

**OPTIMIZATION OF PRODUCTION OF BLACK SOLDIER FLY LARVAE
(*Hermetia illucens*, L) FOR FISH FEED FORMULATION.**

By

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DECLARATION AND APPROVAL

Declaration

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This work is dedicated to my beloved wife Susan Emily Ipala Emukule Nyakeri who despite the loneliness occasioned by my absence, not only trusted and believed in me and gave me the impetus to go on. To my Lovely children Bless Evan Misiga, Praise Moraa and Gift Fave Tosha, for whom I do endeavour to live and strive. To my loving parents Emmanuel Arege and Bilha Arege, who encouraged and supported me all through to this level of education. Above all to God, the creator of all beings, who provided the strength, health and favour to enable me see this output.

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ABSTRACT

Increase in world population has catapulted demand for animal proteins beyond supply and organic waste generation. Conventional livestock production is hindered by land sizes, urbanization and limited and unsustainable supply of fishmeal. Consequently, protein insecurity and poor environmental sanitation are major issues of concern. However black soldier fly larvae (BSFL) bioconversion technology that uses organic waste to produce a nutritive biomass rich in proteins, offers a potential solution to the twin problems. Utilization is however hindered by limited information on BSF production strategies. This study used an experimental research design to evaluate the potential of farming BSFL biomass as an alternative protein source for fish feed. The study's objectives were to; evaluate the performance of different organic waste substrates as attractants on BSF oviposition activity, test and identify suitable organics for sustainable production, optimize the production of BSFL using feeding rates, regimes and substrate combination; and compare the performance of BSFL (BM) and fishmeal (FM) incorporated feeds on tilapia growth. Performance was measured by number of eggs and prepupa, larvae growth rate, substrate reduction, nutrient content, fish growth rate and effect on water quality. Data was analyzed by t-tests and two way ANOVA tests, LSD and Tukey HSD in R statistical package. Fermented mashed maize grain and fruit and vegetable waste were best attractants for the Bongo black soldier fly (BSF) strain (monthly prepupa collection $2.933\text{kg} \pm 0.9$ and $2.2\text{kg} \pm 0.87$ respectively) ($p < 0.05$); while fresh cow manure and frass were the best oviposition attractants in a captive colony (mean egg collection $5.1\text{kg} \pm 2.1$ and $2.5\text{kg} \pm 2.9$ respectively ($p < 0.05$). Proximate analysis of wild collected prepupa recorded 40% crude protein and 33% crude fat. Substrate testing reported that food remains (FR) produced significantly higher prepupa total mean yield and average individual weight of $196.9 \pm 4.0\text{g}$ and $0.101 \pm 0.002\text{g}$, respectively followed by brewers waste (BW) ($154.8 \pm 6.5\text{g}$, $0.078 \pm 0.02\text{g}$), faecal sludge, FS ($138.7 \pm 5.0\text{g}$, $0.070 \pm 0.001\text{g}$) and banana peels ($108.9 \pm 5.6\text{g}$, $0.055 \pm 0.002\text{g}$) respectively ($p < 0.05$). Crude protein content was highest in prepupa fed on FS and BW at $45.4\% \pm 0.1$ and $43.0\% \pm 1.0$, respectively ($p < 0.05$) while the ether extract was more variable ranging from $18.1 \pm 0.3\%$ for FS fed prepupa to 36% for FR fed prepupa. Watermelon, banana peels and pineapples yields, average prepupa weights and CP levels were $2.9 \pm 0.1\text{kg}$, 0.103g , 40.2% ; $2.8 \pm 0.1\text{kg}$, 0.121g , 35.4% and $2.2 \pm 0.09\text{kg}$, 0.101g , 40.0% respectively. Feeding rate 200mg/l/d emerged as the best for yield and average prepupa weights (FR- 255.6g , $0.128\text{g} \pm 0.008\text{g}$; BW- 208.4g ; $0.104 \pm 0.01\text{g}$ and FS 175.8g , $0.089 \pm 0.004\text{g}$ ($p < 0.05$) while feeding rate 150mg/l/d had the highest substrate reduction rate of 84% ($p < 0.05$). Daily feeding (DF) and after four days feeding (AFD) regimes recorded the highest waste reductions ($84.6\% \pm 0.2$; $83.5\% \pm 0.3$ respectively), whereas the biomass was insignificant across the different regimes ($p < 0.05$). Mixing faecal sludge separately with FR, BW and BP respectively at a 30:70 and 50:50 ratio yielded the highest yield ($299 \pm 6.5\text{kg}$, $299 \pm 4.4\text{kg}$ -FR; $270 \pm 4.5\text{kg}$, $261 \pm 3.4\text{kg}$ -BW and $253 \pm 2.7\text{kg}$, $238 \pm 5.0\text{kg}$ -BP respectively. FM and BM feeds recorded no significant differences on final fish average weights ($20.52\text{g} \pm 0.2$ and $18.27\text{g} \pm 5$ respectively); and on water quality indicators ($p < 0.05$). The Bongo BSFL strain can be reared from the wild on identified substrates and production optimized on a feeding strategy of feed rate, regime and substrate combination as an alternative protein source.

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LIST OF ABBREVIATIONS

SDGs:	Sustainable Development Goals
BSF:	Black Soldier Fly
IFIF:	International Feed Industry Federation
MOA:	Ministry of Agriculture
PEM:	Protein Energy malnutrition
GOK:	Government of Kenya
FAO:	Food and Agriculture Organization.
WHO:	World Health Organization
UNICEF:	United Nations Child Education Fund
PAF:	Partnership of African Fisheries
AKEFEMA:	Association of Kenyan Feed Manufactures
ACAF:	Advisory Committee on Animal Feedstuffs
AOAC:	Association of Analytical Chemists
IFT:	Institute of Food Technologists
NRC:	National research Council.
DDGS:	Dried Distillers grain with Solubles, a byproduct of ethanol production
CK:	Cookie meal, a byproduct of the wheat product baking industry
JOOUST:	Jaramogi Oginga Odinga University of Science and Technology
EFSA:	European Food Safety Authority
BSF:	Black Soldier Fly
BSFL:	Black Soldier Fly larvae

CHAPTER ONE – INTRODUCTION

1.1 Introduction

The exponential increase of the world population has resulted in food scarcity in general and protein deficiency in particular (Cochrane et al., 2009). Conventional food production systems such as agriculture, fishing and livestock rearing are unable to cope with the demand (Foley et al., 2011, Mekonnen and Hoekstra, 2012). Efforts to boost productivity via the conventional methods not only generate large quantities of organic wastes but also result in loss of biodiversity due to increased utilization of natural resources and climate change (Mutafela, 2015). As we tend towards 2050, food insecurity, protein malnutrition and the quality of sanitation are expected to decline globally (FAO, 2013). However the situation will be worse in the developing countries of Africa where population growth is exponential and accounted for 98% of the people globally categorized as food insecure in the period 2011-2013 (FAO, 2013). These include East African countries such as Kenya whose population growth rate of 2.4% per annum is ranked among the highest in the world and a third of the population is classified as having chronic food insecurity (FAO, 2013).

To achieve food sufficiency, more renewable and non-renewable resources are required (Mutafaela, 2015). Consequently it is projected that by 2050, food production should be increased by an average of 60 - 70% worldwide (FAO, 2013) and by 300% in Africa (Gabriel *et al.*, 2007). Feed production is on the other hand expected to rise by 70% from current levels to cater for an anticipated doubling of animal protein demand within the same period (Losses and Waste, 2011; IFIF, 2012). This has raised concern on protein security and signaled an increase in the scale of conventional livestock production though animal agriculture is among the top three significant contributors to the most serious environmental problems at every scale from local to the global level (Steinfeld et al., 2006; Merino et al., 2012; FAO, 2016). Not only is the expansion a daunting task to achieve in Kenya where 80% of the people are small scale land holders who prefer the farming of staple food crops and cash crops to livestock farming but also the demand for animal products surpasses supply (MOA, 2004, GOK, 2015). This has resulted in perpetual increase in the prices of protein foods beyond the reach of many and a surge in

diseases linked with protein and energy malnutrition (PEM) (Thurstan and Roberts, 2014; FAO, 2016).

Livestock, poultry and aquaculture farming requires use of fishmeal in feed diets (Merino *et al.*, 2012; Mutafela, 2015, Oonincx *et al.*, 2015, FAO, 2016). Approximately 30% of the fish catch (55.86% wild catch and 44.14% aquaculture) is diverted from human consumption to make fishmeal, a source of protein for animal feeds (FAO, 2016). The competition between humans and animals has led to overexploitation of these fish species and consequently, the cost of feeds has become expensive and unaffordable to most farmers (Merino *et al.*, 2012; Thurstan and Roberts, 2014; FAO, 2016). This has necessitated the search for alternative feedstocks for aquaculture such as plant and edible insects, which in turn can allow more fish to be used for human consumption (Bondari and Sheppard, 1981, Bondari and Sheppard, 1987; Alvarez *et al.*, 2007; Devic and Maquart, 2015; Karapanagiotidis *et al.*, 2014). However of recent, focus has shifted to insect based protein sources which are considered to have additional benefits such as usage of fewer resources like land and water than crop proteins; and are suitable for fish feeds unlike plant proteins which have antinutritive factors (Bondari and Sheppard 1981; van Huis *et al.*, 2013; Nguyen *et al.*, 2015).

Overwhelming amounts of organic waste are generated by the rapidly increasing human population from diverse sources among them agricultural farms, municipal markets, households, supermarkets, industries, animals and human settlements (vanHuis *et al.*, 2013). The waste includes agricultural farm produce, pre and post-consumer food remnants, expired manufactured foods, industrial waste streams, market waste, animal manures and human faeces. In most developing countries, up to two thirds of the generated organic waste is neither collected nor treated (van Huis, 2013; Diener *et al.*, 2009; Taiwo and Otoo, 2013). Though it contains a lot of useful and recyclable nutrients, this form of waste is often considered unworthy and only about 5% is re-used majorly via composting and production of biogas energy (Zurbrugg, 2002; Diener, 2010; HABITAT-UN 2010; Hoornweg and Bhada-Tata, 2012). Transportation costs of the waste to rural areas where it can be used as organic fertilizer or animal feed discourage this option

(Zurbrügg et al., 2012) and high tropical rainfall in Africa increase the moisture content and therefore render the option of incinerating the organic waste into energy uneconomical (UN-HABITAT 2010). For lack of alternatives, much of the solid waste which includes animal and human excreta is often dumped arbitrarily in the streets and drains of urban areas (Mutavi, 2018).

Though organic waste is rich in nutrients, when not managed properly, it can be a source of suffering to humans and the environment in general. For example, improper disposal of human faeces in slum areas is associated with outbreaks of sanitation related diseases such as helminthiasis, dysentery, cholera and schistosomiasis (Who, 2000; UNICEF, 2012; Banks *et al.*, 2014; Clasen *et al.*, 2014; Nguyen et al., 2015). Poor collection and disposal of organic wastes from markets, households and industries located in urban areas causes clogging of drainage systems during flood rains, formation of habitats and breeding grounds for pathogens and disease vectors such as rats, squirrels, wild dogs and other pests, acidification and eutrophication of water bodies, local nutrient overloads and sometimes climate change (UN-HABITAT, 2010; Hoornweg and Badha-Tata, 2012, Gabler and Vinnerås, 2014). In addition, natural rotting of the waste generates harmful and noxious gases such as ammonia, methane, hydrogen sulphide and carbon dioxide, which have negative impacts on human health (Taiwo and Otoo, 2013). Therefore there is need to identify simple and affordable solutions appropriate to African conditions in addition to reducing consumption, embracing reuse and recycling of resources and incorporating value addition in the management of generated wastes (van Huis *et al.*, 2013). An example is though bioconversion process that involves use of biological organisms ubiquitous in nature such as red worms, micro-organisms and insects larvae (van Huis *et al.*, 2013; Nguyen et al., 2015).

In recent times, the use of insects to achieve food and feed security has gained popularity (van Huis, 2013). Some species have been cited and propagated as novel sources of alternate high quality proteins and contributors to global food security, to augment current conventional food production methods (Makkar et al., 2014). Consequently, campaigns on the creation of awareness on the potential role of insects as an innovative food or feed resource to supply needs of an ever increasing human population have

gained momentum (van Huis, 2013). The concept is most relevant among countries within Sub-Saharan Africa whose population growth rate is among the highest in the world, and malnutrition has been classified as acute (M'mboga, 2009; Schönfeldt and Gibson, 2012).

The use of insects is not a new concept for either humans or domesticated animals. Entomophagy is considered an indigenous practice of people from different parts of the world (Ayieko et al., 2016). In Kenya, entomophagy has long been practiced by communities from the Coast and Lake Victoria regions (Christensen et al., 2006; Ayieko et al., 2012; Kinyuru et al., 2012). In nature, adult insects and their larval stages are a food of choice for many domestic animals such as fish and poultry. In Western Kenya, the digging and collection of termite mound chippings for poultry is a common practice (Ayieko et al., 2010). In Ghana, a traditional home has several termitaria, which are harvested and fed to fowls to encourage free range foraging among the birds (Anankware et al., 2015). The concern however is that the practice has largely relied on gatherings and collections from nature, a practice which is not only inefficient, uncontrolled and seasonal but also unsustainable and prone to contamination from the environmental toxins and pathogens (Schabel, 2010; Ayieko et al., 2012; 2016). There is also fear that widespread adoption of insects as an alternative protein source due to the current campaigns may lead to extinction of the commonly utilized species (Gondo et al., 2010). Establishing cost effective and safe production systems for targeted insect species will however forestall the loss of insect biodiversity and also establish a stable market for insects and their products more so in Africa, where usage is low due to lack of insect breeding technologies and inadequate knowledge about their potential (Devic and Maquart, 2015; Kelemu et al., 2015).

Black Soldier Fly (*Hermetia illucens*) larvae have gained popularity both for their ability to decompose organic waste and serve as a source of proteins for domestic livestock (Erickson et al., 2004; Liu et al., 2008, Diener et al., 2009; Humphrey, 2009; Nguyen et al., 2009; Diener et al., 2011b; Canary, 2012, Bullock et al., 2013, Banks et al., 2014). BSF bioconversion technology has been used for the management of animal wastes from confined animal facilities of cows, pigs and chicken with reduction values in the range of

50%-79% being reported on municipal and household waste (Nguyen *et al.*, 2009; Banks *et al.*, 2014; Diener *et al.*, 2011, Newton *et al.*, 2005; Sheppard *et al.*, 1994, Banks *et al.*, 2014; Diener *et al.*, 2009, Lalander *et al.*, 2013). In the process of transforming the waste, the larvae also eliminate bad odours and methane gas usually produced under anaerobic decomposition of organic wastes (Barry, 2004). In addition, BSFL biomass is reported to have up to 44.4% dry matter (DM) crude protein, 23% DM lipids and several important macro and micro nutrients (vaHuis *et al.*, 2013). These values compare favourably with fishmeal and soya beans which combined; supply over 90% of the protein requirements of animal feeds (Yu and Chen, 2009). The two are also consumed by humans and consequently demand has outstripped supply casting doubt on their future continued availability in sufficient quantities (Yu and Chen, 2009). Livestock feeding trials on BSF larval biomass have however yielded promising results signaling the potential to replace fishmeal and soy in animal feeds (Newton *et al.*, 1977; Bondari and Sheppard, 1987; Kröckel *et al.*, 2012; FAO, 2013). Therefore successful development and adoption of Black Soldier Fly larvae farming technology is relevant, has capacity to contribute towards food security, reduce environmental pollution and contribute towards conservation of environmental biodiversity (Van Itterbeeck *et al.*, 2014; Devic, 2016).

Despite the aforementioned potential of BSFL, there is concern on the scarcity of information on Black Soldier Fly rearing as players who have mastered the art of BSFL production tend to hoard information in order to have a competitive edge in the market (Leek, 2017). In addition, there is global concern on utilization of foreign species due to the environmental risks they pose such as decimation of native biodiversity in the event of escape from captivity (Juliano and Lounibos, 2005; Vila *et al.*, 2007; de Groot and Veenvliet, 2011). Consequently, most countries have very stringent rules on the use of foreign materials in accordance with the Catagena protocol Convention on Biodiversity (Secretariat of the Convention on Biological Diversity; 2000). This has hindered the adoption of the technology in some regions due to the rigorous procedures involved to get the necessary approvals. For example, in Denmark it is illegal to utilize, or farm any insect species not native to the country (Leek, 2017). This is the rationale for encouraging the utilization of local found native insect species where possible. Unfortunately, nativity of BSF has not been established in Kenya with current pioneers for BSFL feed and waste

management technology such as Sanergy Ltd obtaining “seed” BSF from South Africa (Kimani, 2015, personal communication).

Though in nature BSFL consume a wide range of organic materials ranging from plant, animal and industrial wastes, not all the materials provide a suitable diet for BSFL in confinement and for commercial production. This is because development time, fecundity, consumption, feed conversion efficiency, mortality, pupal weight and nutritional values of resultant biomass all depend on diet (Tomberlin *et al.*, 2002). Aggrey (2017) noted that BSF populations in compost heaps tended to disappear if large amounts of putrescible wastes such as meat scraps, grass clippings or sawdust were added. Again, though the technology has been applied elsewhere, the substrates used relate more to the waste management role of BSFL in the specific regions where they occur rather than biomass production (Diener *et al.*, 2010). This potentially limits the scope of usage of such substrates not only in terms of application but also to the particular regions and geographical areas. Organic waste materials are also by their nature, highly heterogeneous in nature and variable in terms of moisture and nutrient content and therefore generalized applications of findings is almost impossible (Holmes *et al.*, 2012).

In an insect production facility, the goal is to produce the maximal number of insects in as little time and as inexpensively as possible. For BSFL production, this implies optimization of growth at the larval feeding stages, which directly affects pupa development, adult lifespan and fecundity and viability of eggs produced (Holmes *et al.*, 2012). This is important as larvae growth has been established to depend on diet composition, food ration and feeding frequency. The optimal levels of these parameters need to be established on strain and substrate by substrate basis as consumption and utilization has been found to vary (Kim *et al.*, 2011).

1.2 Statement of the problem.

An increasing human population has led to a rise in both food consumption, organic waste generation increase in price of protein foods and poor sanitation (Mekonnen and Hoekstra, 2012) However, resources for increased food production to meet demand are increasingly becoming scarce and may not meet future needs. For example, conventional

agriculture is at peak efficiency in terms of land and water usage casting doubts on continued sustainability of animal protein production (FAO, 2009; Foley *et al.*, 2011, Mekonnen and Hoekstra, 2012) whereas the continued sustainable availability of fishmeal for animal feeds is not assured due to competition between humans and the animal feed sector (Gabriel *et al.*, 2007). High cost of production occasioned by scarcity of fishmeal and the wide gap between supply and demand has led to increase in prices of animal products beyond the reach of many people; the consequence of which is an increase in the number of cases linked with protein energy malnutrition (PEM) (KNBS, 2014). The increasing population also generates organic waste due to rising consumption levels, whose collection and disposal is wanting in most developing countries and consequent accumulation in open dumpsters and roadside heaps pose environmental problems such as flooding, diseases and unsanitary conditions in general. BSFL farming that utilizes organic waste as substrates has potential to offer a solution to the twin problems of waste management and protein deficiency.

However phobia for foreign species' ability to negatively affect the environment's ecosystem, and variations in environmental conditions that may affect productivity necessitates the search for and utilization of native organisms (Zhou *et al.*, 2013). There is therefore need to establish BSF nativity and establish a technique of attraction for onward captive breeding. Although BSF larvae can survive and grow on many different organic products, parameters such as development time, feed conversion efficiency, mortality, pupal weight and nutrient composition are strongly affected by diet (Zhou *et al.*, 2013). Therefore not all organic materials are suitable for BSF production in captive operations hence the need to identify suitable production substrates for both quality and quantity of product (St. Hilaire *et al.*, 2007; Zheng *et al.*, 2013). Besides substrates, BSFL productivity in captive operations is affected by management practices such as environmental conditions and feeding strategy, whose regulation shifts focus from mere BSF rearing to facilitating efficient consumption and therefore waste reduction and production of sufficient amounts and quality of biomass (Barry, 2004). There is therefore need to establish a feeding strategy on identified substrates that encompasses determination of optimal feed rates, regimes and substrate mixing.

1.3 Objectives of the study

1.3.1 Main objective

To contribute to production and promotion of edible insects use as fish feed diets.

1.3.2 Specific objectives

1. To assess the performance of different substrates in attracting ovipositing black soldier fly.
2. To identify organic waste substrates suitable for the production of black soldier fly larvae.
3. To optimize the production of black soldier fly larvae
4. To compare the performance of black soldier fly larvae and fishmeal incorporated feeds.

1.4 Research hypotheses

1. HO: Black soldier fly attraction does not dependent on the type of attractant substrate
2. HO: There is no difference in the productivity of BSFL on different organic waste substrates
3. HO: BSFL production is not affected by feed rate, feed regime or substrate mixing.
4. HO: There is no difference in the performance of BSFL and fishmeal feeds.

1.5 Justification of the study

In principle, any decaying organic matter may attract BSF females for oviposition as long as it produces volatile compounds (James, 1935). However, different studies have reported variability in attractant performances with some reportedly doing better than others. For example, Bondari and Sheppard (1987) and (Bonso, (2013) advocated for use of carrion and Banks *et al.* (2014) successfully used faecal matter, and Diener *et al.* (2009) reported the use of chicken feed. For Nguyen et al., (2013), rotting agricultural waste is the best attractant in nature. Conspecific chemicals have also been touted as attractants for ovipositing females (Stankus, 2013; Bonso, 2013). According to Sripontan and Chiu (2017) the substrate most abundant in the environment and therefore most likely used as a feed at the larval stage will be the most preferred by the adult BSF as an

attractant for egg laying. These studies were not only conducted in different geographical areas and therefore the findings may not apply to local situations but also show variability in BSF response to oviposition attractants, hence the need to determine suitable oviposition attractants for local strains for optimal performance.

Kenya being a country whose industrial sector is primarily based on agriculture, a number of by-products from agricultural and industrial sectors are available year round, which are usually not utilized for human consumption, but may have a high potential for bioconversion into a nutrient biomass (Alooh, 2015). Indeed agricultural waste constitutes the largest part fraction of organic waste which in turn comprises 44%-66% of the total solid waste, a situation that mirrors the global situation where between 30-33% of the total food produced is wasted between the field and the fork (FAO, 2008; 2011; Alooh, 2015). Economically this accounts for US\$ 750 million per annum of food lost, an amount estimated to feed three billion people in the equivalent time frame if it were accessible (FAO, 2011). Financial constraints have made installation of efficient technologies and machineries for incineration and conversion of the organic waste into energy an unviable option and consequently there is minimal reuse or recycling of this nutrient rich resource (UN-HABITAT, 2010). Consequently, the organic waste is considered worthless, ignored by informal waste recycling sector and mostly dumped in open heaps to undergo natural decomposition and be fed on by scavengers and as a result, becomes a major environmental pollutant and attractant of vermin (Barry, 2004, Henry et al., 2006; UN-HABITAT, 2010; Diener *et al.*, 2011; Hoornweg and Bhada-Tata, 2012; Taiwo and Etoo, 2013). Again, natural composting process emits fugitive emissions and obnoxious odours, wastes land meant for other activities, is limited in the scope and range of wastes that can be processed and requires constant aeration through manual or mechanical mixing (Henry et al., 2006). In addition, the time required to produce finished compost is highly variable, there is lack of market for compost products such as organic fertilizer and biogas due to competition from alternative forms of fertilizer and energy (wind, electricity and solar) and the process does not address the fate of inherent nutrients within the waste (Rouse et al., 2008).

Many species of flies and earthworms cannot process a wide range of organic wastes and need specific nutrition to be effective bioconverters (Latsamy and Preston, 2008; Morales and Wolff, 2010). Again, some flies (such as the common house fly) and microbes are human pests and disease vectors (Sasaki et al., 2000; De Jesús et al., 2004); unlike the black soldier fly which is not known as a pest, pathogen vector or nuisance of any kind to humans or their animals (Erickson *et al.*, 2004). BSFL's have a polyphagous nature enables them to feed on a wide range of decaying organic materials, voracious appetite that facilitates the consumption of large amounts of organic waste during their growth cycle and a robust digestive system that enables them to naturally devour organic materials of both animal and plant origin (Mutafela, 2015). Consequently, BSFL's feeding activity has been noted to reduce the amount of organic waste dumped in open streets and water points by at least 50-60% (Diener *et al.*, 2011), resident nutrients such as nitrogen content by 71%, phosphorus and potassium by 52% each, amount of greenhouse gases generated from the waste through anaerobic respiration such as CO₂, SO₂, methane, ammonia and other noxious gases (van Huis *et al.*, 2013), besides the nutritive biomass (van Huis *et al.*, 2013). BSFL feeding activity also sanitizes remnant feeds and makes it safe for organic farming through antibacterial and antifungal activity (Everest Canary and Gonzalez, 2012; Humphrey, 2009; Banks *et al.*, 2014). In this way a BSF technology manages organic waste by adding value to it and also enables even environmentally hazardous wastes to become a source of income generation and employment creation (Mutafaela, 2015). Added to this is the fact that inclusion of BSFL in pit latrines and sewerage sites has also been reported to decompose the waste and therefore prolong the lifespan of these facilities and improve sanitation (Banks *et al.*, 2014). The reduction ability of waste coupled with BSFL's rich nutrient profile renders them attractive candidates to serve as an alternative source of protein for feeds of domesticated animals and organic waste management tool.

At the moment, a significant obstacle for the use of insects including BSFL in animal feed is the limited quantity of produced insects which does not guarantee a constant supply (Leek *et al.*, 2017). To sustainably produce enough amounts of quality BSFL, suitability of feeding substrates should be investigated as diet has been noted to affect parameters such as quantity and quality of biomass; and development period

(Houshmand et al., 2012). Again, in commercial operations there is need to establish an optimal feeding strategy for the production of the black soldier fly larvae (Rumpold and Schlüter, 2013; Zhou *et al.*, 2013). An optimal feeding strategy that involves identification of suitable feeding substrates, determination of feeding levels, stocking densities, feeding frequencies and substrate combinations which have been found to vary substrate wise (Amalraj et al., 2013), will improve growth performance, survival, food conversion ratios, contribute to minimizing food wastage, reduce size variation, wastefulness in terms of money incurred on feed and consequently increase production efficiency and waste management problems (Rice and Garcia, 2011). The concern however is that the optimal feeding frequency, feeding regimes and substrate mixing and their effect on growth and feed utilization are still largely grey areas in BSFL production.

In Kenya, fishmeal is processed from the silver cyprinid (*Rastrineobola argentea*, locally known as “Omena”) and the Nile perch (Nyandat, 2007). It is estimated that between 50 and 65% of the silver cyprinid from Lake Victoria is used to produce fishmeal (Abila, 2003). The competition between the feed industry and human consumption has led to severe reduction of “Omena” from the Kenyan-Lake Victoria waters to the extent that the country relies on imports from Tanzania and Uganda to supplement her demand (AKEFEMA, 2005). The situation has negatively impacted food security and the livelihoods of lakeside populations because importation makes the product expensive and unaffordable (Nyandat, 2007). In addition, the shortage and uncertainty over fishmeal production has made animal feeds too expensive for farmers and feed cost accounts for over 60% of the total production cost (AKEFEMA, 2005; PAF, 2011; FAO, 2010; Tiu, 2012; Abarike *et al.*, 2013, FAO, 2013).

