

**DEPARTMENT OF MARINE AND FISHERIES SCIENCES
UNIVERSITY OF GHANA, LEGON**

**EVALUATION OF LARVAL MEAL DIET OF BLACK SOLDIER
FLY
(*Hermetia illucens*: L. 1758) ON FINGERLINGS CULTURE OF NILE
TILAPIA (*Oreochromis niloticus*: L.)**

BY

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**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
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DECLARATION

This thesis presented to the Department of Marine and Fisheries Sciences, University of Ghana, is as a result of research work undertaken by me, Christopher Teye-Gaga, supervised by my supervisors. I hereby declare that, except for references to works of other authors who have been duly acknowledged, this thesis is entirely my original research work which has neither been presented in whole or in part to any other university for the award of a degree.

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DEDICATION

This work is dedicated in memory of my mother, Madam Dorothy Matu Adogla, and to my siblings, nieces, nephews and cousins and all those who helped me in diverse ways to come this far in life.

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LIST OF ABBREVIATIONS

ADC	Apparent digestibility coefficient
AOAC	Association of Official and Analytical Chemist
APHA	America Public Health Association
ARDEC	Aquaculture Research and Development Centre
BNARI	Biotechnology and Nuclear Agriculture Research Institute
BoG	Bank of Ghana
BSF	Black soldier fly
BSFL	Black soldier fly larvae
BSFM	Black soldier fly meal
FAO	Food and Agriculture Organization
FM	Fishmeal
GAEC	Ghana Atomic Energy Commission
GDP	Gross Domestic Product
GHS	Ghana Cedis
GSS	Ghana Statistical Service
h	hour
min	minute
MOFAD	Ministry of Fisheries and Aquaculture Development
Nd	No date
NRC	National Research Council
SB	Soybean
SBM	Soybean meal

ABSTRACT

The decreasing availability and increasing cost of fishmeal have called for efforts in evaluating wide varieties of relatively lower cost ingredients that could partially or wholly replace fishmeal. The Black Soldier Fly (BSF), *Hermetian illucens* larvae hold potential in this regard due to their high protein and fat contents. This study was conducted at the BSF Centre of the Ghana Atomic Energy Commission, Accra, and Aquaculture Research and Development Centre at Akosombo, to evaluate the growth performance of fingerlings of *Oreochromis niloticus* (Akosombo strain) using diets with BSF larval meal as an alternative source of protein to fishmeal and to determine the apparent digestibility of nutrients of BSF larval meal in comparison to those of traditional sources of protein, such as fishmeal and soybean meal. The BSF larvae were reared on fruit and vegetable wastes and then solar dried. The BSF larval meal was used to replace fishmeal at 25, 50 and 75% inclusion levels in formulated diets for *O. niloticus* fingerlings. Two other tilapia diets, an on-farm type (ARDEC) and a commercial one (RAANAN), containing 0% BSF larval meal served as controls. In all, five isonitrogenous (380 gkg^{-1} crude protein) and isoenergetic (18 kJg^{-1} gross energy) diets were used in culturing *O. niloticus* fingerlings (initial mean weight $1.3 \pm 0.23 \text{ g}$) for 10 weeks. Apparent digestibility coefficients (ADCs) were determined using chromic oxide as inert marker. The reference diet was formulated to contain 380 gkg^{-1} crude protein and 18 kJg^{-1} gross energy. The reference diet was replaced with test ingredients at 70:30 ratios. Proximate analysis on the whole BSF larvae was found to contain crude protein (37.83%) and crude fat (22.7%) (dry matter basis). All diets were readily accepted by fish. Growth and nutrient utilisation parameters of the cultured *O. niloticus* fingerlings indicated no significant differences ($p > 0.05$) among the various dietary treatments. BSF 25 had the highest final mean weight gain ($33.82 \pm 2.53 \text{ g}$) and the least was BSF 75 ($30.53 \pm 3.95 \text{ g}$). Whole-body observation of harvested fingerlings

showed no abnormalities on the external and internal body. This suggests that BSF larval meal does not exert a negative effect on fish health. Analysis on the cost effectiveness of the various diets used in culturing *O. niloticus* fingerlings showed that producing a kilogram of fish using BSF 75 diet was more cost effective than other diets. Nutrient digestibility of BSF meal compared favourably with those of fishmeal and soybean meal. The apparent digestibility coefficients (ADCs) of nutrients of BSF meal, fishmeal and soybean meal were high ($> 52\%$), implying good utilization of feed for tissue synthesis and metabolic activities. On the basis of final mean weight gained, feed conversion ratio and the cost-effectiveness of diets, it suggests that BSF larval meal may partially replace fishmeal at best 50% inclusion level without affecting fish growth, health and carcass quality.

CHAPTER ONE

INTRODUCTION

1.1 Background

The global population is projected to reach 9 billion by 2050 (FAO, 2012, 2014) leading to an increase in demand for animal products by 60 – 70% (Makkar *et al.*, 2014). Enormous resources will be needed to feed the growing population through an increase in animal production (poultry, swine, aquaculture and livestock). However, resources needed for production are faced with many challenges, such as land and water availability as well as the impact of global climate change (FAO, 2013; Makkar *et al.*, 2014). Globally, aquaculture has consistently been the fastest-growing animal production sector and is expected to make a significant contribution towards meeting the ever increasing demand for fishery products (FAO, 2014).

The Fisheries sector plays a major role in Ghana's socio-economic development. It accounts for 1.4% of Ghana's national GDP (GSS, 2014). An estimated 10% of the population, directly and indirectly, depends on the fisheries sector for livelihood (Kassam, 2014). Fish consumption forms about 60% of animal protein in Ghanaian diet, which amounts to about 22.4% of household expenditure (FAO, 2004; Asiedu *et al.*, 2015). The annual per capita fish consumption in Ghana is 25 kg which is higher than the world and Africa average of 19 kg and 10 kg, respectively (Asiedu *et al.*, 2015). Many Ghanaians prefer fish protein to other animal sources, mainly due to the relative advantages of fish pertaining to cost, nutritional value, and taste (BoG, 2008).

Capture fisheries (marine and inland) which form about 90% of total fisheries production (MOFAD, 2015) has been dwindling in Ghanaian waters over the years. For instance, total capture fisheries production declined from 405,443 metric tonnes to 374,530 metric tonnes

between 2005 and 2014 (MOFAD, 2015); leaving a wide gap between annual fish requirement and total fisheries production. In 2014, Ghana's fish requirement was 1,088,749 metric tonnes as against total fisheries production of 413,077 metric tonnes, leaving a deficit of 675,672 metric tonnes (MOFAD, 2015). The shortfall was partly met through imports (which was reported to be up to the tune of \$157 million dollars per annum) (Agbeko *et al.*, 2014; Frimpong and Adwani, 2015).

In contrast, fisheries production from aquaculture has been growing consistently over the past decades from 1,154 metric tonnes to 38,547 metric tonnes between 2005 and 2014 (MOFAD, 2015). Aquaculture is therefore considered as a sustainable means of bridging the gap between fish demand and fish production. Fisheries production from the aquaculture sector will, therefore, have to undergo a massive expansion. For this reason, an enormous resource would be needed in order to meet the rising demand. The most challenging aspect will be fish feed since it accounts for about 70% of total cost of production (Frimpong and Adwani, 2015; Huis, 2013; Kenis *et al.*, 2014).

In an animal production system, high-quality nutrition is essential to producing economical, healthy and high-quality products (Craig and Helfrich, 2004; Abarike *et al.*, 2012). In fish nutrition, protein is considered as the most important feed component, because it provides amino acids required for synthesising new tissues; replacing worn out tissues and provides energy when other sources of energy such as lipids and carbohydrates are limited (Fitzsimmons, 2005; El-Sayed, 2006; Ng and Romano, 2013). However, dietary protein is the most expensive feed component accounting for over 50% of feed cost in intensive culture (Agbo, 2008; Nguyen *et al.*, 2009). Protein in fish feed is gotten mainly from soybean (plant source) and fishmeal (animal source) (Veldkamp and Bosch, 2015). Soybean meal is the best available plant protein source (El-Sayed, 2004; Ng and Romano,

2013). It has high digestibility, rich in protein with high essential amino acids profile. However, it has low palatability (Sánchez-Muros *et al.*, 2014), and is limiting in methionine and cysteine (Barroso *et al.*, 2012). Also, soybean meal contains some level of anti-nutritional substances such as trypsin inhibitor, haemagglutinin, and antivitamin (Barroso *et al.*, 2012). Sánchez-Muros *et al.* (2014) noted that increasing cultivation of soybean results in deforestation and loss of biodiversity. Besides, land for production of soybean is limited (Veldkamp and Bosch, 2015).

Fishmeal is the single most important and the preferred protein ingredient in formulated feed for culturing fish. This is because it has high level of protein (60 – 75% crude protein), well balanced amino acid profile (especially the essential amino acids), good lipid profile including omega-3 fatty acids, low carbohydrate level, high digestibility and low levels of anti-nutritional factors (Zhou *et al.*, 2004; Tacon and Metian, 2009; Haghbayan and Mehrgan, 2015). These qualities meet the nutritional requirement of fish. However, fishmeal is the most expensive protein component, contributing significantly to the high cost of production in aquaculture industries (Ayoola, 2010; FAO, 2013; Nguyen *et al.*, 2009).

Fishmeal is derived from small pelagic forage fish such as anchoveta and sardinella species. Currently, about 10% of global fish production (i.e. either whole fish or fish remains resulting from processing) is used for fishmeal (IFIF, 2012; FAO, 2013). Although fishmeal is used in other animal production sectors such as poultry and livestock, it has been reported that about 68% of global fishmeal production is used in the aquaculture feed industry alone (Tacon and Metian, 2009).

Globally, aquaculture production has witnessed massive increase from 55.7 to 73.8 million metric tonnes from 2009 to 2014 (FAO, 2016) as a result of an upsurge in demand for

fisheries products. Population growth, rising incomes, and urbanisation are some of the driving factors (FAO, 2016) for the increasing demand for fish. Also, the consumption of fish is considered nutritious and healthier compared to meat, which is associated with increased risk of contracting various diseases (Asiedu *et al.*, 2015). Consumers are therefore shifting from meat to consuming more fish (Asiedu *et al.*, 2015)

An attempt at meeting the ever increasing demand for fishmeal has led to the overexploitation of small pelagic fish resources (Veldkamp and Bosch, 2015).

Most of the world's fishmeal is produced in South America through its catch of anchovy. However, production of anchovy has been dwindling; because it depends on the El Niño climatic cycle (which is also negatively affected by climate change) (FAO, 2013; Huis, 2013). For instance, anchovy catch has declined from 12.5 million tonnes in 1994 to a meagre 4.2 million tonnes in 2010 (IFIF, 2012; FAO, 2013; Huis, 2013).

There is a heightening environmental disquiet over the use of wild fish to produce fishmeal (Nguyen and David, 2013). Tacon and Metian (2009) opined that using large quantities of small pelagic fish species and bycatch of commercial fisheries for fishmeal, do not only lead to increasing fishing pressure but also reduces the quantity of low priced fish available for human consumption especially, for the poor. In addition, it affects other dependent piscivorous species, including birds and mammals.

The increasing demand for fishmeal and the decreasing availability of the commodity globally has led to a sharp increment in the price of fishmeal (Nguyen *et al.*, 2009; Ayoola, 2010; Huis *et al.*, 2013). Globally, the price of fishmeal increased from US \$ 600/metric tonne in 2005 to US\$ 2000 in June 2010 (Sánchez-Muros *et al.*, 2014). This trend is expected to be unremitting for a long time to come; thus threatening the survival of

aquaculture business especially small-scale farmers in developing countries (Ogunji *et al.*, 2006; Barroso *et al.*, 2012).

Due to the growing aquaculture market as well as the high pressures from commercial fishing, it is expected that the demand for fishmeal will soon exceed the annual global production of the commodity (Tacon and Metian, 2009; Vrij, 2013). It is generally acknowledged that using fishmeal for fish feed is unsustainable, thus, posing a challenge to the sustainability and growth of aquaculture industry (Vrij, 2013). There is, therefore, the need to research into alternative, cost-effective, and sustainable sources of protein that have similar essential amino acids, phospholipids and fatty acids as fishmeal (Barroso *et al.*, 2012).

1.2 Justification

The aquaculture sub-sector holds many prospects for Ghana in terms of meeting consumer demand for fish, livelihood for a large number of people who directly or indirectly engage in aquaculture and its value chain. However, aquaculture sub-sector is faced with some challenges which include inadequate capital, seed, and feed (Hiheglo, 2008; Kassam, 2014). Furthermore, the most singular challenge to the aquaculture business is the decreasing availability and rising cost of feed (Cobbina, 2010). Globally, the cost of conventional feed resource keeps on rising. Furthermore, the availability of these feed sources in the future is uncertain (Rao *et al.*, 2012; Makkar *et al.*, 2014).

In Ghana, the average price per kilogramme of imported fish diet is about US \$ 2.0 (Obirikorang *et al.*, 2015b) which is very expensive for most fish farmers, especially the small-scale ones. This makes farmers resort to using a mixture of low-cost and locally available agro-industrial by-products feed sources such as wheat bran, groundnut bran, maize bran, soybean meal and copra cake to feed fish (Agbo, 2008, 2015; Amisah *et al.*,

2009). These ingredients are supplementary and do not meet the nutritional requirements for fish; leading to poor growth and low productivity (Amisah *et al.*, 2009; Attipoe *et al.*, 2009; Obirikorang *et al.*, 2015a). Thus, posing a challenge to the sustainability and growth of aquaculture industry.

Fishmeal is the most singular essential protein ingredient needed by fish; however, it is the most expensive ingredient in formulated feed and thus increasing the cost of production (Nguyen *et al.*, 2009; Hussain *et al.*, 2011).

Insects are increasingly becoming attractive non-conventional protein ingredient option in animal feed formulation and utilisation (Huis, 2013; FAO, 2013). Insect, especially the larvae and pupae are rich in protein (40 - 70% dry weight basis) and are naturally consumed by fish and free range poultry (Rumpold and Schlüter, 2013; Kenis *et al.*, 2014; Huis, 2015).

The Black Soldier Fly (BSF) larvae fed on poultry manure is rich in protein, (42%) and fats (35%) (dry matter basis) (Newton *et al.*, 1977; Sheppard *et al.*, 2002; FAO, 2013; Huis, 2013; Makkar *et al.*, 2014). Live prepupa has higher dry matter content (about 44%) than other insects, thereby, making it easier and less costly to dehydrate and store for long period of time (Newton *et al.*, 2008; Makkar *et al.*, 2014). Besides using BSF larvae in controlling large concentrations of organic wastes and animal manure, they also serve as a high-quality nutritional feedstuff for fish and poultry.

Research has proven that BSF larval meal used as a component of a complete diet, supports healthy growth of rainbow trout (*Oncorhynchus mykiss*) (St-Hilaire *et al.*, 2007a; Stamer *et al.*, 2014), the Nile tilapia, (*Oreochromis niloticus*) (Rana, *et al.*, 2015), the Atlantic salmon (*Salmon salar*) and the blue tilapia (*O. aureus*) (Makkar *et al.*, 2014). According to Sheppard *et al.* (2007) and FAO (2013), BSF meal can replace fishmeal up to at least

25% in a complete diet of rainbow trout or channel catfish (*Ictalurus punctatus*), with no reduction in feed conversion ratio (FCR) or weight gained.

Producing BSF larval meal to feed fingerlings of *O. niloticus* involves cost and benefits. One way of assessing the economic viability of BSF meal is by conducting the cost effectiveness (economic profitability index) of using BSF larval meal as a feed component in the culture of *O. niloticus*.

Digestibility is the ratio of nutrient and energy obtained by an animal from eating a particular food substance during the process of absorption (NRC, 1993; Glencross *et al.*, 2007; Hussain *et al.*, 2011). Measurement of digestibility is a vital aspect of evaluating the efficiency of a feedstuff (Bureau and Cho, 1999; Agbo, 2008; Hussain *et al.*, 2011). This is because a food substance may seem to be an excellent source of nutrient from its chemical composition; but will be of little actual value unless it can be accepted, digested, and absorbed in the targeted fish species (Köprücü and Özdemir, 2005; Agbo, 2008; Hussain *et al.*, 2011). The Apparent Digestibility Coefficient (ADC) of a diet or an ingredient is very necessary for; effective substitution of one feed component for another; formulation of cost-effective feed that maximises fish growth by providing the right quantity of available nutrients and also limits waste produced by fish (Lee, 2002; Köprücü and Özdemir, 2005; Akhter, 2015; Jimoh *et al.*, 2015).

The Nile tilapia was chosen for this study because it is the most commercially important aquaculture fish species in Ghana; constituting over 80% of farmed fish (Kassam, 2014) and globally, it forms about 71% of the world farmed tilapia (FAO, 2012; Ng and Ramano, 2013).

The Black Soldier Flies are naturally available in Ghana and they could be exploited for various purposes such as composting of organic wastes and as a feedstuff for fish,

poultry and livestock. There are established colonies of BSF in Ghana i.e. the BSF Centre at Ghana Atomic Energy Commission, Accra.

Experimentation on the Black Soldier Fly larval meal as a protein component of fish diet could result in some levels of replacement of fishmeal with BSF larval meal; thus cutting down on the cost of feed and invariably, the cost of fish production. This may also lead to availability and affordability of farmed fish to consumers and overall growth in the aquaculture industry in Ghana.

1.3 Objectives of the Study

The primary objective of the study was to evaluate the growth performance of fingerlings of *Oreochromis niloticus* (Akosombo strain) using diets with BSF larval meal as an alternative source of protein to fishmeal.

Specific objectives are to determine:

- (i) The nutritional (proximate and amino acid) composition of BSF Larval meal
- (ii) The culture performance of *O. niloticus* fingerlings (Akosombo strain) fed on diets with BSFL meal as a protein supplement.
- (iii) The cost effectiveness of using BSFL meal as a feed supplement in the culture of fingerlings of *O. niloticus*.
- (iv) The apparent digestibility coefficients (ADCs) of the nutrients and energy of BSF larval meal fed to *Oreochromis niloticus* (Akosombo strain) fingerlings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Current Global Fisheries and Aquaculture Production.

Growth in global fisheries production for human consumption has outpaced population growth by an annual rate of 3.2% compared to 1.6% growth in world population from 1961 - 2013. This remarkable growth has been attributed to aquaculture (FAO, 2016).

The world per capita apparent fish consumption increased from about 14.4 kg in the 1990s to 19.7 kg in 2013 (FAO, 2016). This is as a result of increases in world fish supply, reduction in wastage, better utilisation and improved distribution channels. Also, population growth, urbanisation and rising income have led to growing demand for fish (FAO, 2016).

In addition, global capture fishery production has been stable since the late 1980s. Global total capture fishery was 93.4 million tonnes in 2014; of which 81.5 million tonnes are from marine and 11.9 million tonnes from inland waters (FAO, 2016). The major producing countries are the United States of America, Indonesia, Russia and China which contributed much to this growth (FAO, 2016).

Furthermore, world fishery production from the aquaculture sector in 2014 was 73.8 million tonnes with estimated value of US \$160.2 billion. This total is made of 49.8 million tonnes of finfish (US\$99.2 billion), 16.1 million tonnes of molluscs (US\$19 billion), 6.9 million tonnes of crustaceans (US\$36.2 billion) and 7.3 million tonnes of other aquatic animals including amphibians (US\$3.7 billion) (FAO, 2016). China accounted for 45.5 million tonnes in 2014 or more than 60 percent of global fish production from aquaculture. Other major producers were India, Viet Nam, Bangladesh, and Egypt (FAO, 2016).

Egypt is the biggest aquaculture producing nation in Africa and the only country of the continent that is ranked among the top 25 aquaculture producing nations in the world with a total production of 1137.1 metric tonnes in 2014 (FAO, 2016).

In 2014, an estimated 56.6 million individuals globally were employed in the production sector of capture fisheries. Of this, 36% were full time, 23% part-time, and the remainder occasional fishers (FAO, 2016). Asia accounted for 84% of the global population engaged in the fisheries and aquaculture sector, followed by Africa (10%), and Latin America and the Caribbean (4%). Of the 18 million people engaged in fish farming, 94 percent were in Asia (FAO, 2016).

In 2014, about 19% of people engaged in the primary sector of aquaculture were women. In all, women accounted for 50% of the work force in both the primary and secondary sectors (FAO, 2016).

2.2 Overview of Aquaculture in Sub-Sahara Africa.

Aquaculture production in the Sub-Saharan Africa has witnessed a remarkable growth over the past decades. Production increased over seven folds, from 80,900 metric tonnes to 556,950 metric tonnes from 2004 to 2014. Nigeria is the biggest aquaculture producing nation in the region with total production of about 313,231 metric tonnes, which represents 56% of the total production of the sub-region in 2014, followed by Uganda, 111,023 metric tonnes representing 20% and Ghana, 38,545 metric tonnes representing 7% (FAO, 2017).

This remarkable growth is attributed to; adoption of good governance, investment from the private sector, provision of credit to farmers, involvement of private- capacity building and emphasis on research, the use of new production systems such cages, establishment

of efficient commercial hatcheries, availability of aquafeeds and sound management system (FAO, 2017).

