ASSESSMENT OF REARING TECHNIQUE FOR THE BLACK SOLDIER FLY AND TERMITE COLLECTION TECHNIQUE FOR USE BY SMALLHOLDER POULTRY AND FISH FARMERS IN GHANA.

BY

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DECLARATION

This is to certify that this thesis is the result of original research undertaken by Boafo Hettie Arwoh of the African Regional Postgraduate Programme in Insect Science, University of Ghana, under the supervision of Prof. Vincent Eziah, Dr Eric Cofie Timpong - Jones, and Dr Maxwell Billah. This research has not been included in any thesis or dissertation submitted to any other institution for a degree or any qualification. Authors whose works were used have been duly referenced/ recognised.

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ABSTRACT

Insects offer a cheap source of protein for especially smallholder poultry and fish farmers. Insects contain about 40-70 % protein, 35 % fat, and other micro-nutrients. The black soldier fly (BSF) larvae and termites are promising insects that can replace the animal protein component of livestock feed. The full adoption of their use by farmers is however slow due to insufficient scientific data on the production of BSF larvae and termite collection techniques. In this study, the existing production techniques for BSF larvae and collection of termites were evaluated to recommend the most efficient for adoption by smallholder poultry and fish farmers. Six organic substrates (pito mash, millet porridge mash, pig manure, chicken manure, fruit waste, and waste from roots and tubers) known to be suitable for BSF larvae production were evaluated for their suitability as oviposition attractants and larval development. The substrates were first exposed outdoors to measure the quantity of eggs laid on them by naturally occurring BSF females. The quality of the substrate(s) as larval rearing media was also tested by placing a standard amount of egg mass to measure the individual and total weights of prepupae obtained, total number, and development time. The nutritional profile of the prepupae and the substrates were also determined. Furthermore, the production of BSF larvae under natural oviposition (in garden bins) was assessed by varying the rate of loading substrate (pito mash) and the quantities of the substrate on the overall prepupal harvest. The substrate used significantly influenced the quantity of eggs laid and the development of the resulting prepupae but the substrates most favourable for larval development were not the most favoured by gravid BSF for oviposition. In the oviposition tests, millet porridge mash was the most attractive substrate whereas only a few eggs were recovered from the other substrates. All substrates allowed the successful development of larvae but pig manure was more productive than the others. The crude protein content of the larvae ranged between 35 - 43%, with the shortest development time of 16 days. Applying small quantities of substrates at a constant rate (10 kg per week) in garden bins produced higher prepupal yields than larger quantities (20 kg). Unlike BSF larvae, termites cannot be easily produced but are obtained from chippings of mounds or by trapping using containers with filled organic matter. The commonly used termite collection method was assessed to quantify the amount of termites harvested with commonly used organic matter. Furthermore, indigenous knowledge on the use of termites as poultry feed in Ghana and factors affecting its use were assessed. Containers filled with the four commonly used organic matter (mango seed, maize cobs, dried cow dung, yam peels, and their mixtures) were placed on trails of termites to quantify the daily harvest. Surveys were also conducted in four regions in Ghana to collect information, by the administration of questionnaires, on the use of termites as poultry feed, termite species collected, species not used, and collection methods. Samples of termite species mentioned were collected and identified to the genus level. Twenty-three per cent and 19% of farmers mentioned that termites are always or often used to feed poultry whereas 11% never use termites. A binomial regression analysis showed that termite use was affected by region, sex, education, farm size, and income. Termites collected belonged to eight genera, the main ones being *Macrotermes*, *Trinervitermes*, and *Odontotermes*. Five collection methods are used to obtain termites and involve either breaking mounds or using containers as traps. Collection methods vary with species and region and the abundance of termite genera varies with season. Farmers identified some species as poisonous to poultry. A Kruskal-Wallis test showed that there were significant differences in the quantity of termites collected using different substrates for both *Odontotermes* species and *Macrotermes* species. A mixture of corn cobs and yam peels yielded the highest dry weight harvest of 14.8 g/day in *Macrotermes* species. Likewise, the mixture of mango seed and cow dung gave the highest average yield of 19.40 g/day dry weight of *Odontotermes* species. Termites and black soldier fly larvae are important in indigenous poultry production because they are a readily available and cheap protein source for local farmers.

DEDICATION

I dedicate this thesis to my dad, Mr J.K Boafo, who has always encouraged me to become a scholar, and to my mother and siblings, for their prayers and support.

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To God be the glory for how far He has brought me in this life.

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LIST OF ABBREVIATIONS

ARI	Animal Research Institute
BSF	Black solider fly
BSFL	Black soldier fly larvae
DM	Dry matter
FFA	Fish for Africa
FAO	Food and Agricultural Organization
GDP	Gross Domestic product
IFWA	Insect as Feed in West Africa
IPRI	The International Food Policy Research Institute
MoFAD	Ministry of Fisheries and Aquaculture
MOFA	Ministry of Food and Agriculture
MT	Metric tons
OECD	Organization of Economic Co-operation and Development
USDA	United States Department of Agriculture

CHAPTER ONE

1.0 General Introduction

1.1 Background to study

The increase in world population is the utmost challenge facing world food production systems. Coupled with this, urbanization in developing countries will lead to a shift in food and diet patterns particularly toward livestock products (Delgado, 2003). A rise in purchase power due to increased incomes will accelerate the consumption of meat products (Steinfield *et al.*, 2006). Hence, the demand for livestock products is expected to nearly double in Sub-Saharan Africa and Asia, from 200 kcal per person per day in 2000 to 400 kcal per person per day by 2050 (Thornton, 2010).

Since the 1960s, global meat production has seen a tremendous increase, particularly for poultry meat (Asante-Addo and Weible, 2020). Chicken production increased by nearly a factor of 10, whereas beef production doubled in the same period (Landes *et al.*, 2004; Thornton, 2010). With an estimated mean global consumption of 14.2 kg/capita in 2018 for poultry meat, its demand has surpassed pork as the favoured meat (OECD-FAO, 2019).

Similarly, Ghana has experienced an increase in poultry meat consumption (Asante-Addo and Weible, 2020). Over the past decade, poultry meat consumption has become a very common addition to the Ghanaian diet, the bulk being imported poultry meat (Asante-Addo and Weible, 2020). However, Asante-Addo and Weible (2020) indicated that higher-income earning households tend to consume locally-produced birds more than lower-income earning households due to their palatability and health benefits. Poultry meat consumption increased from 1.7 to 6.1 kg per capita between the year 2000 to 2017, more than double the average for Sub-Saharan Africa (OECD-FAO, 2017). The average probability of consuming chicken in a Ghanaian household was found to be 13.5, 16.9, 25.9, 21.0, and 22.7% for occasional consumers, once a month, 2-3 times a month, once a week, and twice or more a week, respectively (Asante-Addo and Weible, 2020).

Before the mid-1980s, domestic poultry production (both backyard and commercial poultry) was a very vibrant activity in the country. In the past, almost every home in Africa reared scavenging indigenous chicken for home consumption and sold it when surplus money was needed (Sonaiya, 1993). It plays a significant role in the agrarian economy of developing countries and serves as a source of livelihood for unemployed youth and women (Padhi, 2016). Concurrently, aquaculture is the fastest-growing animal-producing sector and provides half of the world's fish for consumption (FAO, 2012a; FAO, 2016). About 8-9% of the protein consumed by humans is obtained from aquaculture (FAO, 2012a). Over the past thirty years, world aquaculture production has increased from 5 million to 65 million tons (World Bank, 2013). The aquaculture sector contributes about 3-5% of the GDP of Ghana and is a source of employment for the populace (Bank of Ghana, 2008). It represents about 11.3% of the total national fish production in the year 2016 and is projected to represent 15% in the next three to five years (MoFAD, 2016). The national production increased from over 32,512 MT per year in 2013 to 52,470.49 MT per year in 2016 and is expected to hit 72,000 tons is expected in the next 3 to 5 years (MoFAD, 2016).

1.2 Justification

The indigenous poultry and aquaculture sector in Ghana have the potential to improve livelihoods, provide employment for the youth and women, and contribute to the economy. However, these two sectors still suffer from basic constraints such as high cost of feed, scarcity of feed, access to high-quality protein sources, and limited knowledge of the cost of investment (Amenyogbe et al., 2018).

Generally, feed represents the highest cost in an animal production system, representing about 70% of the total cost of production (Omole *et al.*, 2005). The two key sources of protein used in livestock feed, fishmeal and soybean meal are economically and ecologically unsustainable. Fishmeal is rapidly declining as a feed source because overexploitation of ocean resources has significantly reduced fish stocks (Naylor *et al*., 2000). Soybean meal, is a stable food resource for both human and animal production systems, instigating both competition and pressure on arable lands (Steinfeld *et al*., 2006). Indigenous poultry production, practised in almost every home suffers from qualitative and quantitative feed shortages (Dankwa, 2004; Pousga *et al*., 2007), leading to low live weight of birds, low egg production, and death and thus reduce family income.

Similarly, smallholder fish farmers due to scarcity and high cost of feed often resort to feeding fish on low-quality feed such as rice bran, brewers waste, maize bran, and anchovies resulting in a slow growth rate and increased susceptibility of fish to diseases and eventual death. The failure of many fish farms and the sale of low-grade fish in Ghana have been attributed to low-quality fish feed and the high cost of feed ingredients (Kassam, 2014; Kaunda *et al*., 2010).

A solution to developing sustainable household poultry farming and aquaculture systems is to use untapped local, easily available, and cheap protein sources. Insects, which are a natural food source for poultry and fish are one source. The protein in insects is comparable to that of fishmeal or soymeal and can also be produced cheaply (Heuzè and Tran, 2013). Insect larvae and pupae are a rich source of proteins (40-70% dry weight), other valuable nutrients such as iron, vitamins A and B, and other essential amino acids (DeFoliart, 1995; van Hius, 2013).

The use of insects as a food and feed source is an old-age practice in Africa, Asia, and Latin America that is gaining popularity in recent times. Local farmers often feed insect larvae and termites to poultry, especially young birds (Pomalégni *et al*., 2016; Sankara *et al*., 2017). Furthermore, scavenging birds are occasionally found feeding on both insect larvae and adults on their own during their feed search. The use of insect protein to replace or supplement conventional protein sources is highly recommended by FAO as an alternative and feasible strategy to reduce food insecurity in the world (FAO, 2010).

Many insects are potentially suitable as animal feed (van Huis *et al*., 2013), however, some of these such as caterpillars, grasshoppers, and mealworms require agricultural products which would otherwise be used for human consumption for their mass rearing. Fly larvae, on the other hand, can be produced cheaply on organic waste streams and simultaneously serve to manage organic waste produced by humans. Fly larvae are potential biodegrading agents capable of reducing a large amount of organic waste and converting them to body mass, which can be used to feed livestock and fish. The remaining digestate is also suitable manure for improving soil fertility. The most promising and commonly used fly larvae species for animal feed is the larvae of the black soldier fly, *Hermetia illucens* Linnaeus, 1758 (Diptera: Stratiomyidae). Black soldier fly larvae are preferred over house fly larvae because they are not known pests of any crop and are not vectors of diseases or a nuisance to humans (Kenis *et al*., 2018).

Termites are also commonly used by farmers throughout West Africa (Chrysostome, 1997). Termites collected from chippings of termite mounds or by trapping from mounds are usually fed to chicks and guinea fowl keets to promote growth and increase egg production.

A preliminary survey in 2015, showed a moderate use of fly larvae and termites as feed by smallholder poultry and fish farmers. About 90% percent of farmers (sample size 1960) surveyed in parts of Ghana used termites and 9% produced fly larvae (house fly) as feed for their poultry (unpublished report by CSIR-ARI). Furthermore, surveys in Benin and Burkina Faso showed that 5.7% and 15.6% of indigenous farmers produced fly larvae respectively, to feed their poultry (Pomalégni *et al*., 2016; Sanou *et al*., 2019). Other farmers also collect fly larvae from decomposing waste when they chance on it to feed their poultry. Similarly, Sankara et al. (2018) reported that 78% of farmers in Burkina Faso use termites as poultry feed.

The full adoption of this novel approach is slow due to insufficient data on production techniques of fly larvae (Kensi *et al*., 2018), concerns about the nutritional quality of the resulting larvae when different substrates are used, and collection (termites) and production (fly larvae) of sufficient quantities to replace the animal protein component of fish and poultry feed. The project 'Insects as Feed in West Africa (IFWA)' was therefore initiated to develop appropriate methods for fly larvae and termites production and utilization in smallholder systems in West Africa, based on waste material. The focus of this study was to evaluate the insect rearing and collection methods used by smallholder farmers, which will be complemented by studies by other researchers on the economic, environmental safety and nutritional acceptability by poultry.

1.3 Broad Objective

Evaluate the production of black soldier fly (BSF) larvae and termite collection techniques.

1.4 Specific Objectives

1. To assess the suitability of different substrate(s) as rearing media.
2. To evaluate the production of BSF larvae under natural oviposition in garden bins.
3. To collect indigenous knowledge on the use of termites as poultry feed.
4. To evaluate termite collection methods

CHAPTER TWO

2.0 Literature Review

2.1 Edible Insects

The consumption of insects by humans and use as livestock feed is an old-age practice commonly seen among the people of Africa, Asia, and Latin America. Perhaps, the earliest record of entomophagy is found in the old testament of the Holy Bible. "Of these, you may eat any kind of locust, katydid, cricket or grasshopper" (Leviticus 11: 22, New International version). John the Baptist was said to be a man whose meat was locusts and wild honey (Matthew 3:4). In Africa, entomophagy was reported as far back as 1685 by Simon van der Stel in his expedition to Namaland, south of Namibia (Waterhouse, 1924; Palmer and Pitman, 1972).

Insect as food is known to supplement the dietary needs of about 2 billion people worldwide (Makkar *et al*., 2014). Many of the edible insects are collected from the forest/wild by local people and was consumed or sold to raise funds to supplement the family budget. Overall, about 1,900 species of insects are consumed by humans (van Huis, 2013). A hundred and four families from 14 orders of insects are consumed worldwide (Malaisse, 2005). A total of 470 species of edible insects are consumed in Africa alone (Kelemu *et al*., 2015). Out of this, 256 are eaten in the Central African Republic, 164 in Southern Africa, 100 in Eastern Africa, 91 in West Africa, and only 9 species in North Africa (Kelemu *et al*., 2015). Commonly consumed species are Orthopterans, Hymenopterans, Coleopterans, Isopterans, Homopterans and Heteropterans, and caterpillars of Lepidopterans. About 30% of total consumed insects in Africa are Lepidopteran, 29% Orthopteran, 19% Coleopteran, 7% Hymenopteran, and 15% belonging to Heteropteran, Homopteran, Isopteran, Dipteran, and Odonata (van Hius, 2005). Popular comestible lepidopteran families include *Saturniidae*, *Notodontidae,* and *Sphingidae* (Malaisse, 2005). In almost every part of Sub-Saharan Africa, the species *Cirina forda*

(Westwood), *Bunaea alcinoe* (Stoll), and *Anaphe panda* are eaten (Kelemu *et al*., 2015). Orthopteran species widely consumed across the African continent include *Schistocerca gregaria*, *Locusta migratoria migratorioides*, *Nomadacris septemfasciata*, *Locustana pardalina,* and *Anacridium melanorhodon melanorhodon* (Kelemu *et al*., 2015).

Alates, soldiers, queens, nymphs, and eggs of *Macrotermes bellicosus*, *Macrotermes subhyalinus*, *Macrotermes falciger,* and *Macrotermes natalensis* are of continent-wide importance in terms of consumption (van Hius, 2003). The honey produced and larvae of *Apis mellifera* Linnaeus and *A. melliferra adansoni* Latreille are the main species of Hymenoptera consumed across the African continent (Takeda, 1990; Muthali and Mughogho, 1992).

Edible insects are an important protein source in the diet of many people in Africa. In Congo, more than 40% of the animal protein in the diets in some parts of the country is insect protein (Gomez *et al*., 1961). On average, the major source of protein is obtained from 300g of caterpillars consumed per week and 96 tonnes annually by households in Kinsasha (Vantomme *et al*., 2004). In the Central African Republic, the protein intake of 95% of the forest people is obtained from insects (FAO, 2004) and sometimes, serves as the sole source of essential proteins available to them (van Huis, 2013).

In contrast to the popular belief that insects are eaten due to the scarcity of food or starvation, research confirms the contrary, that they are a delicacy for many people (DeFoliart, 1999; van Hius, 2003; Kelemu *et al*., 2015). In Uganda, during November when the tettigoniid *Ruspolia differens*, commonly called "nsenene" appears in large numbers, the sale of meat and fish dwindles as there is a preference for the insect (Mulissa, 1997). The season of "nsenene" is waited upon with much anticipation among these people. The edible moths, *Anaphe venata* Butler and *C. forda* Westwood are extensively marketed in Nigeria and purchased for about twice the price of beef (Agbidye and Nongo, 2009; Agbidye *et al*., 2009). In Western Kenya, the Luo people living along Lake Victoria, consume the black ant *Carebara vidua* for its nutritional and medicinal properties (Ayieko *et al*., 2012). Ghana is not left out, as larvae of the palm weevil, *Rhynchophorus phoenicis* known in the Akan language as "akokono" is a traditional delicacy enjoyed by the locals (Anankware, 2016).

Indeed, insect protein is a valuable source of nutrients for many populations across the world and the African continent. They are readily available and a cheap source of protein that can satisfy the need and ease the problem of food insecurity in an ever-increasing population of the world.

2.2 The use of insects as Animal Feed

Information on the traditional use of insects as animal feed is scarce; mostly restricted to anecdotal reports in general articles and reviews (Hein *et al*., 2005; Kenis *et al*., 2014) or technical notes and unpublished thesis (Farina *et al*., 1991; Chrysostome, 1997; Naidoo, 2000; Chrysostome, 2009; Diawara, *et al*., 2013,).

In the available records, termite use has been the most frequently cited as a feed supplement given by local farmers to their poultry. Termites collected from chippings of mounds or trapped using containers are sometimes the only available protein at the disposal of indigenous farmers. In KwaZulu Natal, South Africa, rural farmers feed their local chickens with termites and ants (Naidoo, 2000). The extensive use of termites as supplementary feed by indigenous poultry farmers is reported in Benin and Burkina Faso (Chrysostome, 1997; Chrysostome *et al*., 2009; Diawara *et al*., 2013; Sankara *et al*., 2017). Moreover, Sankara *et al*. (2017) revealed that 78% of the respondents interviewed in parts of Burkina Faso used termites at least occasionally to feed their poultry. In another report of a study conducted in Benin, 5.7% of farmers interviewed produced and used fly larvae (*Musca domestica*) as supplementary feed for indigenous poultry (Pomalégni *et al*., 2016).

However, in several recent studies, experimental works have demonstrated the successful replacement of the protein component of livestock feed (poultry, fish, and pig) with insect protein (Bondari and Sheppard, 1981; Téguia *et al*., 2002; Agunbiade *et al*., 2007; Stammer *et al*., 2014;). The larvae of the black soldier fly (*H. illusecns*) and house fly (*M. domestica*) dominate these studies (Mohammed *et al*., 2017; Zhou *et al*., 2017; Dabbou *et al*., 2018; Wallace *et al*., 2018; Belghit *et al*., 2019). Pupae of the silkworm *Bombyx mori* and larvae of mealworm *Tenebrio molitor* have been proven as an excellent replacement for the protein component of livestock feed (Bovera *et al*., 2015; Jin *et al*., 2016; Asimi *et al*., 2017; Sheikh *et al*., 2018).

Newton *et al*. (1977) successfully reared black soldier fly larvae on dried swine manure. Bondari and Sheppard (1981) demonstrated the possibility of replacing the conventional protein with black soldier fly larvae to feed catfish and tilapia. A diet of 1:1 fish meal and maggot meal ratio resulted in high egg production in old layer (50 weeks old) hens (Agunbiade *et al*., 2007). Furthermore, Téguia *et al*. (2002), showed that the replacement of fish meal at different levels with maggot meal in the starter and grower-finisher diets for broiler resulted in significantly higher final weight gained than in the control diet containing exclusively fishmeal.

2.3 Nutritional value of edible insects

Insects are consumed for both nutrients and medical properties. In general, insects are known to be rich in proteins, fats, fibre, vitamins, and minerals (van Hius, 2013). The nutritional composition of edible insects is highly variable between and within species, the metamorphic life stage consumed, and the diet of the insects (Rumpold and Schlüter, 2013).

The major contribution of nutrients obtained from edible insects is protein, fat, and chitin as this is the major body composition of insects (Roos, 2018). However, insects in any metabolic stage are a good source of various micronutrients that a fully functional insect requires for its metabolism (Roos, 2018).

The protein composition of various insect species generally ranges between 40-70% dry weight (Rumpold and Schlüter, 2013). On dry matter bases, the black soldier fly contains 35-50% (Henry *et al*., 2015; Shumo *et al*., 2019); house fly 50-76% (Hwangbo *et al*., 2009; Pretorius, 2011); mealworm 46 - 70% (Ravzanaadii *et al*., 2012; Zhao *et al*., 2016) and silkworm 50% (Mitsuhashi, 2010) protein. Insect protein is comparable in quality to meat and fish protein (Srivastava *et al*., 2009; Mitsuhashi, 2010; Schabel *et al*., 2010) and protein from *Acheta domesticus is* superior to soy protein (Finke *et al*., 1989). A 100g of caterpillar provided 75% of the daily amount of proteins required by humans (Agbidye *et al*., 2009). When compared to 1 chicken egg, 3 pupae of silkworm had similar nutrient content (Mitsuhashi, 2010).

An overview of the amino acid profile of some common edible insects showed that generally, concerning daily human amino acid requirements, these insects have a high amount of phenylalanine and tyrosine except for methionine (Rumpold and Schlüter, 2013).

Insect fat range from 5% to more than 30% (DeFoliart, 1991) and is higher in pupal and larval stages than in the adult stages (Chen *et al*., 2009). However, others such as the palm weevil, *R. phoenicis,* and maguey grub have a high-fat content of about 62.1% (Omotoso and Adedire, 2007) and 58.55% respectively based on dry matter (Melo *et al*., 2011). The fat content of as high as 77% (dry matter) has been reported in larvae of the butterfly *Phasus triangularis* (Ramos-Elorduy *et al*., 1997).

Insects are not lacking in micronutrients. Generally, the majority of insects contain high amounts of potassium, calcium, iron, magnesium (Schabel, 2010), zinc (DeFoliart, 1992), and selenium (Finke, 2002). Termites are particularly very high in iron (Banjo *et al*., 2006). According to Schabel (2010), caterpillars generally provide the required minerals in abundance. A 100g of caterpillars on average supply 33.5% of the minimum daily required amount of iron needed (DeFoliart, 1992). Also, insects supply several vitamins; bee broods are rich in vitamins A and D, and caterpillars in Vitamins B1, B2, and B6 (Schabel, 2010).

It is noteworthy that the nutritional values of some of the insects considered above are those of wild insects collected. However, as stated earlier the nutritive values of insects highly depend on the feed consumed by the insects and this is likely to vary when insects are reared in the laboratory or semi-farm conditions on organic waste streams.

2.4 Safety of insects as food and feed

The safety of insects as a food and feed source is a priority study area for the total acceptance of entomophagy. Several authors have investigated the safety of insects reared for food and feed (Klunder *et al*., 2012; Charlton *et al*., 2015; Diener *et al*., 2015a; van der Fels-Klerx *et al*., 2016; Quaye *et al*., 2018; van der Fels-Klerx *et al*., 2018; Schrögel and Wätjen, 2019). Contaminants such as veterinary medicines, heavy metals, pesticides, mycotoxins, allergens, and dioxins, occurring mainly in the substrates used in their production or their environment are of concern (van der Fels-Klerx *et al*., 2018).

The contaminant in insect meal varies depending on the substrate used in growing the insects. Insects reared on agricultural waste are likely to contain pesticide residues and mycotoxins, whereas, those grown on animal manure contain veterinary drugs accordingly (van der Fels-Klerx *et al*., 2018). The bioaccumulation of these chemicals is positively correlated with the type of chemical, the concentration in the substrate, the insect species, and the growth phase of the insect (van der Fels-Klerx *et al*., 2016). Copper and zinc are efficiently metabolised by insects as such no correlation has been found between substrate concentration and internal concentration in insects (Maryanski *et al*., 2002; Vijver *et al*., 2003). However, the concentration of cadmium, lead, mercury, and arsenic is reported to be positively correlated with the concentrations in the substrate and the internal concentrations in the insect (Zhang *et al*., 2009; Diener *et al*., 2015; van der Lee and Oonincx, 2016).

Charlton *et al*. (2015), reported that contaminants such as veterinary medicine, heavy metals, pesticides, and mycotoxins were below the recommended maximum concentrations by WHO, European Commission and Codex for black soldier flies, house flies, blow fly, and blue bottle flies. Low levels of mercury were detected in house fly larvae meal produced on pig manure in Ghana. Similarly, no lead contamination was found in house fly larvae meal produced on several substrates (Nkegbe *et al*., 2018a). However, there were concerns with the concentration of cadmium in three housefly samples analyzed (Charlton *et al*., 2015). Bioaccumulation of cadmium was observed in BSF and arsenic in yellow mealworms (Diener *et al*., 2015; van der Fel-Klerx *et al*., 2018).