Various animal and vegetable products have been tested as potential alternative sources of protein and energy for animal feeds worldwide; with the intention to free up more fish for human consumption (Alvarez, 2012; Devic, 2016). These include oilseed cakes (cotton, soybean and sunflower), animal byproducts (bloodmeal and carcass meal), cereal bran (maize, wheat and rice), liver, egg yolk, powdered (skimmed) milk and vegetable oils. However, most vegetable proteins generally lack essential amino acids and

carbohydrates and alter the taste, flavor and aroma of meat products (Forsythe, 2013). Some like wheat and vegetable oil have a deficit supply in Kenya and have to be imported from other countries such as Pakistan, Russia, USA and Malaysia (Nyandat, 2007). Meat by-products on the other hand are either costly, unavailable in large quantities, inconsistent in supply or banned in some countries such as the European Union (Gabriel *et al.*, 2007). The later potentially limits the market scope of fish and poultry products raised on meat containing feeds.

Again in addition to BSFL's feeding activity reducing organic waste content, it also transforms nutrients in the waste into a more valuable, more nutritive and less harmful biomass rich in protein (44.4% of DM), lipids (23% of DM), and other valuable nutrients such as calcium (5-8% DM) and phosphorus (0.6-1.5% DM). Ash content ranges from 11 to 28% DM while the dry matter content of fresh larvae is in the 35-45% range (Newton *et al.*, 2005, St-Hilaire *et al.*, 2007), van Huis *et al.*, 2013). These statistics indicate that BSFL biomass composition compares favourably with that of soya and fishmeal which combined, currently supply over 90% of the protein requirements of animal feeds (Yu and Chen, 2009). The huge potential of the region in terms of climatic conditions and supply of agricultural wastes as raw materials can facilitate sustainability of BSFL production for food and protein security, reduced environmental pollution and biodiversity conservation.

For aquaculture to thrive and bridge the existing gap between demand and supply of animal proteins and be considered the panacea for protein insecurity in the region (Rivera-Ferre, 2012), the role of locally produced fish feed in reducing production cost, ultimately boost fish production and consumption and therefore improve food and protein security cannot be overemphasized (Gabriel *et al.*, 2007). This forms the basis for formulation and evaluation of performance of BSFL feed from by-products and waste materials not suitable for direct human consumption as an alternative to fishmeal based feed whose continued availability is both unreliable and unsustainable (PAF, 2011; Fao, 2013).

1.6 Scope and limitation of the study

Organic waste is of a wide variety that includes sewage, restaurant/kitchen waste, agricultural waste and industrial waste. The study covered all these classes of organic waste. Details in biological behaviour of the adult BSF and the larvae have not been dealt with. The study dealt with the attraction of black soldier flies for oviposition colony in the wild and in an established colony, testing of larvae production substrates, challenges in larvae production, ways to increase larvae production through a feeding strategy under normal environmental conditions. The study did not look at the control of abiotic factors or toxicology of the BSFL biomass product. Although BSFL has many potential benefits, the current study only explored application in food security as a source of protein for fish feed and recycling of nutrients inorganic waste through feeding activity.

1.7 Significance of the study

The study is significant as it makes contributions to the existing body of knowledge on BSF production and those relevant to the global Sustainable development goals (SDGs), Vision 2030 and goals spelt out in the Kenya Government's Big Four development Agenda. At the global scale, the study directly and indirectly contributes to eight out of the seventeen SDG goals. This include: SDG 1 - Poverty eradication in all its forms everywhere; SDG 2 - Food security, improved nutrition and promotion of sustainable agriculture; SDG 3 - Healthy living and promotion of well-being; SD6 - Sanitation for all; SDG12- sustainable consumption and production patterns; SDG 13 – Combating climate change and its impacts; SDG 14 – Conservation and sustainable use the oceans, seas and marine resources for sustainable development and SDG15 –Protection, restoration and promotion of sustainable use of terrestrial ecosystems, forests and halting biodiversity loss (Assembly, 2015). Adoption of BSF rearing technology for biomass production on organic wastes generated by homesteads, restaurants; schools, industries, markets and farms as a source of protein for livestock such as chicken and fish at both small and large scale can contribute to a source of income for most families through the selling of farmed livestock. This contributes to both poverty reduction (SDG1) and also serves to improve environmental sanitation through waste reduction (SDG6). The expected resultant upsurge in fish consumption (“omena” and reared fish), which has

increasingly become unaffordable due to demand from a rapidly rising human population will contribute to food security- a major pillar of both Vision 2030 and the Big Four Agenda of the Government of Kenya (Assembly, 2015)- through lessened protein energy malnutrition (PEM); health improvement of the general populace (SDG2); and also improved healthy living and well-being (SDG3) (Thurstan and Roberts, 2014). The promotion of farming of BSFL as a source of proteins for animal feeds including fish feed that aims at making fish cheaper and affordable to more people is a direct contribution to the pillar of food security. In addition, the promotion of utilization of BSFL biomass instead of farmed crops such as soybeans will ensure less utilization of land, water and reduction of use of aquatic fish used as fishmeal in fish feeds, which will lead to conservation of biodiversity of this overexploited resource (Tacon and Metian, 2008). This contributes to conservation of aquatic biodiversity (SDG-14); and also conservation of limited resources such as land and water (Merino et al., 2012). In addition use of soybeans for animal feeds interferes with human food security (Dicke, 2018). Use of organic waste for BSFL production which otherwise could have been destined for open dumps frees up much land space for other uses. This is especially relevant in Kenya where organic material constitute about 44-65% of the total solid waste generated (NEMA, 2015). Production of edible insects such as BSFL yields less amounts of greenhouse gases hence contributes to reduction of climate change and its impacts (SDG 13) (Oonincx et al., 2010). The study's contributions to sustainable farming of the black soldier fly through identification of feeding substrates and optimization of production through a feeding strategy can contribute to lessening dependency on wild collected larvae and halting biodiversity loss in the terrestrial environment (SDG15) (Schabel, 2010). The development of a feeding strategy and production methods for BSFL is a direct contribution to SDG 12-sustainable consumption and production patterns through sustainable management and efficient use of natural resources through prevention, reduction, recycling and reuse of generated waste. This connects to SDG 2 which aims at creating a circular economy (Horton, 2017). It is anticipated that the findings of the study can benefit the community through dissemination by the outreach mission of the university that can create awareness and increase adoption.

CHAPTER TWO -LITERATURE REVIEW

2.1 The demand for proteins versus supply

A rapid population increase and urbanization have raised concern on two major issues namely food security particularly the supply of proteins, and management of wastes produced from increased consumption (FAO, 2011; The Economist, 2014; Alooh, 2015). Urbanization and development have resulted in preference for diets rich in animal products and increased waste generation per capita (Hoornweg and Bhada-Tata, 2012). Consequently, the demand for animal proteins such as meat and milk is projected to increase by 58% and 70% respectively in 2050 compared to the levels in 2010 (FAO, 2011).

To meet the projected demand increase, more effort and land has been devoted towards increasing production of conventional livestock, poultry and expansion of the aquaculture industry. These actions are counterproductive in that they negatively impact the environment, through environmental degradation and increase in resource use (Foley *et al.*, 2011, Mekonnen and Hoekstra, 2012). Animal agriculture is a significant contributor to serious environmental problems at every scale from local to the global level (Steinfeld *et al.*, 2006). The sector contributes approximately 14.5% of all anthropogenic greenhouse gas (GHG) emissions, produces large fractions of organic waste in addition to being a leader in habitat destruction through soil acidification, erosion, compaction, overgrazing and nitrification (Gerber *et al.*, 2013; Oonincx *et al.*, 2015). Aquaculture on the other hand uses wild caught fish for fishmeal production and therefore diverts a food source that could be consumed by humans. Consequently, the continued availability of fish meal in future is not sustainable due to stiff competition from human consumption; hence the need for an alternative protein sources for livestock feeds, poultry and aquaculture (Logan, 2008; PAF, 2011; FAO, 2012).

2.2 Challenges of organic waste

Globally, poor solid waste disposal ranks as the second most serious environmental problem in urban areas of low and middle income countries after unemployment (UNDP, 1997; Diener *et al.*, 2009; Hoornweg and Bhada-Tata, 2012). The management of solid

waste has undergone evolution from practices of open dumping and burning to programs that lay emphasis on reduction of consumption, reuse and recycling of resources (Mutafela, 2015). Consequently, the disposal of inorganic components like glass, paper, plastic and metal, which are perceived as valuable undergo recycling and the practice has become an important source of income and employment of the informal sector in most developing countries (Diener *et al.*, 2009; UN-HABITAT, 2010; Diener *et al* 2011). The concern however is on organic waste which forms about 50% - 90% (44-65% in Kenya (NEMA, 2015) of the total solid waste and whose reuse or recycling is minimal (Henry *et al.*, 2006, Hoornweg and Bhada-Tata, 2012). This is more so in the developing countries where financial constraints hinder incineration of the waste through installation of efficient technologies and machineries that can convert the organic waste into energy (UN-HABITAT, 2010). Consequently the waste is mainly dumped in open compost heaps to undergo natural composting and be fed on by scavenging animals (Barry, 2004, UN-HABITAT, 2010, Diener *et al.*, 2011, Taiwo and Etoo, 2013).

2.2.1 Organic waste composting

Natural composting process emits fugitive emissions such as dust, stray garbage, noise and obnoxious odours, produces anthropogenic gases which contribute to global warming on a large scale (FAO 2011; Fao, 2013; Alooh, 2015). Open dumpsites also waste land meant for agriculture or other developments whereas the composting process is inefficient for animal products and requires manual or mechanical mixing to stay aerobic (Bullock *et al.*, 2013; Alooh, 2015). Even where it is done in designated sites, the time required to produce finished compost is highly variable and depends on the composition of the waste, aeration frequency, moisture content and temperature (Bullock *et al.*, 2013). Furthermore, there is lack of market for compost products (organic fertilizer and biogas) due to competition from alternative sources such as inorganic fertilizer, wind, electricity, solar and unfair policies and regulations that offer incentives and subsidies to these alternative sources of energy (Rouse *et al.*, 2008). Again, the process does not solve the issue of loss of the inherent nutrients which can be tapped by bioconversion process (Barry, 2004).

In Kenya, the growing human population and urbanization are the main drivers for waste generation (NEMA, 2015). The urbanization has attracted a large population of informal settlements dwellers and the middle class. Despite the existence of laws and policies guiding waste management, lack of prioritization of waste management, weak implementation practices have led to towns and cities being overwhelmed by their own waste (NEMA, 2015). Waste collection is poor and disposal of the collected waste remains is a major challenge as most counties lack proper and adequate disposal sites while the few towns that have designated sites practice open dumping of mixed waste because of lack appropriate technologies and disposal facilities (NEMA, 2014). For instance, in Nairobi County, about 30-40% of the waste generated is not collected and less than 50% of the population is served and for Nakuru, only 45% of the waste generated is collected and disposed at the Giotto open dumpsite. Indeed sites of heaps of uncollected garbage is kind of a permanent feature and eyesore in most parts of the countries' urban centres and compromise both the health of the population as well as the aesthetic value of the towns (Kazungu, 2010; NEMA, 2015). In addition, the organics which constitute about 65% of generated solid waste of most Kenyan towns is a major contributor of greenhouse gases (UNHABITAT, 2010; NEMA, 2015).

About 2.6 billion people in the world are unable to adequately dispose their excreta, of which more than one billion mostly from low income countries practice open defecation (UNICEF, 2012). In Sub Saharan Africa, the estimate is that 40% of the population practices open defecation (Morella et al., 2008), a practice that pollutes groundwater and contributes to contamination of agricultural produce and spreading of diseases such as cholera, schistosomiasis and helminthiasis (Emerson et al., 2004, Nguyen-Viet *et al.*, 2009, Humphrey 2009; UN-HABITAT, 2010). Poor sanitation is more pronounced in slum dwellings of developing countries (HABITAT, 2010), and is thought to be a function of factors such as lack of economic incentives for stakeholders throughout the service chain, high cost of emptying open pit latrines once filled, high population density and therefore lack of land to dig new pit latrines and inability of most local governments to provide sewer lines (HABITAT, 2010). There is therefore need for new ways to

manage the waste and even add value to it such as use of bioconversion process (Mutafela, 2015, Gabler, 2014).

In Kenya, approximately only 40 percent of residents in Nairobi are connected to the sewer system with 54% of the residents making use of different forms of non-sewered sanitation options such as pit latrines. Around 30 percent of the waste emanating from these offerings is poorly handled (IRIN, 2013). The Nairobi City Water and Sewerage Company cannot account for the disposal of over 66% of human waste which most likely is left untreated and poses serious risks to the environment and public health (Mutavi, 2018). Furthermore, about six percent of Nairobians mostly in slum dwellings practice open defecation (IRIN, 2013) with the use of plastic bags being a common method of disposing faeces in areas such as of Mathare, Kibera and Mukuru wa Reuben slums. These bags are discarded to the streets in the wee hours of the morning as “flying toilets” because the few toilets that are there are too expensive; and given the choice between paying for the toilet and buying food, the choice is easily made (WHO, 2006). As a result, lack of access to proper sanitation, including clean water, is a major cause of diarrhoea, the second biggest killer of children in developing countries (UNICEF, 2010). In Nairobi, child mortality in the slums is 2.5 times higher than in other areas of the city (WHO, 2010). In comparison, many slum dwellers in East African cities pay five to seven times more per litre of water than the average North American (WHO, 2010). Therefore there is an urgent need to develop sustainable waste management initiatives to curb this ever-growing hazard. In this respect, the private sector, seeing an opportunity in the waste, is applying bioconversion to generate a living from what others considers an eye sore (Kazungu, 2010). Examples include Sustainable Energy Systems Ltd based in Kiambu County and supported by Kenya Climate Innovation Centre (CIC) involved in vermiculture (the growing of red worms on organic waste) and Sanergy Ltd, Nairobi - a sanitation company whose processing plant is located at Kinanie, in Machakos County.

2.2.2 Bioconversion of organic waste

Bioconversion transfers nutrients in the organics into the body of a living organism that consumes the waste such as earthworms, insect larvae or microbes. The process yields

useful products such as fertilizers and a nutritious biomass (Barry, 2004). However, many species of flies and earthworms cannot process a wide range of organic wastes and need specific nutrition to be effective bioconverters (Latsamy and Preston, 2007) Morales and Wolff, 2010; Zhang et al., 2012). In addition, some flies and microbes are human pests and disease vectors (Sasaki *et al.*, 2000; De Jesús *et al.*, 2004). This is unlike the Black Soldier Fly which is not known as a pest, pathogen vector or nuisance of any kind to humans or their animals (Erickson *et al.*, 2004).

A BSF bioconversion technology in brief involves the artificial growing of BSF larvae on segregated bio-wastes. The reared larvae grow on the waste feedstock from which they extract nutrients and reduce the waste mass. The larvae's polyphagous nature and robust digestive system enables them to feed on a wide range of decaying organic materials both of animal and plant origin whereas the voracious appetite that facilitates the consumption of large amounts of organic waste during their growth cycle (Mutafela, 2015). As a result, the bioremediation potential of BSFL is described as one of the most promising and sustainable methods of managing organic wastes (Zheng *et al.*, 2013). At the end of the process, larvae are harvested and may be post-processed into a suitable animal feed product. The waste residue can also be further processed and potentially sold or used as soil amendment with fertilizing properties. The sanitization of remnant feeds by the antibacterial and antifungal activity of BSFL makes it safe for use in agricultural farming (Humphrey, 2009; Everest Canary and Gonzalez, 2012, Banks *et al.*, 2014; Nguyen *et al.*, 2015). In this way a BSF technology not only manages organic waste but also adds value to it and the process enables even environmentally hazardous wastes to become a source of income generation and employment creation (Mutafaela, 2015). This is especially relevant in Kenya where agriculture constitutes 70% of the economic activity and therefore agricultural waste mainly food waste constitutes the largest part of organic waste (Alooh, 2015). It is estimated that between 30-33% of the total food produced is wasted between the field and the fork, which economically, accounts for US\$ 750 million per annum of food lost, an amount that can feed three billion people in the equivalent time frame if it were accessible (FAO, 2011; FAO, 2008; The Economist 2014; FAO, 2011). Among the organics that pose sanitation problem to the environment and whose management can be facilitated by bioconversion process include post-consumer food

remains from restaurant and homesteads, vegetable and fruit wastes from markets and farms, by products and wastes from food and drink processing industries and animal and human faeces.

2.3 The Black Soldier Fly (*Hermetia illucens*, Linnaeus, 1758) Diptera: *Stratiomyidae*):

2.3.1 Introduction to the Black Soldier Fly.

The Black Soldier Fly is a harmless insect with a potential to solve two of modern agriculture's growing problems namely, serve as an alternative protein source for animal feeds and disposal of organic wastes, byproducts and side streams (Taiwo and Otoo, 2013). The insect is indigenous to the warm tropical and temperate zones of the American continents (Newton *et al.*, 2005). Climate change and human activities facilitated its spread to other continents such as Europe, India, Asia and Australia (Olivier, 2009, Leek, 2017). As a result, the Black Soldier Fly is now native to almost 80% of the world between latitudes 46°N and 42°S (Martinez-Sanchez *et al.*, 2011). In Africa, BSF has only been reported to naturally occur in South Africa, Guinea and Ghana respectively and though used for waste and feed production in Kenya by asocial private enterprise (Sanergy Ltd.), the starter broodstock was imported from South Africa (Drew, 2014, personal communication).

2.3.2 Lifecycle of the Black Soldier fly

The Black Soldier Fly undergoes a complete life cycle (Figure 1), comprising of four live stages: egg/embryo, larva, pupa/imago and adult (Li *et al.*, 2011). Eggs hatch into larvae within 3-4 days of being laid (Diclaro and Kaufman, 2009). Under the right conditions of food, relative humidity and temperature, larvae mature into prepupa in about two weeks. Prepupa, given the right conditions take two weeks to change into pupa in a process called pupation and characterized by development of an embryo within the puparium (casing), stiffness of the body, followed by immobility. Prepupae change into pupa when they find a dry medium to burrow in. In the dry medium, pupa go into a sleeping mode for a duration of at least two weeks during which time, the embryo further develops within their exoskeletal casing. When fully developed, the casing breaks up at the tip to

release an adult fly in a process called emergence (Sheppard et al., 2002). Freshly emerged adult flies have undeveloped, folded wings which gradually unfold within 2-3 hours and also have slightly larger, softer and greenish coloured bodies compared to one day old adults. Adults have a lifespan of 5-12 days during which time they mate and lay eggs (Diclaro and Kaufman, 2009). Eggs are laid in masses of 500-1200 eggs depending on the fertility level of the female, which in turn is dependent on the diet and rearing conditions at the larval stage (Tomberlin et al., 2005). The lifecycle of a Black Soldier Fly from egg to adult is estimated to last about 40-43 days under optimum rearing conditions but under unsuitable rearing conditions, the period can stretch up to six months (Popa and Green, 2012). The longest part of the lifecycle is spent at the larval and pupal stages (Figure 1) (Popa and Green, 2012). In addition, the larval stage determines and influences the longevity of other stages and the productivity of the adult stage (Holmes *et al.*, 2012). It is the most vital stage to humans in relation to its economic significance (Mutafela, 2015).

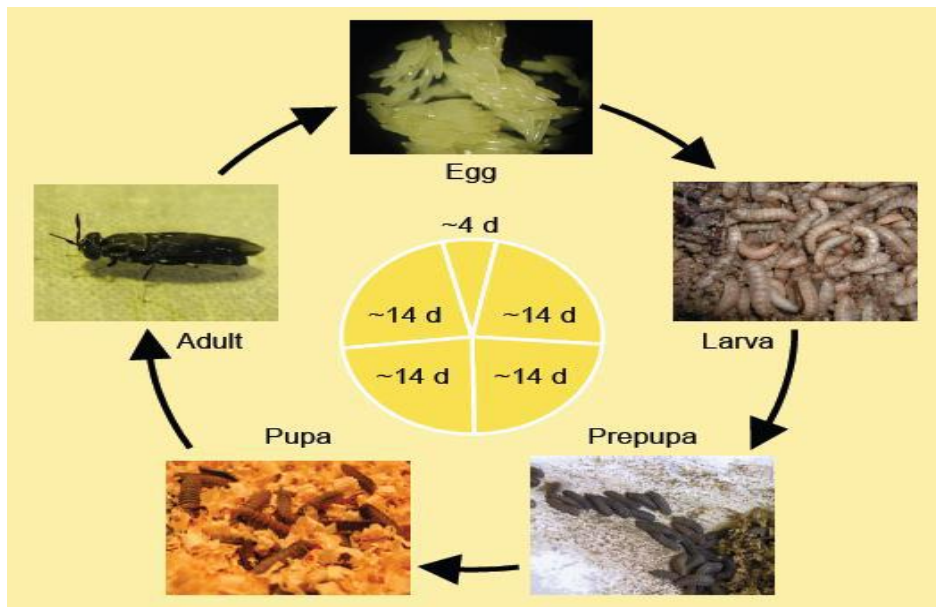


Figure 1: Life cycle of the black soldier fly (source [www: eawag.ch](http://www.eawag.ch))

2.3.3 Morphological, ecological and behavioural description of the black soldier fly

An adult Black Soldier Fly is black in colour, measures 15-20 mm long with two black smoky wings. The adult's body is divided into three distinct parts namely the head,

thorax and abdomen (Mutafela, 2015), with the first segment of the abdomen having two characteristic translucent areas (Tomberlin and Sheppard, 2002) and the terminal segment being elongated to give it an "elbowed" appearance (Diclaro and Kaufman, 2009). The legs are black with a characteristic white colouration near the distal end and the mouth parts are nonfunctional (Sheppard *et al.*, 2002; Tomberlin and Sheppard, 2002; Diener *et al.*, 2011a) (Figure 2). Again, the adult fly exhibits sexual dimorphism in which females are usually larger than the males and have a reddish abdominal tip whereas males have bronze-silvery tip. Again gentle squeezing of the abdomen reveals that the structure of sexual organ in females is long and scissor-shaped and short and fan like in males it is short and fan like (Caruso et al., 2014).

Adult Black Soldier Fly females lay their eggs near or on moist and putrescent organic waste and in the absence of such a medium, mated females cannot lay eggs. BSF adult males do not go near rotting organic wastes and are found resting on nearby vegetation where they way lay passing females to mate with them (Caruso et al., 2014). Adults are generally weak fliers and are as such terrestrial and commonly found resting or basking within heavily shaded vegetation of plants such as daisy family and carrots (Bonso *et al.*, 2013).



Figure 2: An adult Black Soldier Fly

Eggs of Black Soldier Fly (Figure 3) are oval in shape and measure up to approximately 1 mm in length. Freshly laid eggs are white in colour but overtime, the colour progressively changes and darkens to become pale yellow or creamy white. Newly hatched larvae are tiny and white coloured and measure about 0.07 inches long. Over time, the larva's colour progressively darkens to become creamy, dull white, blackish grey and finally dark brown in the course of its development.



Figure 3: Gradual change of colour of Black Soldier Fly mass of eggs on a corrugated plastic pipe.

The larva is torpedo shaped and dorsoventrally flattened on the underside. The body is divided into a small distinct head and a visibly segmented blunt back. The head of a larva contains chewing mouthparts while the segmented back contains pores (spiracles) and a rosette of hairs for breathing and floating respectively (Caruso et al., 2014). The larva's body is covered with a firm, tough skin called exoskeleton, whose toughness improves with age. The last and mature stage of a Black Soldier Fly larva called prepupa (Figure 4) is dark brown in colour and has no mouthparts (Hall and Gerhardt, 2002). Like prepupa, pupa are also dark brown in colour, torpedo shaped and dorsoventrally flattened on the underside. However unlike prepupa which are soft and flexible, pupa are stiff and immobile (Figure 5).

In contrast to the adults, immature feeding larvae prefer aquatic and semi aquatic habitats in their saprophytic feeding stage but as they mature, they prefer drier but moist conditions while prepupa and pupa stages prefer dry terrestrial habitats where they can burrow into (Hall and Gerhardt, 2002). Worldwide, the insect has been reported within latitudes 46°N and 42°S (Martínez-Sánchez et al., 2011); Diener *et al.*, 2011b). Again, an adult fly does not feed and only survives on water whereas an immature larva is a voracious feeder that eats almost all types of organic matter. A mature larva (prepupa) like an adult also does not feed. Instead, it is characterized by flexibility and constant migration as it searches for a dry place to burrow into and change into pupa. Pupae are stiff, immobile and in sleep mode until they change into adults (Hall and Gerhardt, 2002).

Again larvae are photophobic hence found burrowed 2-3cm into the organic material which they feed on while adults are photophilic and conglomerate in the side/direction where light is emanating from (Hall and Gerhardt, 2002).



Figure 4: Black Soldier Fly larvae at different stages of growth; the white ones are the feeding stage while the dark brown ones are the non-feeding prepupa stage.



Figure 5: Black Soldier Fly pupa stage.

2.3.3.1 Ecological requirements for the Black Soldier Fly.

Several studies report that the Black Soldier Fly is sensitive to abiotic factors of the environment (Mutafela, 2015). Their requirements vary with the stage of development and therefore to achieve successful breeding in confinement the conditions should be monitored and regulated to ensure they conform to the requirements. These include

temperature, relative humidity, light, diet and pupation substrate. The ecological requirements are summarized in Table 1 below.

Table 1: Requirements of the Black Soldier Fly at different stages of the lifecycle

Lifecycle stage	Duration (days)	Temp (⁰ C)	% Relative humidity	Light intensity
Eggs	4	above 26	Above 60	-
Larvae under four days old	0-4	26 – 29	65 – 75	Photophobic
Larvae over four days old.	4-14	26 – 35	65 - 75	Photophobic
Prepupa/pupa	10-14	25 – 30	Low	Photophobic
Adults	5-8	27-30	30 – 90	Photophilic. Mating occurs between 60- 200 $\mu\text{mol}/\text{m}^2/\text{s}$ with optimal of 110 $\mu\text{mol}/\text{m}^2/\text{s}$ and wavelength of 450nm-700nm.

(Adapted from Mutafela, 2015).

2.3.4 Other benefits of the black soldier fly.

Reduction of bad odour

The sheer large numbers of BSF larvae, movement and voracious appetite enable them to process organic waste extremely fast, aerate and dry it (Lalander et al., 2013). This prevents production of foul gases which normally occur under anaerobic conditions and is an advantage over other methods of management such as composting (Diener *et al.*, 2011; van Huis *et al.*, 2013).

Low pathogenicity and control of pests, vectors and pathogens

An adult Black Soldier Fly does not transmit pathogens to humans while the larvae stage controls housefly populations on materials it has colonized by preventing housefly oviposition on the same material (Erickson *et al.*, 2004). They accomplish this by secreting a distinctive odour (info-chemical) to drive away houseflies and all other pest flies (Sheppard et al., 1994), outcompeting houseflies and other harmful flies in resource utilization (Beard and Sands, 1973), directly consuming microorganisms and production

of antibacterial and/or fungicidal compounds through *lysobacter* bacteria preset in their guts (Zheng *et al.*, 2013, Erickson *et al.*, 2004). Examples include lauric acid which has proven anti-microbial activity against lipid coated viruses such as HIV and measles viruses, *E. coliform* bacteria, *Clostridium* and pathogenic protozoans such as Coccidiosis and phenolic compounds with broad spectrum antibacterial effect (Sugumaran, 2002; Park *et al.*, 2014).

Unlike the common housefly (*Musca domestica*), the black soldier flies do not transmit pathogens from organic waste to human food unlike Their larvae have only been associated with mild or pseudomyiasis (parasitic consumption of tissue by fly larva) in the equatorial zones, which resulted from accidental swallowing of live larva (Calderón-Arguedas *et al.*, 2009; González *et al.*, 2009). The condition is however associated with mild discomforts (Lee *et. al.*, 1995). The likelihood of disease transfer from BSF to humans in a waste management facility is not known, but with the use of adequate personal protective equipment, the incidence of such transfer is not expected to be significantly worse than current disease vectors present at a landfill (Mutafela, 2015).