In 2014, Production from Inland aquaculture was 547,031 metric tonnes (98%) whilst that of marine/coastal waters was 9,919 metric tonnes (2%). Catfish, tilapia and cyprinid are the main inland species accounting for over 80% of total fish harvest (FAO, 2017).

Despite the massive growth of aquaculture in Sub-Sahara Africa, its contribution to global aquaculture production is insignificant, amounting to only 0.7% in 2014 (FAO, 2017).

2.3 Ghana's Fisheries Sector

Ghana's fishery sector comprises capture fisheries (marine and inland) as well as aquaculture. The marine sub-sector accounts for 70% of total fish supply, while inland and aquaculture sub-sectors contribute 30% of fishery production (MOFAD, 2015). The Fisheries Act (Act 625) is the main legislative instrument that governs the fisheries sector in Ghana.

2.3.1 Overview of Ghana's Aquaculture

Production of fish by means of culturing started in 1953 in Northern Ghana in dugouts and reservoirs (BoG, 2008; Asmah *et al.*, 2009; Obirikorang, *et al.*, 2015a; FAO, 2016). Presently, aquaculture is practised all over the country, predominantly in the central and southern belts. Some of the reasons for the establishment was to close the vast space that exists between fish demand and supply, employment generation and to export the excess produced (Hiheglo, 2008; FAO, 2016).

Eventhough aquaculture in Ghana is synonymous with fish culture, Hiheglo (2008) argues that three forms of aquaculture traditionally existed prior to the introduction of a modern

form of culturing fish. These are “*atidjas* or brush-parks in lagoons and reservoirs; *hatsis* (fish holes) and *whedos* (mini-dams) in coastal lagoons; and *afani* or freshwater clams (*Galatea paradoxa*) in the lower Volta, where young clams are collected and ‘planted’ in ‘owned’ areas of the river (Hiheglo, 2008).

Ghana’s aquaculture production has seen a massive increase from 1,153 metric tonnes in 2005 to 38,547 metric tonnes in 2014 (MOFAD, 2015). This impressive growth has been attributed to the availability of fingerlings, quality feed, increasing production from large-scale cage farm and improvement in data collection (Cobbina, 2010).

Although the aquaculture sector plays an important role in Ghana’s economy, its impact is not fully recognised, because it is enclosed in the overall input of the fisheries sector to the national economy. Data on the exact contribution of aquaculture to employment, food security, and poverty alleviation are unavailable. However, it has been estimated that the production from ponds and culture-based fisheries is worth about US\$ 1.5 million a year (Cobinna, 2010).

From its inception, aquaculture in Ghana has been predominantly inland-based. The main production system has been ponds, dugouts, reservoirs, pens and cages. Ponds are the dominant system in the southern belt of the country accounting for 98% of the existing farms. However, in the northern belt of the country, reservoirs and dugouts are the main systems of fish culture. This has been as a result of the comparatively low rainfall distribution pattern in Northern Ghana (Asmah *et al.*, 2009; Cobbina, 2010).

Pond is the main culture system used by small-scale farmers who form the majority of fish producers. However, over the last decade, cage has become the dominant system for producing fish in terms of volume. Intensive farming of tilapia in cages is being carried out on the Volta Lake by small and large scale operators (Cobbina, 2010; Kassam 2014).

About 73 – 93% of all farmed tilapia is obtained from floating cages of which the vast majority are located in the Asuogyaman and North Dayi Districts of the Eastern and Volta regions, respectively (Asmah *et al.*, 2009; Kassam, 2014; FAO, 2016).

Presently, there are about 5,000 fish farmers operating some 19,000 fish pond and cages (FAO, 2016). Total output from the aquaculture sub-sector for 2013 was 30,000 metric tonnes of fish, out of which nearly 88% came from cage farms (FAO, 2016). Tropo Farms and West African Fish Limited are two large scale commercial farms that are responsible for over 30% of total aquaculture production in Ghana (Kassam, 2014).

Nile tilapia (*Oreochromis niloticus*) is the dominant and the most preferred fish species cultivated in Ghana which constitutes 80% of aquaculture production with current production of 40,000 metric tonnes per year (Kassam, 2014; FAO, 2016). The catfishes, (*Clarias* spp, *Heterobranchus* spp) and *Heterotis niloticus* account for 20% of the fish species cultivated (BoG, 2008; Asmah, *et al.*, 2009; Kassam 2014, FAO, 2016). These species are cultivated in mono and polyculture systems (Kassam, 2014).

Hatchery facilities of the Fisheries Commission at Ashaiman and Kumasi; Aquaculture Research and Development Centre (ARDEC) at Akosombo are the main public sources of fingerling production for fish culture (MOFAD, 2015). Fish Reit and Crystal Lake at Akosombo are among the few private hatcheries. The seed of endemic species such as catfishes is mainly sourced from the wild (Kassam, 2014).

The main supplementary feeds mostly used by small-scale farmers include; maize bran, wheat bran, rice bran and other brans of cereals (Rao *et al.*, 2012). The majority of them practice semi-intensive farming which requires some supplementary feeds and fertiliser inputs to boost natural productivity (Rao *et al.*, 2012). Floating feeds, (which are relatively expensive) are used by large, medium, and also few small scale farmers (Kassam, 2014).

Most commercial feeds in the country are produced by Raanan Fish Feed Ltd, a company based in Prampram, near Accra (Kassam, 2014). Major domestic market and consumption centres include Accra, Kumasi, Sekondi-Takoradi, Tarkwa and Tema.

2.3.2 Challenges of Aquaculture in Ghana

Ghana's aquaculture is still at the developmental stage even though it started 50 years ago. Its full potential is yet to be exploited. There are several challenges that are inimical to the growth of aquaculture industries as reported by Hihego (2008). These include; inadequate supply of quality fish seed and expensive fish feeds, low investment from the private sector as well as inadequate information concerning the economic profitability of aquaculture. However, the aquaculture sector is continually playing a significant role aimed at meeting Ghana's fish requirements (Cobbina, 2010).

2.4 The use of insect to feed fish

Over the last decades, several studies have been carried out on the possibility of replacing fishmeal with insect meal in fish diet with varying degrees of success. Key insects used in these studies include black soldier fly (St-Hilaire *et al.*, 2007a; Stamer *et al.*, 2014), common housefly (Ogunji *et al.*, 2008; Olaniyi and Salau, 2013), silkworm (Kurbanov *et al.*, 2015), grasshopper (Alegbeleye *et al.*, 2012) and krill (Vrij, 2013). In a review by Sheppard *et al.* (2007), a minimum of 25% of fishmeal can be replaced with insect meal in fish feed without affecting weight gained and feed conversion ratio.

In a report by Olaniyi and Salau (2013), fishmeal was replaced with maggot meal at varying inclusion levels in the diet of African catfish (*Clarias gariepinus*). The results showed that fishmeal can be replaced with maggot meal up to 75% without impacting

negatively on fish growth. In another study, the effect of replacement of fishmeal with silkworm (*Bombyx mori*) pupa meal on the African catfish, (*Clarias gariepinus*) fingerlings was determined (Kurbanov *et al.*, 2015). Fishmeal was replaced with silkworm pupa meal at varying inclusion levels and incorporated in the diet of *Clarias gariepinus*. The fingerlings were fed for 40 days. The authors concluded that silkworm pupa meal can be an alternative source of protein to fishmeal in the diet of *Clarias gariepinus* up to 50% without exerting a negative effect on fish growth.

Also in Alegbeleye *et al.* (2012), variegated grasshopper (*Zonocerus variegatus*) meal was used in replacing fishmeal in the diet of African catfish fingerlings at varying inclusion levels. The authors found out that variegated grasshopper can replace fishmeal up to 25% in diets of *C. gariepinus* fingerlings without any adverse effect on growth, weight gain and feed conversion ratio.

2.5 The Black Soldier Fly

2.5.1 Scientific Classification of Black Soldier Fly

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Diptera
Sub-order:	Brachycera
Infra-order :	Tabanomorpha
Super-family :	Stratiomyoidea
Family:	Stratiomyidae
Genus:	Hermetia

Species : *Hermetia illucens*

Binomial Name: *Hermetia illucens*; Linneaus, 1758. (Source: Rana *et al.*, 2015).

2.5.2 Life History of the Black Soldier Fly

The Black Soldier Fly (BSF), (*Hermetia illucens* L: 1758) is a fly (Diptera) of the family Stratiomyidae. It originates from South-eastern United State and spread throughout tropical and warmer temperate regions between about 45°N and 40°S (Diener *et al.*, 2011; Bullock *et al.*, 2013). It has three generations within a year in the southern parts of United States; and it is active between April and November (Sheppard *et al.*, 1994; Tomberlin *et al.*, 2002; Diener *et al.*, 2011).

The adult is a harmless, non-biting, large (about 15- 20 mm long) black fly that resembles wasps but differs from wasps by having only two wings (wasps have four). It lacks a stinger hence cannot bite nor sting (Diclaro *et al.*, 2012). It possesses elongated antennae with three segments. There is a white colouration near the end of each leg (Newton *et al.*, 2005; Diclaro *et al.*, 2012). BSF possesses transparent spots at the first abdominal section. The female's abdomen is reddish in colour whilst the male's abdomen is brownish (Park, 2015). The adult does not possess a mouth part or digestive organs, therefore, they cannot feed (Newton *et al.*, 2005; Park, 2015). Black Soldier fly adult are typically found outdoors near agriculture settings, or near naturally decomposing organic matter such as rotting fruits and vegetables, as well as animal manure for the purpose of oviposition (Sheppard *et al.*, 2002; Bullock *et al.*, 2013).

The larvae occur in dense population (Newton *et al.*, 2005; Park, 2015), has an off-white colour with a small, projecting head containing chewing mouthparts. They can grow up to 27 mm in length, 6 mm in width and weigh up to 220 mg (Sheppard *et al.*, 2002; Diclaro

et al., 2012; Makkar *et al.*, 2014). The larvae are saprophages; a rapacious consumers of a wide variety of organic waste including animal manure, rotting fruits and vegetable, and food wastes, (Newton *et al.*, 2005; Diener *et al.*, 2011) and even human excreta (Banks, 2014). They can also feed on other organic wastes such as leftover restaurant foods, catsup, carrion, coffee bean pulp distillers waste, and fish offal (Veldkamp and Bosch, 2015). They are capable of consuming feed up to their own body weight in a day; from 25 to 500 mg of fresh matter per larva per day (Makaar *et al.*, 2014).

Larvae go through six instars (molting phase) and need about four weeks to reach full maturity (prepupae stage) (Diclaro *et al.*, 2012) depending on factors, such as temperature, the size and number of maggots present, moisture content, and the nutritional quality of the organic matter they consume (Alvarez, 2012; Park, 2015). However, this phase can be extended to sixteen weeks if food is inadequate (Gobbi *et al.*, 2013).

When Black Soldier Fly Larvae (BSFL) reach the end of last larval instar (prepupa stage), they cease to eat; they empty their guts, their mouth parts are sealed and changes to an appendage that aids climbing (Diclaro *et al.*, 2012). Their skin becomes dark and harder with higher chitin content. At this point, they leave the feed source and search for a cool, dark, dry, sheltered place, such as ground vegetation where they bury themselves for protection against predation and desiccation while they metamorphose into adults (Diclaro *et al.*, 2012; Holmes *et al.*, 2012). This innate behaviour makes it possible for self-harvesting (Sheppard *et al.*, 2002; Newton *et al.*, 2008). Pupation takes another two weeks before an adult emerges from the pupal case (Park, 2015).

Unlike other insects, such as common housefly (*Musca domestica*), BSF is not seen as a pest or a mechanical vector for disease because the adult is not attracted to human settlements or foods (Newton *et al.*, 2005; Banks *et al.*, 2014). This is because the adults

do not feed; they survive on the large fat body reserved from the larval stage. However, they take in water (mist) when available (Newton *et al.*, 2008). The availability of water can prolong their lifespan to around 12 days whereas, in the absence of water, they may only survive for around 6 days (Sheppard *et al.*, 2002; Newton *et al.*, 2008; Park, 2015).

2.5.3 Life cycle of the Black Soldier Fly

The Black Soldier Fly undergoes complete metamorphosis. Its life cycle comprises five stages. These are; egg, larvae, prepupae, pupae and adults. These are shown in Figure 2.1

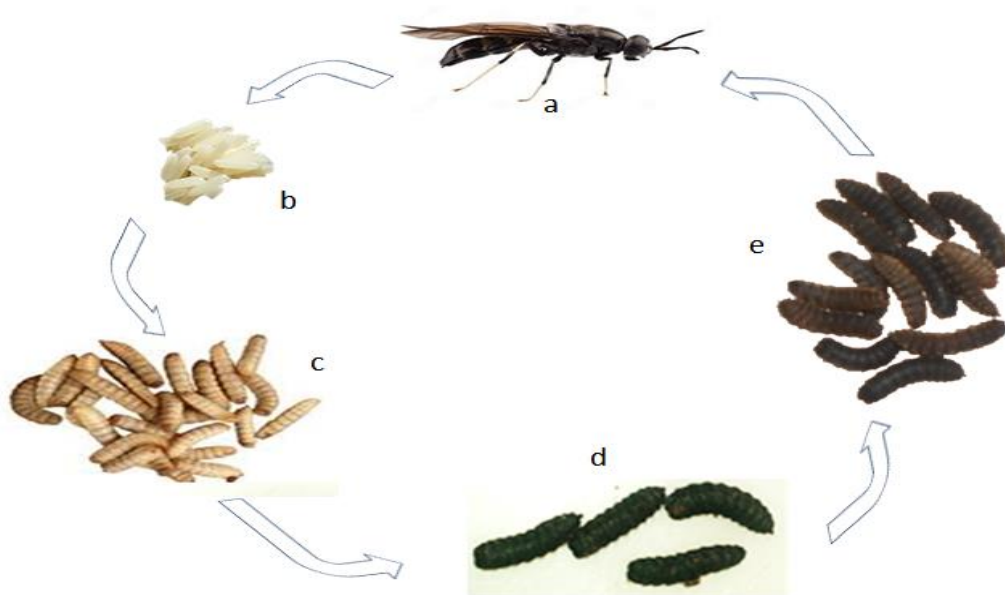


Figure 2.1: Life cycle of BSF (*Hermetia illucens*); (a) adult (b) eggs (c) larvae (d) prepupae (e) pupa

2.5.4 Growth Conditions

The Black Soldier Fly's life cycle is highly influenced by abiotic factors, such as ambient temperatures, relative humidity and light intensity (Sheppard *et al.*, 2002; Zhang *et al.*, 2010; Gobbi *et al.*, 2013). The adult flies are photophilic; thus requiring strong daylight spectra (a minimum light intensity of $100 \mu\text{mol}/\text{m}^2/\text{s}$), relative humidity above 60%, as well as temperatures, ranging from 25°C to 35°C to promote mating (Alvarez, 2012).

Females mate once with one oviposition event in their lifetime (Holmes *et al.*, 2012). Mating takes place whilst in flight; two days after emerging from pupa case as adults. The adult males aggregate in small numbers near a secluded place and display lekking behaviour where a “male intercepts a passing female in mid-air and both descend in copula” in order to mate (Tomberlin *et al.*, 2002; Diclaro *et al.*, 2012; Banks, 2014). Within two days after successful copulation, females oviposit clusters of eggs (one cluster per female in a lifetime) (Tomberlin *et al.*, 2002; Park, 2015) in dry cracks and crevices above and around a moist decomposing organic matter. Each cluster contains approximately 320 - 620 eggs (Banks, 2014). The females die few hours after reproduction (Sheppard 2009, Holmes *et al.*, 2012). In attempting to lay eggs, the females stay away from spots that are anaerobic (Bullock *et al.*, 2013).

The eggs are pale yellow or cream coloured and about 1 mm long hatch within 102-105 h (about 4 days) at optimal ambient temperature of 27 °C, and a relative humidity of 60 – 70% (Booth *et al.*, 1984; Newton *et al.*, 2005; Diclaro *et al.*, 2009; Park, 2015). The newly hatched neonate larvae crawl or fall into the food source. The larvae then begin to feed rapidly in order to accumulate enough fats before they pupate (Bullock, 2013; Banks, 2014). Diener *et al.* (2011) opined that, due to the relatively long period between oviposition and eclosion, the BSF, unlike other species of flies, do not lay their eggs straight into the decaying matter in order to prevent deliquescing of the eggs. Higher quality of feed at the larval stage results in greater number of eggs production and shorter life cycle of the BSF (Tomberlin, 2002; Banks, 2014).

Black soldier fly larvae do not feed well in direct light or in extremely dry or wet conditions. They choose to consume their food source some inches deep. However, they will perform little bioconversion if they are too far below the surface (Avarez, 2012;

Bullock *et al.*, 2013). Optimum temperature range and for efficient feed consumption is 27 to 33 °C (Sheppard *et al.*, 2002) and moisture content of 60 - 90% (Myers *et al.*, 2008; Alvarez, 2012). Larvae leave food source when the moisture content is too high (Alvarez, 2012). The maggots secrete enzymes that make the food digestible prior to ingestion by liquefying the waste as they consume it.

Relative humidity is one of the abiotic factors that affect the growth of BSFL. Decreasing humidity results in increasing rate of weight loss (Bullock, 2013). BSFL develop most rapidly at a relative humidity of 70%. BSF larvae are highly versatile; they are capable of surviving at a minimum temperature of 0 °C and Maximum of 45 °C. However, they become inactive at temperatures less than 10 °C (Bullock *et al.*, 2013; Newton, n.d.). They are able to withstand severe environmental conditions, such as drought and limited availability of food (Gobbi *et al.*, 2013).

The larvae stop feeding prior to pupa stage. Pupation phase takes about 2 weeks where they undergo complete metamorphosis before eclosing as adults. The adults emerge from the pupa case and start the life cycle again (Fig. 2.1) (Banks, 2014; Makkar *et al.*, 2014). The optimal range of temperature for the larvae to pupate is from 25 to 30 °C. The adults live for about 5 - 8 days purposely for mating and laying of eggs (Bullock, 2013).

2.5.5 Economic Importance of BSFL

2.5.5.1 Reducing Manure Contamination

The BSFL have been used in agricultural settings including poultry and pig farms to solve problems of organic waste (Sheppard and Newton 1994; FAO, 2013). BSFL eagerly feed on fresh manure. As they feed, they convert nutrients load in the manure into their biomass (rich in protein and fat) and also reduce the bulk of manure residue by 50-60% or more;

thus reducing pollution potential (Newton *et al.*, 2004). The larvae were able to reduce nitrogen content in confined bovine area by 30 – 50% and phosphorous by 61 – 70% (Newton *et al.*, 2008; Sheppard, 2008; FAO, 2013). Waste consumption rates vary by waste type, moisture content, number and size of the larvae present, and temperature (Alvarez, 2012). About 45,000 larvae are capable of eating up 24 kg of swine manure within two weeks (Newton *et al.*, 2005; Diener *et al.*, 2009; FAO, 2013).

2.5.5.2 Reducing Foul Odour

In addition to reducing the volume of manure, BSF larvae are capable of reducing foul odours emitted by putrefying manure. This is because the larvae suppress bacterial growth and also as they feed, their writhing movement aerates and dries the manure thereby reducing the odour (Diener *et al.*, 2011; FAO, 2013; Huis *et al.*, 2013).

Furthermore, BSF larvae are adept in reducing potential harmful bacteria in manure by modifying the microflora of the manure. For example, BSF larval activity drastically reduced *Escherichia coli* 0157:H7 and *Salmonella enterica* in hen manure (Liu *et al.*, 2008; IFIF, 2012; FAO, 2013).

2.5.5.3 Reducing Populations of Houseflies

Studies have shown that BSFL is capable of reducing the population of the common housefly (*Musca domestica*). In a study by Sheppard *et al.* (1994), BSFL inoculated on poultry and pig manure reduces populations of the common housefly up to 94 – 100%. They are able to achieve this by secreting enzymes to help digest the materials they feed on. Also, their activities raise the temperature of their environment. This therefore, makes the environment unsuitable for house flies to oviposit (FAO, 2013).

2.5.5.4 Biodiesel production

BSFL are found to contain high crude fat (about 35%) which can be converted to biodiesel. In an experiment conducted by Li *et al.* (2011), 1000 larvae reared on 1 kg of cattle manure, pig manure and chicken manure produced 36 g, 58 g and 91 g of biodiesel, respectively (FAO, 2013).

2.5.5.5 Black soldier flies as animal feed

Dried BSF larvae convert manure into their biomass containing protein (42%) and fat (35%) dry matter (dm) which makes them an appropriate source of high-quality feed for both livestock and fish (Newton *et al.*, 1977; Huis, 2013). Live prepupae consist of 44% dm, therefore, can easily be dried and stored for long periods (Newton *et al.*, 2008).

Studies have shown that BSFL meal used as a constituent of complete diet, supports healthy growth in rainbow trout (*Oncorhynchus mykiss*) (St-Hilaire *et al.*, 2007a; Stamer, 2014) and blue tilapia (*Oreochromis aureus*) (Sheppard *et al.*, 2008), channel catfish (*Ictalurus punctatus*) (Pimentel *et al.*, 2004), chicks (Hale, 1973), pigs (Newton *et al.*, 1977). BSF larval meal can replace fishmeal and fish oil at 25% and 38% respectively in rainbow trout diet (IFIF, 2012).

