PRODUCTION AND UTILIZATION OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE AS A COMPONENT OF HIGH-QUALITY FEED IN MALAWI

MSc. (AQUACULTURE) THESIS

ESTHER JOSEPHINE NYIRENDA

LILONGWE UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

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ESTHER JOSEPHINE NYIRENDA

BSc. (Aquaculture and Fisheries Science), Malawi

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DECLARATION

I, Esther J. Nyirenda, declare that this thesis is a result of my own original effort and work. The findings have never been previously presented to Lilongwe University of Agriculture and Natural Resources or elsewhere for any academic qualification. Where assistance was sought, it has been acknowledged accordingly.

Esther J. Nyirenda

Signature: _____

Date: _____

CERTIFICATE OF APPROVAL

We, the undersigned, certify that this thesis is a result of the author's own work, and to the best of our knowledge, it has not been submitted for any academic qualification with the Lilongwe University of Agriculture and Natural Resources or elsewhere. The thesis is acceptable in form and content, and that satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on 02/06/2023.

Major supervisor: Professor Daud Kassam

Signature _____

Date _____

Supervisor: Dr. Bernd Ueberschär

Signature _____

Date _____

Supervisor: Professor Jeremiah Kang'ombe

Signature _____

Date _____

DEDICATION

I dedicate this work to Mr. Aaron Gibson Chitata and Mrs. Josephine Banda Nyirenda, my siblings, and the local farmers in Malawi.

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ABSTRACT

The study was aimed at evaluating the performance of black soldier fly larvae (BSFL) production on various organic wastes and to assess the effect of defatted BSFL meal on *Oreochromis karonage* fingerlings as a cheap-alternative and high-quality fish feed ingredient. In order to assess the influence of rearing waste substrates on BSFL growth performance, waste reduction efficiency, and nutritional composition. five hundred five-day-old larvae were reared in triplicates. The larvae were reared until the onset of prepupal for nutritional analysis and another set of larvae for growth performance and waste reduction efficiency. To assess the effect of defatted BSFL meal replacing fishmeal in *O.karonage* fingerling diets, 12±3g fingerlings were randomly stocked and raised in triplicates in concrete tanks (5 fish/m^2, 15 fish/tank) for 126 days. Growth performance, nutritional utilization, and waste reduction indices were analyzed using One Way ANOVA at a P-value of 0.05. The rearing substrates significantly influenced the nutrient composition of BSFL (36.91±0.36 to 46.83±0.41 % CP), growth rate (0.64±0.70 to 9.40±0.31), development time until onset of pupation (11 to 27 days), and waste reduction efficiency (WRI of 2.84±0.02 to 5.68±0.04 and ECD of 1.07±0.01 to 9.62±0.07). There were no significant differences in crude protein with the highest value in fishmeal-fed fish, survival rate, AFCR, and water quality parameters, while weight gain differed significantly ($p<0.05$) between fish fed on defatted BSFL and fish meal diets. BSFL body nutritional composition is influenced by the rearing substrates. *O.karongae* growth performance, nutrient composition, and utilization were not negatively influenced by the feeds. The results suggest that the defatted BSFL meal can replace fishmeal and is suitable as a protein ingredient in *O.karongae* fingerling feed.

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LIST OF ABBREVIATIONS AND ACRONYMS

AKEFEMA	Association of Kenya Feed Manufactures
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BSF	Black Soldier Fly
BSFL	Black Soldier Fly Larvae
CM	Custom Made
CP	Crude Protein
C. Fat	Crude fat
C. Fiber	Crude fiber
ECD	Ingested Food Conversion Efficiency
FA	Fatty Acids
FAO	Food and Agriculture Organization
GoM	Government of Malawi
SFA	Saturated Fatty Acids
SGR	Specific Growth Rate
WG	Weight Gain
WRI	Waste Reduction Index

CHAPTER ONE

INTRODUCTION

1.1 Background

In African and Asian developing countries, the population increase is remarkable (FAO, 2017). Along with population growth, the demand for animal protein products is also expected to increase. Additional resources will be required to feed the expanding population, such as increased animal output (aquaculture and cattle). Globally, aquaculture has continuously been the fastest growing animal production industry, and 70% of global aquaculture production depends on feed (Tacon and Metian, 2008). However, as the demand for fish for human consumption has risen, the production of fishmeal and fish oil has decreased. Due to its balanced amino acid profile and high digestibility, fishmeal is an ideal protein source in fish feed (Dersjant-Li, 2002). Aquaculture is significantly reliant on a steady supply of fishmeal, which is a key component in commercial fish meals. Aquaculture has increased the demand on wild fish stocks to sustain farmed fish, resulting in the rapid loss of natural stocks (Stankus, 2021). Hence, fishmeal based supplemented feeding practices in aquaculture is a threat to conservation of wild fish population (Rana *et al.*, 2015)

Currently, a large amount of fishmeal is being used in feed formulation for domesticated fish and poultry. FAO (2012) estimated that around 14% of global fish catch was used in the manufacturing of fishmeal over the last decade. However, according to FAO (2014), fishmeal was rapidly depleting and becoming increasingly rare amid continued rise in demand. Due to this supply-demand imbalance, fishmeal prices have risen

globally.Fishmeal is however implicated as a sustainable aquafeed component due to increased price, dwindling supply and competition from other users. This has necessitated increased research into alternative protein sources for fish feed (Ayoola, 2011).

Aquaculturists have relentlessly tried to replace fishmeal and fish oil with conventional plant and animal based protein sources with varying successes (Rana *et al.,* 2015). Different types of plant based protein sources have widely been used and assesd in a number of nutritional studies as ingredients for aquaculture diets such as soybean meal, cotton seed meal and sunflower meal (Dersjant-Li, 2002). Among the plant protein sources Soybean meal is the most nutritive plant protein source. However, the high anti-nutritional factors leads to growth depression in fish at high inclusion levels and low amount of essential amino acids availability limits the inclusion levels in aquafeed (Dersjant-Li, 2002). According to Naylor *et al.,* (2000) carnivourous fish did not respond well to plant protein alternative sources due to lack of essential amino acids and carbohydrates. Therefore, insects have potential to replace fishmeal as the protein source (van Huis *et al.,* 2013).

Insects that can be grown from organic waste products offer a more sustainable source of protein for animal feed due to its cheap way of production and noncompetition with humans. The use of farmed insects as feed ingredients has several advantages, including being high in proteins, energy vitamins, lipids and minerals. The insects possess a greater feed conversion efficiency than livestock and hence consume less feed. And have great acceptance as they are easily adapted by poultry and fish as part of the insect's natural diet and are mainly omnivorous, allowing insect larvae to thrive on a variety of substrates (van Huis *et al*., 2013). The insects for feed should be cheaper to be made than fish meal, easy

to be accessed, and found locally in abundance (Muin *et al.,* 2017). The black soldier Fly, BSF (*Hermetia illucens*) is proved to be an ideal candidate (Bondari and Sheppard, 1981). BSF larvae may provide an effective method to mitigate two large growing concerns in Malawi which are waste management from kitchen and garden waste and the use of fishmeal derived from capture fisheries in aquaculture diets.

Among the rising techniques, Diener *et al.* (2009) describe the culture of the black soldier fly larvae as fish feed and an invention with the added benefit from organic waste. *H. illucens* larvae and pre-pupae have been successfully employed in aquaculture feeds (St-Hilaire *et al*., 2007; Sealey *et al*., 2011; Talamuk, 2016), poultry diets (De Marco *et al*., 2015; Uushona, 2015) and swine feeds (Newton *et al.*,2005; Driemeyer, 2016). Black Soldier fly larvae (BSFL) are high in protein (about 50%) and fats (up to 35%), with an amino acid composition that is acceptable for a variety of fish species (Newton *et al*., 2005; Elwert *et al.*, 2010).

1.2 Description of black soldier fly (BSF)

The insect (black soldier fly), *Hermetia illucens*, (Diptera: Stratiomyidae). is considered as non-pest, does not appear in the list of disease carrying organisms or vectors for pathogens. The adult is a harmless, non-biting, gigantic (approximately 15-20 mm long) wasp-like creature with just two wings (wasps have four). BSF lacks a stinger, it cannot bite or sting (Diclaro *et al*., 2012).). BSF has become established as one of the key insects being industrialized for use in waste management, protein and biofuel production. BSF larvae can be reared on a wide range of organic (waste)-material and reduces the volume of the waste up to 50% of which the waste remains called frass are an excellent fertilizer for plants (Sheppard *et al*., 1994). While many companies have demonstrated success with the mass

production of BSF, key hurdles remain with regards to developing optimal efficiency, safety, and commercial strategy for supplying larger industries such as aquaculture.

1.3 Black soldier fly (BSF) establishment

Fear of foreign species negatively affecting the environment's ecology, as well as changes in environmental conditions that may affect production, mandates the search for and use of native animals (Zhou *et al*., 2013). As a result, there is a need to establish BSF nativity and a technique of attraction for future captive breeding. Although BSF larvae can survive and thrive on a variety of organic items, factors such as development time and feed conversion efficiency must be considered. Diet has a significant impact on mortality, pupal weight, and nutritional content (Zhou *et al.*, 2013). Apart from substrates, management strategies like as ambient conditions and feeding strategy influence BSFL productivity in captive operations. Management strategies and regulation change the emphasis from BSF raising to promoting efficient feed utilization. As a result, waste minimization and the generation of sufficient quantities, and qualities of biomass are required (Barry, 2004).

There has been little emphasis in Malawi on documenting the seasonality, processing, and nutritional value of insects for human consumption or livestock and aquaculture feed (Pechal *et al.*, 2019). Contrary to other countries, such as Kenya, Thailand, and the Netherlands, where substantial research has been done on producing insects for human consumption (RamosElorduy 2009; van Huis 2013; van Huis 2014; Nadeau *et al*., 2015; Müller *et al*., 2016), and for aquaculture and livestock feed (Newton *et al*.,2005; Liu *et al.*,2010; Xia *et al*.,2011; Uuoshona, 2015; Driemeyer, 2016; Nyakeri *et al*., 2017) Malawi has lagged behind. Any effort aimed at bridging the knowledge gap of reliable insect production in Africa as a food/feed stock would substantially enhance the information flow,

supporting in strategically integrating nutritional strategies, providing an innovative source of jobs and revenue for local communities (which incorporates poultry and aquaculture), and thereby enhancing the livelihood of individual stakeholders (Pechal *et al*., 2019).

For years in Malawi, domestic or agricultural waste recycling has long been an issue (Figure 1.1), and the possible employment of BSF larvae in this field has attracted the attention of many researchers.

Figure 1.1. Kuntaya dumping site

Source: *Photograph by Author*

Awareness of the scarcity of feed resources for fish species has led Malawian and German researchers through the German-Malawian project "Ich liebe Fisch" to consider black soldier fly as a new component in fish feed in Malawi. However, information on production of BSF is still limited including the knowledge on proper substrate and its availability in the country, although a preliminary study on effect of culturing substrates and type of BSF culturing technology on BSFL performance and consequent effects has been conducted at Bunda (Goliath and Safalaoh, 2021). The study aims to assess the influence of the different organic waste substrates on the nutritional quality, and consequent effect of BSFL on the growth and feed utilization of *Oreochromis karongae*.

1.4 Problem statement and justification

Malawi's aquaculture sub-sector presents several opportunities in terms of meeting consumer demand for fish, providing a living for a significant number of individuals who are directly or indirectly involved in aquaculture, and its value chain. However, the aquaculture subsector encounters certain obstacles, such as a lack of resources and poor-quality feed (Hegel, 2008; Assam, 2014). One of the most unique challenges to the aquaculture industry is the ever-increasing expense professionally made fish feed (Cobbina & Eiriksdottir, 2010). Worldwide, the cost of conventional fish feed resources continues to rise, accounting for approximately 50-80% of overall production costs in intensive aquaculture (Turchini *et al*.,2010). In addition, the future availability of fish feed supplies is unknown (Rao *et al.,* 2012; Makkar *et al.,* 2014) as far as it is based on fishmeal.

Fishmeal is the only necessary protein element required by fish. However, it is the most costly element in formulated feed, raising production costs (Nguyen *et al.,* 2009; Hussain *et al*.,2011). Aquaculture diverts a food supply that may be consumed by humans by using wild caught fish for fishmeal production as a result of the significant competition from human use, the ongoing availability of fish meal in the future is not sustainable (FAO, 2012). As a result, there is a need for research into alternative, cost-effective, and viable protein sources that provide necessary amino acids, phospholipids, and fatty acids similar to fishmeal (Barroso et al., 2012) in Malawi. The maggots of the BSF have the potential to replace fishmeal without any shortages in the nutritional value of the feed. Black soldier fly larvae (BSFL) have a dry weight of up to 50% crude protein (CP), up to 35% lipids, and an amino acid profile comparable to fishmeal (Elwert *et al*., 2010).

Due to the high expense of fully-fledged feed (industrial pellet/powder feed with fish/soy meal content) for fish farming, farmers in Malawian rural areas primarily use waste from maize use (milled maize husks/ maize bran). This makes small-scale commercial fish production very difficult because maize bran contain about 9.8-14% crude protein (Carvajal-Millan *et al*., 2007. Consequently, the fish's entire growth potential is not being fully used. Therefore, there is a significant need for action in Malawi to discover adequate protein sources for fish nutrition in order to generate full-fledged, but also economical, fish feed for rural aquaculture producers.

