lower AME and AMEn values in partially defatted BSFL meal containing 180 g/kg fat (16.25 and 14.87 MJ/kg, respectively). In their study, the corresponding values for highly defatted BSFL containing 46 g/kg fat were found to be markedly lower (11.55 and 9.87 MJ/kg, respectively). The AME of even the highly defatted BSFL meal was substantially higher than the values of 8.4 to 9.9 MJ/kg DM reported for SBM (Ravindran et al. 2014b). The AME of four samples of full-fat soybeans were reported to be 12.62 to 15.46 MJ/kg DM (Ravindran *et al*., 2014a). The higher AME of BSFL is evidently reflective of its high fat content and retention of fat.

Published data on the ileal AA digestibility of BSFL for broilers are scant. Reports on the AIDC of AA in full-fat BSFL for broilers are limited to only one solitary study (De Marco *et al*., 2015). Schiavone *et al*. (2017) and Mwaniki and Kiarie (2018b) determined the ileal AA digestibility of defatted BSFL for broilers. A study by Matin *et al*. (2021b) reported the SIDC of four samples of BSFL using a precision-fed rooster assay. In the present study, determined values for the AIDC of individual AA in full-fat BSFL ranged from 0.55 (Cys) to 0.90 (Tyr). Cys, Thr, Gly and Ser were the AA least digested in BSFL. The AIDC of Lys, Met, Thr and average AIDC of AA (0.84, 0.88, 0.77, and 0.82, respectively) of our sample were higher than the corresponding values (0.80, 0.81,0.75 and 0.79, respectively) reported for partially and highly defatted samples by Schiavone *et al*. (2017) and were slightly lower than the values (0.87, 0.85, 0.75 and 0.82, respectively) reported for full-fat BSFL by De Marco *et al*. (2015). The discrepancies among the three studies can be speculated to be due to the attributed aspects of BSFL (rearing substrate, processing method) and differences in experimental procedures (methodology, age of birds). Do *et al*. (2020) reported that the AA concentration and digestibility in BSFL were influenced by larval age. It has also been speculated that the high fat content may have negative effects on the nutrient utilisation of BSFL (Schiavone *et al*., 2017). The present findings, however, do not support this contention. Furthermore, this speculation is counter-intuitive because fat is known to slow down the rate of feed passage through the digestive tract (Mateos and Sell, 1981), allowing more time for better digestion and absorption of nutrients in poultry.

The SIDC of Lys, Met, Thr, Val and average SIDC of AA in our BSFL sample were 0.85, 0.90, 0.91, 0.87 and 0.84, respectively. Using the precision-fed rooster assay, Matin *et al*., (2021b) similarly reported generally high SIDC of AA in four samples of BSFL. However, for reasons unknown, a much lower Val digestibility of 0.60 to 0.81 was determined in their samples.

5.5 Conclusions

The present findings demonstrate that the nutritional profile of BSFL, especially the contents and digestibility of AA, closely resembles that of SBM and can be used as a potential SBM alternative in broiler diets. The higher AME contents, compared to SBM, makes BSFL attractive both as a protein and energy source.

Chapter 6: The apparent metabolisable energy and ileal amino digestibility of black soldier fly (*Hermetia illucens*) pre-pupae meal for broiler chickens

Abstract

Two experiments were conducted to determine apparent metabolisable energy (AME), and ileal amino acid digestibility of the black soldier fly pre-pupae (BSFP) for broiler chickens. The BSFP meal contained, on a g/kg dry matter basis: crude protein, 463; crude fat, 251; ash, 61.7; neutral detergent fibre, 173.9; calcium, 8.9 and phosphorous, 7.5. Experiment 1 was an AME assay wherein broilers were fed two experimental diets (a maize-soybean meal basal diet and a test diet containing 250 g/kg BSFP meal) for 7 days from day 23 post-hatch. The AME of BSFP meal was calculated based on the difference between the AME values of basal and test diets. The AME and nitrogen-corrected AME were determined to be 18.2 and 16.7 MJ/kg dry matter, respectively. In Experiment 2, the ileal amino acid (AA) digestibility of BSFP meal was determined using 30-day old broilers by the direct method and the ileal digesta was collected on day 34. The standardised ileal digestibility coefficients of Lys, Met, Thr, Val and average of all AA were determined to be 0.83, 0.89, 0.82, 0.82 and 0.82, respectively. The findings demonstrated that the BSFP meal is a good source of energy and, digestible AA, and is potentially a substitute for soybean meal in broiler diets.

Keywords: Metabolisable energy; amino acid digestibility; broilers; black soldier fly; pre-pupae.

6.1 Introduction

The world population is expected to reach 9.6 billion by 2050, up from the 7.9 billion in 2020 (Leridon, 2020). This population increase will be primarily in the middle class of developing and emerging economies and will be characterised by higher incomes and greater purchasing power. Population expansion and urbanisation are the other drivers of food consumption. They also influence the demand for type of food, which will shift from grains to animal products such as meat, milk, and eggs (Bouwman, 1997). Today chicken meat is the most consumed animal protein worldwide and this trend is projected to continue in the future (Mottet & Tempio, 2017).

The greatest single constraint that limits the future expansion of poultry production is sourcing adequate quantities of quality feed ingredients. Soybean meal (SBM), the traditional protein feedstock, requires large amounts of land and water, contributing to climate change (Mottet and Tempio, 2017). Among the possible options, insects represent a promising alternative protein source due to their nutrient profile and low environmental footprint (van Huis, 2013). Furthermore, insects do not compete with humans for resources and can be reared on a wide range of organic wastes (Diener *et al*., 2011; Makkar *et al*., 2014; Nascimento Filho *et al*., 2021). Therefore, the production and use of insects for animal feed can contribute to reducing food insecurity, and improving human nutrition, both are important to attain the first United Nations Sustainable Development Goal (Moruzzo *et al*., 2021).

Insects can be high-value feed ingredients as they contain 370 to 630 g/kg of crude protein and 70 to 390 g/kg of crude fat on a dry matter basis (Makkar *et al*., 2014; Abd El-Hack *et al*., 2020). Among the insect species, the black soldier fly (BSF; *Hermetia illucens*) is well suited to mass production because of their short life cycle and ease of rearing and productivity (van Huis, 2013). Two stages of BSF, namely larvae (BSFL) and pre-pupae (BSFP), could be used as insect meals. The BSFL are vigorous eaters, cream-coloured and avoid light (Booth and

Sheppard, 1984; May, 1961; Surendra *et al*., 2016). They often spend 2 to 3 weeks feeding before maturing into pre-pupal stage. BSFP are brown in colour and are no longer interested in feeding. Instead, BSFP actively migrate to semi-dry and shady places to pupate (May, 1961; Ushakova *et al*., 2017). When used in conjunction with a suitable apparatus, this migration activity can be used to self-harvest the BSFP, thus reducing labour and the cost of production (Madau *et al*., 2020).

Despite the growing interest in using BSF meal in poultry feeding, the available knowledge about its nutritional value is primarily focused on BSFL, with limited information available for BSFP. Specifically, data on the apparent metabolisable energy (AME) and amino acid (AA) digestibility of BSF meals are crucial for precise feed formulations. Only one published report exists on the AME of full-fat BSFP for broilers (Uushona, 2015). The specific objective of the present chapter was to determine the AME and, for the first time, the apparent ileal digestibility coefficients (AIDC) and standardised ileal digestibility coefficients (SIDC) of AA of full-fat BSFP for broiler chickens.

6.2 Materials and methods

The nutritional evaluation of BSFP meal was carried out in three stages, namely, laboratory evaluation, AME assay, and ileal AA digestibility assay. The experimental procedures for animal trials were approved by the Massey University Animal Ethics Committee (protocol number: MU19/123) and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

6.2.1 Preparation of BSFP meal

The methods used in rearing the BSFL was the same as previously described in Chapter 2, section 2.2.1. The BSFL were allowed to develop into the pre-pupal stage (Chapter 2; Figure 2.23). The BSFP were collected in a self-harvest system with a 35^{o} angle to ensure homogenous age among all BSFP (as discussed in Chapter 2; section 2.7.2. BSFP harvest; Figure 2.26). The BSFP were removed, washed with water and stored at -20°C. The BSFP were then loaded in trays and slowly dehydrated at 65°C in a Unitherm Drier for 24 h, until the BSFP were dry, firm, and shrank in size. The yield was between 350 to 400 g/kg of the fresh weight. The dried material was ground in a hammer mill to pass through a screen size of 5.0 mm and stored at 4 ºC.

6.2.2 Birds and housing

Day-old male broiler (Ross 308) chicks, were obtained from a commercial hatchery and reared following the methods detailed in Chapter 5, Section 5.2.2, which covers bird management and housing.

6.2.2.1 Experiment 1. Apparent metabolisable energy assay

The difference method was used to measure the AME of BSFP meal (Nalle *et al*., 2010). Two diets were formulated: a basal diet, and a test diet containing a 750 g/kg basal diet and 250 g/kg BSFP meal (Table 6.1). On day 23 post-hatch, chicks were individually weighed and assigned to cages (6 birds/cage; 6 cages/assay diet) on the basis of body weight so that the mean body weight of bird per cage was the same (1600 ± 100 g; n = 12) in a completely randomised design. Diets, in pelleted form, were fed for seven days from day 23, with the first three days serving as an adaptation period. During the last four days (27 to 30), feed intake was recorded, and excreta were collected daily, weighed, and pooled within the cage. Pooled excreta from each cage were mixed in a blender and a representative sample was removed and freeze-dried. Dried excreta samples were ground to pass through a 0.5 mm sieve and stored in airtight plastic containers at 4 ºC until analyses for dry matter (DM), nitrogen (N), and gross energy (GE).

Table 6.1 Ingredient composition and, analysis of the basal and test diets used in the AME assay (g/kg; as fed basis), Experiment 1

Item	Basal diet	Test diet[a]
Maize	611.4	-
Soybean meal	351.8	-
Dicalcium phosphate	21.7	-
Limestone	7.8	-
Sodium chloride	2.0	-
Sodium bicarbonate	2.3	-
Trace mineral premix[b]	2.5	-
Vitamin premix[b]	0.5	-
Analysed composition		
Dry matter	885	900
Gross energy (kj/g)	16.2	18.4
Nitrogen (N)	36	42
Crude protein (N×6.25)	225	265
Crude fat	28	103
Crude fibre	2	6
Ash	53	58
Neutral detergent fibre	80	118
Acid detergent fibre	24	48
Indispensable amino acids		
Arg	14.2	14.6
His	5.5	7.0
Ile	9.0	10.8
Leu	17.5	18.9
Lys	12.8	15.0
Met	3.7	4.3
Phe	10.7	11.3
Thr	8.6	10.1
Val	10.7	14.3
Dispensable amino acids		
Ala	10.5	16.0
Asp	24.6	25.9
Cys[c]	3.0	2.8
Gly[c]	9.6	12.6
Glu	40.1	38.0
Pro	12.8	15.3
Ser	10.6	12.3
Tyr	7.9	11.3

[a] Test diet was formulated by substituting 250g/kg (w/w) of the basal diet with black soldier fly pre-pupae meal.
[b] Provided per kilogram of diet: Co, 0.3 mg; Cu, 5 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Zn, 60 mg; choline chloride, 638 mg; *trans*-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamine, 3.0 mg; riboflavin, 12 mg; niacin, 35 mg; calcium pantothenate, 12.8 mg; pyridoxine, 10 mg; cyanocobalalamin, 0.017 mg; folic acid, 5.2 mg; biotin, 0.2 mg; antioxidant, 100 mg; molybdenum, 0.5 mg; selenium, 200 µg.
[c] Semi-indispensable amino acids for poultry.