The conventional practice is to feed fish with insects. However, insects also could be reared on fish. Fish offal (entrails etc.) is among organic waste products that BSF larvae consume. St-Hilaire *et al.* (2007a) compared the nutritional quality of BSF larvae fed on cow manure and fish offal for 24 h. The result showed that lipid content and omega-3 fatty acids were 30% and 3%, respectively higher in larvae fed on fish offal than those fed on cow manure. The author opined that animal manure could be combined with fish offal so

as to increase the omega-3 and omega-6 content in BSFL. Thus BSF larvae can be tailored to provide a nutritional profile to suit a specific dietary need. (IFIF, 2012; FAO, 2013).

Rana *et al.*, (2015) investigated the use of Black Soldier Fly larvae as a replacement for fishmeal. The larvae were fed on vegetable waste, rotten wheat and mustard oil cake for 28 days. They were then used to replace fishmeal in a formulated feed at 0, 25, and 50% inclusion levels and fed to all male *Oreochromis niloticus* fry. The researchers concluded that black soldier fly larvae meal can replace fishmeal up to 50% in the diet of *O. niloticus* without any adverse effect on fish growth or feed intake.

In another study, Stamer *et al.* (2014), substituted fishmeal with black soldier fly (*Hermetia illucens*) prepupa meal at varying levels and incorporated in formulated feed of rainbow trout (*Oncorhynchus mykiss*) and fed for 8 weeks. At the end of the experiment, it was found that black soldier fly meal might be a replacement for fishmeal up to 50% without adverse losses in body weight gain, food conversion ratio, and protein retention ratio.

In a report by Adeniyi *et al.* (2015), BSF prepupae meal was used as a protein supplement to replace fishmeal and incorporated at different levels in the diet of African catfish fingerlings and fed for 12 weeks. The results showed that replacing fishmeal with BSF prepupae meal at 50% gave optimum growth and productivity.

2.6 Rearing of Black Soldier Fly Larvae

2.6.1 Attracting BSF Female to Oviposit

In nature, BSF female lays about 500 eggs in cracks and crevices near a decomposing organic matter since the larvae will then consume this material upon hatching (Tomberlin *et al.*, 2009; Diclaro *et al.*, 2012; Park, 2015).

BSF females in the wild have been attracted to oviposit in flutes of corrugated cardboard (Newton, n.d.). Three strips of corrugated cardboards (about 5 × 2 cm) were glued or tied together at the two end edges. The cardboards are then slightly suspended above (about 3cm) a heaping pile of fresh animal manure (swine, poultry, cow); making sure that the crevasses/flutes of the cardboard are exposed vertically so as to be seen easily by the females. The putrescent odour of the rotting manure attract BSF female to lay eggs in the crevasses of the cardboard (Bullock *et al.*, 2013; Newton, n.d.).

In a BSF colony, a poultry layer meal (corn meal and wheat bran) mixed with 60% water has been reported to produce strong odour and this can serve as an attractant for female BSF to lay eggs (Stankus, 2013). According to Bullock *et al.* (2013), fermented corn kernels, vegetable and fruit scraps, kitchen scraps, rotten potatoes, coffee grounds and other types of waste that release putrid smell can also be used.

Once sufficient eggs have been laid, the strips of cardboards can then be transferred to a container dedicated for the larval nursery (Plate 2). The strip of cardboard suspended above feedstock in the nursery container. Once the eggs hatch, the larvae will fall into the feedstock and start feeding (Newton, n.d.)

2.6.2 Indoor Breeding of BSF

Small scale colonies of BSF have been set up by retaining a small population of the pupae that will develop into adults so as to serve as broodstocks (Stankus, 2013). The required conditions necessary for a successful breeding includes large netted enclosure/cage that will house the broodstocks, light, appropriate temperature and relative humidity, a source of food and corrugated cardboard (Newton, n.d.; Stankus, 2013).

Since the adults BSF mate in flight, they are normally housed in a large net enclosure to ensure enough space for movement and successful mating (Bullock *et al.*, 2013; Stankus, 2013). The cage size could be 2 × 1.5 × 1.8 m (Zhang *et al.*, 2010) or 2 × 2 × 4 m (Ekman, 2014). Ideally, the cage/ netted enclosure that house the adults should be kept in a room where the roofing is interspaced with transparent sheets. This will allow direct sunlight and provide the optimum range of temperature (25 - 35 °C) for mating (Zheng, 2010; Alvarez, 2012). In seasons where there is no or little sunshine, an artificial light has been used (Bullock *et al.*, 2013; Stankus, 2013).

The eggs should be protected from getting in contact with excessive moisture to prevent spoilage. The larvae should be transferred to the larval production or grow-out room. The feed and cardboard in the broodstock cage should be replaced twice weekly (Bullock *et al.*, 2013; Stankus, 2013).

In order for the larvae to pupate to adult, the optimum relative humidity and temperature needed are 30 - 90% and 25 – 30 °C respectively. Since the pupae required a sheltered and cool place to transform into an adult (Diclaro *et al.*, 2012), they could be kept in a dry container filled with chippings of paper or wood shaving. This will provide an artificial sheltered and dry place needed for pupation. The adults should be provided with water. This could be done by the use of a spraying system to spray water (mist) into the cage or a small amount of water could be placed in the cage (Sheppard, 2002; Park, 2015).

The exoskeletons of the pre-pupae and pupae have high chitin content, which is considered anti-nutritional (Ekman, 2012; Kroeckel *et al.*, 2012). Therefore, if the purpose of rearing the animal is to provide high-quality feedstuff for poultry, livestock and fish, the larvae must be collected just before pupation occurs (Newton, n.d.).

2.7 Fish Feed Sources and their Evaluation

2.7.1 Fish Feed Sources

Fishmeal is the major dietary source of protein (constituting 20 - 60% of aqua feeds) due to its high quality of the protein and essential amino acids (EAA) content, valuable fatty acids and high digestibility (El-Sayed, 2006; FAO, 2012; Ng and Romano, 2013).

The increasing cost and decreasing availability of fishmeal had led to strenuous effort by researchers at evaluating wide varieties of relatively lower cost ingredients, that could partially or wholly replace fishmeal over the past decades with some degree of success (El-Sayed, 2004; Glencross *et al.*, 2007; FAO, 2012; Agbo *et al.*, 2015).

Plant-derived resources include soybean meal, groundnut cake, cottonseed meal, protein concentrates and oils, whilst animal origin source includes blood meal (BM), poultry by-product meal (PBM), and hydrolyzed feather meal (HFM) (Bureau and Cho, 1999; El-Sayed, 2004; Glencross *et al.*, 2007; FAO, 2013; Ng and Romano, 2013).

A new innovation of using non-conventional feed resources such as silkworm pupae, *spirulina spp*, black soldier fly larvae, common housefly larvae, corn and wheat gluten, almond cake, sesame cake and brewery waste is gaining prominence in recent years (St.-Halaire *et al.*, 2007a; El-Sayed, 2004; Diclaro *et al.*, 2012; Huis, 2013; 2015).

These food resources, despite having a high amount of crude protein, they are usually lacking in one or more essential amino acids compared to fishmeal. For instance, PBM and HFM are deficient in lysine and MBM in methionine (El-Sayed, 2004; Ogello *et al.*, 2014). Plant based protein source is noted to be deficient in methionine and lysine and has anti-nutritional factors (ANFs) such as phytic acids, trypsin inhibitors and saponin; which restrict their use in formulated diets. Specific processing method may be required to

remove some of these ANFs (El-Sayed, 2004; Mjoun *et al.*, 2010; Ng and Romano, 2013; Agbo *et al.*, 2015).

In evaluating a feed ingredient for aquaculture, several factors need to be considered. These include; ingredient characterization (i.e. the chemical composition, processing method, presence and level/concentration of anti-nutritional factors such as trypsin inhibitor, saponin, glucosinolate and alkaloid); palatability; digestibility; and nutrient utilisation and interference (Glencross *et al.*, 2007; Ng and Romano, 2013).

2.7.2 Digestibility Test

Digestibility test is one of the most important aspects of evaluating the efficiency of a feedstuff. Digestibility is a measure of nutrients and energy absorbed by an animal from a particular feed or ingredient, and therefore is bio-available for growth, reproduction and metabolic processes. Thus, the proportion of nutrients and energy in the ingested feedstuff that is not excreted in faeces (NRC, 1993; Bureau and Cho, 1999; Hossain *et al.*, 2000; Hussain *et al.*, 2011).

Information on the nutrients and energy digestibility of different feed component to a target species helps to optimise feed efficiency; minimise cost; limit wastes produced by the animal and its pollutant effect (Lee, 2002; Köprücü and Özdemir, 2005; Vidal *et al.*, 2015).

2.7.2.1 Methods of Digestibility Test

There are two main methods of assessing diet digestibilities. These are the direct and indirect methods. In the direct method, account is taken of the total quantity of feed given, feed uneaten and faecal output. The digestible value is then estimated as a percentage of

total amount of faecal output per feed ingested (Glencross *et al.*, 2007; Akhter, 2015). The drawback of this method lies in the difficulties and errors involved in determining accurately, the amount of feed intake (ingested) and the faecal production by the fish due to the aquatic medium in which they live. This is because feed and voided faeces lose nutrients immediately on discharge through leaching (Agbo, 2008; Akhter 2015).

The indirect method involves the application of indigestible/inert marker. The marker is introduced in small quantity (0.5 – 1%) and distributed evenly in the diet. The digestibility of the diet is determined by the ratio of the marker in the feed and faeces and is used to calculate digestibility of energy and other nutrients (Glencross *et al.*, 2007).

The indirect method gives apparent digestibility. This is because, faeces is expelled from the body together with other endogenous losses such as a mucosal cell, digestive juice (enzyme) and mucoprotein and microbial bacteria. These endogenous losses are not considered in estimating digestibility. True digestibility takes account of the faecal matter plus the endogenous losses released (Bureau and Cho, 1999; Glencross *et al.*, 2007).

2.7.2.2 The use of Markers in Digestibility Test.

As a result of the marker being ingestible, its role is to bind the faecal matter (undigested matrix) relative to the digestible material. The relative quantities of the marker in the diet and faecal output provide a measure of the digestibility of the diet or its nutrient components (Bureau and Cho, 1999; Glencross *et al.*, 2007).

Chromic oxide (Cr_2O_3) is the most widely used inert marker in digestibility studies and has been used extensively in studies with tilapia. Other markers include; ytterbium oxide (Yb_2O_3), yttrium oxide, (Y_2O_3) and acid indigestible ash (AIA) (Glencross *et al.*, 2007; Akhter, 2015; Orire and Ozoadibe, 2015).

For a marker to be effective, it must be, indigestible (completely recovered in faeces), non-toxic, and inert with no physiological effect on the fish, and should pass through the digestive tracks at the same rate as the digest (Bureau and Cho, 1999; Glencross *et al.*, 2007; Sakita *et al.*, 2015).

2.7.2.3 Methods of ingredient inclusion

Essentially, there are two methods of ingredient inclusion for specific ingredient digestibility assessment. These are the ingredient replacement method (IRM) and the diet replacement method (DRM) (Aksnes and Opstvedt, 1998; Agbo, 2008). In IRM, the test ingredient (the ingredient to be evaluated) is added to replace a portion of an ingredient in a standard diet or reference diet (usually at levels between 25 – 100%) (Glencross *et al.*, 2007; Akhter, 2015). For instance, in a report by Sheppard *et al.* (2007), 25% of fishmeal was replaced with BSF larval meal in a complete diet for rainbow trout.

In DRM, the test ingredient is added to replace a fraction of the reference diet to create a test diet. In most cases, the test ingredient replaces the reference diet at 30%. The digestibility values are then determined for reference and test diet. The digestibility of a particular ingredient is then calculated based on the fraction of that ingredient in the test diet relative the reference diet (Glencross *et al.*, 2007; Akhter, 2015). In this method, the fraction of the reference diet that makes up the test diet must be fully representative of the complete reference diet (Glencross *et al.*, 2007; Akhter, 2015).

2.7.2.4 Methods of Faecal Collection for Digestibility Assessment

The faecal matter released by fish fed a test diet or a test ingredient is collected in order to determine the apparent digestibility of a diet or ingredient by comparing the quantity of

each nutrient consumed with that left in faeces at the end of the digestive process. (Agbo, 2008; Anani, 2015; Obirikorang *et al*, 2015b).

Basically, there are three methods adopted by most researchers for the collection of faecal matter. These are dissection, stripping and collection of voided faeces.

The stripping method is the most widely used technique, where faecal matter is collected from the distal part of the intestine by applying gentle abdominal pressure (Bureau and Cho, 1999; Akhter, 2015).

The advantage of this method is the avoidance of leaching of nutrient from the voided faeces. However, frequently handling of fish for faeces collection poses a stress to the fish. Also, it is difficult to collect faeces from all species of fish especially the juvenile. It is impossible for crustaceans (Bureau and Cho, 1999; Glencross *et al.*, 2007).

It works best for carnivorous fishes with short and simple digestive tracts like the turbot. It is not appropriate for omnivorous and herbivorous fishes, such as tilapia which has long intestines (more than six times its body length) and highly twisted intestine inside the body cavity (Akhter, 2015).

The dissection method involves cutting the lower part of the distal intestine and a careful removal of its content (Kroeckel *et al.*, 2012; Akhter, 2015). This method leads to no leaching of nutrient from faeces and seems to be the best for the collection of faeces of Nile tilapia. The drawbacks include the acquisition of lots of fish in order to get enough faecal matter for analysis. Also, fish are sacrificed each time faecal matter is collected (Akhter, 2015).

In stripping and dissection methods (together called ‘mechanical method’), there is the possibility to underestimate digestibility because of incomplete digestion and also

contamination of digesta (unexpelled faeces) with endogenous material that would otherwise be reabsorbed before the faeces are excreted. Also, the procedure is time-consuming (Bureau and Cho, 1999; Glencross *et al.*, 2007; Akhter, 2015).

A collection of **voided faeces** implies collection of faeces released by fish in water column or settlement (Bureau and Cho, 1999). There are several modifications of this method. These include:

- **Syphoning**, where faecal matter is syphoned slowly from the bottom of a tank.
- **The Guelph system** (Cho *et al.*, 1975) in which settling column is used to separate the faeces, from the effluent water
- **The TUF column** (Ogino *et al.*, 1973) in which faeces are collected by passing effluent water through filtration column and
- **St-Pee system** (Choubert *et al.*, 1979) in which a mechanical rotating-screen is used to filter out faecal matter.

These methods are beneficial by offering a high quantity of faeces with little effort without posing stress to the fish. The downside is the possibility of overestimating digestibility as a result of leaching of nutrients once the faecal matters are discharged in the water (Bureau and Cho, 1999; Glencross *et al.*, 2007).

2.7.2.5 Assumptions in Digestibility Value

1. Digestible coefficients are additive. Thus, digestibility coefficients of individual ingredients used in a diet formulation add up to digestibility value of the diet
2. The marker is inert and has no influence on digestion of food substances
3. Digestibility coefficients ranged between 0% and 100%.
4. Faeces pulled together are of the same characteristic.

(Glencross *et al.*, 2007; Allan and Stuart, 1998)

2.8 Feed Formulation

Feed formulation and preparation are the processes of mixing specific amount of ingredients to form a diet that meets the nutritional requirements of a particular fish species and life stage (Kushwaha, 2013). Formulated feed could be used as complete diet or supplementary diet.

In formulating a nutritionally balanced diet that meets the specific fish requirement, practical consideration such as availability of ingredients, cost, palatability, digestibility and anti-nutritional factors must be taken into account (Glencross *et al.*, 2007; Kushwaha, 2013).

Plant proteins (i.e. soybean based-meal), are typically deficient in methionine and lysine. Therefore, it is necessary to add extra lysine and methionine to feed prepared with plant protein to promote optimal growth of tilapia (Craig and Helfrich, 2004; Mjoun *et al.*, 2010).

2.8.1 Complete and Supplementary Feed

2.8.1.1 Complete Feed

Complete feed is used in intensive culture system where fishes do not have either access to natural productivity of the system or where the natural feed of the system is insufficient to meet their total nutritional requirements (Shiau *et al.*, 2002; Fitzsimmons, 2005; FAO, 2012). This includes cages placed in water with low productivity, intensive recirculating systems and heavily stocked ponds with low natural productivity (Shiau *et al.*, 2002; Fitzsimmons, 2005).

The administered diet must be well-balanced (that provides a proper mix of protein, carbohydrates, lipids, vitamins, mineral, and fibre); a prerequisite for producing high yield and fast growth at least cost (Fitzsimmons 2005; Mjoun *et al.*, 2010; FAO, 2012).

2.8.1.2 Supplementary Feeds

Supplementary feed applies to a semi-intensive system where the natural productivity of the system is capable of supporting the nutritional needs of the animal (FAO, 2012). They contain a high amount of carbohydrates and are usually less costly than complete diets. Application of supplementary feed may result in significant increases in tilapia yield in comparison to fertilised ponds alone (Mjoun *et al.*, 2010). Some serve a dual purpose of fertilising the pond as well as increasing productivity (Fitzsimmons, 1997; Kassam, 2014).

Supplementary feeds can be made up of single ingredients or combinations of ingredients. The most common feedstuffs are agricultural by-products such as rice bran, broken rice, and maize with occasional use of grass and leaves (FAO, 2012).

2.9 The Nile Tilapia (*Oreochromis niloticus*)

Globally, tilapia comes second to carps as the most farmed fish and its production has more than quadrupled over the past decades, yielding over 4.5 million metric tonnes in 2012 (Fitzsimmons, 2013). Among the various species of farmed tilapia, the Nile tilapia, (*Oreochromis niloticus*) is the most commercially important (Ng and Romano, 2013).

The rapid growth of tilapia, globally, is attributed to factors such as tolerance to a wide range of environmental conditions, ability to feed on lower cost diets of plant ingredients, relative ease to spawn in captivity, high resistance to disease, success with polyculture as well as its high marketability (Ng and Rameno, 2013; Godoy *et al.*, 2016).

2.9.1 Nutritional Aspect of Nile Tilapia

2.9.1.1 Natural Feeding of Nile Tilapia

The Nile tilapia is omnivorous and feeds at a low trophic level; on a wide range of dietary sources such as phytoplankton, zooplankton, periphyton, zoobenthos, detritus, and larval fish (El-Sayed, 2004; Mjoun *et al.*, 2010; Ng and Romano, 2013). They feed either by suspension filtering or surface grazing based on the food source (FAO, 2012). *O. niloticus* can adapt to different kinds of food sources based on the culture system and other species they coexist with (Bwanika *et al.*, 2007; FAO, 2012).

2.9.1.2 Nutritional requirements of Nile tilapia.

The nutritional quality of a diet is essential because it influences fish growth, health and waste production (Craig and Helfrich, 2004; Mjoun *et al.*, 2010). The nutritional requirements of *O. niloticus* depend on the size and life stage. Generally, the early juvenile fishes need a diet higher in protein, lipids, vitamins and minerals as well as lower in carbohydrates than the adult fish as they are developing muscle, internal organs and bone with rapid growth. Sub-adult fish (10 - 25 g) require more energy /calories from lipids and carbohydrates for basal metabolism and a less proportion of protein for growth (FAO, 2012). Broodstock may require higher protein and fat levels for, optimum reproduction, spawning efficiency and larval growth and survival (Fitzsimmons, 1997; El-Sayed, 2004; Mjoun *et al.*, 2010).

2.9.1.2.1 Protein Requirement of Nile Tilapia

Proteins are nitrogen-containing substances that are formed by amino acids linked together by peptide bond (Lim and Webster, 2006). They function as the main structural elements of tissues and muscle in the body (Lim and Webster, 2006).

Dietary proteins are continually utilized by fish for growth, maintenance, replacement of depleted tissues, and reproduction functions. Protein requirement as a proportion of the diet decreases as fish approach maturity (NRC, 1993; Shaiu, 2002; Lim and Webster, 2006; Mjoun *et al.*, 2010).

Protein is the most expensive part of fish diet. It is therefore imperative to accurately determine the protein requirements in order to formulate cost effective diet. Dietary protein requirements for optimum growth of Nile tilapia are dependent on the age or size of fish, the energy content of the diet, protein quality (level and availability of essential amino acids) and food intake (Jauncey, 2000; Mjoun *et al.*, 2010) (Appendix I).

Protein quality of a tilapia diet is a function of the combination of essential amino acids in their right quantities (Davis *et al.*, 2009; Mjoun *et al.*, 2010). Therefore, the dietary amino acid profile in a formulated diet is very important. In formulating a diet for tilapia, the amino acid content should be adjusted to meet the fish requirement throughout the growth cycle (Mjoun *et al.*, 2010).