There are a few professional fish feed producers in Malawi such as Apoche farm and MALDECO that have been producing sinking feed using imported fish meal and locally available ingredients. Efforts are being made in utilizing plant protein sources as a component of feed such as soy bean meal but has setbacks such as presence of anti-nutritional factors which results in slow fish growth and low nutrient utilization (Chou *et al*., 2004; Davis *et al.,* 2005; Koumiet *et al.,* 2008) and its usage is in competition with humans. Although the amino acid profile of soymeal is often higher than that of other plant-based feeds, it is nevertheless lacking in lysine, methionine, threonine, and valine when contrasted with an animal protein source (Henchion *et al.,* 2007). Moreover, the massive expansion of soybean cultivation has put pressure on land availability, especially in the tropics, often leading to deforestation and other negative effects for the environment (Foley *et al.,* 2011). Therefore, the production of high-quality proteins through the production of insect larvae would be an option that could be realized in Malawi at low cost through the use of organic substrates and being non-competitive with humans.

The production of BSF larvae for the production of animal protein is a tried and tested process and a realistic way of producing high-quality protein for the production of wholesome fish feed which can be applied in Malawi at low cost using locally available organic resources. In addition to the fish feed being made, the use of BSF will also enhance waste management which is also a sanitation challenge in Malawi as BSF has been demonstrated to add value by reducing organic waste biomass by 50-60% and converting it into high protein biomass (Sheppard *et al*., 1994). However, not all organic materials are ideal for BSF production in captive operations, necessitating the identification of suitable production substrates in terms of both product quality and quantity (St. Hilaire *et al*., 2007; Zheng *et al.,* 2013).

1.5 Objectives

1.5.1 Overall objective

To assess the influence of the various organic waste substrates in the rearing process on the nutritional quality, and growth performance of BSFL and consequent effect on growth and feed utilization of *Oreochromis karongae*.

1.5.2 Specific objectives

1. Assess the growth performance of BSF larvae on different organic waste substrates.
2. To evaluate effect of different organic wastes as feed substrate on BSFL proximate composition
3. To compare growth performance and survival of *O. karongae* fingerlings fed on BSFL meal diets and fishmeal diets

4. To assess nutritional composition of *O. karongae* fingerlings fed on BSFL meal diets and fishmeal diets

1.6 Research hypotheses

1. Ho: There is no statistically significant difference in BSFL growth performance on various organic waste substrates
2. Ho: There is no significant difference in BSF larvae's proximate composition raised on different organic waste substrates
3. Ho: There is no significant differences in growth performance and survival of *Oreochromis karongae* fingerlings fed on BSFL meal and fishmeal diets
4. Ho: There is no significant difference in the body composition of *O. karongae* fingerlings fed on BSFL meal and fishmeal diets

CHAPTER TWO

LITERATURE REVIEW

2.1 Protein demand against supply

A swift raise in population growth and urbanization have raised alarm on two key concerns specifically food security namely the supply of proteins, and management of wastes produced from increased consumption (FAO, 2011: The Economist, 2014; Alooh, 2012). Urnanization and expansion have led to an inclination to foodstuff rich in animal products and intensified waste production. Therefore, the need for animal protein demand for animal proteins such s meat and milk is predicted to rise by 58% and 70% in turn in 2025 in relation to the levels in 2010 (FAO,2011).

More time and land have been put into raising the amount of conventional cattle, poultry and expanding the aquaculture sector in order to satisfy the anticipated growth in demand. Through resource overuse and environmental deterioration, these activities have a detrimental impact on the environment (Foley *et al*., 2011; Mekonnen and Hoekstra, 2012). At all levels, from local to global, animal agriculture significantly contributes to serious environmental issues including pollution (Steinfield *et al*., 2006). Furthermore, aquaculture diverts a food supply that could be utilized by humans by using wild caught fish for fishmeal manufacture. As a result of the increased competition from human consumption, the ongoing availability of fish meal in the future is not sustainable; thus, other protein sources for animal feeds, poultry, and aquaculture are required (FAO, 2012).

2.2 Challenge of fish feed

Fishing is claimed to be the only industry capable of meeting the needs of a growing population (Kassahun *et al*., 2012). In terms of nutrition, fish is one of the cheapest and

most direct sources of protein, micronutrients, and income for many people around the world, particularly in developing nations, due to its widespread acceptance across social, cultural, and religious lines when compared to other animal products (Bene and Heck, 2005; Gabriel *et al.*, 2007). Fish accounts for over 16% of all animal protein consumed globally, and in Africa, up to 5% of the population relies entirely or partially on the fisheries sector for a living (Gabriel *et al*., 2007). Much of the fish consumed in Africa today is sourced from the continent's natural rivers and lakes. However, there is worry that capture fisheries have surpassed their natural limits, and that food security improvements through fish consumption now rely on aquaculture (FAO, 2010). There is a conflict in that aquaculture employs wild caught fish for fishmeal production, diverting a food source that humans can utilize. Given that approximately 30% of wild fish captures are used to generate fishmeal for farmed fish diets, the availability of fish meal in the future is not sustainable (Tiu, 2012; FAO, 2012).

Fishmeal was once a low-cost element in animal feed. However, the rapidly expanding aquaculture industry, which uses fishmeal as a key protein source in compounded feed, has significantly boosted the market and cost for this product. (Olsen and Hasan, 2012). Fish feed today contributes for more than 60% of overall production costs (Association of Kenya Feed Manufactures [AKEFEMA], 2005; FAO, 2010; Tiu, 2012; Abarike *et al*., 2013; FAO, 2022). As a result, aquaculture has become a costly endeavor, limiting the sector's ability to bridge the gap between fish demand and supply (Gabriel *et al*., 2007). Therefore, there is a need for an alternative protein source, preferably produced from by-products and materials not suitable for direct human consumption, as well as local production of fish feed from inexpensive and locally available feedstuffs to diminish

production costs, provide a less expensive way to meet protein requirements, increase food security, and reduce poverty in emerging nations (Hoffman *et al*., 2000; Gabriel *et al*., 2007; Kassahun *et al*., 2007).

Malawi has a variety of organic wastes and byproducts from the agriculture and industrial sectors, which are often not consumed by humans but may have a great potential as a substrate for aquaculture production. These can be employed as ingredients in locally compounded feeds as well as production substrates for currently underutilized animal protein sources, such as farmed edible insects, to be included in compounded feeds. (Van Itterbeeck *et al.*, 2014).

2.3 Insect protein

Insects suited for mass production would need to have specified characteristics such as larval stage duration, pupation synchronization, larvae/pupae weight uniformity, conversion rates, and daily biomass buildup (Peters and Barbosa, 1977; Scriber and Slansky, 1981; Sharaby *et al*., 2010). Protein quality, disease resistance, and substrate cost and composition are all desirable characteristics.

Insect protein incorporation in animal feed studies are typically compared to current protein sources such as fish meal, soybean meal, and groundnut oilcake, among others (Sanchez-muros *et al*., 2016) The majority of the available literature reported the utilization of fly larvae as a protein source when compared with conventional protein sources in efficient broiler and fish production. Calvert *et al.* (1969) researched common housefly larvae (Musca domestica) exploiting poultry waste as a larvae substrate and concluded that dried housefly larvae provided the protein needed for broilers for appropriate growth and development during the early stages of their lives. These scholars were among the earliest

researchers to investigate the applications of insect protein in animal feed. Other authors (Newton *et al*., 1977, 2005; St-Hilaire *et al*., 2007; Sealey *et al*., 2011; Finke, 2012) have come to the conclusion that BSFL offers a beneficial nutritional composition and can be used as a partial replacement for fish meal and other protein sources in animal nutrition.

2.4 Insect protein demand

The demand for insect proteins differs by area. The human food sector is driving demand in Asia and South America. This is particularly prevalent in nations where crickets and mealworms are grown and consumed, such as Thailand, Lao People's Republic, and Mexico (Hanboonsong *et al.,* 2013). Several firms have provided tiny amounts of insect meals to aquafeed makers to date (Alfiko *et al*., 2022). A growing number of investors are investing in various start-up companies that generate insect meals (Rumbos *et al.,* 2019). Insects initially appeared in aquafeeds less than 40 years ago, although tremendous advances in culture, optimizing production, nutritional benefits, and feeding trials have been documented (Daniel, 2018) Insect meal production is expanding quickly in China, Europe, North America, Austria, and Southern Asian countries (Alfiko *et al.,* 2022). The demand for insect protein, mainly as an ingredient in feed and pet food is predicted to reach 500,000 metric tons by 2030, up from the current market of around 10, 000 metric tons globally (Bryne, 20221). In industrial automated mass scale production most often produces at least 1 ton a day of dry insect weight (Smith and Barnes, 2015). Currently only a few thousand metric tons of insect proteins are used in aquafeed (Bryne, 2021).

In Africa, the poultry and fish industries are the primary consumers of insect protein (Schönfeldt and Hall, 2012); Leek, 2017). ICIPE in Kenya, and Chinhoyi University of Technology in Zimbabwe are able to mass produce black soldier flies and GREEiNSECT

in Kenya, a producer of crickets is among a few notable producers in Africa. Despite the region's enormous potential in terms of climatic conditions and agricultural waste supply as raw materials, industry is still in its infancy. Strict legislation in Europe and North America has limited the usage of insects to the aqua feed and pet food markets, with potential prospects for the livestock and poultry sectors (Leek, 2017).

2.5 Aquaculture and fish feed developments in Malawi

Fisheries (captured fisheries and aquaculture) is a key component of rural livelihoods in Malawi and play an important role in food and nutrition security (GoM, 2016). Malawi has an extensive history of fish farming spanning a century, the nation's smallholder aquaculture sector has yet to realize its full potential. Building the sector's capacity can offer a sustainable supply to improve food and nutrition security- a critical achievement for a nation vulnerable to food scarcity (Phiri, 2021).

One of the most important inputs in aquaculture products is feed. Despite on-going interventions, a lack of high quality but affordable feed in the sector persists. Feed supply chains have been neglected to the point where neither affordable nor high quality feed are available for the majority of fish producers. This is one of the main limiting growth factors for the aquaculture sector and needs to be addressed if the industry is to be commercialized and grow in a sustainable manner. In order to promote the sustainability of aquaculture sector, cheap and high-quality feed must be introduced in the sector through sustainable feed supply that can also be accessed by smallholder farms in Malawi.

Local fish feed production is critical to the development and sustainability of aquaculture in Africa (Gabriel *et al*., 2007), especially in Malawi. For aquaculture to flourish and bridge the gap between fish demand and supply, locally produced fish feed plays a critical role in

lowering production costs and making fish farming more appealing to both private and commercial investors and ultimately boost fish production cannot be over-emphasized (Gabriel *et al*., 2007). Development and management of fish feed play a vital role in aquaculture growth and expansion. According to Munthali *et al.*, (2022), 7.4% of fish farms in Malawi used commercial feed and the rest used homemade feed. This hinders the growth of small-scale aquaculture in Malawi.

In order to catalyze aquaculture sector development, there is a need to facilitate public and private sectors investments on a large scale in production of cheap-affordable but high-quality fish feed such as insect protein feed. Improving availability and access to high quality fish feed by manufacturing it within the country using locally available resources would help in developing the smallholder aquaculture farms (Munthali *et al.*, 2022). Lack of available fish farming inputs is stated as one of the main barriers to a greater number of commercial players entering the industry. Fish feed development has not made a significant progress in aquaculture as expected (Gabriel *et al*., 2007) especially in Malawi as most feed is imported from Zambia or purchased from Maldeco (the largest Malawian commercial fish feed producer).

2.6 Dipteran family

The Dipteran order of insects, which includes mosquitoes, black soldier flies, midges, fruit flies, and house flies, is known as the 'real flies' or 'two-winged flies' (Resh and Carde, 2003). The fly to be discussed further from this order is *H. illucens* (BSF).

2.7 The Black Soldier Fly (*Hermetia illucens*, L, 1758) Diptera*:* Stratiomyidae.

2.7.1 Introduction to the Black Soldier Fly

The black soldier fly (*H. illucens*) is native to the warm tropical and temperate zones of the American continents (Newton *et al*., 2005) and has spread over tropical and temperate regions from Argentina to central America (Sheppard *et al*., 1994). Humans and climate change have facilitated the establishment of BSF in Australia, India, Africa, Asia and Europe (Olivier, 2009; Martínez-Sánchez *et al.*, 2011; Gujarathi and Pejaver, 2013; Leek, 2017). The black soldier fly can endure a wide range of environmental circumstances (light, temperature, humidity) and it is currently native to over 80% of the planet between latitudes 46°N and 42°S (Martinez-Sanchez *et al*., 2011).

2.7.2 Life cycle of the Black Soldier Fly

The BSF goes through the entire life cycle (Figure 2.1). Larvae mature into pre-pupa in about two weeks under the correct conditions of food, relative humidity, and temperature. Given the correct conditions, pre-pupa take two weeks to mature into pupa in a process known as pupation, which is characterized by the development of an embryo within the puparium (casing), stiffness of the body, followed by immobility. When pre-pupae locate a dry medium to burrow in, they develop into pupae. Pupae in the dry medium go into a sleeping phase for at least two weeks, during which time the embryo develops within their exoskeletal casing. When fully formed, the casing breaks away at the tip, allowing an adult fly to emerge (Sheppard *et al.*, 2002).