There is no evidence of accumulation of various mycotoxins in distinct insect species when fed with high concentrations of mycotoxins (van Broekhoven *et al*., 2014; Sanabria *et al*., 2017; Sanabria *et al*., 2019; Schrögel and Wätjen, 2019;). It seems insects can metabolize mycotoxins, but further research is needed for confirmation and to identify the metabolites formed (van Broekhoven *et al*., 2017). Likewise, no bioaccumulation of veterinary drugs was found in *H. illuscens* larvae reared on animal manure (Lalander *et al*., 2016), but, Charleton *et al*. (2015), reported the accumulation of nicarbazin in *M. domestica* grown on poultry manure. Information on the accumulation of veterinary drugs is limiting.

The investigation of the effect of BSF larvae meal on poultry meat revealed that there was no significant effect on blood and serum parameters except for phosphorus (Dabbou *et al*., 2018). Red blood cell and white blood cell counts, packed cell volume, monocytes, and basophils were not affected by the inclusion of house fly larvae meal in poultry feed at different inclusion levels (Nkegbe *et al*., 2018b). Likewise, the total cholesterol, uric acid, albumin levels of yolk, and triglycerides were not affected by the inclusion of house fly larvae meal (Nkegbe *et al*., 2018b). Furthermore, insects contain toxins or allergens which may cause allergic reactions or disease in some individuals (Broekman *et al*., 2016). The African silkworm *Anaphe venata* has been associated with the seasonal ataxic syndrome and unconsciousness in Nigerians due to the presence of the enzyme thiaminase which when ingested renders thiamine (Vit B1) inactive (Adamolekun, 1993; Adamolekun *et al*., 1997; Nishimune *et al*., 2000; Moyo *et al*., 2014). In addition, forest insects harvested in the wild may be unwholesome due to poisoning by insecticide treatment. The collection of the desert locust *Schistocerca gregaria* for consumption could be detrimental to health when aerial spraying during outbreaks is used as a means of control.

Poor handling, processing, and storage of insect meals can predispose them to the infestation of microorganisms, rendering them unsafe for consumption. Klunder *et al*. (2012) reported that bacterial levels of dried and roasted domestic house cricket were higher than boiled crickets. Mujuru *et al*. (2014) showed that hot-ash roasting of *G. belina* retained higher levels of *Escherichia coli* and *Staphylococcus aureus* than boiled and open-pan roasted. Also, they observed that solar drying of boiled samples resulted in recontamination by moulds. Several spore-forming fungi, mycotoxigenic fungi, and insect pests have been identified on stored insect meals. *Aspergillus sp*, *Penicillium sp*, *Fusarium sp*, *Dermestes maculatus*, *Sitophilus zeamais*, *Corcyra cephalonica*, *Tribolium confusum*, *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Bracon hebetor*, *Anisopteromalus cavandrae*, *Stathmopoda* species, and mites were collected on mopane caterpillar stored for five months (Mpuchane *et al*., 2000).

Furthermore, due to their richness in nutrients and moisture, insects offer a favourable environment for many microbial organisms (Klunder *et al*., 2012). Pathogenic and non-pathogenic microflorae have been isolated from the gut and body walls of some edible insects. Pathogenic microflorae such as *Staphylococcus aureus*, *Aspergillus tamarrii,* and *Bacillus cereus* were isolated from the body wall and gut of the common housefly *Musca domestica* reared on fresh fish (Banjo *et al*., 2005). Freshly harvested palm grubs contained *Escherichia coli*, *Staphylococcus sp*., and *Klebsiella aerogenes* (Opare *et al*., 2012).

The levels of *Salmonella* sp. and *Klebsiella* sp. recorded in palm weevil exceeded the recommended standard for meat consumption by the food administration manual of the ministry of health for New Zealand (Opare *et al*., 2012). There is, however, no evidence of insects harbouring pathogenic viruses, although they could as vectors in their transmission (van der Fel-Klerx *et al*., 2018).

Nonetheless, proper treatment before consumption has been reported to be effective in eliminating most microbial especially *Enterobacteriaceae*, but to a lesser extent spore-forming bacterium (Klunder *et al*., 2012).

2.5 Farming Edible Insects

Although the exploitation of insects has gone on for thousands of years, only three insect species (honeybee, silkworm, and cochineal) have been fully domesticated. The majority of edible insects are obtained through collection or harvesting from nature.

However, in recent years, with the exigency to find alternate sources of protein to curb food insecurity, the domestication of edible insects has become a necessity. Also, wild collections are seasonal, and their availability is threatened by increased deforestation, agricultural intensification, and environmental pollution due to insecticidal spraying. Moreover, increased demand has led to the overexploitation of wild resources.

Semi-domestication and farming of edible insects are essential for sustainability, continuity, and reduced cost if insects are to become a component of stable diets or as livestock feed ingredients. There is a need for a regular supply of large quantities and high-standard qualities of insects to be produced for use as food and feed.

Insect farming is a rising economic venture. A 14% increase in the livestock feed market between 2011 and 2015 (van Huis and Oonincx, 2017) created a potential opportunity for insects as a resource for this industry. The most common species of insects farmed are *Gryllodes sigillatus*, *Gryllus bimaculatus, Acheta domestius, Tenebrio molitor, Zophobas* *morio, Alphitobius diaperinus, Locusta migratoria*, *Pachnoda marginata peregrina*, *Blaptica dubia*, *Rhynchophorus ferrugineus*, *Hermetia illuscens*, and *Musca domestica* (Durst and Hanboonsong, 2015).

Semi-domestication of insects is ongoing in Vietnam, Laos, and Thailand on a community scale (Dicke *et al*., 2019). Farmers, for example, enhance weaver ant populations by providing stringing ‘ant highways’ from vines to assist movement from tree to tree and also providing household food scraps for ants to enhance growth and nest formation (van Mele and Cuc, 2007; Hanboonsong and Durst, 2014). In recent times, active insect farming is a lucrative venture in Thailand with over 20,000 local people engaged in small-to-medium scale enterprises (Durst and Hanboonsong, 2014). In the USA, the cricket farming industry is a multimillion-dollar business, mostly producing insects for pet and zoo animals (Weissman *et al*., 2012). About 50 million crickets are produced weekly for pet and zoo animals (Weissman *et al*., 2012). Africa known to be among the top continents consuming insects is not left out in insect farming. In recent years, reliance on wild collections in Africa is being discouraged, as such, several institutions have developed production technologies for breeding insects such as crickets, palm weevil grubs, black soldier fly larvae, and house fly larvae. The Jaramogi Oginga Odinga University of Science and Technology has developed protocols for large-scale production of the house cricket, *Gryllus bimaculatus*, and black soldier fly larvae. The rearing technique for palm weevil grubs was developed and disseminated to some rural women in Ghana to empower them economically and improve nutrition (Parker *et al*., 2018). In Bamako-Mali, farming of house fly larvae has been going on for about a decade at the Institut d’Economie Rurale, Centre Régional de Recherche Agricole de Sotuba (Kone *et al*., 1998; Kone *et al*., 2017). The various organic waste streams that support house fly larvae growth and seasonal variations in harvest have been established.

Furthermore, research on black soldier fly larvae and house fly larvae production has been conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) (Ewusie *et al*., 2019) and Animal Research Institute of the Council for Scientific and Industrial Research, respectively. The focus of their research work has been to develop techniques, assess the safety, and improve upon existing techniques for farming insects to help inform farmers on choices to make.

Indigenous farmers in Burkina Faso (Sanou *et al*., 2018) and the Northern Region of Ghana (personal communication with farmers), produce housefly larvae to feed their poultry. Rumen content from livestock is collected, and exposed to natural fly populations for oviposition. The larvae and prepupae developed on the rumen are fed to poultry.

Irrespective of the scale of domesticating insects, the overall benefits are immense. Insect farming has the potential to improve food security, alleviate poverty, and improve the nutrition of poor communities.

2.6 Advantages of farming insect

Farming insects offer some environmental benefits over commercial livestock production. When compared to livestock production, insect production requires less water and land resources, emits lower greenhouse gases, has high feed conversion efficiencies, and can transform low-value organic by-products into high-quality food or feed (van Hius and Oonincx, 2017).

When compared to mealworm production, 1g of chicken protein required two to three times as much land and 50% more water (Oonincx and De Boer, 2012). Similarly, a gram of protein from beef required 8-14 times more land and 5 times more water than mealworm protein production.

Direct quantification of greenhouse gas emissions of five insects, three of which are edible (mealworm, house crickets, migratory locust, flower beetle, and *Blaptica dubia*) showed a lower greenhouse gas emission compared to livestock emissions (Oonincx *et al*., 2010). Oonincx and De Boer (2012), again compared the CO2 emissions between broiler chicken and mealworm protein and showed that broiler chicken emits 32-167% more CO2 than mealworm. Likewise, in Thailand, greenhouse gas emissions from poultry production were 89% higher than from cricket production (Halloran *et al*., 2017). Land use, water use, and greenhouse gas emissions from livestock and insect production are mainly associated with feed production in these systems (van Hius and Oonincx, 2017).

A major reason that puts insects ahead of other livestock as a sustainable source of animal protein is their high feed conversion efficiency (Premalatha *et al*., 2011; Looy *et al*., 2013). Black soldier flies convert about half of their diet into edible protein (Oonincx *et al*., 2015b) in contrast to poultry which converts 33% (Wilkinson, 2011). The Argentinean cockroach and mealworms utilise 51-88% and 22-45%, respectively of their feed into edible protein (Oonincx *et al*., 2015b).

Furthermore, a number of these potential insect species can be grown on organic waste streams, accumulating low-value organic by-products into high-value proteins. An attribute particularly important due to the huge quantities of organic waste generated annually worldwide. The black soldier fly, for instance, is capable of utilizing a wide range of organic waste substances from kitchen waste, animal manure, agricultural by-products, and market waste (Newton *et al*., 2005; Oonincx *et al*., 2015a; Surendra *et al*., 2016). Moreover, it can sterilize the waste by killing bacteria such as *Escherichia coli* and *Salmonella enterica* (Erickson *et al*., 2004; Liu *et al*., 2008). Others, such as the oriental ground cricket and mealworms can be reared on agricultural by-products (Oonincx *et al*., 2015a; Megido *et al*., 2016; Miech *et al*., 2016).

The substrate chosen, however, is based on a legislative framework and food and feed safety issues.

2.7 Nutritional requirements of insects

Nutrition is the chemical requirement by an organism for its growth, tissue maintenance, reproduction, and energy required for these functions (Douglas and Simpson, 2011). Whiles insects can synthesize these chemicals, most are obtained from the food ingested (Behmer, 2009; Douglas and Simpson, 2011). “Insects as a group feed on a remarkably diverse list of organic substances” (Waldbauer, 1968). There is a high specificity in the type of food utilized by a group or species of insects (Waldbauer,1968). Nonetheless, generally, all insects have the same qualitative nutritional needs (Fraenkel, 1953, 1959). However, nutritional requirements vary with age, sex, stage of development, and physiological stress of the insect (Nation, 2001). Insects share much in common with other animals in terms of qualitative nutritional requirements (Thompson and Simpson, 2009). The basic essential macronutrients, protein, carbohydrates, and lipids required by other animals are necessary for insects as well. For example, insects share the same ten essential amino acids needed by humans (Thompson and Simpson, 2009). Nonetheless, there are specificities to their nutritional requirements.

Proteins are especially a limiting nutrient for insects since they are unable to synthesize the essential amino acids (Thompson and Simpson, 2009; Barragan-Fonseca *et al*., 2018). These amino acids act as enzymes for transport and storage and are used for structural purposes (Behmer, 2008). The essential acids are; arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, these are essential dietary requirements for insects (Thompson and Simpson, 2009). These essential amino acids are important in some physiological functions in insects. For example, tyrosine (produced naturally in the body from phenylalanine) is important for the production of phenolic and quinone metabolism, a critical component of the cross-linking of proteins during sclerotization (Thompson and Simpson, 2009). Another essential nutrient required is sterols, which function in membrane formation and production of ecdysone and other moulting hormones (Behmer, 2009; Thompson and Simpson, 2009). Cholesterol is the dominant tissue sterol in insects as well as many other animals.

Water-soluble B vitamins (biotin, folic acid, niacin, thiamine, riboflavin, and pentatonic acid) are also particularly important for insect survival. They are the principal precursors for coenzymes of intermediary metabolism. Phytophagous insects require dietary ascorbic acid for normal function but not for other insects utilizing other types of foods (Behmer, 2009). The notable beneficial fat-soluble vitamins required by insects are tocopherol (E) and retinol (A), they aid reproduction and vision respectively.

Carbohydrates, on the other hand, are non-essential for insects, but needed for energy in the absence of dietary fat or proteins (Behmer, 2009; Simpson, 2009). The ability to digest carbohydrates depends on the digestive capabilities of the insect (Behmer, 2009). However, most insects can utilize sucrose but not all non-sugars such as cellulose and dextrin (Behmer, 2009). Some species of insects such as the screw-worm fly, *Chrysomya*, normally feed on live animal tissues and the wax moth, *Galleria*, survives on artificial diets containing no carbohydrates (Behmer, 2009).

There are exceptions to the extent to which carbohydrate is needed by insects. The desert locust, *Schsitocerca gregaria*, for example, requires at least 20 % of digestible carbohydrates in an artificial diet for optimal growth (Behmer, 2009). Similarly, the flour beetle, *Tenebrio molitor*, develops normally when dietary carbohydrate content is about 70 % but poorly when it drops below 40 % (Behmer, 2009). For some insects such as adult lepidopterans, dipterans, and hymenopterans, carbohydrates are predominantly the main energy source for metabolism. Sucrose acts as a phagostimulant, stimulating feeding in some insects (Thompson and Simpson, 2009).

Likewise, fatty acids are non-essential for many insects. Mosquitoes and some lepidopterans are exceptions to this, requiring polyunsaturated fatty acids, a lack of it resulting in a nutritional disease called “crumpled wings” syndrome (Thompson and Simpson, 2009). In crumpled wing syndrome, the wings of newly emerged adult insects fail to expand making it impossible for the insect to fly. The fatty acid required is arachidonic acid and α -linolenic acid for mosquitoes and lepidopterans respectively (Behmer, 2009).

Insects require potassium, magnesium, and phosphate in greater proportions relative to sodium, calcium, and chloride (Thompson and Simpson, 2009). Zinc and manganese are also important, aiding in the hardening of the mandibular cuticle.

Insects require L-ascorbic acid and vitamin C for growth and development. Ascorbic acid is required in relatively larger amounts than vitamin C for insects (Thompson and Simpson, 2009). Deficiency in ascorbic acid results in abnormalities in moulting. Lipogenic growth factors such as choline and inositol are necessary for the proper growth and development of many insects (Thompson and Simpson, 2009). It is worth noting that insects, however, regulate the uptake of the important nutrients needed to maximize their fitness and for proper functioning. It has been demonstrated that specific proportions and amount of food is ingested to meet the daily nutritional requirement (Behmer, 2009).

2.8 The black soldier fly

The black soldier fly, *Hermetia illucens* (Linnaeus 1758), belongs to the order Diptera and the family Stratiomyidae. It is a ubiquitous insect found in tropical, subtropical regions, and warmer temperate regions of the world (Üstüner *et al*., 2003).

The wasp-like-looking adult measure between 15- 20mm in length, is bluish-black with yellowish-white tarsi and has two lateral translucent spots on the second abdominal segment (Hall and Gerhardt, 2002). They lack a functional mouthpart, therefore do not feed but depend on fat reserves accumulated during the larval stage and require only water during their approximately 5 to 9 days of life on earth (Tomberlin *et al*., 2002; Newton *et al*., 2005).

Furthermore, adults are not known to vector any disease nor do they bite or sting humans (Sheppard *et al*., 2002; Čičková *et al*., 2015). The adults are neither attracted to human habitats nor food crops (Furman *et al*., 1959). The female black soldier fly oviposits near larval food sources rather than directly on the food source (Booth and Sheppard, 1984) and therefore does not come into direct contact with the organic waste to pick up pathogens or transmit pathogens.

The dull whitish larvae, when mature, measure about 20mm long. They are flattened dorsoventrally with a narrow head-bearing mouthpart (Hall and Gerhardt, 2002). Unlike, adults, larvae, are voracious detritivores, consuming large volumes of fresh organic waste streams and converting them to body mass (Hardouin and Mahoux, 2003; Diener *et al*., 2011; van Huis *et al*., 2013; Makkar *et al*., 2014). They consume a wide variety of fresh organic waste, from decaying fruits and vegetables, restaurant waste, animal manure, market waste, fish offals, distillers' grains, and coffee pulp to human excreta (Diener *et al*., 2011; van Huis *et al*., 2013). They have the potential to reduce up to 50% of fresh organic waste material (van Hius *et al*., 2013; Makkar *et al*., 2014), consuming about 25-500 mg of fresh matter per larvae per day (Hardouin and Mahoux, 2003; Diener *et al*., 2011). Moreover, due to the rapidity of decomposition of organic waste by larvae, bacteria growth is suppressed and bad odour is minimized drastically (Diener, 2011). Black soldier fly larvae are also competitors of house fly larvae by having a repulsive effect on oviposition by house fly, thus reducing the population of houseflies where they are found (Furman *et al*. 1959).

2.9 The life cycle of black soldier flies, *Hermetia illucens*

Adults become sexually mature two days after emergence. A male grabs a passing female in mid-air and they descend in copulation (Tomberlin and Sheppard, 2001). A gravid female lays between 400 to 800 eggs in dry crevices near decaying organic matter (Dortmans *et al*., 2017). The oval-shaped, creamy white eggs hatch into larvae in about 96 hours. The larvae undergo 6 larval instars, requiring about 14 days to complete the larval phase (Hall and Gerhardt, 2002). The sixth larval instar called prepupae crawls out of the growing medium in search of a dry suitable protected environment to pupate. The mouthpart transforms into a hook that enhances easy movement out and away from the wet waste and the larvae darken into dark brown or charcoal grey colour (Dortmans *et al*., 2017). Pupation takes approximately 14 days and the adult emerges (Hall and Gerhardt, 2002). During this phase, the pupa becomes stiff and immobile with the posterior end slightly curved. When ready to emerge, the fly breaks off what used to be the head section, crawls out, dries up, spreads its wings, and flies off (Dortmans *et al*., 2017). The adults live for about 9 days under favourable conditions, find a suitable mate, copulate, and the female lays eggs and dies. Important factors for adult survival are natural light and warm temperatures between 25-32^{o}C (Dortmans *et al*., 2017). The entire life cycle from the egg phase to the adult lasts for approximately 40 days (Furman *et al*., 1959, May 1961, Tomberlin *et al*., 2002; Myers *et al*., 2008).

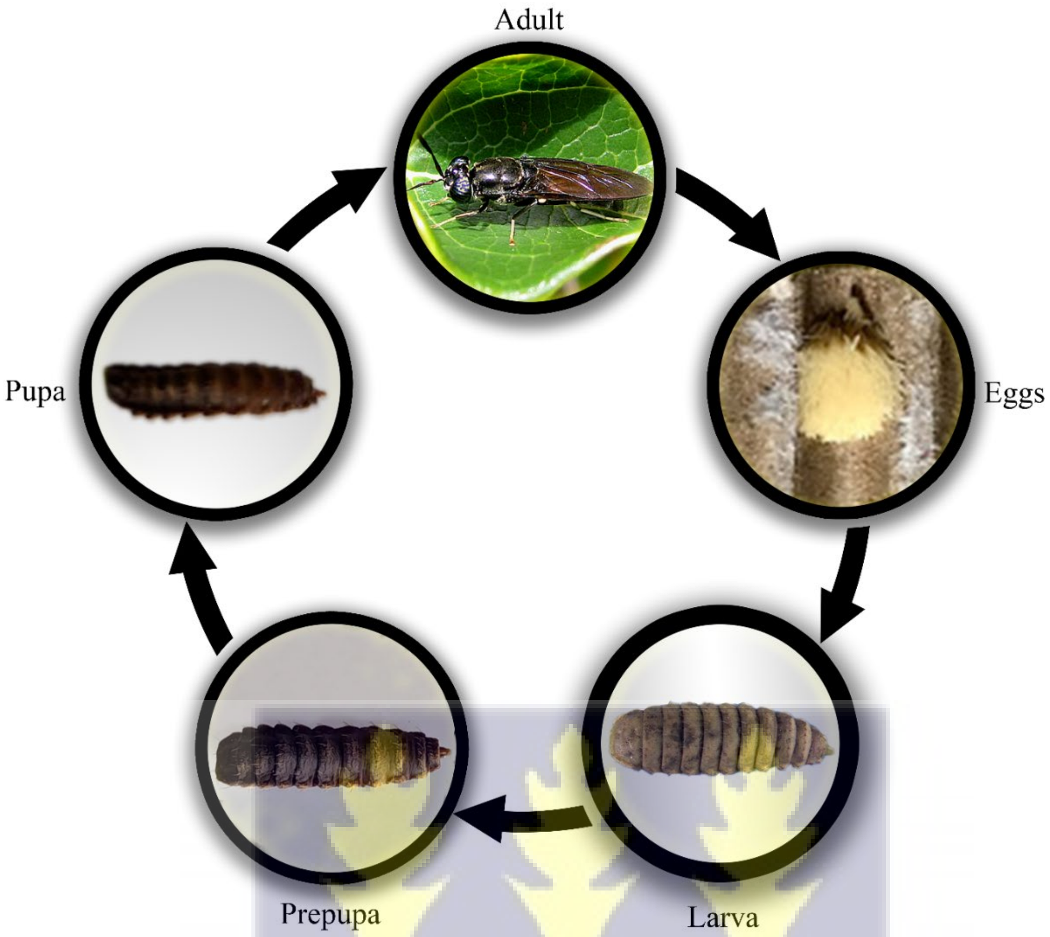

Plate 1: Life cycle of the black soldier fly, Hermetia illucens

2.10 The effect of diet (substrate) on growth, development and nutrient composition of black soldiers fly flies

Good knowledge of the methods for the mass production of black soldier fly larvae is necessary to promote the larvae as an alternate source of protein in livestock feed. Among the demands of mass rearing, the larval diet and nutrition are of great importance (Danieli *et al*., 2019). The black soldier fly larvae are highly polyphagous and able to feed on a wide range of organic waste streams. The nutritional composition of the organic waste stream determines the body composition and growth of the black soldier fly (Tomberlin *et al*., 2002; Barragán-Fonseca *et al*., 2018).

The quality of food (substrate) is one of the major factors that influence the development of the black soldier fly (Barragán-Fonseca *et al*., 2018). Several studies have shown the relationship between the nutrient composition of the organic waste stream on the crude protein, crude fat, crude fibre, ash, dry matter, and weight of the larvae (Newton *et al*., 2005; St-Hilaire *et al*., 2007; Rachmawati *et al*., 2010; Li *et al*., 2011; Oonincx *et al*., 2015b; Barragan-Fonseca *et al*., 2018; Danieli *et al*., 2019; Shumo *et al*., 2019). A highly nutritious diet produces larvae and adults of high body mass and increases the fecundity of the females (Boggs and Freeman, 2005).

Protein levels are especially important for survival and larval development. Oonincx *et al*. (2015b) reported the reduction of the development time of larvae from 37 to 21 days when a low protein diet was replaced with a high protein and fat diet. Larvae fed with swine manure were higher in protein content than larvae fed with cow manure (Newton *et al*., 2005; St-Hilaire *et al*., 2007).

Danieli *et al*. (2019) reported that the crude protein of prepupae reared on three different substrates was significantly different from each other. Shumo *et al*. (2019) also gave an account of the effect of different substrates on the crude protein, ether extract, minerals, amino acid, crude fat, and vitamins of BSFL. The substrate used for the production of larvae affected all the aforementioned proximate factors except the vitamin content of BSF larvae. In the same way, larvae fed chicken manure had higher crude fat content when compared to those grown on pig manure and cattle manure (Li *et al*., 2011).

The survival rate and duration of development have also been reported to be positively correlated with food quality (Newton *et al*., 2005; Gobbi *et al*., 2013; Oonincx *et al*., 2015b; Chia *et al*., 2018). Larval and pupal mortalities between 1-7 % were recorded when BSF was fed hen feed diet alone, but higher (60-80 %) when given a meat diet (Gobbi *et al*., 2013). Likewise, development time was faster on a higher-quality diet than on a lower-quality diet, sometimes taking twice as much time (Gobbi *et al*., 2013; Oonincx *et al*., 2015b; Chia *et al*., 2018). Furthermore, larvae, prepupae, pupae, and adult weights are significantly affected by food quality and quantity (Chia *et al*., 2018; Meneguz *et al*., 2018).

Likewise, the dry matter content of the larvae is affected by the diet but generally ranges between 20 - 44 % for fresh larvae (Sheppard *et al*., 2008; Diener *et al*., 2009; Finke, 2013; Nguyen *et al*., 2015; Oonincx *et al*., 2015a;). However, the micronutrient concentration is to a lesser extent affected by the substrate used as feed (Barragan-Fonseca *et al*., 2017).

2.11 Substrates Suitable for rearing black soldier fly larvae

Black soldier fly larvae are the best-known species for utilizing organic waste streams (van Hius and Oonincx, 2017). Generally, due to the polyphagous nature of the larvae, several organic waste streams can serve as good rearing media for BSF larvae. Of particular importance in choosing a substrate (organic waste stream) is the nutrient composition (Barragán-Fonseca *et al*., 2018), the moisture content (Cammack and Tomberlin, 2007), and the particle size (Palmer *et al*., 2019).