There are 20 amino acids identified for fish growth. Of these 10 essential amino acids are termed essential (indispensable) amino acids (Appendix II) because they cannot be synthesised by fish or are not synthesised in their right amount and therefore must be supplied in fish diet. (Craig and Helfrich, 2004; Lim and Webster, 2006). Of the 10 essential amino acids, lysine and methionine are often the first limiting amino acids (Craig and Helfrich, 2004). Those that can be synthesised in adequate quantity are known as non-

essential (dispensable) amino acids. The essential amino acids include Arginine, Histidine, isoleucine, Leucine, lysine, methionine, phenylamine, Threonine, Tryptophan and valine (El-Sayed, 2006; Ng and Romano, 2013).

2.9.1.2.2 Lipid Requirement for Nile Tilapia

Dietary lipids supply a significant source of highly digestible energy and essential fatty acid needed by fish for normal growth and development (Lim and Webster, 2006; Mjoun *et al.*, 2010).

Lipids especially phospholipids help in the absorption of fat-soluble vitamins; play an important role in membrane structure and maintenance of membrane flexibility and permeability. They serve as precursors for steroid hormones and prostaglandins, improve the flavour of feeds and affect feed texture and fatty acid composition of fish (Lim and Webster, 2006; Mjoun *et al.*, 2010).

In general, the lipid requirements for fish under 2 g represent 10% of the diet and this decrease to 6 - 8% from 2 g above to harvest (Fitzsimmons, 2005). The optimum omega 6 fatty acid (18:2 n-6 or 20:4 n-6) have been estimated to be 0.5% for *O. niloticus* and 1% for *Tilapia zillii* (Lim and Webster, 2006).

Research has shown that dietary lipids have a sparing effect on utilisation of dietary protein (Mjoun *et al.*, 2010). The level of protein in the diet can be reduced from 33.2 to 25.7% by increasing the dietary lipid from 5.7 to 9.4% and carbohydrate from 31.9 to 36.9% (Lim and Webster, 2006). In order to maximise protein utilisation, dietary fat concentration should be between 8 and 12% for tilapia up to 25 g, and 6 to 8% for larger fish (Jauncey, 2000; Mjoun *et al.*, 2010). Tilapia requires n-6 (linoleic) fatty acids and to a lesser extent

n-3 (linolenic) fatty acids. Dietary lipids should supply at least 10% of n-6 fatty acids (Mjoun *et al.*, 2010).

2.9.1.2.3 Carbohydrate Requirement for Nile Tilapia

There is no specific amount of carbohydrate requirement for fish. This is because “amino acid and fatty acid precursors can supply the required glucose via gluconeogenesis” (Mjoun *et al.*, 2010). However, they are supplied in the diet because they are inexpensive sources of energy. In addition, they are used as pellet binders (Fitzsimmons, 2005; Lim and Webster, 2006). Tilapia is capable of efficiently utilising dietary carbohydrate up to 30 – 40%, higher than most farmed fish (Fitzsimmons, 2005; Mjoun *et al.*, 2010).

The ratio of dietary protein to energy is critical in fish nutrition. Diets should contain an optimal amount of lipids and carbohydrates to avoid sparing of protein for energy; thus maximising protein use for growth (Mjoun *et al.*, 2010). Depending on the species, size or age, the optimal ratio of protein to energy (P: E; mg/Kcal) for tilapia ranged from 68 to 125 (Lim and Webster, 2006; Mjoun *et al.*, 2010).

Tilapias do not have the requisite enzymes for the digestion of crude fibre (Lim and Webster, 2006; Mjoun *et al.*, 2010; Obirikorang *et al.*, 2015b). A higher level of dietary fibre > 5% reduces diet utilisation and digestibility in *O. niloticus* (Lim and Webster, 2006; Obirikorang *et al.*, 2015b). Therefore, for maximum growth, crude fibre should not exceed 5% in the diet of *O. niloticus* (Lim and Webster, 2006; Mjoun *et al.*, 2010).

2.9.1.2.4 Vitamins and Mineral Requirement

Vitamins are organic compounds that are required in small quantity for normal growth and reproduction and health (Lim and Webster, 2006). In a semi-intensive system, where there

is some amount of productivity in a fertilised pond, tilapia is likely to meet their vitamin requirements through consumption of natural food. However, in an intensive system in which natural foods are limited, vitamin premix must be supplemented in the diet to sustain normal growth and health (El-Sayed, 2006, 2013; Fitzsimmons 2006; Lim and Webster, 2006; Mjoun *et al.*, 2010) (Appendix III).

Minerals are required for the formation of tissue, metabolic functions such as osmoregulation, acid-base balance and proper functioning of muscle and nerves (Lim and Webster, 2006). However, excessive supplies of certain minerals can cause deficiencies of others and in extreme cases toxicity to fish. For example, high dietary calcium can cause deficiencies in phosphorus, zinc, iron, and manganese. On the other hand, it has been shown that zinc, copper, and selenium, at high concentrations, can be toxic to various finfish species (Jauncey, 2000; El-Sayed, 2006; Lim and Webster, 2006; Mjoun *et al.*, 2010) (Appendix III).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Areas

The study was carried out from October 2015 to June 2017 at the BSF Centre of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC) which lies between Latitude 5° 67' North and Longitude 0° 29' East in Accra, Ghana and at the Aquaculture Research and Development Centre (ARDEC) of the Water Research Institute (WRI) of the Council for Scientific and Industrial Research (CSIR), Akosombo, which lies within Latitude 6° 13' North, and Longitude 0° 4' East in the Eastern Region of Ghana.

The culturing and drying of BSFL were done at the BSFL Centre, GAEC in Accra. Digestibility test and culturing of fingerlings of *O. niloticus* were done at ARDEC, Akosombo.

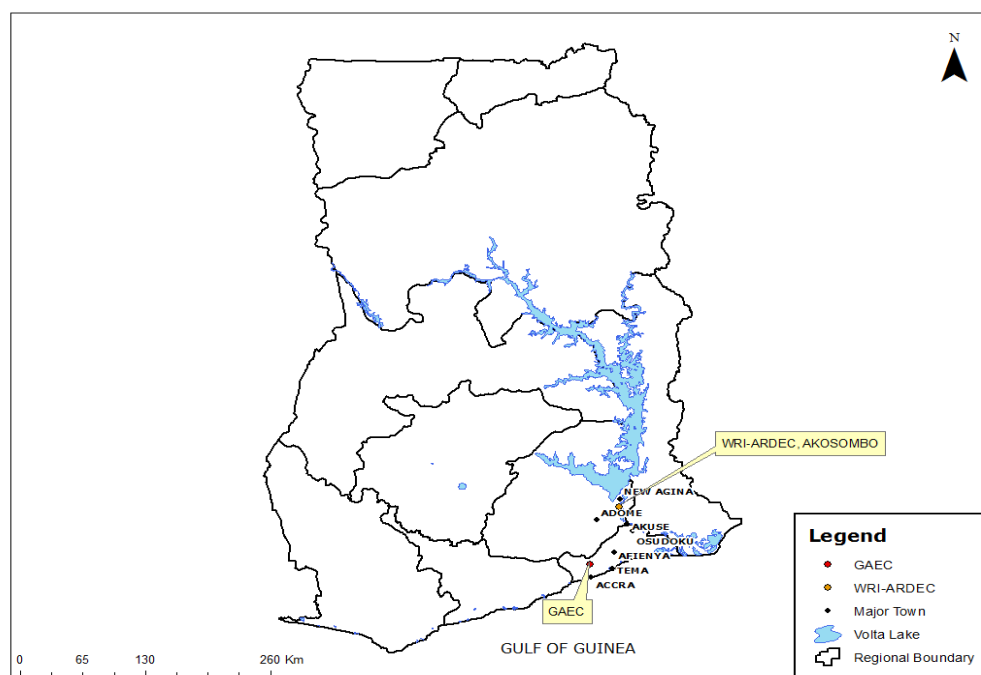


Figure 3.1: Location of the Study Areas

3.2 Formulated Diets for BSF larval culture

The various ingredients used in formulating diet for BSF larval culture are shown in Table 3.1

The formulation is basically composed of fruits and vegetable waste (85%). Ingredient inclusion level ranged from 5 to 22.5%. *Kontomire* (cocoyam leaves) had the highest inclusion level (22.5%) whilst both cassava and plantain had the least (5%).

Table 3.1: Formulated Diets for BSFL Culture

Ingredients	Composition (%)
Biochar	10
<i>Kontomire</i>	22.5
Cabbage	18
Carrot	4.5
Watermelon	20
Pineapple	12
Orange	8
Cassava	2.5
Plantain	2.5
Total	100

Kontomire = cocoyam leaves

Although Biochar is not a feedstock, its addition was to absorb ammonia (NH₃), heavy metals such as Cu, Zn and Cd and to reduce nitrogen losses during composting of manure (Dandie, 2011). In addition, the biochar was to improve oxygen availability to the larvae and hence to stimulate microbial growth and respiration rate (Steiner *et al.*, 2010).

3.3 Culturing of BSF larvae

In this research, the BSF used are native to Accra, Ghana. The eggs were collected from the wild by placing a number of 5×10 cm strips of corrugated cardboard held together by a rubber band and suspended slightly above (about 5 cm) heaps pile of animal manure (chicken, swine, sheep) at the BSF Centre of BNARI, GAEC, Accra for adult females to oviposit. Temperature and humidity of the substrates were taken. The strips of corrugated cardboard were then collected after 24 h. The strips of corrugated cardboard were fitted to the window of plastic containers ($20 \times 5 \times 5$ cm) containing poultry layer meal thoroughly mixed with water (60% moisture).

The eggs hatch between 3 - 4 days after collection. The newly hatched neonate larvae then crawled into the feed substrate and began feeding. The larvae were reared on the chicken feed for 10 days after which the larvae were separated from the feedstuff using a fine mesh with a mesh size of 0.36 mm. About 1200 of BSF larvae were hand counted and then inoculated on 8 kg of the formulated feedstuff (Table 3.1) in a 600 L plastic bin. Sewn muslin cups were used to cover the bins (Plate 2) to prevent houseflies from laying eggs in them. On the 12th day after inoculation, the larvae were removed (picked) from the feedstuff using forceps and then washed in clean tap water to remove any remnant of food particle. The larvae were killed by freezing at -20°C for 24 h.

3.4 Drying of BSF Larvae

Solar drying method was employed in drying the sampled BSFL. Prior to drying, the samples were thawed, washed with running tap water and rinsed in 100 ml distilled water, then spread on plastic trays and placed in the greenhouse solar dryer ($3 \times 3 \times 3$ m) roofed with a transparent sheet at BNARI. The samples were dried for 8 days (between 08:30 - 16:30 h daily). The ambient temperature and relative humidity were taken at 09:30, 12:30

and 15:30 h daily. This was done to ascertain an ideal weather condition for drying BSF larvae in regards to temperature and relative humidity. Dried larvae were weighed, placed in labelled plastic container, stored at a cool dry (room temperature) place for further analysis.

3.5 Nutritional analysis

3.5.1 Proximate Analysis

Proximate composition of all ingredients (including black soldier fly larval meal), used in formulating diets for *O. niloticus* fingerlings, all diets used for both culture trial and digestibility test, whole-body composition of *O. niloticus* fingerlings and faecal samples collected during the digestibility test were analysed for various nutrient components, which included: moisture, ash, crude protein, crude lipid (fats) and crude fibre using AOAC official methods of analysis (1990). The samples were analysed at the Nutrition Laboratory of the Department of Nutrition and Food Science, University of Ghana, Accra, Ghana.

3.5.1.1 Moisture Content

The method is based on drying a sample under controlled pressure and temperature until constant weight is obtained. Moisture content is required to express the nutrient content per dry weight basis. In triplicates, empty containers (glass petri dishes) were dried in an oven for 15 min then transferred to a desiccator to cool. Placing the petri dish on the lid, the weight of the dry empty petri dish was determined using analytical balance. While ensuring sample spread evenly over the bottom of the petri dish, about 2 g of the grounded sample was weighed and then heated in an air oven at 105 °C for 16 h after which it was

cooled in a desiccator. The weight of the samples after drying was taken. The difference in weight was determined.

$$\text{Moisture (\%)} = \frac{[(A + B) - (C)] \times 100}{B}$$

Where A = weight of empty container (g), B = weight of sample (g), C = weight of dried sample + container (g)

As a result of wide variations in moisture content, nutrients of a feed or an ingredient are usually compared on dry matter (DM) basis. % DM = 100 - % Moisture.

3.5.1.2 Ash Content

The ash content is a measure of total amount of minerals present in a feedstuff (AOAC, 1990). The ash content was determined using the ashing method (AOAC, 1990) in which the material was heated to a high temperature to remove all water and volatile substances and to decompose all organic matter. A porcelain crucible was heated in a furnace at 600 °C for 20 min and then it was cooled in a desiccator. The crucible was accurately weighed and about 2.0 g of the sampled was weighed into the crucibles. The samples were then placed in a furnace and then ashed at 600 °C for 6 h. The samples were cooled in a desiccator and then weighed.

$$\text{Ash content (\%)} = \frac{\text{Weight of crucible + ash} - \text{weight of crucible}}{\text{Weight of sample}} \times 100$$

3.5.1.3 Crude Protein content

Crude protein was determined by the Macro Kjeldahl method (AOAC, 1990). In duplicate, about 2 g of the samples were weighed on a filter paper. The filter paper was then carefully

folded (wrapping of sampling) and transferred into a 200 ml Kjeldahl flask. About 1 g of a mixture of catalyst reagents (3 to 4 glass beads, 10 g of chiball, Reese and Williams mixture (0.1 g SeO_2 + 0.25 g CuSO_4 + 9.65 g K_2SO_4) was added. About 25 ml concentrated sulphuric acid (H_2SO_4) was poured into the flask. Care was taken in pouring the acid down sides of the flask. A similar procedure was done for the blank (without sample).

The samples were digested by placing Kjeldahl flask on the Kjeldahl apparatus in an inclined position and heated. The flasks were rotated 180° every 10 – 15 min for even heating and also to avoid breaking the flask. Digestion was continued for 30 min until the digest became clear and the solution allowed to cool. The digests were quantitatively transferred into 100 ml volumetric flasks which were half filled with distilled water. The volumetric flasks were then top up with distilled water to the 100 ml mark.

The digests (samples) were distilled by placing 5ml of 2% boric acid and few drops of mixed indicators (0.06 g bromocresol green, 0.04 g methyl red in 100 ml ethyl alcohol) in a graduated 100 ml erlenmayer flask and placed under the outlet of the condenser such that the outlet dipped into the liquid. About 5 ml of the digest and 5ml of 40% NaOH were placed in the flask/bulb of a steam distillation apparatus. About 50 ml of the distillate were collected in the erlenmayer flask. Each sample was duplicated.

The distillates were titrated with 0.1 N HCl to a faint pink endpoint. Percentage nitrogen was then calculated and converted to percentage protein using the conversion factor of 6.25.

$$\% \text{ N} = \frac{[(a - b) \times \text{normality of HCl} \times \text{molar mass of nitrogen} \times \text{volume of digest}] \times 100}{(1000 \times \text{weight of sample (g)} \times \text{volume of aliquot})}$$

Where a = sample titre

b = blank titre

N = nitrogen

$$\% \text{ Crude Protein} = \% \text{ nitrogen} \times 6.25$$

3.5.1.4 Crude Fat Analysis

Crude fat was determined using the soxhlet method (AOAC, 1990). In duplicate, approximately 2.0 g of samples were placed in different extraction thimbles and plugged with fat-free cotton wool. The extraction thimbles containing the samples were placed in the extraction jackets. A clean dried 100ml round bottom flasks were weighed and about 50 ml of petroleum ether (B.P 40 – 60 °C) was poured into each flask fitted with Soxhlet extraction unit. The round bottom flasks and the condenser were connected to the Soxhlet extractor and cold-water circulation was put on. The heating mantle was switched on, and the heating rate adjusted until the solvents were refluxing at a steady rate. Extraction was carried out for 3 h. The solvents were recovered and the oil was dried in the oven at 100 °C for an hour to remove any residue of petroleum ether in the oil. The round bottom flasks and oil were cooled in a desiccator and weighed and the fat content calculated.

$$\% \text{ Crude fat} = \frac{(\text{Weight of flask + fat}) - (\text{weight of empty flask})}{\text{Weight of sample (g)}} \times 100$$

3.5.1.5 Crude fibre content

Crude fibre was determined using acid-base hydrolysis (extraction with 0.5 M H₂SO₄ and 0.5 M NaOH) method, (AOAC, 1990). The entire residue from the ether extraction was used to determine the crude fibre content. The extracted residue was transferred quantitatively from the thimble into a 600 ml beaker. About 0.5 g of celite or asbestos fibre

was added. Exactly 200 ml of boiling 1.25% sulphuric acid was added and the mixture and boiled gently for 30 min. The beaker was kept covered with a large watch glass and boiling distilled water was added at intervals to maintain volume. The boiled sample was filtered through linen. The residue on the filter was washed with boiling distilled water. The residue was washed back into the beaker with 200 ml of boiling 1.25% sodium hydroxide solution and boiled for 30 min. After boiling and filtering through gooch crucible (prepared with asbestos), it was washed with hot distilled water, and then with 100 ml 1% Hydrochloric acid and finally with hot water. The residue was placed in a crucible and dried in an oven at 105 °C overnight. The dried residue was cooled in a desiccator and then weighed. The content of the crucibles was ignited in a muffle furnace at 600 °C for 6 h. It was cooled in a desiccator and weighed again.

Crude fibre was calculated as follows:

$$\% \text{ Crude fibre} = \frac{A - B \times 100}{C}$$

Where A = weight of dry crucible and sample

B = weight of ignited crucible and ash, C = sample weight (g).

3.5.1.6 Carbohydrate determination

Carbohydrate was computed using the formula [100 – (% moisture + % ash + % crude protein + % crude fat + % crude fibre)] (AOAC, 1990).

3.5.2 Gross Energy Analysis

The gross energy of all the experimental diets was calculated using the physiological fuel values of 23.64, 39.54 and 17.15 MJkg⁻¹ for protein, fat and carbohydrates, respectively (Ali and Asgah, 2001).

3.5.3 Amino acid determination

The amino acid concentration of BSF larval meal was analysed by Evonik Industries (Hanau, Germany) in triplicates following the protocol of a liquid based chromatography (Copper *et al.*, 2001).

3.6 Chromic Oxide Analysis

Chromic oxide content of the test diet, reference diet and as well as the faeces were analysed based on the method of Furukawa and Tsukahara (1966) using spectrophotometry. About 100 mg of sample was weighed into a Kjeldahl flask. About 5 ml of concentrated nitric acid was added to the flask and the mixture boiled (using Kjeldahl apparatus) gently for about 20 min (without boiling dry). After cooling the sample, 3 ml of 70% perchloric acid was added to the flask. The mixture was then gently heated again until the solution turned from a green to an orange colour, after which it was left to boil for a further 10 min to ensure oxidation was complete. The solution was transferred into a 100 ml volumetric flask and diluted to volume with distilled water.

The absorbance of the solution was read using spectrophotometer (Genesys 10S UV-VIS) at 350 nm against distilled water and chromic oxide was computed using the formula (Agbo, 2008).

$$\text{Chromic oxide (\%)} = \left[\frac{(\text{Absorbance} - 0.0032)}{0.2089} \right] \times 100 \quad (\text{Furukawa and Tsukahara, 1966})$$

3.7 Calculations of the ADCs of the Diets and Test Ingredients

The apparent digestibility of dry matter = $[100 - (100 \times \% \text{Cr}_2\text{O}_3 \text{ in diet} / \% \text{Cr}_2\text{O}_3 \text{ in faeces})]$.

The apparent digestibility coefficients (ADC) for the nutrients and energy of the test and reference diets were calculated as follows:

$$\text{ADC}_{\text{nutrient}} = 100 \times [1 - (F/D) \times (D_i/F_i)] \quad (\text{Bureau and Cho, 1999})$$

Where D = % nutrient of diet; F = % nutrient of faeces; D_i = % Cr₂O₃ of diet; F_i = % Cr₂O₃ of faeces.

ADC of the test ingredients (ADC_{ingredient}) was then calculated based on the digestibility of the reference diet and the test diets as follows:

$$\text{ADC}_{\text{ingredient}} = \text{ADC}_{\text{test diet}} [(\text{ADC}_{\text{test diet}} - \text{ADC}_{\text{reference diet}}) \times ((0.7 \times D_{\text{ref}}) / (0.3 \times D_{\text{ref}}))]$$

(Bureau and Cho, 1999)

Where D_{ref} = % nutrient (or kJg⁻¹ gross energy) of reference diet (as fed); D_{ingr} = % nutrient (or kJg⁻¹ gross energy) of test ingredient (as fed).

3.8 Evaluation of Growth Performance of Tilapia fed BSF Larval Meal Diet

10 weeks growth trial was carried out at ARDEC, Akosombo, to evaluate the nutritive value of BSF larval meal in the aspect of feed utilisation, growth performance, and body composition for *O. niloticus* fingerlings.

3.8.1 Pond Preparation

Weeds around and within the earthen ponds used for the experiment were cleared off. The ponds (163 m²) were drained using a generator pump machine and left to dry for about a

week. Afterwards, lime (CaCO_3) was applied at a rate of 8 kg per pond to neutralise the soil pH and to eliminate bacteria, parasites and other pathogens (El-Sayed, 2013). The ponds were later filled with water from the adjacent river Volta through the intake point. The water level at different sections of the ponds ranges between 0.48 m and 1.05 m. This level was maintained throughout the experiment by frequently topping up.