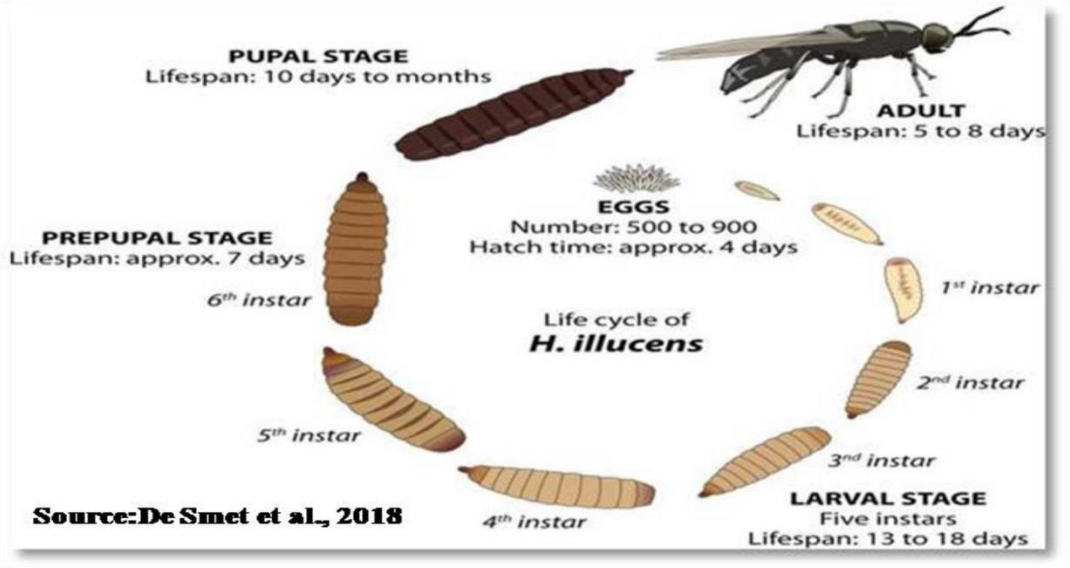

Figure 2.1. BSF life cycle (*H. illucens*).

Source: Outlined by Jeyaprakashsabari and Aanand, (2021).

When compared to one day old adults, newly emerging adult flies have underdeveloped, folded wings that progressively unfurl over two to three hours. They also have slightly larger, softer, and greenish colored bodies. Adults live for 5-12 days and mate and lay eggs throughout that period (Diclaro and Kaufman, 2009). A Black Soldier's Lifecycle under ideal conditions, the lifespan of a fly from egg to adult is projected to be 40-43 days however, given poor raising conditions, the time might last up to six months (Popa and Green, 2012). The larval and pupal stages last the longest in the lifecycle (Popa and Green, 2012). Furthermore, the larval stage dictates and regulates the lifetime of subsequent phases as well as the adult stage's productivity (Holmes *et al.,* 2012). It is the most important stage for humans in terms of economic relevance (Mutafela, 2015).

2.7.3 Ecological requirements for the Black Soldier Fly.

BSF requirements differ depending on the stage of development, thus in order to accomplish successful breeding in confinement, the conditions must be monitored and

maintained to ensure that they meet the requirements. Temperature, relative humidity, light, nutrition, and pupation substrate are all elements to consider. Table 2.1 summarizes the ecological guidelines.

Table 2.1. Ecological requirements of the Black Soldier Fly at different stages of life

Stage	**Duration (days)**	**Temperature (°c)**	**RH(%)**	**Light intensity**
Eggs	4	Above 26	Above 60	-
Larvae 4 days	0-4	26-29	65-75	Photophobic
Larvae 4 days over	4-14	26-35	65-75	Photophobic
Pre-pupa/pupa	10-14	25-30	Low	Photophobic
Adults	5-8	27-30	30-90	Photophilic. Mating occurs between 60-200µmol/m2/s and wavelength of 450nm-700nm

RH (Relative humidity).

Source: Mutafela (2015).

2.7.4 Black Soldier Fly breeding

In the warmer months of the year, a native black soldier fly colony can be formed by enticing wild female BSF to deposit eggs near a high scented food supply (Hamilton and Hess, 2011). A native population can be established in two weeks or less, depending on the number of black soldier flies in the area (Hamilton and Hess, 2011). Females lay eggs on corrugated cardboard, which has little stripes cut out and affixed to an attractant storage container (Sheppard *et al.,* 2002). The cardboard perforations must be exposed so that the female can locate a suitable location to lay her eggs (Hamilton and Hess, 2011).

Small scale BSF colonies can be established by keeping a small population of pupae that will mature into adults to act as broodstocks (Stankus, 2013). Large netted enclosure/cage that will contain the brood stocks, light, proper temperature and relative humidity, a source

of food, and corrugated cardboard are all required conditions for effective breeding (Stankus, 2013). Adult BSF mate in flight, hence they are typically confined in a wide net enclosure to allow for movement and effective mating (Bullock et al., 2013; Stankus, 2013). 2 × 1.5 × 1.8 m (Zhang *et al*., 2010) or 2 × 2 × 4 m (Sheppard *et al.*, 2002; Ekman, 2014) cage sizes have been recommended for effective productivity. The cage/netted enclosure that houses the adults should ideally be kept in a room with transparent sheets interspaced between the roofing. This allows for direct sunlight to penetrate and provides the ideal temperature range (25-35oC) for mating (Zheng, 2010; Alvarez, 2012). Artificial light has been utilized in seasons when there is no or little sunlight (Bullock *et al*., 2013; Stankus, 2013).

To prevent spoiling, the eggs should be kept dry and away from excessive moisture. Transfer the larvae to the larval production or grow-out chamber. The brood stock cage's feed and cardboard should be replaced twice weekly (Bullock *et al*., 2013; Stankus, 2013). Given that pupae need a secluded and chilly environment to develop into adults (Diclaro *et al*., 2012), they could be housed in a dry container packed with paper or wood shaving chippings. This will create an artificially protected and dry environment required for pupation. Water should be supplied for the adults.

2.7.5 Black soldier fly larvae as fish feed

Protein supplementation in fish diets has been researched using BSF larvae and pre-pupae meals for fish species. Bondari and Sheppard (1981) studied the effects of adding BSFL to the diets of channel catfish and tilapia. The aroma and texture of the fish were not affected in the testing, therefore it was still acceptable to consumers. Bondari and Sheppard (1987) evaluated a 10% BSFL substitute of fish meal and showed decreased development rates

for caged channel catfish throughout a 15-week experimental period. The diets employed in that research, however, were not isonitrogenous or isoenergetic, and hence the diets being examined did not provide similar nutritional amounts. Whereas, Fasakin *et al*. (2003) discovered that using defatted fly larvae resulted in greater overall performance than using full-fat fly larvae. There were no significant changes in growth for rainbow trout fed a meal containing 50% BSF pre-pupae for eight weeks compared to the control diet (Sealey *et al*., 2011). This study also included sensory analysis, which revealed that BSF pre-pupae treatments had no significant effect on fish fillet quality when compared to a control. St-Hilaire *et al*. (2007) discovered that incorporating BSFL into rainbow trout meals at a 25% replacement of fish meal had no influence on FCR or weight gain; however, this trial had a low number of repetitions and was conducted over a short period of time. Similarly, juvenile turbot accepted diets containing 33% BSFL, and no impacts on feed intake or feed conversion were observed (Kroeckel *et al*., 2012).

Most of these studies found that only minimal inclusion levels of BSF larvae performed similarly to fish fed conventional feedstuff, which may be attributed to the high protein content of the larvae (Bondari and Sheppard, 1987; Furrer, 2011; Newton *et al.,* 2005b; Sealey *et al.,* 2011; St Hilaire *et al*., 2007a; Zhang *et al*., 2014). High incorporation levels in fish feed (>33%) lowered not only fish development (Kroeckel *et al*., 2012; Newton et al., 2005; St-Hilaire *et al.,* 2007), but also diet palatability and protein digestibility. The type of substrate used to grow BSF larvae and the way of processing may have an impact on their use by fish. BSF, for example, was added at least up to 50% in the diet of Atlantic salmon without impacting growth or fillet quality (Lock *et al*., 2015) Similarly, Belghit e*t*

al. (2019) found that including up to 100% BSF larvae had no deleterious effects on growth in *Salmon salar*.

Although substituting insect meal for fish meal can increase the quantity of fat or modify the type of lipids in fish (St-Hilaire *et al*., 2007), While may thus alter the taste of the fish fillets, a partial inclusion of insect meal (10-50%) in the diet of fish has no effect on fatty acid profiles, scent, or flavor to the amount that consumers detect. (Makkar *et al.*, 2014). For example, there was no variation in organoleptic qualities in Atlantic salmon (Lock *et al*., 2015) or rainbow trout (Sealey *et al*., 2011) fed up to 50% BSF diet.

2.8 Organic waste as a substrate attractant for BSF oviposition

Significant colonies of females have been observed in nature in agricultural settings with significant volumes of decomposing garbage, which match the breeding requirements (Nguyen *et al*., 2009), decaying chicken droppings entice them to oviposit in poultry houses, while manure attracts them in huge cow rearing operations. (Tomberlin and Sheppard, 2002). Though any decaying organic matter may attract BSF females for oviposition (James, 1935), previous studies that used different substrate to attract and lure adult females from the wild to oviposit have had varying degrees of success, with some attractant substrates reportedly performing better than others.

Bradley (1930) observed BSF infestations in pit latrines, and as a result, Lalander *et al*. (2013) and Banks et al. (2014) employed faecal matter to attract wild BSF. The best attractant for Tomberlin (2002) and Diener *et al*. (2009) was wetted chicken feed, however Bondari and Sheppard (1987) and Bonso (2013) advocated for the use of carrion as an attractant. Nguyen *et al*. (2009) observed that wild BSF female flies are attracted to compost piles and other food sources, such as those found in normal thermal compost and

vermicomposting heaps and advocated unidentified decomposing agricultural waste as a natural BSF attractant. In addition to meat, fish, and an existing BSF colony, fermented maize grain (corn), fermented oats, and brewer's hops have all been demonstrated to attract BSF females (Stankus, 2012).

Although there is an agreement that the substrate should cause putrescence in the form of volatile chemicals, investigations conducted in different geographical locations show that different BSF populations respond differently. As a result, adequate oviposition attractants for different strains and raised populations of BSF are required, as this is a critical component that might affect egg collection and thus overall biomass production and/or waste reduction efficiency. The varying attraction reactions to different substrates suggest that BSF females may be looking for a specific food supply for their young, which if not present, may limit oviposition (Sripontan and Chiu, 2017).

2.9 Organic waste as production substrates for BSF larvae

The growth substrate has a considerable influence on major BSFL growth characteristics including as development time, feed conversion efficiency, mortality, pupal weight, and nutritional content (Zheng *et al*., 2012). As a result, commercial scale use of the technology is required, which will necessitate the use of substrates capable of producing quality larvae in a short period of time while reducing losses due to death. Unfortunately, while it is well accepted that the larvae consume a wide variety of organics, the complete spectrum of substrates for growing BSFL, particularly for biomass production on a commercial scale, remains largely unknown (Leek, 2017). Organic waste materials are also highly diverse in nature, with fluctuating moisture and nutrient content, making universal applications of findings nearly impossible. (Holmes *et al*., 2012).

Livestock manures from big, confined animal feeding operations are among them, as are palm kernel waste, pig liver, kitchen waste, rendered fish, and human feces. Hem *et al.,* 2008; Diener *et al*., 2011; Popa and Greene, 2012; Lalender *et al*., 2013; Kalová and Borkovcová, 2013; Nguyen *et al*., 2013; Zhou *et al*., 2013; Bankss *et al*., 2014; Nguyen *et al.,* 2015). Despite the fact that the larvae were able to convert the waste into a more desirable, nutritious, and less hazardous biomass rich in protein (44.4% DM), lipids (23% DM), ash (11- 28% DM), and other valuable elements such as calcium (5-8% DM) and phosphorous (Newton *et al.,* 2005, St-Hilaire *et al.,* 2007, van Huis *et al.,* 2013), the objective was to produce biomass for animal feed rather than waste management. However, the composition of BSFL biomass compares substantially to that of fishmeal and soya, which together presently supply more than 90% of the protein required by animal diets (Yu *et al.,* 2009).

The European Food and Safety Authority (EFSA) has approved the use of fruit and vegetable substrates as having the highest potential for use as feed for insect production due to the low risk of transmitting zoonotic diseases to humans when compared to substrates such as manure, catering waste, or prior foodstuffs containing meat and fish, which are not allowed because insects are considered 'farmed animals' (European scientific committee, 2015). Fruits and vegetables have a significant proportion of post-harvest wastage and losses, as well as various byproducts of the fruit and vegetable processing industries, and hence serve as a viable insect-rearing substrate. (FAO 2011, Kalová and Borkovcová, 2013, Nguyen et *al.* 2015, Parra Paz *et al*., 2015). Furthermore, enormous amounts of fruits and byproducts accumulate at production farms during peak seasons, posing disposal and public health concerns, despite the fact that the only cost to consider

for these substrates is collection, transportation, and some moderate processing, such as cutting into small pieces and removing inorganic materials. (Nguyen et al., 2009; Fila *et al*., 2013).

To be considered a protein source in a feeding diet, an appropriate rearing substrate should contain at least 20% crude protein content (Ramos-Elorduy *et al*., 2002; Munguti *et al.,* 2006; Kassahun, 2012). Food remnants (20%) and a mixture of vegetable and fruit wastes (20%) meet this requirement among all substrates (Munguti *et al*., 2006; Nguyen *et al*., 2015).Kalova and Borkovcova (2013) BSF larvae were fed over a 14-day period, 14 different waste types were tested, and only four of the wastes produced adult flies, including post-consumer food waste, indicating that these diets were most suited to larval growth. These findings are consistent with Barry (2004).

2.9.1 Household waste

The high protein and fat levels can produce ammonia and methane generation, as well as the build-up of volatile fatty acids as a consequence of anaerobic digestion Banks *et al.,* 2011). This fermentation process might theoretically produce biogas, with the remainder of the decomposing material used as soil amendment (Banks *et al.,* 2011).

Waste in the retail industry comprises uneaten and damaged goods from consumers and the food service industry, as well as losses in fresh produce due to transit spoilage and missed due dates (Kantor *et al.,* 1994). It is estimated that in the United States, 26% of all garbage is edible, with fruit and vegetables accounting for 20% of this total (Kantor *et al.,* 1994). Pieterse (2014) discovered that when BSFL were fed 10kg of kitchen garbage per square meter, they harvested 1kg of larvae (wet) per square meter every day. The amount of retail and home garbage is expected to rise in tandem with the human population's

exponential growth. Because of the significant bioconversion of this waste to nutrient-rich larvae biomass, the BSFL can be regarded a successful, and potentially less expensive, alternative protein source for animal feed.

