THE POTENTIAL OF USING DIFFERENT SUBSTRATES IN PRODUCING BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE USED AS A FEED SUPPLEMENT IN LIVESTOCK PRODUCTION

BY

WAMBOGA MILTON

BACHELORS AGRIC SCI & ENTREP (Hons), UCU

REG NO: 19/U/GAIN/20671/PE

A DISSERTATION SUBMITTED TO DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN ANIMAL PRODUCTION

OF KYAMBOGO UNIVERSITY

MARCH 2024

DECLARATION

I Wamboga Milton declare that this thesis is my original and has not been presented for a degree in other University.

………………………………………. ……………………………….

WAMBOGA MILTON **Date**

APPROVAL

This dissertation has been submitted for examination with our approval as University supervisors.

………………………………………… ……………………………

DR. SIMON PETER MUSINGUZI **Date**

Kyambogo University

…………………………………... ……………………………

DR. WILLIAM TINZAARA **Date**

Kyambogo University

…………………………………... ……………………………

MR. STEPHEN KABUGO **Date**

Kyambogo University

DEDICATION

This work is dedicated to my lovely children Ezra Wamboga, Elton Wamboga and Ellery Nabuduwa for whom I do endeavor to live and strive.

ACKNOWLEDGMENTS

I am deeply indebted to my supervisors, Dr. Simon Peter Musinguzi, Dr. Tinzaara William and Mr. Kabugo Stephen for their keen interest, kindness, valuable guidance, encouragement and constructive comments from the initial stage of the research proposal development to completion of the write-up of the dissertation. I also acknowledge Mr. Martin Tenywa and his team of Ento Organic Farm Uganda limited-Makerere University Agricultural Research Institute Kabanyolo-MUARIK, I am forever grateful for the opportunity outside Kyambogo University you gave me to do research with your organization. Great thanks also go to staff of Kyambogo University, Department of Agriculture Production, and all my course mates especially Samuel Echaku for the support and encouragement during the course of this study.

In the special way, I would like to thank Dr. Francis Muzaale for guidance provided in carrying out this study. I will forever be grateful for not only the academic assistance you offered, but also for the various life skills learnt from you. My heartfelt thanks go to my beloved wife Esther, mother and father for the support and encouragement throughout the entire period of this study and friends whom in one way or the other contributed whole heartedly to the successful completion of this study. Above all, I praise and thank the almighty God for keeping me healthy and enabling me to accomplish this study even when things seemed to be hard.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA: Analysis of Variance

BSF: Black Soldier Fly

BW: Brewers' Waste

CF: Crude Fiber

CP: Crude Protein

CRD: Completely Randomized Design

DM: Dry Matter

FAO: Food and Agricultural Organization

FO: Fish Offal

IFIF: International Food Industrial Federation

JFW: Jack Fruit Waste

LL: Length of the Larvae

LSD: Least Significance Difference

LSR: Larval Survival Rate

LW: Laval Weight

MANOVA: Multivariate Analysis of Variance

MS: Mixed Substrates

NPN: Non-Protein Nitrogen

NRC: National Research Council

PW: Pineapple Waste

RC: Rumen Content

SR: Survival Rate

ABSTRACT

A study was conducted on potential of using substrates in producing Black Soldier Fly (BSF) larvae used as feed supplement in livestock production. The objectives were to: establish the nutrient composition substrates; determine the influence of selected substrate types and quantities on growth of BSF larvae; determine the influence of selected substrate types and quantities on protein of BSF larvae. Nutrient composition was determined by testing for crude protein (CP), carbohydrates and crude fat. Growth was determined by measuring weight and length of BSF larvae. Laboratory analysis of protein was used to determine protein of the larvae. Data were analyzed using a two-way Analysis of Variance (ANOVA) and Lowest Significant Difference (LSD) used to separate means. Across substrates, Mixed substrate (MS) recorded the highest crude protein, carbohydrate and crude fat as follows; 7.33%, 45% and 9.60% respectively. Brewers' waste (CONTROL) also recorded high values for crude protein, carbohydrate and crude fat as follows; 12.07%, 58.7% and 9.0% respectively. Brewers' waste (CONTROL), Jack fruit waste (JFW) and Mixed substrates (MS) recorded the highest length of the larvae of 10.06%, 10.30% and 11.33% respectively ($P<0.05$), at 750g and 1000g of substrates. MS and JFW had the highest weight of 0.318g/FM and 0.212g/FM respectively ($P<0.05$), at 1000g of substrate. BW and MS had the highest Survival rate (SR) of 96.19% and 96.01% respectively ($P<0.05$). According to the values, CONTROL had higher CP compared with RC of 19.19% and 17.89% respectively ($p<0.05$). The findings showed that MS and RC significantly influenced growth and protein of BSF larvae respectively.

CHAPTER ONE: GENERAL INTRODUCTION

1.1. Background

Animal production in general, and poultry in particular has got very many important economic and social roles in many countries (Alders, 2004). Provision of additional money and ensuring adequate food are the main reasons for poultry rearing by many resource - poor communities. It is known as an employment source to majority of vulnerable groups in several local areas (Gueye, 2002). Due to faster growth in the sector, poultry rearing is now considered a lucrative business to resource - constrained societies simply because they need slightly low initial capital, small rearing space, and reduced management costs (Omit and Okuthe, 2009). This demand is supported by faster economic growth because of increasing population which has resulted into much desire to feed on animal protein (FAO, 2011). The demand in many developing countries for poultry products is predicted to rise by 70% in order to cater for 9.2 billion people which are likely to occupy the world by the year 2050 (United Nation, 2019); hence putting much pressure on the need to provide enough animal proteins. Globally, silver fish and soyabean are widely used as protein ingredients in the mixing of poultry feeds. However, much of the silver fish produced is used by human beings for food and soyabean cultivation is also limited by land, water and poor yields (Veldkamp and Bosch, 2015). In addition, the Food and Agricultural Organization (FAO) of the United Nations has projected that the globe will have to produce more food, 70% by 2050. However, with respect to animal protein production in particular, the International Feed Industry Federation (IFIF) believes that production of poultry meat, eggs, and pork are likely to double. This poses many challenges to the world capacity to produce adequately enough livestock feeds. In sub -Saharan Africa, silver fish and soya bean are used as a cheap source of food mainly by poor households hence little and highly priced is available for use as ingredients in the manufacture of animal feeds.

In Uganda, the prices for silver fish and soyabean are increasing every year because of competition for proteins between human beings and livestock, in this case affordability by poor livestock farmers is very low (Komi and Nakibugwe, 2017). The increasing scarcity of resources to produce silver fish, and soybean feed ingredients has doubled prices during the past 5 years and yet capital investment in livestock (60 to70%) is feeding thus leaving marginal profits for livestock projects (Komi and Nakibugwe, 2017). Therefore, alternative livestock feeds are needed to reduce amount of the sources in a constituted feed.

Black Soldier Fly (BSF) larvae have become common simply because of their potential to degrade waste, and a cheap protein ingredient for livestock feeds (Banks *et al.,* 2014). BSF larvae bio-conversion technique is being applied in animal waste management particularly from confined animal facilities of livestock, and poultry with reduction value of 5%. Additionally, 50-79% has been recorded as reduction values on municipal and household waste (Banks *et al.,* 2014). In waste transformation process, the larvae also helped to remove bad odor, and methane gas generated under anaerobic decomposition of organic wastes (Barry, 2004). Additionally, BSF larval biomass has been observed to contain 44.4% dry matter (DM) crude protein, 23% DM lipids, and a good balance of essential amino acid (Sheppard *et al.*, 2008). This is associated with better feed conversion efficiency (Veldkamp *et al.*, 2012), with other vital major and minor nutrients (Van Huis *et al.,* 2013), these values are comparable with fish and soybean meals when mixed.

Fish and soybeans provide at least 90% of the animal feeds protein requirements as suggested by Yu and Chen (2009). Although, fish and soybeans are widely eaten by humans, as a result, their demand has outweighed supply hence bringing doubt on their future availability in large amounts (Yu and Chen (2009). Studies on livestock feeding trials on BSF larvae biomass have so far produced good results predicting the ability to substitute fish meal and soybean in livestock feeds (FAO 1, 2013). BSF Larvae are got in the animal waste such as poultry, piggery and cattle manure and can be reared on various substrates (Banks *et al.,* 2014), such as jack fruit waste, pineapple waste, rumen content, fish offal among others. Therefore, successful development of BSF larvae farming technology is key and one of the factors affecting BSF larvae growth. However, this entirely depend on selection of the rearing substrates. Hence there is scanty information on BSF larvae best rearing substrates.

The known feeding amounts for BSF larvae have been reported by recent studies as 100 mg larva^{-1} day^{-1} (Diener *et al.,*2009), 163 mg larva^{-1} day^{-1} (dry base) (Paz *et al.,* 2015) and 200 mg larva^{-1} day^{-1} (Permana *et al.,*2018). Moo and Hudura. (2022) suggested that higher feeding amounts increased BSF larval growth and development but had a negative effect on waste reduction. This study still reported that BSF Larvae produced less waste with reduced feeding rate. Therefore, there is need to implement a standard feeding rate for better bio-conversion process and cost saving to optimize livestock production.

1.2 Problem statement

Fish meal is a highly rich protein ingredient used in chicken feeds but is considered as a limited resource because of problems associated with increasing fishing, and environmental pollution as well resulting from population pressure (Van Zanten *et al.,* 2014). In addition, the fact that fish is also a key source of protein in the human diet, there is competition as a feed source. This is seen by exponential increase of fish meal prices in the market during the recent decade, which has now risen the demand for a new, cost effective, and highly sustainable protein source alternative (Veldkamp *et al.,* 2015). Black soldier fly larval meal has been proven to be an alternative animal protein source for poultry feed. BSF larvae is produced using various substrates, however, there is lack of information of the best substrates that can optimally sustain or ensure optimum production of black soldier fly larvae. Therefore, this study aimed at assessing the different substrates that can optimally be used in production of black soldier fly larvae. Although black soldier fly larvae can be given different substrates, parameters such as length, weight, survival ability of the larvae on the substrates and protein composition of the larvae given different substrates are strongly affected by substrate types and substrate mixing. Besides substrate types, black soldier fly larval feeding is affected by feeding levels (amounts), whose regulation shifts focus from mere black soldier fly larvae feeding to facilitating efficient consumption and therefore waste reduction and production of sufficient amounts and quality of black soldier fly larval biomass (Barry, 2014). Hence, there is need to establish feeding amounts on identified diets that can optimally be used in black soldier fly larval production.

1.3 Objectives of the study

1.3.1 General objective

To assess the potential of different substrates in producing black soldier fly larvae used as a source of animal protein in livestock feed.

1.3.2 Specific objectives

1. To establish the nutrient composition of different substrates for Black Soldier fly larvae feeding.
2. To determine the effect of different types and quantities of substrates on the growth characteristics of black soldier fly larvae.
3. To determine the effect of different types and quantities of substrates on the protein composition of black soldier fly larvae.

1.4 Research hypotheses

1. There is no significant difference in the nutrient composition of the different substrates for BSF larvae feeding
2. There is no significant difference in the types and quantities of the different substrates on the growth characteristics of BSF larvae.
3. The different substrate types and quantities have no significant difference on protein of BSF larvae.