Substrates rich in proteins promotes the growth and development of the larvae and are positively correlated with adult longevity and female fecundity (Cammock and Tomberlin, 2017; Oonincx et al., 2015b). Furthermore, the fat content and fatty acid profile of the resulting larvae are dependent on the substrate (Meneguz *et al*., 2018; Ewald *et al*., 2020).

A moisture content of 70-80 % is optimum for BSF larvae (Myers *et al*., 2008; Cheng *et al*., 2017; Lalander *et al*., 2019), below and above this threshold, the growth and survival of the larvae are negatively affected. The moisture content of substrates can be enhanced when necessary by the addition of water or reduced by dewatering or the addition of bulking agent. To increase the surface area of the substrate to enable easy access to the available nutrients by larvae, the large particle-sized substrate should be shredded. The need for high dietary moisture is due to the morphology of the BSF larvae mouthpart (Kim *et al*., 2010; Purkayastha *et al*., 2017), high moisture makes scraping off food from feeding surfaces easier (Banks, 2014).

For mass production of BSF larvae, several organic waste streams have been tested on their suitability as rearing media. Among these, the most tested are from markets (Rana *et al*., 2015; Barragan-Fonseca *et al*., 2018), animal farms (Sheppard *et al*., 1994; Newton *et al*., 2005; Oonincx *et al*., 2015a; Shumo *et al*., 2019), catering services (Driemeyer, 2016; Surendra *et al*., 2016; Shumo *et al*., 2019), food-processing by-products (Lardé, 1990; St-Hilarine *et al*., 2007; Manurung *et al*., 2016; Permana *et al*., 2018), and brewery distillers (Webster *et al*., 2015; Bava *et al*., 2019; Chia *et al*., 2019; Shumo *et al*., 2019). Larvae effectively processed rotten fruit and vegetable from markets, pig manure, chicken manure, cow manure, food scraps from restaurants, spent coffee grain, coffee palp, and brewers' grain.

Other substrates tested are human faecal matter (Lalander *et al*., 2013; Banks *et al*., 2014) and chicken feed (Diener *et al*., 2009; Gobbi *et al*., 2013; Bava *et al*., 2019).

2.12 Termites

Termites are eusocial insects widely distributed through the tropical and sub-tropical regions of the world (Eggleton, 2000). They are a highly ecologically successful species due to their sophisticated social organization with the unique ability to feed on recalcitrant plant matter such as wood (Khan and Ahmed, 2018). Termites make up about 10 % of total animal biomass and 95 % of soil insect biomass of tropical ecosystems (Jones and Eggleton, 2000). The populations of termites can reach enormous levels as much as 1000 individuals per square meter (Eggleton *et al*., 1996). Over 2,600 species belonging to 281 genera and nine families of termites have been described (Kambhampati and Eggleton, 2000; Engel *et al*., 2009). Africa alone owns 1000 species out of the 2,600 known species worldwide (Lewis, 2003).

Termites have economic importance to man, they are either highly beneficial or highly deleterious. Cellulose being the primary food source for some termites, they seek this food source causing damage to vegetation, buildings, and other man-made wooden structures. Globally, on a per anum basis, the economic losses incurred in controlling termite pests are about $40 billion (Rust and Su, 2012). In Africa (including Ghana), accurate information on economic losses as a result of termite pests is not well documented (Akutse *et al.*, 2011; Ugbomeh and Diboyesuku, 2019). However, due to the enormous damage caused, the perception of people about termites is mostly negative.

Nonetheless, they play very vital roles in tropical ecosystems through plant decomposition, nitrogen, and carbon recycling (Holts and Le Page, 2000). Termites bring about soil formation and nutrient recycling through the consumption of plant necromass. About 50 to 100% of the leaf litter in tropical forests is decomposed by termites (Bignell and Eggleton, 2000; Brauman, 2000). Mounds and soils of termites are used in geochemical prospecting, plastering houses, and making bricks and pots (van Hius, 2017). Mound samples are a good geochemical sample media for mineral exploration (Affam and Arhin, 2005; Arhin *et al.*, 2015) and have aided in gold exploration in parts of Northern Ghana (Arhin and Nude, 2010).

The enzymes found in a termite's digestive system can aid in the production of biofuel from woody biomass (Khan and Amad, 2018). The current biomass conversion technology for fuel and chemicals can be improved by the use of the lignocellulolytic enzyme system in wood-feeding termites (BenGuerrero *et al*, 2015).

Termites also serve as food for many indigenous folks in Africa, Asia, and South America. Swarming reproductive, soldiers and queens are collected and eaten fresh, cooked, or fried. They are also harvested and used as feed for poultry and as bait in fishing. The mushrooms that spring up from the mounds annually are a delicacy. The fungus garden, soldiers, and mounds are used in popular medicine. They are used in the treatment of bronchitis, asthma, whooping cough, tonsillitis, and sinusitis (Alves, 2009; Alves and Dias, 2010).

2.13 Termites as food and feed

Termites are an economically and socially important source of protein consumed in many parts of the world for many generations. The alates, soldiers, and queens, are frequently enjoyed as a delicacy. Indigenous poultry farmers unable to afford conventional protein sources offer termites as an alternative for their poultry (Sankara *et al*., 2018).

Forty-three species of termites are used as food or feed worldwide (Figueirêdo *et al*., 2015). The African continent is very popular with termite consumption. It has been reported in almost all parts of Africa (19 countries) except in Northern Africa (Fombong and Kinyuru, 2018). van Hius (2003) reports that about 14 species of termites from the subfamily *Macrotermitinae* are eaten in sub-Saharan Africa alone. The most consumed termites belong to the family *Termitidae*, representing about 87 % of the total edible termites (Fombong and Kinyuru, 2018). The species frequently recorded as human food and livestock feed is *Macrotermes bellicosus* (van Hius, 2003). Others *M. subhyalinus, Nasutitermes macrocephalus,* and *Pseudacanthotermes spiniger* are equally popular (van Hius, 2003; Fombong and Kinyuru, 2018). Other families commonly consumed are *Hodotermitidae*, *Kalotermitidae*, and *Rhinotermitidae* (Figueirêdo *et al*., 2015).

In a study conducted by Anankware (2016), nine edible insects belonging to five orders were recorded from Ghana with termites representing 45.9 % of this number. The termites consumed in Ghana are predominantly *Macrotermes bellicosus* (Anankware, 2016). The sale of sun-dried termites is common in local markets in many East African towns and villages (Fambong and Kinyuru, 2018). Fried and boiled termites are consumed as snacks between main meals among the Baganda and Bantu-speaking people of Uganda.

The use of termites as livestock feed is not well documented. Most of the literature is found in general reviews on entomophagy, technical reports, and unpublished thesis (Chrysostome, 1997; van Hius, 2003; Dao, 2016; Khan and Ahmed, 2018). A recent study by Sankara *et al.* (2018), reported the use of termites by 78 % of the total number of poultry farmers interviewed in 25 provinces in Burkina Faso. Sogbesan and Ugwumba (2008), demonstrated the possibility of replacement of the conventional fishmeal with termite protein in rearing *Heterobranchus longifilis.* A 50 % termite meal inclusion diet yielded the highest mean weight gain of 9.6 g/fish, the lowest feed conversion ratio (2.9), and the highest protein efficiency ratio of 0.8. The study showed the practicality of using termites as a possible replacement for conventional protein in fish diets.

2.14 Collection of termites

Termite harvesting is mostly done seasonally during the rainy months. In Ghana, *Macrotermes bellicosus* is collected in June and July (Anakware, 2016); in East Cameroon, in March, April, and May (Muafor *et al.*, 2014), and in Kenya, March to May and September to December raining seasons (Fombong and Kinyuru, 2018).

Various trapping/collection methods are used in obtaining termites from their mounds or nests. In the countries where termites are eaten, sexual winged reproductives on nuptial flights are collected during the maiden rains. They are collected in the evenings by placing a basin filled with water right under a source of light. Termites attracted to the source of light, fall into the water-filled basin and get trapped (van Hius, 2003; Chung, 2008; Kinyuru *et al.*, 2010).

In the Democratic Republic of Congo, the emergence hole on the mound is covered with a basket turned upside down. Termites that cling to the bottom of the basket are detached every few minutes by shaking into a container (van Hius, 2003). In another method, an emergence hole is covered with a dome-shaped framework of sticks or elephant grass covered with banana leaves, while all other holes are blocked. An opening at one side of the dome-shaped structure has a light source that attracts flying termites into a receptacle under it.

The women and children of the Central African Republic, push saliva-wet grass blades or tree barks into the open shaft of the mound. Smoke is blown into the opening, causing the soldiers to cling to the grass blades. The blades are pulled out and soldiers are stripped into a receptacle. The ground around the mounds of some species is continuously beaten or drummed to trigger them to emerge and soldiers collected. The queens are collected by digging up the entire mound and in the process potentially destroying the colony.

In Togo and Benin, harvesting is done by making an opening in a termite mound and placing a fibrous and humidified substance in a calabash over the hole (Farina *et al.*, 1991; Chrysostome, 2009). Termites collected by this method are used in feeding poultry.

Some of the substances used are cow dung, maize cobs, maize stalks, and stalks of sorghum. The calabash is protected against excessive heat by covering it with branches and grasses.

2.15 Poisonous termites

Chrysostome (1997), reported that some termites are not suitable for feeding poultry. The humus-feeding termites in the genus *Noditermes* were reported by farmers to be poisonous to poultry. All poultry fed with *Noditermes* died after one week in an experiment conducted by Chrysostome (1997), with the highest mortality recorded for guinea fowls (76.9 %). Reports on the toxicity of termites to livestock seem to be scarce. However, soldier castes are known to secrete toxic substances or have powerful mandibles that are used in defending the colony against intruders.

Defence against termites is a well-known phenomenon. But in the past 25 years, very little work has been done (Šobotník *et al.*, 2010), with the last exhaustive review published by Prestwich in 1984. The task of defence is primarily that of the soldiers and workers in soldierless colonies. The colony is defended by employing either mechanical and chemical defence systems or both (Diyana *et al*., 2018).

The mechanical defence involves soldiers using their heavily sclerotized mandibles to bite or snap an intruder. Complementary to mechanical defence is the release of chemical secretions from the exocrine glands (Šobotník *et al*., 2010). The predominant compounds found in these secretions are terpenes (monoterpenes, diterpenes, and sesquiterpenes), aromatic compounds, quinones, and macrocyclic (Evan *et al*., 1977; Evans *et al*., 1979; Mill, 1983; Prestwich, 1984; Plasman *et al*., 1999). However, across the different species, colonies, and populations, there are extreme variations in the secretions both in quantity and quality (Nelson *et al*., 2001). These chemicals either act as a repellent, irritant, immobilizing agent, anti-healing, or toxins (Mill, 1983; Šobotník and Dahlsjö, 2017). The glandular secretions of soldiers of *Armitermes* spp. contain poisons that are applied topically onto the target (Mill, 1983). A GC/MS analysis separated four compounds from *Macrotermes carbonarius*, three of which were unidentified and one identified as lauric acid methyl esther (Diyana *et al*., 2018). The insecticidal activity of lauric acid has been demonstrated by Mohamed *et al*. (2013), where 100 % mortality within 24 hours was recorded for *Aphis gossypii*. A polycyclic diterpene, acting as an irritant and glue was extracted from the frontal gland of *Trivervitermes* and *Nasutitermes* soldiers (Laurent *et al*., 2005).

The available information indicates that many of these secretions are principally directed against ant predators (Mill, 1983). Nonetheless, they are effective against vertebrate predators such as anteaters (Lubin and Montgomery, 1981), aphids (Mohamed *et al*., 2013), fungal pathogens, centipedes, and other insects (Šobotník and Dahlsjö, 2017). The mechanism involved in the lethality of toxic termites to poultry seems to be unknown or yet to be investigated.

CHAPTER THREE

3.0 Evaluate black soldier fly larvae rearing systems

3.1 Introduction

Larvae of the black soldier fly (BSF) are a readily available source of protein that can be utilised in animal feed to replace non-sustainable and expensive protein sources (Tomberline and van Huis, 2020; van Huis *et al*., 2020). In Africa, in particular, there is advocacy for poultry and fish farmers to include insects, particularly BSF, in their feed to improve the nutrition of their livestock and reduce production costs (Abro *et al*., 2020; Chia *et al*., 2019; Ssepuuya *et al*., 2017), even though there is no data yet on BSF adoption rates by farmers (Abro *et al*., 2020). Black soldier fly larvae can be produced cheaply on a wide range of organic waste materials. Several studies have tested the suitability of many organic waste streams on the growth, development, and proximate composition of BSF, with promising results (Banks *et al*., 2014; Čičkova *et al*., 2015; Danieli *et al*., 2019; Miranda *et al*., 2019).

In most BSF production systems, adults are reared in cages to obtain eggs that are placed on the most suitable and available substrates (Caruso *et al*., 2014, Diener *et al*., 2009). In Ghana, BSF larvae production in the adult production system was tested on a small-scale production at Fish for Africa (FFA), Accra (Devic *et al*., 2014; Anankware, 2016). Eggs collected from caged adults were inoculated onto organic wastes in metallic troughs and larvae were manually removed from the waste after 14 days. Another study conducted on the production of BSF larvae also in Ghana between 2014-2015, by ENTO-PRISE an AgriTT Research Challenge Fund Project, used a bay system. The bay system utilises eggs collected from reared adult flies and inoculated into concrete bays to allow the development of larvae. One edge of the bay was inclined at an angel that led into a trench, where prepupae crawled out and were collected.

However, egg production is a complicated technique that requires specific expertise. Therefore, small systems have been developed for individual farmers or hobby gardeners, consisting of exposing substrates to naturally occurring BSF females for laying eggs (Kenis *et al*., 2018). Koné (1998) developed a similar system in Mali to produce housefly larvae by the exposure of organic substrates to attract wild fly populations for oviposition. They used cement beds which served as substrates holding chambers to attract wild flies to oviposit and develop into larvae. On the fourth day after exposure for oviposition, larvae were separated from the waste by removal of the upper layer and sifting the rest through a colander to release the larvae. The system was later improved as described in Koné *et al*. (2017).

In a simple system such as natural oviposition that relies on wild fly populations, it is crucial to select substrates that are suitable for stimulating oviposition and also meet the nutritional requirement for the development of the larvae. The "preference-performance principle" postulates that gravid female insects prefer to oviposit in substrates that maximize offspring fitness (Jaenike, 1978). Such behaviour is common among phytophagous species (Gripenberg et al., 2010), and it has also been observed in detritivorous flies (Baleba *et al*., 2019). In holometabolous insects such as BSF, where the juveniles are incapable of relocating after hatching and with no parental care, females should favour oviposition substrates that are most suitable for larval development. Egg-trapping efficiency is paramount to the effectiveness of a BSF larvae production system based on natural oviposition (Sripontan *et al*., 2017). Moreover, several studies (Newton *et al*., 2005; Li *et al*., 2011; Oonincx *et al*., 2015a; Barrangen-Fonseca *et al*., 2018) have indicated that the quality of BSF larvae meal is contingent on the nutritional composition of the rearing substrate. The substrate used also affects the survival rate and development duration of BSF larvae (Newton *et al*., 2005; Oonincx *et al*., 2015a).

The literature on the natural oviposition system especially for BSF larvae is limiting. The only documented study was conducted by Nyakeri *et al*. (2016) in Bondo, Kenya, where the ability to attract BSF using an open system rearing bin was studied. However, YouTube and other online blogs (DipTerra.com, insectus.com, bsffarm.com, Protera.com) are flooded with a great variety of household-based BSF larvae treatment rectors developed and promoted by the enthusiastic hobbyist (Diener *et al*., 2015b). The motivation here is to make farmers self-sufficient by treating their farm waste while producing the protein component of feed for the farm animals. The information from the blogs seems to suggest that the production of BSF larvae under natural oviposition is an easy task, needing low-tech and low-cost solutions.

This study, therefore, sought to evaluate the effect of organic wastes on BSF egg trapping efficiency, the development of larvae, and natural oviposition rearing using a bin system.

The result of the study is expected to provide information on the selection of the most suitable substrate and methods for use by smallholder fish and poultry farmers.

3.2 Hypothesis 1: The substrates most attractive for black soldier fly (BSF) oviposition are also those that are most suitable for larval development.

Objectives:

i. To determine the substrate(s) that can maximize oviposition by female black soldier flies (BSF).
ii. To determine the substrate(s) appropriate for enhanced larvae development

3.2.1 Methodology

Substrates used

Six organic waste substances (substrates) collected from markets, livestock farms, and local food processing industries were tested for their suitability as oviposition and larval rearing media. The substrates used were pito mash (waste from a locally brewed sorghum drink), millet porridge mash, pig manure, chicken manure, fruit waste, and waste from roots and tubers (Plate 2). The pito mash used in the study was obtained from a pito processer in Ashaiman and the millet porridge waste from Kisseman (both suburbs in Greater Accra Region). Fruit waste and roots and tubers were from the Madina market (the main foodstuff market in the La

Nkwantanang district of the Greater Accra Region). Pig manure was obtained from Council for Scientific and Industrial Research's (CSIR) Animal Research Institute, whiles, the chicken manure was obtained from a private poultry farm in Ashaiman. All substrates were used in the state in which they were collected, except chicken manure. Chicken manure meal was prepared using 6 kg of chicken manure to 9 litres of water. This measurement was determined through preliminary tests to obtain a moisture content of about 78 %. Black soldier fly larvae are known to prefer moisture content in their diet between 70-80 % (Li *et al*., 2011; Cheng *et al*., 2017; Meneguz *et al*., 2018; Lalander *et al*., 2020). This was also done to match up the moisture content of the other used substrates which ranged between 59 and 80 %. Fruit waste was an amalgamation of five fruits: pawpaw, pineapple, orange, watermelon, and banana in equal proportions. Millet porridge mash was composed of millet grains, chilli pepper, black peppercorns, cloves, and ginger milled together. The mash is the remaining residue after straining out water that has been added to the aforementioned mixture to collect the starchy liquid for making porridge. Samples of each substrate from all the experiments made between September and December 2018 were collected and refrigerated at 4°C The substrates were pooled at the end of the experiment and sampled for analysis to assess the nutritional profile of each substrate used. The samples were dried at 65°C until a constant weight was obtained. The nutritional profile (crude protein, crude fibre, crude fat, ash, and dry matter was determined using the procedure of the Association of Official Analytical Chemists (AOAC) (2016).

Plate 2. Organic substrates tested: (A) roots and tubers; (B) pig manure; (C) pito mash; (D) fruit waste; (E) millet porridge mash (F) chicken manure

3.2.2 Determining the substrate(s) that can maximize oviposition by female black soldier flies

The study was carried out at the University of Ghana school farm at the Botanical gardens between July to September 2018 for seven weeks. A well-shaded area with mango trees and shrubs was chosen as BSF adults take rest in trees where they mate as well. Five-litre plastic bowls were filled with 500 g of each of the substrates to be tested and provided with an oviposition medium made up of corrugated cardboard (Booth and Sheppard, 1984) cut into 10 cm x 6 cm pieces, and taped together to form bundles of three. Four bowls (n=4) per substrate (a total of 24 bowls) were exposed simultaneously at the selected site on the University of Ghana school farm. The bowls were arranged on a roofed stand in a completely randomized order, making sure that the position of the bowl does not influence oviposition. The stand was roofed to shade the substrates from rain. The oviposition media were placed on dried plantain leaves that were placed on the substrates in the bowls (Plate 3A). The dried plantain leaves were to help keep the substrate moist and to avoid wetting the cardboard since BSF females do not lay eggs on wet substrates. Two cardboard bundles were placed on each bowl used in the test. The flutes of the cardboards (Plate 3B) and dried leaves were checked daily for the presence of eggs. Substrates were replaced every five days because, as the days went by, other insects colonized them. A very fine tip needle was used in detaching the egg masses from cardboard for weighing (Plate 3C). Egg masses collected from each bowl were weighed separately and weights recorded.

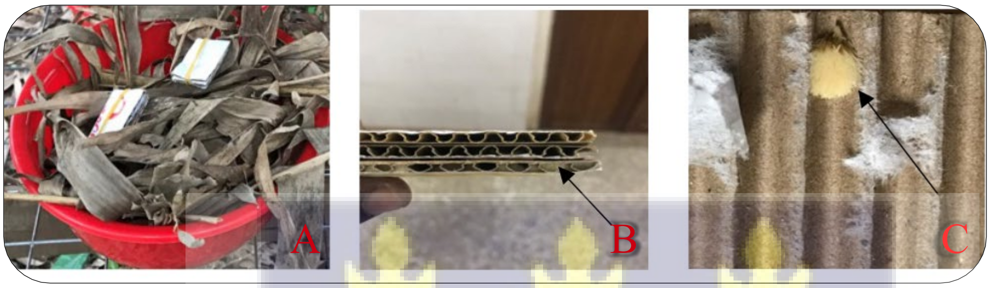

Plate 3. (A) Bowl containing substrate with dried plantain leaves and oviposition cardboards; (B) flute of cardboard containing laid eggs; (C) flute opened to expose eggs

3.2.3 Determining the substrate(s) appropriate for enhanced larvae development

Small plastic bowls (750 ml) were filled with ten grams of the substrates (four replicates of each substrate). Egg masses weighing 10 mg each were placed on 6 cm x 6 cm plastic plates and placed on the respective substrates in the 750 ml plastic bowls to allow eclosion (Plate 4A). The bowls were placed in a screen cage made of muslin in the laboratory to prevent oviposition by other insects (Plate 4B). Dates of eclosion for the substrates (bowl) were recorded and larvae were allowed to develop for four days. On day five, the young larvae were transferred into 10 L bowls filled with 500 g of the substrates (Plate 4C).

Plate 4. (A) plastic bowls used in the incubation of eggs; (B) Incubated eggs in a screen house to prevent oviposition by other insects

The bowls were covered with a muslin cloth and kept in a screen house to prevent oviposition by other insects (Plate 5). A sample of 100 prepupae was selected from each bowl (400 for each substrate) and individual weights were recorded. Prepupae were identified by their characteristic dark brown or black colour. In addition, the total weight of prepupae (prepupal yield) recovered from each bowl was taken, the total number of larvae developed, and the duration of development (date of the first prepupae) for each bowl was recorded.

Samples of prepupae recovered were dried at 65°C and weighed every 24 hours until a constant weight was obtained. The nutritional profile (crude protein, crude fibre, crude fat, ash, and dry matter) of the samples was determined using the procedure of the Association of Official Analytical Chemists (AOAC) (2016). The whole study included three consecutive experiments made between September 2018 and December 2018, with each trial taking a minimum of one month to complete. In total, there were twelve replications per substrate.

Plate 5. Experimental set up in a screen house to prevent alien fly oviposition

3.2.4 Data analysis

All analyses were performed using the R environment for statistical computing (version 3.6.2) (R Core Team, 2018 and 2019). The nutritional profile (crude protein, crude fibre, ash, fat, and moisture) of the substrates was subjected to a one-way Analysis of Variance (ANOVA) ($\alpha < .05$).

Substrates from all the experimental periods (seasons) were pooled at the end of the experiment and sampled for analyses to have an idea of the quality of the substrates.

The effect of different factors (substrate and week of the experiment) on the weight of eggs was examined using a linear mixed effects model (Pinheiro *et al*., 2018). The fixed effect was the substrate while the random effect was the week of the experiment. The penalized quasi-likelihood (PQL) was used to estimate the parameters of the model because data on the weight of eggs were not normally distributed. PQL is a flexible technique that can deal with non-normal data (Venables and Ripley, 2002). The lme function of package nlme (Pinheiro *et al*., 2018) was used for the linear mixed effect model using the maximum likelihood estimator and the glmmPQL function of package MASS (Venables and Ripley, 2002) was used for the linear mixed effect model using penalized quasi-likelihood (PQL) estimator. Similarly, the effect of different factors (substrate and period of the experiment) on the weight of prepupae was examined using a linear mixed effects model (Pinheiro *et al*., 2018). In this second model, the fixed effect was the substrate while the random effect was the period of the experiment. The maximum likelihood (ML) was used to estimate the parameters of the model because the data on the weight of prepupae were normally distributed. The means and confidence intervals of the weight of eggs and the weight of prepupae were computed from the linear mixed-effects models using the package "effects" (John, 2003). The function ggplot of the package ggplot2 (Wickham, 2016). was used to construct the evolution curve of the weight of eggs in the function of the substrates and the weeks. A post hoc test was used for means structuration in the function of the treatment (substrate) for the weight of eggs and the weight of prepupae. The package “emmeans” (Russell, 2020) was used to perform the post hoc tests.

To determine the effect of substrates and experimental periods (seasons) on the crude protein content of prepupae produced, a General Linear Model was used to compute a two-way ANOVA ($\alpha < 0.05$). All the other parameters (fat, ash, and crude fibre) were pooled for proximate analysis due to insufficient sample quantities.

Furthermore, the duration of development (egg to the first prepupae), the total weight of prepupae (prepupal yields) recovered, and the total number of individual prepupae recovered were subjected to a two-way ANOVA to show the effect of substrate on them ($\alpha < 0.05$). In all the Tukey HSD test was used as a Post Hoc test.