2.10 Optimization of production of black soldier fly larvae on organic waste

Establishing a production system with a feeding plan that defines critical substrate elements that affect BSFL feeding behavior, growth, and development is required for BSFL to serve effectively and productively (Devic and Maquart, 2015). These factors include, among others, larvae feeding amounts (feed rate), feeding frequency (regime), substrate types and substrate combination ratios, substrate depth, larvae stocking densities, substrate moisture content, environmental rearing conditions such as temperature and relative humidity, and particle size of substrate (Holmes *et al.*, 2012; Van Itterbeeck, 2014).

Table 2.1. summarizes the optimal rates for BSFL synthesis based on most environmental conditions such as temperature, relative humidity, and substrate moisture content (Fatchurochim *et al.*, 1989; Sheppard *et al*., 2002; Holmes *et al*., 2012). Brits, (2017) indicated an ideal substrate depth of 5-10cm for acceptable bioconversion, however Bullock *et al.* (2013) reported a depth of 20-23cm for adequate bioconversion. The consumption rate of BSF larvae is dependent on the size of the larvae and the type of diet, according to *Diener et al.* (2009): kitchen waste (61mg/larva/day), green banana (103mg/larva/day), vegetable waste (98mg/larva/day). While the study correctly proved that optimal feed rates vary for different substrates, subsequent data have yet to invalidate the expected values. Banks *et al.* (2014), for example, showed an optimal feed rate of 111mg/larva/day on faecal matter vs the recommended 130mg/larva/day but Brits (2017) reported an optimal feed rate of 125 mg/larva/day on kitchen waste substrate. This

demonstrates that feed rate may be related to substrate quality (Liu *et al.,* 2008), implying the necessity to find appropriate feed rates on a substrate-by-substrate basis.

Banks *et al.* (2014) studied BSFL growth on two feeding regimes (lump sum/batch mode and continuous mode) and found significant differences between BSFL groups fed differently. Larvae fed in lump sum mode were larger and heavier than those fed continuously, however, matured at a slower rate compared to those fed on continuous regimes, contrary to Mutafela (2015), who found that continuous regime fed larvae took an average of 2-3 days longer to mature than batch fed larvae despite the fact that the substrates were different. Diener *et al*. (2009) discovered that feeding *H. illucens* larvae at regular intervals (continuous regime) maximizes efficiency. As a result, continuous replacement of feed (continuous feeding regime) has become a regular feeding technique in the literature, despite no demonstrated tangible biological benefit or lack thereof. (Sheppard *et al.,* 2002; Diener *et al.,* 2009.)

2.11 Processing methods of BSFL: Defatting

Processing procedures can have a negative effect on the bioavailability of protein in animal feed. (Choct and Kocher, 2000). Different defatting procedures can be employed on BSF larvae to produce a lipid-rich byproduct that has the potential to be utilized as a biofuel. (Surendra *et al.,* 2016). High fat levels in BSF larvae dilute the potential protein content, removing the oil will enhance the relative protein content remaining in the meal (Shiau *et al.,* 1990). Comment *et al*. (2007) believes that by lowering the fat level of the meal, the crude protein content can be increased to more than 60% due to less dilution of the protein with lipids. Fats have two and a half times the energy density of carbohydrates like starch and a smaller heat increment (Van der Merwe and Smith, 1991).

Defatting the larvae could consequently allow increased BSFL crude protein replacement without limiting its placement in diets due to the crude fat of the larvae. The crude fat content of BSF larvae has been observed to range between 18% (Barroso *et al*., 2014) and 39% (Haasbroek, 2016) Using solvent extraction, earlier defatting efforts allowed crude protein to increase to as high as 64% (Surendra *et al.,* 2016). Other researchers have utilized the pressing method of defatting and obtained crude protein levels of 48% (Kroeckel *et al*., 2012) and 49% (Tschirner and Simon, 2015).

Defatting, an important step prior to protein extraction, can be accomplished mechanically and by aqueous and solvents (Soxhlet and supercritical carbon dioxide) procedures, also known as (Soxhlet and supercritical carbon dioxide) methods. For example, the Soxhlet approach recovered over 100% of the lipids of the yellow mealworm, lesser mealworm, and house cricket, however the aqueous method recovered just 19 to 60% (Tzompa-Sosa *et al.,* 2014). At low extraction temperatures, supercritical carbon dioxide oil extraction of yellow mealworm larvae yielded a high yield of solvent-free oil and a protein-enriched, solvent-free residue (Purschke *et al.,* 2017). Employing the same method on yellow mealworm larvae and house crickets, followed by separation into fine and coarse fractions by air classification, three fractions were obtained: a lipid fraction of triglyceride oils enriched in essential fatty acids, fine flavor-intense, and coarse chitin-rich powder fractions (Sipponen *et al*., 2017). Aqueous extracted oils from yellow mealworms and house crickets all had favorable properties for table oils and oils used as food components (Tzompa-Sosa *et al*., 2014).

According to Boland *et al*. (2013), any use of heat or acid treatment on a component has the potential to cause protein denaturation, with lysine being the amino acid most impacted

by high heat processing and the related Maillard reactions (Parsons, 1996). Processing additionally appears to cause the whole or partial degradation of cysteine, methionine, and tryptophan (Castell, 1986). As a result, in any examination of heat or acid processing on feed ingredients, the effects on nutritional digestibility must be measured. All other production, carcass, and health indicators will be connected to the ingredient's absorption and digestibility.

2.12 Cost effectiveness and feasibility

As a result of the high costs of poultry and aquaculture feed, which can account for up to 80% of total costs, inexpensive and readily available feed is generally out of reach for the majority of smallholders in poor nations (Pechal *et al*., 2019) such as Malawi. This condition necessitates the investigation of alternative feed sources, including the possibility of using insect-derived protein. More importantly, research has demonstrated that the nutritional composition of insect-based protein meals is equivalent to or superior to that of commonly used traditional protein sources such as plant-based soybean meal or fish meal. (Sheppard *et al.,* 1994; Ssepuuya *et al*., 2017)

The use of larval meal as a substitute for fish meal at 50% and 100% reduced tilapia production costs by 18% and 28%, accordingly (Ajani *et al.,* 2004). Fashina-Bombata and Balogun (1997) assessed the cost of larvae production to that of fish meal and discovered that the cost of larvae meal production was below 20% of the cost of fish meal production for a comparable quantity. Nonetheless, this was reached 25 years ago, before the oceans' fish reserves were further decimated. Teye-gaga (2017) reported that the analysis of the The cost efficacy of several diets employed in the culture of *O.niloticus* fingerlings was demonstrated. that BSF at 75% inclusion level diet had 2.54% profit gain, suggesting that

BSF 75 was more cost effective than the other trial diets in generating a kilogram of *O.niloticus* fingerlings. As the world population grows, competition between humans and animals for protein sources will become more evident and prices will reflect the higher demand (Ravindran and Blair, 1993; Nalle *et al*., 2012). Therefore, alternative protein sources must be established for animal feeds in order to keep all protein prices as low as possible.

According to Cockcroft (2018), Insect protein, particularly defatted BSF larvae meal, might be focused on and marketed as a specialty feed, with the extracted oil functioning as a biofuel, resulting in a significantly higher monetary value and consumer evaluation of the ingredient. However, preconceptions, regulatory constraints regulating the use of specific substrates for larvae, and trade prohibitions between countries must all be addressed in order for this fledgling company to prosper financially. (Cockcroft, 2018).

2.13 *Oreochromis karongae*

Culturing species with high growth and reproductive performances may be one of the solutions to high demand for fish. *Oreochromis karongae* (Trewavas, 1941), locally known as "Chambo" is one of the prominent tilapia species preferred for Malawian aquaculture because they exhibit tolerance to harsh conditions such as temperature changes, high salinity and low water quality (Maluwa and Brooks, 1996). Since they are herbivorous and omnivorous, they feed on locally available food and are resistant to diseases (Beveridge and McAndrew,2000). *Oreochromis karongae* has the ability to withstand high stocking densities without affecting growth and possess fast growth characteristics compared to other taxonomic group of tilapia (FAO, 2005). This species is favored by consumers for its good flavor, shiny appearance and its bigger size (Kaunda *et al.*, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out at Lilongwe University of Agriculture and Natural Resources: Bunda College fish farm Figure 3.1, where a pilot plant (Appendix I, a) was built to produce larvae of the black soldier fly, processing of maggots and the feed trial.

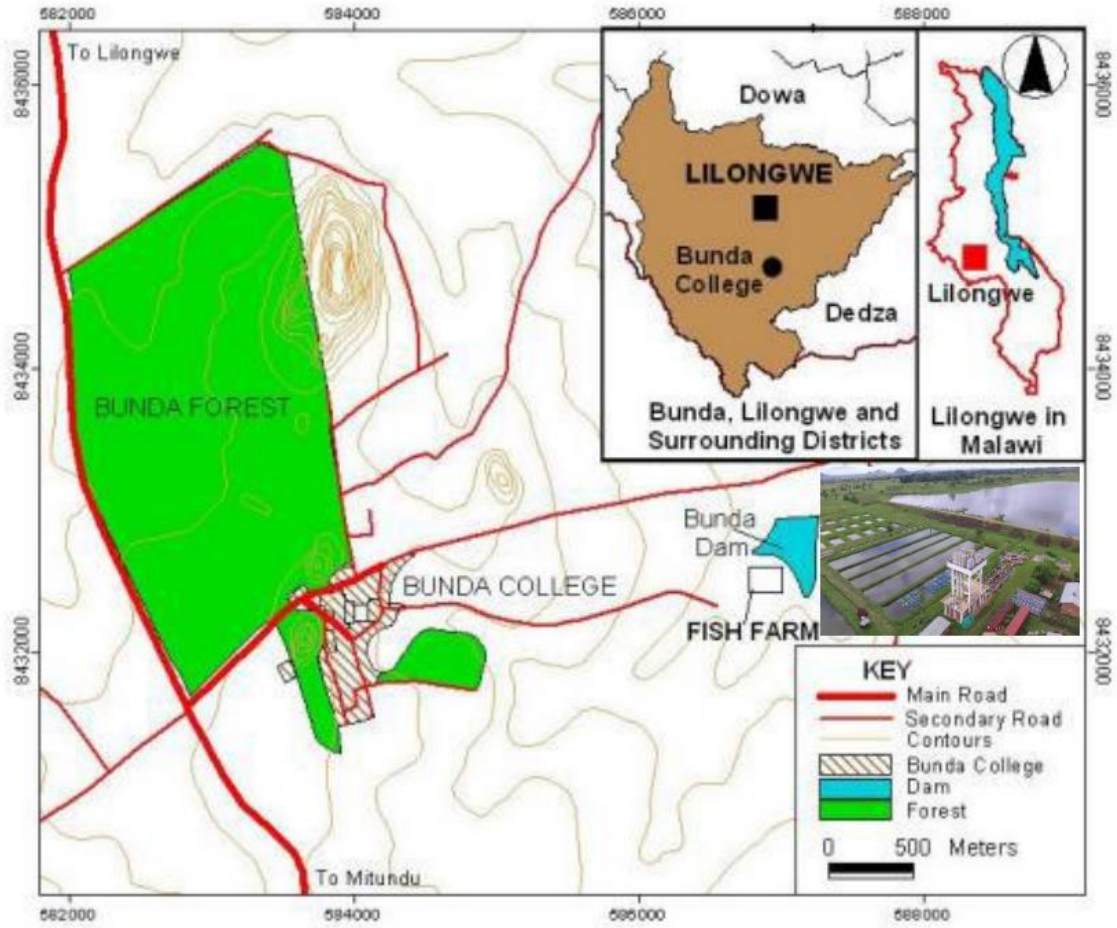

Figure 3.1. Map of the study area. Bunda fish farm, Lilongwe, Malawi.

Source: Outlined by Ndivayele, (2007)

3.2 Experiment I: Production of BSFL

3.2.1. Waste substrates

The four different wastes were collected from different places and stored in different labelled storage plastic bins with lids according to waste type. The wastes were left to ferment before being fed to BSFL. The wastes tested were as follows:

1. **Kitchen waste;** organic food waste were collected from Kumudzi eco-center restaurant, social forestry hostel and restaurants around Bunda campus. Plastic bins were placed in these different places and when the bins were full, the collecting bins were transported to Bunda aquaculture farm for the organic wastes to be fed to the larvae. Prior to storage, the wastes were blended to ease feeding access by the larvae. Kitchen wastes consisted of meat, rice, nsima (corn paste), vegetables, fruits, fish, fish bones and additives, with carbohydrates being prominent. Most of the waste were cooked.
2. **Vegetable waste;** were collected and transported from Bunda farm and surrounding markets to the research area. The wastes were blended before storage. Vegetable wastes were fresh wastes and consisted of lettuce, mustard and cabbage.
3. **Fruit waste;** fresh wastes were collected and transported from Wakawaka market and surrounding farms to the research area. Mangoes were chopped using a chopper before storage into the plastic bin while the other fruits were blended before storage. The fruit wastes consisted of guavas, mangoes, bananas, watermelons.

4. **Fish and meat wastes**; comprised of dried usipa, fresh usipa gut materials and fresh *Oreochomis* fish species. Dried usipa and fresh *Oreochomis* fish were bought from local markets and were minced in a meat grinder at the research site.

3.2.2 Broodstock colony

Nesting plastic containers for oviposition were placed outside under trees/vegetation as well as outside a building block. The nesting plastic containers were filled with different kinds of organic waste (potato peels, kitchen wastes, pig manure: 5 liters) which acted as attractants. The containers were observed for BSF eggs and larvae on a daily basis. Egg traps (Appendix I, b) were placed on the containers for egg collection. After BSF colonization the larvae were transferred to larvarium for further rearing until onset of pupae stage. The pupae were a source of captivate greenhouse brood-stock for the experiment. Prepupal were transferred into the insectarium to pupate (Figure 3.2).