6.2.2.2 Experiment 2. Ileal amino acid digestibility assay

On day 30, the birds used in the AME assay were fasted overnight, randomised, and used in the AA digestibility assay.

The ileal AA digestibility of BSFP meal was determined by the direct method (Ravindran *et al*., 2005). A diet based on dextrose and BSFP meal, as the only source of protein, was formulated to supply 180 g/kg of dietary protein (Table 6.2). The diet contained titanium dioxide as an indigestible marker to calculate the digestibility.

The assay diet, in mash form, was offered *ad libitum* for four days to birds in six cages (6 birds/cage). On day 34, all birds were euthanised by an intracardial injection of sodium pentobarbitone, dissected and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers (Ravindran *et al*., 2005). The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocaecal junction. The digesta from birds within a cage were pooled, frozen immediately after collection, and subsequently freeze-dried.

The diet and dried digesta samples were ground to pass through a 0.5 mm sieve and stored in airtight containers at 4 ^{o}C until analyses for DM, titanium, N, and AA.

Table 6.2 Ingredient composition and analysis of the diet used in the ileal amino acid digestibility assay (g/kg as fed basis), Experiment 2

Item	Value
Black soldier fly pre-pupae meal	450
Dextrose	491
Soybean oil	20
Titanium dioxide	3.0
Sodium bicarbonate	2.0
Dicalcium phosphate	19.0
Limestone	10
Trace mineral premix[a]	2.5
Vitamin premix[a]	0.5
Sodium chloride	2.0
Analysed composition	
Dry matter	897
Gross energy (kj/kg)	192
Nitrogen (N)	30
Crude protein (N×6.25)	187
Crude fat	140
Crude fibre	32.2
Ash	59.9
Neutral detergent fibre	126
Acid detergent fibre	71.8
Indispensable amino acids	
Arg	9.02
His	5.53
Iso	7.97
Leu	12.77
Lys	10.73
Met	3.88
Phe	7.47
Thr	7.34
Val	12.11
Dispensable amino acids	
Ala	14.96
Asp	15.83
Cys[b]	1.39
Gly[b]	10.46
Glu	19.34
Pro	12.54
Ser	8.23
Tyr	12.06

[a] Provided per kilogram of diet: Co, 0.3 mg; Cu, 5 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Zn, 60 mg; choline chloride, 638 mg; trans-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-α-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamine, 3.0 mg; riboflavin, 12 mg; niacin, 35 mg; calcium pantothenate, 12.8 mg; pyridoxine, 10 mg; cyanocobalalamin, 0.017 mg; folic acid 5.2 mg; biotin, 0.2 mg; antioxidant, 100 mg; molybdenum, 0.5 mg; selenium, 200 µg.

[b] Semi-indispensable amino acids.

6.2.3 Chemical analysis

A representative sample BSFP meal that was offered to all the replicated cages, was analysed, in duplicate, for dry matter (DM), gross energy (GE), nitrogen (N), crude fat, crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), ash, minerals, heavy metals, and AA.

The methods employed for these analyses were previously detailed in Chapter 5, Section 5.2.5.

6.2.4 Calculations

The equations employed for calculating the AME and AME corrected for N retention (AMEn) of both basal and test diets, along with the apparent total tract retention (ATTR), Apparent ileal digestibility coefficients (AIDC), and standardised ileal digestibility coefficients (SIDC), were previously reported in Chapter 5, Section 5.2.6.

6.3 Results

The proximate, carbohydrate, mineral, and AA composition of BSFP meal are summarised in Table 6.3. Crude protein was the main component in the BSFP meal, followed by crude fat. Among the indispensable (essential) AA, the contents of branched-chain AA, Leu and Val, were highest, followed by Lys, whereas the lowest content was determined for Met. Glu and Asp were the major dispensable (non-essential) AA, and Cys had the lowest value.

Table 6.3 Proximate and chemical composition of black soldier fly pre-pupae (BSFP; *Hermetia illucens* L.) meal (dry matter basis)[a].

Item, g/kg		**Indispensable amino acids (g/kg)**	
Gross energy (kj/g)	25.1	Arg	20.1
Nitrogen (N)	74.1	His	11.5
Crude protein (N×6.25)	463	Ile	16.1
Crude fat	251	Leu	26.0
Crude fibre	28.3	Lys	22.2
Ash	61.7	Met	9.04
Neutral detergent fibre	173.9	Phe	15.8
Acid detergent fibre	99.8	Thr	16.3
Mineral composition		Val	25.2
Calcium, g/kg	8.9	**Dispensable amino acids**	
Magnesium, g/kg	4.0	Ala	30.6
Potassium, g/kg	9.0	Asp	34.3
Sodium, g/kg	0.76	Cys[b]	4.22
Phosphorus, g/kg	7.50	Gly[b]	22.2
Iron, g/kg	0.08	Glu	36.8
Aluminium, mg/kg	31.1	Pro	26.1
Copper, mg/kg	10.0	Ser	17.2
Manganese, mg/kg	589.4	Tyr	25.8
Zinc, mg/kg	152.1		
Chloride, g/kg	1.68		
Heavy metal content			
Arsenic, mg/kg	<0.10		
Cadmium, mg/kg	0.11		
Lead, mg/kg	0.51		

[a] Dry matter content of black soldier fly pre-pupae meal, 933 g/kg.
[b]Semi-indispensable amino acids for poultry.

The AME and AMEn of BSFP meal and the ATTRC of DM, N, GE and ash are presented in Table 6.4.

Table 6.4 Apparent metabolisable energy (AME) and apparent total tract retention coefficients (ATTRC) of dry matter, ash, nitrogen and gross energy in the basal and test diets and black soldier fly pre-pupae (BSFP; *Hermetia illucens* L.) meal for broiler chickens, Experiment 1[a,b]

Item	
Metabolisable energy, MJ/kg dry matter	
AME basal diet	14.0
Nitrogen-corrected AME basal diet	13.1
AME test diet	15.1
Nitrogen-corrected AME test diet	14.0
AME of BSFP meal	18.2
Nitrogen-corrected AME of BSFP meal	16.7
ATTR coefficient	
Dry matter	0.59
Ash	0.58
Nitrogen	0.58
Gross energy	0.73

[a] Each value represents the mean of six replicates (six birds per replicate).
[b] Measured by total excreta collection between day 27 and 30 post-hatch.

The AA in the BSFP meal were well digested, with an average AIDC of 0.77 and SIDC of 0.82 (Table 6.5). The AIDC of indispensable AA ranged between 0.73 (Thr) and 0.86 (Met). The SIDC of indispensable AA ranged between 0.82 for Thr and Val to 0.89 (Met). Among the dispensable AA, Cys had the lowest AIDC and SIDC of 0.52 and 0.62, respectively.

Table 6.5 Apparent and standardised ileal amino acid digestibility coefficients and contents (g/kg dry matter) of black soldier fly pre-pupae (BSFP; *Hermetia illucens* L.) meal for broiler chickens, Experiment 2[a,b]

	Digestibility coefficient		Digestible protein/ amino acid content	
	Apparent	**Standardised**[c]	**Apparent**	**Standardised**[c]
Crude protein (Nitrogen x 6.25)	0.69 ± 0.01	-	431.8 ± 8.65	-
Indispensable amino acids				
Arg	0.84 ± 0.02	0.87 ± 0.01	5.63 ± 0.66	18.8 ± 0.41
His	0.78 ± 0.02	0.83 ± 0.02	4.17 ± 0.33	10.2 ± 0.24
Ile	0.80 ± 0.01	0.86 ± 0.01	5.31 ± 0.38	14.8 ± 0.20
Leu	0.81 ± 0.02	0.85 ± 0.01	8.66 ± 0.63	23.6 ± 0.35
Lys	0.80 ± 0.03	0.83 ± 0.03	7.64 ± 1.13	19.7 ± 0.69
Met	0.86 ± 0.02	0.89 ± 0.02	2.60 ± 0.34	8.58 ± 0.18
Phe	0.83 ± 0.01	0.87 ± 0.01	5.06 ± 0.42	14.8 ± 0.24
Thr	0.73 ± 0.01	0.82 ± 0.01	6.77 ± 0.35	14.3 ± 0.23
Val	0.78 ± 0.01	0.82 ± 0.01	9.39 ± 0.59	22.1 ± 0.36
Dispensable amino acids				
Ala	0.80 ± 0.01	0.82 ± 0.01	10.6 ± 0.60	27.0 ± 0.30
Asp	0.78 ± 0.01	0.83 ± 0.01	13.0 ± 0.86	30.3 ± 0.55
Cys	0.52 ± 0.02	0.62 ± 0.02	2.29 ± 0.10	2.80 ± 0.08
Gly	0.69 ± 0.02	0.74 ± 0.02	10.7 ± 0.58	17.5 ± 0.42
Glu	0.79 ± 0.01	0.87 ± 0.02	14.2 ± 1.41	34.2 ± 0.75
Pro	0.77 ± 0.02	0.83 ± 0.02	10.3 ± 0.87	23.3 ± 0.57
Ser	0.74 ± 0.01	0.82 ± 0.01	7.01 ± 0.33	15.0 ± 0.22
Tyr	0.83 ± 0.01	0.85 ± 0.01	7.79 ± 0.58	23.5 ± 0.34
Overall mean[d]	0.77 ± 0.01	0.82 ± 0.02	-	-
Sum of all amino acids[e]	-	-	134 ± 10.3	320 ± 6.1

[a]Each value represents the mean of six replicates (six birds per replicate) ± standard deviation.
[b]Measured on d-25 post hatch.
[c]Apparent digestibility values were standardised using the following basal ileal endogenous flow values (g/kg dry matter intake): Arg, 0.29; His, 0.15; Ile, 0.30; Leu, 0.43; Lys, 0.29; Met, 0.09; Phe, 0.31; Thr, 0.50; Val, 0.37; Ala, 0.30; Asp, 0.58; Cys, 0.18; Gly, 0.36; Glu, 0.77; Pro, 0.43; Ser, 0.45; and Tyr, 0.23 (Ravindran, 2021).
[d]Average of 17 amino acids.
[e]Sum of 17 amino acids.