3.8.2 Experimental pond setup

The experiment was conducted in hapas ($3 \times 1 \times 1$ m) set in 3 earthen ponds of the average surface area of 163 m^2 in a completely randomised block design (CRBD). Monofilament nylon gill net was sewn to cover the hapas so as to prevent predators such as birds and frogs from entering the hapas. Each treatment was triplicated. The hapas were made of nylon mosquito netting with mesh size 0.5×0.5 mm. CRBD was used to reduce any large variation (i.e. pond effect) among experimental units since it is a field experiment.

3.8.3 Feed Formulation and Preparation

In all, five isonitrogenous (380 gkg^{-1} crude protein) and isoenergetic (18 kJg^{-1}) diets were used in this experiment. These levels were based on the requirement for *O. niloticus* fingerlings (NRC, 1993; El-Sayed, 2006; Ng and Romano, 2013). Diet I and II were a local on-farm diet (ARDEC) and a commercial feed (RAANAN) respectively, both comprising of 0% BSF larval meal (controls). Diet III, IV and V, (Table 3.2) were formulated by replacing fishmeal with BSF larval meal at 25, 50 and 75%, respectively. Diet I, II and III were formulated using the equation method. Diet I, II, III, IV and V were coded as ARDEC, RAANAN, BSF 25, BSF 50, and BSF 75.

The BSF larvae meal was produced at the Green House of BNARI of GAEC in Accra. All other feed ingredients (Table 3.2) were purchased at Ashaiman, in the Greater Accra Region of Ghana. All ingredients were milled to fine particles size, suitable for easy ingestion by the fingerlings. The weighed feedstuffs were thoroughly mixed with a mixer to obtain a homogeneous mixture and sieved with 0.5 mm mesh.

Table 3.2: Formulated Diets (gkg⁻¹ as-fed) for Growth Trial

Ingredients	BSF 25	BSF 50	BSF 75
Fishmeal	450	300	150
BSFL meal	150	300	450
Soybean meal	60	150	270
Wheat bran	110	150	106
Maize (white)	113	50	10
Cassava flour	107	40	04
Common salt	10	10	10
Total	1000	1000	1000

BSFL meal = Black Soldier Fly larval meal.

3.8.4 Stocking, Feeding and Sampling of Experimental Fish

A total of 1350 mixed sex *O. niloticus* fingerlings with initial mean weight of 1.3 ± 0.23 g were obtained from ARDEC hatchery facility. The fingerlings were randomly distributed in the 15 hapas (90 fingerlings per a hapa).

Mixed sex *O. niloticus* fingerlings were used because this study is organic oriented. Therefore, application of synthetic hormones (to produce all male *O. niloticus*) was avoided.

Before the start of the culture trial, the fingerlings were conditioned in hapas for 10 days and fed with none of the test diets but rather fed with a powdered mixture of wheat bran and fishmeal in a ratio of 1:1 thrice a day. This was to wean them off the commercial feed (RAANAN) they had been feeding on. The fingerlings were starved 24 h before the start of the study to allow for evacuation of previously ingested feed. The experimental diets were administered by hand thrice daily between 008 - 900, 0012 – 0013, and 0016 – 0017 h at 10% of their body weight. The quantity of feed was adjusted bi-weekly throughout the 10 weeks duration of the feeding trials based on new weight gained. Sampling was done fortnightly. In each replicate of the dietary treatments, 25 fingerlings were randomly selected and the weight and length (total length and standard) measured (to the nearest 0.1 cm and 0.1 g) using measuring board and top loading electronic balance.

3.8.5 Growth Performance and nutrient utilisation Indices.

Growth performance and nutrient utilization indices were determined as follows;

- a. Average Daily weight gain (ADG) = weight gain (g) / time (days)
- b. Mean Weight Gain = Initial mean weight - Final mean weight
- c. Relative growth rate (RGR) = (Mean Weight of fish/ Mean initial Weight of fish) x 100
- d. Specific Growth Rate (SGR) = $\ln[(\text{mean final weight}) - \ln(\text{mean initial weight})] / (\text{Time /days}) \times 100$
- e. Condition factor (CF) = $(W_2/L_2^3) \times 100$ where W_2 = Final weight and L_2 = Standard length.
- f. Feed Conversion Ratio (FCR) = (Weight of feed fed) / (Weight gain of fish) (Agbo, 2008)
- g. Feed intake (g) = Total feed intake per fish/no. of days.

3.8.6 Carcass Analysis

Whole body proximate composition was determined for the initial fingerlings prior to stocking and final fingerlings harvested after the experiment.

3.8.7 Hepatosomatic Index

At the end of the growth experiment, 6 fish (2 fish per hapa) from each of the five dietary treatments were randomly selected, weighed, dissected and the liver removed. The liver was then weighed and used to estimate the hepatosomatic index (HSI) (Barnes *et al.*, 2012) as follows:

$$\text{HSI} = (\text{weight of liver})/(\text{body weight of fish}) \times 100 \quad (\text{Barnes } et al., 2012)$$

3.8.8 Health Condition of Cultured fish

At the end of the growth trial, 6 fishes were randomly selected from each feed treatment for whole body observation. The health profile (Table 3.3) were assessed based on criteria adapted from Barnes *et al.* (2012).

Table 3.3: Criteria used at the end of the growth trial for fish health observations

Structure of Tissues	Rating Criteria	Numeric Rating
Eye	Normal	0
	Abnormal	1
Fat	None	0
	< 50% of gut covered	1
	>50% of gut covered	2
	100% of gut covered	3
Fins	No erosion	0
	Light erosion	1
	Moderate erosion	2
	Severe erosion	3
Gills	Normal	0
	Clubbed, frayed, or discoloured	1
Gut	Normal	0
	Slight inflammation	1
	Moderate inflammation	2
	Severe inflammation	3
Kidney	Normal	0
	Abnormal	1
Liver	Normal	0
	Abnormal	1
Pseudobranchs	Normal	0
	Abnormal	1
Opercles	Normal	0
	Short	1
Spleen	Normal	0
	Cysts or enlarged	1

Source: Adapted from Barnes *et al.*, 2012.

3.8.9 Water Quality Parameters.

The physical and chemical qualities of water are essential for optimum fish production.

The quality of water in the experimental ponds was monitored and analysed throughout the culture period to ensure that conducive environmental conditions were maintained.

3.8.9.1 Temperature, Dissolved Oxygen, Turbidity and pH and Conductivity and Total Alkalinity Measurements

The temperature, dissolved oxygen (DO), Turbidity, pH and conductivity were measured *in situ* using Multi Probe Water Quality Metre (YSI 556 MPS). Three readings per parameter were taken in each of the three experimental ponds. The probe was immersed in the pond at different depths and at different sections of the ponds. Samples were taken biweekly at 09:00 h. Total alkalinity was determined *in situ* using Thermo Scientific Orien test kit.

3.8.9.2 Ammonia (NH₃-N)

Ammonia was determined using the Direct Nesslerization Method (APHA, 1999). A 50 ml of the each water sample was measured into a conical flask. A drop (0.05 ml) ethylenediamine tetra acetic acid (EDTA) reagent was added and mixed well; 2 ml of Nessler Reagent was then added and mixed. A 50 ml zero blank and 50 ml standard of 1.00 mg/l NH₃-N were also treated the same way. A reaction process of at least 10 min was allowed with the Nessler Reagent. The presence of a yellow colouration indicates ammonia-nitrogen. The zero blank and standard was used to standardise the Comspec M201 visible spectrophotometer at a wavelength of 420 nm with a 1.0 cm light path cell or cuvette. Ammonia concentration in the sample was measured in mg/l to 2 decimal places.

3.8.9.3 Nitrite (NO₂-N)

Diazotization method was used in the determination of nitrite sample (APHA, 1999). A 10 ml of each sample of water was measured into a test tube and 1 ml of 0.3 M sodium hydroxide solution added. A 1 ml colouring reagent was also added to each sample. A zero

blank and standard of 0.1 mg/l NO₂-N was used to standardise the visible spectrophotometer at a wavelength of 543 nm. The concentration of NO₂-N in mg/l was read with a 1 cm light path cell after 10 min. of colour development. The presence of a pink colouration indicated nitrite – nitrogen.

3.8.9.4 Nitrate (NO₃-N)

Nitrates were determined using the Hydrazine Reduction Method (APHA, 1999). A 10 ml of each sample of water was measured into a test tube and 1 ml 0.3M of NaOH solution added. A 1 ml reducing mixture was also added. The mixture was then heated for 10 min at 60 °C and cooled. About 1 ml of colouring reagent was added and 10 min reaction period was allowed. A zero blank and standard of 1.00 mg/l NO₃-N mixture were used to standardise the visible spectrophotometer at a wavelength of 543 nm with a 1 cm light path cell. Nitrate concentration in samples was measured in NO₃-N to 2 decimal places.

3.8.9.5 Phosphate (PO₄-P)

Phosphate was determined using the Stannous Chloride Method (APHA, 1999) A 100ml of each water sample free from colour and turbidity was measured and 0.05ml (1 drop) phenolphthalein indicator was added and mixed. About 4 ml Molybdate reagent I and 0.5 ml (10 drops) Stannous Chloride reagent I was added with mixing after each addition for colour development. After 10 min a zero blank and standard of 0.50 mg/l PO₄-P was used to standardise the visible spectrophotometer at 690 nm with 1 cm light path cell. Phosphate concentration in the sample was measured as mg/l PO₄-P to 2 decimal places.

3.8.10 Cost Analysis of diet

A simple economic analysis was conducted to assess the cost-effectiveness of diets used in the feed trial. Only the cost of feed was used in the calculations with the assumption that all other operating costs remained constant. Costs of the feeds were calculated using market prices (Appendix IV) of ingredients in Ghana during the year, 2017.

a. Incidence Cost = (cost of feeding/weight of fish produce) (Vincke, 1969)

IC is the cost of feed to produce a kg of fish (relative cost per unit weight gain), and the lower the value, the more profitable using that particular feed (Agbo, 2008)

b. Profit Index = (value of fish/cost of feeding) (Miller, 1976)

3.9 Apparent Digestibility Trial

3.9.1 Experimental Setup

The trial was conducted to determine the Apparent Digestibility Coefficient of Black Soldier Fly Larval meal, fishmeal and soymeal fed to *Oreochromis niloticus* fingerlings in transparent glass units (60 × 33.5 × 37.5 cm) with water holding a capacity of approximately 50 litres. Each treatment was replicated. Mixed sex *O. niloticus* fingerlings (mean weight of 10 ± 1.0 g) at a stocking density of 10 individual per tank were used for the experiment.

Water temperature was maintained at 26.5 – 29 °C throughout the study. The experimental tanks were fitted with aerators to maintain dissolved oxygen (DO) concentration between 5.50 and 7.00 mg l⁻¹. pH ranged between 6.8 and 8.0.

3.9.2 Feed formulation For Digestibility Trial

The Reference diet (Table 3.4) was formulated based on diet substitution approach (Glencross *et al.*, 2007) to contain 380 g kg⁻¹ protein and 18 kJg⁻¹ gross energy. These levels were based on the nutrients requirements for *O. niloticus* fingerlings (NRC, 1993). The diets were formulated on as-fed basis (Table 3.4).

Chromic oxide (Cr₂O₃) was used as an inert marker at a concentration of 0.5%. Test ingredients for an apparent digestibility BSF larval meal (oven dried at 70 °C for 24 h and milled and sieved with 0.2 mm mesh size). Reference diet was mixed with the test ingredient at a ratio of 70:30, respectively. The feed was kept in a clean, cool place (room temperature).

Table 3. 4: Formulated Diets (gkg⁻¹ as-fed) for Digestibility Trial

Ingredients	Reference diet	Test diet I (BSFLM)	Test diet II (FM)	Test diet III (SBM)
Fishmeal	300	210	210	210
BSFL Meal	300	210	210	210
Soybean Meal	150	102.75	102.75	102.75
Wheat Bran	150	102.75	102.75	102.75
Maize (white)	51	35.7	35.7	35.7
Cassava flour	34	23.8	23.8	23.8
Common salt	10	10	10	10
Chromic Oxide	5.0	5.0	5.0	5.0
Test ingredient	-	300	300	300
Total	1000	1000	1000	1000

Test ingredients = Fishmeal (FM), Black Soldier Fly Larval meal (BSFLM), Soybean meal (SBM).

3.9.3 Feeding and Faecal Collection

The fish were acclimated to both the glass tank and the experimental diet for 5 days before collection of faeces began to allow evacuation of the previously ingested material. During the acclimatisation period, faeces collected were discarded. Fish were fed thrice daily (08:30, 12:30 and 16:30 h) at the rate of 5% of their body weight. In about 1 h after dietary administration, experimental tanks were cleaned to remove any uneaten food particles and faecal residues. Faecal samples were collected by syphoning using a flexible rubber tubing of 0.5 cm in diameter. Faeces collected were centrifuged at 3800 rpm for 15 min and stored at -20 °C to prevent decomposition. The collected faecal samples were then later defrosted and oven dried at 70 °C for 24 h, grounded and analysed for their proximate composition and gross energy (GE). The apparent digestibilities of the formulated feeds and test ingredients were then determined by comparing the quantity of each nutrient consumed with that left in faeces at the end of the digestive process (Agbo, 2008; Anani, 2015; Obirikorang *et al.*, 2015b).

3.10 Statistical Analysis of Data

Data obtained from the experiment were expressed as means and standard deviations. Data were analysed using one-way analysis of variance (ANOVA). The statistical analysis was performed using GenStat (version 12). Where significant differences ($p < 0.05$) exist, Duncan's multiple range test (DMRT) was used to compare differences among individual treatments.

The Regression equation was used for Length-weight analysis of *O. niloticus* fingerlings.

CHAPTER FOUR

RESULTS

4.1 Proximate Composition and Amino Acid Profile of BSF Larval Meal

Tables 4.1 and 4.2 present the proximate composition and amino acid profile of the BSF larval meal respectively.

Table 4. 1: Proximate Composition of BSF Larval Meal (% dm basis)

Nutrient	Composition
Moisture	10.50 ± 0.28
Ash	12.23 ± 0.10
Crude Fat	22.70 ± 0.25
Crude Fiber	7.00 ± 0.39
Crude Protein	37.83 ± 0.35
Nitrogen free extract (NFE)	20.26 ± 0.95
Gross energy (kJg ¹)	21.39 ± 2.45

dm = dry matter. Values are mean ± standard deviations in triplicate.

Proximate analysis shows BSF larval meal contain Moisture (10.5%), Ash (12.23%), Crude Fat (22.7%), Crude fiber (7.0%), Crude protein (37.83%), Nitrogen free extract (20.26%) and Gross energy (21.39 kJg⁻¹).

The essential amino acid contents (Table 4.2) ranged from 0.81 to 2.26% with Leucine having the highest (2.26%) whilst the least was Methionine-Cystine (0.81%). In terms of the non-essential amino acids, Glutamine had the highest (3.52%) whilst the least was Serine (1.36%).

Table 4.2: Amino Acid Profile of BSF Larval Meal (as-fed)

Amino Acid	%
Essential	
Arginine	1.49 ± 0.03
Histidine	0.83 ± 0.02
Isoleucine	1.40 ± 0.03
Leucine	2.26 ± 0.04
Lysine	1.74 ± 0.03
Methionine_Cystine	0.81 ± 0.03
Phenylalanine	2.14 ± 0.08
Threonine	1.25 ± 0.02
Tryptophan	N/A
Valine	1.92 ± 0.03
Non-essential	
Alanine	2.30 ± 0.03
Asparagine	2.90 ± 0.04
Glutamine	3.52 ± 0.06
Glycine	1.78 ± 0.03
Proline	1.66 ± 0.03
Serine	1.36 ± 0.03
Tyrosine	N/A

Values are means ± standard deviations in triplicate. N/A= not available

4.2 Proximate Composition of Selected Ingredients

The proximate composition (% as-fed) of the various ingredients used in formulating BSF diets are presented in Table 4.3

Table 4.3: Proximate compositions (% as-fed) of selected ingredients used for formulating diets for growth and digestibility trials.

Ingredients	Dry matter	Crude Protein	Crude Lipid	Crude fibre	Ash	NFE	Gross Energy (kJg ⁻¹)
Fishmeal	95.21±0.04	60.20±0.04	9.74±0.06	5.73±0.06	17.73±0.07	1.81±0.06	18.39±0.09
BSFLM	89.51±0.28	33.87±0.35	20.30±0.25	6.25±0.39	10.95±0.10	18.14±0.95	19.14±2.45
Soybean meal	91.54±0.07	43.25±0.03	8.55±0.06	6.36±0.07	9.54±0.03	23.84±0.04	17.69±0.04
Wheat bran	87.56±0.08	18.42±0.07	4.64±0.06	9.87±0.06	5.71±0.02	48.92±0.06	14.57±0.09
Maize (white)	88.93±0.02	9.31±0.02	3.38±0.06	2.80±0.03	1.39±0.09	71.58±0.03	15.81±0.02
Cassava flour	88.25±0.06	1.48±0.03	0.46±0.02	2.01±0.07	2.62±0.01	81.68±0.04	14.54±0.03

Values are means ± standard deviations in triplicate. BSFLM = Black Soldier Fly Larval meal NFE = nitrogen free extract. GE = Gross Energy, CP = Crude Protein, CL = Crude Lipid, CF = Crude Fibre.

Results on the proximate analysis of all ingredients used in formulating diets (Table 4.3) shows that fishmeal had the highest level of crude protein (60.2%) with cassava flour having the least (1.48%). The ingredient with the highest crude fat content was BSF larval meal (20.30%) whilst cassava flour had the least (0.46%). In terms of crude fibre content, wheat bran has the highest (9.87%) while cassava flour had the least (2.01%). The gross energy ranged from 14.54 kJ g⁻¹ to 18.39 kJ g⁻¹ with fishmeal having the highest (18.39 kJ g⁻¹) while cassava flour had the least (14.54 kJ g⁻¹) (Table 4.3).

4.3 Proximate composition of Diets used for *O. niloticus* culture

The proximate analysis (%) and the gross energy (kJg⁻¹) of diets used in culturing *O. niloticus* fingerlings are shown in Table 4.4.

Table 4.4: Proximate Composition (% as-fed) of Diets used for Growth Trial.

Parameters(%)	ARDEC	RAANAN	BSF 25	BSF 50	BSF 75
Moisture	8.53±0.51	10.03±0.07	11.33±0.18	10.64±0.10	11.25±0.18
Ash	10.74±0.10	8.75±0.07	10.36±0.07	8.53±0.07	7.48±0.07
CP	37.64±0.14	38.64±0.30	37.99±0.33	37.35±0.29	38.07±0.17
Crude fat	11.8±0.11	6.75±0.11	10.20±0.24	12.32±0.01	14.86±0.13
Crude fibre	4.89±0.01	6.56±0.11	7.56±0.53	5.02±0.46	6.73±0.57
NFE	26.4±0.16	29.30±0.09	22.56±0.55	26.15±0.01	21.60±0.60
GE(kJg ⁻¹)	18.09±10.32	16.82±4.47	16.89±7.81	18.18±6.63	18.58±1.41

CP = Crude protein, GE=Gross energy NFE= Nitrogen free extract. Values are means ± standard deviations. Values with different superscript in a row are significantly different from each other (p < 0.05).

Moisture content ranged from 8.53 – 11.25% with BSF 25 having the highest (11.33%) whilst the least was ARDEC (8.53%). Ash content varied between 7.48 - 10.74% with ARDEC having the highest value whilst BSF 75 had the least (7.48%). Crude protein values ranged from 38.64 to 37.35%. RAANAN had the highest crude protein content (38.64%) whilst the least was BSF 50 (37.35%).

Crude fat values ranged between 14.86 and 6.75% with BSF 75 having the highest (14.86%) and the least was RAANAN (6.75 %). Crude fibre content of the diets ranged from 4.89 to 6.75 % with BSF 75 having the highest value (6.75%) and ARDEC had the least (4.89). The values for nitrogen free extract varied from 21.60 to 29.30% with RAANAN having the highest value (29.30%) while the least was BSF 75 (21.60%). The gross energy values ranged from 16.82 to 18.58 kJg⁻¹ with BSF 75 having the peak value (18.58%) and the RAANAN the least (16.82%).

4.4 Growth performance and Nutrient Utilisation

Table 4.5 shows summary of growth parameters and nutrient utilisation of *O. niloticus* fingerlings fed the different diets. There was no significant difference ($p > 0.05$) in all parameters measured at the end of the growth study.

The initial mean weight of *O. niloticus* fingerlings ranged from 1.27 – 1.33 g (Table 4.5) whilst the final mean weight was from 31.83 – 35.15 g. BSF 25 had the highest final mean weight (35.15 g) followed by ARDEC, BSF 50, RAANAN and BSF 75 with 33.80, 33.16, 32.78 and 31.83 g respectively. *O. niloticus* fingerlings fed with BSF 25 had the highest mean weight gained of 33.82 g whilst BSF 75 had the least mean weight gain of 30.53 g. There were no significant differences between mean weight gained of fish fed the various diets.