Immunity boosting

The chitin that makes up the exoskeleton of the prepupa, pupa and adult stages is thought to have immunity-enhancing effects (Liu, 2010). Chicken fed on the chitin have been found to have reduced populations of intestinal *E. coli* and ceacal *Salmonella* (Xia *et al.*, 2011; Liu *et al.*, 2010). This shows the potential of chitin and its products to replace antibiotics in animal feeds as long as hygiene is observed during production to prevent accumulation of microbes from the environment (van Huis *et al.*, 2013).

Reduction of pollution and sanitation of the environment.

BSFL feeding activity reduces the amount of organic waste dumped in open streets and water points by at least 50-60% (Diener *et al.*, 2011a), resident nutrients such as nitrogen content by 71%, phosphorus and potassium by 52% each, amount of greenhouse gases that is generated from the waste through anaerobic respiration such as CO₂, SO₂,

methane, ammonia and other noxious gases (van Huis et al., 2013). The larvae also treat organic leachates that pollute marine and terrestrial environments (Popa and Green, 2012), and clean up oil and grease pollutions that may cause aquatic suffocations by feeding on the spilled oil (Zheng et al., 2013). The inclusion of BSFL in pit latrines and sewerage sites to feed on, decompose and reduce the faecal matter content can help prolong the lifespan of these facilities and therefore contribute to the improvement of sanitation by lessening open defecation (Banks et al., 2014).

Production of sustainable energy.

The oil of BSF can be extracted and be used as biodiesel (Zheng et al., 2012). This can reduce dependence on unsustainable sources such as jatropha plants (Zheng *et al.*, 2012, Li *et al.*, 2011). The black soldier fly's high reproductive capacity and short life cycle put them in a good position compared to the energy plants which need longer life cycles and plenty of land and therefore compete with humans (Zheng et al., 2012).

2.4 Organic waste as attractant substrates for Black Soldier Fly oviposition.

Black soldier fly female adults after mating only lay eggs in the presence of a putrescence producing substrate (Tomberlin and Sheppard, 2002). In nature, large populations of females have been reported in agricultural settings with large amounts of rotting waste that meet the breeding requirements (Nguyen et al., 2009), under poultry houses where rotting chicken droppings attract them to oviposit and in large cattle rearing operations because of the manure (Tomberlin and Sheppard, 2002).

Though in principle, any decaying organic matter may attract BSF females for oviposition (James, 1935), previous studies that have utilized various substrate attractants to attract and lure adult females from the wild to oviposit have had varied levels of success with some attractant substrates reportedly performing better than others. Bradley (1930) noted the infestation of pit latrines by BSF and consequently, (Lalander et al., (2013) and Banks et al. (2014) successfully used faecal matter to attract wild BSF. For Tomberlin (2002) and Diener *et al.* (2009), the best attractant was wetted chicken feed while in the study of Bondari and Sheppard (1987) and Bonso (2013) the advocacy was on use of carrion as an attractant. Nguyen *et al.* (2009) noted that wild BSF female flies

are attracted to compost piles and other food sources such as those occurring in standard thermal compost and vermicompost heaps and therefore proposed unspecified rotting agricultural waste as a BSF attractant in nature. Fermented maize grain (corn) with a gentle smell, fermented oats and brewer's hops have all been shown to attract BSF females, in addition to meat, fish and an existing BSF colony (Stankus, 2012).

Though there is agreement that the substrate should produce putrescence in form of volatile compounds, the studies conducted in different geographical locations indicate variability in the response of different BSF populations. Therefore there is need to determine suitable oviposition attractants for different strains and reared populations of BSF as this is a major factor that can affect egg collection and therefore overall biomass production and/ or waste reduction efficiency. The varied responses to different substrates in terms of attraction point to possibility of BSF females searching for a specific food source for their offspring which if absent may limit oviposition (Sripontan and Chiu, 2017).

2.5 Organic waste as production substrates for black soldier fly larvae

Different types of organic waste have been used for artificial farming of black soldier fly larvae in confinement. These include livestock manures from large confined animal feeding operations; palm kernel waste, pig liver, kitchen waste, rendered fish and human faecal waste (Hem et al., 2008; Canary 2009; Diener *et al.*, 2011; Diener *et al.*, 2011b; Popa and Green, 2012; Lalander *et al.*, 2013; Kalová and Borkovcová, 2013; Nguyen et al., 2013; Zhou *et al.*, 2013; Banks *et al.*, 2014; Nguyen et al., 2015). Though the larvae were able to transform the waste into a more valuable, more nutritive and less harmful biomass rich in protein (44.4% of DM), lipids (23% of DM), ash (11- 28% of DM and other valuable nutrients such as calcium (5-8% DM) and phosphorus (Newton *et al.*, 2005, St-Hilaire *et al.*, 2007, van Huis *et al.*, 2013), the intention was mostly for waste management rather than biomass production for animal feed. However BSFL biomass composition favourably compares with that of fishmeal and soya which combined, currently supply over 90% of the protein requirements of animal feeds (Yu et al., 2009).

This indicates that BSFL biomass rearing on selected organic wastes can suitably serve as animal protein source.

The potential of milled or whole dried larvae as an animal feed supplement has previously been demonstrated. The larvae biomass has been used to supplement fishmeal in experimental feeding of several animal species such as poultry, the mountain chicken frog (*Leptodactylus fallax*), American alligator (*Alligator mississippiensis*), channel catfish (*Ictalurus punctatus*), blue tilapia (*Oreochromis aureus*), juvenile turbot (*Psetta maxima*) and rainbow trout (*Oncorhynchus mykiss*) (Bondari and Sheppard, 1987; St-Hilaire *et al.*, 2007; Kroeckel *et al.*, 2012). There is no record on BSFL biomass use to substitute fishmeal in formulated diets. Again, the production of the tested biomass has relied on animal based organics (animal manures, faecal sludge and carcass) with few such studies utilizing plant feedstocks such as palm kernel meal (Hem *et al.* 2008) and cassava peels (Everest Canary and Gonzalez, 2012; Bullock *et al.*, 2013), and coffee bean pulp (Lardé, 1990). The paradox however is slower development of BSFL is observed on animal feedstocks compared to plant materials (St. Hilaire *et al.*, 2007, Tomberlin *et al.*, 2009, Kulova *et al.*, 2013). For example, Tomberlin *et al.* (2002) reported that growth of BSF larvae on lean decomposing pork at 27°C took 20-30 more days than their growth on decomposing grain under the same conditions. Additionally, the reported BSFL application is in distant places such as Asia and the Americas (Zheng *et al.*, 2013, Sheppard *et al.*, 2002, Bonso *et al.*, 2013; Newton *et al.*, 2005) and therefore limits the scope of usage of such substrates not only in terms of application but also to particular regions and geographical areas. Again the findings have little relevance to the substrates found in tropical and equatorial regions such as agricultural wastes, which equally pose serious challenges to the environment, especially where the main economic activity is agriculture. There is need to expand the scope of potential organic plant wastes from the agricultural sector and harness the large amounts of wasted nutrients in these substrates. For example, no previous study has focused on specific vegetables or fruits despite those that have used unspecified fruits and vegetable waste streams reporting excellent development rates and low mortalities (Carruthers, 2014).

Major BSFL growth parameters such as development time, feed conversion efficiency, mortality, pupal weight and nutrient composition are strongly affected by the growth substrate (Zheng et al., 2012). Therefore commercial scale application of the technology will demand usage of substrates that can yield quality larvae within a short duration and reduce losses through mortality is a necessary. Unfortunately, though generally known that the larvae consumes a wide range of organics, the full range of substrates for rearing BSFL especially for biomass production on commercial scale are still largely undetermined (Leek, 2017). Organic waste materials are also highly heterogeneous in nature and variable in terms of moisture and nutrient content and therefore generalized applications of findings is almost impossible (Holmes et al., 2012).

The use of fruit and vegetable substrates received endorsement from the European Food and Safety Authority (EFSA), as having the highest potential for use as feed for insect production due to low risk of transmitting zoonotic diseases to humans compared to substrates such as manure, catering waste, or former foodstuffs containing meat and fish which are not allowed since insects are considered ‘farmed animals’ (Committee, 2015). Fruits and vegetable commodities have a high proportion of post-harvest wastage and losses and several byproducts of the fruit and vegetable processing industries and are therefore a sustainable insect-rearing substrate (FAO, 2011, Kalová and Borkovcová, 2013, Nguyen *et al.* 2015, Paz et al., 2015). However despite this potential, little research has been done on the BSF rearing on the substrate hence the interest in this study. In Kenya no legal restriction exists on usage of fruit and organic waste such as spoilt fruits and vegetables. Past studies have revealed that vegetables and fruits in market organic waste constitute 65% and 44% respectively in most parts of Kenya (NEMA, 2015). Again, large quantities of fruits and their byproducts accumulate at production farms in peaky seasons and present disposal and public health concerns whereas the only cost to be considered for these substrates relates to collection, transportation and some moderate processing such as cutting into small pieces and removal of inorganic materials (Nguyen et al., 2009; Fila et al., 2013).

Additionally, fruits and vegetable (bananas, pineapples and avocados, and watermelons, kales), contain high nitrogen free extract (NFE) content which can serve as energy sources (Pardo et al., 2014) and low amounts of acid detergent fibres (ADF) and neutral detergent fibres (NDF) whose recommended level in a feeding substrate should be less than 300g/kg of dry matter (Council, 1993; Sang-Min and Tae-Jun, 2005; Kassahun, 2012). In addition, the selection of avocado was also mitigated by a high fat content (refer to Table 2), which is known to be a source of metabolic energy and can therefore spare the protein amount for growth and development (Sang-Min and Tae-Jun, 2005; Emebu and Anyika, 2011). Finally, studies that have used “unspecified” fruit and vegetable waste streams for BSFL growth reported excellent development rates and low mortality on such diets (Nguyen *et al.*, 2015). The concern is on lack of specific fruits and vegetable substrates for BSFL production.

A suitable rearing substrate should contain at least 20% crude protein content to be considered a source of protein in a feeding diet (Ramos-Elorduy et al., 2002; Munguti et al., 2006; Kassahun, 2012). Among the selected substrates for this study only food remains (20%) brewers waste (26%) and mixture of vegetable and fruit wastes (20%) met this criterion (Munguti et al., 2006; Nguyen *et al.*, 2015). The three substrates were therefore selected on this basis amid others such as ease of availability and low cost that relates mainly to transportation from collection sites. Performance of food waste and faecal sludge in previous studies also mitigated for their selection. Fritz (2015) reported that among the four substrates used in the study food remains produced the heaviest prepupa and highest yield followed by brewers waste with faecal sludge in third place. Nguyen et al., 2015 compared development rate, size and mortality of BSFL fed on poultry, feed, pig liver, pig manure, fish renderings, and kitchen food waste and reported that larvae fed on kitchen food waste had the fastest growth, heaviest biomass and yields attributed to higher calorie content. Kalova and Borkovcova (2013) fed BSF larvae 14 different waste types over a 14 day period and only four of the waste streams resulted in adult flies during this period among them post-consumer food waste suggesting that these diets were the most suited to larval development. These findings concur with those of Barry (2004), hence the justification for the usage of the substrate in the current study.

The usage of faecal matter in the study of Banks *et al.* (2014); Fritzi (2015) and Diener *et al.* (2009) was based mainly on the need to improve sanitation rather than biomass production. This was also the main consideration for selecting the substrate in this study, coupled by the good performance as a production substrate at Sanergy as reported by Fritzi (2015). Its availability in large quantities in almost all places further supported its use as this could guarantee sustainable BSFL production.

2.6 Optimization of production black soldier fly larvae on organic waste

For BSFL to serve effectively in the dual roles of biomass production and attendant waste reduction, establishment of a production system with a feeding strategy that specifies important substrate factors that affect BSFL feeding behaviour, growth and development is necessary (Devic and Maquart, 2015). These include aspects such as 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 size of substrate particles among others (Holmes *et al.*, 2012; Van Iterbeeck, 2014).

Optimal rates for BSFL production on most environmental parameters such as temperature, relative humidity and moisture content of the substrate have been determined as summarized in table 1, section 2.5 above (Fatchurochim *et al.*, 1989; Sheppard and Newton, 1995; Sheppard *et al.*, 2002; Barry, 2004; Tomberlin *et al.*, 2009; Zheng *et al.*, 2012; Holmes *et al.*, 2012). Brits, (2017) reported an optimal substrate depth of 5-10 cm while Bullock *et al.* (2013) and (Bullock *et al.*, 2013) reported a depth of around 20 cm to 23cm for adequate bioconversion. Differing values have also been expressed on feed rates and feeding regimes.

According to Diener *et al.* (2009), the consumption rate of BSF larvae depend on the size of larva and the type of feed. The study subsequently predicted that a feeding rate of 100 mg of chicken feed at 60% moisture as the optimal tradeoff feed rate between nutrient rich prepupa and high waste consumption in the shortest time span and also proposed optimal feed rates for various feed sources: kitchen waste (61mg/l/d), vegetable waste (98 mg/l/d), green banana (103 mg/l/d), pig manure (158 mg/l/d); poultry manure (175

mg/l/d) and human faeces (130 mg/l/d). Whereas the study accurately established that optimal feed rates vary for different substrates, the predicted values have so far not been contradicted by latter findings. For example, Banks *et al.* (2014) reported an optimal feed rate on faecal matter of 111mg/l/d against the recommended 130mg/l/d while (Brits, 2017) reported an optimal feed rate of 125 mg/larva/day on kitchen waste substrate. This shows that the feed rate may be related to the quality of the substrate (Liu *et al.*, 2008) and hence the need to determine optimal feed rates on substrate by substrate case. The determination of optimum feeding rate on the substrates used for BSFL growth is critical from both economical and biological standpoints (Aydin *et al.*, 2011) and will contribute towards a feeding strategy that can improve growth performance, survival, food conversion ratios, reduce size variation and waste management problems; and contribute to minimizing food wastage and money incurred on feed and consequently increase production efficiency (Rice and Garcia, 2011).

The study of Banks *et al.* (2014) compared BSFL growth on two feeding regimes (lump sum/batch mode and continuous mode) and reported significant differences between BSFL groups of different feeding regimes. Larvae fed on lump sum mode were larger and heavier than those fed continuously but with slower maturation periods compared to those fed on continuous regimes contrary to the study of Mutafela (2015) where continuous regime fed larvae took an average of 2-3 days longer than the ones in batch feeding though the substrates were different. Diener *et al.* (2009) showed that *H. illucens* larvae feed most efficiently when fed at regular intervals (continuous regime). Consequently continuous replacement of feed (continuous feeding regime) has somehow developed as a standard protocol for feeding in literature though with no reported tangible biological benefit or lack of it (Sheppard *et al.*, 2002; Diener *et al.*, 2009), which shows the need to clarify the effect of feeding regime, a subject of the current study.

In addition, optimal feed rates and feed regimes not only relate more to the use of BSFL as a waste management tool for cow, chicken and pig manures; municipal organic wastes and human faeces (Sheppard *et al.*, 1994; Newton *et al.*, 2005; Diener *et al.*, 2011; Lalander *et al.*, 2013; Banks *et al.*, 2014). There is limited information on the optimal

regimes or feed rates when the larvae process agricultural wastes and on production systems designed for biomass production.

Again, most research on BSFL rearing even for waste management of the different materials have mostly dwelt with single substrates as feed source (Fritz *et al.*, 2015). Though (Diener *et al.*, 2011b) has applied BSF technology in the management of mixed municipal market in Indonesia, a typical waste stream is highly variable, being made of several different components and its characteristic is dependent on the life style of producers (Bonso, 2013). Therefore, the waste mix in a developed country is different from that of a developing country and generally from place to place. For example, the organic percentage of waste in Nairobi Kenya is 65% and 34% for waste from San Francisco United States (UNHABITAT, 2010). Again, most of these researches were done in developed countries with only few in developing countries. The only known applications of the soldier fly technology in the tropics are the bioconversion of palm kernel meal in the Republic of Guinea and market waste in Indonesia (Hem *et al.*, 2008; Flechet, 2008, Diener *et al.*, 2010). There is the need to try the usage of this technology especially on the role of mixed substrates in optimizing both dry matter reduction and growth and development of black soldier fly larvae for biomass production elsewhere. The concern however relates to the scarcity of information on these necessities of BSFL production. The little that has been published relates to distant places such as Asia and the Americas (Sheppard *et al.*, 2002; Newton *et al.*, 2005; Bonso *et al.*, 2013; Zheng *et al.*, 2013) and has little relevance to the climates, conditions and substrates found in tropical and equatorial regions. This lack of knowledge concerning tropical and equatorial regions and substrates needs to be filled.

2.7 The fish feed challenge

Past global strategies to solve food problems have mainly aimed at increasing agricultural production at both the household and national levels and ensuring safe storage of harvests (Brown and Kane, 1995). These strategies have however, not been successful in the third world, mostly because of declining land sizes, stagnant farming technology, poor infrastructure and an ever-expanding population. As a result, fishing is touted as the only

sector with the potential to cope with the demands of the rising population (Kassahun *et al.*, 2012).

Nutritionally, fish is one of the cheapest and direct sources of protein, micro nutrient and income to many people in the world and especially in developing countries courtesy of its wide acceptability across social, cultural and religious backgrounds compared to other animal products (Bene and Heck, 2005; Gabriel *et al.*, 2007). Fish represents almost 16% of all animal protein consumed globally and in Africa, as much as 5% of the population depends wholly or partly on the fisheries sector for their livelihood (Gabriel *et al.*, 2007; FAO, 2013). Much of the fish currently consumed in Africa mostly comes from the natural rivers and lakes in the continent. However, there is concern that the capture fisheries have reached their natural limits and improvements of food security through fish consumption now rely on aquaculture (FAO, 2010). The paradox is however that aquaculture uses wild caught fish for fishmeal production and therefore diverts a food source that could be consumed by humans. Given that about 30% of wild fish catches are used to produce fishmeal for farmed fish feeds, the continued future availability of fish meal is not sustainable (PAF, 2011; Tiu, 2012; FAO, 2012).

In Kenya, fishmeal is processed from the silver cyprinid (*Rastrineobola argentea*, locally known as “Omena”) and the Nile perch captured from Lake Victoria and the Indian Ocean waters (Bokea and Ikiara, 2000; Nyandat, 2007). It is estimated that between 50 and 65% of the silver cyprinid from Lake Victoria is used to produce fishmeal whose supply is about 30, 000 tonnes per annum (Abila, 2003). The growing demand for the use of “omena” for fishmeal use as well as for domestic consumption has led to its severe reduction from the Kenyan-Lake Victoria waters to the extent that the country relies on imports from Tanzania and Uganda to supplement her demand (AKEFEMA, 2005). Consequently, fishmeal supply is considered the major constraint facing the animal feed industry as a whole (Bokea and Ikiara, 2000). The situation has negatively impacted food security and the livelihoods of lakeside populations as importation makes the product expensive and unaffordable (Nyandat, 2007). The exorbitant cost of fish feed now accounts for over 60% of the total production cost (AKEFEMA, 2005; PAF, 2011; FAO, 2010; Tiu, 2012; Abarike *et al.*, 2013, FAO, 2013). This has made aquaculture an

expensive venture and therefore hindered the potential of the sector to bridge the gap between fish demand and supply (Gabriel *et al.*, 2007). There is therefore need for an alternative protein source, preferably produced on by-products and materials which are not suitable for direct human consumption and local production of fish feed from inexpensive and locally available feedstuffs to reduce the cost of production, provide a cheaper means of meeting the protein requirements, improve food security and reduce the level of poverty in developing countries (Hoffman *et al.*, 2000; Gabriel *et al.*, 2007; Kassahun *et al.*, 2012).

A number of organic wastes and by-products from agricultural and industrial sectors are available in Kenya, which are usually not utilized for human consumption, but may have a high potential for aquaculture. These can be used both as ingredients for locally compounded feeds and as production substrates for currently underutilized animal protein sources such as farmable edible insects for inclusion in the compounded feeds (Van Itterbeeck *et al.*, 2014). The transformation of these locally available by-products low in protein into high quality fish protein can be a major contribution to improving the protein supply for the local human population.

Global enthusiasm for insect farming is growing as its diverse range of potential commercial and environmental benefits become well recognised (Devic, 2016). Insects which can be produced on organic waste products provide a more sustainable source of protein for animal feed. Use of farmable edible insects as feed ingredients is associated with certain advantages: they are rich in proteins, fat, energy, vitamins and minerals; have higher feed conversion efficiency compared to livestock and therefore use less feed; less land than crop production; have great acceptance from poultry and fish as part of their natural diet and are mostly omnivorous and therefore grow on different substrates (van Huis *et al.*, 2013). However despite the tremendous potential to be used as a feed item of many livestock animals, they are currently not widely used perhaps due to inadequate knowledge about their potential (Devic, 2016).

The use of BSFL as a fish feed due to the need to find a replacement for fishmeal in animal feeds has been considered. To date, studies of fish species fed black soldier fly

larvae have only been performed with rainbow trout, *Oncorhynchus mykiss*; channel catfish, *Ictalurus punctatus*; and blue tilapia, *Oreochromis aureus*. The studies report that the larvae can replace up to 25% of fishmeal use with zero adverse effects. However no work has been reported on the potential of BSF larvae as a complete alternative replacement for fishmeal.

2.8 Challenges in the use of insects as a food and feed resource

Operations for the production of BSF have been faced with different challenges which mostly relate to the use of the larvae as feed rather than as an agent of waste management (Mutafaela, 2015). To start with, Black Soldier Fly production requires a warm environment. This requirement has proved difficult and energy consuming to sustain in the temperate climates and during winter periods (Holmes *et al.*, 2012). Use of greenhouses to ensure continued production during the cold seasons within the tropics and equatorial climates has made the enterprise expensive (Holmes *et al.*, 2012).

The duration of the life cycle ranges between several weeks to several months depending on temperatures, quality and quantity of the diet. This makes prediction of production a challenge (Veldkamp *et al.*, 2012). The continued lack of legal framework and specific legislations on the use of insects discourages investment in the sector (Leek, 2017). For example within the European Union (EU), strict sanitary regulations, a lack of guidelines on the mass rearing of insects, lack of clarity on which insect types are authorized for the market, and prohibition of some common types of substrates for insect production have also hindered progress in the acceptance and establishment of the insect market (van Huis *et al.*, 2013). This is in contrast to countries in Africa where there is virtually no restriction on the kind of substrates used (Leek, 2017).

Issues of feed quality due to the potential of BSF to bio-accumulate toxins and heavy metals from pesticides, chemical fertilizers, herbicides and other chemicals sprayed on production substrates and genetic engineering technologies presents another challenge (Diener *et al.*, 2009). High sodium levels in processed food stuffs have also proved problematic. Most of these accumulate in ecosystems and in larva, and at higher

concentrations may be toxic both to the larvae and the consuming animals along the food chain (van Huis *et al.*, 2013). This therefore limits the potential sources of suitable substrates. Another concern involves acceptance and perception of insects. Insects that fall under the category of ‘flies’ are commonly perceived as filthy and unsanitary (van Huis *et al.*, 2013; Mutafaela, 2015). This is perhaps because society associates them with houseflies which are a known health risk. This is the basis for the EU restrictions on the use of insects as feed ingredients of animals destined for human consumption (Leek, 2017). The generalization is affecting even harmless flies like BSF and is largely due to lack of awareness. Lack of collaboration among experts in the field to make necessary explanations to the naïve public and create awareness on potential of insects as a food and feed resource has contributed to poor acceptance and persistence of the wrong perceptions (Smith and Barnes, 2015). However the perceived benefits of insects such as sustainable production, lowered dependence on imported protein sources and lower environmental impact are mitigating for improved change of attitude towards broad acceptance and are considered more important than the perceived risks such as microbiological contamination, chemical residues in the food chain and lower consumer acceptance of animal products (Verbeke, 2015).

Healthy risks from a variety of pathogens, parasites and diseases are a major challenge in insect production systems (Leek, 2017). Knowledge of disease and health management in intensive insect rearing is still limited and population crashes sometimes involving the whole colony do occur (Leek, 2017). For instance in Georgia, a parasitoid wasp of the *Trichopria* genus has been reported to infect 21-32% of Black Soldier Fly pupae (Mutafaela, 2015). Current mitigation measures involve minimizing the health risks by ensuring bio-security in a breeding colony, use of very ‘clean’ substrates and separate housing of the different stages of the breeding stock to avoid cross infection between the different stages (Leek, 2017). In addition, predators such as rats, mongooses and lizards do feed on larva and adults and can therefore significantly contribute to diminishing of populations and returns.

The quantity of insect currently produced is a significant obstacle for the use of insects in animal feed as it does not guarantee a constant supply. Consequently, the prices for insects and insect meals are presently very high, and cannot compete with other protein sources in this respect. This makes insect protein too expensive compared with common sources of proteins used in poultry diets (Leek, 2017). At current prices, BSF is at par with fishmeal which over recent years has all but disappeared from most livestock diets. However with increase in campaigns to increase adoption of insect rearing technologies, supply is expected to rise and with it, reduction of price (Rumpold and Schlüter, 2013).

2.9 Demand for insect proteins

The demand for insect proteins varies from one region to another. In Asia and South America, the demand is from the human food industry. This is especially in countries such as Thailand, Laos's Peoples' Republic and Mexico where crickets and mealworms are produced and consumed (Hanboonsong et al., 2013). In Africa, the demand for insect protein is primarily from the poultry and fish industries (Leek, 2017, Schönfeldt and Hall, 2012). However production is still at infancy despite the huge potential of the region in terms of climatic conditions and supply of agricultural wastes as raw materials. In Europe and North America, strict legislations has restricted the use of insects to the aqua feed and pet food markets with future prospects for livestock and poultry sectors (Leek, 2017).

2.10 Theoretical Framework

The use of edible insects as a source of cheap proteins has been practiced by many ethnic groups world over, and the practice has received support from Food and Agriculture Organization (FAO) as a viable alternative protein source for both human food and animal feeds (FAO, 2012). Sustainable utilization of the resource is however dependent on successful development of farming strategies (Ramos-Elorduy, 1997; Scriber and Slansky, 1981; Tchuinkam et al., 2011; Oonincx et al., 2015). This was a major aim of this study with emphasis on the Black Soldier Fly.

The need to investigate growth and larval biomass composition under different diets emanates from findings that BSF development and biomass quality in terms of nutrient content varies across diet types (Tomberlin *et al.*, 2005). Optimization of BSF larvae

production was based on three premises: that feedstock composition influences both the quantitative performance and larval composition (St-Hilaire *et al.*, 2007, Bullock *et al.*, 2013), and that the amount of food (feed rate) and feeding frequency (feeding regime) influences the growth characteristics of larvae (Banks, 2014, Li *et al.*, 2008, Sheppard *et al.*, 1994, Tomberlin *et al.*, 2002). Finally, the choice of the larvae stage of the insect was informed by the fact the larvae stage determines not only the characteristic of the adult and pupal stages of the insect but is also the most relevant with respect to waste management and protein production. It is the stage where the whole concept of bioconversion occurs.

CHAPTER THREE -MATERIALS AND METHODS

3.1 Introduction.

This chapter presents a comprehensive description of how the data was obtained, processed, analyzed and interpreted to fulfill the specific research objectives. The methodology elements that are considered in this section include the research design, study area, the sampling procedure and size and methods of data analysis.