1.5 Significance of the study

The findings of the research can be used in making recommendations for farmers on suitable types of substrates that will be used to rear black soldier fly and boost production of larvae. This can be a cost-effective strategy for farmers to produce protein to supplement animal feeds to produce more meat, milk, eggs at relatively lower cost. Therefore, feeding animals on black soldier fly larvae will help farmers especially in the villages to cut on the costs of feeding hence encouraging farmers to keep more animals and improve on their incomes and standards of living.

1.6 Scope of study

The research was conducted from the month of April – August, 2022. The study focused on the potential of using different substrates in producing BSF larvae used as a feed in livestock production. Specifically, the study was conducted at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). Laboratory analysis was carried out at Makerere and Kyambogo Universities.

CHAPTER TWO: LITERATURE REVIEW

2.1 Black soldier fly history and biology

Black Soldier Fly belong to insect order Diptera (true flies) in the Stratiomyidae family. It originates from North and South America, having a native range stretching from South America to central United States. In addition, because of worldwide movement, trade and climate change, BSF is now seen on the entire universe across tropical and temperate regions according to Barragan-Fonseca *et al.* (2017). Luckily, BSF is not a pest, even outside its native range, it does not carry diseases and adults do not sting or bite but also the larvae are better at decomposing wastes in organic form (Barragan-Fonseca et *al.*, 2017). BSF are holometabolous insects which undergo four stages in their life cycle (egg, larvae, pupae, adult) (Figure 1). According to Tomberlin *et al.* (2002), 3 flies' generations are produced each year. While Barragan-Fonseca *et al.* (2017) stressed that the flies are produced with a life cycle of 40 days during the year under controlled laboratory conditions.

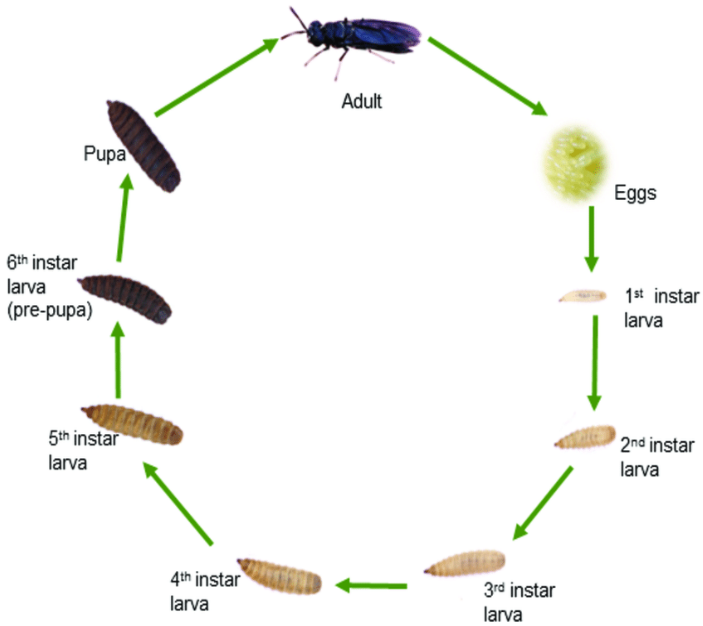

Figure 1: Life cycle of the Black soldier fly

In addition, adult flies look like wasps, are long; 15-20 millimeters in length according to Barragan-Fonseca et *al.* (2017), adult larvae survive for only 9 days.

At this period, adult larvae do not feed, but only focus on mating. Female flies lay above 500 eggs per clutch but only fertile eggs hatch within 4 days according to Sheppard *et al.* (2002). On average, BSF has a larval development period of 24 days, and its larval molts seen at 4 days, but if the flies are provided with favorable conditions, only 16 days are enough for them to develop as suggested by Barragan-Fonseca et *al.* (2017). The larvae are generalist decomposers, and can easily thrive on many various substrate types, such as fish offal, jack fruit, pineapple waste, rumen content, animal manure, carrion and among others as suggested by Tomberlin *et al.* (2009). BSF larvae grows to an average weight of 0.158g (Barragan-Fonseca et al., 2017). However, at prepupal stage, the BSF reduces feed intake and its cuticle begins to change color to black as it prepares for pupation. The pre-pupae move away from their substrate and congest themselves into a separate container for easy collection. The pre-pupae will weigh slightly lower than the larvae because of decline in feeding but rich in calcium. The accumulated calcium in the cuticle adds strength to the yet developed puparium. The pupal stage lasts about 14 days, later turns to adult fly with reduced calcium content according to Barragan-Fonseca *et al.* (2017).

2.2 Nutrient requirements for growth of black soldier fly larvae

Black soldier fly (BSF) larvae feed on many organic substrates; substrates of plant origin, are however, said to be lacking some nutrients which are very important to ensuring faster growth of BSF larvae. Therefore, a mixture of different substrates (plant and animal) can be used to ensure adequate larval biomass production on low value, and thus low-cost substrates (Spranghers *et al.*, 2018) (Figure 2). The same authors stressed that low value substrates might be improved by the larvae into high valuable protein rich biomass with increased content of essential amino acids. This was confirmed by Spranghers *et al*. (2020) in the study of amino acids, its composition, and requirements. Additionally, the nutrient concentration of any substrate used in BSF larvae rearing should therefore have the necessary nutrient requirements for its growth as nutrient composition of a feed affect larval development time, weight, and yield. For example, greater nutrient concentration significantly influences individual BSF larval weight (Karol, 2018).

The nutrient concentration of diet affects the growth and performance of BSF larvae since it influences the behavioral and physiological adaptations of the insect. In addition, with respect to nutritional demands, diet balancing for example protein to carbohydrate ratio is equally important since the two macro-nutrients highly regulate food intake. Therefore, the protein to carbohydrate ratio does not only influence growth and development but also BSF body composition, reproduction, and aging among other aspects. In addition, optimum dietary protein to carbohydrate ratio will vary among BSF species and can change with respect to species depending on development phase, and sex because of various physiology demands. However, according to Spranghers (2019), BSF larvae requires as low as 10% protein; 2% fat and 2% minerals. Protein content of less than 10% on dry matter would result into a reduction in larval protein.

De Haas *et al.* (2006) observed that high food quality protein increases larval development time and survival in many insects. In addition, Oonincx *et al.* (2015) reared BSF larvae on vegetable waste by-products, and found out that larvae that were given diet substrates highly rich in protein recorded faster development period (21 days) as opposed to those given low protein diets (37 days).

Predatory flies fed on substrates that were highly rich in protein recorded an increase in development time, and survival rate (Simon *et al.,* 2011). The high survival rate of these flies was attributed to increased larval development times which permitted enough period for nutrients storing. However, Oonincx *et al.* (2015), reared larvae on vegetable by products that were rich in protein, fats and fed chicken feed (rich in fat) as control diet, this study revealed that larvae given substrate high in both protein and fat, and larvae given chicken feed, recorded shorter development period; 21 days as opposed to larvae given protein diets alone; 37 days. Nguyen *et al.* (2015). The study still reported increased weight and length on diets that were richer in both protein and fat.

Additionally, Nguyen *et al.* (2015), fed larvae on low protein and fat diets, these diets made larvae to have longer development times and low weight. Although, very rich fat diet, 20 – 36% DM crude fat still contributed to slow growth and low weight gain and thus were observed to be harmful to larval, and adult survival. In addition, growth and survival rates were also affected by excess macro nutrients such as protein other than low nutrient levels. For example, soluble distillers' grains (SDGs), meat, liver and fish meal recorded increased mortalities; 78%, 80%, 43, and 53% respectively, and had slow growth (Tschiner and Simon, 2015). This is attributed to extremely high energy use for the metabolism of too much nutrient or by high ammonia accumulation. Extremely high protein is removed as uric acid or as ammonium compounds, and in many forms. Dipteran insects excrete water soluble allantoin and abrupt degradation product of uric acid is changed to urea by the enzyme urease to ammonia (Green and Popa, 2012). Therefore, BSF larvae reared on highly rich protein diets, had an increase in N-mineralization, and possibly high fecal ammonium concentration.

On the other hand, BSF larvae have no enzymes to help in fiber break down, therefore, larvae gut microbes and microbes in bio waste hydrolyze fiber, and provide more nutrients for larval development. Additionally, carbohydrates or simple sugars, plus other metabolites result in breakdown of fiber (Gold *et al*., 2018). Barragan-Fonseca (2018), suggested a diet rich in proteins and carbohydrates as the main driver for BSF larval treatment process performance; in addition, high fiber content in diet can decline performance by reducing the nutrient availability and BSF larvae development. This can result into reduced dry weight waste reduction and increased larval development period on bio-waste that is high in fiber such as manures, rumen waste and some fruits like jack fruit. Fortunately, treatment of such waste with microbes before feeding to the BSF larvae can degrade fibers (Gold *et al*., 2018).

Besides diet and larval density, there are many other factors that may affect survival of the larvae, particularly temperature. The influence of temperature on development has so far been observed on several dipteran species according to Couret *et al.* (2014). However, other factors such as temperature may also influence survival rate and larval development period more than larval density for example in *Musica domestica* (Barnard and Geden, 1993). Surrounding temperature mainly influence metabolic, and development periods of BSF according to Jarośík *et al.* (2004). Development period is high at increased (>32^{o}C), or low, (< 16^{o}C) temperatures, but 26-27^{o}C is the optimal temperature for BSF larvae (Holmes *et al.,* 2016).

2.3 Feed quality effect on larval growth characteristics

2.3.1 Larval food quality

According to Moreau *et al.* (2006), larval food quality is key in determining fitness of plant feeding insects, especially those that do not feed at adult stages, this is also applicable to BSF that keeps its food nutrients during the larvae stage. Additionally, the larvae are highly attracted to feed on substrates which are highly rich in fat to make a fat body required to accomplish larval development (Nguyen *et al.,* 2015). This explains why food quality is paramount for BSF Larvae growth rate, and is directly proportional to survival rate, length, and duration of larvae stage (Gobbi *et al.,* 2013).

BSF larval body composition varies across diets particularly in macro-nutrient amounts (Barragan-Fonseca *et al.*, 2017), and to a smaller extent in micro-nutrient amounts according to Spranghers *et al*. (2016) and Barragan-Fonseca *et al.* (2017)

According to Liu *et al.* (2008), BSF larvae weight gain is influenced by over relying on bacteria as diet, this has also become food for many dipterans as suggested by Spiller (1964). For example, Yu *et al.* (2011) isolated the bacterium *Bacillus subtilis* from the BSF larvae gut, and found out that these promote the growth of con-specific larvae simply by fermentation of their substrate. This is because *B. subtilis* is able to digest protein in the substrate, and supply phosphorus as reported by Guohui *et al.* (2010). Zheng *et al.* (2013b) classified 78% bacterial genera obtained from larval, prepupal, pupal, adult, and egg samples of BSF. The results showed that 42% and 33.4% of the most dominant phyla were Bacteroidetes and proteobacteria respectively. Therefore, these findings indicated an increased diversity of bacterial species that associates with BSF. Although, diet is most likely to affect microbial flora content as stated by Jeon *et al.* (2011).