Figure 3.2. Insectarium where BSF colony was kept (left) and love cage with "egg traps", top corners (right).

Source: *Photograph by Author*

3.2.3 Experimental layout

Production cages (0.80m width x 1.0m height) with wooden frames, covered with gauze as mating cages were placed in the greenhouse / insectarium (6.80m x 3.0m) (Appendix I; a). In the insectarium sunlight and artificial light (100 watts “Mira Pro2” TiRoled bulbs) were made available to facilitate mating and egg laying. In the top corners of the cages “egg traps” (Figure 3.2, right) were placed to collect the eggs. Thermo-hygrometer (Appendix I, c) (KlimaLogg Pro. TFA Dostmann GmbH and Co.KG, Zum Ottersberg 12, D-97877 Wertheim, Germany) was placed in the greenhouses to measure temperature and humidity.

Black soldier fly larvae experiment was conducted in two phases. In both phases BSFL were reared in triplicates in plastic containers (50cm x 30.4cm x 26.1 cm) with netted lids (Figure 3.3) to keep away other insects and predators in a complete randomized design (CRD). Rearing containers were kept at 30 °c - 33.86 °c and 61% - 65% humidity. Then 500 5-day old larvae were introduced randomly into one-liter rearing substrates. Substrates were being added upon checking in each rearing container if food was consumed. Water was sprayed daily when the substrate seemed to get dry to keep the conditions moist and maintain substrates humidity in a larvarium (7.40m by 4.30m) (Appendix I, a).

Figure 3.3. Inside the larvarium where rearing containers were placed in a complete randomized design.

Source: *Photograph by Author*

3.2.3 BSF larval nutritional composition

The first phase of larval replicates was reared according to substrates and it was aimed for proximate composition and fatty acids profiling. During this first phase, larvae were not handled for weight data to avoid handling stress until onset of prepupae stage marked by brown/black color of the larvae (Nguyen *et al*., 2013). The trial was terminated based on emergence of prepupae stage. Larvae were separated from substrates and pooled per substrate, cleaned with running tap water, dried with paper towel and batch weighed using an electric scale (KERN 572, Germany). (Appendix I, c)

3.2.4 Drying of BSF larvae

Chimney solar dryer (Figure 3.4) was used in drying BSFL samples fed on different substrates. The samples were weighed before drying, then killed with hot water, cleaned with running tap water, and rinsed in distilled water. The cleaned samples were placed on drying trays and placed in the chimney solar dryer under the sun where an outdoor thermo-hygrometer was placed to measure temperature and humidity during the drying days.

Figure 3.4. A Chimney solar dryer used in drying BSFL.

Source: *Photograph by Author*

This procedure was carried out in order to determine the best weather conditions for drying BSF larvae in terms of temperature and relative humidity. The larvae were chimney dried at a mean temperature of 45°C for three days. Drying of larvae was necessary in this experiment to prevent lipid oxidation, enzymatic degradation and microbiological spoilage (Rahaman, 2007) and by evaporation of water nutrients get concentrated in the product which means dried larvae have a higher protein content compared to fresh larvae. In general, the production of pelleted feed needs to use dried and subsequently milled larvae. After chimney drying, dried larvae were weighed, placed in labelled zip-lock bags and stored in a cold, dry (room temperature) location for additional examination. The larvae samples were sent to Germany for the analyses of dry matter (DM) (AOAC, 2005), ash (AOAC, 2005), crude protein (CP) (AOAC, 2005) and fatty acids (FA). The analytics were conducted at TeLA Technische Lebensmittel- und Umweltanalytik GmbH, Geestland, Germany and Gesellschaft für marine Aquakultur (GMA), Büsum, Germany.

3.2.5 BSF larval growth performance and waste reduction efficiency on different substrates

To investigate larval growth performance, 30% of each replicate's larvae were batch weighed every three days with the electrical scale (KERN 572, Germany) to avoid handling stress on the larvae. Sampling started at 9 days old. Larvae were separated from substrates by tong picked per replicate, cleaned with running tap water, dried with paper towel and batch weighed. Data sampling ended in each container when pre-pupal stage was reached (evidenced by the larvae's dark/brown hue), (May, 1961). The trial was terminated when 60% of larvae had turned into prepupae. The Diener *et al.* (2009) approach was used to compute the following:

1. Rate of larval growth (g d-1);

$$Larval\ growth\ rate(GR) = \frac{[final\ larval\ average\ weight(g) - initial\ larval\ average\ weight(g)]}{number\ of\ days\ of\ the\ trial(g)} \quad (1)$$

2. Efficiency of Conversion

To assess larval efficiency in feeding substrate consumption and metabolization, the entire final biomass (larvae + pupae) and residual substrates were weighed. The waste reduction index (WRI) and the efficiency of conversion of the ingested feed (ECD) were calculated to determine the waste consumed by the larvae and the substrate conversion efficiency into usable biomass. The following indices were calculated:

i. Waste reduction index (WRI)

$$waste\ reduction\ index(WRI) = \frac{(W-R)/W}{days\ of\ trial(d)} * 100 \quad (2)$$

W is the overall amount of diet given (g), and R is the residual amount of the diet (g), per wet weight

ii. Efficiency of conversion of ingested food (ECD)

$$Efficiency\ of conversion\ of\ ingested\ food = \frac{B}{W-R} * 100 \quad (3)$$

B is the overall amount of larval and pupal biomass(g), W is the total amount of diet given, and R is the residue of the diet (g).

3.3 Experiment II: Feed trial using *O.karongae* fingerlings

3.3.1 Experimental design and data collection

For four months, the feeding trial was carried out. The fingerlings of *Oreochromis karonage (O.karonage)* were obtained from Bunda Aquaculture fish farm. The feeding trial compared growth perfomance on different dietary treatments and was conducted in concrete tanks (Figure 3.5) (2m x 1.5m x1m) at 5 fish/m^2 (15 fish/tank). There were four experimental feed (Table 3.1).

There were two Germany industrial feed types used in the study, two from Beskow; one type with fishmeal, and another type with Hermetia meal, replacing fishmeal. The other two feed types were Bunda feed and a defatted Hermetia larvae meal replacing fishmeal (Hermetia meal from Germany, Baruth) called Bunda-Hermetia feed which were formulated (Table 3.1). *O.karongae* fingerlings (12g±3g) were randomly selected, weighed and randomly distributed among 15 concrete tanks (Figure 3.5).

Prior to the start of the feeding trial the fingerlings were acclimatized for two weeks to the environmental conditions and were fed commercial pellet crumbles. Every day, the fish were fed twice a day at 9 and 14 hours, at 5% body weight. The amount of diet that was provided to each concrete tank of fish was measured and recorded daily. Each diet was fed to three replicate tanks of *O.karongae,* and assignment of diets among the tanks was randomly done. The quantity of feed was adjusted after every two weeks throughout the length of the feeding trial based on new weight attained.

Figure 3.5. Concrete tanks used for feeding trial.

Source: *Photograph by Author*

Table 3.1. Feed composition used in the experiment

Ingredients (%)	Fishmeal CM	BSFL meal CM	Bunda feed	Bunda-Hermetia BSFL feed
Fishmeal	17.50	-	10	-
BSFL meal	-	17.50	-	10
Soybean meal	-	-	41.2	41.2
Poultry-meat meal	8.0	8.0	-	-
Mineral Premix	-	-	0.2	0.2
Vitamin premix	-	-	0.2	0.2
Rape seed grist/oil	4.50	4.50	-	-
Wheat	32.10	32.10	-	-
Maize flour	-	-	48.4	48.4
Poultry blood meal	8	8	-	-
Hydrolized feather meal	5.00	5.00	-	-
Fish oil	6.00	6.00	-	-
Aqua-Mix/F 0.5	6.50	6.50	-	-
(H)Monocalciumphosphat	0.40	0.40	-	-
Aqua-Mix/F 0.5	1.50	1.50	-	-
(H)Monocalciumphosphat	2.00	2.00	-	-
Aqua-Mix/F 0.5	9.00	9.00	-	-
(H)Monocalciumphosphat	4.50	4.50	-	-
Total	100	100	100	100

- means absent ingridients

The following procedure was used to determine and adjust the amount of feed needed by the fish based on average body weight, (ABW) (Nandlal and Pickering, 2004);

$$ABW(g) = \frac{Total\ weight\ of\ sampled\ fish(g)}{Total\ number\ of\ fish\ sampled} \quad (4)$$

$$Daily\ feed\ ration(DFR) = ABW * \#\ of\ fish\ stocked * \%\ food\ requirement \quad (5)$$

3.3.2 Fish sampling

Sampling was done every two weeks (Egna & Boyd,1997) after stocking. 70% of the fingerlings were randomly scooped out of the tanks employing a hand net in each replication of the nutritional regimens. To remove excess water, the fish were patted dry with a paper towel. Individual weight using an electric scale (KERN 572, Germany) and length (total length and standard) a measuring board were determined to the nearest 0.1g and 0.1cm respectivly. After measurements fish were put back in the tanks. At the final stage of the experiment, the fish were physically counted to assess survival rates and the fish were externally examined for any abnormalities. Data was recorded and stored in Microsoft Excel

3.3.3 Body composition analysis

Ten fish were killed at the start of the experiment and kept frozen until the completion of the experiment for proximate analysis. Five of the fish were slaughtered, pooled, and examined for nutritional composition at the end of the experiment from each experimental diet. Proximate composition of fish carcasses before and after being fed experimental diets were established using AOAC (2005) standard laboratory procedures. The following components were analyzed:

a) The crude protein content was calculated by multiplying N by 6.25 factors and using the automatic Kjeldahl procedure..

b) The Soxtec Tecator System was used to assess crude lipid, and dry matter was estimated based on weight loss after 24 hours of drying at 100°C..

c) The ash sample was burned in an oven at 600°C until it reached a consistent weight. Fiber analysis was performed utilizing the fiber cap approach, which comprises alkali and acid digestion..

d) The peak retention duration of amino acids was compared to a known standard using HPLC (Jasco, CO-2056 Plus, Intelligent Column Oven). The following fomulae were used:

$$\%\ Ash = \frac{weight\ of\ crucible + ash - weight\ of\ crucible}{weight\ of\ sample} * 100 \qquad (6)$$

$$\%\ Nitrogen \qquad (7)$$

$$= 100$$

$$* \frac{(a-b) * normalityof\ HCl * molar\ mass\ ofN * volume\ of\ digest}{1000\ * weight\ of\ sample\ (g) * volume\ of\ aliquot}$$

Where a = sample titre, b = blank titre and N = nitrogen

$$\%\ Crude\ Protein = \%\ N\ * 6.25 \qquad (8)$$

$$\%\ Crude\ fat = \frac{(weight\ of\ fat) - (weight\ of\ empty\ flask)}{weight\ of\ sample\ \ (g)} * 100 \qquad (8)$$

$$\% \, Crude \; fibre = \frac{A - B}{C} * 100 \quad (9)$$

Where A is the weight of the dry crucible and sample, B is the weight of the lit crucible and ash, and C is the weight of the sample (g).

3.3.4 Growth performance and nutrient utilizatioon indices.

Specific growth rate (SGR), weight gain (WG), protein efficiency ratio (PER), and average weight of fish are all growth performance and nutrition utilization indicators that were calculated after harvest based on Stickney (1994) and feed conversion ratio (FCR) based on Agbo, (2008), in experiment I and II. Condition factor (k) was calculated based on Nash *et al*. (2006) .The parameters were determined as follows;

$$Average \; Daily \; weight \; gain(ADG) = \frac{weight \; gain \; (g)}{time(days)} \quad (10)$$

$$Mean \; Weight \; Gain = final \; mean \; weight - initial \; mean \; weight \quad (11)$$

$$Relative \; growth \; rate(RGR) \quad (12)$$

$$= \frac{mean \; final \; weight \; of \; fish - mean \; initial \; weight \; of \; fish}{mean \; initial \; weight \; of \; fish} \quad (13)$$

$$Specific\ growth\ rate(SGR)$$

$$= \frac{\ln(mean\ final\ weight) - \ln(mean\ initial\ weight)}{time\ (days)} * 100 \quad (14)$$

$$Condition\ factor\ (k) = \frac{W_2}{{L_2}^3}$$

K for condition factor, W for final weight (cm), and L for standard length (cm).

Apparent Feed Conversion Ratio (FCR) calculated based on Agbo (2008).

$$FCR = \frac{weight\ of\ feed\ fed}{weight\ gain\ of\ fish} \quad (15)$$

Survival rates for all the treatments were calculated based on OlaniyiAfolabOpasola *et al*. (2013)

$$Survival\ rate\ (\%) \quad (16)$$

$$= \frac{\#\ of\ fish\ at\ the\ end\ of\ the\ experiment}{number\ of\ fish\ stocked} * 100$$

$$Protein\ efficiency\ ratio = \frac{wet\ weight\ gain(g)}{total\ protein\ intake(g)} \quad (17)$$

3.3.4 Water quality parameters

Throughout the culture period, the water quality in the experimental units was monitored and analyzed to investigate environmental influences on the experiment.. Temperature was measured using a thermometer. Dissolved Oxygen, pH and Ammonia (NH3-N) concentration were monitored by JBL Combiset (JBL GmbH and Co. KG 67141 Neuhofen Diesselstraβe 3 Germany). In each of the experimental tanks, three readings were taken for each parameter. Water samples were taken daily at 09:00 and 16:00 hours.

Water smples for ammonia were collected and analyzed in the aquaculture laboratory at the farm. Five ml of water were put in 2 small glass jars collected from each tank of which the glass jars were rinsed three times with the tank water. One of the glass jar held the 5 ml of the tank water that was a control. Four drops of JBL ammonia reactive agents from two different reactive agents vails and 5 drops from the third vail (vails labelled 1,2,3) were added to the second glass jar of the 5ml of the collected water distinctly, and each time the distinct reactive agents were added the glass cylinder was shaken to blend the water with the reagents and was observed for 15 minutes. After 15 minutes the cylinders were placed against a color chart with glass jar 2 (with reactive agents) placed on colorless side of the chart and the control on the colored side of the chart. The jars were moved across the color chart until the experimental jar color was similar to that of the control jar, and the levels of ammonia were obtained based on the color.