6.4 Discussion

Feed represents two-thirds of the total cost of poultry production. The most consequential components in poultry feed formulations are energy and protein (Ravindran, 2013). Broiler producers rely on grains to provide 60 to 70% of the energy supplied in poultry feeds, and the remainder comes from lipids and protein (van der Klis *et al*., 2010). Soybean meal (SBM) is the most widely used protein source due to its exceptional characteristics, such as relatively high CP content, an excellent AA profile and high AA digestibility. Although expanding the global poultry industry increases the demand for SBM, limited resources (*i.e*., land and water) restrict further expansion of soybean production. Thus, there is an urgent need to evaluate alternatives to SBM. On the other hand, insects such as BSF not only provide a potential replacement for SBM, but also can be reared on organic waste streams (i.e., food waste, animal manure and human faeces), which relieves the pressure on limited global resources (Newton *et al*., 2005; van Huis, 2013; Banks *et al*., 2014).

Two life stages (larval and pre-pupal) of BSF could be used as feed ingredients for poultry. Although BSFP meal provides similar nutritional value to BSFL, previous studies (De Marco *et al*., 2015; Schiavone *et al*., 2017; Moula *et al*., 2018; Do *et al*., 2020; Matin *et al*., 2021a; Matin *et al*., 2021b) have focused only on the BSFL meal. The other merits of BSFP have largely been overlooked. For instance, BSFP do not require a labour-intensive harvest. Unlike the BSFL, BSFP leave their feeding site, making it possible to be easily collected, separate from their rearing substrates, without the need for additional labour. Another characteristic of the BSFP is that they do not eat, and their guts are naturally empty of any food residues. This unique behaviour may offer an advantage over the BSFL in disease transmission and microbial loads. Recent evidence has suggested that replacing conventional dietary proteins with BSFP meal improved the gut microbiota in monogastrics and fish (Huyben *et al*., 2019; Abd El-Hack *et al*., 2020).

The protein content of BSFL and BSFP meals are lower (486.3 (Chapter 5) and 463 g/kg DM, respectively) than those in animal protein sources, such as fish meal (500 to 600 g/kg DM), and meat and bone meal (495 to 590 g/kg DM) (Bryden *et al*., 2009; NRC, 2012; Heger *et al*., 2016). On the other hand, the nutritional profile in both BSF meals is superior to plant protein sources such as canola meal, sunflower meal, and grain legumes (van der Poel *et al*., 2013; Heger *et al*., 2016; Wiltafsky *et al*., 2016; Iji *et al*., 2017). Furthermore, the BSF meals do not contain high fibre and/or anti-nutritional compounds, favouring the use of high inclusion levels in replacing SBM (Ravindran and Blair, 1991).

Published data on the composition of BSFP meal are scant. The analysed composition of BSFP meal in this study was found to be lower in fat and protein contents (251 and 463 g/kg DM) compared to the values (320 and 486 g/kg DM) for BSFL reported by Mahmoud *et al*., (2023). On the other hand, Uushona (2015) reported substantially lower values for fat (156 to 194 g/kg DM) and protein (255 to 261 g/kg DM) in the BSFP meal. The wide variability in the nutrient composition of BSF meals (BSFL and BSFP) is recognised, depending on the age of larvae or pre-pupae, and the rearing substrate (Liu *et al*., 2017). Generally, BSFP tends to be lower in fat content than the BSFL, as they have stopped eating and start mobilising their body fat, which is reflected in their fat content. Overall, insect meals are characterised by their relatively high-fat contents compared to those reported for full-fat soybeans (177 to 192 g/kg; Ravindran *et al*., 2014a) and SBM (10.9 to 20.5 g/kg; Ravindran *et al*., 2014b).

The digestibility of fat in BSFP meal was not measured in this study. Also, there are no data in the literature. However, the data on BSFL meal indicated high fat digestibility. One can assume the fat digestibility in BSFP meal is also high and it can serve as a good source of energy in poultry diets.

BSFP meal has a lower crude fibre content (26.4 g/kg) compared to BSFL (62.3 g/kg). This finding contrasted with the usual expectation that fibre content increased from the larval to the pre-pupal stage (Parry and Weldon, 2021). Crude fibre in insects is an indicator of chitin content, the main component of the exoskeleton (Lovell *et al*., 1968). Chitin is insoluble in water and acid, inert, and nutritionally unavailable to poultry (Razdan and Pettersson, 1994). However, it has been reported to have no adverse effects on poultry performance (Kobayashi and Itoh, 1991).

Soetemans *et al*. (2020) reported that the chitin levels in BSFP, BSFL, and adult flies were 31, 36, and 29 g/kg DM, respectively, with only the puparium exhibiting a high chitin concentration of 240 g/kg DM. This suggests that while BSFP and BSFL have relatively low chitin content, the puparium, which is not typically included in poultry feed, has significantly higher chitin levels. The lower crude fibre and chitin content in BSFP meal, compared to BSFL, makes it potentially more digestible and nutritionally beneficial for poultry.

To the author's knowledge, no published data are available on the complete mineral profile of the BSFP meal. In the present study, among major minerals, K, Ca, and P were present in the highest concentrations. The contents of Ca and P are crucial owing to their roles in skeletal health. Among the trace minerals, Mn and Zn were found in the greatest concentrations and it appears that the BSFP accumulated these two minerals. The Ca to P ratio was different between larvae and BSFP meals. The BSFP meal was higher in Ca but lower in P, while the ratio was opposite for the BSFL meal. The Ca and P contents determined for BSFP meal in the current work were higher than those observed for SBM in the literature (NRC, 2012; Ravindran *et al*., 2014b).

Energy is the costliest component in poultry diets and accurate information on the AME of feed ingredients is crucial. In the current study, the AME and AMEn of full-fat BSFP meal were determined to be higher (18.44 and 16.94 MJ/kg DM, respectively) than the values (17.40 and

16.52 MJ/kg DM, respectively) reported by Uushona (2015). Interestingly, the values for BSFP were lower than those of BSFL (19.1 and 18.0 MJ/kg DM, respectively) reported in Mahmoud *et al*. (2023). These differences in AME values can be explained based on the fat content of meals. For instance, the fat content of BSFP meal in this study was higher than that reported by Uushona (2015). Similarly, the fat content of the BSFL (320 g/kg DM; Mahmoud *et al*. 2023)) was higher than the BSFP meal (251 g/kg DM) in this study. However, it is noteworthy that the AME and AMEn values of BSF meals (BSFL and BSFP) were considerably higher than those reported for SBM. Reported AME values of SBM range from 12.62 to 15.46 MJ/kg DM and AMEn from 11.72 to 14.21 MJ/kg DM, respectively (Ravindran *et al*., 2014a).

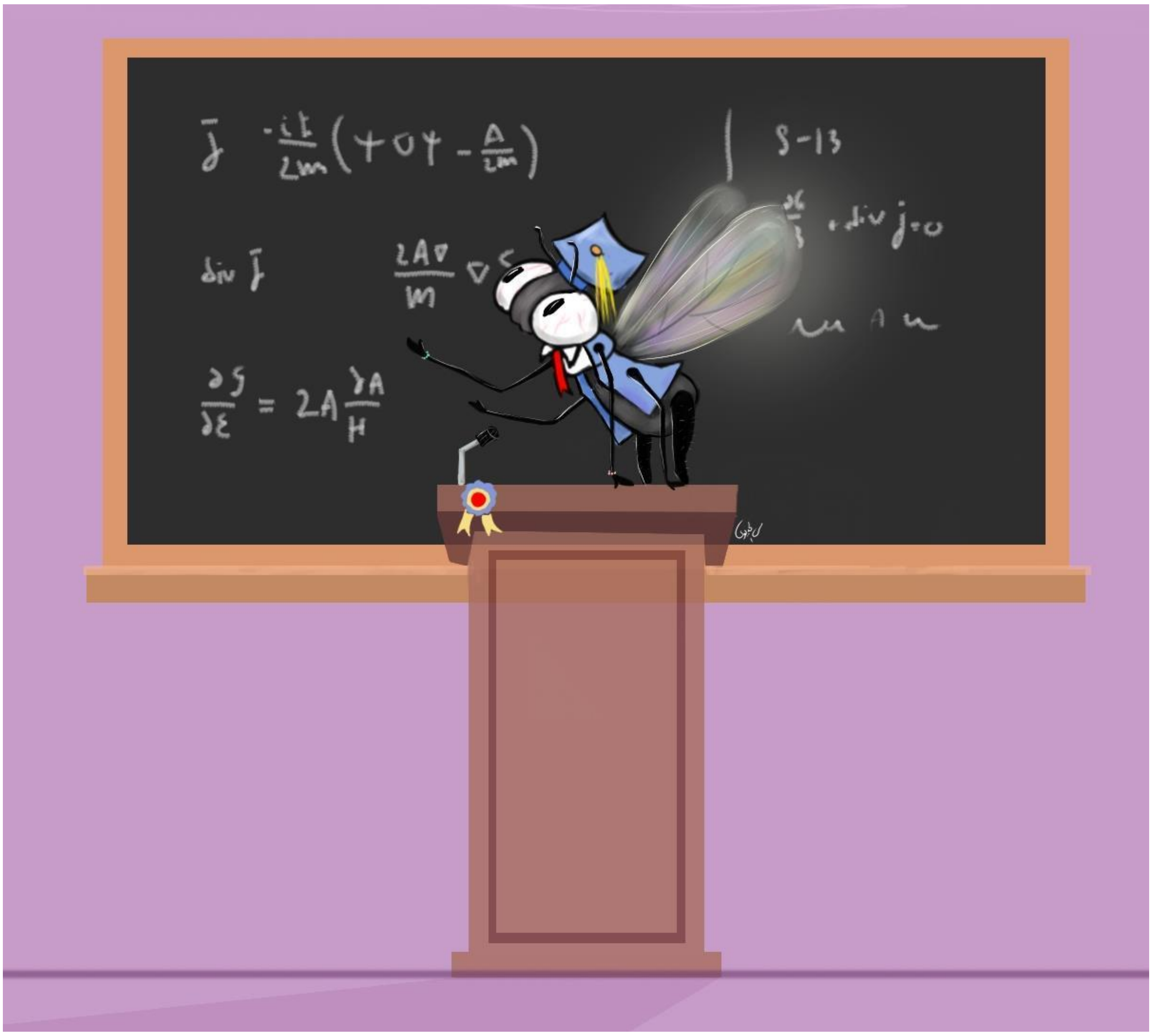
The current study is the first to report the AIDC and SIDC of BSFP meal for broilers. The values for the AIDC of individual AA in BSFP meal ranged from 0.52 (Cys) to 0.83 (Tyr). The AIDC of Lys, Met, Thre and average AIDC of AA were 0.80, 0.86, 0.73, and 0.77, respectively. Cys, Gly, Thr and Ser were the AA least digested in BSFP meal. The lower AIDC of these four AA in the BSFP meal most likely reflect their predominant presence in the endogenous protein (Ravindran, 2021).