3.2.5 Results

3.2.5.1 Proximate analysis of substrates

The crude protein and the moisture content for all six substrates were significantly different ($p < 0.0001$). Pito mash had a significantly higher crude protein content when compared with the other substrates, except roots and tubers (Table 1). Fruit waste recorded the highest moisture content. Likewise, the ash, crude fibre, and fat contents of the different substrates were significantly ($p < 0.0001$) different (Table 1). The ash content of millet porridge mash was not significantly different from pito mash, however, the other substrates had significantly different ash content. Chicken manure had higher ash content compared to the other substrates. A significantly different crude fibre content was obtained for roots and tubers. Millet porridge mash and fruit waste had significantly different crude fibre content from pito mash, pig manure, and chicken manure. The fat content of millet porridge mash, pito mash, and fruit waste was significantly different from those of pig manure, roots and tubers, and chicken manure (Table 1).

Table 1. The nutritional composition of the various substrates was tested.

Substrate	**Crude protein ± SE (%)**	**Ash ± SE (%)**	**Fat ± SE (%)**	**Crude fibre ± SE (%)**	**Moisture ± SE (%)**
Millet porridge	20.8 ± 0.4^{b}	1.8 ± 0.1^{e}	13.8 ± 0.1^{a}	$20.\ 0 \pm 0.7^{b}$	73.9 ± 0.1^{d}
Pito mash	30.6 ± 0.3^{a}	3.2 ± 0.2^{de}	12.0 ± 0.2^{a}	31.8 ± 0.3^{a}	79.9 ± 0.1^{b}
Fruit waste	8.1 ± 0.4^{e}	10.4 ± 0.6^{c}	12.4 ± 1.2^{a}	15.7 ± 1.0^{b}	87.1 ± 0.4^{a}
Pig manure	16.1 ± 0.2^{c}	16.3 ± 0.1^{b}	6.7 ± 0.9^{b}	35.1 ± 2.3^{a}	70.8 ± 0.3^{e}
Roots and tubers	3.3 ± 0.1^{f}	4.3 ± 0.1^{d}	8.1 ± 0.1^{b}	1.9 ± 0.2^{c}	59.1 ± 0.1^{f}
Chicken manure	13.0 ± 0.3^{d}	23.8 ± 0.7^{a}	7.7 ± 0.5^{b}	35.7 ± 1.3a	78.0 ± 0.4^{c}
df	5	5	5	5	5
F- value	1028	503.6	14.82	126.1	1381
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $p < 0.05$).
SE= Standard Error

Determining the substrate(s) that can maximize oviposition by female black soldier flies

The type of substrate significantly (P< 0.001) influenced the quantity of eggs laid (Table 2). The estimate of the variance explained by the week of the experiment (random effect) is distinguishable from zero (5.06), then the period of the experiment affected the weight of eggs laid. Only 14.74 % of the total variance of the random effect is attributed to the week of the experiment effect.

Table 2. Effect of the substrate on the weight of eggs: detailed results of the linear mixed effects model

Source of variation	**Estimate**	**SE**	DF	t-value	***p*-value**
(Intercept)	2.842857	2.649670	156	1.072910	0.285
Fruit waste	-0.110714	3.541258	156	-0.031264	0.975
Millet porridge mash	28.796429	3.541258	156	8.131694	**<0.001**
Pig manure	1.346429	3.541258	156	0.380212	0.704
Pito mash	-1.700000	3.541258	156	-0.480055	0.632
Roots and tubers	-0.453571	3.541258	156	-0.128082	0.898

Df: Degree of freedom; SE= Standard Error; t-value: Student statistics value; *p*-value: Probability value.

Females laid significantly more eggs on millet porridge than on other substrates (Table 3 and Figure 1).

Table 3. Estimate of the weekly mean weight of eggs laid and confidence intervals (CI).

Substrates	The weekly mean weight of eggs ± SE (mg)	CI_lower	CI_upper
Chicken manure	2.8 ± 1.3^{b}	-2.3	8.0
Fruit waste	2.7 ± 1.37^{b}	-2.4	7.9
Millet porridge mash	31.6 ± 4.9^{a}	26.5	36.8
Pig manure	4.2 ± 2.5^{b}	-0.9	9.3
Pito mash	1.1 ± 0.7^{b}	-3.9	6.3
Roots and tubers	2.4 ± 2.1^{b}	-2.7	7.5

Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $p < 0.05$)
CI: Confidence interval; SE: Standard error of the mean

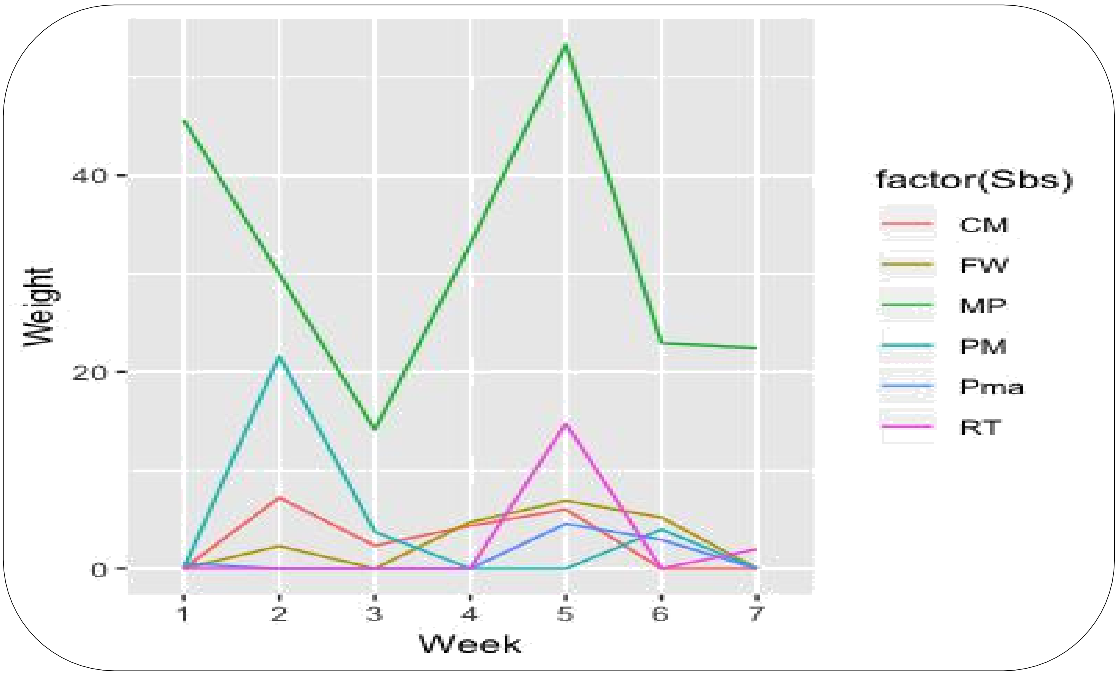

Figure 1. Evolution curve of the weight of eggs in the function of the substrate and the week

Legend: CM: Chicken Manure; FW: Fruit Waste; MP: Millet Porridge; PM: Pig Manure; Pma: Pito mash; RT: Roots and tubers.

3.2.5.2 Determine the substrate(s) appropriate for enhanced larval development

The global result of the linear mixed effects model revealed that the substrate significantly ($P < 0.001$) influenced the individual weight of prepupae (Table 4). The estimate of the variance explained by the period of the experiment (random effect) is distinguishable from zero (150.06), meaning that the period of the experiment influenced the weight of the prepupae. Only 29.62 % of the total variance of the random effect is attributed to the period of experiment effect. The detailed result of the linear mixed effects model (Table 4) showed that all substrates were significant ($P < 0.001$), while the total weight of prepupae was greatest on pig manure (Table 5).

Table 4. Effect of the substrate on the weight of prepupae: detailed results of the linear mixed effects model

Source of variation	Estimate	SE	DF	t-value	*p*-value
Fruit waste	38.9	1.2	7201	32.71452	<0.001
Millet porridge mash	54.0	1.2	7201	45.40229	<0.001
Pig manure	123.4	1.2	7201	103.91715	<0.001
Pito mash	113.0	1.2	7201	95.11055	<0.001
Roots and tubers	21.9	1.2	7201	18.46500	<0.001

DF: Degree of freedom; SE: Standard error; t-value: student statistics value; p-value: probability value

Table 5. Estimate of the means and confidence intervals (CI) of the weight of individual prepupae

Substrate	**Means ± SE (mg)**	**CI_lower**	**CI_upper**
Chicken manure	81.4 ± 12.1^{f}	69.9	92.9
Fruit waste	120.3 ± 10.3^{d}	108.9	131.7
Millet porridge mash	135.4 ± 8.9^{c}	123.9	146.8
Pig manure	204.9 ± 2.4^{a}	193.4	216.3
Pito mash	194.5 ± 9.6^{b}	183.0	205.9
Roots and tubers	103.4 ± 15.1 ^{e}	91.9	114.8

Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $p < 0.05$)
SE: Standard error

The total prepupal yield of pig manure was significantly higher in comparison to the other substrates, while chicken manure and fruit waste had comparable prepupal yields (Table 6). Millet porridge was also significantly higher in total prepupal yield when compared to chicken manure, fruit waste, and roots and tube.

Table 6. Effect of substrate on total prepupae yields, the total number of individuals surviving to prepupae, and the time for development from egg to prepupae stage

Substrates	**Total prepupal yields** ± SE **(grams)**	**Total number of individuals Surviving** ± SE	**Development time** ± SE **(days)**
Chicken manure	23.4 ± 3.4^{d}	262.6 ± 10.5^{b}	17.1 ± 0.5^{c}
Fruit waste	30.4 ± 2.1^{d}	313.5 ± 26.0^{ab}	22.6 ± 0.8^{a}
Millet porridge	46.4 ± 3.4^{b}	353.3 ± 14.3^{a}	15.8 ± 0.2^{c}
Pig manure	59.8 ± 4.5^{a}	348.9 ± 34.6^{a}	16.7 ± 0.3^{c}
Pito mash	44.0 ± 4.0^{bc}	252.7 ± 21.2^{b}	16.3 ± 0.2^{c}
Roots and tubers	31.9 ± 2.4^{cd}	$303.8 \pm 20.4a^{b}$	18.9 ± 0.9^{b}
Df	5	5	5
F-value	20.19	33.57	4.57
P -value	< 0.001	< 0.001	0.001

Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $p < 0.05$)
SE: Standard error

The trend in total prepupal yield in decreasing order was pig manure > millet porridge > pito mash > roots and tubers > fruit waste > chicken manure.

The development time and the total number of individuals that survived to prepupae were also significantly ($p < 0.0001$ and $p = 0.001$, respectively) affected by substrate type. Fruit waste and roots and tubers had the longest development time of 23 and 19 days, respectively when compared to the others (Table 5). The development time was not significantly different for millet porridge, pig manure, and pito mash, recording a lower number of days to reach prepupae. Prepupal development time increased in the order, millet porridge < pito mash < pig manure < chicken manure < roots and tubers < fruit waste.

The total prepupal survival on millet porridge and pig manure was comparable but significantly higher than on pito mash and chicken manure. Fruit waste and roots and tubers were similar in total prepupal survival.

There were significant ($P < 0.0001$) interactions between the time that the experiment was conducted and substrate type on the total prepupal survival and also on the duration of development from egg to prepupae. However, there was no significant interaction between the time the experiment was conducted and substrate type on total prepupal yields.

Furthermore, the substrate type had a significant ($p < 0.001$) influence on the crude protein accumulated by developing larvae. The crude protein content of pito mash was highest, but not significantly different from roots and tubers (Table 7). Fruit waste had a higher crude protein than pig manure, while pig manure and chicken manure were similar. Crude protein for prepupae reared on the different substrates was in the order pito mash > roots and tubers > millet porridge > fruit waste > chicken manure > pig manure (Table 6).

The ranges of crude ash, fat, crude fiber and moisture were 7.72 - 19.79 %, 25.59 - 41.32 %, 9.95-12.26 %, and 57.50 – 61.83 %, respectively (Table 7).

Table 7. The nutritional composition of black soldier fly prepupae reared on different substrates

Substrate	Parameter (Mean ± SE)				
	Crude protein ± SE (%)	Ash ± SE (%)	Fat ± SE (%)	Crude fibre ± SE (%)	Moisture ± SE (%)
Millet porridge mash	40.7 ± 0.9^{bc}	7.7±1.8a	32.0±2.4a	12.3±0.8a	61.0±0.5a
Pito mash	43.4 ± 1.5^{a}	11.5±1.5a	41.3±0.2a	10.2±1.4a	60.2±0.2a
Fruit waste	39.4 ± 1.37^{c}	8.8±0.4a	38.1±4.0a	11.4±0.2a	60.6±0.5a
Pig manure	34.7± 0.5^{d}	15.1±1.5a	31.8±0.8a	9.9±0.3a	57.5±1.3a
Roots and tubers	42.6 ± 1.8^{ab}	12.4±1.1a	40.6±1.1a	10.4±0.3a	59.3±1.3a
Chicken manure	36.2 ± 2.6^{d}	19.8±3.8a	25.6± 2.2a	10.4±0.3a	61.8±3.0a

Means in the same column followed by the same letter are not significantly different (Tukey's HSD, $p < 0.05$).
SE: Standard error

3.2.6 Discussion

Jaenike (1978) indicated that there is a strong selection pressure on adult insects to oviposit on or near their source of food to maximize offspring fitness. Such behaviour is common among phytophagous species whose juveniles are incapable of relocating after oviposition (Gripenberg *et al.*, 2010), and it has also been observed in detritivorous flies (Baleba *et al.*, 2019). It is expected that the choice of food by the adult (mother) meets the requirement for the growth and development of the offspring. Based on this principle, the study tested whether oviposition preference of wild BSF populations correlated with optimum development of larvae on some selected substrates.

The results from the study indicated that the type of substrate used had a significant influence on egg laying, final individual prepupal weight, the total number of individuals recovered, the total weight of prepupal recovered, development time, and crude protein content of recovered prepupae.

The most efficient substrate acting as bait to lure gravid female BSF to oviposit was millet porridge mash. Two recent studies (Sripontan *et al.*, 2017; Ewusie *et al.*, 2019) compared the effect of substrates as an attractant for oviposition by BSF. The study by Sripontan *et al.* (2017) reported that fruit waste was most efficient in attracting gravid BSF females for oviposition, while Ewusie *et al.* (2019) reported pig manure as the most efficient. However, none of them tested millet porridge. According to both studies, the field populations of BSF in the study site were pre-exposed to fruit waste and pig manure respectively, and therefore possibly accounting for their preference as oviposition substrate among the substrates used. Jaenike (1983) reported that prior exposure by dipterans to a food source may subsequently influence their preference as an oviposition medium/site. Perhaps, the success of millet porridge mash as a lure in the current study was due to the pre-exposure of the field populations to chilli, an ingredient in millet porridge mash. In the experimental farm where the study was conducted, farm workers cultivate chilli pepper and extract the seeds for planting. The remaining mesocarp after seed extraction is sun-dried and milled into powder for use in culinary activities. During sun drying of the mesocarp, larvae of BSF were; usually found colonizing the chilli (personal observation; communication by the farmers). Some insects are also known to be attracted to spices, especially peppers (Wilson, 2003). However, the small numbers obtained with the other substrates, including pig manure and fruit waste, which were considered by Sripontan *et al.* (2017) and Ewusie *et al.* (2019) as successful lures, maybe simply be because the substrates were tested simultaneously and millet porridge may have been too attractive, overshadowing the attractiveness of the other substrates.

The fruit mix used by Sripontan *et al*. (2017) differed from the combination of fruits in this study. While the authors from the former study included apples but excluded oranges, this study included oranges and excluded apples. Since testing was not done for the individual fruits on their effectiveness in acting as bait for BSF oviposition, it may be hasty to conclude that the exclusion of apples and inclusion of oranges in this study accounted for the differences seen between the two studies on fruit waste acting as the best lure.

Although chicken manure was not the most attractive substrate, nonetheless, some quantity of eggs was laid on it. This corroborates studies by Booth and Sheppard (1984). No eggs were, however, deposited on chicken manure in the studies by Ewusie *et al*. (2019) and Sripontan *et al*. (2017).

In another study, Ganda *et al*. (2019) exposed a large number of substrates to house flies and BSF, although they did not count the eggs laid on each substrate, the quantity (weight) of larvae collected from the substrates provided a good indication of the attractiveness of the substrates since our study showed that differences among substrates are much lower in larval performances than in oviposition rates. Ganda *et al* (2019) obtained much more BSF larvae from agri-food wastes such as maize bran and soybean bran than from pig or chicken manure, which corroborates the results of this study.

Like millet porridge mash, pito mash seems not to have been reported as an attractant used in collecting BSF eggs. Pito mash, in the presence of the other substrates, had the lowest luring ability for BSF oviposition.

Although all substrates were suitable for larval growth, the most efficient substrate was pig manure. Pig manure recorded the highest individual prepupal weight, while chicken manure recorded the least. Prepupae that had grown on pig manure were twice as heavy as those reared on chicken manure. The total prepupae and the total number of prepupae surviving on pig manure were 25% and 59% more than on chicken manure. However, the development time was similar on both substrates. Both manures have been reported as suitable substrates for rearing BSF larvae (Miranda *et al*., 2019; Oonincx *et al*., 2015a; Zhou *et al*., 2013). Oonincx *et al*. (2015a) reported better survival of BSF on pig manure than on chicken manure, while Miranda *et al*. (2019) reported the contrary. In addition, Zhou *et al*. (2013), recorded the highest larvae weight reared on chicken manure among the different substrates tested. The differences are attributable to the differences in the type of chicken and pig manures used. In particular, the quality of chicken manure to produce fly larvae may depend on whether or not it contains litter, and which type of litter. The chicken manure used in this study was from a deep litter house whereas Zhou *et al*. (2013) used manure from a battery cage. Manure from a deep litter house has a mixture of wood shavings, chicken dropping, feathers, and feed leftovers, while manure from a battery age is mainly made up of pure chicken dropping due to the nature and design of battery cages. Manure from the deep litter system was produced by broilers while manure from the battery cage system is from layers. The differences in the manure used could account for the differences observed in the two studies. In particular, the wood shaving contained in the manure from the deep litter system is probably a low-quality additive compared to straw litter (Koné *et al*., 2017) or rice bran (Sanou *et al*., 2018).

The results obtained on fruit waste were moderate but the development time was longer on this substrate. The weight and development time for the prepupae recovered from fruit waste (120 mg and 22.3 days respectively) were similar to the values (120 mg and 22.0 days, respectively) reported by Meneguz *et al*. (2018). Among the substrates tested, pito mash had the highest crude protein content and this was translated into the crude protein content of the final prepupae obtained.

High dietary protein is reported to be important in the growth and development of BSF larvae (Cammack and Tomberlin, 2017; Meneguz *et al*., 2018; Oonincx *et al*., 2015b). In addition, pito mash had a high moisture content, crude fibre, and fat content. Furthermore, the second highest mean individual prepupal weight was recorded on pito mash following pig manure. The third highest total prepupal yield and development duration were recorded in pito mash. There is no previous documentation on the use of pito mash in the production of BSF larvae. A shorter development time for BSF has been attributed to high dietary protein and fat content (Oonincx *et al*., 2015b). Similar findings were documented for yellow mealworms when fed on a high protein and a high-fat diet, (Oonincx *et al*., 2015b). High dietary moisture content has been reported to be necessary for growth. Cammack and Tomberlin (2017) demonstrated that moisture impacted larval development and adult life history more than the protein and carbohydrate contents of the diet. The requirement for high dietary moisture has been attributed to the morphology of the mouthpart of BSF larvae (Kim *et al*., 2010; Purkayastha *et al*., 2017), increased dietary moisture making it easy for scraping off food from feeding surfaces (Banks, 2014). Larvae of BSF may have performed well on pito mash due to the aforementioned qualities, although it was not successful as a lure for egg trapping of BSF.

Although generally, waste from roots and tubers had the lowest protein, moisture, fibre, and fat content, BSF performance was satisfactory. The mean prepupal weight, total prepupae yield, and the total number of individuals were moderate. In addition, the proximate profile of the resulting prepupae was good, especially the crude protein content. However, the development time was significantly longer than all substrates except for fruit waste. Food quality is known to affect the rate of development and survival in insects (de Haas *et al*., 2006). A longer development time of BSF larvae was observed when fed low protein vegetable diets than when larvae were fed high protein diets (Nguyen *et al*., 2013; Oonincx *et al*., 2015b). Green *et al*. (2003) and Barragan-Fonseca *et al*. (2018) made similar observations in *Phormia regina*

(Meigen, 1826) and *H. illucens,* respectively. All these studies confirm the possible reason accounting for the longer development time of BSF larvae reared on waste from roots and tubers and fruits.

Millet porridge mash, the preferred substrate for oviposition by wild populations of BSF, generally ranked among the best three in growth, development, and nutrient composition of the prepupae recovered. It resulted in the third highest individual prepupal wet weight but far from pig manure and pito mash, the second highest total prepupae yield, and the highest survival rate. It was also in these three substrates that BSF larvae developed the fastest. Several authors reported that high protein and fat diets supported a higher growth rate in BSF and faster development than low-protein and low-fat diets (Nguyen *et al*., 2013; Oonincx *et al*., 2015b; Tschirner and Simon, 2015). Conversely, Ujvari *et al*. (2009) reported that very high crude fat (20-60%) may be detrimental to larval survival. A diet balanced in amounts of crude protein, fat, and calories seems to be more essential for short development duration and higher larval weight (Nguyen *et al*., 2013).

Based on the preference-performance principle (Jaenike *et al*., 1978; Gripenberg *et al*., 2010; Baleba *et al*., 2019), the most efficient egg trapping substrate should record the best growth and the development of larvae, but this was not the case. Millet porridge mash was by far the most attractive substrate for oviposition, however, its performance as a larval substrate was lower than pig manure, especially when individual prepupal weight is considered. Interestingly, the high survival rate of larvae in millet porridge mash partly compensated for the small size of the prepupae.

While most substrates were very poor in attracting females for oviposition, all allowed the successful development of larvae. Differences in larval performance among substrates were rather comparatively marginal. This suggests that BSF larvae production systems based on adult rearing for egg production can run with a large variety of substrates, thus the selection of the substrates to be used will largely depend on their availability and cost. In contrast, in systems based on natural oviposition, the choice of the substrate is much more critical and the addition of attractants should be considered, as practised in house fly larvae production systems (Kone *et al.,* 2017; Ganda *et al.*, 2019).

3.2.7 Conclusion

The study demonstrated that the substrate most favourable (pig manure) for larval development was not the most favoured by gravid BSF for oviposition. While some substrates were very poor (pito mash) in attracting females for oviposition, all allowed the successful development of larvae. The differences in the growth and development of larvae among substrates were rather minor.

3.3 Testing the effect of loading rate and weight of substrates on prepupal yields

Hypothesis 2: *There is a threshold in the substrate supply, where larval yields do not increase further.*

3.3.1 Introduction

Industrial production of BSF is being developed worldwide, many of which are, however, unpublished (Kenis *et al*., 2018). The available patented production systems (owned by big companies) have not scientifically been peer-reviewed and also omit some important production information. Producing fly larvae on a small scale on individual farms using simple systems for on-farm use is feasible. The simplest of these systems is natural oviposition, where substrates are exposed (usually in bins/ containers) to attract wild gravid females of BSF and extract the developed larvae/prepupae to use as feed for poultry or fish. While not considered to be efficient enough to support the daily production of BSF larvae and the recycling of waste, it can still be employed by farmers or backyard poultry producers in regions where wild BSF are found in high densities (Kenis *et al*., 2018).

The main concern of small-scale farmers looking to adopt black soldier fly larvae production technology is the constant supply of larvae and the production of sufficient quantities to either replace or supplement the protein source in animal feed. An efficient black soldier fly (BSF) organic waste conversion system aims to achieve a high waste reduction efficiency and obtain maximum yields in larval biomass (Diener *et al*., 2009).

The main hindrance to the adoption by smallholder farmers is the ability to design and construct an efficient and economically viable BSF larvae recycling bin and to determine the total quantities of substrates needed to operate the bins over long periods.

The maximum amount of substrate that can be utilized by larvae over some time and the cyclic fluctuations when producing over long periods require further investigation to assist farmers looking to adopt the technology.

The study aimed, for one system, to determine the effect of the total amount of substrate and the frequency of its addition to a BSF rearing bin on the larval yield to optimize continuous larval production under natural oviposition. The results obtained in this study will then be discussed for their implication in determining the optimal loading rates of substrates in different systems.

3.3.2 Methodology

The BSF rearing bins used in the study were constructed with wood, measuring 1.22 m X 0.5 m, with the extreme ends inclined at 35°. The ends of the rearing bins had the inclined angles enter into holes, with each hole measuring 0.02 m in diameter and having a PVC pipe with a collecting bucket where prepupae crawl out of substrate to pupate (Plate 6).

Two different tests were carried out: i) applying different loading rates of the substrate to the rearing units and ii) applying different weights of the substrate to rearing units to assess their overall effects on the prepupal harvest. There were three treatments, set in a completely randomized design and replicated twice for each study. The experiment was conducted during the dry and wet seasons, with each lasting for three months. At the end of the first season, bins were completely emptied of their content, cleaned, and left fallow for two weeks before the next season trial was conducted.

Pito mash was used to attract adult BSF for oviposition because it can be purchased in large quantities needed for the trial.