3.4 Statistical Analyses

All Data were analyzed using One Way ANOVA (P value of 0.05 set as a threshold), followed by a comparison of means using Tukey test, except for survival rates data were subjected to Kruskal Wallis test. All statistical tests were done using statistical package IBM SPSS (Version25). Microsoft Excel (2019) for windows was used to create graphs All data is provided in the form of means and standard error (SE).

CHAPTER FOUR

RESULTS

4.1 Experiment I: Production of BSFL

4.1.1 Evaluation of BSF larvae performance on various substrates

The growth rate of black soldier fly larvae (BSFL), waste reduction and bioconversion of the rearing substrates by BSFL varied significantly ($p<0.05$), (Table 4.1). Growth rate was highest (9.40±0.39) in kitchen waste (KW) substrate compared to the other rearing substrates and lowest in fishmeal (FM) substrate (0.64±0.70). Waste reduction index (WRI) describes larval ability to reduce feeding substrates. Larvae were able to reduce KW substrate (5.68±0.04) better than any other substrates. The lowest WRI was recorded in vegetable waste (2.84±0.02). The highest mean value of efficiency of conversion of ingested food (ECD) was in KW (9.62±0.007) which was found to be the best substrate in being converted into valuable biomass. Fruits (F) and vegetable (V) substrates were not significantly different from each other but were significantly different from fish and meat substrate (1.07±0.001) which was the lowest.

Larvae fed on vegetable waste took longest time (27 days) for first prepupae to emerge than any other substrates. Larvae fed on kitchen waste substrate took shortest days (11 days) for prepupae to emerge.

Table 4.1. Growth performance, Waste Reduction Efficiency of BSFL reared on different experimental substrates.

	F	V	FM	KW	p-value
Time needed for pre-pupal onset (days)	23	27	15	11	-
Growth rate (g d^{-1})	3.99 ± 0.23^{b}	2.71 ± 0.10^{c}	0.64 ± 0.70^{d}	9.40 ± 0.39^{a}	0.01
WRI % (d^{-1})	3.03 ± 0.09^{c}	2.84 ± 0.02^{d}	4.72 ± 0.04^{b}	5.68 ± 0.04^{a}	0.02
ECD%	3.61 ± 0.001^{b}	3.68 ± 0.003^{b}	1.07 ± 0.001^{c}	9.62 ± 0.007^{a}	0.01

Significant differences exist for means (±SE) values in the same row with different superscripts ($p<0.05$); F= Fruits, V= Vegetables, MF=Meat & Fish, KW= Kitchen Waste, GR=growth rate, WRI=waste reduction index, ECD= Ingested food conversion efficiency

Data on average total final biomass of larvae and prepupae fed on different organic substrates (Figure.4.1), showed that substrates had an effect on final biomass with kitchen waste recording the highest biomass (110.44g±4.67) and, fish and meat the lowest (10.11g±1.12).

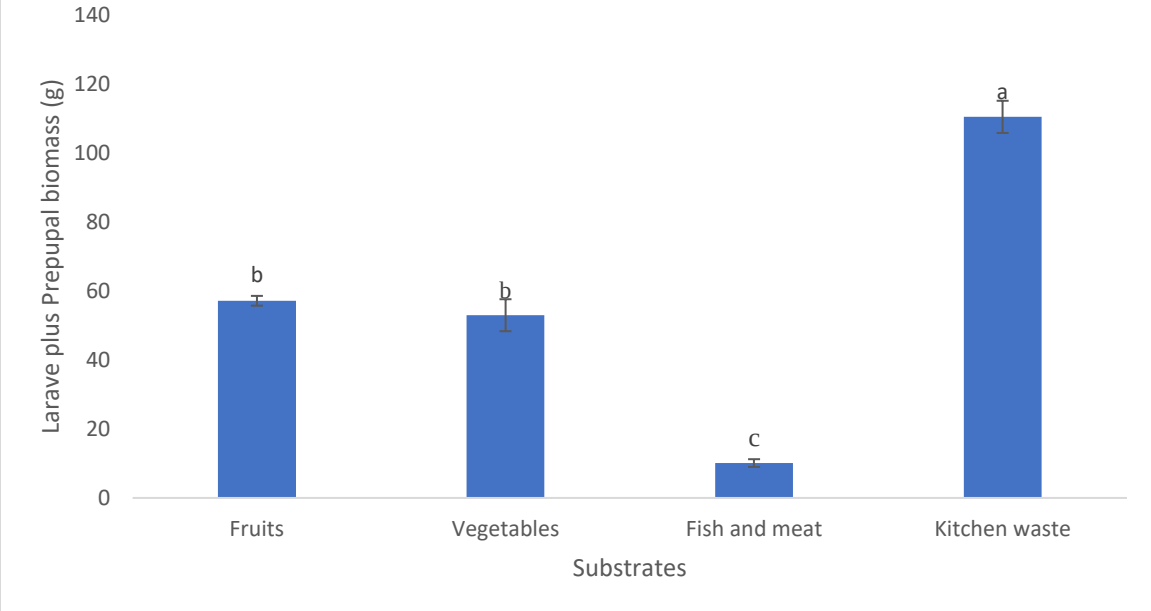

Figure 4.1. Average final biomass of BSFL and prepupae from different organic substrates

4.1.2 Nutritional composition of whole BSFL

Main BSFL nutritional composition (Table 4.2.) were analyzed from whole BSFL and had varied significantly ($p<0.05$) among treatments in different parameters. Highest energy content was recorded in kitchen waste (27.91±0.48) fed larvae but was not significantly different from larvae fed on fruits and fish and meat substrates and was lowest in larvae fed on vegetable substrate (20.77±0.40). Highest carbohydrates content was significantly comparable in larvae fed on fruit waste and kitchen waste substrates and lowest content was in meat and fish substrate fed larvae (13.25±0.38). The ash content was highest in vegetable substrate fed larvae (21.64±0.51) and was lowest in larvae fed on kitchen waste substrate (8.83±0.24). Highest protein content was in vegetable substrate fed larvae (46.83±0.41) and lowest protein content was in kitchen waste substrate fed larvae (36.91±0.36). Highest lipids content was in kitchen waste substrate fed larvae (35.28±0.78) and decreased significantly in vegetable substrate fed larvae (16.34±0.38).

Table 4.2. Proximate analysis of macronutrients of whole BSF-larvae (stage 4) reared on different substrates.

	F	V	MF	KW	p- value
Energy MJ/kg	$26.44±0.26^{a}$	$20.77±0.40^{b}$	$26.80±0.44^{a}$	$27.91±0.48^{a}$	0.01
% Total Carbs	$19.46±0.48^{a}$	$15.19±0.42^{b}$	$13.25±0.38^{c}$	$18.97±0.40^{a}$	0.01
% Ash	$10.47±0.37^{bc}$	$21.64±0.51^{a}$	$10.52±0.51^{b}$	$8.83±0.24^{c}$	0.01
% Protein	$40.77±0.62^{c}$	$46.83±0.41^{a}$	$43.16±0.66^{b}$	$36.91±0.36^{d}$	0.01
% Lipids	$29.31±0.32^{c}$	$16.34±0.38^{d}$	$33.08±0.52^{b}$	$35.28±0.78^{a}$	0.01

Significant differences exist for means (±SE) values in the same row with different superscripts ($P<0.05$); DM=dry matter. F= Fruits, V= Vegetables, MF=Meat and Fish, KW= Kitchen Waste

From Table 4.3, vegetable fed larvae recorded highest saturated fatty acids content (54.9) and lowest was recorded in fish and meat fed larvae (36.4) which was the opposite trend for unsaturated fatty acids in meat and fish fed larvae. Omega-3-fs registered highest and were comparable in kitchen waste and vegetable fed larvae (3.0).

Table 4.3. Fatty acids content of whole BSFL reared on different substrates (data expressed as g/100g DM, mean)

	F	V	MF	KW
Saturated fatty acids	47.3	54.9	36.4	49.2
Unsaturated fatty acids	52.7	45.1	63.6	50.8
Monounsaturated FA	18.9	28.3	30.1	15.9
Polyunsaturated FA	33.8	16.8	33.5	34.9
Omega-3-fs	2.3	3.0	2.2	3.0
Omega-6-fs	31.5	13.7	31.3	31.9

4.2 Experiment II: Feed trial using *Oreochromis karongae* fingerlings

4.2.1 Efficiency of utilization of feed and growth performance

Table 4.4. results showed the apparent feed conversion ratio, condition factor and survival rate to be not significantly affected by different experimental diets. Apparent protein efficiency ratio (APER) showed that fish fed on BSF meal custom made (CM), Bunda feed and Bunda-Hermetia BSF feed had lowest APER and significantly varieed ($P<0.05$) from custom made fed fish which recorded the highest APER (1.24±0.05). Fish fed on Fishmeal CM were significantly different ($P<0.05$) in final weight, weight gain, average daily weight gain and relative growth rate from the fish fed on Bunda feed. However, these parameters were not significantly different from fish fed on BSF meal CM and Bunda-Hermetia BSF feed. Relative growth rate indicated that Bunda fed fish gained approximately 345.6± % of their initial weight in 126 days and were significantly different from fishmeal custom made fed fish which gained approximately 448.81±19.50% of their initial weight in 126 days.

Table 4.4. Growth performance and feed utilization efficiency of *O. Karongae* fed on variousdiets.

	Fishmeal CM	BSF meal CM	Bunda feed	Bunda Hermetia-BSF feed	p-value
I ABW (g)	11.3±0.12	11.3±0.12	11.3±0.12	11.3±0.12	0.897
Final ABW(g)	62.7±2.24[a]	55.4±2.27 [ab]	51.04±0.73[b]	54.9±1.45 [ab]	0.009
WG (g)	51.31±2.24 [a]	43.97±2.27 [ab]	39.61±0.73 [b]	43.49±1.50 [ab]	0.009
Average daily WG	0.43±0.02 [a]	0.37±0.02 [ab]	0.32±0.01 [b]	0.36±0.01 [ab]	0.009
Relative growth rate	448.88±19.56[a]	384.66±19.87 [ab]	346.5±17.30[b]	380.52±13.09[ab]	0.023
SGR(%)	1.42±0.03 [a]	1.31±0.03 [ab]	1.20±0.01[b]	1.30±0.02 [ab]	0.007
Condition factor	2.99±0.02	2.85±0.09	2.89±0.11	3.07±0.08	0.334
AFCR	2.02±0.18	1.91±0.17	2.13±0.02	2.06±0.11	0.868
PER	1.24±0.05 [a]	1.04±0.05 [b]	1.01±0.06 [b]	1.01±0.03[b]	0.012
Survival rate (%)	88.9±2.22	95.6±2.22	95.6±2.22	91.1±4.44	0.289

Means (±SE) values are for triplicate feeding groups. Means in the same row followed with different superscripts are significantly different ($P<0.05$). rows with no letters indicate no significant differences; CM (custom made), ABW (Average body weight), AFCR (Apparent feed conversion ratio), PER (protein efficiency ratio), WG (weight gain)

The fish growth trends (Figure 4.2) for the experimental period showed that fish fed on BSF meal custom made had highest growth rate in the first five weeks compared to the other four treatments and came second in the last weeks of the experiment. After week five, fish fed on fishmeal custom made had highest growth rate until the end of the experiment.

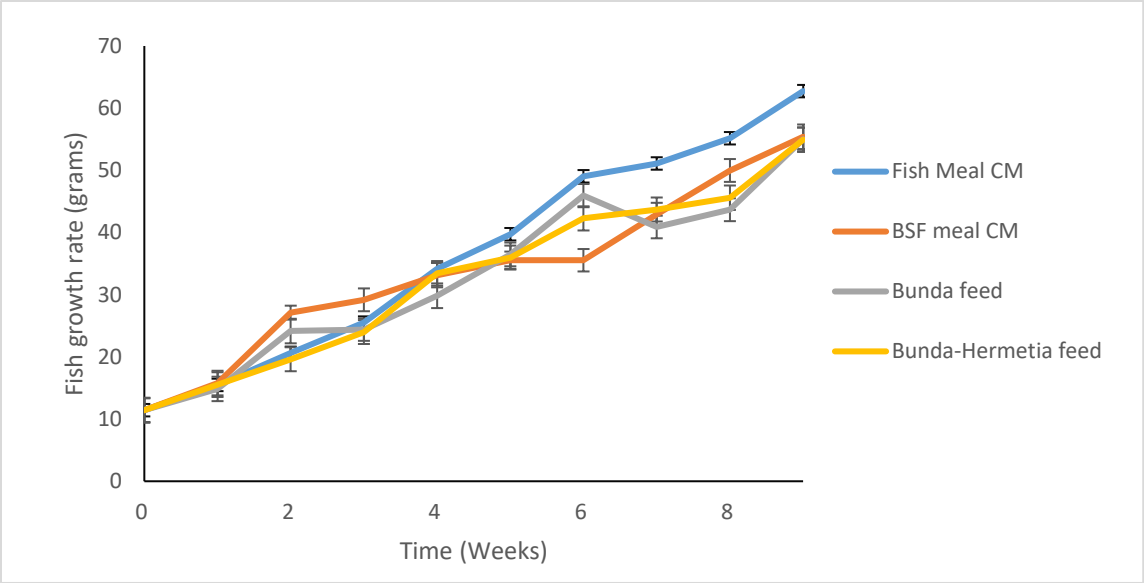

Figure 4.2. Fish growth trends fed on different experimental diets.