In addition, BSF larvae likes feeding on a moist substrate as reported by Tomberlin *et al*. (2002). However, early studies have indicated that substrate moisture content (52 – 70%) lead to the highest adult weights (Barragan–Fonseca *et al.,* 2017).

2.3.2 Larval density

Larval density also influences growth and development of BSF larvae, previous studies have also indicated that 10 l/gram of substrate decreased waste accumulation and increased larval weight (Diener *et al.,* 2009). Although other scientists have only focused on densities between 0.3 – 80l/gram of substrates but majority of these studies have so far shown that larvae reared at lower substrate amounts, had higher weights and reduced development period as opposed to larvae reared at increased substrate amounts (Barragan – Fonseca *et al.,* 2017). In addition, Banks *et al.* (2014) also reported that as more food is made available per larva, larval development and length and weight also increase, but more waste accumulates.

According to Green *et al.* (2002), reduced larval densities are good at increasing growth rate. In many insects, larval aggregations have advantages such as heat provision, which enhances food assimilation, and protect the larvae against lower temperatures, and probably predators (River and Dahlem, 2013). In addition, weight gain of the larvae can also become influenced by dependence on bacteria as food. Furthermore, increased larval densities are always associated with increased bacterial densities which can permit larvae to get good access to bacterially recycled foods, thus leading to higher nutrient uptake. Increasing larval density can increase the output of insect farming. In addition, Para Paz *et al.* (2015), mentioned that larval density can significantly affect bioconversion process in BSF. High larval density is very important; therefore, it is recommended that overcrowding avoided, especially if the main objective of BSF farming is to obtain biomass (Rivers and Dahlem, 2013). However, increased larval densities can result into reduced quality of substrates simply by accumulation of larval waste materials according to Green and Popa. (2012), and this may require high energy expenditure since the larvae use more energy interacting with each other (Jannat and Roitberg, 2013).

2.3.3 Substrate characteristics

Substrate characteristics is one of the factors that may affect performance of insects. For instance, thick substrate layers, such as for meat, swine, fish and liver meal decrease larval food intake hence increased larval development and lower survival rate (Nguyen *et al.,* 2013). Similarly, Larde (1990). reported better performance of BSF larvae on highly uniform and drier substrate. It is also known that moisture in poultry feed (60-70%) is appropriate for the larvae. But since there is variation in organic waste composition, it is extremely hard to control food moisture thus needs to be determined both under laboratory and field conditions where the rate of evaporation is never constant. Kalová and Borkovcová. (2013), reported that BSF larvae given substrates having extremely high moisture (greater than 90%) survived, but did not perform well.

2.3.4 Larval crowding

Larval crowding is known as one of the major factors influencing development time, and rate, just as it has been observed in many dipteran species (Jannat and

Roitberg, 2013). Unfortunately, larval density has been reported in few studies. Although, considering the container sizes used in various studies (Oonincx *et al.,* 2015), taking assumption that food is 1 – 2 centimeters layer thickness, and average density of 1.4l/cm squared is computed (minimum, 0.1; maximum, 3.33 l/cm2). Sheppard *et al.* (2002) reported a density of 2.5 l/cm2 of surface area for BSF given chicken diet, so as to attain optimum growth. Banks *et al.* (2014) reported related development period; 28 – 30 days in BSF larvae given human - fecal at various quantities; 0.02, 0.2, 0.31 l/cm squared, attained the greatest pre-pupal weight; 0.32 g FM, and least Food Conversion Ratio; 10.4 at the least density. Density is a key factor for dipterans, since it influences larval aggregation which is a trait for the biology of these species according to Rivers and Dahlem. (2013). Larval aggregation has effects for example generation of heat and coprophagy leading to higher nutrient utilization.

2.3.5 Humidity

Studies have indicated the suitable humidity (60-70%) for BSF farming; however, they can also perform well in a wide range (25-99%), (Barragan- Fonseca *et al.,* 2018). For example, humidity in the range (60-70%) yielded egg eclosion rates between 72-86%, and adult emergence rates of 93%. However, humidity values that are below this range can result into adult emergence rates to plummet 25%; egg eclosion 8%, and adult emergence 16%. However, no difference was observed between humidity ranges for the hardier larval and pupal stages indicating that humidity is not vital for larval growth and feeding rates as confirmed Holmes *et al.* (2012).

2.3.6 Temperature

Temperature has significant effect on BSF larval development and can drop *E. coli* counts with highest suppression observed at 27°C (Liu *et al.,* 2008). According to Zheng *et al.* (2013b), reported providencia as being responsible for vertical transmission in BSF, this was detected in eggs and adult fly. The chances of bacteria to be left in successive BSF life phases are high. This need to be determined if observed by testing of the first bacterial load followed by testing diversity in the entire fly's population before introducing in the substrate. By doing this, any inadvertent disease transmission can be remediated, and remove specific bacteria that expose danger to both livestock and human-beings. In addition, BSF produce chemicals that stop other flies from ovipositing eggs on substrate already occupied by larvae, that leads to complete decline of the housefly species (Bradley and Sheppard, 1984).

2.3.7 Toxic feed contaminants

Diener (2010), reported that cadmium, Zinc and lead were detected in the pre-prepupae stage of BSF when larvae were fed organic waste. However, neither of the three metals significantly had effects on life cycle characteristics especially on pre-pupal weight, development period and rate and sex ratio nor on the bilateral symmetry of the adult flies. Unfortunately, cadmium observed in pre-pupae potentially restrict its use in animal feed production. However, Diener (2010) stressed that neither lead nor zinc accumulated in larvae or prepupae which may signify that it poses concerns about their use in livestock feed. However, potential hurdles like the bio-accumulation of insecticides, disposable medical drugs, heavy metals as well as toxic substances can potentially be managed in large scale rearing setups through quality control of BSF farming substrates as suggested by Van der Spiegel *et al.* (2013).

2.4 Determinants of nutritional composition of BSF larvae

Knowing nutrient composition of BSF larvae is vital before using it as protein supplement in livestock feeds because it is now widely used as animal feed. However, many researchers have conducted studies to ascertain how certain aspects of larval development and farming could cause changes in nutrient composition of the larvae. Many studies have so far revealed that compositional changes are inherent and change as larvae develops. For example, the young larvae contain a large amount of protein content and gradually decreases as it grows additionally, dry matter (DM), and ash components becomes much as age of the larvae increase (Rachmawati *et al.*, 2010). Besides, larval composition can be influenced by diet type fed on. For example, BSF larvae given swine manure contain much protein as opposed to BSF larvae given cattle or poultry waste (Barragan – Fonseca *et al.,* 2017). Thus, the following aspects need to be put in consideration when designing best practices for larval rearing on large-scale livestock feed production.

2.4.1. Protein content

This is a key aspect to consider for example, best fish meals constitute 55 – 75% crude protein. If insect meal is to be used as protein ingredient or supplement in livestock feed, then its protein should be equal or more ingredients will be required to hike the overall protein content. Unluckily, insects are reported to have minimal crude protein as opposed to fish meal. BSF Larval protein is in the range CP37% - CP63% DM, as reported by Barragan Fonseca *et al,* (2017). Five-day old larvae tested the greatest crude protein in the life cycle at 61%. Unfortunately, these are short in length, small, contain much moisture, and cannot be applied in decomposition of waste materials (Rachmawati *et al.,* 2010). However, mature larvae can be applied efficiently in waste utilization, and are easily harvestable but its overall crude protein content drops to below 40% (Barragan – Fonseca *et al.,* 2017). The larvae substrate may increase or decrease the final crude protein content. The greatest protein was reported in larvae that consumed liver (CP62.7%), and fish (CP57.9%) as their main diet source. But these dietary substrates are scarce and likely lead to increase in overall costs. According to Barragan-Fonseca *et al.* (2017), BSF larval diet substrates include chicken diet (47.9%), swine waste (43.4%), cattle waste (42.1%), and food waste (41.7%), chicken manure (40.1%), fresh fruits and vegetables (38.5%). In addition, many research finding have shown that BSF larval amino acid profile approximates with anticipated values for animal feeds (Newton *et al.*, 1977). BSF larvae have a matching profile to soybean meal which is a second largely consumable protein source next to fish for animal feeds.

BSF larvae have a joint cysteine, and methionine concentration of 3.39 g/kg on an as is basis Cysteine; 1.02g/kg, methionine; 3.37g/kg). This is sufficient for many animals. Makkar *et al.* (2014) recommends addition of cysteine, and methionine to pig diets. However, arginine might be a limiting amino acid for uricotelic animals, birds, and reptiles (Finke, 2013). For those that have a complete urea cycle, can make arginine. Although, uricotelic animals do not make arginine during uric acid production, implying that arginine should be got from the diet (Ines *et al.,* 2010). Finke, (2013), recorded arginine content of BSF larvae of 12.3 g/kg as compared to the known requirement for rats at 4.3 g/kg (NRC, 1995). However, increasing concentrations of dietary lysine may worsen the situation because of an 18 antagonistic relationship that exist in- between these two amino acids (Ines *et* *al.,* 2010). BSF larvae have got higher amount of lysine; 6-8% of the overall protein content (Sheppard *et al.,* 2008) hence arginine content can be strictly assessed to deter any deficiencies. The only amino acid that is not reported in BSF larvae is Taurine, according to initial research studies but can be added to make complete feed for mammals.

2.4.2 Chitin content

Protein is only determined by testing the quantity of nitrogen that is contained in a substrate, since nitrogen is barely gotten in other nutrients hence quantity of nitrogen in a protein is approximately the same; %Nitrogen x 6.25 % = %CP, is applied to compute the quantity of protein that is available in the substrate. Lamentably, invertebrates pose a serious problem for determining protein because they are associated with chitin. Chitin is a sophisticated molecule containing nitrogen that is structurally similar to cellulose (Caligiani *et al.,* 2018). Chitin is an essential component of an insect exoskeleton and gives much strength, and durability, however, determination of chitin is still a major challenge. Different methodologies are being applied to approximate the quantity of chitin contained in an insect. Values for chitin estimation in BSF larvae (<1-90% DM), with many studies averaging around 5-7% (Caligian *et al.,* 2018). The different methods for computing chitin composition, have a significant effect on the tilted protein value, hence research scientists must come to a 19 consensus on how to well determine chitin, and protein. Irrespective of methods applied to estimate chitin percentage, BSF larvae are equivalent to many insects in their chitin concentration (Finke, 2007). The same study estimated the chitin composition of various insects and observed an average chitin composition of 19.7 mg/kg as is. Ines (2013), applying the same method to estimate chitin, found out that BSF Larvae were 21 mg/kg as is, a value comparable to that of domestic crickets (Finke, 2013). Since the amount of chitin in the exoskeleton has got an important role in its digestibility, therefore BSF Larvae digestibility should correlate with that for a cricket.