Applying different loading rates of substrates to the rearing unit

There were three treatments: For the first treatment (T1), bins were filled with 5 kg of pito mash on the first day and 5 kg of pito mash was added to the bins twice a week. In the second treatment (T2), the bins were initially filled with 10 kg pito mash and 10 kg pito mash was added once a week. In the third treatment (T3), the bins were filled with 20 kg of pito mash and 20 kg of pito mash added every two weeks. At the end of every two weeks, 20 kg of pito mash was added to all treatments.

Applying different weights of substrates in rearing unit

In this second test, the treatments are as follows:

1. Treatment (T4):10 kg pito mash was initially added to the bin and the same quantity was added every week.
2. Treatment (T5): 10 kg of pito mash on the first day, with 10 kg added twice per week (i.e. 20 kg is added to the bin per week)
3. Treatment (T6): 10 kg of pito mash added initially and 10 kg added every fortnight.

At the end of the test, different amount of pito mash was added to the different treatment bins. After filling each bin (for both trials) with the proposed treatment, egg collection units, which were made up of strips of corrugated cardboard measuring 10 cm x 10 cm x 6 cm, were placed in packs of four on sticks and dried plantain leaves in the bins, just above the substrates (Plate 6A). Each bin had five of the oviposition cardboards.

Plate 6. (A) BSF rearing bins with oviposition cardboards; (B) BSF rearing bins with the treatments arranged in a completely randomized design

The bins were inspected daily for the presence of eggs and/or larvae. The aforementioned substrates addition protocol was followed. The bins were monitored until prepupae emergence in the buckets under the bins (Figure 6B). The prepupae collected were sent to the ARPPIS lab for weight measurements.

3.3.3 Data analyses

Daily harvest data were grouped into the weekly harvest to obtain longitudinal data or repeated measures on the same experimental unit (bin). On these longitudinal data, linear mixed-effects models (Pinheiro and Bates, 2000) were applied to assess the effect of loading rate (treatment) and time on the weekly harvest of black soldier fly larvae. In these models, loading rate and time were considered fixed factors whereas the experimental unit (bin) was considered a random factor. The least-square means of the weekly harvest of black soldier fly larvae were estimated from the above-mentioned models [using R package *lsmeans*: Lenth (2016)] and used to (i) establish summary tables and (ii) draw figures showing the evolution trend of the weekly harvest of black soldier fly larvae according to the factors considered. Models were fitted using the R package *nlme* (Pinheiro *et al*., 2018). The models as previously defined were applied on cumulative weekly harvest to evaluate the effect of the treatments. Regarding the effect of substrate weight on the harvest of black soldier fly larvae, collected data were transformed to obtain larval weight per kg of substrate. The new data were grouped by week, allowing an application of longitudinal linear mixed effects models. When significantly different, means were separated using the Tukey test. The analysis was performed using R packages "*multcomp"* (Hothorn *et al*., 2008) and "*multcompView*" (Graves *et al*., 2015). All analyses were conducted in R statistical environment version 3.5.3 (R Core Team, 2019).

3.3.4 Results

The presence of prepupae was recorded on the twentieth day after administering treatments. There was no significant (P = 0.957) effect of the loading rate on the quantity of prepupae harvested in the dry season. However, there was a significant (P < 0.008) effect of the duration of exposure to the substrate on prepupae yields. The interactive effect of loading rate and day of exposure to the substrate was also significant (P < 0.023).

In contrast, the loading rate had a significant (P = 0.007) influence on the total prepupae harvest in the wet season. Treatment 1 (T1) differed significantly from treatment 2 (T2). There was no significant (P = 0.435) effect of duration of exposure, likewise, no significant (P = 0.187) interaction was observed for the duration of exposure to substrate and loading rate on the prepupal harvest.

Generally, a larger weekly harvest of prepupae was recorded in the dry season than in the wet season (Table 8).

Table 8. Weekly harvest of black soldier fly prepupae (mean ± SE) during the dry and wet seasons

	Mean Larval weight (g)	
Loading rate	**Dry season**	**Wet season**
T1	143.16 ± 42.44	21.72± 6.79b
T2	124.85 ± 39.49	163.05± 53.06a
T3	90.37 ± 26.17	95.31± 30.35ab

For the wet season, means with different letters indicate significant differences at P < 0.05

T1: 5 kg (initially), 5 kg added in that same week and 5 kg added twice a week until the end of the experiment; T2: 10 kg (initially) and the same 10 kg added every week; T3: 20 kg (initially) and the same 20 kg added every two weeks.

Prepupal yields were larger when bins were loaded at 10 kg per week in both seasons (Figure 2). Larger yields were recorded a month after exposing substrates in the dry season than in the wet season. In the dry season, T1 consistently recorded the larger daily harvest until day 56 with the highest peak on day 35 (Figure 2a). However, it consistently recorded the lowest harvest in the wet season (Figure 2b). Similarly, in the dry season, the daily harvest in treatment 2 (T2) peaked on day 35. Treatment 3 (T3) had the largest record of harvest on day 67 in the dry season (Figure 2a). Generally, in the dry season, there were two peak harvests recorded on days 39 and 53, 39 and 80 for T1 and T2, respectively. In the wet season, the largest daily harvest for T2 was on day 80, and for T3, on day 53 (Figure 2b).

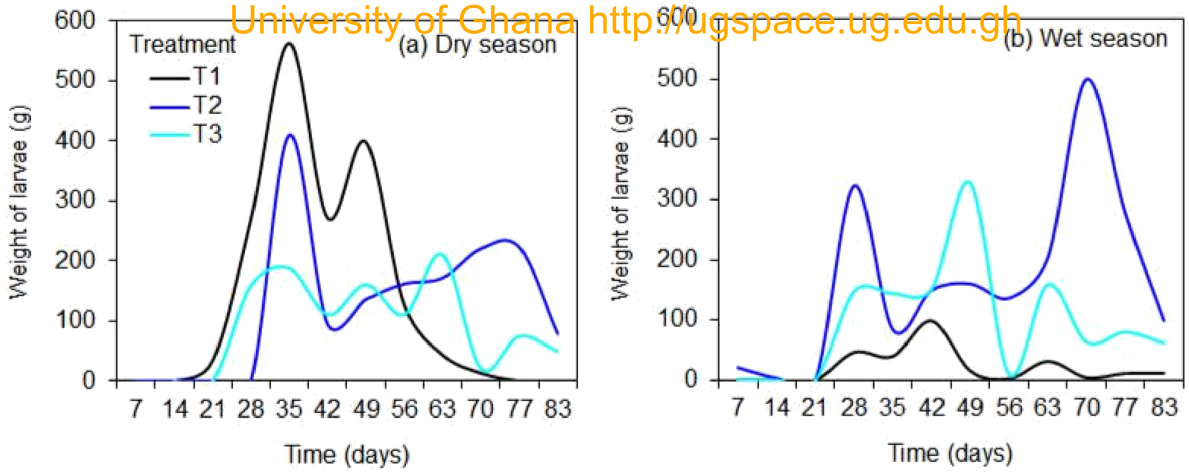

Figure 2. Trends of the weekly harvest of black soldier fly larvae according to the treatment applied and climatic season considered.

Legend: T1: 5 kg (initially), 5 kg added in that same week and 5 kg added twice a week until the end of the experiment; T2: 10 kg (initially) and the same 10 kg added every week; T3: 20 kg (initially) and the same 20 kg added every two weeks.

No significant differences were observed in the cumulative daily harvest of prepupae in the dry (P = 0.633) and wet (P = 0.126) seasons. Similarly, the interaction between loading rate and duration of exposure was not significant in both seasons (dry: P = 0.421 and wet: P = 0.746). On the contrary, time had a significant influence on the harvest of prepupae in both dry (P = 0.000) and wet (P = 0.000) seasons.

Cumulative prepupae harvested increased steadily from day 21 until day 77 in both seasons (Figure 3a). Treatment 1 recorded a larger harvest in the dry season but a consistently lower harvest in the wet season.

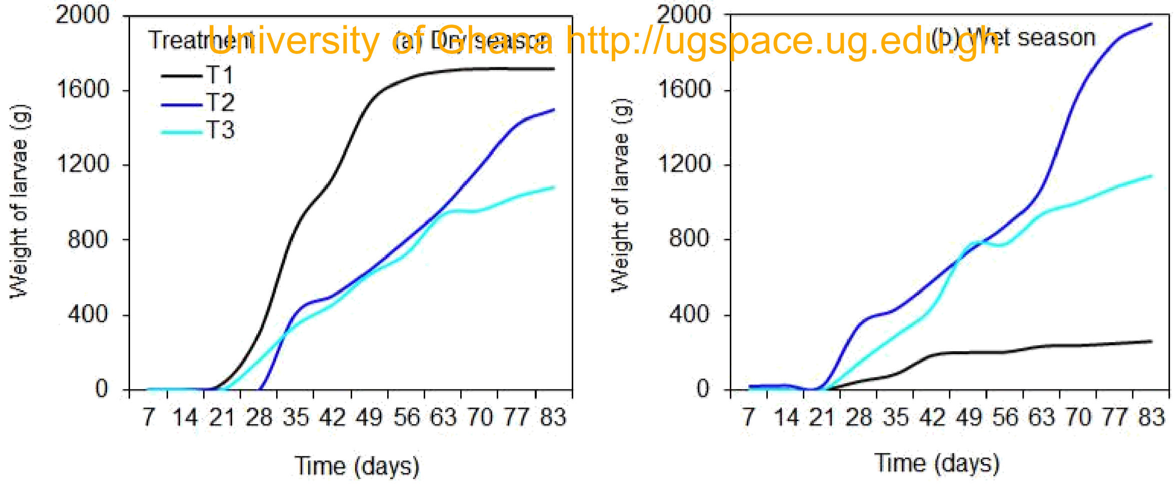

Figure 3. Trends in the cumulative weekly harvest of black soldier fly prepupae according to the treatment applied and climatic season considered.

T1: 5 kg (initially), 5 kg added in that same week and 5 kg added twice a week until the end of the experiment; T2: 10 kg (initially) and the same 10 kg added every week; T3: 20 kg (initially) and the same 20 kg added every two weeks.

A linear mixed effect model also shows that the weight of substrate had no significant (P = 0.842) effect on the weekly harvest of BSF prepupae per kilogram of the substrate in the dry season. Correspondingly, there was no significant (P = 0.653) interaction between the weight of substrate added and the duration of exposure on the overall harvest of prepupae. In the wet season, the weight of substrate significantly (P = 0.036) influenced the quantity of prepupae harvested. T5 was significantly different from T6 (Table 9). Likewise, there was a significant (P = 0.048) interaction between the weight of substrate and duration of exposure on the prepupal harvested.

The largest harvest per kg of the substrate in the dry season was recorded on days 39, 67, and 74 for T4, T5, and T6 respectively (Figure 4a). Loading substrate at 10 kg per two weeks yielded a larger harvest in both seasons (Figure 4). Likewise, the yields when bins were loaded at 10 kg once per week were similar in both seasons. Larger yields were however obtained when bins were loaded at 10 kg twice per week in the dry season than in the wet season (Figure 4).

Table 9. Weekly harvest of black soldier fly per kilogram of substrate for the different treatments and climatic seasons considered

Treatment	Dry season		Wet season	
	Mean (g)	Standard Error (g)	Mean (g)	Standard Error (g)
T4	1.79	0.69	1.79ab	0.69
T5	2.36	0.53	0.78b	0.51
T6	1.98	0.83	2.93a	0.56

For the wet season, means with different letters indicate significant differences.

T4: 10 kg (initially) and 10 kg added once every week; T5: 10 kg (initially) and 10 kg added twice every week, T6: 10 kg (initially) and 10 kg added every two weeks.

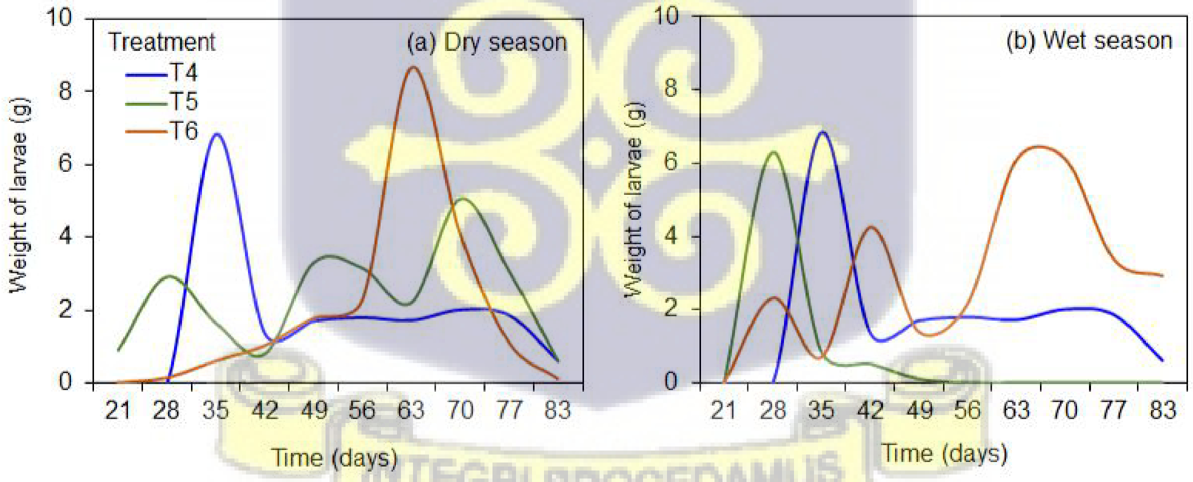

Figure 4. Trends of the weekly harvest of black soldier fly prepupae per unit (kg) of substrate according to the treatment applied and climatic season considered.

T4:10 kg (initially) and 10 kg were added once every week; T5: 10 kg (initially) and 10 kg were added twice every week, T6: 10 kg (initially) and 10 kg were added every two weeks.

The cumulative daily harvest was largest for treatment 5 than for the other two treatments in the dry season (Figure 5a). However, in the wet season, treatments 4 and 6 recorded a larger harvest than treatment 5. The daily harvest increased from day 21 until day 84 for all seasons.

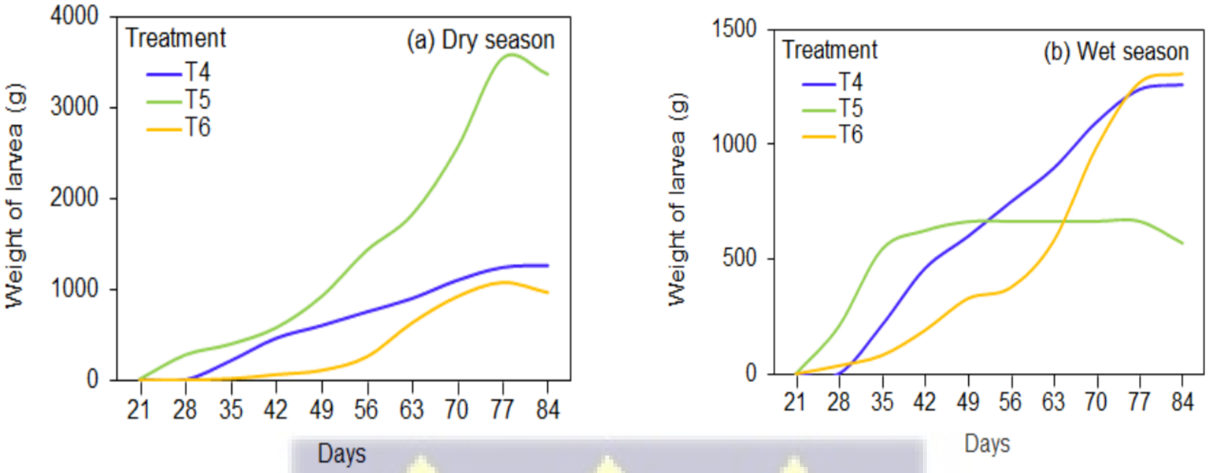

Figure 5. Trends of the cumulative daily harvest of black soldier fly prepupae when different weights of substrates are added to bins in different seasons

3.3.5 Discussion

This study shows that producing BSF larvae under natural oviposition in garden rearing bins is feasible in Southern Ghana, as in other parts of the world (Sheppard *et al*., 1994; Newton *et al*., 2005, Hem *et al.* 2008; Bullock *et al*., 2013; Rana *et al*., 2015; Nyakeri *et al.* 2016). Such systems are also frequently described in videos and popular descriptions on the internet. However, the number of scientific publications testing and comparing the performances of such systems is very limited, which does not allow a proper comparison of our results with others.

In the systems used, the first harvest of prepupae was on the 21st day following the addition of pito mash to bins. In similar rearing bins in Kenya (Nyakeri *et al*. 2016), the first batch of prepupae was harvested on the 23rd day. There were high variations in yields in the different seasons and between the treatments. Nyakeri *et al*. (2016) in Kenya also observed variations in yields during six months but found few differences between seasons, except for the first month of testing. However, Nyakeri *et al*. (2016) mainly tested the effect of different substrates on the production of BSF prepupae and the specific quantities of substrates used were not stated.

In this study, higher yields were obtained in the dry season, when temperatures ranged from 29-35°C. These temperatures are favourable for BSF activities (Chia *et al*., 2018). In addition, as the mating of BSF is dependent on the availability of light rays, increased mating is expected in the dry season, where there is enough sunshine than in the wet season when it is mostly cloudy. Moreover, in the wet season, although a shed was built over the bins, in heavy storm conditions, water gets inside some of the bins, wetting the substrate. This may have increased the moisture content of the substrate, changing the quality, and thereby reducing the efficient utilization by larvae.

In contrast, in studies on house fly production through natural oviposition in Mali (Koné *et al*. 2017) and Burkina Faso (Sanou *et al*. 2019), much higher yields were obtained in the rainy season than in the dry season. This difference is possibly due to differences in the biology of the two fly species (van Huis 2020). On the other hand, the dry season in the unimodal rainfall model of Mali and Burkina Faso is much drier, longer, and with more extreme temperature than the mild dry seasons in Ghana, and it is possible that, in the conditions of Burkina Faso and Mali, the BSF would also be more abundant in the rainy season.

Generally, there were cyclic fluctuations (peaks and troughs) in prepupal yields across the period of testing for all treatments in the two seasons. These cyclic fluctuations occurred possibly as a result of oscillating changes in BSF larvae population density inside the bins over space and time. The heterogeneous population of larvae of various ages and sizes co-existing in a confined space increases larva-to-larva interaction, with younger larvae competing for feed and space with older larvae and prepupae, triggering the latter to egress the bins.

There were clear peaks of crawl-off for all the treatments. Two clear crawl-off peaks each on days 39 and 53 were experienced when the bins were loaded with 5 kg of pito mash twice per week. Likewise, when bins were loaded with 10 kg pito mash per week, two clear crawl-off peaks on day 39 and 74, was observed. On the other hand, there were four clear crawl-off peaks on days 39, 53, 67, and 74 when bins were loaded with 20 kg pito mash every two weeks. The peak crawls off corresponded with weeks 4, 8, 10, and 11 when a generation is expected to have completed the cycle from egg to prepupal stage. Clear crawl-off peaks were observed by Sheppard *et al*. (1994), in a layer hen cage manure management system using naturally occurring black soldier flies. Oviposition occurred whenever new substrates were subsequently added to the bins, the different treatments caused a difference in the maturities of larvae and this accounted for the clear crawl-off among the treatments.

For all treatments, the largest yields were recorded on day 39 (T1 = 580 g, T2 = 400 g, and T3 = 210 g), approximately one month after set up in the dry season. In a study on the production of house flies using natural oviposition, larger yields were obtained in the first cycle when substrates were not colonized (virgin), but smaller yields and for some substrates, no yields were recorded in the second cycle after the addition of substrate to the residual substrate (Ganda *et al*., 2019).

Furthermore, it was observed in this study that, the yields increased with the subsequent addition of substrates. Usually, eggs were found deposited a day or two after substrate addition to the bins, on hatching, the neonates compete for food and space as mentioned above, possibly driving the rate of the cyclic fluctuations. The fresh substrate and consequent neonates possibly changed the conditions of the substrate by increasing the moisture and heat, causing prepupae to leave the substrate in search of a dry place to pupate. The peak yields (day 39, 53, 67, and 74) corresponded to a week after substrate addition in the bins.

Prepupal yields were greater when bins were loaded with 5 kg of substrate twice per week, likewise, when bins were loaded with 10 kg per week. On the contrary when loaded with 20 kg per fortnight yields of prepupae decreased. Black soldier fly larvae were able to better handle loading substrates in smaller quantities at a constant rate than larger quantities, falling behind utilizing larger quantities additions to the bins in the dry season. Loading bins with 5 kg pito mash twice per week yielded 27 % and 67 % more prepupae than loading at 10 kg per week and 20 kg every two weeks respectively, between 21 to 42 days (peak period) in the dry season. During production, it was noted that larvae were found feeding just about 10 cm below the top of the substrate. The larvae were unable to get deeper into the substrate to digest it, adding substrates in larger quantities prevents its optimal use, leading to the formation of a heavy impenetrable mud-like sludge. The optimal depth of substrate for BSF larvae has been reported to be between 10-15 cm, above this, the bottom may become anoxic (Kenis *et al*., 2018).

However, a different pattern was observed in the wet season, where higher yields were recorded when bins were loaded with 10 kg pito mash every week, while consistently very low values were recorded when bins were loaded with 5 kg pito mash twice every week. Black soldier flies tend to oviposit on substrates already containing their larvae (Booth and Sheppard, 1984). It was observed that in bins with higher activities of larvae, more eggs were deposited. The activities of larvae were consistently low when bins were loaded with 5 kg pito mash twice every week due to unknown reasons, subsequently, fewer eggs were laid and therefore resulting in lower yields. In this season, a larger yield (500 g) was obtained in the last month (month 3) of testing, unlike in the dry season in the first month (400 g) when bins were loaded with 10 kg piot mash per week. A possible reason for this observation could be because the season was coming to an end and therefore there was relatively less rainfall and more sunlight.

In the second test, the weight of substrate influenced the weekly prepupal harvest in the wet season, however, this was not the case in the dry season. Nonetheless, there were variations in the yields among the treatments in the dry season. As expected, the largest amount of substrate applied per week (loading at 10 kg twice per week) resulted in the largest harvest (2.36 g/kg of substrate) in the dry season. This is similar to findings by Diener *et al*. (2015), where 4.5 kg substrates (labelled high) provided per day produced a higher prepupal harvest (252 g/ day), whereas, 1.5 kg substrate/day (labelled low) yielded lower (134 g/day) harvest. In their study, larvae were fed to the bins on daily basis unlike in this study where supply was dependent on the natural fly population.

A different pattern in yield was observed in the wet season when bins were loaded with 10 kg piot mash twice per week; the treatment recorded small yields and produced prepupae only in the first month of production. A higher yield (2.93 g/kg substrate) was recorded in bins loaded with 10 kg pito mash every two weeks in this season. Nonetheless, in all two seasons, and for all treatments, a steady prepupae production was not achieved. A similar observation was reported by Diener *et al*. (2015). They reported strong fluctuations in daily harvest, just as in this study. Diener *et al*. (2015) found no clear reasons to attribute to their observations. However, they suspect the fluctuations could be attributed to exogenic (humidity, temperature, solar radiation) or endogenic (pheromones) in nature.

The natural oviposition system used in the study produced 22 to 163 g of fresh larvae per 10 kg of the fresh substrate, which is equivalent to ca. 10 g/kg in fresh weight or 20 g/kg in dry weight (considering a DM of 40% in BSF and 20% in pito mash, see Chapter 3.2.5). This yield is rather low when compared to other systems. In Accra, a small-scale BSF production system produced, on average, depending on the substrate, between 18 and 115 g of dried larvae per kg of the dry substrate (Kenis *et al*., 2018). But larger, more controlled systems can produce higher yields (Pastor *et al* 2015; Wang *et al*. 2017). Larger yields can also be produced with the natural oviposition of house flies, such as the one developed in Mali by Koné *et al*. (2017). This system produced on average 124–144 g of fresh larvae per kg of the dry substrate in 3 days, with a maximum of 427 g per kg. Few yield data are available from other similar BSF natural oviposition systems. An exception is that of Rana *et al*. (2015) in Bangladesh. They obtained a production of 186, 134, and 48 g/kg (fresh weight) with rotten wheat, rotten vegetables, and mustard oil cake, respectively.

3.3.6 Conclusion

Although the production of prepupae was low with the BSF garden bin, the study demonstrated the possibility of producing BSF under natural oviposition in Ghana. Applying small quantities of substrates (10 kg per week) at a constant rate seems to be more effective than a larger amount of substrate (20 kg twice per week) at one time. The optimum period for the garden bin to support the continuous production of BSF larvae was within two months of loading bins. High variations in yields were observed between the different seasons and the different treatments. The season when testing was conducted strongly influenced larval yields.