The SIDC of Lys, Met, Thr, Val and average SIDC of AA in BSFP meal (0.83, 0.89, 0.82,0.82 and 0.82.) were lower than the values (0.85, 0.90, 0.91, 0.87 and 0.84) reported for BSFL by Mahmoud *et al*., (2023). Using precision-fed rooster assay by Matin et al. (2021b) reported similar high values of SIDC of AA in four samples of BSFL except for Val values were much lower (0.60 to 0.81) for unknown reasons. On the other hand, the SIDC of Thr was greater in the BSFP meal.

6.5 Conclusions

BSFP meal has a nutritional profile and digestibility of AA which compares favourably to SBM for use as a poultry feed ingredient. The AME of BSFP, on the other hand, is higher than that reported for SBM. The high AME values and fat content in BSFP meal makes it an attractive alternative for SBM to supply protein and energy. Furthermore, the self-harvesting aspect of BSFP can be advantageous to the insect meal industry by reducing the labour costs.

Chapter 7: General Discussion



7.1 Introduction

Between the significant annual food loss and waste (FLW) (Gustavsson *et al*., 2013; Wang *et al*., 2023) and the inefficiencies inherent in current organic waste management methods—such as open dumps, landfills, composting, and uncontrolled incineration—the utilisation of insect bioconversion of organic waste has emerged as a compelling solution. Among the various insects considered for waste management, the black soldier fly (BSF; *Hermetia illucens* L., Diptera: Stratiomyidae) stands out due to its various advantages (Chapter 1). BSF technology offers an efficient and environmentally clean method to decompose organic waste, recover lost nutrients, and convert waste into valuable resources such as sustainable animal feed ingredients, organic fertilisers, biodiesel, and several other applications (Figure 7.1).

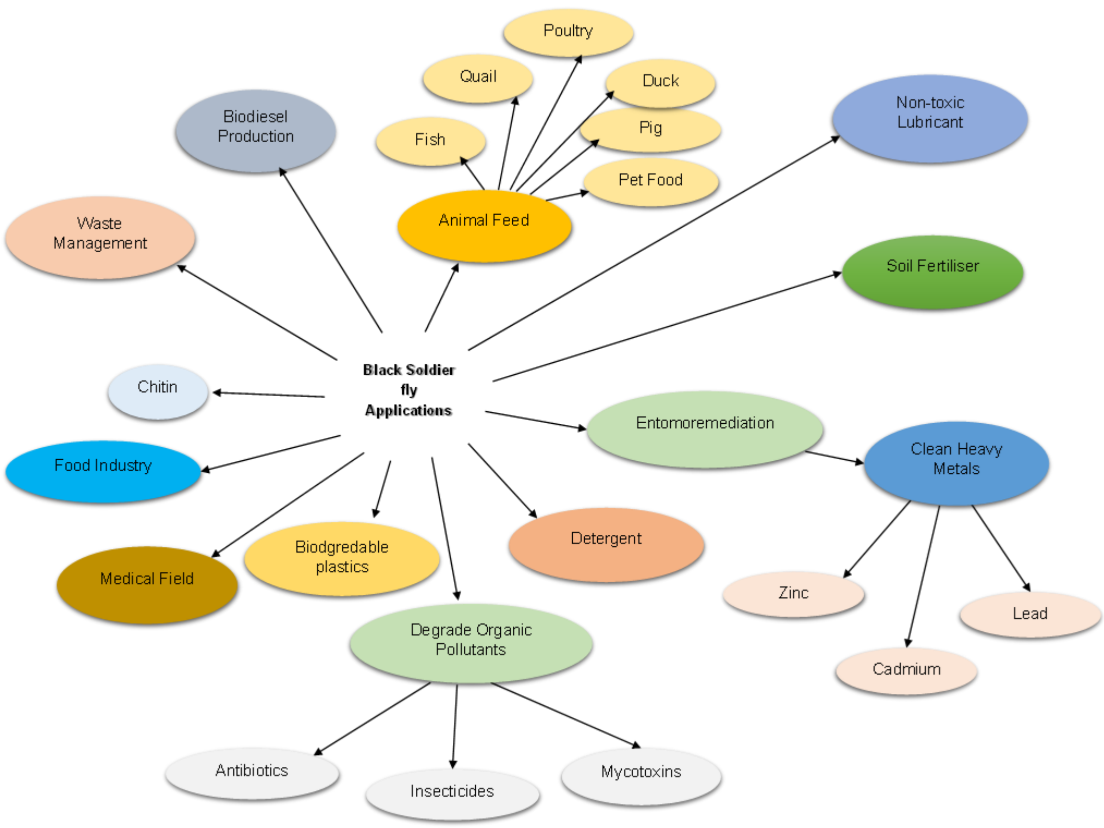


Figure 7.1 Potential applications of the black soldier fly (BSF; *Hermetia illucens*) products and by-products for food, feed and environment. Image by Mahmoud. A. E licensed under CC BY-ND 4.0)

Over the past decade, BSF bioconversion technology gained significant traction globally, with numerous operations integrating these applications. However, much of the knowledge and production techniques remain either underdeveloped or are dominated by large-scale operations, thus preventing the technology from reaching its full potential, particularly for smaller entities (Joly and Nikiema, 2019). Additionally, much of the existing research had focused on process engineering rather than on the practical implementation of BSF technology (Caruso *et al*., 2014; Dortmans *et al*., 2017; Surendra *et al*., 2020). It is therefore essential to bridge these knowledge gaps and develop methodologies tailored to the needs of small and medium-sized operations. My thesis research was designed to fill the gaps in (1) the knowledge and methods of BSF breeding and insect meal processing, derived from the production of 450 kg of BSFL, which is the first research scale that is relevant to small and medium-sized operations, (2) factors such as substrate type, moisture, and compaction, which influence the non-feeding stages of BSF (3) disease management in the BSF colony; the thesis reported the first parasite case within BSF colonies to ensure healthy population dynamics, and (4) study the nutritional potential of two developmental stages of BSF (larval and pre-pupal) as replacements for soybean (SBM) meal in poultry feeds.

7.2 Upscaling BSF bioconversion technology for small and medium scale production

An important limitation to the commercialisation of insects is the need to enhance the efficiency and scalability of BSF technology. Several companies like Ÿnsect, Protix, Entomo Farms, AgriProtein, EnviroFlight, Entosystem and JM Green have showcased the potential of large-scale BSF production systems. However, the focus should now be on developing more efficient, cost-effective, and scalable methods tailored to small and medium-scale BSF production. This emphasis on innovation is essential to drive the sustainable growth of the BSF industry.

Small and medium-scale operations offer several advantages, particularly in terms of on-site bioconversion of waste materials. This localised approach can enhance the economic feasibility of the technology and contribute to its sustainability. Notably, one advantage is the reduction in the transportation of waste materials, which is often required in large-scale operations. The transportation of waste materials to feed the BSF colony can contribute up to 18% of cost associated with the overall production process (Salomone *et al*., 2017; Smetana *et al*., 2021) along with associated costs for the price of fuel and vehicles. Moreover, small insect farms hold promises for future food security, aligning with the principles of a circular economy paradigm (Cadinu, 2020). Embracing this sustainable approach can pave the way for a more resilient and environmentally friendly food production system.

To achieve appropriate commercial and technological thresholds for profitability, it is imperative to develop innovative techniques for small and medium-scale BSF production. While the concept of BSF bioconversion technology may appear straightforward, its successful implementation demands a profound understanding of various complex stages (Figure 7.2). This involves understanding of the BSF's life cycle, specific rearing requirements for each stage, and seamlessly transitioning from one stage to the next. Key elements such as facilitating mating, efficient larval rearing, formulating balanced diets for larval growth and waste reduction, and processing insect biomass into usable BSF meals are critical components (Pleissner and Smetana, 2020). However, several challenges arise when striving to maintain year-round BSF colony production while efficiently managing the inherently labour-intensive work involved in the colony (Drew and Pieterse, 2015). To overcome these hurdles and promote small and medium-scale BSF production, a practical manual was outlined in Chapter 2. This manual provides a guided step-by-step approach to establishing and managing a BSF colony from the egg to larval and pre-pupal stages for meal processing.

Previous research has highlighted that labour makes up two-thirds of the total production cost of insect farming (Joly and Nikiema, 2019). Thus, it becomes crucial to develop efficient methods for minimising the labour requirements. Several applications were utilised in Chapter 2 to address this challenge and reduce the time spent on colony maintenance. For instance, at the adult stage, a continuous fly release method was implemented. This approach sustained egg production for up to 4 months and decreased the need for frequent cage changes, which typically occurred every cycle (~15 to 30 days). By releasing flies into the same cage on a weekly basis, the necessity for frequent cage changes was markedly reduced. Moreover, by allowing natural gender ratios to occur, the production process was further simplified, avoiding additional labour-intensive tasks.

Another aspect tackled was enhancement of adult longevity. Different feeding options for BSF adults, including sugar and water or fresh milk tested. While fresh milk was found to extend the BSF lifespan, it proved less practical due to spoilage and higher cost compared to sugar and water. Therefore, using sugar and water as a food source was preferred, as it remained stable for a longer duration, necessitating only weekly replacement or supplementation. The introduction of sugar and water increased the longevity of both male and female BSF. For instance, male's lifespan doubled from 12 to 24 days, and that of the female nearly doubled from 15 to 27 days.

Chapter 2 also addressed the challenges in achieving year-round egg production. The study encompasses an exploration of diverse factors that have the potential to influence BSF egg production. These factors include fly density, gender ratio, longevity, attractants, egg traps, as well as ambient temperature and lighting conditions. A critical consideration is the provision of adequate lighting for the mating and breeding of adult BSF. This aspect becomes crucial as

BSF adults might not receive sufficient natural sunlight for mating, leading to diminished egg production (Tomberlin *et al*., 2002; Zhang *et al*., 2010).

To overcome this hurdle, the utilisation of artificial light, in tandem with natural light, has been investigated, particularly during winter months or in regions with limited sunlight, such as New Zealand. Remarkably, this approach has demonstrated effectiveness in ensuring a consistent and stable egg production rate. Notably, it was observed that the implementation of artificial lighting resulted in an increase in egg production, with a remarkable 492% improvement. Importantly, the impact of artificial lights was most pronounced during overcast days. During sunny days, sunlight further amplified egg yields, demonstrating an extraordinary increase of 2052% compared to artificial lights.