2.4.3 Fat composition and metabolizable energy

Many studies have revealed that crude fat in BSF larvae ranges between,7-39%DM. According to Barragan-Fonseca *et al*. (2013), fresh fruits and vegetables generated the larvae with the minimum crude fat; 6.63%, next to chicken feed; 14.6%, liver; 25.1%, swine manure; 26.4%, chicken manure; 27.9%, fish; 34.6%, cattle manure; 34.8%, and restaurant waste; 39.2%. Fat content will drop through the production process of BSF meal. The fat reduction process can be achieved mechanically or chemically by defatting and permit feed manufacturers to adjust protein and fat contents separately (Schiavone *et al.*, 2017).

Finke (2013) reported that 72% of the fatty acid profile constituted of saturated fats, together with lauric acid being the most abundant (51.2%), Finke still found out that the BSF larvae were complying with NRC standards with respect to linoleic (16.9%), linolenic (0.65%) acids but these values are considered greater in fish meal, therefore, more extra 20 can be advantageous. As it has been discussed for some nutrients, diet directly influences the final fatty acid profile of the larvae. Whereas for metabolizable energy, BSF larvae like all larval stages of hemimetabolous insects possess a much greater energy content than adult insects and nymphal (Finke, 2002). Finke (2013), established BSF larvae being 5,139 kcal/gram, dry matter. As supplement BSF larvae can simply be used as defatted meal or can be mixed with least fat; plant-type substrates for livestock. For livestock that feed larvae as whole, this represents a much energy-robust diet, and can only be given together with less fat insects.

2.4.4 Ash composition

BSF larvae have higher ash levels in comparison to majority of the insects. Finke (2013) recorded the ash content as 35 gram/kg as is, whilst some common insects did not exceed 12 g/kg as is. Ash is made up of purely inorganic substances that remain after burning organic molecules, many of the inorganic substances mainly constitute of minerals. As earlier stated, the calcium composition of BSF larvae is much higher possibly due presence of calcium carbonate impregnated into the exoskeleton matrix (Johannsen, 1922). However, it is not confirmed that adding ash to the matrix can contribute to lowest cuticular digestibility that has been encountered with this species. Possibly calcium carbonate would prevent chitinase from binding together chitin and instead facilitates its breakdown. In addition, other minerals, for instance Mg, P, Fe, Mn, as well as Zn are also present in the BSF Larvae nutritional composition profile and therefore contribute to the increased ash content (Finke, 2013). The mineral that has values well below average as compared to many feeder insects is sodium.

2.4.5 Vitamin content

According to Finke (2013), the vitamin profile of the BSF Larvae is comparable to most feeder insects. Water soluble vitamins such as B vitamins are largely considered to constitute the standards of the laboratory Rat (Finke, 2013). In addition, B6, and B12 do not closely meet the standards (Finke, 2013). Unluckily, many insects do not have fat soluble vitamins. BSF larvae are no exception with vitamins, retinol, cholecalciferol and α-tocopherol and all these recorded are below 50% of NRC standards (Finke, 2013). With these low values, it is now important that these nutrients can be provided by additional ingredients

CHAPTER THREE: MATERIALS AND METHODS

3.1 Description of study area

An experiment was conducted at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). MUARIK is Located on spatial coordinates 0˚27'60"N, 32˚36'24" E at an altitudinal range of 1250 m to 1320 m above sea level (Yost and Eswaran, 1990). The study site is located in Nangabo sub county in Wakiso District, Wakiso district is about 14 km north of Kampala, Uganda's capital city. Kabanyolo is also part of the Lake Victoria basin that receives an average annual precipitation of 1218 mm and slightly drier periods in June to July and December to February. The average annual temperature is 21.5˚C (Komutunga and Musiitwa, 2001).

3.2 Experiment design and treatment structure

The experiment used a completely randomized design (CRD) that was replicated 4 times. The treatments composed of six substrates: Two of plant origin (jack fruit and pineapple waste) and two of animal origin (rumen content of cattle and fish offal). Substrates of both plant and animal origin was integrated/mixed in the ratio of 1:1 to form mixed substrates and the control experiment used was brewers waste (breweries spent grains). Breweries waste was selected because of its superior nutrition (Table 4.1). Each substrate constituted four levels. These all together totaled to 24 treatments. The substrates that were fed to the larvae were then varied in four levels or feeding amounts at 250g, 500g, 750g and 1000g while the number of larvae added to each level of substrate was constant with each feeding level receiving 2500 larvae. Substrates were weighed and placed into containers (Figure 3), the containers were initially covered with a mosquito net to stop other flies from dropping eggs in the substrates (Figure 4). Mosquito net was later removed from the containers, and taken to raised wooden structure in a temperature-controlled room at 21.5 - 24˚C as recommended by Chia *et al.* (2018)

Figure 2: Weighing of black soldier larvae on sensitive scale

Figure 3: BSF larval neonates in substrate containers covered with net.

Figure 4: **Inspecting the growing BSF larvae on a raised wooden structure in a temperature-controlled room.**

3.3 Management of substrates

Substrates were selected on the basis of cost, nonuse by humans, need for disposal to improve sanitation, nutritional content, moisture content (60 – 70%), palatability (ability to attract black soldier fly larvae) and sustained local availability in large quantities with a plan for future use for large scale industrial BSF larval production in Uganda.

Plant substrates included pineapple waste and jack fruit waste. These were collected from fresh farm produce markets around Wakiso district and Kampala capital city. Animal type substrates included rumen content and fish offal. Fish offal was collected from the fresh fish sells markets at the waste disposal points in Wakiso and Kireka towns while rumen content was collected from the municipal abattoir of Wakiso and Kampala. Plant/ animal mixed substrates included a combination of the above (pineapple waste, jack fruit waste, rumen content and fish offal).

Breweries waste included breweries spent grains. This served as reference substrate/control experiment, it was collected from Uganda breweries Limited in Kampala

3.4 Data collections methods

3.4.1 Nutrient composition of different substrates for use in larval rearing

During determination of nutrient composition of substrates, all substrates were first poured separately in a shredder and shredded for 10min to obtain uniform particles of 1 - 2mm in size, 100g of each substrate was measured and spread in a petri dish and taken to solar dryer and placed on a wooden table, left to dry for a week. However, to get the mixed substrates, equal weight (1000g) was measured from each shredded substrates and placed in a plastic container, the substrates were then thoroughly mixed by hand to obtain a uniform mixture. 100g of the mixture was measured put and spread on a petri dish and taken to dry under solar dryer for a week, after this period all the substrates were removed from the dryer and those that still had moisture like fish offal (Fish offal contains much fat which holds moisture and cannot easily dry under fluctuating temperatures of solar dryer) were then taken for further drying to the laboratory oven, dried at a constant temperature of 70˚C for about 48 hours until a constant weight was achieved to obtain dry matter (DM). A sample of the DM was used in the testing of crude protein, carbohydrates, and crude fat as discussed below.

3.4.1.1 Crude protein determination

During determination of crude protein, the principle of Kjeldahl was employed using fine crushed samples. Two grams (g) of a substrate-sample were weighed and put into micro-kjeldahl flask for digestion. 5ml of concentrated Sulphuric acid was added to a sample in micro-kjeldahl flask. Five grams(g) of selenium tablets were then added to act as a catalyst and a mixture heated using special laboratory heaters (digester), slowly initially and later rapidly for about 45minutes until the digest turned color to pale green and then cooled. 10mls of the digest was added into the apparatus via a funnel, and 10mls of sodium hydroxide (NaOH) from the measuring cylinder was then added, so that ammonia is not lost. Distillation by Markham distillation apparatus was started to steam up the reagents to remove ammonia which was present. Same procedures were followed for blanks but instead of pure sample, distilled water was used. Distilled alkaline ammonium borate formed was removed and 50mls of 2% baric acid containing screened methyl red indicator was added to titrate distillate with 0.1m Hydrochloric acid. Titration to first appearance of purple color was done. The titre value which is the volume of the acid was noted (Hirpa *et al*., 2015). Crude protein was calculated using the formula:

Crude proteins (%Cp) = (Net titre × normality of HCl × dilution factor × 14×6.25×100) ÷ (weight of a sample) (g) ×1000)

3.4.1.2 Carbohydrates percentage

Carbohydrate percentage was determined by difference for example sum total of %moisture, %ash, %crude protein, %crude fiber, and %crude fat subtracted from 100% for all samples (Hirpa *et al.,* 2015).

% carbohydrate = 100% - (%DM + %ash + %CP + %Cf + %CF)

3.4.1.3 Crude Fats

Fat or ether extract was determined by solvent extraction gravimetric technique described (Kirk and sawyer, 1980) 5 grams of the sample was wrapped in a porous paper and put in a thimble. The thimble was put in a soxhlet reflux flask and then mounted into a weighted extraction flask containing 200ml of petroleum ether. The upper part of the reflux flask connected to a water condenser. The solvent (petroleum ether) was heated, boiled, vaporized and condensed into a reflux flask filled up and siphoned over, carrying down its oil extracts to the boiling flask. This process was allowed to go over repeatedly for 4 hours before the defatted samples were removed. The solvent was recovered, and oil extracts left in the flask. The flask containing oil extracts was dried in the oven at 60 ˚C for 30 minutes to remove any residual solvent, cooled in the desiccator and weighed. The weight of the fat or oil extract was determined by difference and calculated as percentage of weight of sample analyzed (Chinyere, 2014).

% Fat = Weight of flask + Fat – Weight of flask /Weight of sample x100

3.4.2 Effects of different types and quantities of substrates on growth characteristics of black soldier fly larvae

Growth characteristics of BSF larvae was established in terms of weight, length, and survival of the larvae on the substrates. Four 4 plastic containers each measuring were added 250g, 500g, 750g and 1000g of shredded substrates respectively including the corresponding replicates. The neonate larvae of 5-6 days were picked from Ento Organic Farm (U) Ltd (insect breeding centre)- MUARIK. The larvae were counted and weighed using a sensitive scale and each substrate container was added 2500 larvae. The containers were then carried and taken in a temperature-controlled room at 21.5 - 24˚C as described by Chia *et al.* (2018). The BSF larvae were to fed on the substrates for 8 -10 days and after this period, the neonate larvae had much protein and were big and easy to harvest. A sample of 50 larvae was picked from each substrate using forceps and washed with tap water. The larvae were then placed in different Petri dishes, labeled using a masking tape, five (5) larvae were picked from each. These were used in the determination of weight and length. For weight determination, each of the 5 larvae from different Petri dishes were measured using a sensitive scale and recorded in grams (Figure

7). To determine length, each of the 5 larvae was measured using a thread and then placed on a ruler (HACO school ruler, made in Kenya) the length was taken and recorded in millimeters (Figure 7). In addition, survival rate was determined by counting the number of the larvae that remained in the substrate after a rearing period of 10 days and divided by the original number of larvae introduced into the substrates multiplied by 100. Survival rate= final number/initial number x100.

Figure 5: Picking the grown BSF larvae in substrates.