CHAPTER FOUR

4.0 Identification of Other Insect Species that may Populate the Rearing Substrates

4.1 Introduction

Producing BSF larvae under natural oviposition (uncontrolled system) exposes the substrates to invasion by other organisms. Naturally, different insect species other than black soldier flies will be attracted to the different substrates either to feed or to oviposit. The yields of BSF larvae will subsequently be altered due to competition for resources, and the quality of the meal, due to variation in nutritive values of the different insects. The presence of *Calliphoridae* flies and BSF were reported in a house fly natural oviposition system (Ganda *et al*., 2019). Furthermore, pupae and larvae of BSF may be attacked by natural enemies in such a system. However, very little is known about the natural enemies of BSF. Generally, animals that feed on flies (lizards, frogs, birds, and arachnids) are likely to feed on BSF larvae. However, Devic and Maquart (2015) recorded the presence of the parasitoid *Dirhinus giffardii* (Silvestri, 1913), in a cultured colony of BSF larvae in Ghana. Parasitising pupae of BSF, *D. giffardii* are known to cause about 72% reduction in broodstock of a BSF colony in a system based on adult rearing and egg production (Devic and Maquart, 2015). This parasitoid is known to be a generalist parasite of dipteran flies and lepidopteran moths (Noyes, 2014) and has been employed as a biocontrol agent in controlling tephritid flies (Mohamed, 2007; Mehmood *et al*., 2018).

The objective of this study, therefore, was to identify other insects, especially natural enemies that will be attracted to the BSF breeding substrates.

4.2 Methodology

4.2.1 Collection of other insect species attracted to the different rearing substrates

Yellow sticky traps were placed on the muslin cloth covering BSF rearing bowls and also around the experimental setup to collect other insects attempting to oviposit on the substrates. The yellow sticky traps were also applied to bowls containing prepupae of black soldier flies. BSF larvae rearing bins and bowls set on the University of Ghana School farm were also examined for the presence of other insects and these were also collected. Larvae collected were reared to adults before identification. Insects suspected to be parasitoids found visiting pupal bowls were hand collected and preserved in 70% ethanol. *D. giffardii* are known to feign death when touched (Dresner, 1964; Narendran and Amareswara, 1987), making them easy to capture.

Species were characterized morphologically using identification keys by Bland and Jaques (2010) and Scholtz and Holm (1985).

4.2.2 Assessing the incidence of parasitism in a BSF production system

A total of 1000 individual two-day-old pupae were collected from the Animal Research Institute (ARI) Black Soldier Fly Production Facility. This colony has been running for the past 5 years. The pupae were placed in four bowls of 250 individuals each and left uncovered for 5 days. This was done since it has been reported that *D. giffardii* prefers among other reasons to oviposit in older pupae, between two to three days old for better survival (Wang and Messing, 2004). Two of the bowls were placed inside adult cages and the other two were placed outside the adult cages in the rearing facility of ARI.

Each pupa after 5 days was placed individually in plastic containers, covered with a mesh lid to allow ventilation, and kept under ambient temperatures and relative humidity in the laboratory for 4 weeks. Pupae were kept for 4 weeks because, this parasitoid takes approximately 16 to 20 days to complete its life cycle from egg to adult in tropical Africa (Silvestri, 1913). The number of BSF adults that emerged was recorded.

4.3 Data Analysis

Adult emergence rate was calculated by the formula:

$$\frac{\text{Total number of adult emerged}}{\text{Number of pupae}} \times 100$$

4.4 Results

Generally, it was observed that the attempt to invade substrates by other insects was during the first five days of the life of BSF larvae, but decreased as larvae grew older. Substrates attracted other insects especially when they were still fresh, however, as BSF larvae fed on them, the frequency of invasion decreased.

Insects from six different orders and sixteen families were collected (Table 10). The majority of insects collected belonged to the order Diptera. Two species, *Drosophila melanogaster* (*Drosophilidae)* and *Musca domestica* (*Muscidae)* formed the majority of insects collected. Several insects in the order Coleoptera were also collected on the substrates and traps.

The hymenopteran, *Dirhinus giffardii* (Chalcididae), and a Diptera of the family Syrphidae were collected from the traps on the prepupal boxes and directly by hand, but not from the pupae or larvae.

Table 10. Other insect species collected from the different substrates

Order	Family	Species	Total number collected	Location/ substrate
Diptera	Calliphoridae	*Lucilia caesar*	3	Pito mash
		Lucia illustris	1	Pito mash
	Drosophilidae	*Drosophila melanogaster*	>100	Fruit waste
	Drosophilidae	*Drosophila melanogaster*	>100	Millet porridge
	Drosophilidae	*Drosophila melanogaster*	>100	Pito mash
	Drosophilidae	*Drosophila* sp.	23	Fruit waste
	Syrphidae	*Ornidia obesa*	4	Prepupae
	Muscidae	*Musca domestica*	143	Pig manure
	Muscidae	*Musca domestica*	20	Fruit waste
	Muscidae	*Musca domestica*	59	Millet porridge
	Muscidae	*Musca domestica*	137	Chicken manure
	Muscidae	*Musca domestica*	10	Pito mash
	Anthomyiidae		4	Fruit waste
Coleoptera	*Hesteridae*		2	Pito mash
	Scarabaeidae	*Kheper* sp.	1	Millet porridge
	Scarabaeidae	*Ancognatha* sp.	1	Roots and tubers
	Staphylinidae	*Tachyporus* sp.	7	Fruit waste
	Staphylinidae	*Tachyporus* sp.	10	Roots and tubers
	Phalacridae		1	Fruit waste
	Dytiscidae		1	Fruit waste
	Pselaphidae		4	Pito mash
Hemiptera	*Lygaeidae*		25	Fruit waste
Hymenoptera	*Chalcididae*	*Dirhinus gifarrdii*	9	Prepupae
Isoptera	*Kalotermitiidae*		1	Pito mash
Dermaptera	*Forficulidae*		18	Millet porridge

The rate of parasitism recorded for BSF pupae placed both indoors and outdoors was zero percent (Table 11). Eighty-five pecent of the total pupae incubated emerged as adults and 14.7% died from unknown causes.

Table 11. Percentage of adult emergence of BSF larvae from enclosed and exposed pupae

Sample	N	Emergence (%)	Parasitized	Death due unknown Causes (%)
1(outdoor)	250	85.2	0	14.8
2(outdoor)	250	84.4	0	15.6
3(indoor)	250	86.0	0	14.0
4(indoor)	250	85.6	0	14.4
Mean		**85.3 ± 0.3**		**14.7 ± 0.3**

Adult emergence = 85.3 ± 0.34%

4.5 Discussion

The most important insect collected was the hymenopteran, *Dirhinus giffardii* (Silvestri 1913), a known parasitoid of the pupae of BSF (Devic and Maquart, 2015), and reported for the first time in the BSF colony in Ghana. According to the authors, this parasitoid can hinder egg production in an adult and egg production system, reducing future broodstock by almost 72%. *D. giffardii*, is a generalist parasitoid, known to parasitise several species of Diptera (Tephritidae, Muscidae, and Glossinidae) and Noctuid lepidopterans (Noyes, 2014). The species was first recovered from puparia of the fruit fly, *Ceratitis anonae* Graham by Silvestri (1913) in Nigeria. It has been employed as a biological control agent against tephritid fruit flies an economically important pest group of commercial fruits (White and Elson-Harris, 1992; Billah, 2003; Wang and Messing, 2004; Mohamed, 2007). However, in this study, the parasitoid was collected in the vicinity of pupae, but not found parasitizing the pupae.

The common fruit fly, *Drosophila melanogaster*, was the most numerous species collected from the study. They are generalist feeders and can breed on a wide variety of rotting fruits, vegetables, and other plant matter (Markow and O'Grady, 2008; Markow, 2015). Adult *D. melanogaster* in nature, tends to form temporary aggregates on fermenting fruits, where they feed, mate, and oviposit (Reaume and Sokolowski, 2006) and the fruits also serve as a source of nutrition and habitat for developing larvae (Wertheim *et al*., 2005). This could perhaps explain the large numbers on the fruit waste used in this study. Ethanol appears to be of particular significance for the common fruit flies. This is because fruit flies prefer food containing ethanol odours over those not containing ethanol odours (Schneider *et al*., 2012). Besides, ethanol-enriched food sources are preferred as oviposition sites (Devineni and Heberlein, 2013). The affinity for pito mash and millet porridge can be because they contain alcohol or have alcohol due to fermentation.

Musca domestica, the common house fly, is a popular cosmopolitan pest invading farms and homes (Learmount *et al*., 2002; Khan *et al*., 2012; Khamesipour *et al*., 2018). It is a well-known vector of life-threatening diseases such as cholera and typhoid fever in humans (Nassiri *et al*., 2015; Tsagaan *et al*., 2015). The preferred oviposition substrate for house flies was animal manure, and they only resort to other decaying material when faecal matter was unavailable (Dahlem, 2003; Khamesipour *et al*., 2018), supporting their large numbers in the chicken and pig manure used in the study. Moreover, pig and chicken manure have been reported to be good rearing substrates for house fly larvae (Larrían and Salas, 2008; Khan *et al*., 2012; Ganda *et al*., 2019). Although *M. domestica* is not necessarily a bad source of protein for livestock or a contaminant in a BSF larvae production system, their presence is not encouraged due to their disease vector status. The protein content of house flies is reported to be between 28.63 and 76.3 % (Pieterse and Pretorious, 2013; Hussien *et al*., 2017; Gadzama *et al*., 2019).

Some studies have suggested that the presence of BSF larvae limits house fly oviposition (Furman *et al*., 1959; Sheppard, 1983; Bradley and Sheppard, 1984; Miranda *et al*., 2019). It was observed in this study, that house flies attempted to oviposit usually when BSF larvae are less than 6 days old, but as BSF larvae grew older (beyond 8 days), house fly oviposition was rare. A similar finding was reported by Miranda *et al*. (2019), where the authors observed that house fly survival was low when house fly eggs were placed on a substrate treated with BSF larvae for the first 8 days.

Several coleopteran families were also collected in the substrates tested. However, the total individual numbers were few compared to the number of dipterans. Three of these species (Histeridae, Pselaphidae, and *Tachyporus* sp) are known predators of other insects (Schoman *et al*., 2008; Balog *et al*., 2013; Mazur *et al*., 2014), but not reported as predators of BSF larvae. Diener *et al.* (2011) reported the presence of *Ornidia obesa* (Diptera: Syrphidae) when rearing BSF larvae under natural oviposition. They reported a daily harvest of 8 g of *O. obesa*, whereas, the harvest of BSF prepupae was 252 g/day. Considering this value, the effect of *O. obesa* on the BSF rearing system is negligible. This finding corroborates this study, where 4 individuals of *O. obesa* were collected. However, since these insect species tend to share similar larval development with BSF larvae and are found to be associated with decaying material makes them a possible threat to the BSF production system (Diener *et al*., 2011).

A hemipteran, *Lygaeidae*, and *Forficulidae* (Dermaptera), which are known predators of other insects were also collected (Boukary *et al*., 1997; Saha *et al*., 2016). The low numbers of the Coleopteran families and other insects collected can be coincidental since none of these insects has been reported in relation to the black soldier fly.

4.6 Conclusion

Competition with BSF larvae by other insects in a BSF larvae rearing system has not been widely reported. During the experiment, *Musca domestica* and *Drosophila melanogaster* were the predominant species collected from the rearing system. The fact that more than 100 individuals of these species were collected makes them potential protagonists in organic waste treatment with BSF larvae. Although *M. domestica* is highly nutritious, they pose a potential risk for humans and animals due to its disease vector status.

The pupal parasitoid reported to parasitising black soldier fly was recorded but it was not found parasitizing black soldier fly pupae.

CHAPTER FIVE

5.0 Termites as supplementary protein sources for poultry in Four Regions of Ghana

5.1 Introduction

Indigenous Africans, Asians, Latin Americans, and Australians commonly use termites as food and feed for livestock. Termites are rich in nutrients for both humans and animals (Redford and Dorea, 1984; Sogbesan and Ugwumba, 2008; Ntukuyoh *et al*., 2012). Forty-three termite species belonging to four families are used by humans as food and feed for livestock (Figueirêdo *et al*., 2015).

The consumption of termites by humans has been well recorded in literature (Harris, 1971; Paoletti *et al*., 2003; Wilsanand, 2005; Silesh *et al*., 2009; Ntukuyoh *et al*., 2012; Raubenheimer and Rotham, 2012; Figueirêdo *et al*., 2015). On the contrary, reports on termites as livestock feed are scanty and mostly limited to anecdotal reports in general articles and reviews (Hein *et al*., 2005; Kenis *et al*., 2014; van Huis *et al*., 2017) or technical notes and unpublished thesis (Farina *et al*., 1991; Vorsters *et al*., 1994; Chrysostome, 1997; Chrysostome, 2009; Diawara, 2013).

Sankara *et al*. (2018), also highlighted that about 78% of farmers in Burkina Faso use termites to feed their poultry at least occasionally. Termites are a vital protein supplement for indigenous African poultry farmers that have no other affordable source of protein at their disposal. In West Africa, farmers obtain termites from chippings of mounds and/or by trapping using organic matter, placed in buckets, pots, or open-top gourds, inverted in the vicinity of the termite nest (Farina *et al*., 1991; Vorsters *et al*., 1994; Chrysostome, 2009). Using this method generally does not result in the collection of large quantities of termites and therefore, termites collected are often fed to chicks and keets, for which proteins are essential for their survival. Accessibility to termites is seasonal, therefore, farmers have to travel long distances at certain times to obtain them.

Reports by farmers in Benin suggest that some species of termites are unsafe as poultry feed (Chrysostome, 1997). Consumption of *Noditermes* species by two poultry species resulted in death. It was also noted in Burkina Faso, that some species of *Cubitermes* are poisonous to domestic fowl chicks but not to ducks and guinea fowls (Diawara, 2013).

In Ghana, verbal communication with farmers rearing indigenous poultry indicates the tremendous use of termites in their feeding regime. However, documentation of this indigenous practice seems to be absent in literature. There is, therefore, a crucial need to collect indigenous knowledge on the practices to allow the evaluation of the different techniques to disseminate the most efficient ones and improve the availability of protein feed among smallholder farmers.

5.2 Methodology

5.2.1 Study area

The study was carried out through two surveys in four regions in Ghana. The objective was to assess termite utilization as poultry feed in the country. Furthermore, the factors that influenced the use of termites were assessed. The termite species used as feed and the species indicated as toxic were identified. Also, the seasonality of termites and a description of the methods used in obtaining termites were studied.

The surveys were conducted in the Volta, Northern, Upper East, and Upper West Regions to determine the use of termites as feed for indigenous poultry. The selection of the four regions was purposefully done based on their dominance in indigenous poultry production (FAO, 2014).

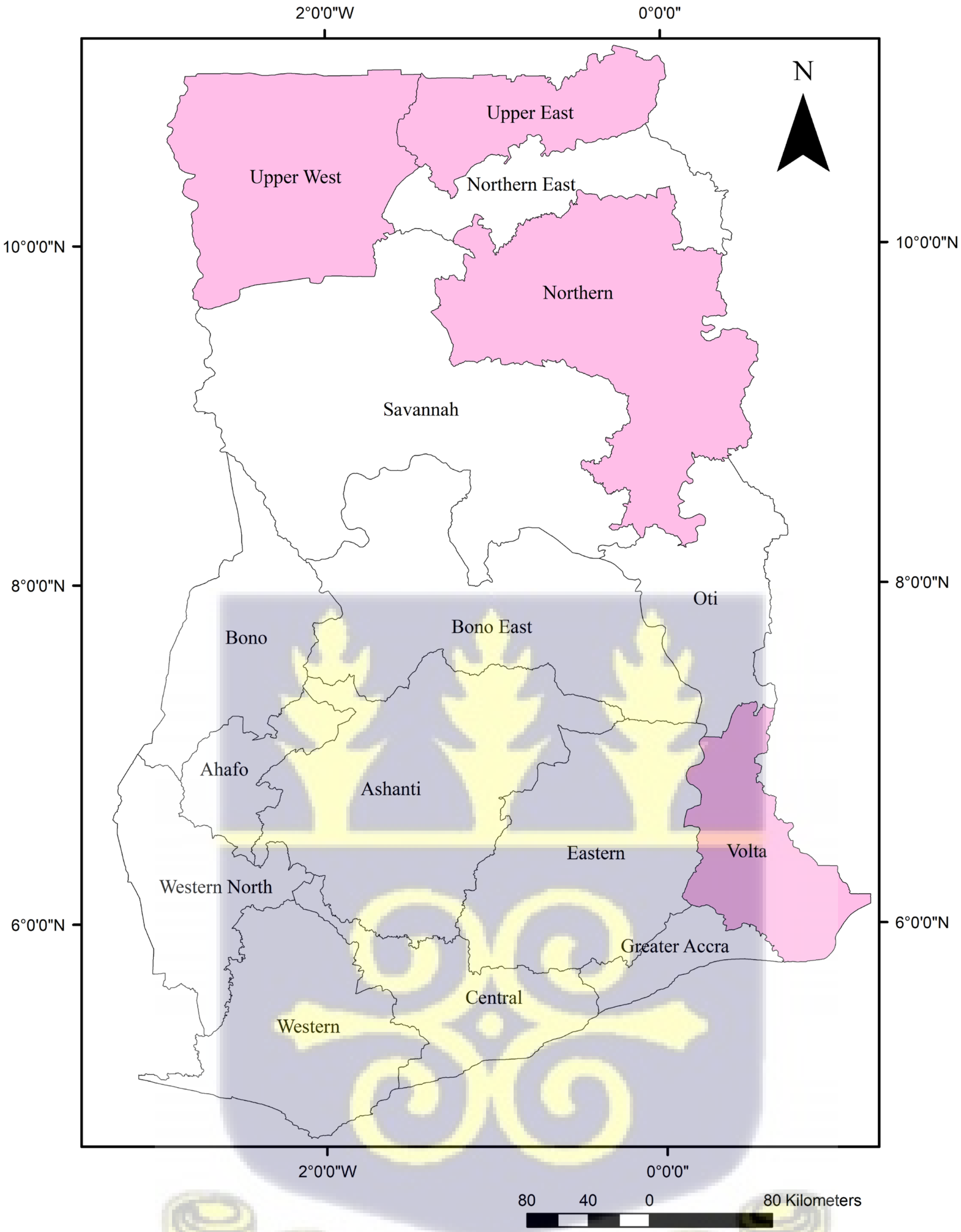

Plate 7: Map of Ghana showing the four regions where the study was conducted

Geographically, the Volta Region lies in the eastern part of the country at latitude 3o 45' N and longitude 8o 45' N. Covering a total land area of 20572 km2, the region stretches from the Gulf of Guinea and runs through all the vegetational zones found in the country (MOFA, 2010). The vegetation varies from coastal strand mangrove swamps, woodland savannah, and savannah grassland to deciduous forest. The region has a tropical climate, characterized by moderate temperatures between 12 to 32°C for most of the year. Agriculture is the predominant socio-economic activity, with livestock production being second to crop production (FAO, 2014). The three other regions are located in the Northern belt of Ghana, covering approximately half of the total land surface. They are characterized by mainly savannah woodland with grassland vegetation and scattered drought-resistant trees such as baobab, acacia, and shea tree. There is a single rainy season, that last for six months with a prolonged dry, cold, and hazy harmattan season. Indigenous poultry production is a common activity in these regions, accounting for most of the local poultry found in the Ghanaian market (FAO, 2014).

5.2.2 Surveys to ascertain the use of termites in the four regions

A large-scale general survey was conducted to obtain information on poultry farming in Ghana in the framework of the project "Insect as Feed in West Africa - IFWA". A structured questionnaire was administered to 1960 farmers, randomly selected from 31 districts in the four study regions between October and December 2015. Most farmers interviewed were indigenous poultry producers and a few commercial producers.

Field enumerators from the Ministry of Food and Agriculture (MOFA), trained on the objectives and expectations of the study aided in the administration of the questionnaires. The questionnaire addressed inter alia, the characteristics of the respondents, and the use of termites as poultry feed. The main questions asked in this study on termites were; "do you use termites to feed poultry? what are your reasons for using or not using termites? and what factors affect the use of termites as poultry feed?"

A second specific survey to obtain more detailed data on the practices of termite usage as a feed supplement for poultry was later carried out. The focus was on villages, that from the first survey, most frequently fed termites to their poultry. The questionnaire addressed the species of termites used as feed, the species not used, the effect of feeding the toxic/ poisonous species, how the termites are obtained, and the availability in the different seasons. A sample collection of the termites mentioned were sent to the laboratory for taxonomic identification.

5.2.3 Identification of the termite species collected

The samples of termites collected were taxonomically identified to the genus level using identification keys by Bouillon and Mathot (1965) and Sands (1998). Termites were identified to only the genus level because, in West Africa, most genera cannot be identified to the species level with certainty and also because congeneric species typically have similar local names (Korb *et al*., 2019).

5.3 Data Analysis

The data from the large-scale general survey was used to determine the factors affecting the use of termites as poultry feed by farmers. The poultry farmers were grouped according to the frequency with which they use termites. The non-users were categorized as farmers who “never” or “seldom” use termites as feed for their poultry. While the users were farmers who sometimes, often, or always used termites as feed. A binomial regression analysis was used to examine the different factors that affected the use of termite by indigenous poultry farmers. The different factors were region, sex, age, religion, educational background, annual income from poultry farming, years of keeping poultry, and farm size. A total of 1232 responses were used in the analysis after respondents that did not answer at least one of the questions correctly were removed. Prior to adapting the model to the results, a step-by-step selection of variables was made to avoid comparisons between explanatory variables in the model. A stepwise regression incorporated both backward and forward approaches, and finally considered only significant explanatory variables in the final model. The selection of variables and the adjustment of the binomial regression to the data were carried out using R3.3.4 (2017), while the VGAM (2015) package was used for the generalized linear model function.

For the specific survey, the data from the four regions were used to build a termite species relative frequency matrix based on citations by the farmers. Eight genera of termites identified from the study were used in the analysis. A principal component analysis was used to examine the relationship between the region and termite genera (four regions and eight genera). Furthermore, data from this survey was used to build a termite collection methods relative frequency matrix based on responses from respondents. Five collection methods were identified, and this was subjected to a principal component analysis to determine the relationship between regions and the collection method used in obtaining termites.

5.4 Results

5.4.1 The use of termites as supplementary feed for poultry

The general survey revealed that 10.68% of the total number of respondents had never used termites in feeding their poultry (Table 12). Twenty-three percent always gave termites while 19% often fed termites to their poultry. The most frequent answer was “sometimes”; 42.68% of the total number of respondents.

Table 12. The percentage of farmers from the four regions using termites in feeding their poultry (n = 1817)

Region	**Never**	**Seldom**	**Sometimes**	**Often**	**Always**
Northern	21.8	2.7	50.0	11.3	14.2
Upper East	5.5	4.5	35.2	29.2	25.6
Upper West	7.1	2.4	39.2	29.7	21.6
Volta	9.7	8.5	47.0	6.6	28.3
Total	**10.7**	**4.6**	**42.7**	**19.3**	**22 7**

When evaluating the factors that significantly influenced the use of termites, the stepwise selection allowed for the selection of five factors: region, sex, education, farm size, and annual income from poultry farming (Akaike information criterion = 1033.03 for the saturated model and 983.09 after the selection of the five factors). The detailed results of the binomial regression model revealed that the use of termites is influenced by the region where the farmer resides (Table 13). Male respondents were more likely to use termites. The use of termites was negatively correlated with higher education (tertiary and University education) and annual income from poultry farming, in contrast, farm size was positively linked to the use of termites. Farmers with higher education tend to operate larger farms and feeding termites becomes impractical.

Table 13. Results of the binomial linear regression showing the factors affecting the use of termites

Factors	**Estimate**	**Std. Error**	**Probability**
(Intercept)	0.610	0.356	>0.05
Region Upper East	1.517	0.250	**<0.001**
Region Upper West	1.551	0.266	**<0.001**
Region Volta	0.780	0.235	**<0.001**
Sex male	0.483	0.232	**<0.05**
Education middle	-0.307	0.335	>0.05
Education none	-0.073	0.255	>0.05
Education non-formal	-0.119	0.551	>0.05
Education senior high school	-0.199	0.357	>0.05
Education tertiary	-0.958	0.371	**<0.01**
Education university	-1.881	0.404	**<0.001**
Farm size	0.077	0.033	**<0.05**
Income from poultry	-0.00024	0.00008	**<0.01**

5.4.2 Identification of the termite species cited by respondents

More than 105 local names of termites were recorded from the specific survey because different names exist for different termite species in the different local languages. Moreover, different local names are given to the different caste members (queen, soldiers, workers, and nymphs). Termites are generally described by their colour, size, shape of the head, type of feed utilized by the termite, and nature and colour of the mound. Taxonomic identification led to eight genera of termites; *Macrotermes, Odontotermes, Trinervitermes, Microtermes, Cubitermes, Allondontermes, Microcerotermes and Amitermes* (Plate 8).

The relative frequencies of the different species of termites mentioned by farmers per region are shown in Table 14. More than 90 % of all the farmers in all four regions cited *Trinervitermes* species as termites used in feeding poultry. The termite species used mostly in the three other regions in the north (Northern, Upper East, and Upper West regions) were *Macrotermes*, *Odontotermes,* and *Trinervitermes* species. The use of *Cubitermes*, *Amitermes,* and *Microtermes* was reported to a lesser extent. *Amitermes* was mentioned by over 50 % of the farmers in the Northern region but by far less than 25 % in the other regions. *Odontotermes*, *Cubitermes,* and *Microtermes* were not reported in the Volta Region whereas *Allodontermes* and *Microcerotermes* were cited only in this region.