Table 4.5: Evaluation of Growth parameters and Nutrient Utilisation of *O. niloticus* Fingerlings Fed Different Diets for 10 Weeks.

Values

	ARDEC	RAANAN	BSF 25	BSF 50	BSF 75
Initial weight(g)	1.27±0.15	1.30±0.20	1.33±0.29	1.28±0.33	1.30±0.35
Final weight(g)	33.80±4.73	32.78±1.00	35.15±2.63	33.16±2.12	31.83±3.70
Weight gain(g)	32.53±4.68	31.48±1.04	33.82±2.53	31.88±2.36	30.53±3.95
ADG (g)	0.46±0.07	0.45±0.01	0.48±0.04	0.46±0.03	0.44±0.06
SGR	2.04±0.09	2.01±0.11	2.04±0.13	2.03±0.21	2.00±0.24
Survival rate (%)	90.74±1.70	87.41±6.70	85.56±5.88	91.85±1.70	89.26±5.25
CF	3.78±0.44	3.59±0.10	3.77±0.25	3.70±0.08	3.74±0.16
HSI (%)	0.68±0.32	0.70±0.20	0.68±0.13	0.66±0.15	0.62±0.13
FCR	1.70±0.04	1.72±0.15	1.61±0.18	1.63±0.23	1.84±0.33
Feed intake	59.56±6.6	59.26±6.48	61.60±4.92	58.74±3.69	57.68±2.00
PER	1.46±0.15	1.41±0.21	1.41±0.03	1.44±0.08	1.38±0.13

are means ± standard deviations. ADG = Average daily growth; FCR= Feed conversion ratio, SGR = Specific growth rate; HSI = Hepatosomatic index; CF = Condition factor; PER = Protein efficiency ratio. Absence of a letter indicates no significant difference between all the means.

The average daily growth (ADG) of *O. niloticus* fingerlings fed different dietary treatments ranged from 0.44 to 0.48 gday⁻¹. BSF 25 had the highest average daily growth (0.48 gday⁻¹) while BSF 75 was the least (0.44 gday⁻¹). The specific growth rate (SGR) was highest in ARDEC and BSF 25 with the same value of 2.04% while the least was observed in fish fed BSF 75 with 2.00% (Table 4.5). The highest survival rate was seen in BSF 50 with 91.85%, closely followed by ARDEC, BSF 75, RAANAN and BSF 25 with 90.74, 89.26, 87.41 and 85.56%, respectively.

The feed conversion ratio (FCR) ranged from 1.61 to 1.84. The lower the FCR, the more efficient the diet was utilized for growth. In the present study, BSF 25 had the least FCR of 1.61 followed by BSF 50, ARDEC, RAANAN and BSF 75 with 1.63, 1.70, 1.72 and 1.84, respectively. The highest feed intake was seen in BSF 25 with 61.60 g followed by ARDEC, RAANAN, BSF 50 and BSF 75 with 59.56, 59.26, 58.74 and 57.68 g

respectively. The higher the protein efficiency ratio (PER), the more efficient, the protein was utilised for growth. The highest PER was observed in ARDEC (1.46) whilst the least was BSF 75 (1.38).

The hepatosomatic indices (HSI) of *O. niloticus* fingerlings ranged from 0.62 to 0.70% (Table 4.5). Fingerlings fed RAANAN had the highest HSI (0.70%) whilst the least was BSF75.

4.5 Growth response of *O. niloticus* fingerlings fed different diets

Figure 4.1 shows a graphical illustration of the growth trend of *O. niloticus* fingerlings fed on the different diets over the 10 weeks culture trial.

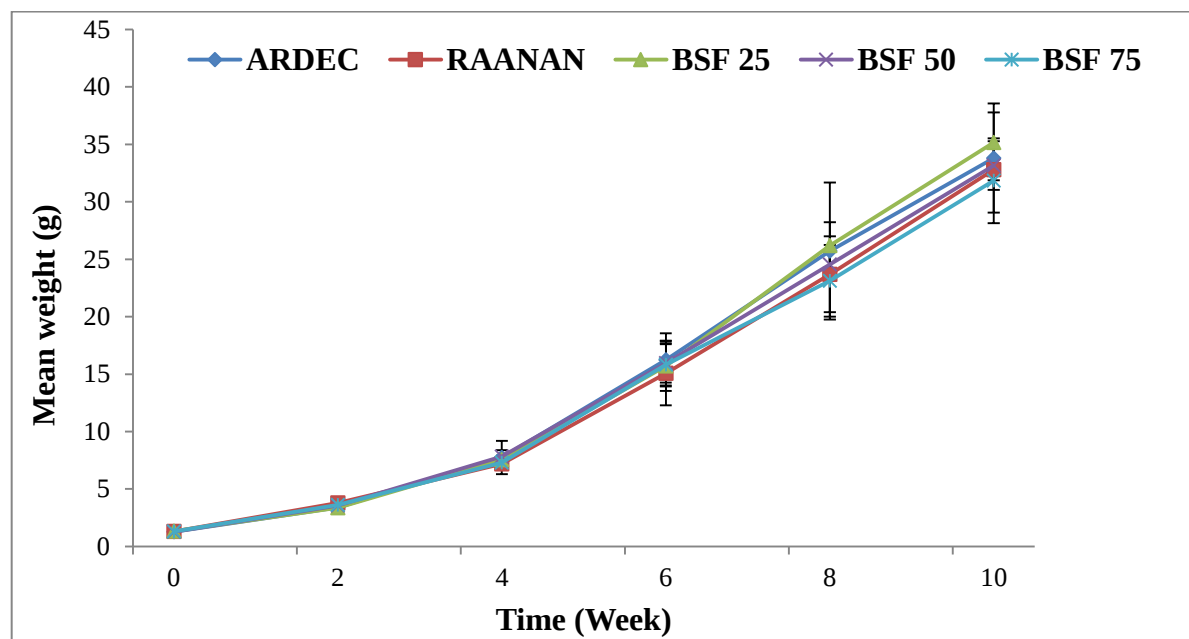


Figure 4.1: Growth trend of *O. niloticus* fingerlings fed different diets

There was a gradual growth of *O. niloticus* fingerlings for the first 4 weeks. The growth became rapid from the 6th week up to the 10th week in all treatments. BSF 25 had the highest final mean weight of 35.15 g followed by ARDEC, BSF 50, RAANAN and BSF

75 with 33.8, 33.16, 32.78, 31.83 g, respectively. However, there were no significant differences ($p > 0.05$) between the final mean weight of fish fed various dietary treatments.

4.6 Carcass Analysis

The whole body proximate composition and gross energy of *O. niloticus* fingerlings are presented in Table 4.6.

Table 4.6: Whole-body composition (%) and gross energy (kJg⁻¹) of cultured *O. niloticus*

%	Initial	ARDEC	RAANAN	BSF 25	BSF 50	BSF 75
MC	74.93±0.11	72.03±0.20	72.41±0.04	72.44±0.17	71.65±0.59	71.86±0.32
Ash	1.30±0.21	2.62±0.04 ^d	2.32±0.04 ^c	1.60±0.14 ^a	2.62±0.04 ^d	1.92±0.03 ^b
CP	13.95±0.19	16.28±0.17 ^a	17.15±0.14 ^b	17.10±0.18 ^b	17.15±0.31 ^b	16.23±0.33 ^a
CL	5.23±0.17	6.29±0.03 ^b	5.19±0.18 ^a	6.53±0.04 ^b	7.00±0.14 ^c	7.98±0.17 ^d
CF	2.42±0.04	1.82±0.11 ^c	1.10±0.07 ^b	1.15±0.07 ^b	0.82±0.04 ^a	1.29±0.01 ^b
NFE	2.18±0.23	0.97±0.21 ^a	1.84±0.46 ^b	1.20±0.09 ^{ab}	0.76±0.07 ^a	0.72±0.22 ^a
GE(kJg ⁻¹)	5.74±7.45	6.50±6.33 ^a	6.42±2.41 ^a	6.83±4.44 ^b	6.96±13.96 ^{bc}	7.12±10.84 ^c

MC = Moisture content, CP = Crude protein, CL = Crude lipid, CF = Crude fiber, NFE = Nitrogen free extract, GE = Gross energy. Values are means ± standard deviations in triplicate. Values of different superscript in a row are significantly different ($p < 0.05$). The absence of a letter indicate no significant difference between treatments.

Generally, the results for the whole-body composition of *O. niloticus* fingerlings showed that moisture content, crude fibre and nitrogen free extract of initial fingerlings prior to the beginning of the culture experiment were higher than fingerlings fed the dietary treatments. However, crude ash, crude protein, crude lipid and gross energy values were higher for experimental fish than the initial fingerlings (Table 4.6).

The moisture contents of the experimental fish ranged from 71.65 to 72.44% and there were no significant differences ($p > 0.05$) between the dietary treatments. The ash contents

differed significantly between the dietary fish groups ($p < 0.05$). Both ARDEC and BSF 50 recorded the highest ash content of 2.62%, whilst the least was BSF 25 (1.60%) (Table 4.6).

Crude protein content of the cultured fish ranged from 17.15 to 16.23% and the highest value was found in fish fed BSF 75 (7.98%) but was not significantly different ($p > 0.05$) from those of BSF 25 (17.10%) and RAANAN (17.15%). Values for crude fibre ranged from 0.82 to 1.82.42% and the highest significant value ($p < 0.05$) was seen in ARDEC (1.82%). The peak value for nitrogen free extract between the dietary group was observed in RAANAN (1.84%) but this value was not significantly different ($p > 0.05$) from that of BSF 25 (1.20%).

The gross energy (kJg^{-1}) content of whole-body composition differs significantly between dietary groups. Values ranged from 6.50 to 7.12 kJg^{-1} and the highest significant value ($p < 0.05$) was seen in BSF 75 (7.12 kJg^{-1}).

4.7 Health condition of cultured fish

Based on the scale used in ranking health conditions of fish (Table 3.3), whole-body observation on fish sampled from each dietary treatment showed no abnormalities in the eyes, fins, gills, kidney liver, Pseudobranchs, Opercles and spleen. There were no abdominal fats observed in the visceral of all fish examined.

4.8 Length - Weight Relationship of *O. niloticus* Fingerlings

The length- weight relationship is given by the formula $W = aL^b$ (Pauly, 1983) where W = body weight of fish a = exponent of the regression equation, L = total length or standard length of fish, b = weight at unit length.

The results for linear square regression of log body weight (BW) on log total length (TL) (i.e. $\log BW = \log TL + \log a$) for *O. niloticus* fingerlings fed different various dietary treatments are illustrated in Figures 4.2 to 4.8.

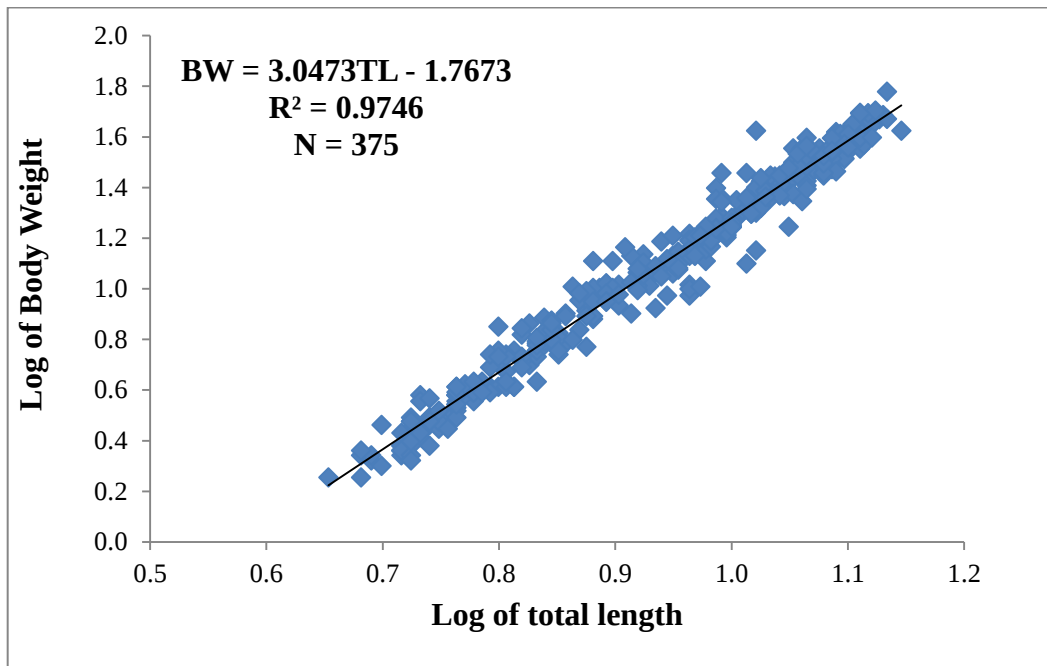


Figure 4.2: Length – weight relationship of *O. niloticus* fingerlings fed ARDEC.

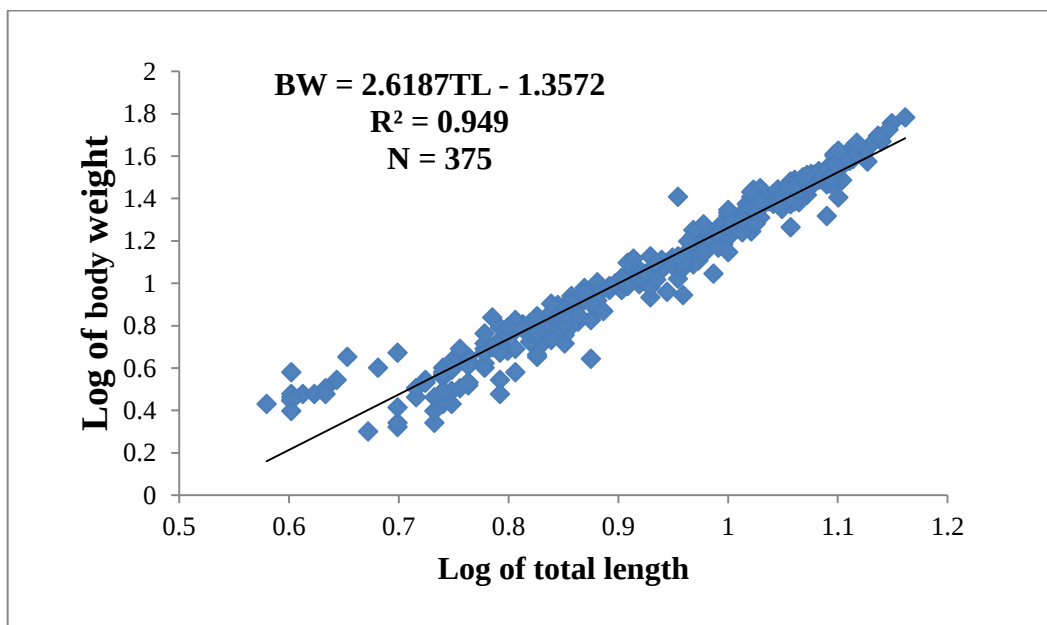


Figure 4.3: Length – weight relationship of *O. niloticus* fingerlings fed Raanan

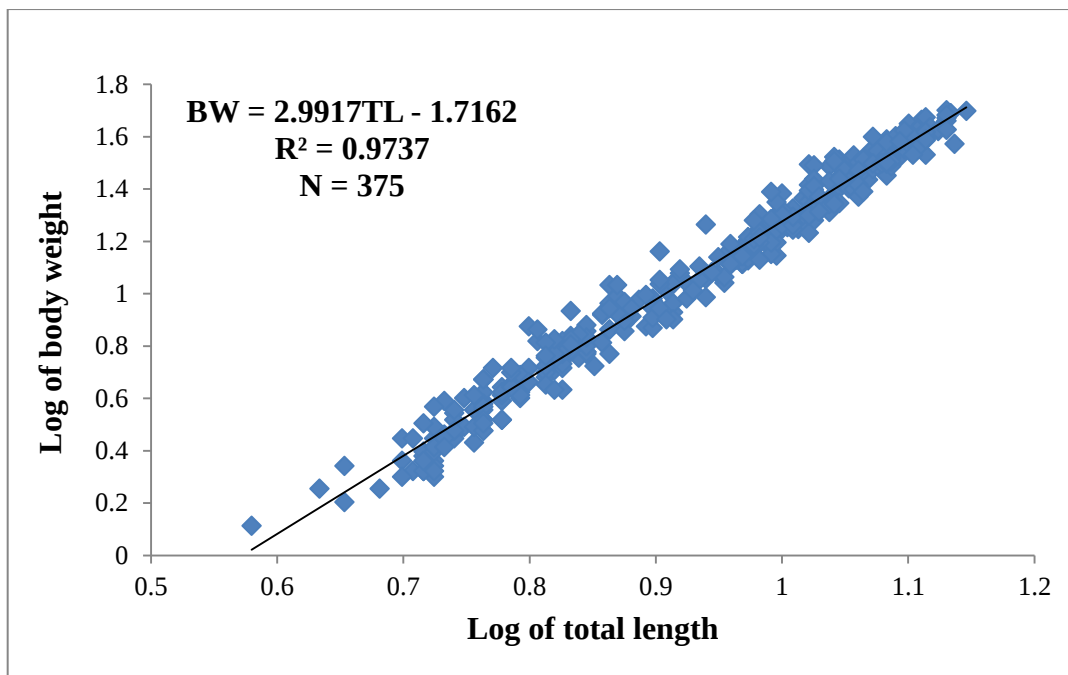


Figure 4.4: Length – weight relationship of *O. niloticus* fingerlings fed BSF 25

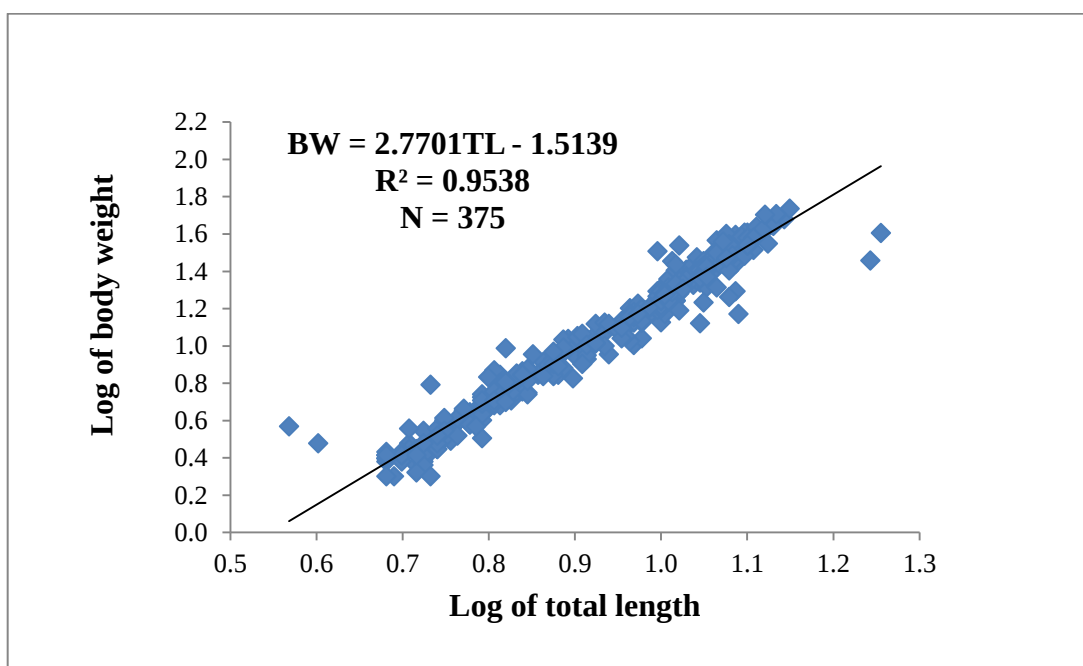


Figure 4.5: Length – weight relationship of *O. niloticus* fingerlings fed BSF 50

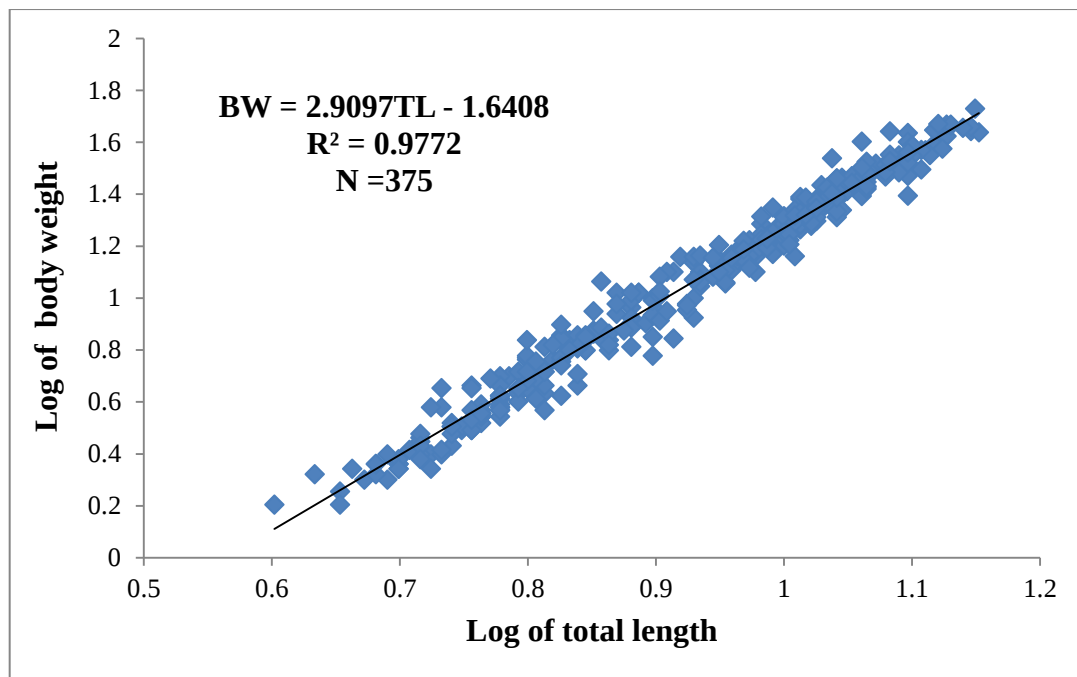


Figure 4.6: Length – weight relationship of *O. niloticus* fingerlings fed BSF 75

BW = Body weight, TL = Total length.