4.2.2 Body proximate composition analysis for whole fish samples

There were significant differences ($p<0.05$) between initial and final body composition of the whole fish samples (Table 4.5). Highest crude protein (69.98±0.01), lowest fat (23.26±0.01) and moisture content (2.69±0.06) was recorded in initial fish that were not exposed to the experimental diets. There was no statistically significant change in crude protein in fish fed on different experimental diets. However, fish fed on fish meal custom made (CM) recorded highest crude protein content (63.94±0.01 %), crude fat content (26.18±0.02) and lowest in ash content (9.51±0.02). Bunda-Hermetia BSF fed fish recorded lowest crude protein (23.96±0.0) and highest ash content (14.13±0.0

Table 4.5. Whole body composition of initial fish and fish fed on different diet

	Fishmeal CM	BSF meal CM	Bunda feed	Bunda Hermetia-BSF feed	Initial	p-value
Moisture(%)	6.06±0.06[b]	4.49±0.06[c]	12.82±0.06[a]	3.77±0.06[d]	2.69±0.06[e]	0.001
Ash (%)	9.51±0.02[c]	9.87±0.06[c]	10.08±0.01[b]	14.13±0.01[a]	14.01±0.01[a]	0.001
C. fiber (%)	0.03±0.00[b]	0.04±0.00[a]	0.04±0.00[a]	0.02±0.00[c]	0.04±0.00[c]	0.001
C. fat (%)	26.18±0.02[a]	25.63±0.03[b]	25.42±0.01[b]	23.96±0.01[c]	23.26±0.01[c]	0.001
CP (%)	63.94±0.01[b]	61.84±0.01[b]	61.41±0.01[b]	61.18±0.01[b]	69.98±0.01[a]	0.001

Mean (±SE) values are for triplicate feeding groups. Means in the same row followed with different superscripts are significantly different ($P<0.05$); CM (custom made), CP (crude protein), C. fiber (crude fiber), C. fat (crude fat)

4.2.3 Water quality parameters

Mean water temperature, dissolved oxygen (DO), and Ammonia are shown in Table 4.6. The mean values for water quality parameters during the entire feeding experiment were not significantly different ($p>0.05$) among the treatments. The mean temperature ranged from 24.02±0.78 °C to 24.48±0.07 °C. DO ranged from 6.21±0.08 to 6.52±0.11. pH ranged from 7.45 to 7.63 and Ammonia was 0.04±0.02 to 0.03±0.02 across all the treatments.

Table 4.6. Water quality parameters collected during feeding trial (Mean± SE)

	Fishmeal CM	**BSF meal CM**	**Bunda feed**	**Bunda Hermetia-BSF feed**	**p-value**
DO (mg/l)am	6.42± 0.08	6.52±0.11	6.40±0.09	6.40±0.11	0.740
Pm	6.21±0.08	6.48±0.06	6.33±0.08	6.35±0.07	0.369
Temp.(°C)am	24.32±0.09	24.02±0.78	24.12±0.70	24.32±0.80	0.874
Pm	24.48±0.07	24.2± 0.09	24.26±0.10	24.26± 0.10	0.360
pH	7.45-7.49	7.57-7.58	7.46-7.48	7.58-7.59	
Ammonia(mg/l)	0.03±0.02	0.03±0.01	0.03±0.01	0.04±0.01	0.992

Means (±SE) values are for triplicate feeding groups. DO: Dissolved Oxygen, CM (custom made)

CHAPTER FIVE

DISCUSSION

5.1 Experiment I: Production of BSFL

In fish, there are local constraints on feed in aquaculture industry such as cost of feed importation, poor quality of local ingredients, competition with humans and other animals, and sustainability putting pressure on potential fish feed producers to find cost effective and sustainable alternative animal protein sources as feed components to substitute expensive feedstuffs derived from marine elements (FAO, 2018). According to Barroso *et al.* (2014) and Henry *et al.* (2015), insect meals from dipterans such as the common housefly (*M.domestica*) or the black soldier fly (*H.illucens*) have nutritional characteristics similar to fishmeal which makes them an ideal substitute for conventional feed ingredients. The current study assessed how different rearing substrates influenced the larval growth rate of BSF and development time, efficacy in waste reduction and bioconversion and nutritional composition.

5.1.1 Assessment of BSFL performance on different substrate

BSFL growth rate and conversion efficiency through WRI and ECD were the means of determining the BSF larval growth performance on different waste substrates in the present study. Waste conversion into valuable biomass is affected by type and quality/nutrient composition of the substrate. Growth of insect larvae is influenced by many factors such as access to substrate, texture of the substrate and presence of essential nutrients in the substrates (Lalander *et al.,* 2019). According to Cohen (2004) shortages or even lack of essential nutrients led to reduced growth and lower survival rates, and even when the nutrients are available, the nutrients must be bioavailable to the insect larvae.

The larvae of the black soldier fly used in this investigation good performance on kitchen waste substrate compared to any other substrates can be attributed to being heterogenous, nutritionally balanced and better structural properties as kitchen waste consisted of corn paste known as nsima, vegetables, fruits, meat, fish bones, rice, and additives which were thoroughly cooked. Tschirner and Simon (2015) reported that heterogenous of a substrate improves its nutritional quality which is attributed to highest kitchen waste fed larval weights and yields. The results are comparable to the results reported by Nguyen *et al*. (2015) evaluated the development rate, size, and mortality of BSFL fed on poultry feed, pig liver, pig manure, kitchen food waste, fish renderings and pig liver, finding that the latter had the fastest growth, the heaviest biomass, and the highest yields. According to Tomberlin *et al.* (2002), BSFL consume diets with greatest fat content during larval stages in order to build up a fat body which is necessary to complete development and survive as adults long enough to mate and lay eggs. Similarly, Nguyen *et al*. (2015) reported that probably the reason kitchen waste substrate had the best performance to any other substrates apart from fish and meat waste, was high content of fat and calorie which might have been responsible for producing heavy larvae.

Fruits, vegetables, and fish and meat are reported to be homogenous in nature. And have nutritionally imbalanced composition as they contain lignocellulose and grease respectively, such that BSFL grow at a slower rate and attain lower body weights (Nyakeri *et al.,* 2017). However, vegetable substrate had high crude protein content, the low carbohydrate content and average higher lignocellulose content which has poor biodegradability (Hubbe,2014) are a limiting factor for an efficient digestion of these substrates. It is likely that the reduced carbohydrate content had a negative impact on

prepupal weight and development rate. Whereas dietary fiber has been known to cause poor growth, it causes delayed feeding and reduced feed intake, digestibility and nutrient utilization (Tomberlin *et al*., 2009; Tschirner and Simon, 2015; Nyakeri *et al*., 2017).

Although the consistency and physical texture of the substrate were not tested in the present study. Visual evidence in the present study observed that fish and meat substrate was quite thick and greasy affecting the substrate consistency. This might have limited BSFL mobility and access to the available nutrients as larval feed intake was reduced leading to lighter larvae. According to Spranghers *et al*. (2017) slow development of larvae was attributed to high amount of grease in the substrate which was also the case in the current study, with fish and meat substrate as observed in the rearing containers. Furthermore, grease does not favor the feeding of BSFL (Nyakeri *et al*., 2017).

Waste Reduction Index (WRI) illustrates amount of food the larvae has consumed through-out its feeding stage. The highest WRI was observed in kitchen waste, this can be attributed to larvae heavily consuming kitchen waste which was cooked making it more palatable to be consumed in a shorter period reducing the waste at higher rates. The current results contradict with results obtained in a study done by Nguyen *et al*., (2015) on ability of BSFL to recycle food waste. Nguyen *et al*., (2015) reported highest WRI in fruits and vegetable waste and the reason was attributed to larvae consuming food for a longer time as they took longer to develop to pre-pupae stage. In the current study low WRI on both vegetable waste and fruit waste can be attributed to larvae's inability to heavily and thoroughly feed on the vegetable and fruit waste.

Food Conversion Efficiency (ECD) outlines the ability of the larval to convert the consumed food into their biomass. Higher ECD index means better efficiency in

transforming the rearing substrate into larval biomass. The nutritional composition of the rearing substrate is the main factor that affects the feed conversion efficiency of BSFL. Oonincx *et al.* (2015), observed that diets with high protein content resulted in higher ECD. In the current study, the highest ECD index was highest in kitchen waste fed larvae although the protein content was the lowest, showing that BSFL was more efficient in kitchen waste conversion into useful body biomass.

5.1.2 Nutritional body composition of whole BSFL and larval developmental time.

The chemical composition of the substrate has an impact on the nutritional profile of the larvae. Bieners and Graham (2019) reported that Larval composition is influenced by feed type with heavier larvae producing significantly more fat/lipids than lighter larvae which is consistent with the current study; Kitchen waste fed larvae being heaviest with total biomass of 110.44g and with more lipid content of 35.28%.

In the present study larvae fed on the different substrates had protein content in mature larvae between 36.1% to 46.83% dry weight in line with research findings by Jucker *et al.* (2017), Tinder *et al.* (2017), Sprangers *et al. (*2017), Shumo *et al. (*2019), Lalander *et al*. (2019). The highest protein content was found in vegetable waste fed larvae (46.83% DM) which is similar to what Jucker *et al.* (2017) yielded in respect to other substrates used, despite being a bit higher than found in this study. Results obtained for crude protein on the larvae reared on kitchen waste are consistent with that obtained by Shumo *et al.* (2019) who also obtained lowest protein contents in kitchen waste fed larvae (33%). In the present study ash contents are higher (8.83-21.64) than the results obtained according to Barroso *et al*. (2014), which was 9.3% in larvae and 19.7% in pre-pupae, and Jucker *et al.* (2017) which was 5.6%-14.3%. The varied results can be attributed to differences in processing

methods of larvae, age of the larvae at harvesting and composition of particular substrates used (Ojewola *et al.*2005).

According to Gobbi *et al.* (2013), Oonincx *et al.* (2015), Spranghers *et al.* (2017) slower development in larval is attributed to poor substrate feed quality provided, in specific poor protein contents in the substrate diets. Which is not the case in this study as substantial differences were detected among the treatments: larvae reared on kitchen waste substrate, with lowest content of protein, had the shortest development period and pre-pupae emerged on the 11^{th} day of the trial. The faster development observed on larvae reared on kitchen waste substrate could be attributed to the bioavailability of the nutrients and a better energy balance from protein, lipids and carbohydrates, as the energetic demand should be compensated by high carbohydrates and lipids content found in the kitchen waste substrate needed by the larvae for biomass production.

Vegetable waste fed larvae with highest protein content had the longest development period. Emergence of pre-pupae took 27 days in vegetable waste fed larvae unlike in Spranghers *et al.* (2017) who used similar type of substrate. Even though the amount and quality of protein in the diet appears to be the main factor influencing development time in insects (Friend 1958, House 1961, Oonincx et al. 2015), the slower larval development noted on vegetable substrate could be due to a lack of energy balance from lipids and carbohydrates. The longer developmental time can also be attributed to BSFL preference to feeding low cellulosic substrates and delay their feeding on high cellulosic substrates (Manurung *et al*., 2016).

Fatty acid composition of BSFL is affected by fatty acid composition in the substrates and larval weight (Lalander *et al.*, 2019*;* Elward *et al.*, 2019). In this study, larvae fed on

vegetable waste had higher amount of fatty acids and were lower in fruits fed larvae than that reported by Jucker *et al*. (2017) who used the same types of the substrate. According to Ewald *et al*. (2019) larvae with high saturated fatty acid are associated with higher larval weights which contradicts the current findings as found in larvae fed on vegetable waste, and lower unsaturated fatty acid. It has been reported that low amount of n3 PU FA decreases nutritional quality of insect meal inclusion especially when full fat meals are used (Bovera *et al.,* 2016*;* Renna *et al.,* 2017). This poses as a challenge of using insect meals intended for animal and fish feed as n-3 is essential for optimal growth and reproduction especially in BSFL fed fish (Tocher, 2015; Ewald *et al,* 2019). It was observed in the current study that an increase in saturated fatty acid led to a decrease in unsaturated fatty acid content and vice versa in BSFL which is similar to what Liu *et al.* (2017); Ewald *et al.* (2019) found. This implicates a possibility of modifying fatty acid profile in larval diet by incorporating fish offal and algae to increase fatty acid levels as suggested by Oonincx *et al*. (2015), Sprangers *et al.* (2017).

5.2 Experiment II: Feed trial using *O. karongae* fingerlings

The current study completely replaced fishmeal with defatted BSF meal in the diets without affecting growth performance and nutrient utilization of juvenile *O. karongae* when compared to fishmeal-based diets. *O.karonage* fish indicated no significant differences among various experimental diets in weight gain, relative growth rate, and specific growth rates suggesting that complete BSFL meal replacement of fishmeal for *O.karonage* fish is ideal without reducing growth. The results are comparable with a study done by Muin *et al.* (2017), where fishmeal was replaced completely with BSF meal in Nile tilapia and observed slight differences on weight gain of fish. However, specific growth rate, food

conversion ratio and crude protein content were not significantly different among diets and dietary protein was better at 100% inclusion level. In gilthead seabream (*Sparus aurata*) partial replacement of fishmeal by *H. illucens* prepupae meal did not significantly reduce fish growth rate (Karapanagiotidi *et al.* 2014). Madibana *et al*. (2020), reported that a partial dietary replacement of fishmeal with BSF meal did not affect feed utilization and growth performance of juvenile dusky kob (*Argyrosomus japonicus*). Jahan *et al.* (2021), reported highest fry production at harvest in nursing common carp (*Cyprinus carpio)* when fishmeal was replaced at 100% with BSF meal. In rainbow trout (*Oncorhynchus mykiss*) the growth of fish fed enriched *H. illucens* diets were not significantly different from fish fed a fishmeal-based control diet (Sealey *et al.* 2011).