3.2 SUBSTRATE ATTRACTANTS FOR BLACK SOLDIER FLY OVIPOSITION.

3.2.1 Research design of the study

The study applied the experimental research design. The method is used to establish a cause and effect relationship between two variables or among a group of variables (De Vaus, 2006). The experimental research design has an experimental group and a control group and study subjects are randomly assigned to the two groups. The researcher administers the treatment (independent variable) to the experimental /test group (manipulation) and not to the control group and both groups are measured on the same dependent variable(s) of interest to gauge the difference due to the treatment and tries to control for confounding variables (De Vaus, 2006). Experimental research is often used where there is time priority in a causal relationship (cause precedes effect), there is consistency in a causal relationship (a cause will always lead to the same effect), and the magnitude of the correlation is great (Kirk, 2013). Therefore true experiments unlike quasi experiments must have control, randomization, and manipulation; and in addition, may employ power analysis in order to determine appropriate sample sizes at the desired probability of making a Type I or Type II error, making them highly fixed even before the data collection starts (Anastas, 1999).

The design was selected for its ability to allow the researcher to maintain control over all factors that may affect the result of an experiment, support the ability to limit alternative explanations and to infer direct causal relationships in the study. The method also provides the highest level of evidence for single studies (Creswell, 2018). The study had different experimental procedures corresponding to the study objectives.

3.2.2 Wild black soldier fly attraction for oviposition

3.2.2.1 Study site

Attraction of the wild Black Soldier Fly was done at Jaramogi Oginga Odinga University of Science and Technology main Campus in Bondo within the School of Agricultural and Food sciences demonstration farm. Bondo, located at latitude 0° 14' 19.00" N and Longitude 34° 16' 9.98" E, is characterized by generally high temperature ranges with mean minimum and maximum temperatures of 15°C and 30°C respectively with a mean annual temperature of 22°C, an altitude of approximately 1250 m above sea level and is ecologically located in the semi-humid to transitional lower midland zones (LM2, LM3 and LM4). The area receives an annual rainfall amount that ranges from 800 to 1600 mm per annum in two rainy seasons and has high relative humidity with mean evaporations being between 1800mm to 2000mm in a year courtesy of the nearby Lake Victoria (Achonga et al., 2011).

3.2.2.2 Source, preparation of attractant substrates

Maize grains were bought from Bondo market and soaked in water for three nights to ferment and then roughly ground in a mortar using a pestle into a mash. Omena was also bought from Bondo open air market vendors and soaked in water overnight to make it wet. Cow and chicken manures were obtained from the animal farm at JOOUST. The two were mixed in a ratio of 1:1 and thoroughly mixed using a concrete mixer before use. The fruits and vegetable mixture was obtained from collection bins of the County government of Siaya located in Bondo open air market and used in the state collected.

Though the Black Soldier Fly is reported to be found within the tropics, its existence within Kenya had not been reported as at the start of this study. The first challenge was therefore to determine its nativity in Kenya and consequently establish a production colony. To achieve this, different literature sources that report the attraction of adult Black Soldier Fly females by putrescence of moist organic materials of various kinds were used. For example, Bondari and Sheppard (1987) reported that adults of the species are attracted to rotting carrion. Nguyen *et al.* (2009) also describes the attraction of Black Soldier Fly females to agricultural settings where organic waste provides sites for their

reproductive needs (Bondari and Sheppard, 1987, Nguyen-Viet *et al.*, 2009). The study simulated the described natural conditions in an attempt to lure Black Soldier Fly females from the wild if available to oviposit and initiate a confined colony.

3.2.2.3 Experimental design for attraction of wild black soldier fly

A quiet area within the demonstration farm of the School of agricultural and Food Sciences covered with eucalyptus and *Lantana camara* flowers was selected as according to literature, adult Black Soldier Flies are shy, weak fliers that prefer to rest in quiet highly shaded vegetation (Nguyen *et al.*, 2009). Four similar larvae feeding structures of size 1m by 0.5 m by 0.5 m (Figure 6) later modified (Figure 7) were constructed and separately half filled with garbage of different types (Table 2), to serve as an attractant for adult females. The containers were distributed along a single row at a distance of approximately 5m from one another.



Figure 6: A metallic feeding and attraction structure constructed and fitted with side pockets for collection of prepupa.



Figure 7: A wooden feeding and attraction container fitted with plastic basins for collection of prepupa

Table 2: Substrates used to attract wild adult Black Soldier Flies

Feeding structure	Type of attractant substrate used
FS1	Mashed maize grains
FS2	Silver cyprinid (<i>Rastrineobola argentea</i> , local name: “omena”)
FS3	Vegetables and fruit wastes from the market (kales, <i>Solanum nigrum</i> , cabbage leaves, tomatoes, rotten water melons rotten oranges, rotten bananas and their peelings).
FS4	Animal manures (mixture of chicken and cow manures).

Key: FS: feeding structure

Corrugated flexible plastic tubes 0.5 inches wide in diameter were placed on top of the substrate attractants and on the sides to serve as an oviposition medium (Figure 8). Each structure was then covered with a lid, raised at a slanting angle to allow the entrance of the adults into the structure to lay eggs. The 4th side of the structure was slanted at an angle of 35° to allow the satiated larvae an easy way out of the growing substrate onto a plastic bucket placed nearby. Every day at 4 pm, the number of adult flies within the containers was monitored and plastic pipes were taken out of the structures and observed for the presence of laid eggs and then returned to their place in the feeding structures. Twice a week, fresh supplies of attractants were replenished. Every day at 8 am for six

months, prepupa that had fallen into the attached buckets were collected and weighed per attractant and recorded for later analysis. The day's collection from all the buckets was pooled together for use as emergence broodstock as per the method of Holmes *et al.* (2012). However 30% of the collection from FS3 taken for proximate nutrient analysis for later use in feed formulation.

3.2.3 Black soldier fly attraction for oviposition in a captive colony

3.2.3.1 Study site

Attraction of wild Comparison of attractants for oviposition activity of BSF reared in captivity was done at EnviroFlight LLC, Ohio USA. EnviroFlight LLC is a private enterprise involved in the large-scale production of Black Soldier Fly larvae for the production of animal feeds for the pet industry. The company is based at Yellow Springs, Ohio and uses frass tea, as bait attractant for ovipositing females. Frass tea is prepared by soaking frass (remnant of larvae feed) in water for 2-3 nights. The company however was in need to diversify baiting attractants as the frass lacked consistency in egg production. This coupled with the need to compare performance of different strains on attractant substrates that were available and had been used in Kenya formed the basis of the study.

3.2.3.2 Source of attractant materials.

Five attractants namely cow manure, fruits mix (pineapples, bananas, watermelon and avocado), a commercial, rotten fish, frass tea and sweet scenting commercial liquid were selected for the trial. Cow manure was obtained from a livestock farm near EnviroFlight LLC. All the fresh fruits and commercial scent were bought from a fruit market at Yellow Springs town. The frass tea was made by overnight soaking frass from previous feeding remnants in water. The attractants used were the same as those used in oviposition attraction of wild populations in Bondo except the commercial sweet smell and frass and were meant to compare response of wild and domesticated strains of BSF populations.

3.2.3.3 Experimental design for egg collection

Two cages with adult BSF emergence were selected in an insect insectarium and two compartments (out of four compartments) within the cage were selected for the

oviposition experiment. The selected compartments were those not adjacent to each other in order to avoid interference of putrescence of baiting attractants. Each of the baiting attractant was put into a separate 50ml plastic container to the half mark level (Figure 8). The containers were then covered with a nylon net that allows the spread of odour but prevent the insects from seeing or touching the trapping material within as per the method of Sripontan and Chiu (2017). A lid with twenty equidistantly perforated holes was then used to secure the nylon material to the plastic container (Figure 8). A block of wooden pieces stuck together by rubber bands was put on top of the container containing the attractant substrate and each day at 4pm, a pair of containers containing different attractants and the block of wood was separately put inside the selected compartments of a cage (Figure 9).



Figure 8: Rubber bound wooden blocks used to trap eggs oviposited by the black soldier fly

After 24 hours, the attractant and the block of wood with laid eggs were removed, and the eggs scratched from the woods with a pen knife. The eggs were weighed using a weighing scale. This was repeated until all the attractant pairs had been tried to give a total of eight replicates per attractant. Care was taken to ensure uniform distribution of the attractants within the compartments of the cages.



Figure 9: Rubber bound wooden pieces on a plastic box containing attractant inside a cage.

3.3 TESTING OF PERFORMANCE OF LARVAE FEEDING SUBSTRATES.

3.3.1 Black soldier Fly larvae growth performance on organic waste substrates

3.3.1.1 Study site

The identification of suitable organic waste substrates was done at Sanergy Ltd, Nairobi, Kenya. Sanergy Ltd located about 45 km, south east of Nairobi City, within Machakos County, in an area that is dry most times of the year, sunny, and sparsely populated. Sanergy Ltd. is a social enterprise based in Nairobi, Kenya, dedicated to building healthy, prosperous communities by making hygienic sanitation affordable and accessible to residents of urban informal settlements. The company takes an innovative system-based approach to build out an entire sustainable sanitation value chain cycle that has three main components namely:

1. Building a dense network of high-quality, low-cost sanitation facilities, called Fresh Life Toilets to collect urine and feces separately and operate without water in Nairobi's slum dwellings. The toilets are distributed to local entrepreneurs who charge users.
2. Collecting the waste from the toilets in sealed 30 litre capacity cartridges on a regular basis from the operators and exchanging them with clean empty ones. This way, the waste is safely removed from the community and between November 2011 and May, 2014, 6,200 metric tonnes of waste had been collected and safely removed from the

community. This facilitates hygienic sanitation for approximately 33,000 residents (Sanergy, 2014).

3. Converting the waste at a centralized facility, into a number of high-margin products, predominantly organic fertilizer which is sold to Kenyan farms and additional by-products such as biogas, biochar as well as dried BSFL meal for the feed industry. The BSFL biomass is raised on a mixture of faecal sludge from the Fresh Life Toilet network and organic waste. However, low productivity, high mortality and longer development periods on the substrate have been witnessed. The study therefore compared the performance of this substrate with that of other locally available substrates with an aim of blending them with the primary substrate to boost production.

3.3.1.2 Selection and sourcing of feeding substrates

Potential production substrates were selected on the basis of sustained local availability in large quantities, cost of purchase, non-use for human consumption, need for disposal to improve sanitation and published proximate nutritional content. The substrates that were used include food remains (FR), faecal sludge (FS), brewers' waste (BW) and banana peels (BP). Faecal sludge substrate was obtained free of charge from the daily collections at the processing facility of Sanergy LTD, located at Kinanie, Machakos County Kenya. Banana peels and food remains were also obtained free of charge from the nearby Kinanie market as garbage collections, with the only costs related to their acquisition being transport costs. Brewers waste was obtained from EABL breweries production site in Ruaraka at a cost of 5 shillings per kilogramme. The substrates were packed in air tight gunny bags and transported to the production facility at Kinanie.

3.3.1.3 Substrate preparation

Except for shredding of banana peels by hand into small sizes, all the substrates were used in feeding experiments on "as is basis" (in the collected form without any modification or treatment). The performance of the substrates was evaluated in terms of the effect on growth rate, development period and nutrient composition of the larvae in larvae feeding experiments.

3.3.1.4 Source of larvae.

Larvae for this part of the study were sourced from a BSF colony previously established from a Black Soldier Fly colony maintained at the Sanergy Ltd production site at Kinanie in Machakos County. Corrugated plastic pipes were put in the adult colony and stale food remains was used as an attractant medium as per the standard operating procedure at Sanergy (Sanergy, 2014). Eggs laid within two days were collected and transferred to a cylindrical plastic vessel containing a 15% protein chick mash thoroughly mixed with water to moisture content of 60% (Figure 10).



Figure 10: Plastic containers with BSF eggs on corrugated pipes in the hatchery.

The container with eggs was transferred to a nursery section with controlled conditions (temperature, 30°C, relative humidity, 70%) for hatching. To accelerate their growth, newly hatched neonates were allowed to feed on the commercial chick mash until they were 5 days old (Tschirner and Simon, 2015). On the 6th day, the larvae were sieved through a mesh screen of diameter 1.2 mm and those that passed through were considered of the same size and weight. Sampling was done from among these by initially counting the larvae into five groups each containing 2,000 larvae, using a pair of forceps to handle the delicate larvae. Each group was then weighed on an electronic weighing scale to determine the average collective weight of 2,000 larvae, and consequently the mean initial weight per larvae. The average weight of a cohort was then used to divide the rest of the larvae into 12 cohorts to ensure triplicate treatment per substrate. For control, an extra container with substrate but no larvae was included for each substrate.

3.3.1.5 Larval feeding and sampling.

Using a uniform feeding rate of 100 mg/larva/day (Diener *et al.*, 2009), substrates were weighed and distributed to the feeding containers. Each larval cohort was then evenly spread on the allocated substrate surface. The feeding containers were randomly placed on a floor surface and covered with a mosquito netting to keep off other fly species from ovipositing on the substrates (Figure 11). Using the formula: $n = \frac{z^2 \cdot p \cdot q \cdot N}{e^2(N-1) + z^2 \cdot p \cdot q}$ where, $N = 2000$ (population of larvae in a container) $z = 1.96$ (desired confidence level is 95% and value obtained from table) $p = 0.5$ (sample proportion). $q = 0.5 \{(1-0.5) \text{ i.e. } 1-p\}$ $e = 10\%$ or 0.1 (approximate precision rate or acceptable error) (Kothari, 2004), 100 larvae were randomly counted from each container and collectively weighed on every 4th day. The total weight obtained was divided by the number of larvae to obtain an average weight of each larva. The weight gain after every 4 days was calculated by comparing the obtained average larval weight with the previous mean larval weight. The sampled larvae from each substrate treatment were pooled together and sun dried and stored in airtight zip lock bags for nutrient analysis as per the method described by (Intl, 1995). After sampling, fresh ration of food was added to the containers. The feeding was continued for a total of 16 days, by which time most larvae (white/cream in colour) had changed into black/brown prepupae (Tomberlin *et al.*, 2009). All the prepupa and any larvae were then harvested by sieving them through a 5-mm diameter mesh screen and the residue material dried at 105°C for 24 hours to determine its dry mass. The final prepupa weight was taken to be the average weight of the biomass recorded on the day of harvest. All the feeding experiments were done under the ambient conditions of temperature ($28 \pm 2.0^\circ\text{C}$) and relative humidity ($65 \pm 5.0\%$) of the environment and the moisture content of the feedstock.



Figure 11: Plastic containers used for feeding larvae in larvarium

3.3.2 Black soldier Fly larvae growth performance on fruit and vegetable substrates

The comparison of BSFL growth performance on vegetable and fruit wastes was done at EnviroFlight LLC, Ohio USA. EnviroFlight LLC uses a mixture of Dried Distillers grains (DDGs) and Cookie Meal (CM) in a ratio of 1:1 as primary production substrates. The need to diversify the range of feeding substrates beyond DDGs and CM mixture and adopt fruit and vegetable substrates as approved by Committee (2015) was the basis of the current study. The chosen substrates were also available in Kenya albeit in waste form and therefore results obtained could be compared and equivalent substrates utilized.

3.3.2.1 Source of larvae.

Fifteen bins of 3rd instar larvae from the EnviroFlight nursery ready for launching into large feeding bins were selected and the larvae were sieved through a 1.2 mm screen mesh to remove the debris content (frass content from previous feedings in the nursery). All the sieved larvae from three bins were separately weighed to determine their collective weight. The sum of the total weight of larvae from the three bins was averaged to determine the average weight of a bin and thereafter the average larvae weight before feeding was started.

3.3.2.2 Source of substrate, preparation and larvae feeding.

To satisfy, the legislative requirements in USA, that requires the production of insects destined for feed or food on exclusively food or feed grade materials, approval of fruit

and vegetable substrate raised insects for the feed market (Committee, 2015), the substrates selected for this study included ripe avocado, bananas, kales, pineapples and watermelons all purchased from Fairborn town, Ohio USA. For control, the substrate being used at EnviroFlight LLC (a mixture of dried distiller's grains with solubles (DDGS) and cookie meal (CM) at a ratio of 1:1) was included. Though the study intended to utilize organic waste forms of the substrates, legal restrictions prohibited the usage of these materials, necessitating purchase of the above substrates. The purchased substrates were separately chopped into small pieces using a knife in different feeding basins (Figure 12). Then six feeding basins were selected and clearly labelled according to the intended respective feeding composition. Using a feed rate of 150mg/l/d, and the number of larvae per group (approximately 33,000), 4.95 kg of each substrate was weighed and distributed into 18 feeding bins such that each substrate treatment was replicated thrice including the control. For the control, 0.125 grams of CaCl_2 and 0.0625 grams of calcium propionate were added as calcium enrichments and preservative respectively as is the practice at EnviroFlight LLC. Thereafter, 1000 ml of water was added and a small hand rake used to mix it with the components. This was followed by introduction of the 33000 larvae to each treatment. During feeding management practices included frequent monitoring of water content and feeding behaviour of larvae. Where water content was observed to be too high to cause larval migration to the top of the bin, frass from previous feedings was added to moderate the water level, otherwise, the substrates were used in their "as is states". Daily, the feedstocks in all the treatments were agitated by a rake to ensure uniform aeration and feeding within the substrates.

3.3.2.3 Larvae feeding and sampling

Using the formula, $n = z^2 \cdot p \cdot q \cdot N / e^2(N-1) + z^2 \cdot p \cdot q$: where, $N = 33000$ (population of larvae in a container) $z = 1.96$ (desired confidence level is 95% and value obtained from table) $p = 0.5$ (sample proportion). $q = 0.5 \{(1-0.5) \text{ i.e. } 1-p\}$ $e = 10\%$ or 0.1 (approximate precision rate or acceptable error) (Kothari, 2004), 100 larvae were randomly counted from each container and collectively weighed on every 4th day as per the method of Tomberlin et al. (2002) and Myers et al. (2008) and thereafter returned to their containers. The total weight obtained was divided by the number of larvae to obtain an

average weight of each larva. The weight gain after every 4 days was calculated by comparing the obtained average larval weight with the previous mean larval weight. After sampling, fresh food rations were provided and this was continued until the first turned prepupa was noticed within a bin and at this point, addition of fresh food was stopped and larva allowed feed for four more days and for the feed to dry up to facilitate easy sieving during larvae and frass separation. Upon sieving, both larvae biomass and frass mass were recorded. The effect of the different treatments on the nutrient content of prepupa was ascertained by proximate nutrient analysis as per procedures described in AOAC (1995). Harvested Black Soldier Fly larvae were sun dried for 4 days in a greenhouse and then ground into powder using a kitchen blender. For CP analysis, micro-Kjeldahl method was used (Feldsine et al., 2002). 0.5 g of sample was weighed into test tubes using ash less paper. 5g of a catalyst (90% K_2SO_4 and 10% $CuSO_4$) was added into the test tubes. For a control, blank test tube containing only the catalyst was included. Fifteen millilitres of concentrated sulphuric acid was then added and the mixture transferred into an acid hydrolyser for 3 h. After digestion, each sample was then titrated and the crude protein (CP) percentage content determined by multiplying the nitrogen content by the factor 6.25. For the analysis of ether extracts (EE), 2 g of ground sample was transferred into labeled crucibles. Seventy percent pure diethyl ether was added to each sample and then transferred to an ether extractor machine. After extraction, the ether extract was then dried in an oven at 110°C for 30 min before weighing to determine the net weight of the extract.



Figure 12: A representation of each of the substrate treatments used at EnviroFlight LLC.

3.4 OPTIMIZATION OF PRODUCTION THROUGH A FEEDING STRATEGY.

3.4.1 Study site:

This was done at Sanergy Ltd and involved three parameters namely: variation of feeding amounts (feeding rate), feeding frequency (feeding regime) and substrate combination whose effect on larvae growth parameters was investigated.

3.4.2 Source of substrates

The substrates used in the study included brewer's waste (BW) collected from East African Breweries (EABL) Nairobi, faecal sludge (FS) donated by Sanergy Ltd from their daily collections from the toilets they have established in the sprawling Mukuru and Kibra slums, post-consumer restaurant food waste (FR) and banana peels obtained from the eateries located at Kinanie trading centre and Kinanie market respectively. Black Soldier Fly larvae were obtained and prepared for feeding experiments as previously described in section 3.1.2.4 and 3.1.2.5 above.

3.4.3 Effect of feeding rate

Comparison of larval growth rate was done on the principal substrate (FS), Food remains (FR) and brewers waste (BW) -best performers in feed identification experiments - under four different feeding rates namely 100, 150, 200 and 250 mg/larva/day. Larvae sampling

and weighing was done as previously described on the 4th, 8th, 12th and 16th days respectively. The total amount of substrate required before the next feeding was calculated on the basis of the feeding rate and total amount of larvae and thereafter weighed and distributed to the respective feeding containers. Fresh food was done to the respective treatments after every four days. The feeding was done for 16 days but harvesting was delayed until prepupa (recognized by a change of colour of the integument from white/cream for larvae to dark/brown for prepupa) were observed (Tomberlin *et al.*, 2009). All the feeding treatments were done in triplicates. The effect of the different treatments was analyzed quantitatively in terms of weight gain, larvae growth rate and duration of development, and qualitatively in terms of protein/lipid ratio of the produced larvae.

3.4.4 Effect of feeding regime

Effect of feeding regime was done on the principal substrate (FS) under different feeding regimes namely daily feeding (DF), after four days feeding (AFD), weekly feeding (WF) and lump sum feeding (LF). Substrate distribution was done according to the individual feeding regimes: daily feeding (DF), after four days feeding, (AFD), weekly feeding, (WF) and lump sum feeding (LF). Fresh food was distributed according to the designate feeding regime: daily for DF, after every four days for AFD, once a week for WF and only once at the beginning for LF. The total amount of food distributed into each basin was calculated on the basis of number of larvae, feeding rate of 200mg/l/d and number of days to the next feeding period. After food distribution, each larval cohort was evenly spread on the allocated feedstock ration. In all the feeding regimes, feeding was done for 16 days followed by harvesting when prepupa (recognized by a change of colour of the integument from white/cream for larvae to dark/brown for prepupa) were observed (Tomberlin *et al.*, 2009).

3.4.5 Effect of substrate combination

In order to determine an ideal co-substrate for FS (the primary substrate at Sanergy Ltd), three co-substrates (food remains, brewers waste and banana peels) were combined with faecal sludge in three different ratios (30:70; 50:50; and 70:30) and compared to the

control diet (100% faecal sludge). An earlier study by Fritz (2015) at Sanergy had combined faecal sludge with the co substrates at a ratio of 50:50. Diener et al. (2010) combined market waste with FS at the same ratio. Therefore ratios close but in either side of this reference ratio were selected for comparison and compared to the control diet (100% faecal sludge). The amount of food substrate fed to 2000 larvae was provided in four batches of four days each and was calculated proportionately according to the feeding rate of 200mg/l/d and ratio of mixing the principal substrate (FS) and the co-substrate and each weighed accordingly. The two were then thoroughly mixed in large plastic bowls to obtain homogeneity before distribution into the feeding basins (Figure 13). In all the mixing treatments, feeding was done for 16 days but harvesting was delayed until prepupal (recognized by a change of colour of the integument from white/cream for larvae to dark/brown for prepupa) were observed (Tomberlin *et al.*, 2009).



Figure 13: Faecal sludge-Co-substrate mixing.

3.4.6 Larvae feeding, sampling and harvesting

Larvae feeding was done separately according to the respective treatment being investigated (feeding rate, feed combination and feeding regime), under the existing conditions of temperature ($28 \pm 2.0^{\circ}\text{C}$) and relative humidity ($65 \pm 5\%$) of the environment and the moisture content of the feedstock. Each larval cohort was spread

evenly on its allocated substrate for feeding in triplicates. The feeding containers were then randomly placed on a floor surface and covered with a mosquito netting to keep off other fly species from laying on the substrates (Figure 14).



Figure 14: Set up of experiment at Sanergy Limited.

For all treatments, sampling was done on every 4th day by randomly counting 100 larvae from each container as described in section 3.2.2.3 (Kothari, 2004). The sampled larvae were collectively weighed on an electronic scale and thereafter, returned to their respective feeding containers. The total weight obtained was divided by the number of larvae to obtain an average weight of each larva. The weight gain after every 4 days was calculated by comparing the obtained average larval weight with the previous mean larval weight. The final prepupa weight was taken to be the average weight recorded on the day of harvest. On the last day of sampling, all the prepupa and any remaining larvae in a treatment were separately harvested by sieving through a 5mm diameter mesh screen and supplemented by manual picking of small sized larvae that may have passed through the sieve together with the residue, to minimize losses. The biomass and residue weight obtained were weighed and recorded. Thereafter, the weight of total feed provided and that of total residues obtained were used to calculate the reduction effect of a treatment. The performance of the different treatments was evaluated using the recorded parameters of periodical larval weights, total prepupal/larval harvests in grams and calculation of feed conversion and reduction efficiency. In addition effect of the different treatments on

the nutrient content of prepupa was ascertained by proximate nutrient analysis as per procedures described in AOAC (1995) described in detail in section 3.2.2.3 above.

3.4.7 Material consumption and reduction efficiency

The efficiency of the BSFL to consume and therefore reduce organic matter content in the fed substrates was determined by calculation of waste reduction efficiency, feed conversion into increased body mass efficiency (feed conversion rate, FCR) and bioconversion rate, as described previously (Banks *et al.*, 2014; Diener *et al.*, 2009):

$$\text{Substrate Reduction}(\%) = \frac{\text{Total feed added} - \text{Residue feed after treatment}}{\text{Total feed added}} \times 100$$

$$\text{Feed Conversion Rate (FCR)} = \frac{\text{Feed consumed}}{\text{Total prepupal biomass}}$$

$$\text{Bioconversion rate } (\%) = \frac{\text{Total prepupal mass}}{\text{Total feed added}} \times 100$$

3.5 FISH FEED FORMULATION AND FEEDING TRIALS

3.5.1 Nutritional analysis of wild source prepupa

The potential of the wild sourced prepupa as a protein source for fish feed was determined by subjecting a sample of pooled prepupa fed on vegetable and fruit wastes (section 3.1.2); the most abundant and acceptable substrate according to EFSA (committee, 2015) to proximate analysis to determine nutrient composition according to method described by Association of Analytic Chemists, AOAC (1995) as described in 3.2.2.3 above.

3.5.2 Fish feed formulation

This study was conducted at Jaramogi Oginga Odinga University of Science and Technology (JOUST) main campus in Bondo and combined different ingredients to formulate a BSF diet (BM) and a fishmeal diet (FM). The ingredients included; maize

flour mill sweepings, milled rice bran, brewer's waste, cassava flour, milled fishmeal or milled BSF larvae. The selection of the ingredients was done on the basis of their local availability in sufficient amounts, cost, need for disposal and nutrient profile. Preference was given to those that are byproducts or waste products, whose incorporation in feeds could also enhance good sanitation (Madu et al., 2003). Two whole isonitrogenous feeds were formulated namely a BSF feed and fishmeal feed. The inclusion level of each ingredient in the feeds was calculated using the Pearson's square method based on their crude protein content (Pandey, 2013), to obtain a feed whose composition is represented in Table 3 below. Vitamins and minerals premixes were sourced commercially and supplemented in the feed in the required amounts to ensure the final feed product meets the National Research Council (NRC) recommended amounts for tilapia (NRC, 1993).