Another important aspect of small-scale BSF operations that was addressed is the management of post-larvae feeding residues, also known as frass. Residue or frass production is often overlooked as a feed efficiency measure in BSFL rearing systems (Lopes *et al*., 2022). However, it plays a crucial role in assessing the overall efficiency of the production process and minimising wastage. High frass production can indicate an inefficient process, which signifies substantial amounts of unconsumed or undigested organic materials. Therefore, evaluating frass production should be accompanied by other process parameters, such as bioconversion efficiency, material reduction, and final larval weight, to gain a comprehensive understanding of the system's performance. Studies have shown that frass and post-composting processes are hotspots for GWP, contributing to a significant portion (69%) of the total GWP associated with BSFL meal production (Mertenat *et al*., 2019). Hence, effective management of frass and residual is crucial for reducing the environmental impact of the production process. Lopes *et al*. (2019) reported frass conversion efficiencies on a dry matter (DM) basis for different feed sources, ranging from approximately 15% for poultry feed to 52% for human faeces. Feeding BSFL with food wastes resulted in a frass conversion efficiency of around 45%

of the input DM, while poultry manure had a conversion efficiency of about 40% (Lopes *et al*., 2019). Klammsteiner *et al*. (2020) also reported frass production efficiencies ranging from 28% to 43% in BSF colonies. These findings highlight the importance of considering the feed source and optimising frass conversion efficiency to minimise waste production. In the current thesis, Chapter 2 showcased a practical and sustainable approach to the utilisation of the residual generated from the BSF colony. Approximately two-thirds of the residual material was effectively utilised in various applications, demonstrating its value. One of its uses was as an attractant for adult oviposition. The residual material from the BSFL was subjected to natural fermentation in a warm environment for a few days. The resulting fermented product was then placed into breeding cages, leading to a remarkable increase (1067%) in egg yield compared to the use of fermented kitchen waste. Additionally, the residual material served as pupation substrates, facilitating the transformation of the pupal into the adult stage, as discussed in Chapter 3.

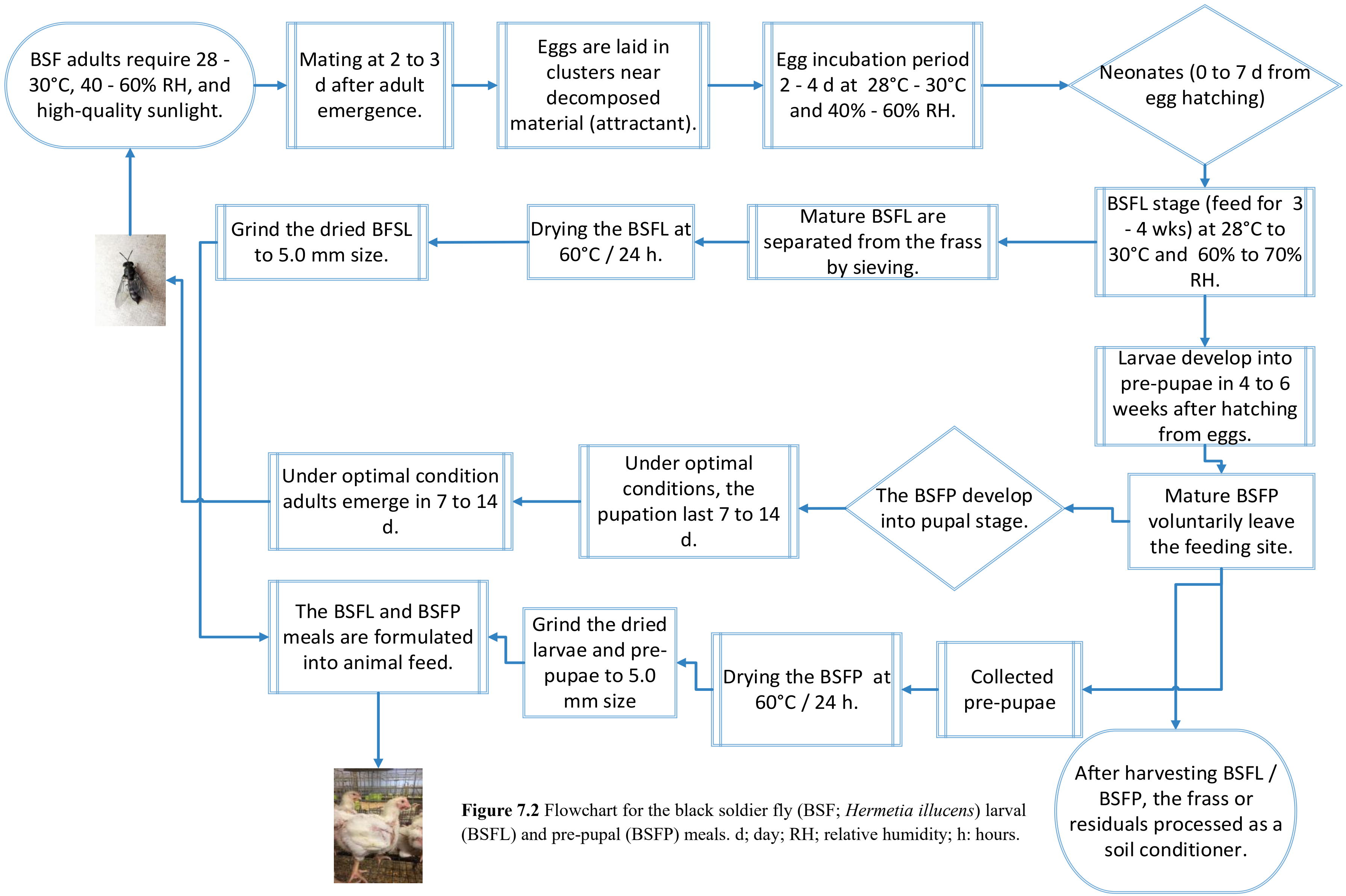


Figure 7.2 Flowchart for the black soldier fly (BSF; *Hermetia illucens*) larval (BSFL) and pre-pupal (BSFP) meals. d; day; RH; relative humidity; h: hours.

7.3 Maintaining healthy colonies and disease management in BSF facilities

Maintaining a healthy colony and controlling disease or parasite outbreaks is the next step after establishing and sustaining BSF bioconversion technology. While BSF offers numerous benefits, there are concerns about mass rearing and conditions in extensive production facilities. The ideal rearing ambience for the BSF colony requires specific conditions, including high temperature (25 to 28°C) and humidity (50 to 70%). These conditions can also create opportunities for pests and pathogens, increasing the risk of disease, especially when proper hygiene protocols are not in place (Müller *et al*., 2017). Despite BSF's excellent reputation for disease resistance, it is important to recognise that several insects used for food and feed can be susceptible to diseases under mass rearing conditions. For example, the mite *Varroa destructor* significantly impacts Western honeybee colonies (*Apis mellifera*) (Bauer *et al*., 2018), the virus *Acheta domesticus densovirus* (AdDV) can severely affect mass-reared house crickets (*A. domesticus*) (Herren *et al*., 2023), and the microsporidium *Nosema bombycis* causes the highly lethal disease 'pébrine' in the domestic silkworm (*Bombyx mori*) (Pan *et al*., 2013).

Although BSF has generally been known for its disease resistance, there is still limited knowledge about potential risks or diseases that may affect BSF colonies. The pathogens that can be present at BSF facilities may include nematodes, microsporidia, *Apicomplexa* parasites, and others. Additionally, there is a potential risk of competition for food sources and the spread of pathogens by other insects, such as houseflies and grain mites, which can act as vectors for diseases in BSF production facilities (Müller *et al*., 2017). However, research findings have indicated that BSF may play a beneficial role in mitigating housefly infestations. Studies have shown that the presence of BSF in manure sites can inhibit wild colonies of houseflies, and laboratory colonies of houseflies tend to avoid laying eggs in feeding sites dominated by BSF

(Bradley and Sheppard, 1984). This suggests that housefly infestation may not be a significant issue as long as BSF populations dominate the feeding sites.

In Chapter 4, an infestation of red mites in the BSF colony was identified; there were noticeable anatomical damage on the BSFL and BSFP, causing discolouration of the skin from light cream to dark brown. The parasitic behaviour of the mite distressed the BSFL due to repeated bites and feeding on their body juices. The presence of feed for the BSFL reduced the impact of mite damage on days 1 and 2. However, it was less effective as they developed to days 3 and 4, where no significant difference was observed in the survival. Furthermore, the presence of red mites raises human health concerns, as they can potentially transfer viruses to handlers in the BSF colony and cause skin irritation. Economically, the impact of red mite infestation can be severe for the insect protein industry, leading to the complete destruction of the colony and incurring significant costs for re-establishment, premises disinfection, and sterilization. Moreover, the risk of mite transmission to poultry may limit the use of live larvae as poultry feed.

The red mite infestation may result in a total loss of the BSF colony, requiring rebuilding the colony in a mite-free environment. Several methods to control red mites were discussed in Chapter 4 and include chemicals, natural oils, inert substrates, and physical control such as temperature and lighting. However, the best approach is to prevent the infestation from happening in the first place through careful hygienic procedures, including the examination of the feeds used, especially grains. In addition, it is recommended to keep the BSF colony away from wild birds or poultry farms. Finally, safe, periodic inert dusting or natural oil spraying can help control mite infestation.

While BSF was once considered immune to pests and diseases, these findings underscore the necessity for expanded research to assess potential risks across various colony stages, including pupal and adult phases. Towards the end of this PhD research, another type of unidentified mite appeared during both the adult and neonate stages (Figure 7.3). Unfortunately, due to time constraints, a thorough investigation of this phenomenon was not pursued.

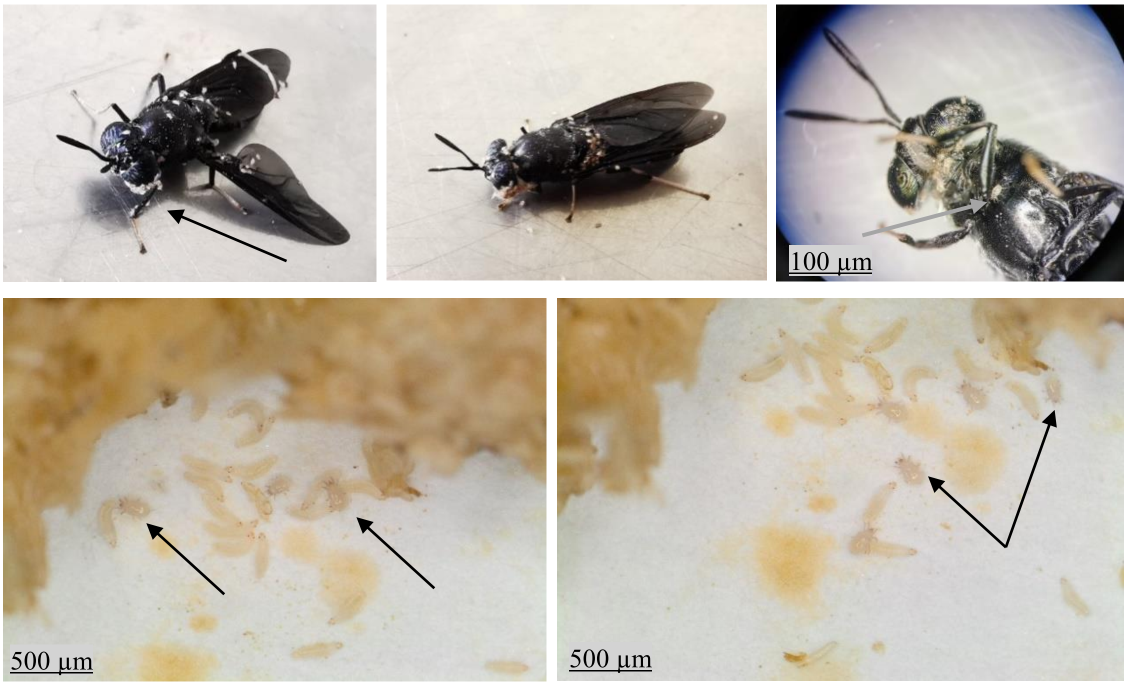


Figure 7.3 Observation of unidentified mites attached to adult and near neonates of black soldier fly (BSF; *Hermetia illucens*). Arrows point out to unidentified mites.