Figure 6: Preparing for BSF larvae data collection

Figure 7: Data collection on black soldier fly larvae

3.4.3 Effects of different substrate types on the protein composition of black soldier fly larvae

During determination of protein composition of BSF larvae (crude protein), the larvae were separated from substrates, cleaned using tap water. Ten (10) larvae were picked from each substrate and its corresponding replicates using forceps as stated by Spranghers *et al,* (2017), placed on petridish and labeled using a masking tape. The larvae were then dipped into boiling water for 2-3 minutes and then put back on petri dish, and placed on the table under a solar dryer for a week (Figure 9) After this period, the larvae that still had moisture was dried in oven at a temperature of 60–70 °C for about 48 hours, the dry larvae were ground to powder using a laboratory mortar. The larval powder was then kept for further analysis in a refrigerator at -20°C. Larval samples were analyzed to establish their proximate composition, as dry matter, crude protein, crude fat, and ash content according to AOAC (2002). Dry matter composition of larvae was determined by oven drying the samples at 105°C until constant weight and content was determined as the difference in weight before and after oven drying. Crude protein content, nitrogen content was determined following the Kjeldahl method and the value was multiplied by a conversion factor of 4.76 (Janssen *et al.,* 2017) to get the crude protein value. Fat content of larvae was determined by diethyl ether extraction in a fat extraction unit (SER 148, Velp Scientifica, Usmate Italy) following the Randall technique which involves dipping of substrate sample in a hot solvent to ensure faster solubility, washing off solvent after boiling, replacement by evaporation, and condensation of the solvent. Ash content was determined by ignition of samples at 55°C in a muffle furnace. Four replicates samples were analyzed for each substrate.

Figure 8: BSF larval drying under a solar dryer

3.5 Statistical data analysis

During data analysis first and foremost, the data were analyzed by Two-way Analysis of Variance (ANOVA) to determine the significant differences between substrates, and levels of substrates. Least significant difference (LSD) was used to separate means between substrates and levels of substrates at 95% confidence interval (CI). Data were then presented in tables and graphs. For the data that were heavily skewed to either left or right upon normality test by Shapiro wilk's (Ghasemi and Zahediasl, 2012), was used to transformed such data.

CHAPTER FOUR: RESULTS

4.1 Nutrient composition of different substrates for BSF larval feeding

There was no interaction between substrates and levels of substrates. However, substrates and its levels were highly significant (prob<0.0001) and each contributed to the growth parameters (length, weight) and survival rate of BSF larvae. (Table 4.2).

Table 4.2: Multivariate interaction of feeding substrates with growth parameters

Term	d.f	Wilk's lamdda	Rao F	n.d.f	d.d.f	F-prob
Sub	5	0.0007	58.32	20	150	P< 0.0001
L of sub	3	0.2461	6.95	12	119	P< 0.0001
Sub*L	15	0.0629	3.06	60	178	P<0.0001

*SUB=Substrates, L= Levels of substrates, Sub*L Substrate and Levels of substrate*

The Mixed substrate (MS), Fish offal (FO) and Control (CONT) recorded the highest crude protein and fat. Pineapple waste (PW) and Jack Fruit waste (JFW) also recorded high values for carbohydrate. Rumen content (RC) recorded the lowest crude protein (Table 4.1).

Table 4.1: Proximate analysis of nutrients composition of selected feeding substrates for BSF larvae.

Substrates (g)	Nutrient composition (DM)		
	Carbohydrate (%)	Crude protein (%CP)	Crude Fat (%CF)
PW	48.60	4.20	3.80
JFW	50.50	4.20	5.60
RC	31.20	1.00	5.30
FO	24.30	29.40	15.80
MS	45.00	7.30	9.60
CONT	58.7	12.10	9.00

CONT =Control, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO =fish offal, MS= mixed substrates.

4.2 Effects of types and quantities of substrates on growth characteristics of BSF larvae

There was significant ($p<0.001$) difference between length of the larvae; the highest larval length was recorded in Mixed substrate and the lowest was recorded in Fish offal. The Larval weight also showed a high significant difference ($p<0.001$); the highest larval weight was recorded in Mixed substrate and the lowest was registered in Fish offal. There was a high significant ($p<0.001$) difference between Survival rate of the larvae; the highest survival rate and lowest was recorded on Mixed substrates and Fish offal respectively (Table 4.3).

Table 4.2: Effect of substrate types on growth and survival of BSF larvae.

SUBSTRATES	Larval length (mm)	Larval weight (g/FM)	Larval survival rate (%)
CONT	10.06	0.205	96.01
PW	9.62	0.168	72.96
JFW	10.30	0.212	70.09
RC	10.01	0.171	85.16
FO	9.36	0.156	34.15
MS	11.30	0.318	96.01
LSD $_{(5\%)}$	1.566	0.348	04.42
P-VALUE	P<0.001	P<0.001	P<0.001

CONT =Control, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO =fish offal, MS= mixed substrates.

There was significant (p=0.03) difference between levels of substrates which varies according to the substrates. The larval survival rate showed several trends, whereby it generally increased from feeding levels 250g to 1000g in Control, Jack fruit waste, Rumen control and Mixed substrate. Fish offal decreased from 250g to 1000g (Table 4.4).

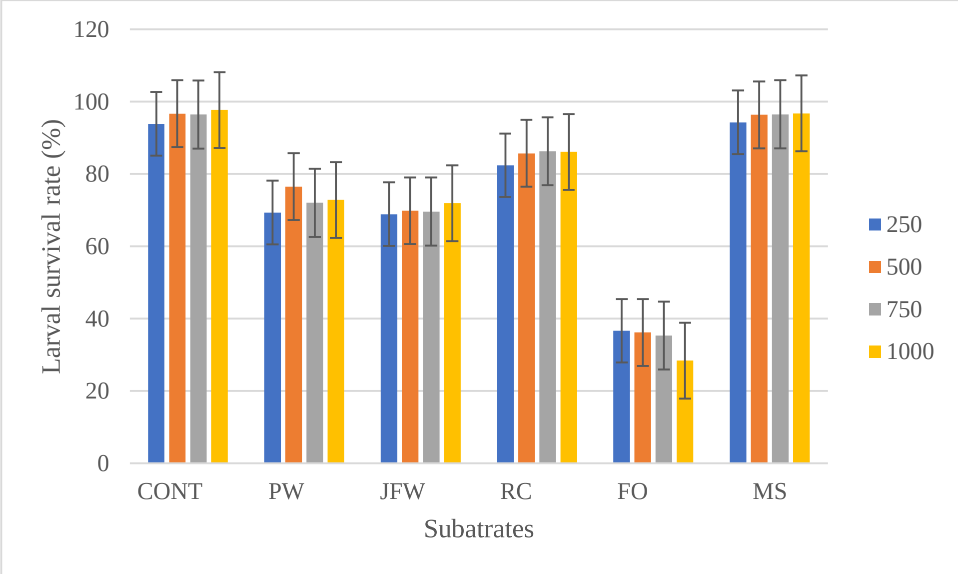

Figure 9: Substrate amounts influenced survival rate of BSF larvae

CONT= Reference substrate, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO= fish offal, MS= mixed substrates.

There was high significant ($p<0.001$) between levels of substrates which varies according to the substrates. The larval length showed several trends, whereby it increased from feeding levels 250g to 750g across levels of substrates; decreased from 750g to 1000g among the levels of substrates. The highest larval length was attained at feeding level 1000g, and the lowest was observed at level 250g across substrate levels (Figure 3).

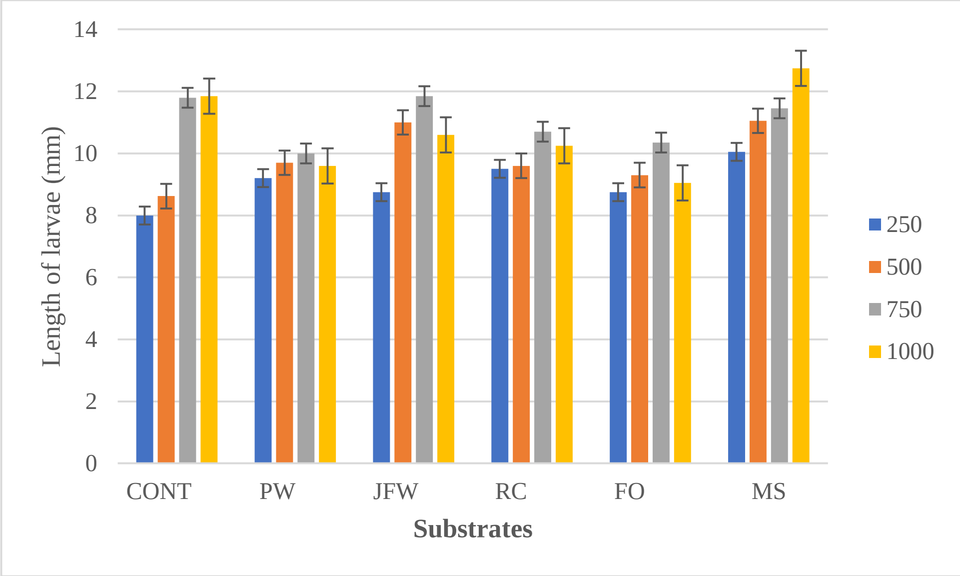

Figure 10: Larval length influenced by substrate amounts

CONT =Reference substrates, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO =fish offal, MS= mixed substrates.

There was high significant ($p<0.001$) difference between levels of substrates which varies according to the substrates. The larval weight showed several trends, whereby it generally increased from feeding levels 250g to 1000g across levels of substrates; The highest larval weight was generally achieved at 750g, and the lowest was achieved at feeding level 250g (Figure 4).

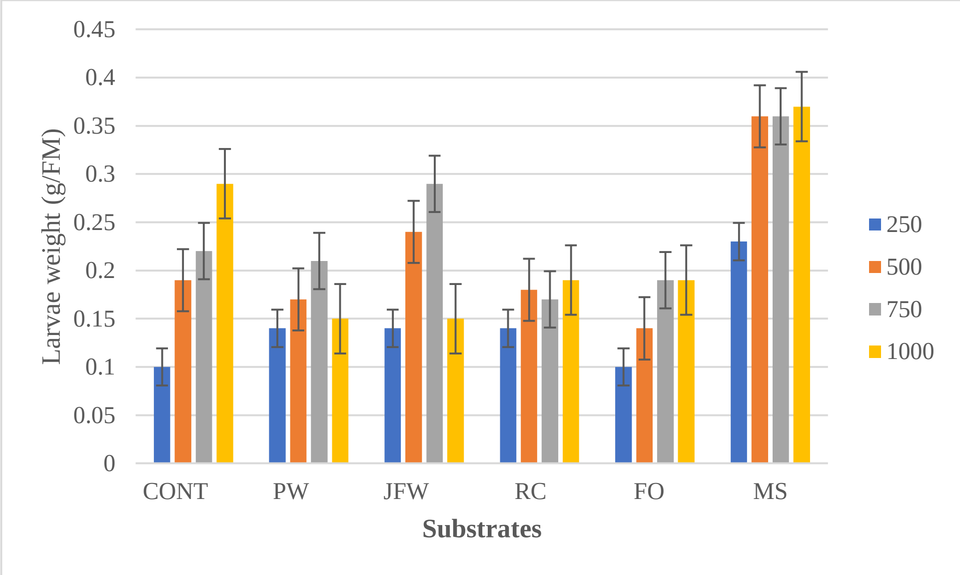

Figure 11: Larval weight influenced by substrate amounts

CONT =Control, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO =fish offal, MS= mixed substrates.