Table 3: Percentage composition of ingredients in the formulated feeds

Ingredients	Diet	
	BM	FM
BSF	62	0
Fishmeal	0	38.5
Rice bran	12.4	20.1
Maize floor sweepings	12.4	20.4
Binder (cassava)	500g	500g
Brewers' waste	12.7	20.5
Total	100	100

3.5.3 Feed pelleting

All plant feedstuffs were precooked to remove any potential anti nutritional factors present (Hecht, 2007) and then milled using a hammer mill into powder form, and then accurately weighed in the correct proportions as determined by Pearson's square method. Cassava flour and ethoxyquin were added at a rate of 150 mg per kg of diet as a binder and preservative respectively (Pandey, 2013). The ingredients were manually mixed thoroughly to form a homogenous mixture followed by addition of warm water to form dough. To ensure pellet floatability on water, cooking liquid oil was added using a handheld sprayer (Pandey, 2013). Pelleting was done using a locally fabricated meat mincer machine. The pellets were then sundried to a final moisture content of 6-10%, and

then cut into 1 cm sized pieces using a sterile scalpel and stored in in a cool dry place at room temperature for fish feeding (Pandey, 2013).

3.5.4 Fish feeding

For acclimatization, before commencement of the feeding experiment, juvenile tilapia fish acquired from a nearby fish hatchery were fed on a commercial diet for two weeks. At the end of the two week period, a random sample of 30 fish were individually weighed using an electronic scale according to Khan et al. (2013) and thereafter, the fish were randomly distributed into 12 pond lined cages (1m-L \times 1m-W \times 1m-H divided into two categories (FM and BM) (Figure 15). The BM and FM groups represented cages in which fish were fed on fishmeal and BSF meal feed respectively. The tanks were arranged in three rows with each row having four tanks. The BM and FM labeled tanks were distributed in a randomized complete block design (RCBD) such that each row had two tanks for each treatment to give a total of six tanks for BM and another six for FM. (i.e. each feed treatment had six replicates). Each tank was stocked with fish at a stocking density of 30 fish per tank. The fish were hand fed twice daily, between 8.00-9.00 am and 15.00-16.00 pm, at 3% body weight throughout the experiment for 8 weeks as per the method of Khan *et al.* (2013). Applying the Kothari (2004) formula described in section 3.2.2.3, fish sampling was done weekly by randomly selecting 23 fish from each tank and individually weighing and measuring their length. Thereafter the feeding ration was adjusted weekly on the basis of the previous obtained weekly mean weights. The experimental cages were inspected daily to remove any dead fish (usually found floating). The parameters used to evaluate the performance and feed characteristics included periodical fish weights, final fish mean weight, feed floatability time and duration of feed stability on water, feed shelf life, colour of the feeds and effect on water parameters (Mustapha et al., 2014).



Figure 15: Pond liner tanks used during the fish feeding trials.

3.6 Data collection and statistical analysis

Data was collected and recorded in tables and computed into averages using Microsoft excel for all parameters measured. The data was subjected to two-way ANOVA and t-tests to determine significant differences between treatments and between groups within the same treatments. Least significant difference (LSD) and Tukey HSD were used for mean separation between treatments at 95% confidence level. All the statistical analyses were conducted in the R studio (Team, 2015; Team, 2016). Data was presented in form of graphs and tables.

CHAPTER FOUR –RESULTS

4.1 PERFORMANCE OF SUBSTRATE ATTRACTANTS

4.1.1 Attractant substrate performance in the attraction of wild Black Soldier fly

On day two to day four, different insect species were observed frequenting the different attractant wastes. These included the common housefly, green bottle fly, blowflies and sandflies among others. However, no BSF was observed during this period. The first BSF was observed on Day five on the plastic pipes of the feeding structure with vegetable wastes and the mashed maize grain. A mass of eggs oviposited inside the corrugation grooves were also observed on the same day. On the 14th day, white cream larvae were seen wriggling on plastic pipes. On the 23rd day, the first prepupa was observed in the collection bins.

Comparison of the monthly collection of prepupa produced over a period of 6 months from the different attractant substrates showed that the highest collection was obtained from mashed maize grains, FS1 (17.6 Kg), followed by vegetable and fruit wastes, FS3 (13.2 Kg), Omena remains, FS2 (10.1 Kg) and lastly animal manures, FS4 (5.0 Kg) (Figure 16). ANOVA analysis of the monthly prepupa means per attractant substrate showed significant differences at the 95% confidence level (Table 4). The LSD grouping of the treatment means is shown in Table 5.

Table 4: Summary of 2 way ANOVA comparison of attractant prepupa yield

Source of variation	Df	Sum of squares	Mean square	F-value	P-value	Significance
Attractant substrate	3	14.1	4.7	46.18	8.2e-08	***
Residuals	15	1.5212	0.1014			
Totals	18					

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

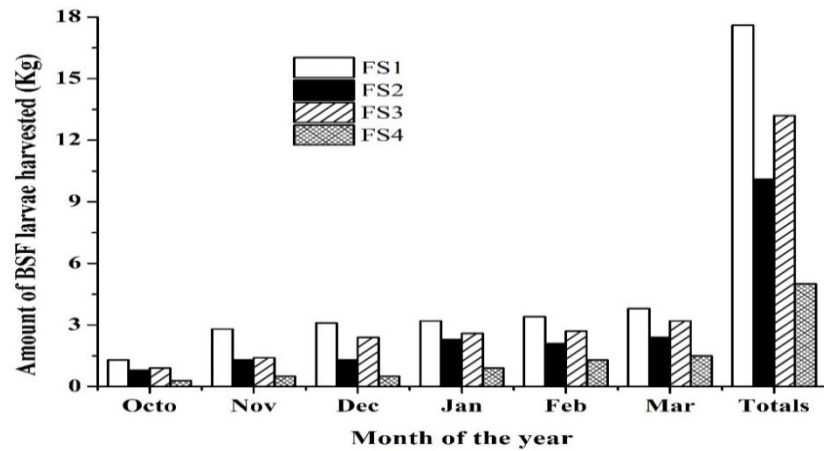
Table 5: LSD t-test for yield response across the substrate treatments (mean±SD)

Substrate	Prepupal yield (g)	LCL	UPCL
Mashed grain	2.933 ^a ±0.8664	2.6562221	3.210445
Market waste (fruits and vegetables)	2.200 ^b ±0.8694	1.9228888	2.477111
Omena	1.683 ^c ±0.6676	1.4062221	1.960445
Animal manures	0.8330 ^d ±0.4844	0.5562221	1.110445

[†]Alpha: 0.05; DF Error: 15

*Critical Value of t: 2.13145; Minimum Significant Difference: 0.3918944.

[†]Treatments with the same letter are not significantly different.



Key:

FS1: Mashed maize grains

FS2: Silver cyprinid (*Rastrineobola argentea*, local name: “omena”)

FS3: Vegetables and fruit wastes from the market (Kales, *Solanum nigrum*, cabbage leaves, water melon, oranges and bananas and their peelings).

FS4: Animal manures (mixture of chicken and cow manures).

Figure 16: Amount of wild BSF larvae harvested from October to March on different attractant substrates

4.1.2 Attractant substrate performance in the attraction of confined black soldier flies.

The results indicate that cow manure and frass tea were more attractive to BSF and stimulated more egg-laying activity in the Black Soldier Fly followed by frass tea, fruits, fish and scent respectively (Figure 17).

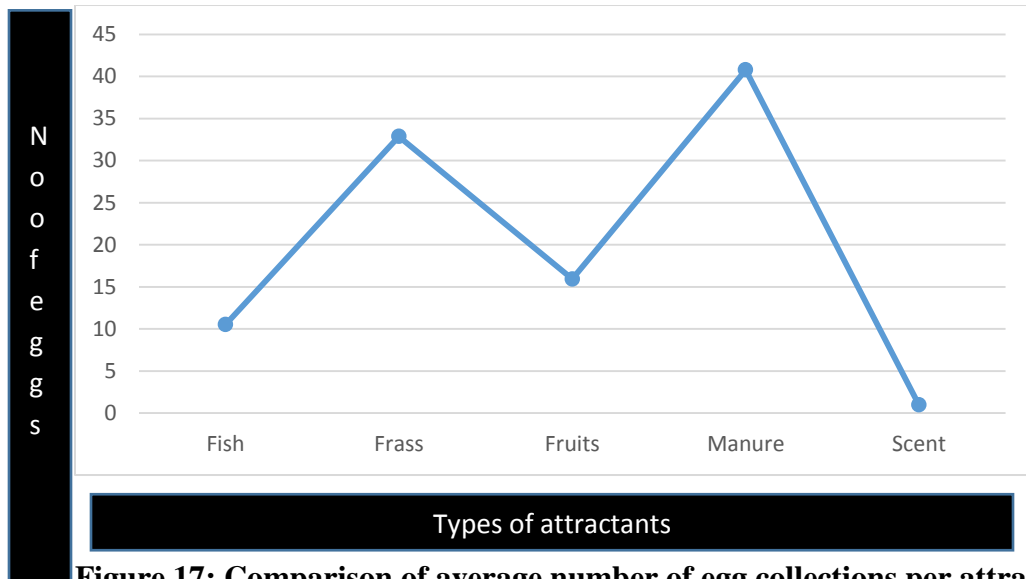


Figure 17: Comparison of average number of egg collections per attractant.

An ANOVA analysis of the result showed that the performance of the attractant substrates was significant at 95% confidence level (Table 6) while mean separation by LSD test showed that the difference was insignificant between cow dung manure and frass on one hand and the performance of fish, fruits and scent on the other (Table 7). Commercial sweet scent emerged as the least egg laying stimulant. However wide variability within and between the substrates was evident as indicated by the standard deviations.

Table 6: ANOVA summary of performance of the attractant substrates at EnviroFlight

Source of variation	Df	Sum squares	Mean squares	F-value	Pr(>F)	Significance
Attractant	4	108.96	27.239	7.753	0.000153	***
Cages/tanks	1	0.06	0.060	0.0017	0.896989	
Residual	34	119.86	3.525			
Total	39					

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 7: LSD test for number of eggs from attractants at EnviroFlight (Mean \pm SD).

Substrate	Parameter						Alpha,0.05; Tc= 2.032;
	No. of eggs	Rep	LCL	UCL	Min	MAX	
Fish	1.317875 ^b \pm 1.788	8	-0.031	2.667	0.0560	4.179	Min. significant difference =1.908
Frass	2.522912 ^{ab} \pm 2.941	8	1.174	3.872	0.0272	7.584	
Fruits	2.002375 ^b \pm 0.870	8	0.653	3.351	1.0730	3.218	
Manure	5.096850 ^a \pm 2.125	8	3.748	6.446	1.4580	7.892	
Scent	0.124625 ^b \pm 0.083	8	-1.224	1.474	0.0350	0.274	

*Treatments in the same column with the same letter are not significantly different

4.2 PERFORMANCE OF FEEDING SUBSTRATES ON LARVAE GROWTH.

4.2.1 BSF larvae growth and nutrient content on organic waste substrates

At the same feeding rate of 100 mg/larva/day, FR gave significantly higher average prepupal weights of 0.101 \pm 0.002g while BP gave lowest average prepupal weights (0.055 \pm 0.002g), ($p < 0.05$). The prepupal weights for BW and FS were 0.078 and 0.070g, respectively. Similar trends were also observed for the total prepupal and larval yield where FR gave significantly higher yield (196.9 \pm 4.0) followed by BW (154.8 \pm 6.5), FS (138.7 \pm 5.0) and lastly BP (108.9 \pm 5.6) (Table 8).

Table 8: Summary of weight, yield, crude protein and fat content of BSF prepupa fed on different organic waste feedstocks (Mean \pm Standard deviation).

Feedstock	Prepupal weight (g)	Prepupal yield (g)	Crude Protein (%)	Ether Extract (%)
Food waste (FR)	0.101 \pm 0.002 ^a	196.9 \pm 4.0 ^{a†}	36.1 \pm 0.1 ^b	35.9 \pm 0.1 ^a
Banana peels (BP)	0.055 \pm 0.002 ^c	108.9 \pm 5.6 ^c	34.9 \pm 0.6 ^b	38.0 \pm 0.3 ^a
Brewers waste (BW)	0.078 \pm 0.007 ^b	154.8 \pm 6.5 ^b	43.0 \pm 1.0 ^a	27.2 \pm 1.0 ^b
Faecal sludge (FS)	0.070 \pm 0.001 ^b	138.7 \pm 5.0 ^b	45.4 \pm 0.1 ^a	18.1 \pm 0.3 ^c

[†] Mean values with the same letter within a column are not significantly different ($p < 0.05$).

*Weight and yield measurements were done on wet weight

[‡] Nutrient content was based on dry matter.

The nutrient content of larvae from different substrates and at different growth stages is represented in Figure 18 below. In terms of nutrient profile of the harvested BSF larva, significantly higher CP level was recorded in BSF prepupae produced on FS and BW at 45.4 ± 0.1 and $43.61 \pm 1.0\%$, respectively. However, the prepupae produced on FR and BP recorded lower CP content (Table 8). In contrast to CP levels, significantly higher ($p < 0.5$) crude fat content was observed for prepupae produced on BP and FR ($38.0 \pm 0.3\%$ and $35.9 \pm 0.1\%$ respectively), compared to those reared on BW ($27.2 \pm 0.3\%$), and interestingly those reared on FS which had a significantly lowest ($p < 0.001$) crude EE content ($18.1 \pm 0.3\%$). Again, between day 4 and 16, the CP content of BP prepupa decreases unlike in BW, FR and Fs where CP content increases until day 12 and then decreases. The results in figure 18 show that the CP content decreased with increase in age of the larvae, irrespective of the rearing substrate used. The CP content of larvae reared on FS and BW was consistently greater than EE content across all the stages of development while the inequality of the CP and EE content reduced in older larvae (Figure 18).

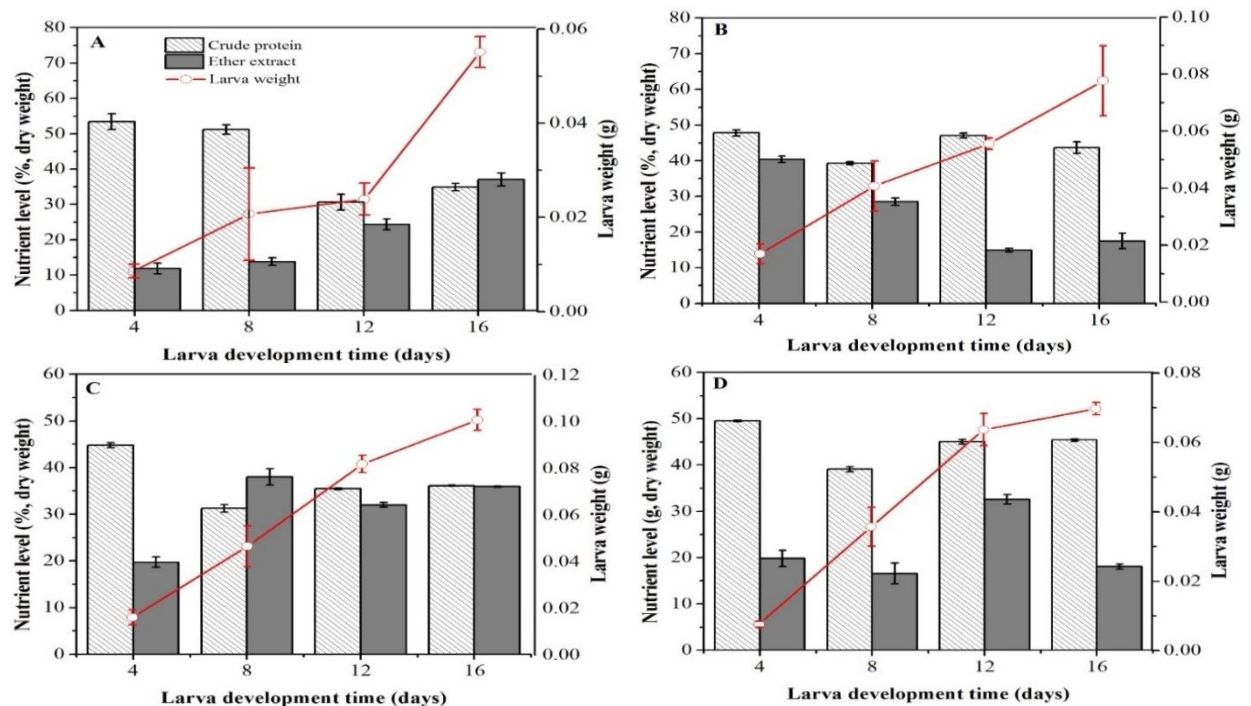


Figure 18: The change in crude protein and ether extract of BSF larva during growth in different organic waste feedstocks: A, banana peelings; B, brewer's waste; C, food remains; and D, faecal sludge. Larval growth is presented by a red line showing change in weight with time.

An analysis of the effect of the individual substrates on the mean larval weight over time showed varied response of BSFL for the different substrates at different stages of growth (Figure 18). Whereas the larvae's response to food remains and brewers' waste was consistent throughout the entire feeding period, noticeable growth on faecal sludge and banana peels was as from the 4th day onwards. BSFL reared on banana peels showed the least growth during the whole lifecycle (Figure 19).

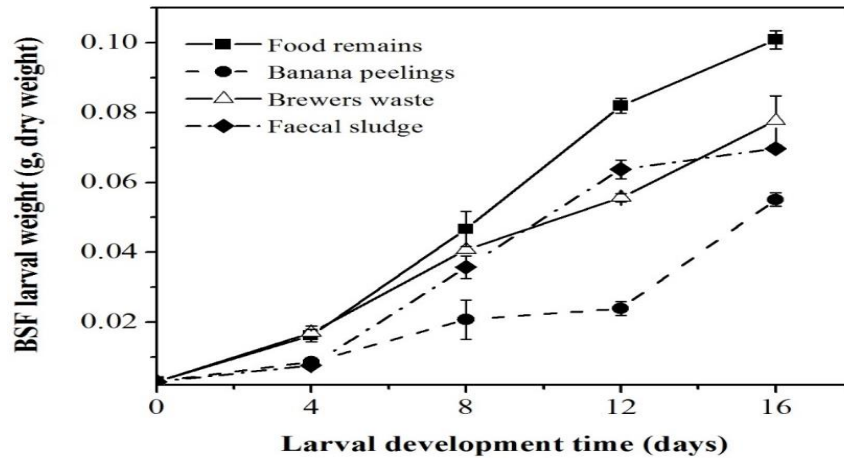


Figure 19: Growth of BSF larvae on four different waste feeding treatments.

4.2.1.1 Substrate reduction and bioconversion efficiency of organic waste substrates

The results on the efficiency of the BSF larvae to consume and therefore reduce the waste load of the different substrates are shown in Table 9. Substrate conversion rates ranged between 44.7-81.8% for the feedstocks studied. FR ranked as the best reduced substrate with very little residue (18.2%), mostly consisting of the larval excreta being observed, which resulted in a high bioconversion rate. In contrast, high residue content was observed for FS substrate in this study as shown in Figure 20. The lowest FCR was obtained on FR substrate again whereas the highest was recorded on BP.

Table 9: Substrate reduction, bioconversion rate and feed conversion rate of *Hermetia illucens* larvae fed on different organic waste substrates.

Substrate/Feed resource	Substrate reduction (%)	Bioconversion rate (%)	FCR [‡]
FR	81.8	20.8	2.6
BW	44.7	16.6	2.7
BP	52.4	11.7	4.5
FS	50.3	14.9	3.4

[‡] Results based on wet weight basis

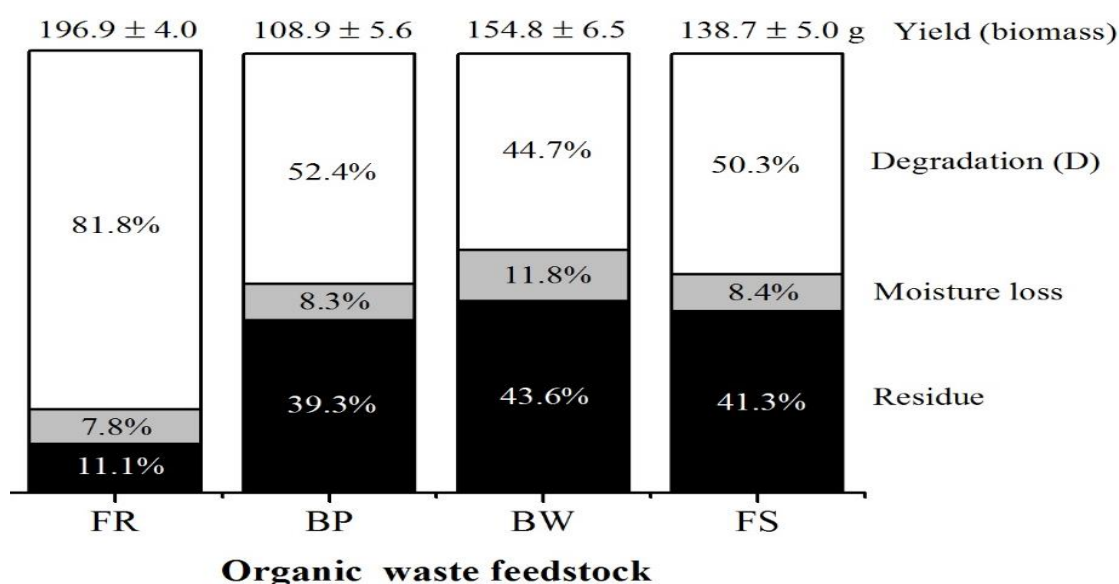


Figure 20: Waste reduction values of BSFL on organic waste substrates

[†] FCR feed conversion ratio

4.2.2 BSF larvae nutrient content and growth on fruit and vegetable substrates

The nutrient content of the prepupal product, especially the fat and protein content were strongly affected by the diet consumed. The fat content was highest on avocado diet followed by banana diet and lowest on kales diet. However the CP content was highest on prepupa from kales diet followed by those from watermelon, pineapples, avocado and lastly banana diets. The dry matter content was significantly high in the avocado and banana diets respectively and low on pineapple, watermelon and kales diets in that order. Ironically, larvae fed on kales had the highest CP content while those fed on avocados had the highest fat content. The fibre content was however highest on banana raised prepupa and lowest on those raised on avocado diet (Table 10).

Table 10: Proximate analysis of the nutrient content of prepupa harvested from different vegetable and fruit substrates.

Substrate	%DM	%Moisture	%CP	%EE	%ADF	% Ash
Watermelon	26.8	73.2	40.2	31.5	6.0	12.7
Pineapples	28.28	71.72	40.0	37.2	8.5	11.7
Bananas	34.99	65.01	35.4	44.6	10.8	8.79
Avocados	36.22	73.78	37.1	51.5	5.1	2.47
Kales	23.68	76.32	47.8	20.2	9.8	15.6

[‡] Results on dry matter basis

The average prepupal weight and biomass yields obtained from the different substrates were significantly different at the 95% confidence level (Table 11). Prepupa fed on the control diet (DDGs+ Cookie meal) produced both the heaviest average mass and highest biomass yield. Among the plant substrates, bananas produced the heaviest prepupa followed by watermelon, pineapples, avocados and lastly kales. Prepupa harvested from kales had both the least average mass and total biomass yield (Table 11). There was a significant difference in terms of days taken to mature across the treatments

4.2.2.1 Substrate reduction efficiency, bioconversion rate and feed conversion ratio

The results on the efficiency of the BSF larvae to consume and therefore reduce the waste load of the different substrates are shown in Table 11. Substrate reduction rates ranged from 41.8% for the control feed to 78.5% for avocado among the feedstocks used in the study. Among the fruits category, the greatest amount of residue was obtained on pineapple treatment while avocados yielded the least amount of remnant wastes.

Table 11: Mean prepupal weight, substrate reduction, prepupal yield, BCR and FCR of larvae reared on fruit and vegetable substrates (Mean± Standard deviation).

	DC	WM	PA	BA	KA	AV	F - value	P - value	Sig
Prepupal yield (kg)	3.4 ^a ±0.53	2.9 ^{a,b} ±0.14	2.2 ^c ±0.09	2.8 ^{a,b} ±0.18	1.5 ^c ±0.10	2.329 ^b ±0.10	7.16	0.004	***
Av. prepu weights (g)	0.20 ^a ±0.003	0.103 ^c ±0.003	0.101 ^c ±0.001	0.121 ^b ±0.00	0.070 ^c ±0.005	0.091 ^d ±0.007	627.2	<0.001	***
Frass (kg)	5.24 ^a ±0.23	3.08 ^b ±0.08	3.61 ^b ±0.06	3.48 ^b ±0.07	3.26 ^b ±0.05	1.94 ^c ±0.24	12.3	0.005	***
Days to maturity	10.33±0.58	16.33±1.53	17±1.00	15.7±0.58	18.6±0.6	17.0±1.0	23.97	2.88e-05	***
Substrate reduction (%)	41.8	65.8	59.9	61.3	63.8	78.5			
FCR	1.11	2.04	2.45	1.97	3.83	3.05			
Bioconversion rate (%)	37.8	32.2	24.4	31.1	16.7	25.8			

* Means within a row having different letters were statistically different at $p < 0.05$

*Replication was not important in all the treatments as P value was above 0.005 except for average prepupa weight where it was 0.0032.

Key:

DC- DDGS and cookie meal

WM-water melon,

KA-Kales

BA-Banana

AV-Avocado

PA-pineapples

Across all the fruit substrates, larvae were unable to consume the outer peel layers and contributed to the remnant composition. During early days, there was little consumption of kales but this improved with time as the kales underwent decomposition. Kales yielded the lowest bioconversion rate while the control diet had the highest. Among the fruits, bananas and watermelons had higher bioconversion rates compared to pineapples and avocados. The feed conversion ratio on the other hand was lowest for the control diet followed by banana diet and was highest on the kales diet.

4.3 OPTIMIZATION OF BLACK SOLDIER FLY PRODUCTION

4.3.1 Effect of feeding rate on growth, prepupal weight, biomass yield, and maturation

As shown in Figure 21, feed rate had an effect on the growth rate of larvae fed on the principal homogeneous substrate (FS) as from the 4th day onwards with higher feed rates resulting in faster growth and vice versa (Figure 21). At day eight, 100mg/l/d performed significantly below other feed rates. As from day eight to day 16, the performance of 100mg/l/d and 150mg/l/d was significantly below that of 200 and 250mg/l/d with the performance at 200mg/l/d and 250 mg/larva/day not being significantly different (Figure 21).

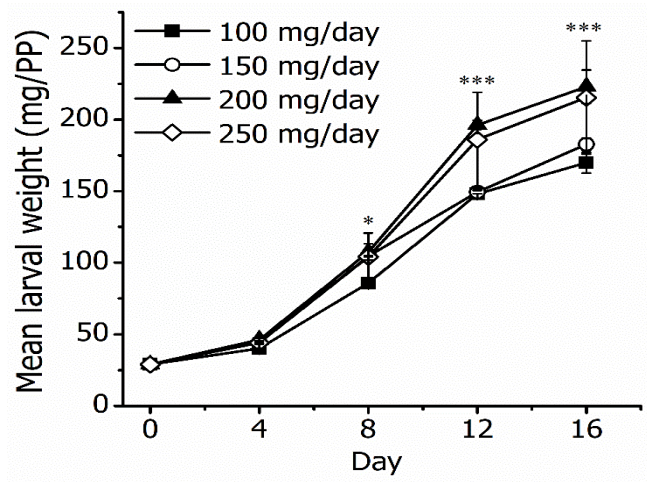


Figure 21: The influence of feeding rates on the growth of BSFL fed on faecal sludge.