Another aspect of a healthy colony is prevention of genetic degradation. In Chapter 2, the breeding cages maintained a density of 800 to 1000 flies per cage. This population density sustained a robust and healthy colony over numerous generations throughout the study, which extended beyond 3 years. Notably, our observations revealed the absence of inbreeding issues or colony collapse, defying the common challenges encountered in captive insect colonies. Typically, genetic degradation due to close relative breeding has led to collapses (Eriksson and Picard, 2021; Rhode *et al*., 2020).

Rhode *et al*. (2020) demonstrated that maintaining as few as 500 flies could establish a stable colony, effectively circumventing inbreeding concerns. This outcome aligns closely with our findings presented in Chapter 2. Notably, our colony remained robust without the introduction of genetic diversity from wild populations throughout the study duration. This resilience can be attributed to the maintenance of high population density within the breeding cages (≥ 800 flies). Furthermore, the nourishment provided to the BSFL likely played a crucial role in enhancing adult health and egg productivity, as the diet was rich in protein (ranging from 171 to 395 g/kg DM) and fat (exceeding 66 g/kg DM). Such dietary richness has previously been associated with improved development in both BSFL and adults, leading to heightened fecundity (Barragan-Fonseca *et al*., 2017).

7.4 Optimisation of non-feeding stages (pre-pupal to adults)

Pupation is a complex process involving substantial morphological and physiological transformations, and the vulnerability of pupae during this stage is well-documented. Pupae lack movement and defence mechanisms, making them susceptible to predation and other environmental stressors (Wen *et al*., 2016). However, it is not uncommon for small-scale BSF farmers to forego substrates to avoid additional costs (A.E.M., unpublished data). Studies on BSF have shown that the absence of substrate negatively impacts pupation time and adult emergence rates (Holmes *et al*., 2013). Additionally, research on other insects, such as *Tribolium freemani* (Hinton) (Coleoptera: Tenebrionidae), indicates that both mechanical and chemical stimuli from conspecifics can delay pupation (Kotaki and Fujii, 1995). Although BSF are not known for positive thigmotaxis (movement towards tactile stimuli), the lack of a suitable substrate can adversely affect their development. When pre-pupae come into direct contact or rub against each other due to inadequate substrate, pupation is delayed (Holmes *et al*., 2013). Therefore, the use of appropriate pupation substrates is crucial.

Moist substrates consistently improved pupation and adult emergence percentages and reduced mortality at both stages. The optimal moisture level varied across the substrates used, but generally, 10% moisture appeared to yield the best results. However, substrates like spent wheat middlings, which absorb water, could face mould issues if moisture exceeds 10% (Figure 7.4). A trade-off is observed with substrate depth; pupation tended to avoid higher moisture levels (10% and above). Thus, a depth of 3 cm for pupation substrates can still be effective while saving on cost and substrate volume.

Furthermore, balancing moisture levels, substrate types and cost are essential. While five substrates performed well in this study, spent wheat middlings—a by-product—offered a cost-effective alternative to more expensive substrates like vermiculite, which costs approximately US $4 per kg. The findings suggest that using effective pupation substrates can encourage small farmers to incorporate them without significantly increasing production costs, while maintaining optimal moisture levels. It is essential to emphasise the importance of sterilising spent wheat middlings prior to using them as a pupation substrate to prevent potential diseases. Studies have reported that BSF colonies can be susceptible to mites, houseflies, and other parasites, which can act as disease vectors in BSF production facilities, as discussed in Chapter 4. The risk of encountering these challenges is higher under poor hygienic conditions. Therefore, proper storage conditions, such as maintaining at -20°C, and sterilisation are crucial to ensure substrate quality.

Another factor to consider is that compaction did not alter pupation percentages or adult emergence rates. Although it was previously reported that substrate compaction may reduce the ability of oxygen to penetrate, causing hypoxia and failure to pupate (Bubnov *et al*., 2015; Shi *et al*., 2021), this was not the case in my study. It may be that the use of manual compaction did not harden the substrate enough. Therefore, reusing substrates with minimal impact from compaction can still provide benefits, provided some level of sterilisation is applied.

Another notable finding was the uneven distribution of pupae within the substrate. A significant number of pupae tended to migrate to the top layer of the substrate, because this region had relatively lower moisture levels compared to deeper layers. This observation raised the possibility of moisture stratification within the substrate, with higher moisture towards the bottom and relatively drier conditions towards the top. Based on these results, it is recommended to maintain a moisture level of 10% for spent wheat middlings to avoid mould growth and achieve successful pupation and adult emergence. However, it is essential to consider the specific characteristics of each substrate when determining the optimal moisture level for pupation to ensure optimal performance and minimise potential issues with mould development.

Figure 7.4 Mould growth on spent wheat middlings (also known as larval colony residual) due to excessive moisture content (> 10%) in pupation substrate for black soldier fly (BSF; *Hermetia illucens*) pupae.

7.5 Utilisation of BSF meals in poultry diets

The BSF holds significant promise in the realm of animal feed, particularly in the production of BSF meals. This application gains particular significance in the domain of poultry feeding, where the two distinct developmental stages of BSF (BSFL and BSFP) emerge as valuable potential ingredients for insect-based feeds. With the growing interest in integrating BSF into poultry diets, there emerges a clear and pressing need to comprehend its nutritional value. This importance is underscored by the fact that energy accounts for a substantial portion of the cost of poultry diets. As such, possessing accurate data on the apparent metabolisable energy (AME) of BSF meals stands as a fundamental necessity.

Unfortunately, the existing body of published literature on these aspects remains sparse, even in the face of extensive research efforts directed at BSFL production, their overall nutritional composition, and their feeding value (Makkar *et al*., 2014; Abd El-Hack *et al*., 2020). It is worth noting that published reports on the digestibility of full-fat BSF meals for broiler chickens are limited (De Marco *et al*., 2015; Uushona, 2015). This highlights a significant gap in our understanding of these pivotal nutritional dimensions, even within the expansive exploration of BSF's potential applications in the animal feed sector.

In Chapters 5 and 6, experiments were conducted to assess the AME, nitrogen-corrected AME (AMEn), apparent amino acid digestibility coefficients (AIDC), and standardised amino acid digestibility coefficients (SIDC) of the BSFL and BSFP meals.

The AME and AMEn values of full-fat BSFL (Chapter 5) exceeded the corresponding values for BSFP (19.10 and 18.00 vs. 18.44 and 16.94 MJ/kg DM, respectively). These findings are higher compared to those reported by De Marco *et al*. (2015) for BSFL (17.38 and 16.60 MJ/kg DM, respectively), while the values for BSFP greater than those determined by Uushona (2015) (17.40 and 16.52 MJ/kg DM, respectively). However, for partially defatted BSFL with a fat content of 180 g/kg, the AME and AMEn values were lower at 16.25 and 14.87 MJ/kg DM; respectively, in reported by Schiavone *et al*. (2017). A markedly lower AME and AMEn values of 11.55 and 9.87 MJ/kg DM, respectively, was observed in highly defatted BSFL containing 46 g/kg fat in the same study.

A striking observation that the AME of both BSF meals distinctly surpassed the values documented for soybean meal (8.4 to 9.9 MJ/kg DM, and 12.62 to 15.46 MJ/kg DM, as reported by Ravindran *et al*., 2014a and 2014b, respectively). This disparity may be largely explained by the higher fat content of the insect.

The primary value of BSF meals is to supply the protein and amino acids. Chapter 8 provided determined values for the AIDC and SIDC of individual AA in the full-fat BSFL meal. The values for Lys, Met, Thr, and Val, and the average SIDC were determined to be 0.85, 0.90, 0.91, 0.87, and 0.84, respectively. In a parallel study employing the precision-fed rooster assay, Matin *et al*. (2021b) similarly reported similarly higher SIDC values in four BSFL samples.

In Chapter 6 represents the first report on the amino acid digestibility of the BSFP meal. The SIDC for Lys, Met, Cys, Gly, Thr, Val and average of all amino acids were measured to be 0.80, 0.86, 0.73, 0.77, and 0.80, respectively.

7.6 Research limitations and future perspectives

Despite the depth of understanding achieved in the literature and the current research, there are number of areas that could be improved and present avenues for future exploration:

- The investigation into red mite infestation was limited to natural infestations within a specific time-frame. Extending the observation periods in future research may lead to better understanding of the mite's impact on BSF production. Additionally, exploring the spread of infestation to other stages of the colony would provide insights into managing and preventing mite infestations effectively.
- Due to limited time and funding, the thesis concluded with the digestibility studies reported in Chapters 5 and 6. While these studies provide valuable information, it is acknowledged that conducting feeding trials with broiler chickens using BSFL and BSFP as feed sources could be a promising avenue for future research.
- Furthermore, it would be beneficial to explore using BSF colony residues as feed for other insects, particularly earthworms. Since the residues from the BSF colonies are not

fully composted due to the short cycle of BSF (approximately 14 to 28 days on average), their direct use as a soil conditioner may be limited in terms of providing high microbial activities. However, by using the residues as feed for earthworms, they can be further composted and converted into vermicompost.

7.7 Conclusions

In conclusion, this doctoral thesis has made novel contributions to the understanding of various aspects of BSF bioconversion technology and its application for poultry feed. The study adopted a multidisciplinary approach to understanding the biology of the BSF and essential steps to establish and maintain a healthy colony for the year-round production of eggs. Furthermore, the non-feeding stages namely, pupal and adult emergence stages, were enhanced and tested with different conditions and affordable pupation substrates. Additionally, the research explored the processing of insect meals from two stages of BSF life, namely BSFL and BSFP, and evaluated their potential as feed sources for broiler chickens. The overall contribution of this thesis was to address several important knowledge gaps in BSF production and processing, providing cost-effective solutions particularly beneficial for small and medium-scale farmers.