Amitermes sp. *Allondontermes sp.* *Cubitermes sp.* *Macrotermes sp.*

Microtermes sp. *Microcerotermes sp.* *Odontotermes sp.* *Trinervitermes sp.*

Plate 8. Termites species collected from the four regions

Table 14. Percentage of farmers that used the eight termite genera to feed poultry in the four regions

Region	***Trinervitermes***	***Macrotermes***	***Odontotermes***	***Amitermes***	***Microtermes***	***Cubitermes***	***Allodontermes***	***Microcerotermes***
Upper West	94.4	83.0	71.9	10.1	25.8	51.7	0.0	0.0
Northern	96.8	76.6	74.4	58.5	0.0	10.6	0.0	0.0
Upper East	96.9	92.3	99.2	7.7	17.7	30.0	0.0	0.0
Volta	95.0	40.0	0.0	25.0	0.0	0.0	10.0	10.0
Mean	95.8	73.0	61.4	25.3	10.9	23.1	2.5	2.5

A Principal Component Analysis revealed that *Macrotermes*, *Odontotermes*, *Microtermes,* and *Cubitermes* were positively correlated with the first axis (Figure 6A), while *Allodontermes* and *Microcerotermes* were negatively correlated with the same axis. The second axis is positively correlated with *Trinervitermes* and *Amitermes* and negatively with *Microcerotermes* and *Cubitermes* (Figure 6A): these first two axes accounted for 93.16 % of the variation in the data matrix. The projection of the regions in Axis 1 and 2 (Figure 6B) indicates that, in general, *Macrotermes*, *Odontotermes*, *Microtermes*, and *Cubitermes* were frequently mentioned in the Upper East and/or Upper West Regions. On the other hand, termites of the less frequently cited genera *Allodontermes* and *Microcerotermes* were cited more in the Volta Region. *Amitermes* were predominantly mentioned in the Northern region, whereas, *Trinervitermes were* abundantly used in all four regions.

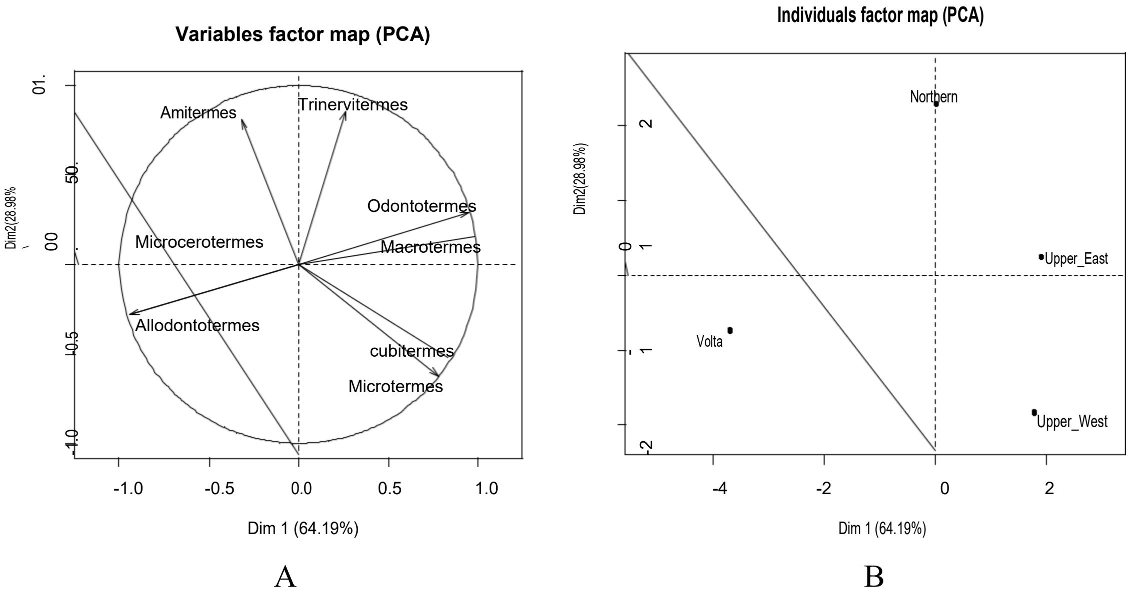

Figure 6. Principal component analysis results for the description of relationships between termite genera and regions. (A) correlation circle of termite genera. (B) projection of the regions in the first factorial plane formed by axes 1 and 2 defined by the termite genera

5.4.3 Termite collection methods

Farmers described five collection methods used in obtaining the termites. When setting traps to harvest termites, farmers usually set them in the evening between 6 - 6:30 pm and harvest the termites from the traps in the morning between 5 - 6 am. The harvesting is done very early because, during the hot afternoon, the termites move deeper into the underground chambers of the mounds making them inaccessible.

Method 1: Removal of part of the termitarium

The base around the mound is loosened by digging with an axe or hoe, and then the whole mound is removed (Plate 9A). Method 1 is usually used on small or medium-sized mounds. A small opening is made on the top of the mound which causes soldiers to rush out and they are then brushed off with a branch before the mound is dug out. The above routine is done because the soldiers are thought to be toxic/ injurious by farmers. The species usually harvested by the above method is *Trinervitermes* species because they build relatively short and small mounds. Some farmers go to sprinkle water on mounds at night during the dry season to boost the harvest in the mornings. Harvesting using this method is destructive and mounds can only be re-harvested after they have been rebuilt.

Plate 9. Collection methods used in harvesting termites: (A) method 1; (B) method 2; (C) method 3; (D) method 4; (E) method 5

Method 2: Inversion of a container with organic matter over a termite nest

The topsoil covering the mound to be harvested is removed to expose the tunnels and trails (Plate 9B). A container filled with moistened organic matter is turned upside down onto the trails and harvested after 12 hours. The organic matter in the container is secured with stalks of millet, sorghum, or a flexible stem of a shrub to prevent them from falling out when the container is inverted or turned upside down onto the trails. The organic matter often used are mango seeds, dried cow dung, millet stalks, maize cobs, fresh leaves, groundnut husk and leaves, sorghum stalks, baobab fruits, watermelon parts, neem leaves, yam peels, banana peels, and donkey dung. Some of the common containers used are earthen pots, open-top gourds, open-top jerrycans (usually 1litre), and old buckets (Plate 10). The size of the container used by the farmer is usually dependent on the flock size of the poultry. The above method is considered a more sustainable approach, as the same nest can be harvested over a period of time because the colony is not destroyed. *Odontotermes* species and *Macrotermes* species are usually harvested using this method. *Odontotermes* species are harvested by Method 2 because the nests are subterranean and can only be accessible using a lure. *Macrotermes* species on the other hand make hard and huge mounds making it impossible to harvest the whole mound, therefore using the container method is more appropriate.

Method 3: Filling a hole made in the mound with fresh leaves to lure termites

It involves filling a hole made in the mound with fresh leaves and collecting the termites that get attached to the leaves after a few hours (Plate 9C). Fresh leaves from any plant are placed in a hole made in the mound to be harvested and covered with the mound debris. The leaves are then pulled out between 2 - 3 hours together with the termites attracted to feed on the leaves and fed to the birds.

Method 4: Removal of part of the mound to collect soft rebuilt part full of termites

Method 4 is achieved by simply removing part of the termite mound and returning after about 5 hours to collect the newly rebuilt part of the mounds (Plate 9D), which is full of termites (usually workers who farmers believe are most suitable as feed).

Method 5: Fixing a basket with leaves in a hole made in the mound to trap termites

A hole is dug in the termitarium of *Macrotermes* species until the fungus garden is reached. Farmers identify fungus gardens by their characteristic whitish colour. A basket filled with leaves and parts of the broken mound is placed in the hole made and left for 24 hours (Plate 9E). The basket now containing termites (mostly worker caste) is fed to poultry.

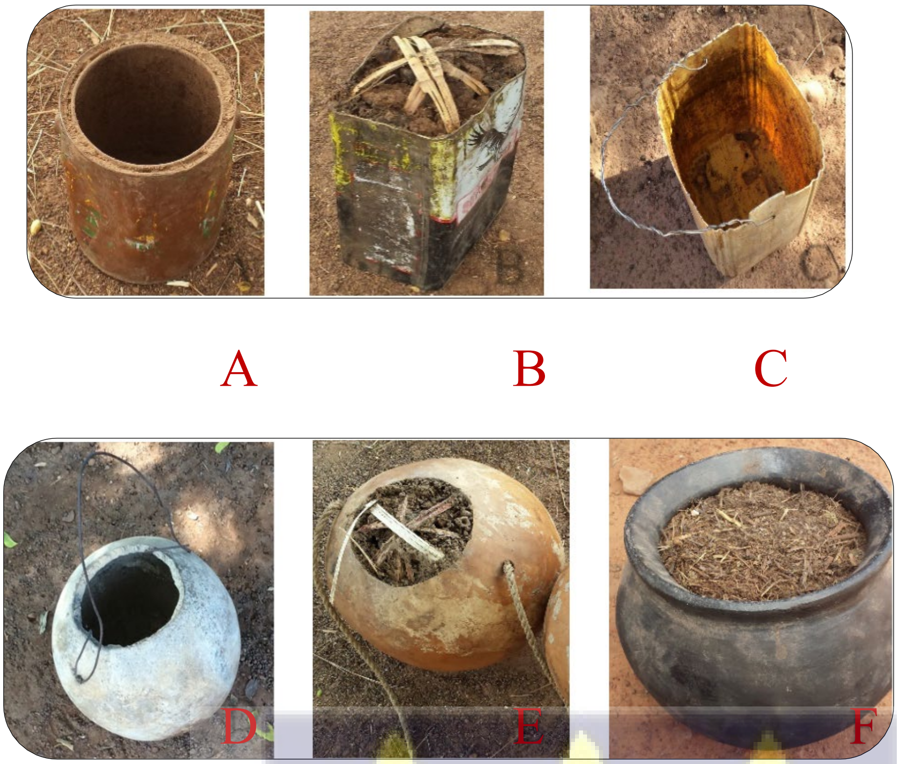

Plate 10. Containers used in harvesting termites: (A, B, C) jerry can; (D, F) open top gourd; (E) earthen pot

The collection method employed is dependent on the targeted species. *Macrotermes* species are collected using Methods 3, 4, and 5 mostly due to the hardiness of their mound, making it almost impossible to remove the whole mound. Termites that build smaller mounds such as *Trinervitermes* species and *Cubitermes* species are harvested using Method 1. Termites that build subterranean nests such as *Odontotermes* species are collected using Method 2, however, this method is also commonly used for *Macrotermes* species too.

5.4.4 Relationship between collection method and region of study

The results of the principal component analysis carried out to show the diversity in termite collection based on the regions show that the factorial plane formed by axes 1 and 2 explains 98.2 % of the difference in the data matrix. The correlation between these two axes and the methods brings to the conclusion that methods 3, 4, and 5 are positively correlated with the first axes (Figure 7A). The second axis is positively correlated with Method 1 but negatively with Method 2 (Figure 7A). The projection of the regions in the factorial plane formed by axes 1 and 2 (Figure 7B) reveals that Methods 3, 4, and 5 are predominately used in the Upper West region for harvesting termites. Method 1 is the only method deployed in the Volta region whereas Method 2 is commonly used in the other three regions. Method 2, in addition, is the dominant method in the Upper East and Northern regions (Figure 7B). The other three methods are seldom used except for Method 3 which is used in the Upper West region.

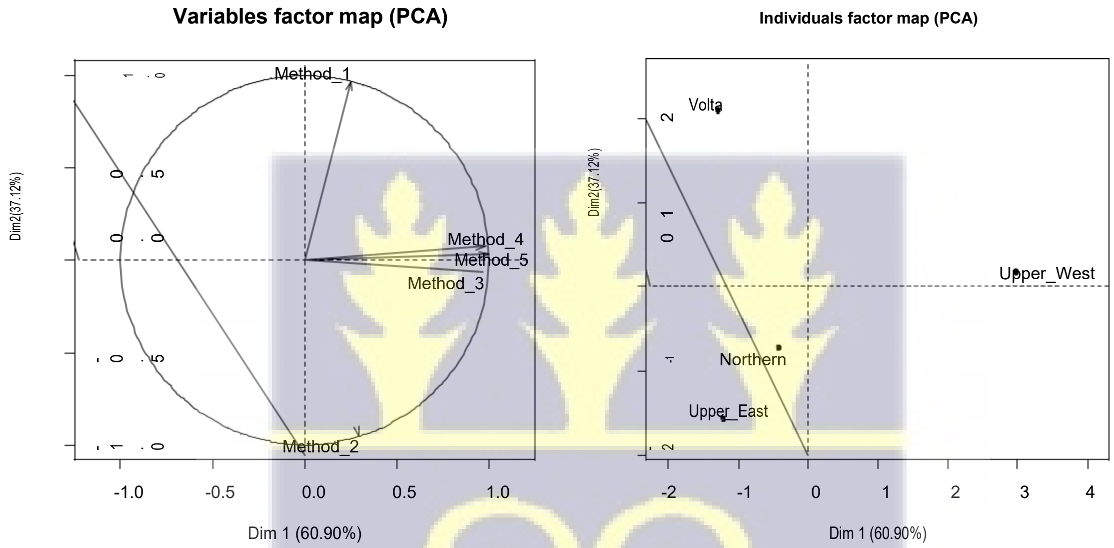

Figure 7. Principal component analysis for the description of the relationship between termite species collection method and regions. (A) correlation circle of collection methods. (B) projection of the regions in the first factorial plane formed by axis 1 and 2 defined by the collection method.

Overall, 76.6 % of respondents from the specific survey used Method 2, while 1.2 % employ Method 4 in collecting termites (Table 15).

Table 15. Percentage of farmers employing the different collection methods of harvesting termites

Collection method	Method 1	Method 2	Method 3	Method 4	Method 5
Percentage of farmers using methods	41.3	76.6	13.2	1.2	2.4

5.4.5 Seasonal availability of termites

The species collected was dependent on the time of year or the season (chi-square = 217.17, df = 6, p-value $< 2.2 \times 10^{-16}$). In the rainy season, *Trinervitermes* species were mostly collected because their mounds became moist and easy to remove (Figure 8). The other species were collected throughout the year, however, *Odontotermes* species were mainly collected in the dry season.

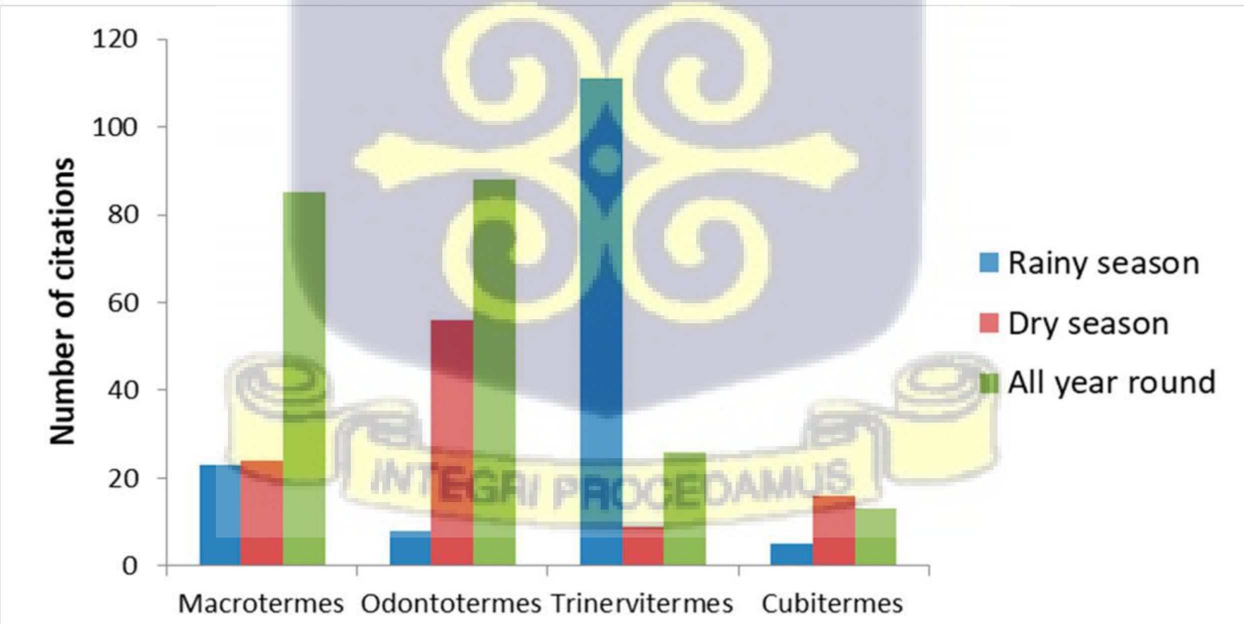

Figure 8. The relationship between the four major termite species collected and seasonal availability

5.4.6 Poisonous/ toxic termite species

Four termite types were reported as injurious to poultry (Table 16). Soldiers of both *Trinervitermes* species and *Macrotermes* species, some species of *Cubitermes* and *Amitermes* were reported as unsuitable. In addition, it was reported that species that erect mounds under the *Naudea latifolia* (Smith) shrub locally called "gulungu" are not used as feed. Farmer indicated that birds younger than four weeks old were generally more susceptible to toxic species than older birds. The overall result of feeding toxic species is death. Other symptoms mentioned included weakness, dullness, reduced feed intake, paralysis, and difficulty in excretion. The symptoms mentioned as a result of poisoning, however, were inconclusive.

Table 16. Toxic species of termites and their symptoms of illness usually to chicks

Termites	Symptoms
Soldiers of *Macrotermes*	Death due to choking
Cubitermes (some species)	Dizziness, weakness, reduced food intake (weight loss), diarrhea, indigestion, and death
Soldiers of *Trinervitermes*	Dullness, dizziness, weakness, paralysis and death
Amitermes	Difficulty in excretion and death

5.5. Discussion

5.5.1 The use of termites as supplementary feed for poultry

The data obtained from the study shows that termite use as feed for indigenous poultry is a popular practice in the four regions investigated. The proportions of farmers using termites in this study were very similar to those observed by Sankara *et al.* (2018) in a study in Burkina Faso, that showed that 78% of poultry farmers used termites to feed poultry at least occasionally. The use of termites among highly educated farmers was low probably because these farmers had large commercial poultry farms, making feeding the birds with termites less practical. The large flock of birds under semi-intensive or intensive systems kept by these farmers would require large amounts of termites to feed their birds. Obtaining large quantities of termites may be difficult and unsustainable as termites are currently harvested from the wild. The predominant reasons given for feeding termites were that it promotes growth, improves the immunity of the poultry, easily accessible and cheap source of protein. The main reasons why termites were not provided were lack of time and the scarcity of termites during some seasons, although some mentioned health complications associated with using termites.

Generally, farmers with large poultry farms did not provide termites because they use industrial feed, and obtaining large quantities of termites for a large number of poultry heads is not feasible.

5.5.2 Termite species identified in the survey

Significant variations were seen in the termite species collected in the different regions. *Trinervitermes* species were mentioned in all the regions of the study (more than 90 % of all farmers interviewed). *Trinervitermes* species was probably used extensively due to its abundance and the ease of obtaining workers by simply breaking the mound. The use of this species as poultry feed was mentioned in studies by Chrysostome (1997) in Benin and Diawara (2013) in Burkina Faso. In the Volta region, *Trinervitermes* species was by far the most commonly used species, possibly due to their availability as the more humid conditions of the regions favour their abundance, compared to the three Northern regions, which experience a longer dry season each year. The unfamiliarity with the use of *Odontotermes* species as poultry feed by farmers in the Volta region raises some curiosity as farmers in the three Northern regions have reported that *Odontotermes* species is the best termite to feed because no caste member is known to be injurious to birds when eaten. *Macrotermes* species were reported more frequently in the three Northern regions than in the Volta region. Farmers in the Volta region were aware of the use of *Macrotermes* species as a feed for poultry but harvested them by breaking the mound and seem to lack the trapping techniques used in the other regions. *Macrotermes* species have been reported to be used as food by humans and feed for livestock (Figueirêdo *et al*., 2015; Kelemu *et al*., 2015). This species has also been reported by Deblauwe and Janssens (2008) to be a delicacy for chimpanzees. Perhaps, the affinity for this species is due to its large size and abundance on the African continent (Figueirêdo *et al*., 2015).

5.5.3 Termite collection methods and availability during the different seasons

The study revealed that five collection methods were used in obtaining termites. While their respective use depends on the targeted termite species and region, the two most common methods were Method 1 (breaking the mounds) and Method 2 (inverted container). Methods 1 and 2 are also popularly used in Burkina Faso (Diawara, 2013; Dao, 2016; Ouédraogo, 2016). Different termites are collected in different seasons of the year. During the rainy season, *Trinervitermes* species are mainly collected, possibly due to the ease of breaking wet or soft mounds. *Odontotermes* species and *Macrotermes* species are collected throughout the entire year, although collecting the former is predominant in the dry season. *Odontotermes* species are obtained with baited inverted containers (Method 2) and this method is particularly well adapted to the dry season, in the rainy seasons, termite tracks are washed off by the rains. *Macrotermes* and *Odontotermes* species are mainly collected using baits and elaborate trapping methods in contrast to *Trinervitermes* species, which are collected by removing the mound, thereby destroying the whole mound in the process. Trapping termites using baited containers were reported by farmers in Togo (Farina *et al*., 1991) and Benin (Chrysostome *et al*., 2009).

The duration taken for regeneration of mounds after removal was unknown to the farmers, however, farmers were aware that, if the queen is not removed, mounds will be rebuilt. Harvest and collection for all species are usually done in the early hours of the morning (between 5 am to 7 am), as termites move deeper into mounds in the hot afternoons. When Method 2 (inverting baited container on trails) is used, the trap is set in the evening (around 6 pm) and collected the next morning. According to the farmers, termites are sensitive to noise and therefore minimal sound should be made when the traps are being collected. Some reported that the collector must tiptoe when getting close to the traps, or else the termites will move back into the nest.

Containers of various sizes, shapes, and materials are used for trapping termites when using the baited container technique (Method 2). The size of the container is usually dependent on the size and age of the flock. Larger and younger flocks require bigger containers as more termites are needed for feeding. Containers are usually made from earthenware, plastic, calabash, or aluminium material; however, farmers advise the use of earthenware as it remains cooler than containers made of other materials. To maintain a good microclimate for the termites, baited containers are covered with leaves after placing them on termite mounds.

5.5.4 Poisonous/toxic termite species

A few termite species were reported by farmers as injurious to their poultry. However, the mechanisms of toxicity would need to be investigated and verified through specific studies. Injurious species described by farmers include *Amitermes* and *Cubitermes* species and soldiers of both *Trinervitermes* and *Macrotermes* species. Farmers mentioned that mainly chicks and keets were vulnerable to these species. Prestwich (1976) described the chemical mechanisms used by termites in defending their mound against intrusion. Termite employs mechanisms such as biting with simultaneous injection of toxins, releasing irritants or oily chemical secretions from their frontal gland, ejection of sticky solutions which irritate and mechanically immobilise small assailants, and release of a topically active poison using the labrum. *Trinervitermes* species secrete toxins and irritants, while *Amitermes* and *Macrotermes* bite and simultaneously inject toxins. Farmers reported that soldiers of *Macrotermes* species bite and get stuck in the throat of chicks and *Trinervitermes* species release sticky secretions that cause the death of chicks. Indeed, the toxicity of *Trinervitermes* species, also mentioned by Diawara (2013) is most likely due to the diterpenes and monoterpenes that are released from their snout to deter ants and predators (Nutting *et al*. 1974; Eisner *et al*., 1976; Braekman *et al*., 1984). The injurious effect on chicks and keets is probably because their throats are more sensitive to the bites and also due to an undeveloped immune system.

Farmers also described various symptoms of illness associated with feeding poisonous termite species, however, the mechanisms involved in the reported death or symptom of illness following the consumption of certain termite species are unknown.

Some techniques have been devised by farmers to avoid the collection of large quantities of soldiers in the catch. When collecting *Trinervitermes* species, a small hole is made on the mound to cause soldiers to rush out to defend the colony. The soldiers are then brushed from the mound with the aid of a branch before the mound, now containing mainly workers are removed. In addition, farmers avoid collection from old mounds and believe that new mounds containing more grass have less poisonous castes because workers are predominately more in such mounds. According to the farmers, the adverse effect of *Trinervitermes* species can be reserved by soaking leaves of *Vernonia amygdalina* (bitter leaves) in water and given to birds to drink.

5.6 Conclusion

Termites as supplementary protein are considered very important by indigenous farmers in indigenous poultry production because they are an easily available protein. About 85% of the farmers interviewed use termites as feed for their poultry. Factors that affect the use of termites are region, sex, farm size, educational level, and income from poultry farming. Male respondents and farmers with small farm sizes usually use termites as feed. Higher educational levels and higher income earning farmers are unlikely to use termites as feed usually because they have larger flock sizes and therefore use commercial feed. The species collected belonged to 8 genera, *Macrotermes*, *Trinervitermes*, *Cubitermes*, *Odontotermes*, *Microtermes*, *Allondontermes*, *Amitermes* and *Microocertermes*. The abundance of the different termite species varies with season. *Trinervitermes* species were collected mostly in the rainy season, while the other species were collected throughout the year. Farmers obtain termites using 5 methods, the method chosen is determined by the species to be collected. Some species are considered unwholesome as feed for birds; soldier caste of *Trinervitermes* and *Macrotermes*, Cubitermes, and some species of *Amitermes*. These species can cause death in poultry.