Days denoted by *, ** and *** indicate significant difference (ANOVA followed by Tukey post hoc tests) at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

When different feeding rates were adopted in place of the standard feeding rate recommended by Diener *et al.* (2009) for the other substrates (FR, BW and FS), a significant increase in prepupal weight and prepupal yield for all the homogeneous substrates used in the study including the control treatment was observed. Increase of feeding rate from 100 to 200 mg/larva/day resulted in increase in the prepupal weight and prepupal yield (Table 12 and Table 13). Across the other homogeneous substrates, the prepupal biomass yield increased with increase in feeding rate with higher biomass being obtained from larvae fed at 200mg/l/d and 250mg/l/d feed rates compared to 100 and 150 mg/l/d feed rates (Tables 12 and 13). However the effect of 200 and 250 mg/l/d was not different across the substrates especially in terms of days to maturity, substrate reduction and bioconversion rate as shown in Table 12; and prepupal weight and yield (Table 13; Figure 22). The amount of food provided also had an effect on the time taken by larvae to mature. For example at 250mg/l/d and 200mg/l/d, a significant proportion of the population had turned into prepupa by the 15th and 16th days respectively compared to 18th and 20th for those fed on 150mg/l/d and 100mg/l/d respectively. Substrate reduction in this study declined with increase in the feeding rate (81% \pm 8.9 for 100mg/l/d and 54% \pm 1.2 for 250mg/l/d but FCR and bioconversion rates show that conversion of the substrates into larvae biomass was more efficient between feeding rates 100mg/l/d and 200mg/l/d and poorest at 250mg/l/d (Tables 12).

Table 12: The mean prepupal yield, days to maturity, substrate reduction, bioconversion rate and FCR of BSF grown on faecal sludge at different feeding rates (Mean \pm Standard deviations).

Parameter	Feeding rate (mg/larva/day)				Sign. [†]
	100	150	200	250	
Prepupal yield (g)	124 \pm 3.0 ^d	140 \pm 1.3 ^d	176 \pm 3.3 ^b	190 \pm 1.2 ^a	***
Days to maturity	20 ^a	18 ^b	16.7 ^{bc}	15 ^c	***
Substrate reduction (%)	81 \pm 8.9 ^a	84 \pm 0.3 ^a	57 \pm 1.1 ^b	54 \pm 1.2 ^b	**
Bioconversion rate (%)	21 \pm 2.5 ^b	29 \pm 0.3 ^a	21 \pm 0.8 ^b	23 \pm 0.6 ^{ab}	*
Feed conversion rate	2.4 \pm 0.02 ^c	2.9 \pm 0.03 ^b	2.8 \pm 0.05 ^b	3.9 \pm 0.09 ^a	***

[†] ***, **, * denoted *p* value <0.01, <0.01, <0.1, respectively

[‡] Means within a row having different letters were statistically different at *p*<0.05. (ANOVA, Tukey post hoc tests).

Table 13: Effect of different substrate feeding rates on prepupal weight and prepupal yield (mean± Standard deviation).

Substrate	Feeding rate (mg/larva/day)			
	100	150	200	250
Food remains (FR)				
Mean prepupal weight (g)	0.098 ^c ±0.002	0.110 ^b ± 0.009	0.128 ^a ± 0.008	0.128 ^a ± 0.002
Prepupal yield (g)	196.6 ^c ± 4.0	219.9 ^b ± 14.3	255.6 ^a ± 9.6	256.1 ^a ± 5.6
Brewer`s waste (BW)				
Mean prepupal weight (g)	0.075 ^d ±0.007	0.085 ^d ± 0.004	0.104 ^b ± 0.001	0.103 ^b ± 0.009
Prepupal yield (g)	140.2 ^f ± 6.5	170.8 ^e ± 8.90	208.4 ^b ± 23.8	206.7 ^b ± 23.6
Faecal sludge (FS)				
Mean prepupal weight (g)	0.068 ^e ±0.001	0.073 ^d ± 0.002	0.089 ^d ± 0.004	0.095 ^c ± 0.010
Prepupal yield (g)	123.3 ^g ± 5.0	140.7 ^f ± 19.3	175.8 ^e ± 12.1	190.2 ^c ± 13.7

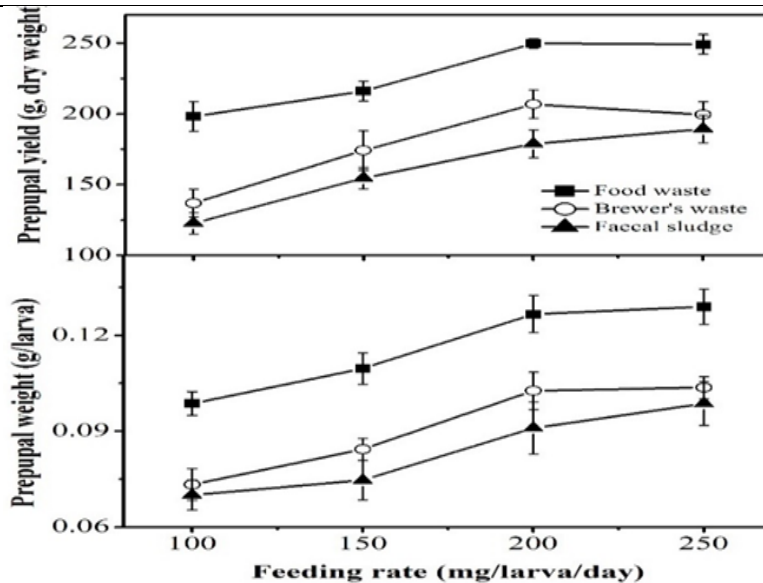


Figure 22: Effect of feeding rates on different homogeneous substrates on *Hermetia illucens* mean prepupa weight and yield.

4.3.2 Effect of feeding regime on larval growth, prepupal biomass and residence period.

As shown in Table 14 below, feeding regimes had significant effects in terms of maturation period, substrate reduction, feed conversion ratio and bioconversion rates. In terms of maturation period, Larvae fed on regimes with shorter feeding intervals (DF and AFD) took 16 days to mature while those fed on regimes with longer feeding intervals

took longer periods to mature (17 and 20 days for WF and LF respectively). Substrate reduction was also higher on DF and AFD regimes (84.6% and 83.5% respectively) compared to 79.2% and 77.1% for WF and LF regimes respectively. However there was no difference in terms of feed conversion rate between DF, AFD and WF while LF had the best feed conversion rate of 2.56.

Table 14: The effect of feeding regimes on mean (\pm SD) prepupal yield and maturation period of BSF grown on faecal sludge, and the corresponding substrate reduction, bioconversion rate (BCR) and feed conversion rate (FCR).

Parameter	Feeding regime				<i>p</i> value
	AFD	DF	WF	LF	
Prepupal yield (g)	201 \pm 7.4	194 \pm 3.1	202 \pm 6.1	204 \pm 2.1	0.56
Days to maturity	16 ^c	16 ^c	17 ^b	20 ^a	<0.001
Substrate reduction (%)	83.5 \pm 0.3 ^b	84.6 \pm 0.2 ^a	79.2 \pm 0.1 ^c	77.1 \pm 0. ^d	<0.001
Feed conversion rate (%)	3.15 \pm 0.12 ^a	3.04 \pm 0.05 ^a	3.04 \pm 0.06 ^a	2.56 \pm 0.1 ^b	0.003
Bioconversion rate	26.6 \pm 1.1 ^b	27.9 \pm 0.4 ^{ab}	26.1 \pm 0.5 ^b	30.2 \pm 0.9 ^c	0.019

[‡] Means within a row having different letters were statistically different at the indicated $p < 0.05$ (ANOVA, Tukey post hoc tests)

Figure 23 below show the effect of feeding regimes during the growth cycle of the larvae. The figure shows that there was no significant effect of feeding regime on the larvae growth rate between day zero and day four with differences becoming discernible as from day four onwards. Between day four and day 8, there is significant better performance for DF followed by LF whereas the performance of both WF and AFD is low and significantly different from DF and LF. After day 8, the performance of DF starts to decline but is still superior to the rest of the regimes. The effect of LF on the other hand dips below that of both DF and AFD whose performance increases between day 8 and 16. Between day 12 and day 16, the effect of LF regime improves to catch up with DF, AFD and WF regimes (Figure 23).

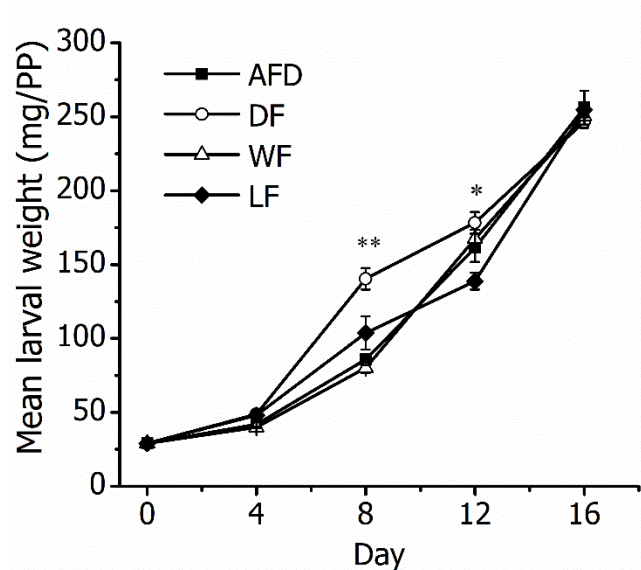


Figure 23: The influence of and feeding regime on the growth of BSFL fed on faecal sludge. Days denoted by *, ** and *** indicate significant difference (ANOVA followed by LSD tests) at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

4.3.3 Effect of feed combination on growth, prepupa yield and maturation period.

As shown in Table 15 and Figure 24 below, growth rate, prepupa yield and maturation period improved in all the treatments where the principal substrate (FS) was combined with a co-substrate irrespective of the combination level. However for most treatments, an increase in the amount of the co-substrate resulted in reduced yield of prepupa biomass, except for the FS-FR mixture. For the FS-FR, FS-BP and FS-BW mixes, the 30% and 50% part portions produced higher biomasses compared to the FS alone, which however were not significantly different from each other (Table 15). In terms of substrate reduction and bioconversion efficiency, the combination of FS-FR produced the highest reduction across all mixing ratios followed by FS-BW and lastly FS-BP mix. For FR and BW mixed with FS, the reduction levels declined with increase in the amount of FS from 30% to 70% unlike in the FS-BP treatment where reduction levels increased with increase in the amount of FS. Bioconversion efficiency was again highest for FS-FR mix and lowest on FS-BP mix across all mixing ratios. The development period for the larvae

fed on combined feedstocks shows that the larvae fed on FS-FR at 30:70% and %0:50 mix ratio took the shortest period to mature (14 days) and increased when the amount of the FS was increased to 70%. The period taken by those fed on FS-BW mix showed no significant variation across the different mix ratios. However the period taken by larvae fed on FS-BP mix increased with increase in the amount of BP in the mix from 17.7 days for 70:30 to 24.3 for the 30:70 mix ratio FS: BP respectively (Table 15).

4.3.3.1 Nutrient content of prepupa fed on combined feedstocks

The study analyzed the content of the proteins and lipids in samples of the harvested prepupal from different treatments. The results are summarized in Table 15. In general, the CP values from mixed substrates combinations were not significantly different (46.1% for prepupa fed on FS-FR mixture, 47.8% for those fed on FS-BW mixture and lastly 43.2% for prepupa fed on FS-BP mixture) and were consistent with those of pure fecal sludge substrate (46.2%). The crude fat content was consistent and not significantly different for prepupa fed on pure FS, FS-FR; FS-BW (18.4%; 18.2% and 19.6% respectively). However the crude fat content was highest (22.7%) and significantly different in prepupa fed on the FS-BP combination at $P < 0.05$ (Table 15).

Table 15: Influence of faecal sludge supplementation with other organic waste feedstocks on BSFL biomass production, substrate reduction, time taken to larval maturity and harvested BSFL nutrient composition. All results are presented as means \pm SD. ‡

Substrate supplementation [†]	Prepu pal yield(g)	Days to maturity (days)	Substrate reduction (%)	BCR	Crude protein (%)	Ether extract (%)
Faecal sludge						
100%	201 \pm 7.4	22.3 \pm 0.3	83.5 \pm 0.3	3.15 \pm 0.12	46.2 \pm 0.7 ^a	18.4 \pm 0.5 ^a
Food remains						
30%	295 \pm 4.4 ^a	14.0 ^a	92.5 \pm 0.2 ^a	4.62 \pm 0.10 ^a		
50%	299 \pm 4.4 ^a	14.0 ^a	88.0 ^b	4.67 \pm 0.07 ^a	46.1 \pm 0.4 ^a	18.2 \pm 0.4 ^a
70%	263 \pm 5.1 ^b	16.0 ^b	88.2 \pm 0.6 ^b	4.12 \pm 0.08 ^b		
Brewer's waste						
30%	270 \pm 4.5 ^a	15.3 \pm 0.3	63.6 \pm 0.03 ^b	4.22 \pm 0.07 ^a		
50%	261 \pm 3.4 ^a	15.0	69.7 \pm 0.04 ^a	4.08 \pm 0.05 ^a	47.8 \pm 0.5 ^a	19.6 \pm 0.5 ^a
70%	196 \pm 7.3 ^b	15.0	63.1 \pm 0.04 ^b	3.07 \pm 0.11 ^b		
Banana peelings						
30%	253 \pm 2.7 ^a	24.3 \pm 0.3 ^a	56.1 \pm 0.6 ^c	3.95 \pm 0.04 ^a		
50%	238 \pm 5.0 ^a	20.0 ^b	63.1 \pm 0.1 ^b	3.72 \pm 0.08 ^a	43.2 \pm 1.1 ^b	22.7 \pm 1.5 ^b
70%	197 \pm 5.1 ^b	17.7 \pm 0.3 ^c	67.1 \pm 0.6 ^a	3.07 \pm 0.05 ^b		

‡ Means within a column having different superscript letter are statistically different at $p < 0.05$

[†] FS, faecal sludge; FR, food remains; BW, brewer's waste; and BP, banana peelings

‡ Nutrient content was based on dry matter.

Figure 24 below shows the effect of different feedstocks combinations at different ratios on the growth rate of BSF larvae. BSF larvae raised on combined feedstocks grew faster than those raised on pure faecal sludge alone irrespective of the combination level or substrates combined. For most of the treatments where FS was combined with the co-substrates, combination level of 30% co-substrate to FS yielded the greatest growth rate followed by 50% and lastly 70% across all treatments and development stages with

growth rate at 70%:30% co feedstock to faecal sludge treatments being almost the same as for 100% faecal sludge. The performance of the 30:70 and 50:50 combinations were not significantly different across the different substrate treatments. However higher levels of the co-substrate above 50% lowered the BSFL growth rates to levels comparable to the effect of 100% FS substrate (Figure 24).

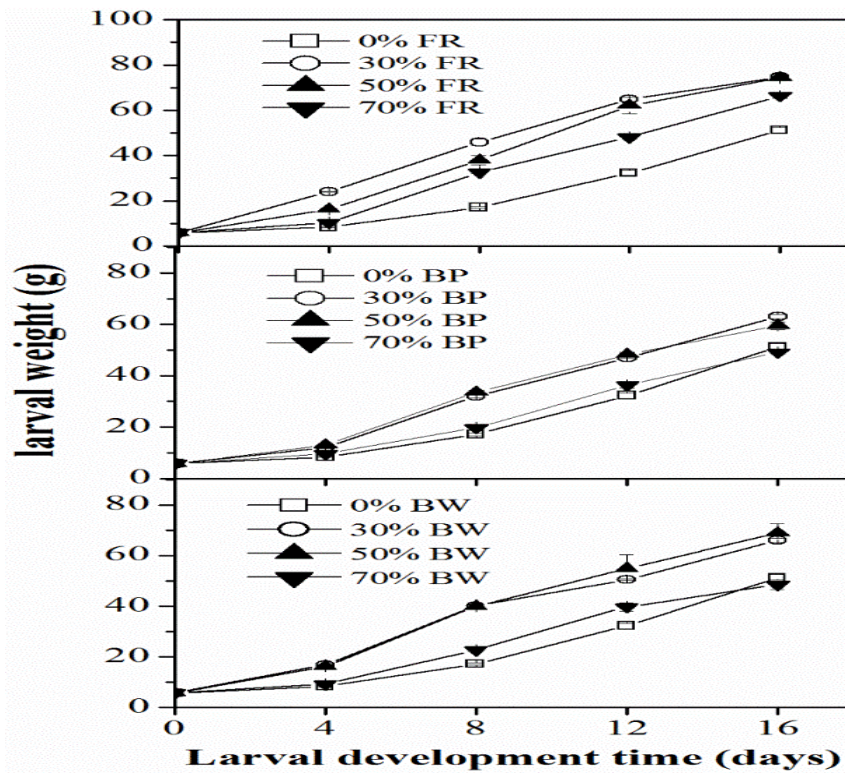


Figure 24: Growth of BSF larva grown on faecal sludge (FS) supplemented with different levels (0, 30, 50 and 70%) of other organic waste feedstock: food remains (FR), banana peelings (BP) and brewers' waste (BW).

4.4 FEED FORMULATION AND FISH FEEDING

4.4.1 Nutrient content of wild sourced prepupa

The proximate analysis results for protein, lipid, minerals and vitamin composition of the harvested wild sourced black soldier fly prepupa are represented in Table 16. The prepupa crude protein and crude fat content, the most important nutrients in animal feeds were 38.98% and 32.2% respectively.

Table 16: Nutrient content of wild BSF larvae

Parameters	Wild BSF larvae
Proximate Analysis	
Crude protein (%)	38.98
Crude fibre (% ^{w/w})	12.36
Ash (% ^{w/w})	14.61
Fat (% ^{w/w})	32.62
Free fatty acids (mgNaOH/g)	42.075
Mineral Analysis	
Manganese (%)	0.56
Copper (%)	0.006
Sodium (%)	3.07
Iron (%)	0.57
Calcium (%)	0.10
Potassium (%)	2.27
Vitamin Analysis	
Vit B1 (Thiamine) (mg/100g)	0.24
Vit B2 (Riboflavin) mg/100g	2.2
Vit E (mg/100g)	1.3

[‡] Nutrient content was based on dry matter.

4.4.2 Fish feed formulation and pelleting

Though the two feeds were formulated to be isonitrogenous in composition and of similar diameter, there were some differences in some observable characteristics as detailed in Table 17 and in Figure 25 below. The t-test of BM feed's floatability and shelf life were significantly different from those of FM feed whereas the stability in water of both feed types was not significantly different.

Table 17: Physical characteristics of the formulated BM and FM feeds

Parameter	Type of feed		F=t ² statistic	Pr(>F)
	BM	FM		
Floatability (s)	50.6	40.5	6.9946	0.00232
Colour	Dark brown	Light brown		
Stability in water (s)	21.3	20.2	-0.249	0.816
Shelf life	23 days	32 days	-4.276	0.01374

**Figure 25: Sample of formulated Fishmeal on the left and BSF feed pellets on the right.**

In terms of nutrient composition of the feeds as determined by proximate analysis, BM feed had a higher CP, crude fat and crude fibre contents but was low in amount of carbohydrates and metabolizable energy (Table 18).

Table 18: Proximate analysis contents of FM and BM formulated feeds

Parameter	Type of feed	
	BM	FM
Crude protein (%m/m)	21.5	18.5
Carbohydrates (%m/m)	31.6	42.6
Crude fat (%m/m)	20.2	13.3
Crude fibre (%m/m)	9.3	3.3
Metabolizable energy (kcal/kg)	3454.1	4260.6
Phosphorus (%m/m)	1.7	1.6
Total ash (%m/m)	10.3	6.8
Minerals contents		
Calcium (ppm)	111686.9	9926
Copper (ppm)	10.37	23.06
Iron (ppm)	1844.1	2407.26
Magnesium (ppm)	2065.1	1228.9
Manganese (ppm)	380.9	166.24
Phosphorus (ppm)	5832	5077.0
Potassium (ppm)	6461.1	5160.1
Sodium (ppm)	464.1	1090
Zinc (mg/kg)	133.21	65

4.4.3 Effect of feed on fish growth

ANOVA done on the on weight gain of fish samples from the two diets showed significant differences with respect to feed type, cage and age of the fish (Table 19), whereas the separation of the means is shown in Table 20 below.

Table 19: ANOVA on effect of feed on growth weight of fish

Sources of error	Df	Sum squares	Mean squares	F-value	Pr(>F)	Significance
Feed	1	128.1	128.12	1845.821	5.21e-15	***
Cage	11	11.1	0.0091	0.1311	0.9658	
Residual	10	0.694	0.0694			
Total	22					

The LSD mean separation by Tukey HSD method showed the significant difference between the two feed types were only in week eight of the study (Table 20) with FM and BM feeds having no significant difference between week one and seven.

Table 20: LSD means separation for growth weight of fish on BM and FM feed types (Mean± Standard deviation).

Age	Feed type	Df	LSD mean±SD	lower.CL	upper.CL
1	BM	11	5.89 ^a ±0.774	4.495240	6.591426
1	FM	11	6.24 ^a ±0.681	5.359285	7.127382
2	BM	11	6.61 ^b ±0.879	5.561907	7.658093
2	FM	11	8.42 ^b ±1.019	7.539285	9.307382
3	BM	11	7.63 ^{ab} ±1.421	6.581907	8.678093
3	FM	11	9.93 ^{ab} ±0.903	9.049285	10.817382
4	BM	11	9.02 ^c ±1.370	7.975240	10.071426
4	FM	11	12.48 ^c ±1.298	11.599285	13.367382
5	BM	11	11.35 ^{bc} ±1.173	10.298574	12.394760
5	FM	11	14.06 ^{bc} ±1.001	13.182618	14.950715
6	BM	11	13.50 ^{cd} ± 0.728	12.451907	14.548093
6	FM	11	16.56 ^{cd} ±1.763	15.675951	17.444049
7	BM	11	15.40 ^{cd} ±0.965	14.350240	16.446426
7	FM	11	18.7 ^{cd} ±1.508	17.815951	19.584049
8	BM	11	18.27 ^d ±1.229	17.221907	19.318093
8	FM	11	20.5 ^c ±0.969	19.632618	21.400715

[‡] Means within a column having different superscript letter are statistically different at $p < 0.05$

A graphical representation of the fish weights against time in weeks is represented in Figure 26 below. The graph shows at the growth trend of fish fed on the FM and BM diets were similar throughout the feeding period but with BM fed fish trailing FM fed fish, although the test fish for both treatments started at the same level (average initial weight) (Figure 26).

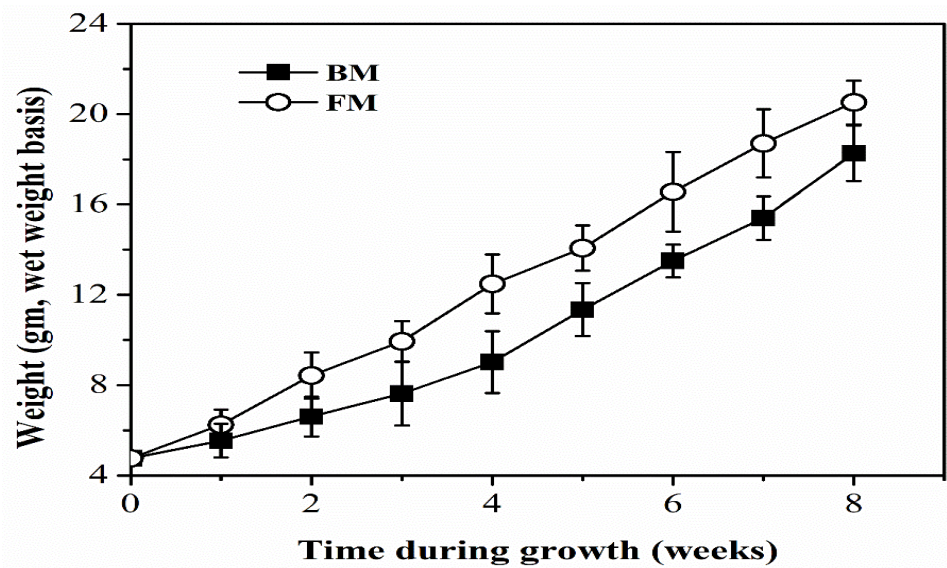


Figure 26: The growth rates of fish fed on BSF feed (BM) and fishmeal (FM) formulated feeds.

4.4.4 Effect of feed on water parameters

The physical-chemical parameters of water between the two feed treatments were not significantly different (Table 21). Though differences were noted between water parameters of the two feed types, these were insignificant at 95% confidence interval. Temperature values recorded for water in which BM feed was administered showed slight variations compared with those fed with FM. Changes in pH recorded in this study ranged from 6.7 to 6.8 and indicated that it was slightly acidic for both feeds. Turbidity (TDS) recorded for the experimental period for FM feed was above 50 NTU and below 50 NTU for BM whereas conductivity was slightly higher for BM feed than that for FM feed (Table 21). The parameter that showed the biggest variation in the two feed treatments occurred in the nitrite (15.7 ± 4.94 SE for FM feed and 7.6 ± 1.1 for BM feed type).

Table 21: Effect of feed type on the water quality parameters (mean±SD)

PARAMETER	FM	BM	P-value
Temp °C	21.4±0.11	21.28±0.41	0.858
PH	6.9±0.094	6.7±0.15	0.208
DO ₂ (mgL ⁻¹)	2.93± 0.26	2.89±0.23	0.901
TDS (NTU)	68.13±12.62	44.85±11.0	0.149
Nitrite-N (mgL ⁻¹)	15.7±4.94	7.6±1.1	0.109
Ammonia-N (mgL ⁻¹)	223.7±13.67	245.8±14.79	0.275
Conductivity	109.39±3.7	113.24±4.07	0.482

CHAPTER FIVE - DISCUSSION.

5.1 PERFORMANCE OF OVIPOSTION ATTRACTANT SUBTRATES

5.1.1 Sightings and ovipositing by wild BSF

At the start, sightings of ovipositing adult Black Soldier Flies in the feeding containers were a rare event. During this time, the commonly observed flies in the attractant medium were the common housefly, blowflies and the green bottle fly. Consequently, only a handful of prepupa was moving into the harvesting buckets across the attractant substrates. This could be attributed to initial low numbers of female adults within the locality, or due to incomplete rotting of attractants to produce a strong putrescent smell to attract the females in large numbers. Furthermore, the initial competition with the other fly species in the vicinity could also account for the low numbers of self-harvesting prepupa.

With time, the amount of prepupa harvested started to increase gradually as the number of BSF adults seen ovipositing also increased. As this happened, the number of non BSF flies sighted on the medium gradually decreased. It is possible that the initially hatched larvae sent out species specific chemicals that attracted more females to the attractant. This in turn led to more egg production and consequently the boost in the prepupa harvest. Generally, BSF larvae send out species specific chemicals called pheromones which are recognized by those of their kind (Newton et al., 1977; Bradley and Sheppard, 1984). Consequently, an existing colony has been touted as the best attractant for oviposting female BSF due to pheromones secreted by larvae (Stankus, 2013). In addition, the rise in numbers of prepupa collected can be attributed to maturation and pupation of early generations of prepupa into adults, which boosted the oviposting BSF numbers, increased larvae amounts and gradually overcame competition from the other fly species.

The observation is supported by other succession ecology studies. Stankus (2013) observed the housefly to be the primary colonizer of a new food source. Forensic entomology research on cadavers also record the housefly to be the primary colonizers

followed by several species of beetles as secondary colonizers, with a colony of BSF becoming visible after several weeks (Erickson *et al.*, 2004). The BSF delay is attributed to the comparatively longer time taken by the species between laying eggs and development into prepupa (43 days for BSF compared to one week for houseflies). However once the BSF become established, a monoculture develops to the near complete exclusion of houseflies and beetles (Stankus, 2013, Erickson *et al.*, 2004). It is thought that BSF larvae secrete antimicrobial compounds to discourage other fly species from laying eggs on the same medium (Newton *et al.*, 1977; Bradley and Sheppard, 1984; Erickson *et al.*, 2004; Choi *et al.*, 2012; Zheng *et al.*, 2013; Park *et al.*, 2014).