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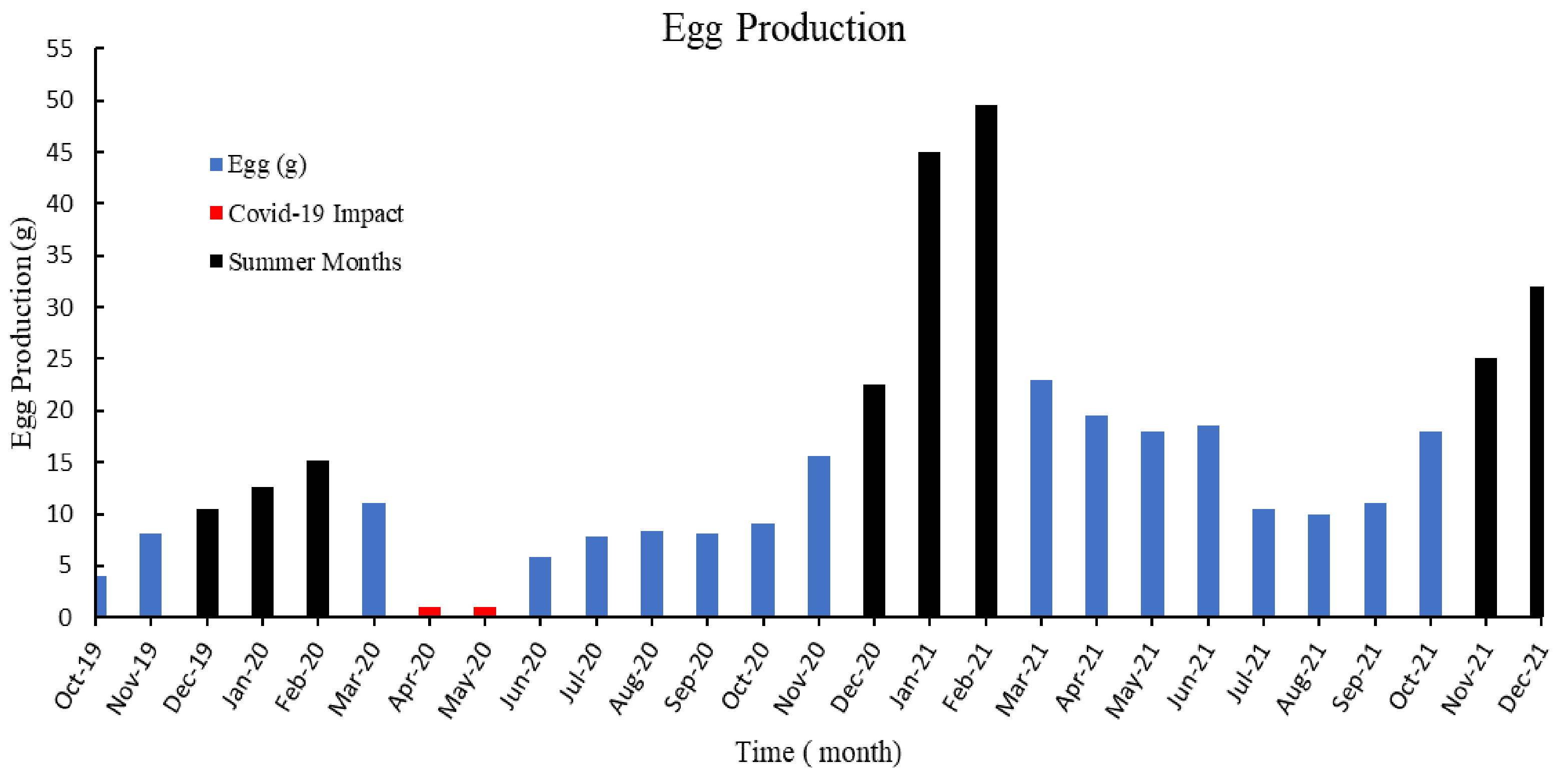
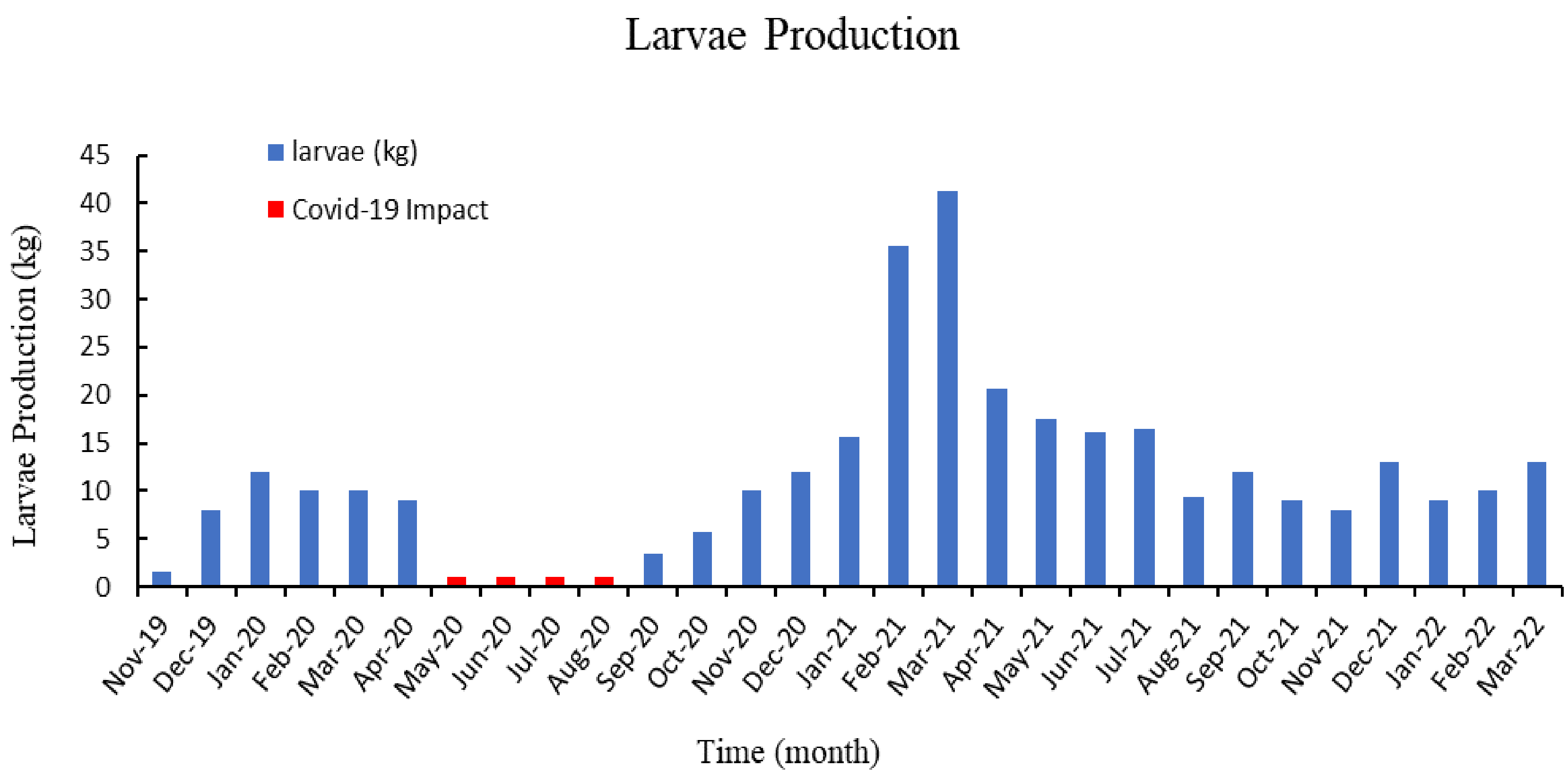
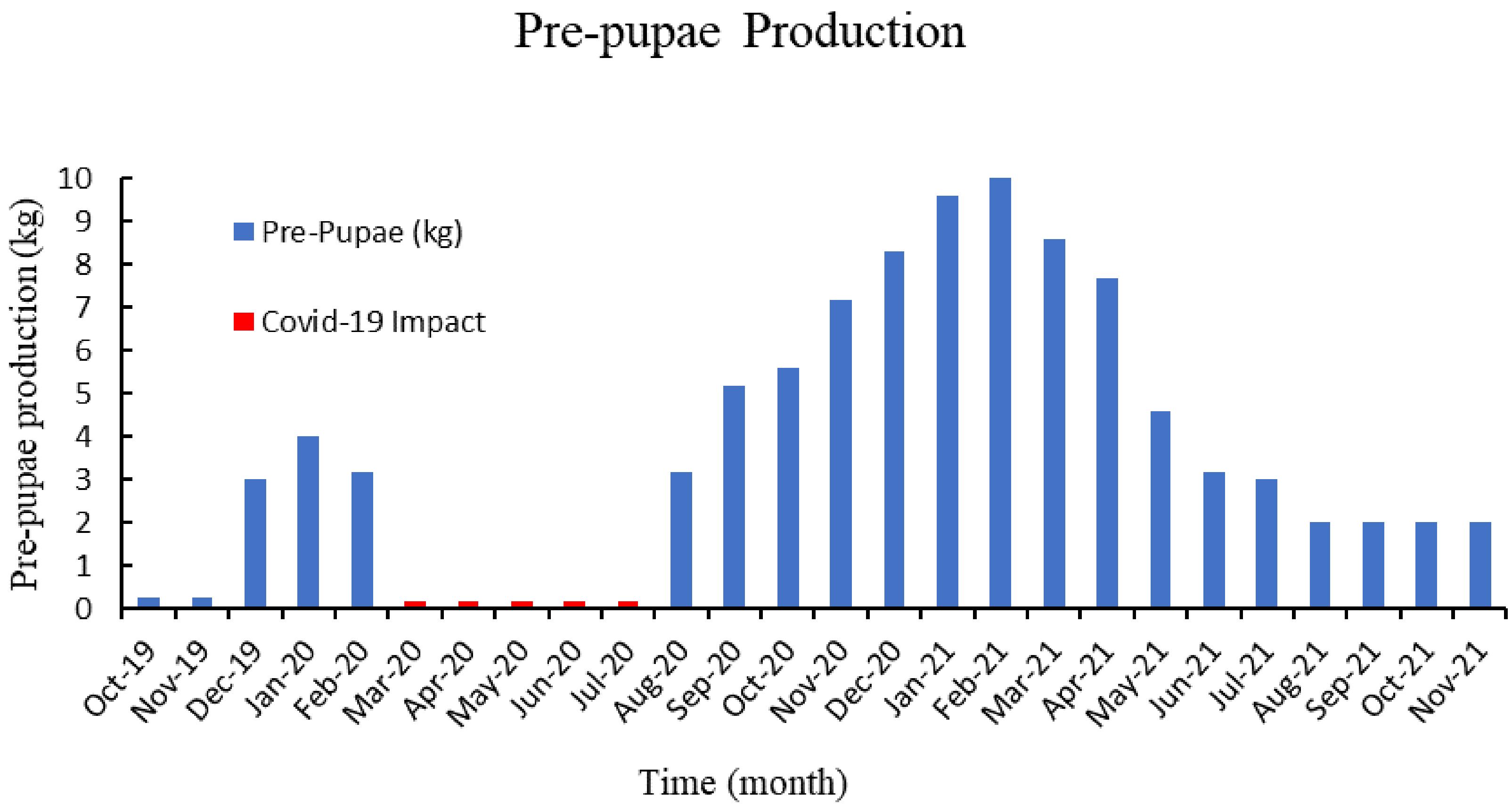
Appendix A. Egg production of the black soldier fly (BSFL; *Hermetia illucens*) colony from October 2019 to December 2021 (Chapter 2).