The regression equations for each dietary treatment are as follows:

ARDEC: BW = 0.0171TL^{3.0473} (R² = 0.9746)

RAANAN: BW = 0.0439TL^{2.6187} (R² = 0.949)

BSF 25: BW = 0.0192TL^{2.991} (R² = 0.9737)

BSF 50: BW = 0.0306TL^{2.7701} (R² = 0.9538)

BSF 75: BW = 0.0229TL^{2.909} (R² = 0.9772)

From the regression equations above, fish fed ARDEC and BSF 25 diets showed isometric growth whilst the rest showed allometric growth.

Table 4.7: Length-weight relationship, regression coefficient, and conditional factor parameters of *O. niloticus* fingerlings fed different diets

Treatment	a	b	R ²	K
ARDEC	-1.77	3.05	0.97	2.36
RAANAN	-1.36	2.62	0.95	2.29
BSF 25	-1.72	3.00	0.97	2.34
BSF 50	-1.51	2.78	0.95	2.23
BSF 75	-1.64	2.91	0.98	2.28

a = intercept of regression line, b = slope of regression line, R² = regression coefficient, K = condition factor

4.9 Water Quality Parameters in the Experimental Ponds

The water quality parameters in the pond are summarised in Table 4.8.

The temperature in the experimental ponds ranged between 29.30 and 31.2 °C and the mean temperature was 30.24±0.60 °C. The pH ranged from 5.79 to 6.37 and the mean was 6.14±0.15. The turbidity values ranged from 7.46 to 29.4 NTU and the mean was 18.10±8.10 NTU. Conductivity varied from 78.00 to 164 µS/cm and the mean was 118±21.34 µS/cm.

Table 4.8: Summary of water quality parameters of the ponds.

Parameter	Minimum	Maximum	Mean	Standard Deviation
Temperature ($^{\circ}\text{C}$)	29.30	31.2	30.24	0.60
pH	5.79	6.37	6.14	0.15
Turbidity (NTU)	7.46	29.4	18.13	8.10
Conductivity ($\mu\text{S}/\text{cm}$)	78.00	164	118.47	21.34
Ammonium-Nitrogen (mg/l)	0.18	0.51	0.34	0.09
Nitrite-Nitrogen (mg/l)	0.00	0.01	0.00	0.00
Nitrate-Nitrogen (mg/l)	0.00	0.01	0.01	0.01
Phosphate-Phosphorus (mg/l)	0.01	0.08	0.12	0.04
Total Alkalinity (mg/l)	36.00	78.5	55.87	10.77
Dissolved Oxygen (mg/l)	2.40	4.40	3.46	0.70
Sulphite (mg/l)	1.00	4.88	3.41	1.02

The ammonium-nitrogen in the ponds ranged from 0.18 to 0.51 mg/l and the mean was 0.34 ± 0.09 mg/l. The values obtained for nitrite-nitrogen ranged between 0.01 mg/l and 0.00 mg/l. The highest value for nitrate- nitrogen was 0.01 mg/l whilst the minimum was 0.00 mg/l. The phosphate-phosphorus in the pond ranged between 0.01 to 0.08 mg/l and the mean value was 0.12 ± 0.04 mg/l. The total alkalinity ranged from 36.00 to 78.5 mg/l and the mean value was 55.87 ± 10.77 mg/l. Dissolved Oxygen (DO) in the pond ranged from 2.40 to 4.40 mg/l and the mean value was 3.46 ± 0.70 mg/l. Sulphite content in the pond varied from 1.00 to 4.88 mg/l and the mean value was 3.41 ± 1.02 mg/l.

4.10 Cost Analysis of Diets used for Growth Trial.

The cost (based on current market price) of various ingredients and feeds as well as the amount of feed (kg) of each treatment used for the growth trial are shown in Appendix iv and v, respectively. Cost per kg of feed includes labour constituting 10% of the cost of feed production.

Table 4.9: Cost Effectiveness of diets fed to *O. niloticus* fingerlings.

Diet	Cost per kg of feed (GHS)	Feed input (kg)	Cost of feed (GHS)	Harvested biomass (kg)	Estimated value of biomass (GHS)	Incidence Cost (GHS)	Profit Index
ARDEC	3.19	15.12	47.74	8.27	99.22	5.77	2.08
RAANAN	4.60	14.59	67.11	7.73	92.70	8.69	1.38
BSF 25	2.68	15.18	44.44	8.11	97.30	5.48	2.19
BSF 50	2.43	15.09	39.98	8.22	98.69	4.86	2.47
BSF 75	2.31	14.64	36.18	7.67	92.04	4.72	2.54

Cost per kg of feed includes labour constituting 10% of the cost of producing the feed. Incidental cost = (cost of feeding/weight of fish produce) Profitability index = (value of fish/cost of feeding). GHS = Ghana Cedi. The selling price of fish = GHS 12.00 per kilogramme fish. (1 US Dollar = 4.33 GHS in May 2017).

The cost analysis (Table 4.9) shows that it was more expensive to use RAANAN to produce a kilogram of tilapia (8.69 GHS) than other dietary treatments. BSF 75 had the highest profit gain (2.54) followed by BSF 50 (2.47), BSF 25 (2.19), ARDEC (2.08) and RAANAN feed was the least (1.38). This suggests that it was more cost effective to use BSF 75 feed in producing a kg of *O. niloticus* fingerlings than the other feeds.

4.11 Apparent Digestibility Test

4.11.1 Proximate Composition of Digestibility Test Diets and Faecal Samples

Table 4.10 shows the proximate composition (%) and gross energy (kJg⁻¹) of the test diets and the faecal samples for the apparent digestibility test.

Table 4.10: Proximate composition (%) and gross energy (kJg⁻¹) of test diet and faecal samples

%	Test Diets			
	Reference diet	BSF diet	FM diet	SB diet
DM	91.93±0.06	92.82± 0.21	93.72±0.21	93.29±0.29
Ash	11.51±0.07	11.28 ±0.11	12.37±0.05	10.77±0.07
CP	37.76±0.10	36.58 ±0.00	42.73 ±0.14	38.18±0.11
CF	7.08±0.07	6.97± 0.14	6.92 ±0.06	7.30 ±0.10
CL	12.67±0.04	15.56 ±0.04	11.09±0.14	9.78±0.03
NFE	22.9±0.00	22.43±0.15	20.61±0.60	27.26±0.34
GE (kJg ⁻¹)	17.87±0.01	18.65 ±0.04	18.02±0.02	17.57±0.04
Faecal Samples				
	Reference Diet	BSF Diet	FM Diet	SB Diet
DM	75.92±0.03 ^c	74.62 ±0.07 ^a	75.53±0.06 ^b	75.60±0.04 ^b
Ash	3.28±0.06 ^b	3.131±0.03 ^{ab}	4.33±0.02 ^c	2.91±0.15 ^a
CP	12.65±0.27 ^b	16.41±0.02 ^c	11.74±0.01 ^a	12.76±0.02 ^b
CF	2.61±0.04 ^a	2.53±0.00 ^a	3.42±0.05 ^c	3.20 ±0.03 ^b
CL	4.43±0.14 ^b	5.88 ±1.71 ^c	3.46±0.11 ^a	3.43±0.07 ^a
NFE	52.95±0.54 ^b	46.67±0.19 ^a	52.58±0.15 ^b	53.30 ±0.02 ^b
GE(kJg ⁻¹)	13.82±0.03 ^c	14.21±0.00 ^d	13.16±0.02 ^a	13.51±0.03 ^b

BSF = Black soldier fly, FM = Fishmeal, SB = Soybean, DM = Dry matter, CP = Crude protein, CF = Crude Fiber, CL = Crude lipid, NFE = Nitrogen free extract, GE = Gross energy. Values are means ± standard deviations in triplicate. Values of different superscript in rows are significantly different ($p < 0.05$).

Generally, nutrients and gross energy composition were significantly different ($p < 0.05$) among the various test diets and faecal samples. Also, nutrients and gross energy composition were higher for the test diets than that of the faecal samples.

4.11.2 Proximate composition of Digestibility Test Diets

Dry matter content ranged from 91.93 to 93.72%. The highest value was observed in FM diet (93.29%) whilst the least was Reference diet (91.93%). The ash content varied between 10.77 and 12.37% (Table 4.10). The highest value was recorded for FM diet (12.37%), whilst the least was for SB diet (10.77%). The highest crude protein was observed in FM diet (42.73%), whilst BSF diet had the least (36.58%). Crude lipid ranged from 9.78 to 15.56%. The highest value was found in BSF diet (15.56%) and the least in SB diet (9.78%). The highest crude fibre value was observed in SB diet whilst FM diet (6.92%) was the lowest. The nitrogen free extract (NFE) ranged from 21.63 to 27.26%, and the peak value was observed in SB diet (27.26%), while the least was FM diet (20.61%). The gross energy value for BSF diet (18.65 kJg^{-1}) was the highest, whilst the least was SB diet (17.57 kJg^{-1}).

4.11.3 Proximate Composition of Faecal Samples.

Dry matter content ranged from 74.62 to 75.92% (Table 4.10). The Reference diet gave a value of 75.92%, which was significantly higher ($p < 0.05$) than faecal samples of the other treatments. Faecal samples from fish fed FM diet gave the highest ash content (4.33%), whilst the least was SB diet (2.91%). The highest protein content was observed in the faecal samples of fish fed BSF diets (16.41%) whilst the least was FM diet (12.76 %). The crude lipid content of faecal samples for fish fed BSF diet gave the highest value (5.88 %),

whilst FM diet gave the least (3.46%). Faecal samples of fish fed FM diet gave the highest significant ($P < 0.05$) crude fibre content of 3.42%, whilst the BSF diet was the least (2.53%). In terms of nitrogen free extract, faecal samples of fish fed BSF diet gave the least value of 46.67%. Values obtained from the faecal samples of fish fed Reference diet was not significantly different ($p > 0.05$) from those of FM and SB diets. The highest gross energy was obtained from the faecal samples of fish fed BSF diet (14.21 kJg^{-1}), while the least was FM diet (13.16 kJg^{-1}) (Table 4.10).

4.11.4 Apparent Digestibility Coefficient of Test Diet and Test Ingredients.

The apparent digestibility coefficient of the test diets and test ingredients is shown in Table 4.11.

For the test diets in general, the Apparent Digestibility Coefficients (ADCs) of dry matter, ash, crude lipid, crude protein, crude fibre, and gross energy were significantly different ($p < 0.05$) among the various dietary treatments (Table 4.11).

The ADC of dry matter ranged between 56.64 and 57.81%. The highest value was observed in the Reference diet (57.81%), which was not significantly different ($p > 0.05$) from FM and SB diets. The ADC of crude ash differed significantly among the test diets with SB diet having the highest value of 88.35%, whilst the least was FM diet (84.83%) (Table 4.11).

Table 4.11: Apparent Digestibility Coefficients of Test Diets and Test Ingredients

%	Test Diets			
	Reference diet	BSF diet	FM diet	SB diet
DM	57.81±0.49 ^b	56.64 ±0.50 ^a	56.64±0.50 ^{ab}	56.87±0.53 ^{ab}
Ash	87.98±0.29 ^b	87.83±0.29 ^b	84.83±0.16 ^a	88.35±0.37 ^b
CP	85.87±0.50 ^b	80.33±0.05 ^a	88.08±0.17 ^c	85.59±0.25 ^b
CF	84.45±0.57 ^c	84.11±0.22 ^c	78.83±0.23 ^a	81.07±0.13 ^b
CL	85.24±0.60 ^b	83.42±0.32 ^a	86.46±0.32 ^c	84.89±0.45 ^b
GE (kJg ⁻¹)	67.35±0.46 ^a	66.58±0.06 ^a	68.33±0.30 ^b	66.83±0.40 ^a
Test Ingredients				
		BSF meal	Fish meal	Soybean meal
DM	-	52.2±0.58 ^a	54.02±1.64 ^a	54.70±1.77 ^a
Ash	-	89.15±1.00 ^b	81.1±0.41 ^a	93.32 ±2.11 ^c
CP	-	65.92±0.17 ^a	91.33±0.42 ^c	85.02±0.75 ^b
CF	-	83.21± 0.81 ^c	62.61±0.90 ^a	72.29±0.47 ^b
CL	-	80.78±0.78 ^a	90.17±0.36 ^b	83.69±1.99 ^a
GE(kJg ⁻¹)	-	64.91±0.20 ^a	70.54.53±1.0 ^b	65.6±0.99 ^a

BSF = Black soldier fly, FM = Fishmeal, SB = Soybean, DM = Dry matter, CP = Crude protein, CF = Crude Fiber, CL = Crude lipid, NFE = Nitrogen free extract, GE = Gross energy. Values are means ± standard deviations. Values of different superscript in rows are significantly different ($p < 0.05$).

The ADC of crude protein was significantly different from the various dietary groups. The highest ADC was recorded for FM diet (88.08%) closely followed by References diet, SB diet and BSF diet with 85.87, 85.59, 80.33%, respectively (Table 4.11). The peak ADC value for crude lipid was found in FM diet (86.46%), whilst BSF diet gave the least value (83.42%).

The reference diet had the highest ADC value for crude fibre (84.45%) and FM diet had the least (78.83%). The ADC of ash was significantly lower in FM diet (84.83%) than the

other dietary treatments. The ADC for gross energy ranged from 66.58 to 68.63%. The ADC of gross energy for FM (68.63 kJg⁻¹) was significantly higher ($p < 0.05$) than the other feed treatment groups (Table 4.11).

In the case of test ingredients, the ADC of dry matter was highest (57.70%) for soybean meal (SB) but not significantly different ($p > 0.05$) from that of BSF meal (52.20%) and Fish meal (54.02%). Soybean meal had the highest ADC for ash ($p < 0.05$) followed by BSF meal and fishmeal with 89.15% and 81.1%, respectively (Table 4.11)

The ADC for crude protein was highest for Fishmeal (91.33%), followed by Soybean meal (85.02%) and BSF meal (65.92%). Fishmeal had a higher ADC for crude lipid (90.17%) than Soybean meal (83.69%) and BSF meal (80.78%). The ADC of crude fibre for fishmeal (62.61%) was significantly lower ($p < 0.05$) than that of BSF (83.21%) and soybean (72.29%). The highest ADC of gross energy was observed in fishmeal (71.53 kJg⁻¹) followed by soybean meal (65.6 kJg⁻¹) and BSF meal (64.91 kJg⁻¹) (Table 4.11).

CHAPTER FIVE

DISCUSSION

5.1 Culture and Drying of BSF larvae

The BSF larvae were reared on fruit and vegetable wastes (Table 3.1). This was meant to make efficient use of these organic resources by converting them to nutrient rich protein for use in animal feed (Diener *et al.*, 2011; Huis, 2013). Fruit and Vegetable wastes are readily available throughout the year. One type of fruit or vegetable could be substituted for another in a season of scarcity.

The average mean weight of larvae sampled was 0.24 ± 0.03 g which was slightly higher than the 0.22 g reported by other researchers (Sheppard *et al.*, 2002; Diclaro *et al.*, 2012; Makkar *et al.*, 2014). The greenhouse solar dryer was used in drying the larvae. The average ambient temperature and relative humidity recorded in the solar dryer during the drying period were 45.37 ± 5.32 °C and $25 \pm 8.72\%$, respectively.

Solar drying method has several advantages over direct Sun drying method. This includes;

- Protection of foodstuff from rain, dust, insects and other animals since it is enclosed in the dryer.
- The higher temperature in the room deters insect, ensure faster drying rate, thereby preventing spoilage by microorganisms (Weiss and Buchinger, 2004).

The Solar drying method is also cheaper than oven drying method.

5.2 Nutritional composition of BSF Larvae

The proximate composition of BSF larval meal is highly influenced by the diet that the maggot fed on (Sheppard *et al.*, 2005; Nguyen *et al.*, 2013). In the present study, the BSFL were reared largely on fruits and vegetable waste (Table 3.1). The crude protein and crude

fat obtained were 37.8% and 22.4% (dry matter basis), respectively (Table 4.1). These values were relatively lower than the reported value of 42 and 35% (dry matter basis) for protein and fat, respectively for BSF larvae raised on poultry manure (Sheppard *et al.*, 2002; Newton *et al.*, 2008; Park, 2015). According to Nguyen *et al.* (2013) and Spranghers *et al.* (2016), fruits and vegetables have lower energy (calories), protein, as well as fat contents compared to other organic substrates, such as poultry manure, kitchen waste and pig manure; hence larvae raised on fruits and vegetable wastes tend to have lower nutritional profile. The ash and fibre contents of 12.23 and 7.0%, respectively, observed in the present study were within the range of 11- 28 and 7.0%, respectively, reported in Makkar *et al.* (2014) and Park (2015).

The values for the amino acids composition (Table 4.2) were lower than values obtained by other researchers (Sheppard, 2007; Kroeckel *et al.*, 2012; Makkar *et al.*, 2014; Sanchez-Muros *et al.*, 2014). This variability in amino acid could be due to the organic substrates on which the larvae were cultured (Nguyen *et al.*, 2013).

5.3 Growth parameters and feed utilisation

All the diets formulated were readily accepted by fish (*O. niloticus*). Also, the final weight gain, average daily growth (ADG) and specific growth rate (SGR) among the different dietary treatments for the 10 weeks growth trial were not significantly different. This could suggest that the various diets formulated provided similar nutrients to the fish (*O. niloticus*). BSF 25 had the highest final mean weight gain followed by ARDEC, BSF 50, RAANAN and BSF 75. This indicates that BSF feeds performed favourably compared to the commercial diet RAANAN and farm-made diet ARDEC. Nevertheless, reduction in weight was observed in diets with increasing inclusion level of BSF meal. This could be attributed to lower feed intake as a result of reduced palatability. According to Kroeckel

et al. (2012), reduced palatability is often noticed in diets in which fishmeal is substantially replaced with alternative protein source especially if it contains anti-nutritional factors (ANFs). Although this study did not determine the types and concentration of ANFs for the various test ingredients such as BSF meal and soybean meal, it had been reported that substantial increase in soybean meal in fish diet could progressively depress fish growth (Glencross *et al.* 2007; Obirikorang *et al.* 2015b).

The present study is in agreement with other reports by Newton *et al.* (2007), St-Halaire *et al.* (2007a) and Stamer *et al.* (2014) on feed utilization by the rainbow trout.

The feed conversion ratio (FCR), and protein efficiency ratio (PER) values of fish fed BSF diets were not significantly different ($p > 0.05$) from the control groups (ARDEC and RAANAN). Lower FCR implies better utilisation of feed for tissue synthesis and metabolic activities. In the present study, BSF 75 had the highest FCR (1.84), whilst BSF 25 had the lowest (1.61). It was observed from the results that FCR increased with increasing inclusion of BSF larval meal. This phenomenon could be due to increasing level of chitin content (a component of exoskeleton of invertebrates) in the BSF diets, which had been reported to decrease feed intake, growth and digestibility in carp, tilapia (*O. niloticus* × *O. aureus*), turbot, and Atlantic salmon (Kroeckel *et al.*, 2012; Ekman, 2014; Stamer *et al.*, 2014).

Another important factor that might have affected feed utilisation is the quality of crude protein. Eventhough diets used for culture experiment were isonitrogenous, increasing inclusion levels of BSF meal and decreasing level of fishmeal reduces the quality of dietary protein. According to Lim and Webster (2006) and Mjoun *et al.* (2010), the protein quality of fish diet is a function of the essential amino acids in their right proportion. The BSF

meal had a far lower amino acid composition especially the essential amino acids compared to fishmeal as reported by Kroeckel *et al.* (2012).