5.1.2 The efficiency of substrates in attraction of ovipositing black soldier fly.

In this study, higher production was observed in the substrate consisting of mashed maize grains (FS1) and vegetable wastes (FS3) compared with other substrates (Figure 16). The variation in the amount of larvae harvested could be due to the differences in the intensity and quality of odours produced by the decomposing food, as well as the nutritional quality of the substrate materials. Previous studies have also reported that the growth of BSF larvae is better when plant materials are used as substrates compared to animal substrates (St-Hilaire *et al.*, 2007; Tomberlin *et al.*, 2009). This is attributed to the fact that BSF harbor microbes in their gut that produce enzymes that aid the digestion of plant substrates and not those of animal based substrates (Liu *et al.*, 2008).

The attraction of wild Black Soldier Flies by Bonso (2013) using rat meat, fresh fish, fresh human faeces and wet chicken feed found that rat meat carcass was the best attractant among these though Tomberlin and Sheppard (2002) and (Diener *et al.*, (2009) had successfully used chicken feed (Bonso, 2013). However the stench was a public nuisance that necessitated the location of the production facility away from human habitats and wearing of nose masks (Bonso, 2013). In a separate study, Stankus (2013) found that fermented grain did well as an attractant compared to meat and fish which also attracted unwanted vermin such as rats and mongooses. Other studies report that fermented oats, corn, and brewer's hops all attract BSF females (Stankus, 2013). In the current study the superior performance of the mashed maize grains can also be attributed

to fermentation process that releases the necessary volatile compounds and further confirms the superiority of plant based attractants.

In the attraction of ovipositing females in a captive colony, comparatively more eggs were laid using cow manure and frass tea substrates than on fruits and fish and commercial sweet scent (Table 6 and Figure 17). This shows the varied effectiveness of smells produced by different substrates as earlier reported by Sripontan and Chiu (2017, Oliveira et al., (2015) and Oliveira et al., (2016). It is thought that the attraction performance of a substrate is related to the kind of volatile compounds it emits in its putrescence. Though not completely characterized, compounds such as ethanol, acetic acid, ethyl acetate and acetaldehyde have been found in successful attractants of BSF and those of adults of the vinegar fly (Zhou *et al.*, 2013; West and Hardy, 1961). These compounds are probably absent in sweet smelling scents such as sugary smells that attract bees (*Apis mellifera*) (Sripontan *et al.*, 2017), which can explain the poor performance of the sweet smelling scent and fresh fruits used in the study.

The superior performance of cow manure and frass can be attributed to fermentation process. The gut of the cow contains microbes that carry out fermentation process to yield volatile compounds responsible for fresh manure smell while the overnight soaking of frass in water could have fermented remnant sugars in larvae feeds into putrescent volatile compounds. The necessity for fermentation was also reported in the study of Sripontan *et al.* (2017). In this study, household food wastes, chicken manure, pig manure and dairy manure proved relatively slow in attracting ovipositing adults Black Soldier Flies compared to fermented fruits (Sripontan *et al.*, (2017). However the actual fruit composition is not specified in the study. In addition to fermentation, the efficiency of frass can also be attributed to the pheromones left by feeding larvae, which are easily recognized by conspecific females, which led to the report by (Stankus (2013) that the best BSF attractant is an already existing colony.

The studies were carried out in geographically different locations (USA-Tomberlin et al., (2002); Indonesia-Diener et al., (2009); Ghana- Bonso et al (2013) and Thailand -

Sripontan and Chiu (2017) and therefore possibly involved different BSF strains of BSF. This raises the possibility that different strains of the Black Soldier Fly may be responsive to different substrates akin to findings of other insect studies. (Oliveira et al., 2015) reported that the larval stage diet affected the feeding preference of adults of the vinegar fly, *Drosophila melanogaster*.

An alternative explanation by Sripontan *et al.* (2017) suggests that BSF adults are more likely to be attracted to ovipost by an attractant which is abundant in the environment and therefore most probably utilized by the feeding larval stage. However this assertion is not supported by this study's finding for both wild and captive strains of BSF. For wild BSF at JOOUST in Bondo, the most common substrate in the environment was cow and chicken manure from the livestock farm barely 20 metres away from the site of study while for confined BSF at EnviroFlight, the most abundant substrate was frass used in the feeding of BSF larvae. The study therefore is in supports the position that different strains of BSF have different oviposition attractants and the role of fermentation in producing necessary volatiles for attraction of oviposting black soldier fly adults. In conclusion, the substrates identified in this study for attraction of oviposting adult females (fermented maize grains and fruit and vegetable waste for wild BSF; and cow manure and frass tea for confined BSF) are all abundant in nature, freely available or cheap and therefore their use is sustainable.

5.2 ASSESSMENT OF LARVAE FEEDING SUBSTRATES

5.2.1 Organic waste substrate performance on larvae growth

The high prepupal yield growth rate and average biomass observed for food remains (FR) and brewers' waste (BW) (Table 8) in this study can be attributed to superior nutritional content of the diets (Nguyen, 2010). Comparatively, kitchen food left overs - consisting of a mixture of ugali (corn paste), rice, vegetables and meat/fish bones – was highly heterogeneous and nutritionally superior to the other substrates used. The brewers' waste (BW) is also a mixture of spent grains and yeast cells used for grain fermentation, and proximate composition shows that it contains 26.8% crude protein, 12.2% crude fibre and

high amounts of metabolisable energy (Munguti *et al.*, 2006), which explains its superior performance in the measured parameters (Oonincx *et al.*, 2015).

Documented biochemical analysis of faecal sludge (FS) shows that it constitutes of 2-25% nitrogen content depending on factors such as diet, a high microbial load of 25–54% mostly bacteria, 25% carbohydrate and 2–15% fat (Banks, 2014). At Sanergy Ltd, the substrate is collected from the Mukuru slum in Nairobi, whose inhabitants live on an income below 1USD /day and therefore the staple diet of the majority most probably consists of the cheapest foods in the market- cereals and green vegetables- which point to lower end of the protein content of the faecal sludge substrate from the inhabitants. Furthermore, the substrate is mixed with saw dust at the point of collection to moderate moisture content and accumulated in heaps before use in larval feeding. As a consequence, the relatively low protein content coupled with anoxic conditions due to stockpiling of the substrate before use, and addition of sawdust, could have resulted in the inferior performance of BSFL grown on FS compared to BW and FR substrates. It has been reported that the performance of BSFL is higher in fresh human faeces compared to pit latrine faeces (Banks, 2014; Banks *et al.*, 2014). The stock piling encourages development of anoxic conditions which in turn discourage feeding of BSFL (Banks *et al.*, 2014) and would have therefore accounted for the slower growth rate observed on FS in the early stages in this study (Figure 19). Again, the sawdust mixed with FS has high contents of cellulose and lignin; both of which delay BSFL feeding until they start to decay or undergo microbial fermentation (Manurung *et al.*, 2016). The latter appreciable performance of FS substrate after the 4th day (Figure 19) can therefore be attributed to the late decomposition of sawdust and perhaps consumption of microbes in the waste by later stage larvae.

Comparatively, banana peels (BP) showed poor performance as feedstock for BSFL (Figure 19). Documented proximate analysis of banana peels shows that they contain crude protein of 7.2-8.6%, crude lipids 7.9%-8.5%, crude fiber 11.3%, nitrogen free extracts 53.8%-62.7% and ash 7.8%-10.9% irrespective of variety or locality of collection (Munguti *et al.*, 2006). The poor performance of the substrate can therefore be

attributed to its low crude protein to carbon content ratio and high crude fiber content (Tschirner and Simon, 2015). The low protein content may have adversely affected both the larval growth rate and the total prepupal yield while high dietary fibre is known to cause poor growth as it causes delayed feeding, reduced feed intake, digestibility and nutrient utilization (Tomberlin *et al.*, 2009).

5.2.1.1 Organic waste substrate preference at different stages of growth

According to Diener *et al.* (2009), the preference for a substrate at a particular stage of growth may be related to the structure of the feedstock. This could have accounted for the high larval growth rate response for brewers' waste and food remains, between days four to day eight (Figure 19). The fine structure coupled with nutrient composition perhaps is the basis why special starter diets are recommended in insect production systems (Oonincx *et al.*, 2015). This explains why the fibrous banana peels only stimulates significant growth when the larvae are almost mature, because at this stage, the larval mouthparts have hardened enough to feed on the large particles of the substrate. Consequently, identification of the most suitable substrate at each stage of growth is necessary to reduce development time and maximize feedstock utilization. Based on the reported results and in the absence of a specialized starter diet, BSF feeding on a menu consisting of brewers' waste for the 1st four days since leaving the hatchery is recommended. The feeding regime can be followed by use of food remains, faecal sludge and finally banana peels in the last few days before larvae molt into prepupa though more research is required on the suitability of different substrates and substrate cocktails that are optimal at different growth stages to reduce the development period of BSFL.

5.2.2 Performance of different vegetable and fruit substrates.

As explained earlier, legal restrictions prevented the use of waste forms of fruits and vegetables for BSFL production at EnviroFlight LLC, USA. In the study, the control diet (DDGs and Cookie meal) outperformed all the fruit and vegetable substrates in terms of average weight gain of the prepupa and maturation period. This can be attributed to superior nutritional quality of the substrate especially in terms of the protein, carbohydrate, metabolisable energy and fat contents when compared to the content of

fruit and vegetable substrates (Table 23 below), though the performance in terms of total yield of prepupa was comparable to that of watermelon and banana substrates which also have relatively high crude protein and carbohydrate (NFE) content respectively (Table 23). Again, the control diet was a combination of two substrates (Dried Distillers grain with Solubles (DDGS) and Cookie meal) which further renders support to substrate mixing to improve nutritional quality and performance (Everest Canary and Gonzalez, 2012; De Haas, 2006). DDGS is a byproduct of ethanol production that is made up of corn and grain from which much of the starch has been extracted, but which still retains high levels of protein, fibre and oils hence quite nutritious. Proximate analysis content reveals its richness in proteins, fats and energy content compared to levels in vegetable and fruit substrates which is not only low but also unbalanced, for example avocado - energy rich in terms of fats but low in protein amounts (Arukwe et al., 2012). High fat content has been reported to slow down BSFL growth (Barry, 2004). This additionally explains the average performance of avocado.

In conventional animal production, the energy content of a feed directly determines growth rates efficiencies whereas in insects, the amount and quality of proteins has been found to directly influence larval development in insects (Gobbi et al., 2013), Oonincx *et al.*, 2015, House, 1961). This is because conventional animals require both energy for maintenance of body temperature and proteins for growth and in the presence of sufficient proteins in the diet, they minimize losses that would occur during conversion of proteins into energy for maintenance of body temperature and in so doing, preserves the proteins solely for growth. This is unlike in insects which do not need energy to maintain a constant body temperature and therefore do not need to convert proteins into energy (Herrera et al., 2013; Lundy and Parrella, 2015). Therefore in insects, dietary protein is used mainly for growth and body maintenance and its absence or limited availability adversely affects growth rate and yields (Kassahun et al., 2012; Nalwanga et al., 2009). When limited in a diet, other nutrients such as fats or carbohydrates have to be converted into proteins which not only causes losses but also delays growth. Optimal performance in terms of growth rate and maturation period is ideal when there is balanced composition of nutrients as it prevents losses incurred during interconversion (Nguyen, 2010).

Though all the plant substrates generally performed well, the performance of watermelon and bananas proved slightly better compared to pineapples and avocados among the fruit category (Table 11). For watermelon, this can be attributed to the large portion of the edible mesocarp portion while for the bananas; the edible sugary part could have been the reason. The sugary portion of bananas majorly contains reducing sugars that are not only sweet but also readily absorbed and assimilated. Though pineapples also contain the juicy sugary portion, the presence of fibres could have hindered consumption especially by young larvae. For avocados, though they contain the soft fat mesocarp portion, losses due to conversion of the fats into energy and proteins for growth during its metabolic events to compensate for their low content in the substrate could have accounted for its comparatively poor performance. High fat content has previously also been associated with poor development in BSFL by Barry (2004) and Nguyen *et al.* (2015).

Across all the fruits, the outer layer (epicarp) was not consumed by the larvae probably because it is tough and mainly composed of approximately 11% fibrous lignin ((Femenia *et al.*, 1998). Low consumption of kales was also noted in this study as evidenced by the frass content (Table 11), and can be attributed to the high cellulose and lignin content of the substrate. Previous studies have also recorded poor BSFL consumption of plant materials such as stems, grasses and mature leaves due to inability of BSFL larvae to digest high lignin and cellulose content (ur Rehman *et al.*, 2017). Herranz *et al.* (1983) also reported the preference of BSFL for pig manure and poultry (which contains less than 2% lignin) over cow manure, which contains as much as 10% lignin. Slow growth has also been observed among insects that feed on tree leaves compared to those that feed on forbs and seeds. Whereas tree leaves contain higher levels of poorly digestible compounds such as cellulose, lignin and tannins, forbs and seeds typically have high levels of readily digestible carbohydrates, protein, nitrogen and water forbs and low in fibrous material (Scriber and Slansky Jr, 1981). This indicates that lignin and cellulose content in a substrate is an important factor to consider in selecting suitable BSFL growing substrates though the poor performance of kales can also be attributed to its low calorie content (Nguyen *et al.*, 2015).

5.2.3. Nutrient content of prepupa from different substrates and stages of growth

The crude protein and lipid content of the prepupae from different substrates generally reflected the nutrient content of the feedstock used (Table 8 and 10). For example among the fruit and vegetable substrates, kales had the highest crude protein content whereas avocado had the highest fat content and watermelon pupa the least (Table 23) and both are respectively reflected in the prepupa product (Table 10). In addition, the observed protein content of the biomass produced from all the tested substrates ranging from 34.9-45.4% for organic wastes (Table 8) and 35.4-47.8% for fruits and vegetables (Table 10) is sufficient to recommend the use of the larval product as protein source in the feed industry according to Liu *et al.* (2008). The obtained values for crude fat in the range of 20.2-44.6% (Tables 8 and 10) are consistent with those reported elsewhere, such as 15-25% for larva fed on poultry manure (Arango Gutiérrez *et al.*, 2004), 25% for swine manure (Newton *et al.*, 2005), 35% for cattle manure (Newton *et al.*, 1977) and 42-49% for oil rich food waste (Barry, 2004). Again, the crude fat content seems to be dependent on the diet used for larvae production while the CP content is fairly constant and may be related to genetics (Table 8 and 10). St Hilaire *et al.* (2007) also reported that the fat quality dependent on the diet type, where elevated levels of omega-3 fatty acids, which are essential for fish and human health, was observed in larva fed on fish scraps.

The link between composition and diet suggests the possibility of growing BSF larvae on specialized diets in order to customize resultant biomass nutrient content to meet needs of targeted animals (St Hilaire *et al.* (2007). All the substrates in the study yielded prepupa products that had high fat and crude protein contents (Tables 10). This shows that BSFL larvae are efficient at bioconversion and valorization process as they are able to convert low nutrient feedstocks into high value biomass with sufficient nutrient quantities.

The decreasing trend in the CP content as larvae age increases (Figure 18), can be explained by the effect of sclerotization, a process which causes enzymatic degradation of proteins to build up the chitin layer of the exoskeleton (Aniebo and Owen, 2010). Large variation in crude protein composition throughout the course of larval development with the same decreasing trend as larvae age increases has been reported elsewhere. According to Rachmawati *et al.*, (2010), CP level was highest in five day old larvae

(61%) and decreased to 44% in 15 day old larvae and 42% in 20 day old larvae. Recently, Liu et al (2017) reported highest CP content in one day old larvae of 56%, which then declined to 38.2% for 12 day old larvae. The same trend has been reported in other fly species. Aniebo and Owen (2010) noted that the nutritional value of house fly larvae varied greatly with the age of harvest due to significant reduction in the protein content. A CP content adjusted for chitin content in BSF prepupa also came to the same conclusion (Diener *et al.*, 2009). Correction for chitin in the CP amount of prepupa was however not done in the current study.

In this study, a batch system of production was adopted whereby harvesting of all larvae was done on the 16th day when prepupae were observed. Perhaps harvesting on the 12th day would have resulted in a better tradeoff between average weight and optimal biomass yield, which has minimal prepupa and hence maximal digestibility (Bosch et al., 2014). The batch system is desirable where the goal is to produce larvae of optimum nutritional quality, whose exoskeleton are not hardened enough like those of pre-pupae and pupae; which is anti-nutritional due to its high chitin content (Diener *et al.*, 2009). The down side of the system however is that harvesting BSF larvae prior to pupation adds complexity to the process as the larvae do not get to self-harvest and therefore some other means of harvesting them have to be explored. In the current study, harvesting was effected through manual sorting and sieving through a wire mesh screen, and more efficient methods of harvesting such mature larvae prior to pupation need to be explored especially for mass production facilities.

5.2.4 Waste reduction and bioconversion efficiency of organic waste substrates.

High residue contents were realized in this study for fecal sludge (FS) and brewers' waste (BW) substrate (Figure 20). This can be due to the high content of sawdust added to FS to reduce the water content and excess amount of feed for BW. Material reduction obtained in the present study compared well with those reported for swine manure, municipal organic waste, chicken manure, and fresh human wastes. Similar to the current results, Kalová and Borkovcová (2013) also found that best substrate reduction occurred on plant materials in comparison to other substrate sources. The higher degradations

observed in plant materials can be attributed to the ability of BSF larvae to secrete enzymes from gut microbes that can degrade plant materials.

Bioconversion rates generally indicate the efficiency of consumption of a substrate by the larvae. In contrast, a feed conversion ratio (FCR) indicates the proportion of digested food that is assimilated and therefore ends up as biomass, and the lower its value, the higher the efficiency of conversion of substrate to biomass. Indeed, a higher FCR indicates that the substrate is digestible but of little nutrient value and therefore largely excreted. As shown in Table 5, bioconversion rates ranged from 11.7 to 20.8 % for organic waste substrates and 16.7 to 37.8 for fruit and vegetable substrates (Tables 9 and 11 respectively). These values are higher than those described in literature for the substrates used and therefore show effectiveness of BSFL to both consume and reduce the substrate biomass (Banks, 2014; Banks *et al.*, 2014; Diener *et al.*, 2011a; Newton *et al.*, 2005; Sheppard *et al.*, 1994). The ability of the larvae to consume FR, BW and FS can be attributed to diet structure, nutrient composition and moisture content. Interestingly, FR and BW had low FCRs (2.6 and 2.7 respectively) and correspondingly higher bioconversion rates of 20.8 and 16.6 respectively. This implies that the two substrates are not only effectively consumed but also highly assimilated into larval biomass and that use of BSF technology can effectively serve the dual roles of waste management and biomass production when the two are used as substrates.

Moderate values of bioconversion rate and FCR (14.9 and 3.4 respectively) were obtained for faecal sludge in this study (Table 9). This indicates that BSFL were fairly effective in reducing the substrate quantity and transformation of nutrients consumed into biomass. The low digestibility of the substrate due to addition of sawdust, coupled with poor nutritive value in terms of low protein content, could have accounted for this observation. The low nutrient content of the FS substrate due to the aforementioned factors: stock piling, addition of sawdust and source vegetarian population) triggered compensatory feeding of the larvae whereby the larvae are forced to consume more of the substrate in order to extract and accumulate enough essential nutrients for growth and maintenance (Manurung *et al.*, 2016).

For banana peels, the low bioconversion rate of 11.7% and high FCR of 4.5 shows that the substrate is neither preferred as a feed nor is the little consumed being converted into biomass. This explains the low growth rate on this substrate (Figure 19). This is supported by growth rate plasticity and compensatory feeding studies that explain that BSFL can potentially consume organic material of varied nutritional composition; they take a longer period to develop to maturity when fed on nutrient-poor substrates (Banks *et al.*, 2014; Manurung *et al.*, 2016). In the long run, they end up consuming more of the substrate due to lengthened feeding period in order to extract sufficient nutrients for biomass increase. This consequently leads to a high FCR as most of the consumed feedstock contains little nutrients of value. This has also been observed in other compensatory feeding studies (Banks *et al.*, 2014; Manurung *et al.*, 2016).

Material reduction obtained in the present study (Figure 20, Table 11) compares well with reported values such as 39-56% for cow manure, (NC State University, 2006, 58 % for dairy manure (Myers *et al.*, 2008), and 50% for chicken manure (Sheppard *et al.*, 1994) and 37.3-43.2% for chicken feed (Diener *et al.*, 2009). For other feedstuff, higher degradation values have been achieved. For example, Diener *et al.* (2011) reported a reduction of 70 % for mixed organic waste, (Gobbi *et al.*, 2013) also obtained a 70% degradation on hen feed while Lalander *et al.* (2013) reported a reduction of 75 % reduction on fecal sludge. Again as in the present study, Kalová and Borkovcová (2013) found that best reduction occurs on plant materials with an average reduction of 66.53%. The higher degradations observed in plant materials can be attributed to the ability of BSF larvae to secrete enzymes and harbor microbes that degrade plant materials. The lower degradation values for faecal sludge in this study can be attributed to the presence of saw dust conventionally added to reduce the moisture content of fresh faeces. Saw dust contains mature cellulose which made the substrate less degradable to the larvae as it is not consumed by the larvae.

In conclusion, based on FCR and bioconversion values and nutrient content food remains and brewers waste rate as the best substrates among the organic waste category (Table 9)

whereas watermelon and bananas rate as the most suitable substrates in the fruit and vegetable category (Table 11).

5.2.5 Sustainability of BSFL production on tested substrates

All the substrates used in the current study are easily available. For instance, large amounts of food wastes are generated by various producers along the food production chain such as farms, groceries, warehouses, learning institutions, municipal markets and eateries (FAO, 2013). The producers of the waste have few options and end up disposing them in dumping places and landfills (Everest Canary and Gonzalez, 2012). The use of food waste in BSFL farming is economically feasible and sustainable, due to its widespread availability and low cost implication which only relates to the collection cost. As for brewers' waste, the substrate is both easily and cheaply available in many parts of the country where local brews are distilled, and beer is manufactured. At the current price of KShs 450 per 90kg bail, the substrate is cheap and therefore its use as a substrate is also economically feasible. However BW as a BSF substrate faces competition from the livestock feed industry in areas where zero grazing is practiced. The use of fruits (bananas, watermelons, pineapples and avocados) and vegetables as BSFL production substrates is feasible especially during seasons of overproduction. This is due to their short shelf life (Ahvenainen, 1996). However alternation of use of the fruits and vegetables can greatly contribute to ensuring sustainability of BSFL production.

5.3 OPTIMIZATION OF BLACK SOLDIER FLY LARVAE PRODUCTION

5.3.1 Effect of feeding rate on larvae productivity

Feed rate has no effect on larvae growth between day 0 and day 4 showing that the amount of food provided across all the feeding rates was sufficient. However as larvae grew, feed rate effect on growth became apparent with higher feed rates resulting in faster growth and vice versa (Figure 21). This shows that the food requirements of the larvae vary with time over their growth cycle with older larvae consuming more food than younger larvae and therefore different feed rates should be applied for different larval ages during feeding. However the increment from one feeding amount to another should be done gradually to avoid starvation of older larvae, and overfeeding and wastage

of food by young larvae. This can be avoided by observation of critical signs such as crawling of immature larvae off the feed when underfed and presence of unconsumed food in the feeding container during the next round of feeding (Diener *et al.*, 2009). Overfeeding not only in wastage of feedstock but also contributes to the development of anorexic conditions within the unconsumed layers of the substrate, production of awful smells and attraction of vector and pest flies (Mutafela, 2015).

In this study, low feed rates (100 and 150mg/l/d) resulted in comparatively low average larvae weights and yields compared to higher feed rates (200 and 250mg/l/d) (Tables 12; 13). This can be attributed to intraspecific competition that caused starvation and restricted development and possible mortality at the lower feed rates while the availability of more nutrients at the higher rates led to faster growth and biomass gain (Mitchell-Foster *et al.*, 2012, Ojeda-Avila *et al.*, 2003; Brits (2017). Many studies have shown that insects are significantly affected by intraspecific competition in many forms such as feed restriction and overcrowding (Diener *et al.*, 2009; (Dmitriew and Rowe, 2011; Mitchell-Foster *et al.*, 2012).

The higher individual larvae weights and total collective weights of larvae fed on higher rations of feedstocks (200mg/l/d and 250mg/l/d) (Tables 12,13) compares well with the study of (Tomberlin and Sheppard, 2002) but deviates from that of Diener *et al.* (2009). For (Tomberlin and Sheppard (2002), a feed rate of 200mg/l/d on chicken feed resulted in the largest individual larvae weights while for Diener *et al.* (2009), the optimal feed rate for biomass gain on chicken manure was 100mg/l/d. Other subsequent studies have also shown that optimal feed rate may be substrate dependent: chicken manure (100mg/l/d), (Diener *et al.*, 2009); swine manure (107mg/l/d) (Newton *et al.*, 2005), and faecal matter 111mg/l/d (Banks *et al.*, 2014). According to Liu *et al.* (2008), the appropriate amount of a resource to provide the larvae will vary depending on the quality of the resource, which in turn is determined by parameters such as the amount and quality of nutrients, particle size, moisture content and structure of the feedstock (Diener *et al.*, 2009, Lin, 2016).

Interestingly, biomass gained was non-significant for the 200 and 250 mg/l/d showing that feed rates above 200 mg/l/d may be wasteful. This is supported by bioconversion values obtained in the study and the study of Brits (2017) that reports that Black Soldier

Fly larvae can only bioconvert significantly to a certain capacity at given rearing conditions before maturation and further resource investment in the insect's growth gradually lead to diminished returns (Brits, 2017).

In this study relatively low reduction values were obtained at the higher feed rates than at lower rates (Table 12) Substrate matter reduction efficiency is meant to give an indication of whether the substrate is sufficiently fed on and therefore reduced by the larvae to achieve suitable biomass within an acceptable duration. A low substrate reduction value indicates wastage of the substrate either because it is not consumed or because it is in excess. A very high reduction value on the other hand indicates possible starvation of the larvae at the feeding ration being used (Mutafela, 2015). When obtained values are considered alongside the prepupal weights and BCR rates obtained at feed rates of 200mg/l/d and 250mg/l/d show that these feed rates represent excessful feeding and therefore wastage across all the feed substrate (Mutafela, 2015), which however can be saved for use to raise more prepupa without compromising quality of product. This is supported by the fact that unconsumed layers of feedstock were obtained at feed rate of 250mg/l/d though again higher BCR values were obtained in this study compared to literature (Diener, 2010; Zheng *et al.*, 2012; Bonso, 2013; Banks *et al.*, 2014), which can be attributed to inefficient harvesting methods that caused loss of some larvae.

The shorter maturation period (15 and 16 days respectively) and higher biomass content for larvae fed on higher feed rates (200 mg/l/d and 250 mg/l/d) compared to those provided with lower feed rates (100 and 150mg/l/d) (Table 12). This can be attributed growth plasticity- ability of the larvae to regulate growth according to feed availability (Tomberlin *et al.* 2002, Tomberlin *et al.*, 2009; Myers *et al.*, 2008; Yu *et al.*, 2011). BSF larvae have been reported to undergo intermittent growth depending on the amount of food available. When plenty, they undergo continuous growth and lessen development period and in periods of starvation, they go into dormancy only to resume growth when food is available. This explains the variable length of BSF lifecycle from 43 days to six months. In the current study larvae fed at the higher feed rates should be harvested between day 13th and 14th to maximize the quality of a larval-derived product as larvae are

at their largest size and stage, most nutritious because the chitin is not yet fully formed and the amount of prepupa in the total harvested biomass is limited (Park *et al.*, 2014, (Gobbi *et al.*, 2013).