Appendix B. Larvae production of the black soldier fly (BSFL; *Hermetia illucens*) colony from October 2019 to December 2021 (Chapter 2).



Appendix C. Egg production of the black soldier fly colony from October 2019 to December 2021 (Chapter 2).



Appendix D. Chemical composition of the diets used in this study as feeding substrates for black soldier fly (BSF; *Hermetia illucens*) larvae. The diets include restaurant waste (RW), restaurant waste and coffee mixture (RWC), and coffee grounds (SCG) (Chapter 2).

Parameters*	Wheat middlings (broll)	Restaurant Waste (RW)	Restaurant Waste and spent coffee grounds (RWC)	Restaurant Waste and soybean oil (RWO)	Spent coffee grounds (SCG)
Dry Matter (g/kg)	412	341	279	442	545
Ash (g/kg)	52	46	38	40	42.0
Crude protein (g/kg)	171	365	272	192	199
Fat (g/kg)	67	285	103	332	80.0
Sum (crude protein + fat)	238	650	375	524	279
Neutral Detergent Fibre (g/kg)	450	101	288	426	614
Acid Detergent Fibre (g/kg)	167	42	125	219	415
Lignin (g/kg)	42	22	36	94	145
Gross Energy (MJ/kg)	19	25	21	26	21

*Values are presented on a dry matter basis

Appendix E. Repeat Measures Analysis of experimental diets on the black soldier fly (BSF; *Hermetia illucens* L.) larvae live weight on days 7, 14, and 21 from the hatching day of the eggs* (Chapter 2).

	Experimental Groups							*P* Values		
Day	RW7	RW10	RW	RWC	SCG	RWO	SE	Diet	Age	Diet *Age
7	$^{4}0.102^{A}$	$^{4}0.102^{A}$	$^{4}0.051^{B}$	$^{4}0.034^{C}$	$^{4}0.011^{D}$	$^{4}0.036^{C}$	0.002	<0.0001	<0.0001	<0.0001
10	$^{2}0.143^{A}$	$^{2}0.139^{A}$	$^{3}0.119^{B}$	$^{3}0.085^{D}$	$^{3}0.041^{E}$	$^{3}0.104^{C}$				
14	$^{1}0.161^{A}$	$^{1}0.146^{B}$	$^{2}0.147^{B}$	$^{2}0.125^{B}$	$^{2}0.077^{D}$	$^{2}0.110^{C}$				
21	$^{3}0.132^{B}$	$^{3}0.118^{B}$	$^{1}0.208^{A}$	$^{1}0.186^{A}$	$^{1}0.104^{B}$	$^{1}0.174^{A}$				

^{ABCD}Means within the rows with different superscripts are different from each other ($P < 0.05$). The level of significance was set to $p = 0.05$.
^{1234}Means within columns with different superscripts are different from each other ($P < 0.05$). The level of significance was set to $p = 0.05$.
*The diets used are Rw7: the eggs were hatched on a wheat middlings diet for seven days and then moved to a restaurant waste diet for 14 days; RW10: the eggs were hatched on a wheat middlings diet for ten days and then moved to restaurant waste for another 14 days; RW; the larvae were fed entirely on a restaurant waste diet for from hatching to 21 days; RWC: the larvae were fed a mixture of restaurant waste (75%) and spent coffee grounds (25%) from hatching 21 days; RWO: the larvae were fed on a mixture of 35% frass (larvae residual) + 60% restaurant waste + 5% soy oil from hatching to 21 days; SCG; the larvae were fed on a spent coffee grounds diet from hatching to 21 days.

Appendix F. Feed conversion ratio (FCR), waste reduction index (WRI), efficiency conversion of digested food (ECD) and substrate reduction (SR) of black soldier fly (BSF; *Hermetia illucens* L.) larvae reared on organic wastes* (Chapter 2).

Parameters	RW7	RW10	RW	RWC	SCG	RWO	*P* Value
Feed conversion ratio (FCR)	1.77 ± 0.30	1.57 ± 0.24	2.17 ± 0.23	1.44 ± 0.23	2.10 ± 0.18	1.77 ± 0.18	0.247
Waste reduction index (WRI) (g / day)	2.34 ± 0.16 ^{BC}	2.92 ± 0.05 ^{BC}	3.21 ± 0.05 ^{A}	3.15 ± 0.06 ^{A}	1.87 ± 0.20 ^{C}	1.85 ± 0.14 ^{C}	< .0001
Efficiency conversion of digested food (ECD)	0.038 ± 0.01^{AB}	0.03 ± 0.01^{AB}	0.04 ± 0.00 ^{A}	0.03 ± 0.00 ^{AB}	0.02 ± 0.00 ^{AB}	0.02 ± 0.00 ^{B}	<. 0001
Substrate reduction (SR)	0.49 ± 0.03 ^{BC}	0.61 ± 0.01^{AB}	0.67 ± 0.01^{A}	0.66 ± 0.01^{A}	0.39 ± 0.04 ^{C}	0.39 ± 0.03 ^{C}	< .0001

*ABC Means within the rows with different superscripts are different from each other ($P < 0.05$).

The level of significance was set to p = 0.05. Values are presented as a mean ± SE; n = 3. The diets used are Rw7: the eggs were hatched on a wheat middlings diet for seven days and then moved to a restaurant waste diet for 14 days; RW10: the eggs were hatched on a wheat middlings diet for ten days and then moved to restaurant waste for another 14 days; RW; the larvae were fed entirely on a restaurant waste diet for from hatching to 21 days; RWC: the larvae were fed a mixture of restaurant waste (75%) and spent coffee grounds (25%) from hatching 21 days; RWO: the larvae were fed on a mixture of 35% frass (larvae residual) + 60% restaurant waste + 5% soy oil from hatching to 21 days; SCG; the larvae were fed on a spent coffee grounds diet from hatching to 21 days.

Appendix G. Life cycle parameters and development time of black soldier fly (BSF; *Hermetia illucens* L.) at different stages when fed on wheat middlings (Chapter 2).

Larvae weight*	Mean ± SD	n
Larva 2 d old	1.05 ± 0.12	4
Larva 3 d old	6.28 ± 1.43	4
Larva 5 d old	37.2 ± 0.58	4
Larva 7 d old	74.1 ± 18.6	4
Larva 9 d old	87.2 ± 19.9	4
Larva 11 d old	124 ± 13.8	4
Larva 13 d old	142 ± 12.5	4
Larva 15 d old	163 ± 1.80	4
Larva 17 d old	179 ± 3.52	4
Larva 19 d old	199 ± 6.15	4
Larva 21 d old	226 ± 3.29	4
Larva 23 d old	185 ± 2.52	4
Pre-pupae	151 ± 26.9	4
Pupae	120 ± 8.23	4
Development time		
Egg development time	2.22 ± 0.44	9
Larvae development time	22.5 ± 0.87	21
Pre-pupae development time	14.3 ± 1.15	21
Pupa development time	9.08 ± 0.90	12
Total cycle (d)	43.8 ± 1.58	21
Feed conversion rate (FCR)	2.74 ± 0.21	16
Substrate reduction ratio	78.6 ± 1.53	16
Bioconversion ratio	4.49 ± 0.33	16
Drying yield (g/kg)		
Larvae	314 ± 34.1	21
Pre-pupae	357 ± 3.30	21

* Each value represents the mean of four replicates (20 individual larvae/pre-pupae or pupae per replicate).

Appendix H. Main activities involved in maintaining the black soldier fly (BSF; *Hermetia illucens* L.) colony* (Chapter 2).

Work schedule and daily activities in the BSF facility	Check done
Egg collection from glasshouse and/or indoor colony Place eggs in Petri dishes (Hatching Table) Label dishes with date + write ID (indoors) or GH (glasshouse) Place Petri dishes in a moist box for 2- 3 days (Hatching Table) Place old Petri dishes with eggs on rearing trays with wet feed (Hatching Table) Move small hatching box to big trays after 7 days (Rearing Table) Label the rearing tray with date + ID (indoor egg source) or GH (glasshouse adult colony) Add 1 cup of wet feed to all rearing boxes (based on previous diet consumption) OR ½ cup of minced food waste on each tray Add water in attractant boxes for the adult colony (if needed) Add drinking water for the adult colony (if needed) Fill/ check the humidifier water level	
Weekly Activities	
Harvest BSFL at age 18-21 days old based on size/colour (3-5% pre-pupae) Harvest pre-pupae to place in pupation box for two weeks Replace attractant boxes for the adult colony Release emerging adults in the breeding cages Collect food waste from the restaurant and mince it Stock the dry feed (i.e., wheat middlings, chicken feed) Clean the floor in the insect room Check room corners for pre-pupa escapees Wipe surfaces in the insect room with disinfectant Wipe the floor in the insect room with water and chlorine	
Monthly Activities	
Cleaning and disinfecting the insect rearing room by placing an insecticide bomb overnight, then ventilation for two days Replace or add a new adult mating cage Place fresh and dead flies inside breeding cages	

*A copy of this list was placed in the rearing units and glasshouse

Appendix I: Statement of contribution (Chapter 4**).**

The Poultry Red Mite (*Dermanyssus gallinae*) Poses a Risk in Rearing Black Soldier Fly (*Hermetia illucens*).

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Amira El sayed Abdalla Mahmoud
Name and title of main supervisor:	Professor Peter Tozer
In which chapter is the manuscript/published work?	Chapter 7

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Amira contributed to experimental design, data collection, data analysis, initial and subsequent draft writing, and addressing feedback from supervisors. This involvement extended to preparing the final manuscript, responding to journal feedback.
Prof. Murray Potter contributed to data analysis, manuscript writing and responding to the reviewers.
Prof. Ravi Ravindran provided inputs to the writing, final manuscript and responding to the reviewers.

Please select one of the following three options:

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  Mahmoud, A. E., Morel, P. C. H., Potter, M. A., & Ravindran, V. (2023). Poultry red mite (Dermanyssus gallinae) poses a risk in the rearing of black soldier fly (Hermetia illucens). Journal of Insects as Food and Feed, 9(1), 55-63.

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Appendix J: Statement of contribution (Chapter 5**)**.

Chapter 5: The Apparent Metabolisable Energy and Ileal Amino Digestibility of Black Soldier Fly (*Hermetia Illucens*) Larvae Meal for Broiler Chickens

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STATEMENT OF CONTRIBUTION DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Amira El Sayed Abdalla Mahmoud
Name and title of main supervisor:	Professor Peter Tozer
In which chapter is the manuscript/published work?	Chapter 8

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

Amira contributed to data collection, data analysis, initial and subsequent draft writing, and addressing feedback from supervisors. This involvement extended to preparing the final manuscript, responding to journal feedback.
Prof. Ravi Ravindran guided in experimental design, data analysis, manuscript writing and responding to reviewers, leading to acceptance.
Prof. Murray Potter provided inputs to the final manuscript.

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