5.7 Assessment of indigenous termite collection methods

5.7.1 Introduction

The collection methods mentioned by farmers from the specific survey conducted were tested for their efficiency in luring termites. The study focused on the collection of termites with the aid of a container filled with organic matter inverted over the trails of termites (Method 2). Method 2 was chosen because, among the methods cited by the farmers, it was the most environmentally friendly and sustainable. The emphasis of the study was on the different types of substrates used, as it was one of the most important factors that affected the recruitment of individuals (termites) to a food source (Lima and Costa-Leonardo, 2014; N'dri *et al.*, 2018).

The study was conducted to enable the quantification of harvest or catches when different organic waste materials are used. Farmers engaged in this practice made it known during the survey that the quantities collected are unknown. In addition, they mentioned that large numbers are not obtained, the reason for feeding only young poultry which cannot fend for themselves. Therefore, it became necessary to test substrates to enable the quantification of harvest obtained using termite harvesting Method 2.

The objective of this study was therefore to test the ability of the frequently used substrates in luring (baiting) *Macrotermes* and *Odontotermes* species for collection.

5.7.2 Methodology

The test was conducted in Tongjin, a community in the Tolon-Kumbungu District of the Northern Region of Ghana together with the farmers. The test was done in the dry season with morning temperatures as low as 18°C and rising as high as 37°C during the afternoons. Harvesting of termites from mounds was carried out in the dry season mainly due to flooding of the nesting area during the rainy season. Furthermore, termites move deeper into the ground during the rainy seasons, and obtaining them becomes difficult. Farmers also said in addition, that during the rainy season food is abundant and therefore birds have enough to eat.

The four frequently used substrates by the farmers and a combination of these substrates (making a total of eight) were used in the study. The substrates were mango seed, corn cobs, dried yam peels, and dried cow dung. The combinations were mango seed + dried cow dung, corn cobs + dried yam peels, dried cow dung + corn cobs, and dried cow dung + dried yam peels.

Two termite species, *Odontotermes* species and *Macrotermes* species are mostly collected by farmers using the above method, thus they were chosen for the study. Eight nesting sites each (16 in total for the two species) for each of the termite species (*Macrotermes* and *Odontotermes* species) were therefore chosen and labelled. Sixteen earthen pots representing the selected *Odontotermes* and *Macrotermes* nesting sites (8 per species), with a volume of 7.55 m^3 were given corresponding nesting site numbers. The pots were filled with the eight substrates and moistened with water (Plate 11). The volumes of the substrates were standardized because, the substrates by their nature had different textures and densities, as a result, the same weight did not fill the same volume of the pot. In addition, by practice, the farmers fill the pots to the brim, but the same weight of the different substrates did not achieve this.

Plate 11. Pots filled with substrates for trapping termites in the field

The pots were then planted on the corresponding nesting sites, covered with fresh neem leaves and grasses and the set up left for 24 hours (Plate 12). The substrates were rotated each day so that all nesting sites received all eight substrates.

Plate 12. Harvesting of termites. (A) pots buried on Macrotermes species mound; (B) pot buried on Odontotermes species nest

Trapped termites were then collected each morning and separated from the substrates by sifting through sieves of different mesh sizes and with the aid of water (Plate 13). The dried weight of termites was taken after 5 hours of sun drying on aluminium plates. The termites were sun-dried because the test took place under field conditions (far from the lab) where oven drying was impossible.

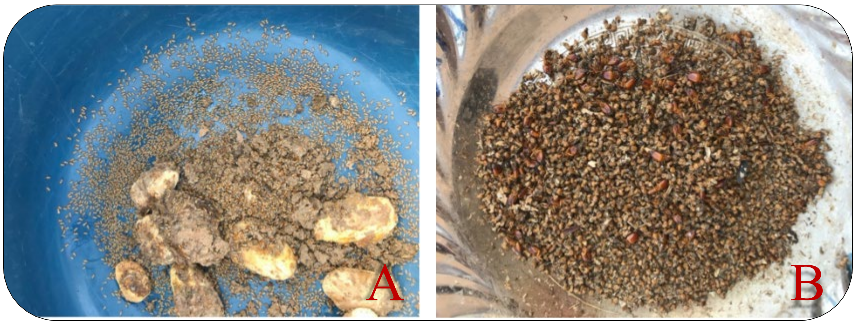

Plate 13. Termites collected from the trapping. (A) Odontotermes species (B) Macrotermes species

5.8 Data Analysis

Data from the study were subjected to the non-parametric Kruskal Wallis test after it failed the Shapiro-Wilk normality test using R software. The pairwise multiple comparison Nemenyi test was used in separating the significant pairs or the source of significance at $P < 0.05$. The means of the daily harvest and standard errors were calculated using R software version 3.6.2. (2019-12-12).

5.9 Results

5.9.1 *Macrotermes* species

A Kruskal-Wallis test showed that there were statistically significant differences (chi-square = 22.59, p-value = 0.002) between the type of substrate used and the quantity of termites harvested (Table 17). The combination of corn cobs + yam peels resulted in the highest yield of harvested termites, while the use of yam peels alone resulted in the lowest yield of 1.27 g.

Table 17. Mean weight of Macrotermes species harvested using the different substrates

Substrate	Mean dry weight (g) ± standard error
Corn cobs + yam peels	14.8 ± 4.5^{ab}
Mango seed	14.17 ± 6.3^{ab}
Corn cobs	10.8 ± 4.6^{ab}
Cow dung + corn cobs	10.6 ± 3.3^{b}
Mango + cow dung	3.5 ± 2.0^{ab}
Cow dung	1.4 ± 0.8^{a}
Yam peels	1.3 ± 0.3^{ab}
Cow dung + yam peels	1.2 ± 0.3^{ab}

Means in the same column followed the same letter are not significantly different (Nemenyi test, $p < 0.05$).

5.9.2 Odontotermes species

There were statistically significant differences between the substrates and the total harvest of *Odontotermes* species (chi-square= 21.29; p-value = 0.003) (Table 18).

Table 18. Mean weight of Odontotermes species harvested using the different substrates

Substrate	Mean dried weight (g) ± standard error
Mango seed + cow dung	19.4 ± 5.7b
Cow dung	18.6 ± 4.5b
Cow dung + corn cobs	15.4 ± 3.9b
Cow dung + yam peels	14.5 ± 3.8ab
Mango seed	11.1 ± 2.8ab
Corn cobs	7.6 ± 3.7ab
Corn cobs + yam peels	7.2 ± 3.3ab
Yam peels	0.3 ± 0.2a

Means in the same column followed the same letter are not significantly different (Nemenyi test, $p < 0.05$).

Overall, a combination of mango seed + cow dung resulted in the highest catch of *Odontotermes* species, while on the other hand, yam peels alone gave the least with a mean of 0.3g (Table 18).

5.10 Discussion

Termites are both herbivores and decomposers feeding on a wide range of living, dead or decaying plant material (Bignell and Eggleton, 2000; Traniello and Leuthold, 2000). Reports indicate that termites prefer some plant materials over others (Wood and Sands, 1978; Aihetasham and Iqbal., 2012; Rasib *et al*., 2014). The quantity of termites recorded differed with plant species used as baits in a choice and no-choice feeding preference experiments (Basu, 2011; Rasib *et al*., 2014; N'dri *et al*., 2018).

The data obtained from this study indicate that using different substrates (organic matter) as bait resulted in differences in the total quantities of termite harvested. This was true for both *Macrotermes* and *Odontotermes* species.

Among the factors influencing the food choice of termites are the palatability, moisture content, nutritional content (sugars, starch, proteins), chemical profile (phenols, terpenoids), hardness, and the effect the food has on their gut microfauna (Wolcott, 1951; Akhtar and Jabeen, 1981; Nagnan and Clement, 1990; Rasib, 2005; Rasib, 2008; Rasib *et al*., 2014).

The substrate giving the most harvest for *Macrotermes* species was the combination of corn cobs and yam peels. It can be seen looking at the catch using only yam peels that, the *Macrotermes* species were attracted to the corn cobs. In addition, the combination of corn cobs and cow dung was very effective, but cow dung alone had a low yield. Indeed, Ouédraogo *et al*. (2004), reported the preference of maize straw over cattle dung by *Macrotermes* species, although, in their case, the straw was used instead of the cobs. Generally, termites are known to prefer a diet with a lower C/N ratio than their body tissues to avoid the urgent removal of excess. Ouédraogo *et al*. (2004), however, reported that despite the higher C/N ratio of corn straw than cow dung used, it was preferred over cow dung. They reasoned that the symbiotic microfauna of this species could compensate for the very low food quality of corn straw.

Similarly, the combination of mango seed and cow dung yielded the highest mean weight for *Odontotermes* species. Cow dung seems to be a very effective lure among the substrates for *Odontotermes* species in this study. In combination with all the other substrates, cow dung yielded a high collection of *Odontotermes* species (cow dung + mango seed = 19.40g; cow dung + corn cobs = 15.38g; cow dung + yam peels = 14.46g). Moreover, using cow dung alone resulted in the second-highest harvest obtained. The preference for herbivore dung by *Odontotermes* species were reported by Cheik *et al*. (2019). In their study, elephant dung was preferred over plant litter and cellulose baits (acacia leaves, elephant grass, and cardboard) used. Mammalian dung is believed to be attractive to termites perhaps because it is an already "preprocessed" plant material by the mammalian herbivore and their endosymbionts (Freymann *et al*., 2008). In addition, Johnson and Whitford (1975) reported that subterranean termite species such as *Odontotermes* prefer cattle dung which has a larger surface area over small twigs and leaf litter. Other studies also suggest a preference for mammalian dung especially elephant dung by *Odontotermes* species over other food sources used as bait (Coe, 1977; Buxton, 1981).

On the contrary, some other authors believe that termites in general do not show a clear preference for mammalian dung but rather opportunistically exploit dung when it becomes available as a food source (Ferrar and Watson, 1970; Rouland *et al*., 2003; Ouédrago *et al*., 2004; Freymann *et al*., 2008). These studies reported that about half of the 11 fungus-growing members of the subfamily *Macrotermitinae* showed a preference for plant food and 33% had no clear food preference at all. The possible explanation given for this observation among fungus-growing termites, although dung is highly nutritious, perhaps is a means to avoid the contamination of the fungus garden by other microorganisms (entomopathogenic fungi and bacteria) which could be hypothetically present in herbivore dung (Freymann *et al*., 2008).

Nonetheless, the study by Cheik *et al*. (2019) clearly showed that *Odontotermes* species was highly attracted to cow dung.

Using yam peels alone was not very effective for both species. Although, without doubt, yam peels are nutritious (Akinmutimi *et al*., 2006; Yusef *et al*., 2017), the rejection by both species of termites could be a result of the size of the peels. Compared to all the other substrates, yam peels came in very small pieces, averagely about 5 cm in length and 0.3cm wide. This agrees with studies by Johnson and Whitford (1975) which showed that termites preferred cattle dung with a large surface area over small twigs and surface litter. Basu (2011) also showed that *Macrotermes bellicosus* and *Odontotermes aff. pauperans* preferred to feed on larger wood species of *Crossopteryx febrifuga* and *Piliostigma thonningii* than on smaller pieces of the same wood species. The preference for different substrates could also be due to the nutritional needs of the fungus cultivated by the two species. The selection of a suitable fungal symbiont is dependent on the substrate provided by the termite (Rouland-Lefèvre, 2000; Nobre and Aanen, 2012). It has been reported that there is specificity in the species of *Termitomyces* cultivated by *Macrotermes* species and less specificity in *Odontotermes* species (Aanen, 2006; de Fine Licht *et al*., 2006; Aanen *et al*., 2007). *Odontotermes* species are associated with a broad range of *Termitomyces* species (Aanen, 2006; de Fine Licht *et al*., 2006; Aanen *et al*., 2007). The less specifically cultivated *Termitomyces* have a broader potential substrate rate than the former (Dangerfield and Schuurman, 2000; da Costa *et al*., 2018). This could be why most of the substrates yielded good results for *Odontotermes* species than *Macrotermes* species.

Furthermore, the differences in texture of the substrate could account for the differences seen in the selection by the two termite species. On examining the extent of breakdown of the substrates used, it could be said that perhaps cow dung was chosen due to the texture making it easy for *Odontotermes* to utilize it. It was observed that *Odontotermes* species attacked and chewed almost entirely the pith (inner white and foam-looking part) of the cobs but not the hard-outer pole. On the contrary, *Macrotermes* species utilized corn cobs better by consuming the inner soft pith and devouring almost the entire cob. However, the quantity of *Odontotermes* species collected when corn cobs were used was also quite high considering the hard nature of the substrates as compared to the type of mandibles possessed by this termite. With the mango seed, both species fed on the outer endocarp because the seeds were mostly freshly eaten, and the endocarp had some fresh pulp on them. No visible sign of eating was seen on the yam peels, but some sheetings were seen. Moreover, in combination with other substrates, the termites were seen mostly feeding on the other substrates than on the yam peels.

Generally, when compared to *Odontotermes* species, yields of *Macrotermes* species were lower, with the highest recorded weight of 14.80g and a low 1.2g. As the days progressed, the quantities of termites collected reduced. A possible reason for this is the continuous harvesting from the same nests during the study. Farmers are not faced with this challenge because unlike in the study, harvesting is done once at a nesting site and left fallow for a few days before the farmer returns to the same spot.

5.11 Conclusion

Farmers have used containers filled with moistened organic matter to trap termites for centuries. Farmers report insufficient quantities obtained to feed the entire flock, however, they are unable to quantify the yields. This study quantified the yields obtained with different substrates for *Odontotermes* and *Macrotermes* species. There were differences in the substrates acting as a lure/ bait for termites. Generally, yields were low, although there are no available studies to compare with this study. *Odontotermes* species were attracted by different substrates (mango seed + cow dung) than *Macrotermes* species (corn cobs + yam peels). A combination of substrates produced better results than single substrates.

CHAPTER SIX

6.0 General discussion

This thesis reports on a series of studies investigating the insect rearing systems used by smallholder poultry and fish farmers in parts of Ghana. The already existing black soldier fly larvae production and termite collection methods were evaluated. The research presented addressed the egg-trapping efficiency of the substrates and the influence of the substrate on some growth parameters. In addition, the effect of the substrate loading rate and quantity on the total larval harvest (yields) were examined. Indigenous knowledge on the use of termites as poultry feed and the evaluation of termite collection were also assessed.

Six substrates (millet porridge mash, pito mash, chicken manure, pig manure, fruit waste, and root and tubers waste) were examined for their effectiveness as a lure for oviposition and suitable for the growth and development of larvae. The total yields of larvae obtained in a garden bin system were examined when the rate of supply of substrate and quantities of the substrate was varied. Furthermore, the study sought to gather information on types of termite species used as feed for poultry, unwholesome species, factors influencing the use of termites, and collection techniques.

The results of the studies presented in this thesis suggest that the substrate used significantly influenced the oviposition and development of black soldier fly larvae. The substrates preferred for oviposition were not necessarily the most favoured for the development of the larvae. Millet porridge mash was the most preferred for oviposition. All substrates successfully supported the development of the larvae, although pig manure was by far the best. The finding also suggests that in a natural oviposition system, the choice of the substrate is much more critical than in an adult rearing system where a varied number of substrates can be used.

Furthermore, loading smaller quantities (10 kg per week) of substrates yielded significantly more than larger quantities (20 kg) at a time.

Information from respondents indicated that more than 80% used termites as poultry feed, while 11% never used termites. Termites collected belonged to three main species, *Macrotermes*, *Odontotermes,* and *Trinervitermes* species. Two main collection methods are used in the collection of termites and this is species dependent. Four species of termites are considered unwholesome as feed for poultry; soldiers of both *Macrotermes* and *Trinervitermes*, *Amitermes,* and some species of *Cubitermes*. The subjection of the collection methods mentioned showed that *Odontotermes* and *Macrotermes* are attracted to different substrates (mango seed + cow dung and corn cobs + yam peels, respectively). Termites are a very important, cheap, and easily accessible protein for indigenous poultry farmers.

6.1 General Conclusions

The first part of the study evaluated black soldier fly larvae rearing systems by studying the various substrate(s) that can effectively lure gravid adults for oviposition and promote the development of larvae. The hypothesis postulated was that "*The substrates most attractive for black soldier fly (BSF) oviposition are also those that are most suitable for larval development*". A linear mixed effect model was used to examine the effect of substrate on the quantity (weight) of eggs deposited by gravid BSF and also on larval developmental parameters (individual prepupal weight etc). The results indicate that the substrate inherently favoured by gravid BSF females was not necessarily the most suitable for larval development. Although some substrates were very poor in attracting females for oviposition, all the substrates were successful in enhancing larval development, with minor differences in larval performance. Millet porridge was by far the most attractive for oviposition (31.63 mg/day), however, it was not the most suitable for development (135.38 mg prepupal weight), when compared to pito mash (1.1 mg egg/day and 194.5 mg prepupal weight). The proximate composition of the recovered prepupae ranged between 35-43% for all the substrates tested. This suggests that, in a production system in which adults are reared and eggs placed on substrates, many substrates are suitable for larval development and the lowest valued and the most easily available substrate should be used.

The second hypothesis was that "*There is a threshold in the food supply, where larval yields do not increase further*". This study was carried out by the evaluation of BSF larvae production to determine the amount of substrate needed and the rate of addition of substrate to maximize yields in a natural oviposition system. The results indicate that generally when compared to a similar study, yields obtained were low. It was shown that there is a threshold of substrate supply where larval yields do not increase further. The yields of larvae did not vary after 70 days, even when substrates were added. In all loading rates tested, maximum yields were obtained in the first month of testing in the dry season. Overall, loading substrate at 10 kg twice per week resulted in higher yields in both seasons. Furthermore, strong cyclic fluctuation in daily harvest was observed for all treatments. A steady state of production of larvae was not achieved in this study.

A sample of other insect species attracted to the different substrates species resulted in the collection of mainly *Musca domestica* and *Drosophila melanogaster.* The pupal parasitoid, *Dirhinus giffardii*, reported as a threat to the BSF larvae rearing system was recorded. However, no pupal parasitism was detected in this study. Some predatory Coleopterans were recorded; however, their overall individual numbers were low. The colonization of the substrates by other insect species was predominant when BSF larvae were less than 5 days old.

The second part of the study assessed the use of termites as poultry feed by administering a questionnaire to collect information on this indigenous practice. The study showed that termites as supplementary protein is considered very important by indigenous farmers in indigenous poultry production. Eighty-five percent of the farmers interviewed were engaged in this practice. The factors that influenced the use of termites were region, sex, educational level, farm size, and income from poultry farming. Male farmers and farmers with small farm sizes were more likely to use termites as feed. Farmers with higher education (tertiary level) and high income from poultry farming, rarely used termites as feed. The termite species used belonged to eight genera, with *Macrotermes*, *Trinervitermes,* and *Odontotermes* being the major species. Furthermore, the termite species used were region and season-dependent. Termites were obtained with five collection methods, that involve breaking mounds or trapping with containers. Farmers mentioned four species (soldiers of both *Trinervitermes species* and *Macrotermes* species, some species of *Cubitermes*, and *Amitermes*) as toxic or poisonous to poultry. Trapping termites using containers resulted in low yields. *Odontotermes* species and *Macrotermes* species are attracted to different substrates. Substrate combinations; mango seed + cow dung (*Odontotermes* species) and corn cobs + yam peels (*Macrotermes* species) seem to be better as a lure than single substrates.

6.2 Recommendations

Based on some observations made and findings from this study, the following recommendations are made for further work.

- Considering the low yields obtained with garden bins compared with other studies, a further test is needed. In most cases, farmers would not use single standard substrates but a varying mixture of waste material, which would add to the complexity of testing. A combination of substrates, therefore, needs to be tested in the same way to assess which loading rate would maximize yields. In addition, an evaluation of the yields when the wild population of BSF is enhanced in the testing vicinity as reported in other studies is needed.
- The study revealed that some termite species were poisonous, however, the causes of toxicity were not determined. Further studies should be conducted to verify the toxicity reports by farmers. The toxins responsible and the mechanisms involved need to be studied.

- The nutritional profile of the various termite species mentioned must be evaluated to ascertain the claim by farmers that the *Odontotermes* species is the most nutritious.
- A comparison should be made between the proximate composition of termites and the black soldier fly.
- Considering the efficiency of trapping termites with containers, the effect of different container sizes on termite harvest should be evaluated.
- The effect of the different seasons and continuous collection from the same mound on termite harvest should be investigated.
- The existing trapping techniques need to be improved and a study of the time for regeneration of the mound after collection needs to be determined.
- The Ministry of food and agriculture should adopt the use of insects as feed in the national strategy in solving feed shortages in Ghana for poultry and fish production.
- Food and drugs authority should formulate policies and legislature for the use of insects as feed, providing clear regulations.
- The government must support research on the use of insects as feed as part of their food security and rural development programs.

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LIST OF APPENDICES

Appendix 1. Effect of loading rate on the weekly harvest of black soldier fly larvae: results of linear mixed-effects models

Source of variation	DF	Dry season		Wet season	
		F-value	p-value	F-value	p-value
Treatment	2	0.04	0.957	5.35	**0.007**
Time (Day after exposure)	1	7.46	**0.008**	0.62	0.435
Treatment × Day after exposure	2	3.99	**0.023**	1.72	0.187

Appendix 2. Descriptive statistics of the weekly harvest of black soldier fly larvae according to treatment applied and climatic season considered

Treatment	Dry season		Wet season	
	Mean (g)	Standard Error (g)	Mean (g)	Standard Error (g)
T1	143.16	42.44	21.72b	6.79
T2	124.85	39.49	163.05a	53.06
T3	90.37	26.17	95.31ab	30.35

Appendix 3. Effect of substrate on development time: results of a two-way ANOVA

	df	Mean square	P-value
Substrate	5	76.51	3.48e-16 ***
Time	1	56.33	5.87e-06 ***
Substrate: time	5	16.13	2.93e-05 ***

Significant: 0 '***'

Appendix 4. The effect of substrate on the total yield of prepupae harvested in the study

	df	Mean square	p-value
Substrate	5	2108.9	9.40e-12 ***
Time	1	2190.8	2.41e-05 ***
Substrate : time	5	122.0	0.336

*** Significant at 0 level

Appendix 5. The influence of substrate on the total number of individuals that survived to prepupal stage

	df	Mean square	P-value
Substrate	5	21049	0.001343 **
Time	1	2581	0.456927
Substrate: time	5	24614	0.000396 ***

*** Significant at 0 level and ** at 0.001 level

Appendix 6. Questionnaire of the specific survey

IFWA TERMITE SURVEY (GHANA)
Sample Data Sheet

Name of Enumerator/ Scientist……………………………….
Date…………………

GPS………….……..

1. Village/Town

Review

2. Name of Farmer

3. Telephone

4. Termite species used

Sample ID

5. Description of termites used

6. Collection or trapping methods

7. Toxic termite species

8. Description of toxic species		Sample ID

9. Reason for being termed toxic/ poisonous

10. Any effect on the poultry when fed?

11. re there any variations in the termite species collected in the different seasons?

Appendix 8. Phonetic transcriptions of termite genera in the four investigated regions

Region	Local name	Genus name	Caste
Volta			
	Babanoe	*Trinervitermes*	Workers
	Babataga	*Macrotermes*	All members
	Babatsifome	*Microtermes*	All members
	Babatsoe	*Trinervitermes*	Soldiers
	Babanulabe	*Trinervitermes*	Soldiers
	Babanto	*Trinervitermes*	Soldiers
	Babasoe	*Trinervitermes*	Workers
	Babagye	*Trinervitermes*	Soldiers
	Babayentor	*Trinervitermes*	Soldiers
Upper West			
	Kpele/Kpolo	*Odontotermes*	All members
	Tambiezie	*Trinervitermes*	Soldiers
	Zuzie	*Trinervitermes*	Soldiers
	Kontontambire	*Trinivertermes*	Soldiers
	Moree	*Odontotermes*	All members
	Guno	*Cubitermes*	All members
	Yawzugboli	*Macrotermes*	Soldiers
	Yawmaa	*Macrotermes*	Queen
	Zugboli	*Macrotermes*	Soldiers
	Dadigre	*Cubitermes*	All members
	Dadiga	*Cubitermes*	All members
	Yaokpele	*Trinervitermes*	Workers
	Feeli	*Trinervitermes*	Soldiers
	Tien	*Macrotermes*	All members
	Feelidjuron	*Cubitermes*	All caste
	Feelikokoo	*Trinervitermes*	Soldiers
	Tambituo	*Trinervitermes*	Soldiers
Upper East			
	Kunfio/fio	*Trinervitermes*	Soldiers
	Kunkwio	*Macrotermes*	All members
	Tua/Toa	*Microtermes*	All members
	Morka	*Odontotermes*	All members
	Kotonko	*Trinervitermes*	Soldiers
Northern			
	Tambiezie	*Trinervitermes*	Soldiers
	Mochereba	*Trinervitermes*	Workers
	Tambie pielegu	*Amitermes*	All members
	Worikogu	*Odontotermes*	All members
	Tambiegun	*Amitermes*	All members
	Tambietuo	*Trinervitermes*	Soldiers
	Tambie gbungara	*Cubitermes*	All members
	Yoblezie	*Macrotermes*	Soldiers
	Gbutegba	*Trinervitermes*	Soldiers