5.3.2 Effect of feeding regime

The analysis of results across the feeding regimes reveals significant differences between the regimes in terms of maturation period, substrate reduction and bioconversion efficiency (Table 14). The longer maturation periods were recorded on WF and LF regimes (17 and 20 days respectively) compared to 16 days on both DF and AFD regimes. The prolonged development period on WF and LF can be attributed to gradual deterioration of diet quality due to competition between resident microbes and the larvae. These competition causes nutritional imbalance in these diets whereas food in the DF and AFD regimes is relatively fresh food, devoid of this competition and therefore has nutritional balance (Zheng *et al.*, 2013, Sherman *et al.*, 2000). Lumping also encourages the development of anorexic conditions (Eby and Morgan, 1977). However because BSF larvae consume more during the late instars of growth, larvae are forced to consume more in order to extract enough essential nutrients for growth. The increase of consumption rates in response to low concentrations of critical nutrients such as proteins is known as compensatory feeding (Lindroth *et al.*, 1993). This may explain the high bioconversion rates observed on LF and WF of 26.1% and 30.2% respectively compared to 26.6% and 27.9% on DF and AFD respectively (Table 14); though the corresponding FCR values are not significantly different especially for DF, AFD and WF. This confirms that the large part of the food consumed due to compensatory feeding in was nutritionally poor and therefore did not contribute to biomass of the larvae.

The longer development period on regimes with longer feeding intervals observed in the current study (Table 14) concurs with the findings of Banks *et al.* (2014) among larvae fed on faecal sludge. In Bank's study, shorter intervals between feedings led to faster maturation and therefore shorter development periods (Banks *et al.*, 2014). However Mutafela (2015) reported that larvae fed on periodical regimes took an average of 2-3 days longer to mature compared to those on lumpsum mode. Whereas Mutafela (2015)

attributed the longer development periods to lowered metabolism of BSFL as an adjustment to less food during the periodical feeding regimes, the present study attributes the short maturation period to the better quality of the fresher feeds compared to lumped feeds as fresh food can be said to be nutritionally balanced and therefore results in faster development within a shorter time (Zheng *et al.*, 2013). This position is supported by findings on other insects that report that the number of days necessary to complete development in insects generally varies with available resources, with those developing on low quality resources requiring more days and vice versa (De Haas *et al.*, 2006).

In the first four days of feeding, feeding regime has no appreciable effect on larval growth (Figure 23). This is probably because the quality of the food in terms of nutrient composition is almost the same within all the four feeding regimes and sufficient in amount for the larvae within this period. The difference between feeding regimes as from day 4 onwards can be attributed to gradual change in quality of nutrients and quantity of food in the feed over time (given that larvae consume more as they age). With time, microbial competition sets in for the AFD, LF and WF regimes which decrease the quality of the food in terms of nutrient composition. This explains the lower performance of AFD, WF and LF in terms of growth rate compared to DF between day 0-6 (Figure 23). The slight better performance of LF over AFD and LF between days 4-10 can be attributed to the fact that large quantity of the feedstock supplied at the start of the feeding, which made the feed in this regime to take longer to reduce and deteriorate in quality compared to AFD and WF.

In terms of total prepupal harvests continuous feeding regime (DF) produces the least larval biomass (194 ± 3.1 g) compared to the batch feeding regimes (AFD: 201 ± 7.4 g), WF: 202 ± 6.1 g, LF: 204 ± 2.1 g). In the study of (Mutafela, 2015), continuous feeding mode also recorded lower prepupal weights compared to the batch mode. This is probably because larval feeding response is directly proportional to the amount of supplied food. Larvae are stimulated to eat more on regimes that provided large amounts of food all at once, whereas in the regimes where food was provided at regular intervals, the interval periods may not have been close enough to guarantee a continuous steady

supply of food (Mutafela, 2015). Again, a possible explanation can be the effect of compensatory feeding where deterioration of quality due to lumping is compensated for by increase in quantity of the feed consumed. Additionally, the additional feeding days on WF and LF (Table 14) led to increase in DM content hence the higher yields observed. In conclusion, BSFL fed on fresh food (DF and AFD) developed into smaller prepupae faster whereas those fed on weekly and lump-sum regimes grew slowly to become larger prepupae. Though development periods are different among the various feeding regimes, the study however shows that BSFL are capable of growing and consuming organic material of varied nutritional quality and still develop into nutritively valuable prepupa. These data provide insights into the generalist nature of BSF larvae and their ability to utilize a variety of resources for development.

The substrate reduction values obtained in the current study indicate that all the regimes were effective at inflicting waste reduction though reduction values decreased with increase in feeding intervals (Table 14). Regimes with shorter feeding intervals (DF and AFD) produced higher waste reductions and vice versa (Table 14). The higher values of waste reduction on DF and AFD may be related to both the drying effect of the larvae as a result of aerating movements since the quantity was small compared to those in WF and LF; and the feeding activity of the larvae. Longer intervals between feeding restrict feeding and therefore aeration effect of BSFL which in turn slows down drying through loss of moisture. This is corroborated with BCR findings in Table 14. The feed conversion rate (FCR) values however reveal inverse proportionality to feeding intervals and increased from LF>WF>AFD>DF (Table 14). This shows that the longer the interval between feedings, the better the conversion of the feed into biomass. This implies that the higher reduction values obtained on DF and AFD were either not due to feeding or if due to feeding, they were poorly digested and assimilated into biomass (Banks *et al.*, 2014), Brits, 2017). Indeed in lump-sum regime, the BCR is lowest which translates to higher residual biomass probably caused by water content but highest FCR showing very high efficiency at digestion and bioconversion of food into biomass. Again continuous feeding in periodical regimes accelerates the passage of food through the gut, reduces

digestibility of the ingested food, with much of the food ending up as waste (Lindroth, 1993), which can explain the high FCR for DF, AFD.

Considering the duration to maturation, growth rate and final biomass produced under the four feeding regimes across all substrates, regimes with short intervals between feedings (DF and AFD) seem well suited for biomass production within a short period and organic waste reduction as supported by the prepupa yields, high substrate reduction levels and short maturation periods (Table 14).

5.3.3 Effect of feedstock combination

The study compared the effect of homogeneous faecal sludge substrate and heterogeneous substrates composed of faecal sludge and co-substrates on larvae growth, prepupa yield, maturation period and composition. The aim was to test whether the performance of faecal sludge as a feed can improve when mixed with other co-feedstocks at different combination levels in terms of consumption and biomass production. In all the treatments where faecal sludge was combined with a co-substrate, the performance was better than pure faecal sludge in terms of larval weight, prepupal yield and shortened maturation period (Table 15). This therefore supports substrate mixing to improve nutritional quality of a feedstock as advocated by Everest Canary and Gonzalez (2012) and Bullock et al. (2013). Fritzi (2015) also found that larvae developed faster and became fatter on a mixture of market waste and faecal sludge compared to faecal sludge alone. Combining feedstocks has the effect of pooling diets and results in a diet with both balanced and increased nutrient composition which in turn improves both growth rate and weight gain and shortens growth period (Everest Canary and Gonzalez, 2012, Tschirner and Simon, 2015).

The best performing combination on both average larvae weights and total prepupal weight at harvest was that of FS: FR followed by FS: BW and lastly FS: BP across all feeding ratios (Table 15). The superior performance of FR-FS and BW-FS mix in terms of total prepupa harvests and maturation period over pure FS can be attributed to their high protein content contributed by the co-substrate (FR and BW). According to Oonincx *et al.* (2015), a food waste diet with 22% crude protein and 9.5% fat content resulted in

the fastest development and greatest survival rate of black soldier fly larvae. FR was a mixture of foods such as rice, spices, cooking fat and animal products with an estimated crude protein value of 20% while BW is a mixture of starch grains and yeast cells with a crude protein content of 26.7% (Table 23). This is also supported by studies on larvae of other studies. For example larvae of noctuid caterpillars (Lepidoptera: Noctuidae) and acridid grasshopper nymphs (Orthoptera: Acrididae) fed on carbohydrate biased diets took 0.375–6.875 days longer to mature than those feeding on the other diets (Deans et al., 2015). Nash and Chapman (2014) also found the development of larvae of the (Nash and Chapman, 2014) *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) to be significantly slow when reared on a low-protein diet though the carbohydrate content remained constant. This suggests that the protein level of a feed is the key nutrient for larval development with levels of approximately 20% to 30% being considered sufficient (Arif et al., 2012), though better performance can be obtained on nutritively balanced diets. The fat and carbohydrate levels can however be similar to or lower than protein levels since BSFL are omnivores (St-Hilaire *et al.*, 2007); Nguyen *et al.*, 2015), Gobbi *et al.*, 2013; Tschirner and Simon, 2015).

The performance of the different mixing ratios across all substrate combinations show that the best mix ratio for biomass production is at the 30:70 level and 50:50, whose performance in terms of yields, maturation period and bioconversion rate are not significantly different (Table 15). In this study, co feedstock amounts above 50% in the mix, led to lowered biomass yield across all the treatments. However with the intention to utilize as much faecal sludge as possible as a method of managing the waste, the mix ratio of 50:50 emerges as a compromise for both efficient waste reduction and biomass production.

Waste reduction reduced with increase in amount of faecal sludge in the substrate across the treatments except BP-FS where it increased with increase in amount of BP and can be attributed to drying up effect rather than consumption by BSFL. Comparatively higher bioconversion values were obtained on combined feedstocks than on pure faecal sludge alone (Table 15). These show that combining substrates improves substrate digestion and assimilation into larval biomass. Again, the exception case of FS-BP feedstock

combination increasing maturation period beyond that of pure FS (Table 15) can be explained by the fiber content due to cellulose and lignin in the peels and variation of particle sizes of the resultant feed mix. Particle size has a significant effect as it influences larvae's ability to uptake nutrients (Diener, 2009). Large particles of feedstock require more energy for the larvae to feed than small particles and also create variable nutrient zones which often cause significant variation in the size of larvae (Brits, 2017). BSFL are also reported to delay feeding activity on cellulose and lignin materials until it decays (Li *et al.*, 2011, Olivier 2009). Higher proportions of these in a feedstock therefore reduce the consumable portion hence delay growth. The high fiber content could therefore have contributed to reduction of consumable feedstock in the FS-BP diet and consequently, changed the allocated ration per larvae for this treatment.

5.3.3.1 Nutrient content of prepupa fed on combined feedstocks

Given that the value of a feed for animals is determined by the protein and fat ratio (Olivier, 2009), the study analyzed the content of the two in samples of the harvested prepupal from different treatments. The study reports that the CP values from mixed substrates combinations are higher than those from pure faecal sludge substrate except for FS-BP combination (Table 15). However the crude protein content (43.2% -FS-BP, 46.1% FS-FR and 47.8% on FS-BW) was well within reported literature values which ranges from 37 to 48% DM (Barragan-Fonseca *et al.*, 2017). Unlike for the single substrate study (Table), the fat content for the mixed feedstock study was more stable and consistent, except for the FS-BP treatment where the EE content of prepupa was significantly different from prepupa of other substrates (Table 15). Whereas (Oonincx *et al.* (2015) reported that a mixture of food waste from food production industry fed on BSF showed little variation in nutrient content of the larvae, most studies have reported variation of fat contents on BSFL fed on different diets. For example, Mutafela, (2015) reported a fat content of 13% from larvae fed on manure substrate and 38% fat content from larvae reared on fruits stream waste. St-Hilaire *et al.* (2007) found that mixing wastes in different ratios (dairy cow manure and fish offal) resulted not only in significantly larger prepupae than when reared on dairy manure alone but also with differing fatty acid contents depending on the ratio of the two waste types. Barragan-

Fonseca et al., 2017) reported the most variation on fats ranging from 7% to 39% DM on different substrates. This not only indicates the variability of the fat content depending on the substrate used but also on the possibility to alter the contents of the resulting prepupae depending on what percentages of fat and protein are needed.

Perhaps the high fat content and reduced crude protein content obtained from prepupa reared on FS-BP and the single feedstocks can be attributed to nutrient stress in the diet. Previous studies report on the effect of stress in a feeding medium on prepupa fat composition. In the study of Brits (2017), larvae showed significant increases in fat and decreases in protein when stressed with higher levels of intraspecific competition. Larvae of other insects have also been reported to induce compensatory feeding for more fat-depositing nutrients when subjected to stress via larval density or feed restriction to help alleviate the extra stress on the larvae (Green et al., 2001; Raubenheimer and Simpson, 2003); Ojeda-Avila et al., 2003). Stress however reduces the value of the resultant larval biomass as it reduces the protein to fat ratio (Olivier, 2009).

In conclusion, the study reports that larvae fed on different feeds may have different nutrient profiles of protein and fats. Though growth rate of larvae is directly proportional to the feed rate, but inversely proportional to substrate reduction efficiency, there is a threshold amount of food supply where larval weight gain does not significantly increase further. The study recommends the use of the utilized substrates for BSFL production and combining faecal sludge and the co-substrates at a ratio of 50:50, a feeding rate of 200mg/l/d and either after four days or daily feeding regimes as an optimal feeding strategy for both waste management and waste reduction.

5.4 FEED FORMULATION AND FISH FEEDING

5. 4.1 Nutritive value of wild prepupa harvested from vegetable and fruit waste mixture.

Proximate analysis of wild BSF fed on a mixture of fruit and vegetable wastes shows that the larvae had a crude protein and lipid content of 39.98 % and 32.62 %, respectively. In addition, other important nutrients in the biomass were zinc, calcium, iron and vitamin B

complexes (Table 16). The crude protein level was below reported average literature values of 44.0% (St-Hilaire *et al.*, 2007) within levels reported elsewhere such as (Spranghers *et al.* (2017) (40.2%), Sheppard and Newton (1994) and (Yu *et al.* (2011) (40%) and Tomberlin *et al.* 2002 (42%). The value also compares favourably with the protein sources currently used in animal feeds such as fishmeal and soybean with crude protein values of 55.1% and 47.9% respectively (Robinson and Li, 1994; Munguti *et al.*, 20006). The fat content obtained in the study also compares well with values reported in literature: Yu *et al.* (2011) -30-35%; Sheppard and Newton (1994) -35% and Liu *et al.* (2008)- 28.4%.

The favourable comparison of CP and fat content of wild sourced BSF in Bondo area show the potential of utilizing the obtained biomass to supply proteins for use in animal feeds such as fish feed and therefore contribute to food security through enhanced production of fish meat. By using organic waste substances as substrates, the BSF technology can contribute to considerable reduction of production costs and ultimately make these products more available and cheap in the market.

5.4.2 Fish feed formulation and pelleting

Only one size of feed pellets were produced for both BM and FM feeds. This is because the fabricated meat mincer used for pelleting could only produce feeds of one size (diameter). The formulated feeds however differed in some physical parameters such as colour (Figure, 25), floatability, stability in water and shelf life (Table 17). The darkness of BM feed compared to FM feed can be attributed to the chitin content of the BSF prepupa exoskeleton in BM feed. The longer floatability period observed on BM feed of 50.6 seconds compared to FM's floatability period of 40.5 seconds can be attributed to the higher fat content of the BM feed of 20.2% compared to 13.3% in FM feed (Table 18). High fat content is reported to increase buoyancy and consequently feed floatability in water (Chevanan *et al.*, 2009). Again, though the two feeds were formulated to be isonitrogenous in composition, proximate analysis of the feed products revealed differences nutrient composition (Table 18). This confirms the inefficiency of the Pearson's square method of feed formulation.

5.4.3 Effect of feed on fish growth

Analysis of variance (ANOVA) of weekly fish weights of fish fed on FM and BM feed is given in Table 19. The table shows that there was a significant difference in the weights of fish fed on FM and BM feeds. Separation of weekly means of fish fed on BM and FM feeds is represented in Table 20, which showed the differences to be in only week eight of the study.

When the weekly average weights of fish fed on BM and FM were plotted against time, fish growth was slow on both FM and BM feeds in the first two weeks of feeding (Figure 26). The slow growth can be attributed to the fact that only one size of feed was pelletized using the fabricated meat mincer used in the study. Therefore the fish were fed a diametrically uniform feed throughout the experimental feeding period. Consequently, the feed pellets might have been big for the fingerlings and contributed to reduced feed intake which could have caused the slow growth during this period. Otherwise the growth trend of the fish by weight was similar throughout the feeding period, which therefore shows that both feeds were suitable for tilapia feeding.

Comparatively lower growth rates on both diets were obtained in this study than in other previous studies, a fact attributable to several conditions of the studies. The average temperature of the waters in the current study was 21 °C for both feeds. These values were generally below the optimal growth temperatures for tilapia fish (25-27°C) (De Silva and Anderson, 1994, Hecht and Jones, 2009; Mjoun et al., 2010). The feeding regime adopted in the current study where fish were fed twice in a day (morning and evening), and at a percentage of their body weight might also have been a contributory factor to the reduced growth rate. Fish grow faster if they are fed to satiation multiple times per day instead of at specific times, and on a given ration as in the current study (Buurma and Diana, 1994). Sealey et al. (2011 and St. Hilaire *et al.* (2007) fed two and three times per day respectively, until apparent satiation. Furthermore the starting weights of the fish differed significantly between the studies (~25 g in St.-Hilaire *et al.*, 2007, 145 g in Sealey *et al.*, 2011 and 4.6 g in this study). Last is the fact that the pellets in this study were extruded in a fabricated meat mincing machine while compression pelleting

was implemented in the studies of St.-Hilaire *et al.* (2007) and Sealey *et al.* (2011). The low pressure applied may have made the feed porous and therefore shortened their floatability period on water and therefore reduced consumption. Inability to make pellets for different stages of fish and the method of preparation of the pellets together may have led to reduced feed intake and therefore slow growth.

The similar trend (Figure 26) and lack of significant differences between fish fed on BM and FM feeds (Table 20) shows that biomass of BSFL can be used to completely replace the use of fishmeal in feeds. Use of BSF larvae meal as a protein source in feeds of warm water fish species has been documented by previous studies. The studies reported the suitability of BSF meal for other fish types such as the Rainbow Trout (Bondari, Sheppard, 1981, Sealey *et al.*, 2011, St-Hilaire *et al.*, 2007), Channel Catfish (Sheppard, *et al.*, 1994) and cat fish (Stankus, 2013). However St. Hilaire *et al.* (2007) reported a reduction in the content of Omega-3 fatty acids (St-Hilaire *et al.*, 2007). This seems to be the first study using a complete BSF meal on Nile tilapia feeding and therefore no comparison would be made with previous studies.

In the current study, there was wide variability of final fish weights in both the control and experimental fish tanks. At the end of the study period, some fish were approximately 30cm long while others were as short as 12 cm long, possibly at the juvenile stage. A possible explanation is the concept of compensatory growth ((Buurma and Diana, 1994); characterized by hierarchy establishment in the fish pond and consequently more feed consumption by the dominant fish which results in high variability of sizes. The establishment of hierarchies in fish tanks and resultant variability of fish sizes was also reported by (Puvanendran *et al.*, 2003). However due to the black nature of the feeding tanks and murky nature of the water posed a serious disadvantage in that it was difficult to observe the feeding behaviour and quantify feeding as some feeds sank and it was difficult to measure the amount that sank and remained un-eaten.

5.4.4. Effect of feed on physico-chemical water parameters

The physical-chemical parameters of water between the two feed treatments were not significantly different (Table 21). Nitrites that showed the highest variation in the two feed treatments (15.7 ± 4.94 SE for FM feed and 7.6 ± 1.1 for BM feed type). While most of the other parameters were statistically similar and within the recommended ranges of optimum growth of tilapia fish and generally within the tolerance limits of warm-water fish species (Boyd, 1979) the average water temperature range of 21°C was slightly below the optimum range for survival of *O. niloticus* of $22\text{--}27^{\circ}\text{C}$ (Stickney, 1986; Mjoun et al., 2010). However the temperature level was similar for both treatments indicating that water was not affected negatively by either treatment. Changes in pH ranged between 6.7 and 6.9 for BM and FM treatments respectively showing they were slightly acidic but within the optimal range of between 6 to 9 (Tacon and Nates, 2007 (Tacon and Metian, 2008), Stickney, 1986).

Turbidity (TDS) recorded for the experimental period for FM feed was above 50 NTU and below 50 NTU for BM, though the difference was insignificant ($P > 0.05$). According to Tacon and Metian (2008), turbidity levels of below 50 NTU have no effect on the growth rate of Nile tilapia. The same study however found that turbidity of above 50 NTU reduced growth rate of the Jamaica Red, and by extrapolation, might have affected the growth rate of tilapia reared on the FM feed. The higher turbidity value of FM feed waters can be attributed to the low fat content of the feed that reduced floatability of the feed and with this feed intake since tilapia are top feeders (Hecht and Jones, 2009). However conductivity was slightly higher for BM feed but was not significantly different from the FM value (Table 21). Generally, the ineffectiveness of the feed to affect the water quality parameters can be attributed to the periodic replenishment of the water in the polythene lined water tanks. Besides, use of BSF meal as an alternative protein source for fish does not conflict with human food security interests (Abarike et al., 2013). In conclusion, this study demonstrated successful utilization of BSF as an alternative protein source for tilapia fish and therefore supports its use to replace fishmeal in tilapia nutrition.

CHAPTER SIX –CONCLUSION AND RECOMMENDATIONS

The black soldier fly larvae have a wide range of applications ranging from those that benefit the environment (waste management, reduction of bad odours, land fill usage, greenhouse gas emissions, and environmental degradation) to food security benefits, making one of the most beneficial insects. However sustainable realization of the benefits depends on successful development of breeding strategies especially in mass rearing set ups in industrial scales. This is essential considering the demand for food security and the ever increasing amount of organic waste that the society produces. This will not only contribute to food security directly but also indirectly by decreasing the demand for currently used fish farming ingredients such as “omena” and monoculture plants such as soybeans, which can be left for direct human consumption. To

In objective one, the study evaluated the use of different substrates to lure the black soldier fly adults to oviposit both from the wild and in captive conditions. The former being necessitated by global concern on utilization of foreign species due to the environmental risks they pose such as decimation of native biodiversity and the latter for purposes of increasing production to useful levels. The study established the nativity of BSFL in Bondo area of Kenya, and reported that BSF adults respond variedly to different substrates when used as oviposition attractants. The wild Bondo strain was most responsive to fermented mashed maize grains and market waste composed of fruit and vegetables, while the captive breed was most receptive to fresh cow dung manure and fermented frass. This gave insight into possible differences in strain responses that can be attributed to either prior exposure of the species to the abundant substrate in the environment or the role of fermentation in producing relevant volatile chemicals in the substrate.

In objective two the study took cognizance of the fact that though BSF larvae can survive and grow on many different organic products, not all organic materials are suitable for BSF production in captive operations in terms of quantity and quality of the product. Consequently, the study evaluated a number of potential production substrates in terms of growth rate of larvae and quality of nutrient content (crude protein: lipid ratio). The study reports that post restaurant food remains, industrial brewers’ waste and faecal

sludge, watermelons, bananas and pineapples are all suitable production substrates for BSFL larvae in captive breeding which produce BSFL biomass with crude protein levels suitable for use in fish feed.

Objective three of the study was concerned about optimization of BSFL production in captive breeding through establishment of a feeding strategy. The study assessed BSFL production on identified substrates at different feeding levels (feeding rate), feeding frequencies (feeding regime) and feed combination. Feeding rate 200mg/l/d was most effective with respect to both biomass production and waste reduction across the different substrates used in the study while daily feeding and after four days feeding regimes as they resulted in highest prepupa yields, high substrate reduction levels and short maturation periods. Combined feeds also performed better than single substrate feeding on parameters such as biomass yield, individual larval weights, nutrient composition of biomass and shortened maturation period with the best performing mixing ratio for faecal and utilize co substrates being reported at ratios of 30:70 and 50:50 co substrate to faecal sludge respectively. However with the intention to utilize as much faecal sludge as possible as a method of managing the waste, the mix ratio of 50:50 emerges as a compromise for both efficient waste reduction and biomass production.

With respect to objective four of the study, proximate analysis of wild collected prepupa fed on vegetable and fruit waste mixture showed sufficient crude protein content to enable usage in fish feed formulation. Fish feeding trials of the formulated feeds on tilapia showed no significant differences in the performance of fishmeal and BSF prepupa formulated feeds in the growth rate of fish and effect on water parameters. Therefore BSF reared on organic waste can effectively replace the use of fishmeal in tilapia feeds.

RECOMMENDATIONS FOR FUTURE STUDIES

The study recommends for future studies, the following:

1. A study to determine the nutrient content of BSFL fed on different organic substrates in terms of aminogram and fatty acid content to facilitate more accurate use of larvae in feed making.

2. Further studies to determine of the efficacy of the BSF feed on different kinds of fish such as the carnivorous fish types and when supplemented with premixes of lacking nutrients.
3. Research on how to safely utilize substrates such as faecal sludge for production of BSFL larval biomass without endangering the lives of humans and the feed species of animals.
4. A repeat of the wild attraction of black soldier fly in other parts to determine complete geographical mapping of the insect in Kenya. Again, characterization of volatile chemicals in putrescence of attractant substrates will greatly contribute towards artificial production of the same to enhance production even in areas where natural forms of the attractants are lacking or when production is out of season.
5. BSF feeding on other categories of organic wastes in order to establish a complete data base of eligible substrates for black soldier fly larvae production.
6. A study to determine the cost of production of BSF prepupa and compare the economic efficiency of BM based feeds to alternative feeds.

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APPENDICES

APPENDIX 1: PUBLICATIONS

1. Nyakeri, E., Ogola, H., Ayieko, M. and Amimo, F. (2016). An open system for farming Black Soldier Fly larvae as a source of proteins for smallscale poultry and fish production. *Journal of Insects as Food and Feed*, 2017; 3(1): 51-56.
2. Nyakeri, E., Ogola, H., Ayieko, M. and Amimo, F. (2017). Valorization of organic waste material: Growth performance of wild black soldier fly larvae (*Hermetia illucens*, Linnaeus, Diptera: Stratiomyidae) reared on different organic wastes. *Journal of Insects as Food and Feed*, 2017; 3(3): 193-202.
3. Nyakeri, E., Ogola, H., Ayieko, M. and Amimo, F. (2017). Comparison of the performance of different baiting attractants in the egg laying activity of the black soldier fly (*Hermetia illucens* L.). *Journal of Entomology and Zoology Studies* 2017; 5(6): 1583-1586.

MANUSCRIPTS SUBMITTED FOR PUBLICATION.

1. Nyakeri, E., Ayieko, M. and Amimo, F. Establishing an optimal feeding strategy for Black Soldier Fly larvae (*Hermetia illucens*, L) for biomass production and organic waste reduction. Submitted to *Journal of Entomology*: eISSN: 1812-5689; pISSN: 1812-5670.

Status: **Accepted** with corrections.

2. Nyakeri, E., Ayieko, M. and Amimo, F. A Manual Guide for Production of Black Soldier Fly larvae in Kenya. Submitted to *Unified Journal of Agriculture and Food Science*.

Status: **Accepted** for publication.

MANUSCRIPTS UNDER PREPARATION

1. Nyakeri, E., Ayieko, M. and Amimo, F. A comparative performance of two fish feeds: growth performance of fish fed on Black Soldier Fly prepupa and fishmeal formulated feeds.
2. Nyakeri, E., Ayieko, M. and Amimo, F. Growth performance and nutrient content of Black Soldier Fly larvae reared on vegetable and fruit wastes.