4.3 Effects of different types and quantities of substrates on Protein composition of BSF Larvae

There was high significant ($p<0.001$) difference between substrates. Control and Rumen content recorded the highest mean crude protein of 19.92 and 17.89%, respectively. Pineapple waste and Jack fruit waste also recorded values for larvae crude protein but Pineapple waste out performed Jack fruit waste; Fish Offal substrates recorded the lowest mean crude protein (Figure 5).

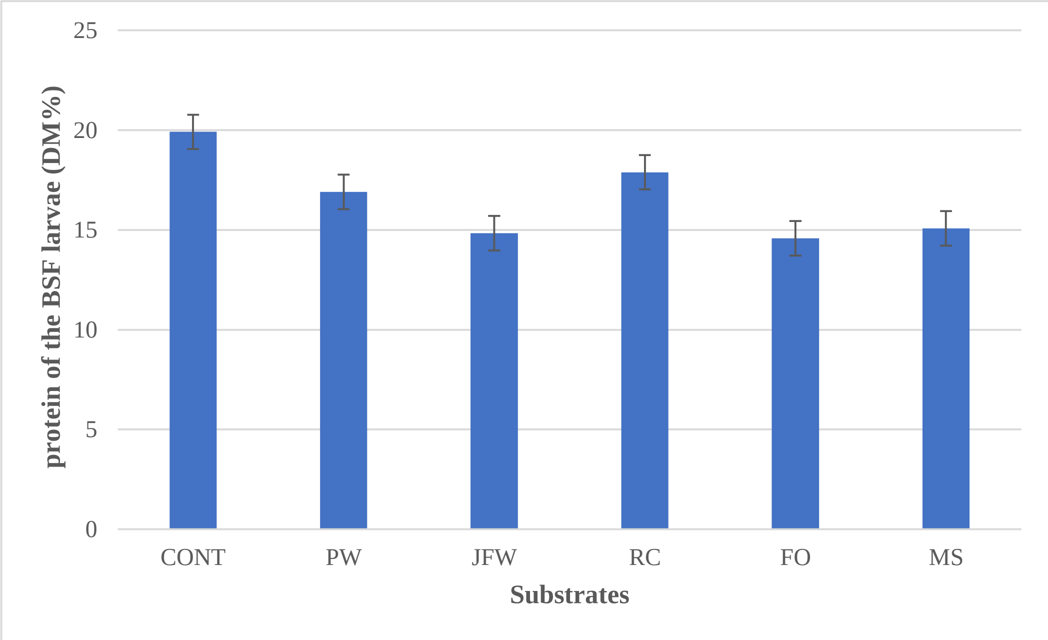

Figure 12: Crude protein of BSF larvae fed selected substrates

CONT =Reference substrate, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO= fish offal, MS =mixed substrates.

There was high significant ($p<0.001$) difference between levels of treatments which varies according to the substrates. The highest larval crude protein recorded was 20.74% and 19.48% in Control and Rumen content that increased from feeding level 250g to 1000g respectively. The trend also revealed an increase in larval crude protein in Control from 500g to 750g; Pineapple waste, Jack fruit waste and Mixed substrates produced the larva having the highest crude protein at level 500g that increased from feeding level 250g to 500g; larval Crude protein levels decreased from 750g to 1000g in Pineapple waste, Control and Mixed substrate while Jack fruit waste and Fish offal larval crude protein increased at same levels. Fish offal recorded the highest crude protein at 1000g which later increased from 250g to 1000g. The highest and lowest feeding levels were achieved at 500g and 1000g respectively (Figure 7).

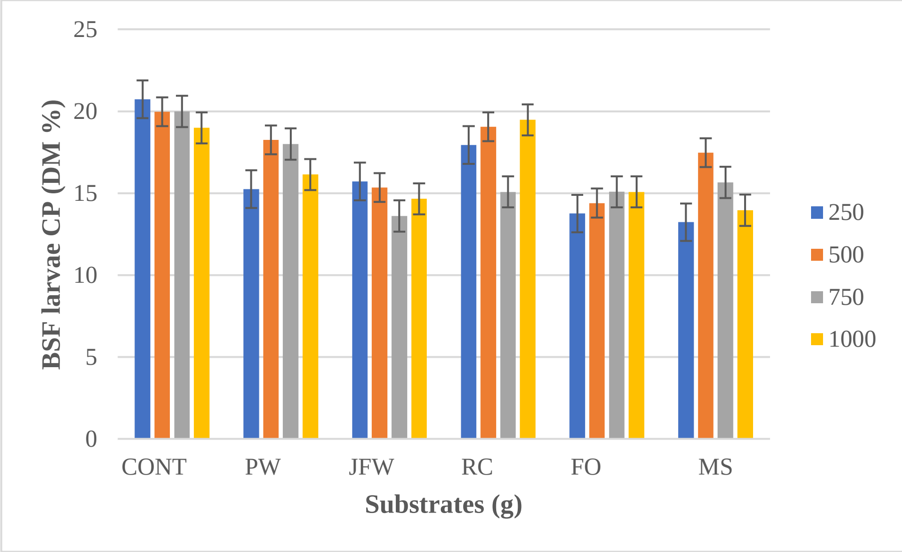

Figure 13: Substrate amounts on protein composition of BSF larvae

CONT= Reference substrate, PW= pineapple waste, JFW= jack fruit waste, RC= rumen content, FO= fish offal, MS= mixed substrates

CHAPTER FIVE: DISCUSSIONS

5.1 Nutrient composition of different substrates

The results show that the plant-based substrates (Pineapple Waste, Jack Fruit Waste) and Mixed Substrates recorded the highest carbohydrate percentage compared to animal-based substrate. The bulk of BSF diet in nature is mainly carbohydrate (Barragan-Fonseca, 2018; Gold *et al.,* 2018). Studies have shown that dietary carbohydrate are used to control enzyme processes and tissue development (Cohen, 2015). Therefore, the substrates with high carbohydrates content are more desirous if these can combine with other nutrients such as protein and fats in their recommended proportions

The highest protein of Mixed Substrate is attributed to substrate mixing, Nguyen *et al. (*2013). The BSF larvae requires a minimum of about 10% protein as a source of amino acids for larval development (Spranghers, 2019). However, when selecting a substrate for BSF larval nutrition, the quantity of protein is key for larval development (Barragen-Fonseca, 2018).

The results indicate that Fish Offal and Mixed Substrates recorded the highest crude fat. The highest fat in the Mixed substrates is also attributed to substrate mixing, Nguyen *et al. (*2013), fats or lipids provide fatty acids, or mono diglycerides for uptake by the gut cells for use in larval metabolism. Many studies have reported that fats may not affect BSF larval development except when given in excess but the minimum crude fat requirement for BSF larvae is 2% (Gold, 2018).

5.2 Effects of types and quantities of substrates on growth of BSF larvae

Survival rate (%) of the larvae was highly associated with protein content of larvae (prob<0.001). The highest survival rate of the larvae on Mixed substrate may have been attributed to the high crude protein of the substrate (figure 9). This finding is in line with Nguyen *et al*. (2013). Rumen content recorded higher values than Fish Offal among the animal-type substrates. This is may have been attributed to the numerous bacteria that come from the rumen along with the substrate; these bacteria increased decomposition of the substrate and generated more nutrients Li *et al.* (2011). However, Fish Offal substrate recorded the lowest survival rate of the animal-type substrates, this is may have been caused by excess fats in the feeding substrate which may have interfered with uptake and absorption of nutrients (Nguyen *et al.*, 2013). Plant-type substrate also registered high Survival rate in this study. However, Jack fruit waste recorded slightly lower values than Pineapple Waste. This could have been attributed to high fiber content of the Jack fruit that might have limited the larvae from feeding on the substrates, this study matches with the study of Tomberlin *et al*. (2009).

Contribution of substrate amount/levels to larval survival rate on the substrates

The high survival rate of the larvae obtained at high feeding level (1000g) across all substrates with exception of Fish Offal is possibly due to the highest crude protein content in the substrates that might have increased with additional substrate amounts from 250 to 1000g. However, the lowest survival rate recorded from the larvae fed on Fish Offal is caused by excess crude fat (Table 2) in the feeding substrates that could have limited larvae aggregation, mobility, aeration, feeding, growth and hence survival. This finding is in agreement with Nguyen *et al. (*2013), the study reported high survival rate, growth, and decline in developmental time of larvae fed on higher protein diets. However, research have shown that increased fat amounts, 20-36% Crude fat DM) can be harmful to the larvae and possibly adult survival, and reproduction. The decreasing survival rate of the larvae on Fish offal may have been caused by increasing fat levels in the substrate that increased with additional substrate amounts (Figure 9).

In comparison with other studies, the mean larval survival rate (96%) of this study is above the results of Oonincx *et al*. (2015), recorded the highest survival rate of 78.9±13.2 using vegetable waste as feeding substrate under controlled conditions.

There was a positive correlation between larval weight and larval length (prob<0.001, R=0.7774). The highest mean weight and length of the larvae (Figure 10 and 11), fed on Mixed substrates may have been attributed to high nutritional quality of the diet generated by mixing the substrates; this finding is in line with Nguyen *et al.* (2013), this study registered a greater weight and length on high protein diet substrates. Additionally, larvae fed on Jack fruit waste substrate also recorded higher weight and length. This is may have been attributed to high fat in the substrates that attracted the larvae to feed quickly and grow bigger and longer. More so, the lowest values recorded by Fish offal substrates is probably due to high production of ammonium compounds that may have been caused by increased fat amount in the substrates that may have limited feeding and ultimately reduced larvae weight, and length. This finding is in line with Nguyen *et al.* (2015), the study reveals that extremely high fat amounts in diets (20 – 36% DM crude fat) contributed to slow growth (weight and length).

Contribution of substrate amount/levels to larval length

At 250g of substrate (Figure 9), the larvae were shortest; this is because 250g of substrate was not enough for the larvae. This finding agrees with Nguyen *et al.* (2013), this study reported an increase in larval length and weight, were proportional to the amount of feed given. The increasing length of the larvae fed on 250 and 750g of substrates may been be attributed to increasing nutrient amounts that increased with additional substrates. However, the decline of larval length at 1000g of substrates may have been caused by accumulation of waste in the feeding substrates; this finding is in agreement with Banks *et al.* (2014), this study reported that as more substrate was supplied to larvae, it positively affected both development, length and weight in BSF, but negatively affected waste reduction efficiency.

Contribution of substrate amounts/levels to larval weight

Increase in mean larval weight from feeding levels 250g to 1000g in Mixed substrates is attributed to high nutritional superiority of the substrates that may have driven the BSF larvae to increase feeding and obtain much nutrients to grow, this finding matches with the findings of Leong *et al.* (2016). The study reported that weight gained by BSF larvae generally increased as the substrate quantities of palm oil waste increased (1g/day to 25g/day). In comparison, lower feeding levels used in this study; feeding at level 250g generally resulted in comparatively low average larvae weight compared to higher feeding levels. This may have been attributed to intraspecific competition that caused starvation, limited growth, and development as well, Mitchell- Foster *et al.* (2012). However, the lower larval weight of BSF larvae fed on Fish Offal at 1000g of substrate may have been attributed to increased ammonium compounds caused by increasing fat amounts in the substrates that increased by additional substrates and thus compromising feeding and therefore decline in weight of the larvae. This finding is agreeing with Nguyen *et al. (*2015), increased fat mounts in diets; 20-36% crude fat contributed to reduced growth and low weight larvae.