Further processing of larvae meals may be beneficial as it may improve palatability, nutrient availability, and utilization hence better performance (Fasakin *et al.* 2003). Fasakin *et al.* (2003), stated that Clariid catfish fed defatted maggot meal that replaced fishmeal completely performed better than full fat maggot meal fed fish, and was favorably comparable with fish fed fishmeal-based diet. In the present study, the no significant difference in performance of BSFL diets and fishmeal diets is attributed to the larvae being defatted. Lock *et al*. (2015) reported a difference in performance of two insect meal diets that were processed differently, with insect meal (A) that replaced fishmeal at 100% performed comparably to fishmeal diet.

Protein Efficiency Ratio (PER) values were significantly different among experimental diets. Protein efficiency ratio values found in this study were greater than in a study on *O.niloticus* fed with different levels of BSFL meal diets by Muin *et al.* (2017). Better protein utilization (digestion, absorption and synthesis) was achieved in this study.

According to Karapanagiotids *et al.* (2014), protein efficiency ratio indicates protein quality in feedstuff and higher values are needed for better utilization in fish. The varying values of PER among studies are attributed to the quality of dietary protein used in specific studies (Muin *et al.* 2017).

Feed conversion ratio was not significantly different among the diets. A low feed conversion ratio value indicates a high-quality feed and efficiency of the fish to convert food into its body biomass. The present study yielded the FCR which are in the recommended tilapia ranges of 1.5 - 2.0 (Watanabe *et al.*, 2002; Rahman and Arifuzzaman, 2021) indicating that good feed utilization was obtained among the diets. The FCR results reported by other studies are higher than the present study. Muin *et al.*, (2017), reported FCR values above 2.5.

Condition factor (k) shows physiological state of the fish, higher condition factor (k>1) is an indicator of good the fish's physiological condition and lower value is an indicator of poor physiological condition of the fish (Ogunji *et al.*,2008). Condition factors in all treatments were above one. Kroeckel *et al. (*2012) found that an increase in *Hermetia* meal fed to juvenile turbort (*Psetta maxima*) led to decreased condition factor values. Unlike in the present study BSF meal inclusions were not significantly different and did not affect physiological state of fish.

BSF meal diets did not affect survival rates of the fish. Reduced survival rates were due to predation by birds and survival rate values were highest in BSFL meal diets. Similar findings were reported by Kroeckel *et al. (*2012), Muin *et al.* (2017) who achieved 100% survival rates which support that BSFL meal does not negatively affect survival of fish.

Fish carcass body proximate composition at the end of the experiment showed that dietary treatments affected protein content and fat deposition in the fish carcasses. According to Fawole *et al*. (2007) knowledge of proximate profiles such as protein content, lipid, ash and other nutrients is crucial to ensure that they are within the range of dietary requirement and commercial specifications. Fish fed on fishmeal custom made diet had highest crude protein content but crude protein did not significantly vary among the treatments in the study. The results obtained are consistent with results reported by Kroeckel *et al.* (2012), in which prepupae BSF meal replaced fishmeal for juvenile turbot (*Psetta maxima*). In the current investigation, the fish that were not fed experimental diets had the greatest level of crude protein and least crude fat content. The results are consistent with results obtained by Fasakin *et al.* (2003) in the experiment with defatted maggot meal in catfish. According to Hossain (2012) higher content of crude protein and crude fat in fish reflects a feed source enriched in these nutrients which imply BSFL meal being enriched in crude protein and crude fat.

The results obtained on proximate composition were lower in moisture, and ash content but higher in fat content compared to the results reported by Mchazime and Kapute (2018) on a wild captured tilapia species known as *O. shiranus*. The ash values obtained were lower than results reported by Longwe *et al.,* (2016) on *O. karongae* raised in pond, however, were greater than the results obtained by Fawole *et al*. (2007). Size of fish is one of the major factors that influence ash content, in that smaller sized fish species such as in this current study with average weight range of 53.4±1.45 - 62.7±2.24 grams, exhibit higher ash content due to the higher bone to flesh ratio (Daramola *et al*., 2007). High ash content is thus consistent with bony fish such as tilapia species (Devi and Sarojnalin, 2012). Lower

moisture content implies lower water activity which determines storage shelf life of fish (Daramola *et al.,* 2007). This can suggest a fish product with long shelf life.

Fat generally varies much more widely than other proximate components of fish, and usually reflects differences in the way fat is stored in particular species (Ababouch, 2005). The fibre values obtained in the current study are lower than the values reported by Fawole *et al*. (2007). Lower fibre and ash values suggests proper utilization of nutrients in the fish. The variation among the studies noticed could have been due to variation in age, sex and environment (Huss,1995). Overall, the current results suggests that BSFL meal has high nutrient content similar to fishmeal, and the fish fed on BSFL meal can be recommended for consumption.

5.3 Water quality parameters

Tilapia fish species are capable of withstanding water quality conditions and physical handling than other fish species that would pose serious challenges. Analysing water quality parameters is ideal to determine impact of increased feed and fish biomass on water quality (DeLong *et al.,* 2009). Optimum growth of tilapia is achieved at 27 to 29°C, the acceptable range is between 25°C to 32°C, as higher temperature makes it difficult to maintain dissolve favourable oxygen concentration. Recommended dissolve oxygen is 5.0 to 7.5 mg/L, chronical low dissolve oxygen of below 3.5 mg/L affects growth and feed conversion. Ammonia and pH levels must be maintained below 1.0 mg/L and between 6.5 and 9 respectively (Lim and Webster, 2006; Timmons and Ebeling, 2007). Water quality parameters were within the recommended range for tilapia production through-out the study. Temperature was around 24°C which was still in the recommended range of 24°C

to 32°C for tilapia species (Moyo & Rapata, 2021). The water quality parameters did not influence growth performance of the experimental fish.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study demonstrates that there was a significant difference in the growth performance of black soldier fly larvae fed various organic waste substrates. The larvae developmental time, growth rate, Waste Reduction Index (WRI) and Food Conversion Efficiency (ECD) variability. Overall, kitchen waste fed larvae showed the best ECD together with the absolute highest biomass production and the shortest developmental period. The black soldier fly larvae were able to successfully reduce various organic wastes and convert it into valuable body biomass which is an ideal fish feed ingredient.

Furthermore, there was a significant difference in proximate composition of the black soldier fly larvae fed and raised on diverse organic waste substrates. This imply that different organic wastes influenced the nutritional composition of black soldier fly larvae. In the current study, there were no significant variation in growth and survival of *O. karongae* fingerling fed on diets where fishmeal was replaced at 100% with deffated BSFL meal. Therefore, supporting BSF meal as an ideal alternative protein source to fishmeal used in feed for *O. karongae* fingerlings in aquaculture.

The whole body composition analysis of *O. karongae* fingerlings fed on BSFL meal diets and fishmeal diets suggests that defatted BSFL meal can be used to replace fishmeal completely as an ingredient in fish feed in *O. karongae* fingerlings.

6.2 Recommendation

Findings from the current study provide a platform for local farmers, and food and feed industries to frame feed formulation strategies.

1. Fermentation and/or cooking of substrates before being fed to BSFL enhances digestibility efficiency significantly.
2. Kitchen waste is an ideal rearing substrate for BSFL production, shortest developmental period with high growth rate and mostly has balanced nutrients required by larvae to complete its growth cycle.
3. To utilize fruits and vegetables, the most prominent organic wastes in Malawi, as substrates, they should be blended to achieve body mass equivalent to that acquired from a higher nutrition diet but with a longer feeding period to reach the pre-pupal stage.
4. Defatted BSFL meal is an ideal component in the feed for *O. karongae* fingerlings

For further trials, studies should be conducted on the following areas:

1. To assess impact of BSFL meal on reproductive performance of different fish species
2. To investigate fish growth response to full fat BSFL meal
3. To assess the economic feasibility of BSF mass production and cost analysis of diets
4. To assess the substrate composition for BSFL rearing.
5. The effects of processing methods of larvae on storage duration and its consequent effects on nutrient composition and utilization of BSF meal

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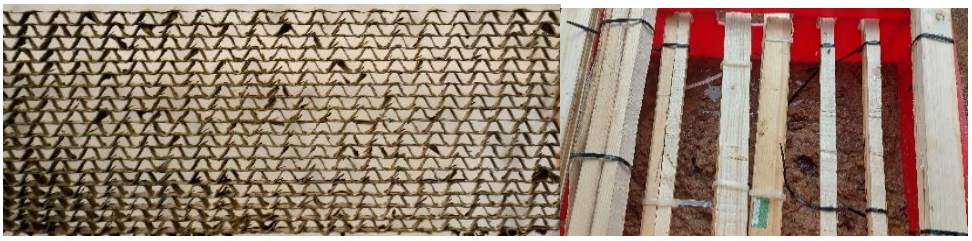
APPENDICES

Appendix I. List of figures used in the study

a. The pilot plant; insectarium (Left) and larvarium (right).
Source: *Photograph by Author*



b. Egg traps used for female BSF to lay eggs.
Source: *Photograph by Author*

c. The Thermo-hygrometer for temperature and humidity measurements (left) and electronic scale for measurements (right).
Source: *Photograph by Author*

Appendix II. Data analysis for BSFL growth performance and nutrient composition on different organic substrates

a. One-way ANOVA data for BSFL growth performance fed on different organic substrates

		Sum of Squares	df	Mean Square	F	Sg.
GR	Between Groups	51.217	1	51.217	15.267	0.00
	Within Groups	13.419	4	3.355		
	Total	64.636	5			
WRI	Between Groups	5.790	3	1.930	240.155	0.02
	Within Groups	.064	8	0.008		
	Total	5.854	11			
ECD%	Between Groups	104.946	3	34.982	72.285	0.00
	Within Groups	3.872	8	0.484		
	Total	108.818	11			

B. One-way ANOVA data for BSFL nutritional composition fed on different organic substrates

		Sum of Squares	df	Mean Square	F	Sig.
Energy	Between Groups	214.537	3	71.512	62.433	0.001
	Within Groups	27.490	24	1.145		
	Total	242.027	27			
carbs	Between Groups	80.846	3	26.949	85.963	0.001
	Within Groups	2.508	8	0.313		
	Total	83.354	11			
Ash	Between Groups	731.493	3	243.831	196.187	0.001

	Within Groups	29.828	24	1.243		
	Total	761.321	27			
protein	Between Groups	157.058	3	52.353	99.443	0.001
	Within Groups	4.212	8	0.526		
	Total	161.270	11			
Lipids	Between Groups	647.707	3	215.902	191.906	0.001
	Within Groups	9.000	8	1.125		
	Total	656.707	11			

Appendix III. Data analysis for *O. karongae* fingerlings growth performance and feed utilization efficiency and body composition fed on different experimental diets

a. One-way ANOVA data for *O.karongae* fingerlings growth performance and feed utilization efficiency

		Sum of Squares	df	Mean Square	F	Sig.
IW	Between Groups	0.00	3	0.000	0.000	0.897
	Wiin Groups	3.928	8	0.393		
	Total	3.928	11			
FW	Between Groups	340.759	3	85.190	6.159	0.009
	Within Groups	138.310	8	13.831		
	Total	479.069	11			
WG	Between Groups	340.759	3	85.190	6.159	0.009
	Within Groups	138.310	8	13.831		
	Total	479.069	11			
AVWG	Between Groups	0.024	3	0.006	6.159	0.009
	Within Groups	0.010	8	0.001		
	Total	0.033	11			
RGR	Between Groups	22275.384	3	5568.846	4.588	0.023
	Within Groups	12138.935	8	1213.894		
	Total	34414.320	11			
SGR	Between Groups	0.073	3	0.018	6.783	0.007
	Within Groups	0.027	8	0.003		
	Total	0.100	11			

Condition Factor	Between Groups	0.000	3	0.000	1.888	0.334
	Within Groups	0.000	8	0.000		
	Total	0.000	11			
Survival Rate	Between Groups	189.630	3	47.407	1.600	0.249
	Within Groups	296.296	8	29.630		
	Total	485.926	11			
FCR	Between Groups	0.069	3	0.017	0.306	0.868
	Within Groups	0.564	8	0.056		
	Total	0.633	11			
PER	Between Groups	0.247	4	0.062	5.732	0.012
	Within Groups	0.108	10	0.011		
	Total	0.355	14			

b. One-way ANOVA data for *O.karongae* body composition after being fed different diets

		Sum of Squares	df	Mean Square	F	Sig.
Moisture	Between Groups	311.830	5	62.366	13723.559	0.001
	Within Groups	0.055	12	0.005		
	Total	311.884	17			
Ash	Between Groups	70.446	5	14.089	21675.660	0.0001
	Within Groups	0.008	12	0.001		
	Total	70.454	17			
fiber	Between Groups	0.000	5	0.000	40.862	0.001
	Within Groups	0.000	12	0.000		

	Total	0.000	17			
Crude fat	Between Groups	20.766	5	4.153	6615.754	0.001
	Within Groups	0.008	12	0.001		
	Total	20.774	17			
Crude protein	Between Groups	164.523	5	32.905	311727.000	0.001
	Within Groups	0.001	12	0.000		
	Total	164.524	17			

Appendix IV. One-way ANOVA data for water quality parameters

		Some of Square	df	Mean of Square	F	Sig.
Ammonia	Between Groups	0.000	4	0.000	0.062	0.992
	Within Groups	0.002	10	0.000		
	Total	0.002	14			
DO (am)	Between Groups	0.046	4	0.012	0.496	0.740
	Within Groups	0.234	10	0.023		
	Total	0.280	14			
DO(pm)	Between Groups	0.118	4	0.030	1.201	0.369
	Within Groups	0.246	10	0.025		
	Total	0.365	14			
Temp(pm)	Between Groups	0.169	4	0.042	1.036	0.436
	Within Groups	0.408	10	0.041		
	Total	0.577	14			
Temp(am)	Between Groups	0.202	4	0.051	0.296	0.874
	Within Groups	1.706	10	0.171		
	Total	1.908	14			