Generally, the findings shows that the optimum weight that was obtained at level 750g/2500 larvae of 0.313g was highest in comparison with Oonincx *et al.* (2015) findings (0.158±0.02 and 0.13±13.2) of the experiment conducted using vegetable waste as feeding substrate conducted under laboratory-controlled conditions

5.3 Effect types of substrates and quantities on protein composition of BSF larvae

The highest crude protein means of larvae fed on Rumen content substrate may have been attributed to numerous ruminal bacteria that continue to breakdown the substrate to release more protein and hence improving the uptake and digestibility of the substrate. BSF larvae fed Jack fruit and pineapple waste recorded high protein values. However, Pineapple waste recorded slightly higher protein than Jack fruit waste, this is attributed to low fiber content of Pineapple fruit compared Jack fruit, studies have reported that higher fiber content in the substrate can cause slow growth because it reduces the amount of feed, decrease feeding and possibly feed intake as well as digestibility and nutrient utilization, this finding matches with the study of Tomberlin *et al*. (2009). However, the slightly lower value registered for Mixed substrate may have been attributed to increasing amount of fat in the substrate (Figure 12), Other studies have reported that increased fat amounts in diets reduce BSF larvae growth (Barry, 2004). Finally, the lowest larval mean of BSF larvae given Fish Offal substrate is attributed to too much fat in the substrate that decreased feed intake and nutrient absorption as result of reduced mobility of the larvae to search for food with in the substrate.

Contribution of substrate amounts to protein of BSF larvae

Rumen Content produced larvae with highest crude protein at 1000g of substrate, this may have been attributed to the increasing number of rumen bacteria that associates with the ruminal content. Possibly, bacteria concentration increases with additional feeding amounts and thus the highest protein was achieved 1000g of the substrate. Additionally, bacteria decompose the substrate gradually and rate of decomposition and release of nutrients is slow and this explains why the optimum crude protein of the BSF larvae was achieved at a highest feeding level (1000g) of the substrate. This finding is in agreement with Yu *et al.* (2011), reported that bacterium *Bacillus subtilis*, that was removed from the BSF larval gut, promoted growth, and development of con-specific larvae by slowly fermenting their food. Possibly because *B. subtilis* has the potential to digest the non-protein nitrogen (NPN) in the substrate and provide true protein (bacteria protein). Additionally, Pineapple Waste, Jack Fruit Waste and Mixed Substrate recorded highest protein at 500g, the proximate analysis of these substrates indicated crude that was well below the minimum requirements of BSF larvae meaning that the BSF larvae had to feed more substrate to derive more crude protein to compensate for the low protein and hence the optimum protein of the BSF larvae attained at feeding level 500g. However, the decline in crude protein from 750g to 1000g is attributed to accumulation of larval waste, this finding matches with the study of Banks *et al.* (2014), The study findings reported that as more substrate was made available per larva, larval development time increased and weight decreased, waste reduction and larval protein content decreased. However, the lowest crude protein of BSF larvae fed on Fish Offal at 1000g of substrate is attributed to increasing concentration of fat in the substrate that may have increased with additional substrate amounts and this greater fat content in the substrate could have also reduced mobility of the larvae to search for food.

In comparison with other studies, the crude protein (15-20%) of this experiment is below the findings of Evan (2018) who got the highest crude protein (30-40%) using vegetable waste as feeding substrate to produce BSF larvae and pre-pupae used as feed for fish under controlled conditions. High crude protein in other studies may have been attributed to the different methodologies employed like the feeding regimes, weighing the larvae at certain time interval to check for weight gain and establish the food substrates to add, substrate agitation by raking to ensure uniform aeration, and feeding within the substrate. Also considering the fact that the substrates used in this study were different from other studies and the fact the study was conducted under un-controlled conditions.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

BSF larvae have many uses, beginning from beneficial to environmental; waste management, removal of odors, reduction of greenhouse gases in the atmosphere and degradation of the environment, to benefits of food security hence making it one of the vital insects. However, suitable realization of benefits is dependent on right selection of feeding substrates. This is important while considering demand for feed security, and escalating quantities of waste that community produces. BSF larvae will contribute to food security directly, and also indirectly by reducing demand for currently used livestock farming ingredients, such as silver fish and soya bean which can be left for human consumption.

In objective 1, all substrate that were in line with the nutrient requirements of BSF larvae performed better in terms of growth parameters and nutrient composition. Substrates with high carbohydrates content are more desirous if these can combine with other nutrients in the required proportions.

In objective 2, the study revealed that though BSF larvae can survive and grow on various substrates, not all substrates are suitable for BSF larvae production but suitability depends on quantity of substrates. Consequently, the study evaluated the potential of feeding substrates in terms of survival, length, and weight of the larvae. The study reports that Jack fruit waste, Pineapple waste, Rumen content and Mixed Substrates are all suitable for growth of BSF larvae with exception of Fish Offal. The study further reveals that feeding quantities or levels entirely depends on substrate composition in terms of crude proteins. For example, substrates with low crude protein will attract larvae to feed more to attain higher growth. Generally, the findings showed that the optimum length and weight was obtained at level 750g/2500 larvae of the substrate

With respect to objective 3 of the research, proximate analysis of BSF larvae fed on different substrate types and substrate mixture indicated enough crude protein for use in livestock supplementation. But quantity of the protein in the larvae depends on the composition of feeding substrates in terms of crude protein: crude fat ratio, therefore, high crude protein: low crude fat ratio produces larvae with high protein and vice versa but excess fat is detrimental to larvae and its crude protein. The study further reveals that crude protein of the larvae drops at higher feeding levels especially when the larvae is fed on substrates containing high protein and low fat. The study still indicates that BSF larvae produce more protein at low feeding level and less protein at highest feeding level hence the highest crude protein was achieved at 500g/2500larvae of substrate. Conclusively, in respect to the above objective, BSF larvae becomes a suitable supplement for protein in livestock production.

6.2 RECOMMENDATIONS

The study recommends that, among the substrates used to feed the BSF larvae, Rumen Content (RC), is the best option for farmers intending to rear BSF larvae for feeding livestock so as to cut on the costs associated with feeding.

From the results of this study, future research studies are needed in the following areas;

- A study of BSF larvae feeding on other substrates/combinations of substrates so as to have a complete data base of the best substrates for BSF larvae farming.

- There is need to conduct further study on feeding Black soldier fly larvae on high fat diets and its implications on growth.
- There is need for a study on feeding BSF larvae on Rumen content at varied feeding amounts, under different feeding regimes at different growing stages
- There is need to do research to establish the standard length under quality nutrition and optimum feeding of BSF larvae
- There is need for a study to establish the nutrient composition of BSF Larvae reared on various substrates in form of amino-gram and fatty acid composition to facilitate more accurate usage of BSF larvae in feed supplementation.
- There is also need for a study on proper management of BSF larvae neonates since these determine the future growth traits.

REFERENCES

Alders R. (2004). Poultry for profit and pleasure. FAO Diversification Booklet No. 3. Rome.

AOAC (2002). Official methods of analysis of AOAC International. 17th edition Arlington, Virginia United States of American.

Barnard D, Geden C. (1993). Influence of larval density and temperature in poultry manure on development of housefly (Diptera: Muscidae). Environmental Entomology, 22: 971 – 977

Banks I.J, Gibson WT, Cameron M.M. (2014). Growth rates of Black soldier fly larvae fed on fresh human feces and their implication for improving sanitation. Tropical Medicine International, Health 19: 14 – 22

Barragan- Fonseca, K.B, Dickie M, Van Loon. (2017). Nutritional Value of Black soldier fly (Hermetia illucens) and suitability as animal feed – a review. Journal of Insects Food Feed 3: 105 – 120

Barragan-Fonseca, KB. (2018). Dissertation, Flies are what they eat, Wageningen University and Research, the Netherlands

Barry T. (2004). Evaluation of economic, social and biological feasibility of bio converting food wastes with black soldier fly (Hermetia illucens)

Bosch G, Zhang S, Oonicx DGAB, Hendriks WH. (2014). Protein quality of insects as potential ingredient for dog and cats. Journal Nutrition Sciences 3 (e 29): 1 – 4

Bradley S W, Sheppard DC (1984). House fly oviposition inhibition by larvae of *Hermetia illucens*, the black soldier fly. Journal of chemical ecology, 10: 853 – 859

Chia SY, Tanga CM, Osuga IM, Mohammed SA, Khamis FM. (2018). Effects of waste stream combinations from brewing industry on performance of BSF, Hermetia illucens. Wiley-Black well, peer J6: e588

Cligiani A, Mareglia A, Leni G, Baldassare S, Maistrllo L, Dossena A, Sforza S. (2018). Composition of black soldier fly prepupae and systematic Food Resource International, 105: 812- 820

Cohen, AC. (2005). Insects Diets, CRC press, Taylor and Francis group, Broken sound park way NW, USA

Couret J, Dotson E, Benedict MQ. (2014). Temperature, larval diet and density effects on development rate and survival of Aedes aegpti (Diptera: Culicidae). Plos **1**, 9, e87468

De Haas EM, Wagner C, Koelmans AA, Kraak MH, Admiraal W. (2006). Habitat selection by chironomid larvae; fast growth requires fast food. Journal of Animal Ecology*,* 75: 148-155

Diener S. (2010). Valorization of organic solid waste using black soldier fly, Hermetia illucens. Swiss Federal Institute of Aquatic Science Technology (Eawag). ETH Zurich

Diener S, Zurbrugg C, Tockner K. (2009). Conversion of organic material by black soldier fly larvae; establishing optimal feed rates. Waste Management Resource 27 (6): 603 – 610

FAO, World Livestock. (2011). Livestock in Food Security; Food and Agriculture Organization of UN (United Nations). World Population Prospects 2019: Highlights; United Nations Department of Economic and Social Affairs Available online: https://www.un.org/development/desa/publications/world-population prospects-2019-highlights.html. (Accessed on 1 March 2021)

FAO. (2013). World Food Programme – The state of food insecurity in the world. The multiple dimensions of food security, Food and Agricultural Organization. Rome

Finke MD. (2002). Complete nutrient composition of commercially raised invertebrates used s food for insectivores. Zoo Biology, 21 (3):269 – 28

Finke MD. (2013). Complete nutrient content of four species of feeder insects. Zoo Biology, 32: 27 – 36 processed Ruspolia nutidula (edible grasshoppers). Food Science: Nutrition 5 (1)

Ghasemi, A, Zahediasl, S. (2012). Normality test for statistics: A guide for non Statisticians. International Journal Endocrine**,** 10: 486 – 489

Gobbi P, Martinez-Sanchez A, Rojo S. (2013). The effects of larval diet on adult life history traits of black soldier fly larvae, Hermetia illucens (Diptera: stratiomyidae), European Journal of Entomology, 110: 461 – 468

Gold M, Jeffery K, Tomberlin, Diener S, Zurbrugg C, Alexander M. (2018). Waste management and chemicals in Black soldier fly, A review Biovision foundation, 147: 8005

Green P, Popa R. (2012). Enhanced ammonia content in composite leachate processed by soldier fly larvae. Applied Biochemistry and Biotechnology, 166: 1381 – 1387

Green PW, Simmonds MS, Blancy WM. (2002). Does the size of Larval groups influence the effects of metabolic inhibitors on development of phormia regina larvae? European Journal of Entomology 99: 19-22 Gueye. (2000). The role of poultry in Poverty alleviation, Food security and promotion of gender Equality in Rural Africa. Outlook on Agriculture 29 (2): 129 – 135

Gueye, E.F. (2002). Family poultry research and development in low – income Food deficit countries: approaches and prospects. Outlook on agriculture 31(1): 13 – 21.

Guohui Y, Chuyan N, Guobao H, Lin Z, Qiang X, Ping C. (2010). Isolation and Identification of bacteria producing enzymes from the gut and skin of black Soldier fly (Hermetia illucens) larvae. Chinese Bulletin of Entomology, 47: 889 – 894.

Hirpa, L, Nigussie, Dechassa, R, Setegn, G and Bultosa, G. (2015). Chemical quality of common beans is influenced by genotypes and aluminum rates under two soil linning regimes. Africa journal of food agriculture and nutrition, volume (15): 975 – 987.

Holmes L, Vanlaerhoven S, Tomberlin J. (2016). Lower temperature threshold of black soldier fly larvae (Diptera: Stratiomyidae) development. Journal of insect as food and feed, 2:255 – 262Holmes LA, Vanlaerhoven SL, Tomberlin JK. (2012). Relative humidity on effects on life-history of Hermetia illucens (Diptera: stratiomyidae). Environmental Entomology, 41: 971 – 978

Ines J, Fernandes M, Murakami AE. (2010). Arginine metabolism in unicotelic species Animal Sciences Maringa, 32 (4): 357 – 366

Jannat KNE, Roitberg DB. (2013). Effects of larval density and feeding rates life traits Anopheles gambiae spp (Diptera: culicidae), Journal Vector Ecology 38: 120 – 126

Jarosik V, Kratochvil L, Honesk A, Dixon A. (2004). A general rule for dependence of developmental rate on temperature in ectothermic animals. Proceedings of Royal society of London. B: Biogical Sciences, 271: S219 - S221

Kalova M, Borkovcova M. (2013). Voracious large Hermetia illucens treatment of selected types of biodegradable waste. Acta Universitatis Agriculture et silviculturae Mendalianae Brunensis, 33: 307 – 310

Kirk, R. E. (2013). Experimental design: procedures for the behavioral sciences 4^{th} edition. Thousand Oaks, CA: Sage

Komi F, Nakibugwe D. (2017). INSFEED – integrating insects in poultry and fish feed in Kenya and Uganda. IDRC project Number: 107839

Komutunga, ET and Musiitwa, F. (2001). Climate in: Mukiibi, J.k, Ed, Agriculture in Uganda volume 1: general information fountain publishers, Kampala, 21 – 32.

Larde G. (1990). Recycling of Coffee Pulp by Hermetia illucens (Diptera: stratiomyidae) larvae. Biological wastes, 33: 307 – 310

Leong SY, Rahman S, Kutty M, Malakmad A, Tan, CK. (2016). Feasibility study of biodiesel production using lipids of a Hermetia illucens larvae fed with organic waste. Waste Management, 47: 84 – 90

Li, Q, Zhang, L, Qiv, N, Cai, H, Tomberlin, J.K, Yu, Z. (2011). Bioconversion of dairy manure by black soldier fly (Diptera: stratiomydae) for biodiesel and sugar production. Waste management, 31: 1316 - 1320

Liu Q, Tomberlin JK, Brady JA, Sanford MR. (2018). BSF Larvae reduce Escherichia coli in dairy manure, Environmental Entomology, 37: 1525- 1530

Liu Q, Tomberlin JK, Brady JA, Sanford MR, Yuz. (2008). Black soldier fly (Diptera: stratiomyidae) larvae reduce Escherichia Coli in dairy manure. Environmental Entomology, 37: 1525 – 1530

Markkar HP, Trans G, Heuze V and Ankers P. (2014). State of the art on use of insects as animal feed. Animal Feed Science Technology. 197: 1-33

Moreau J, Benrey B, Thiery D. (2006). Assessing larvae food quality for pytophagous insects: are the facts as simple as they appear? Functional

Ecology, 20: 592 – 600

Moo CY, Hadura AH. (2022). Effect of feeding rates on growth performance and waste reduction efficiency of black soldier fly larvae (Diptera stratiomyidae). Tropical life science resource 33 (1): 179 – 199

National Research Council. (NRC). 1995. Nutrient requirements of laboratory animals Washington DC. National Academy Press

Newton GL, Booram CV, Barker RW, Halc O. (1997). Dried Hermetia illucens larvae meal as supplement for swine. Journal Animal Science. 44: 395 – 400

Nguyen TT, Tomberlin JK, Vanlaerhovens. (2013). Influence of resources on Hermetia illucens larvae development. Journal of medical entomology, 50: 898-906

Nguyen TT, Tomberlin JK, Vanlerhoven. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environmental Entomology, 44**:** 406 – 410

Nyakeri E, Ayieko M, Amimo F. (2018). Performance of two fish feeds. Growth performance of fish feed of Black Soldier Fly prepupa and Fish Meal formulated feeds.

Omiti MJ, Okuthe, SO. (2009). An Overview of the Poultry Sector and Status of Highly Pathogenic Avian Influenza (HPAI) in Kenya; Africa/Indonesia Team Working Paper No. 4; International Food Policy Research Institute (IFPRI): Washington, DC, USA, 2009; p. 117.

Oonincx D, Van Broekhoven S, Van Huis Loon JJA. (2015). Feed conversion, survival and development and composition of four insect species on diets composed of food by products. PloS 1,10: e0144601

Paz, A.S, P, Carrejo, N.S, Gomez Rodriguez CH. (2015). Effects of larval density and feeding rates on the bio-conversion of vegetable waste using black soldier fly larvae Hermetia illucens (L), (Diptera: Stratiomyidae). Waste and biomass valorization, 6: 1059 – 1069

Permana A, Ramadhani EP. (2009). Growth of black soldier fly larvae (Hermetia illucens) larvae fed on spent coffee ground. IOP conference series. Earth and Environmental Science doi: 10. 1088/1755 Rachmawati R, Buchori D, Hadayat P, Hem S, Fahmi (2010). Perkembbangan dan kandungan nutrisi larvae *Hermetia illucens* (Linnaeus) (Diptera: stratiomyidae) pada bungkil kelapa sawit. Journal Entomology Indonesia, **7:** 28 – 41

Rivers DB, Dahlem GA. (2013). The Science of Forensic Entology, wiley- Black well, Chichester, UK.

Sheppard, D.C, Tomberlin, J.K, Joyce, J.A, Kiser, B.C, and Summer, S.M. (2002). Rearing methods for the black soldier fly (Diptera: stratiomyidae). Journal of Medical Entomology, 39: 695- 698

Sheppard DC, Newton GL, Burtle G. (2008). Black soldier fly prepupae a a compelling alternative to fish meal and fish oil. Alternative feeds for for aquaculture. The national marine fisheries services.

Simon P, Kruger R, Ribeiro P. (2011). Influence of diets on rearing of predatory flies of housefly larvae. Arquivo Brasileiro de Medicina Veterinaria eZootecnia, 63: 1414 – 1420

Spranghers T, Ottoboni M, Klootwijik C, Ovyn A, Deboosere B, De Meulenaer B. (2016). Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrate. Journal of Science of Food and Agriculture**,** 97: 2594 - 2600

Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Debroosere S, De Meulenaer B, De Smet S (2017) Nutritional composition of black soldier fly larvae (Hermetia illucens) prepupae reared on different organic waste substrates. Journal of Science of Food and Agriculture**,** 97: 2594 – 2600, 97(5): 2594 – 2600

Ssepuuya G, Mukisa, Nakibugwe D. (2017). Nutritional composition, Quality and self-stability of processed Ruspolia nutidula (edible grasshoppers). Food Science: Nutrition 5 (1): 103-112 Tomberlin JK, Sheppard DC, Joyce JA. (2002). Selected life-history traits of black soldier flies (Diptera: stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95: 379-386

Tomberlin, J.K, Adler, P.H, Myers, H.M. (2009). Department of black soldier fly Research progress on the larvae and prepupae of black soldier fly Hermetia illucens used as animal feed stuff China Bull Entomolgy. 38, 930 - 934

Tschirner M, Simon A. (2015). Influence of different growing substrates and processing on nutrient composition of black soldier fly larvae destined for animal feed. Journal of insects as Food and Feed, 1: 249 – 259

United Nations. (2019). World population prospects, highlights of the United Nations department of Economic and Social Affairs. Available online: https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html (accessed on 1 March 2021)

Van Broek hoven SV, Oonincx DGAB, Van Huis A. (2015). Performance and feed conversion efficiency of 3 edible meal worm species (Coleoptera; tenebrionidae) on diets composed of organic by products. Insect pyhsiology 73:1-10

Van der Spiegel M, Noordam MY, Van Der Fels – Klerx HJ. (2013). Safety of novel source (Insects Micro algae seaweed, duckweed and rape seed) and legislative aspects for their application in food and feed production Comprehensive reviews in food science and food safety 12: 662 - 678

Van Huis A. (2015). Edible insects contributing to food security; Agriculture Food secure. Doi: 10.1186/ s40066-015-0041-5

Van Huis A, Van itterbeeck J, Klunder HC, Mertens E, Holloran A, Muir G, Vantomme. (2013). Edible insects-future prospects food and feed security Food and Agricultural Organization Rome

Van Zanten HHE, Oonincx DGAB, Mollen horst, Bikker p, Meerburg and G deboer IJM. (2014). Can the environmental impact of livestock feed be reduced by using waste feed housefly larvae? Proceedings of the 9th International conference on life cycle assessment.

Veldkamp T, Bosch. (2015). Insect a protein rich feed ingredient in pig and Poultry diets, Animal Front 5:45 – 50

Veldkamp T, Van Duinkerken G, Van Huis A, Lakemond, C. M. M, Ottevanger E bosch G, Van Boekel T. (2012). Insects as a sustainable feed ingredient in pig and poultry diets. A feasibility study = Insecten als duurzame dierroedergrondstof in varkens – en pluimvee voeders: een haalbaarh eidsstdie (Wageningen UR livestock research)

Yost D, Eswaran H. (1990). Major land resource area of Uganda, world soil resources, conservation services-USDA, Washington DC 227p

Yu, G, Chen, Y, Li, Cheng P. (2009). Research progress on larvae and prepupae of black soldier fly Hermetia illucens used as animal feed stuff. Chin. Bull Entomology 46: 41 – 45

Zheng L, Crippen TL, Singh B, Tarone AM, Dowd S, YuZ. (2013b). Survey of bacterial diversity from successive stages of black soldier fly (Diptera: stratiomyidae) by using r DNA pyrosequencing. Journal of Medical Entomology, 50: 647 – 658