Despite the fact that there is no specific universally accepted period of time for feeding trial, Barnes *et al.* (2014) opined that the duration for culture evaluation of different diets must be long enough for any potential significant differences to manifest. In a report by Anani (2015), significant differences in growth performance of *O. niloticus* fed different dietary treatments were observed after 16 weeks. In a study by Barnes *et al.* (2012), significant differences in growth performance of juvenile rainbow trout became apparent after 10 weeks culture period. In another report by Abarike *et al.* (2012), no significant differences were observed in the growth parameters of *O. niloticus* fry fed different agro-industrial by-product for 8 weeks culture period. However, Obirikorang *et al.* (2015b) reported significant differences in growth and nutrient utilization parameters for *O. niloticus* fingerlings cultured for 8 weeks. Thus a longer duration of this study could have led to some significant differences in the growth parameters.

Survival rate was similar among the different dietary treatments ($p > 0.05$). BSF 50 had the highest survival rate (91.85%), whilst the least was BSF 25 (85.56%). This suggests that BSF meal did not pose negative effects on the fish survival. The mortality recorded could be due to stress during sampling since most mortality was mostly observed a day after length and weight measurement. The results in the present study are in agreement with other reports by Abarike *et al.* (2012), Karapanagiotidis *et al.* (2014) and Anani (2015).

5.4 Health condition of cultured fish

At the end of the experiment, whole body observation on fish sampled from each dietary treatment shows no physical deformities in the eyes, fins, gills, kidney liver, pseudobranchs, opercles and spleen. There were no abdominal fats observed in the visceral of all fish examined. The high nutritional quality of the diets, appropriate culture environment and good management procedures/ practices could have accounted for the excellent health status of the fish. It also suggests that, inclusion level of BSF larval meal did not cause health defects to the cultured fish. These observations are in line with those reported by Anani (2015) and Barnes *et al.* (2012).

The hepatosomatic index (HSI) of fish give information about the liver energy reserves, metabolic activities, feed intake and environmental impact on the fish (Nunes *et al.*, 2011). Poor environment (i.e. polluted water) affects feed intake and fish usually have a small liver with less energy reserved in them whereas favourable environmental conditions and availability of feed causes an increase in HSI value. Daily increase in body weight is related to increase in HSI. Seasonality and reproductive cycle of fish also affect the HSI value (Nunes *et al.*, 2011; Lock *et al.*, 2013).

The highest HSI was observed in RAANAN (0.70%) while the least was BSF 75 (0.62%). Nevertheless, there were no significant differences among the dietary treatments ($p > 0.05$). This suggests that the HSI of *O. niloticus* fingerlings were not affected by the inclusion of BSF larval meal as a replacement for fishmeal nor the levels of inclusion. The values of HSI obtained in the present study was similar to that of Obirikorang *et al.* (2015b) who reported that HSI was not affected by replacing fishmeal with soybean diet or the inclusion levels in the diet of Nile tilapia fingerlings.

5.5 Whole-body Composition

The results on the whole-body composition of *O. niloticus* fingerlings showed that moisture content, crude fibre and nitrogen free extract of initial fingerlings prior to the beginning of the culture experiment were higher than fingerlings fed the dietary treatments. However, values for crude protein, crude fat, ash and gross energy were higher for fish fed on the dietary treatments than the initial fish. This indicates that dietary treatment might have had an influence on the carcass quality of the cultured fish. Similar trends have been reported by Kroeckel *et al.* (2012), Anani (2015) and Obirikorang *et al.* (2015b).

The crude protein contents of BSF 25 and BSF 50 were higher ($p < 0.05$) than that of BSF 75 but similar to the control diet RAANAN. This could be attributed to decreasing feed intake as result of reducing palatability. Kroeckel *et al.* (2012), Ogunji *et al.* (2008) and Stamer *et al.* (2014) observed reduced feed intake with decreasing level of fishmeal in the diet of turbot, Nile tilapia and rainbow trout respectively. Also, the disparities could be due to reduced quality of amino acids especially the essential amino acids. Fishmeal contains a superior quality of essential amino acids compared to BSF meal (Kroeckel *et al.*, 2012). Increasing inclusion levels of the BSF meal and decreasing level of fishmeal reduces the quality of dietary protein and may affect digestibility as well.

5.6 Length-weight relationship

Length- weight relationship, condition factor, growth, recruitment and mortality are an important quantitative aspect of fish biology (Anani and Nunoo, 2016; Migiro *et al.*, 2014). The values of b were 3.05, 2.62, 2.99, 2.78, and 2.90 for ARDEC, RAANAN, BSF 25, BSF 50 and BSF 75, respectively (Table 4.7). These values are close to the ideal fish shape of $b = 3$ (Datta *et al.*, 2013; Offem *et al.*, 2009) and fall within the range of 2 – 4

recommended for freshwater fishes (Martin, 1949; Migiro *et al.*, 2014; Anani and Nunoo, 2016).

The condition factor (K) is a reflection of the physiological state of the fish in relation to its well-being (Migiro *et al.*, 2014). It gives information on gonadal maturation, the degree of feeding and feeds utilisation. Condition factor greater than 1.0 implies good health condition of fish (Anani and Nunoo, 2016). From the K values of *O. niloticus* fed ARDEC, RAANAN, BSF 25, BSF 50, and BSF 75 were 2.36, 2.29, 2.34, 2.23 and 2.28, respectively. These values were greater than 1.0.

Good water quality parameters of the ponds and the high nutritional quality of the diets might have contributed to the high values of b and K. This data also suggest that fish from all dietary treatments were in good health condition and that BSF larval meal did not exert any negative effect on fish health.

5.7 Effect of diet on Water Quality

Water quality parameters values recorded throughout the culture period were within the recommended optimum range for the culture of *O. niloticus* (Popma and Masser, 1999; El-Sayed, 2006; Mjoun *et al.*, 2010; Bhatnagar and Devi, 2013).

The suitable water quality parameters recorded in the experimental pond could be attributed to good pond management practices such as administering high-quality feed which might have resulted in high feed intake and digestibility; avoidance of feed waste by giving just the right amount of diet based on the recommended feeding rate. Also, water levels in the ponds were maintained by constantly topping up, thus replenishing the culture system with freshwater.

5.8 The Cost Effectiveness of Diets

Analysis on the cost effectiveness of the various dietary treatments (Table 4.9) in the present study shows that BSF 75 had the highest profitability index (2.54) whilst RAANAN had the lowest (1.38). This implies that it was more profitable feeding *O. niloticus* fingerlings with BSF 75 than other diets, although higher FCR and reduced weight gain were observed. Generally, profitability decreases with increasing levels of fishmeal inclusion as a result of it being the most expensive ingredient in the formulated diets (Appendix IV). The cost of fishmeal per kilogramme was GHS 3.6 whereas that of BSF larval meal was GHS 2.38.

The results obtained in the present study confirms the general view held by other authors that fishmeal is the most expensive ingredient in formulated fish diet, accounting for the high cost of aquaculture feed (Glencross *et al.*, 2007; Hussain *et al.*, 2011; Nguyen *et al.*, 2013).

5.9 Apparent Digestibility Test

Generally, apparent digestibility coefficients (ADCs) of nutrients for all the test diets were high (> 52%) (Table 4.12). The ADCs of nutrients for the Reference (basal) diet were slightly higher than those of BSF, FM and SB diets, except gross energy. In addition, lower ADCs were obtained for the test ingredients (BSF, FM and SB meals) compared to the test diets. Also, the ADCs of protein for BSF meal (65.92%) (Table 3.3) was lower than values obtained for fishmeal (91.33%) and soymeal (85.02%). However, ADCs of crude lipid for BSF meal (80.78%) was significantly lower ($p < 0.05$) than those of fishmeal (90.17%) and soybean meal (83.69%).

The ADC of protein for BSF diet (80.33%) and BSF meal (65.92%) compare favourably to the ADCs of 81.1% and 63.1% recorded for *Hermetia* (BSF) diet and *Hermetia* meal, respectively for turbot as reported by Kroeckel *et al.* (2012). However, the ADCs of lipid and gross energy for BSF diet (83.42% and 66.58 kJg⁻¹, respectively) and BSF meal (80.78% and 64.91 kJg⁻¹, respectively) in the present study were lower than reported values (92.8% and 75 kJg⁻¹ respectively) and (78% and 54 kJg⁻¹ respectively) for *Hermetia* diet and *Hermetia* meal respectively for turbot by Kroeckel *et al.* (2012). However, the ADCs of lipid and gross energy for BSF diet (83.42% and 66.58 kJg⁻¹, respectively) and BSF meal (80.78% and 64.91 kJg⁻¹, respectively) in the present study were lower than the reported values for *Hermetia* diet (92.8% and 75 kJg⁻¹, respectively) and *Hermetia* meal (78%, 54 kJg⁻¹, respectively) by the same authors. This could be due to the differences in chemical composition of the other ingredients used in formulating the diets. According to Glencross *et al.* (2007), the ADCs of individual ingredients used in a diet formulation add up to digestibility value of the diet. This phenomenon could also be due to increasing level of chitin content in the BSF meal, which had been reported to decrease feed intake, growth and digestibility in carp, tilapia (*O. niloticus* × *O. aureus*), turbot, and Atlantic salmon (Ekman, 2014; Kroeckel *et al.*, 2012; Stamer *et al.*, 2014). Additionally, digestibility of a diet or an ingredient could be influenced by the method employed and solvent used in extracting the oil (Guimarães *et al.*, 2008).

The ADCs of dry matter, crude protein and gross energy for BSF meal observed in the present study is comparable to other non-conventional animal protein source such as blood and meat meal fed to *Labeo rohita* fingerlings (Hussain *et al.*, 2011) and housefly maggots meal fed to *Oreochromis niloticus* (Ogunji *et al.*, 2008), but slightly lower in crude lipids. This disparity could be attributed to the differences in the origin, composition and processing methods of the ingredients.

The ADCs of dry matter (54.2%), crude protein (91.33%), crude lipid (90.17%), and gross energy (71.53 kJg^{-1}) for fishmeal in the present study are similar to values reported by Hussain *et al.*, (2011) for *O. niloticus* and Sklan *et al.* (2004) for hybrid *O. niloticus* $\times$ *O. aureus* but marginally lower than values obtained by Zhou *et al.* (2004) for cobia (*Rachycentron canadum*). This could be attributed to differences in fish species. According to Sklan *et al.* (2004), the apparent nutrient digestibility value of an ingredient varies between species as a result of the nature of the digestive tract and the type of digestive enzyme secreted.

The ADCs of nutrients for soybean meal in the present study are in line with values reported by Hussain *et al.* (2011) for *Labeo rohita* but marginally lower than values obtained by Köprücü and Özdemir (2005) and Obirikorang *et al.* (2015b) for *O. niloticus*. The differences may be due to the processing method of the ingredient.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Proximate analysis of whole BSF larvae showed that, BSF larvae reared on fruits and vegetable wastes contain moisture (10.5%), crude protein (37.83%), crude lipid (22.7 %), crude fiber (7.0%), crude ash (12.23%) and gross energy (21.39 kJg¹) on dry matter basis.

Growth parameters indicate no significant differences ($p > 0.05$) in final mean weight gain, average daily growth, specific growth rates, and survival rate. Nevertheless, BSF 25 had the highest final mean weight gain of 35.15 g while BSF 75 (32.78 g) was the least. In terms of feed utilisation parameters, no significant differences were observed among the dietary treatments. Fingerlings fed on BSF 25 utilised the diets most efficiently as it resulted in the least FCR of 1.61 whilst the highest was observed in BSF 75 which had FCR of 1.84.

Analysis of whole- body composition has shown that initial fingerlings prior to the study had higher moisture content (74.93 ± 0.11) and nitrogen free extract (5.74%) than the experimental fingerlings. However, the experimental fish had higher crude protein, crude lipid, crude, nitrogen and gross energy than the initial fingerlings.

Water parameters recorded during culture period are all within the recommended levels for *O. niloticus* culture. Whole-body observation of harvested fingerlings had shown no abnormalities on the external and internal body. No visceral fats were observed. High survival rate (85.56 – 91.85%) was seen in all the dietary treatments. This may suggest that BSF larval meal posed no negative effect on fish health.

Analysis of the length-weight relationship has shown that, the b values of the dietary treatments ranged from 2.62 to 3.05 which are close to ideal fish shape $b = 3$ and fall within the recommended range of 2 – 4. The condition factor (K) ranged between 2.36 to 2.28 (> 1) which indicates good health condition in relation to their culture environment.

Economic analysis of the diets had shown that it was more cost effective using BSF 75 in producing *O. niloticus* fingerlings than the other dietary treatments as it gave the highest profitability index of 2.72.

The ADCs of the test diets as well as the test ingredients were high ($> 52\%$), indicating good utilization of feed for tissue synthesis and metabolic activities. BSF larval meal compared favourably with fishmeal and soybean meal even though BSF larval meal had lower protein and lipid digestibility values than fishmeal and soybean meal. Generally, ADCs of the test diets were higher than those of the test ingredients. The ADCs of BSF meal, fishmeal, soybean meal were: dry matter 52.2%, 54.02%, 54.70%; ash 89.15%, 81.1%, 93.32%; protein 65.92%, 91.33%, 85.02%; crude fiber 83.21%, 62.61%, 72.29%; lipid 80.78%, 90.17%, 83.69%; gross energy 64.91, 71.53, 65.6 (kJg^{-1}), respectively.

Results obtained for growth and nutrient utilization parameters, the cost effectiveness of diets, health status of fish, as well as apparent digestibility test in this study suggests that BSF larval meal may be an important protein supplement for the culture of *O. niloticus* fingerlings production.

Based on final mean weight gain, feed conversion ratio and cost effectiveness of diets, the results suggest that BSF larval meal may partially replace fishmeal at best 50% inclusion level without exerting a negative effect on the fish growth, health and carcass quality.

6.2 Recommendation

- This work is regarded as a baseline study for the utilization of BSF larval meal as a substitute for fishmeal. Future research should consider longer culture duration, preferably from first feeding fry to the grow-out to ascertain the full implications of using BSF larval as a substitute for fishmeal. This should include; growth, health condition of fish, economic and commercial viability of BSF larval meal as a replacer for fishmeal.
- The crude protein content of BSF larval meal (about 36 - 44%) is significantly lower than that of fishmeal (> 60%), making it difficult to formulate a fish diet that contains high-level protein (> 35%) using BSF larval meal as the main protein supplement. Future studies should consider increasing the crude protein content of BSF larval meal through defatting.
- Education of fish farmers on BSF larval meal diets through stakeholders meeting.
- Distribution of BSF larval meal diets to fish farmers for trials. Feedbacks from farmers could help in determining the commercial viability of the diets.
- In order to ascertain the commercial prospect of BSF larval meal diets, sensory analysis and survey on consumers' acceptability of *O. niloticus* cultured on BSF larval meal diet should be carried.
- The idea of alternative sources of protein should be seriously exploited by the Ministry of Fisheries and Aquaculture Development (MOFAD) particularly to BSF larval meal.

REFERENCES

- Abarike, E. D., Attipoe, F. Y. K., & Alhassan, E. H. (2012). Effects of feeding fry of *Oreochromis niloticus* on different agro-industrial by-products. *International Journal of Fisheries and Aquaculture*, 4 (8), 178-185. Retrieved from <http://www.academicjournals.org/IJFA>. DOI: 10.5897/IJFA11.028
- Adeniyi, Victoria, O., Folorunsho, & Yemi, C. (2015). Performance of *Clarias gariepinus* (Burchell, 1822) fed dietary levels of Black Soldier Fly, *Hermetia illucens* (Linnaeus, 1758) prepupa meal as a protein supplement. *International Journal of Research in Fisheries and Aquaculture*, 5(3), 89–93.
- Agbeko, E., Kwarfo-Apegyah, K., & Akongyuure, D. N. (2014). Optimization of dugout fisheries for climate change adaptation in Northern Region , Ghana. *Journal of Energy and Natural Resource Management*, 1(1), 63–68.
- Agbo, N.W. (2008). Oilseed meals as Dietary Protein Sources for juvenile Nile Tilapia *Oreochromis niloticus* (L). PhD Thesis. University of Stirling, Stirling, UK.
- Agbo, N. W., Madalla, N., & Jauncey, K. (2015). Mixtures of oilseed meals as dietary protein sources in diets of juvenile Nile tilapia (*Oreochromis niloticus* L.). *Journal of Science and Technology (Ghana)*, 35(3), 11–24.
- Akhter, Z. (2015). Utilization and Growth Performance of Nile Tilapia Fed Asian Latrine Fly Maggots. MSc Thesis. Norwegian University of Life Science.
- Aksnes, A., & Opstvedt, J. (1998). Content of digestible energy in fish feed ingredients determined by the ingredient substitution method. *Aquaculture*, 161, 45–53.
- Allan, G. L., Stuart, J. R., (1998). Fish meal replacement in aquaculture feeds : Sub-program administration. *NSW Fisheries Final Report Series*, 7(93), 9–11.
- Alegbeleye, W. O., Obasa, S. O., Olude, O. O., Otubu, K., & Jimoh, W. (2012). Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L .) for African catfish *Clarias gariepinus*. *Aquaculture Research*. 42, 412–420. <https://doi.org/10.1111/j.1365-2109.2011.02844.x>
- Ali, A., & Al-Asghar, N.A. (2001). Effect of feeding different carbohydrate to lipid ratios on the growth performance and body composition of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Animal Research*, 50, 91-100.
- Alvarez, L. (2012). The Role of Black Soldier Fly, *Hermetia illucens* (L.) (Diptera : Stratiomyidae) in Sustainable Waste Management in Northern Climates. *Electronic Thesis and Dissertations*. University of Windsor, Ontario Canada. (402pp)
- Amisah, S., Oteng, M. A., Ofori J. K., (2009). Growth performance of the African catfish, *Clarias gariepinus*, fed varying inclusion levels of *Leucaena leucocephala* leaf meal. *J. Appl. Sci. Environ. Manage*. Vol. 13(1) 21-26.

- Anani, F. A., & Nunoo, F. K. E. (2016). Length-weight relationship and condition factor of Nile tilapia, *Oreochromis niloticus* fed farm-made and commercial tilapia diet. *International Journal of Fisheries and Aquatic Studies*. 4(5), 647–650.
- Anani, F. A. (2015). Evaluation of Farm-Made and Commercial Fish Diets for Hapa Culture of Nile Tilapia (*Oreochromis niloticus* L.) in Ghana. PhD Thesis. Department of Marine and Fisheries Sciences, University of Ghana. <http://ugspace.ug.edu.gh>, (10120933).
- AOAC (1990). Official Methods of Analysis. 15th edn. Association of Official Analytical Chemists, Arlington, Virginia.
- APHA (1999). Standard methods for the examination of water and waste water. American Public Health Association.. 20th ed. New York:.
- Asiedu, B., Failler, P., & Beyens, Y. (2015). The performance of tuna processing fishery sector to sustainable fish trade and food security in Ghana. *Journal of Energy and Natural Resource Management*. 2(1), 8–14.
- Asmah, R., Awity, L., & Ofori, J. K. (2009). *Review of National Policies and Programmes on Aquaculture in Ghana 2009 Contract number : 213143 SARNISSA : Sustainable Aquaculture Research Networks in Sub Saharan Africa Duration : 36 months Title : Ghana Review on National policies and Programmes on Aquaculture Research*.
- Attipoe F.Y.K, Nelson, F.K., & Abban, E.K. (2009). Evaluation of three diets formulated from local agro-industrial by-products from production of *Oreochromis niloticus* in earthen ponds. *Ghana J Agric Sci.*, 42, 185-191.
- Ayoola A.A. (2010). Replacement of Fishmeal with Alternative Protein Sources in Aquaculture Diets. MSc Thesis. North Carolina State University.
- Bank of Ghana. (2008). the Fishing Sub-Sector and Ghana's Economy, 1–34. Retrieved from http://www.bog.gov.gh/privatecontent/Research/SectorStudies/fisheries_completerpdf.pdf
- Banks, I. J. (2014). To assess the impact of black soldier fly (*Hermetia illucens*) larvae on faecal reduction in pit latrines. PhD Thesis. London School of Hygiene & Tropical Medicine, 1–231. <http://researchonline.lshtm.ac.uk/1917781/>
- Barnes, M.E., Brown, M.L., & Rosentrater, K.A. (2012). Replacement of Fish Meal with High Protein Distillers Dried Grain in Juvenile Rainbow Trout Diets. *J Aqua Feed Sci Nutr.*, 4(3-4), 39-47.
- Barnes, M. E., Brown, M. L., Bruce, T., & Sindelar, S. (2014). Rainbow Trout Rearing Performance , Intestinal Morphology , and Immune Response after Long-term Feeding of High Levels of Fermented Soybean Meal. *North American Journal of Aquaculture* 76:333–345. <https://doi.org/10.1080/15222055.2014.920748>
- Barroso, F. G., Haro, C. De, Sánchez-muros, M., Venegas, E., Martínez-sánchez, A., &

- Pérez-bañón, C. (2014). The potential of various insect species for use as food for fish. *Aquaculture*, 422–423. <https://doi.org/10.1016/j.aquaculture.2013.12.024>
- Bhatnagar, A., & Devi, P. (2013). Water quality guidelines for the management of pond fish culture. *International Journal of Environmental Sciences*, 3(6), 1980–2009. <https://doi.org/10.6088/ijes.2013030600019>
- Booth, D. C., and C. Sheppard. (1984). Oviposition of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae): eggs, masses, timing, and site characteristics. *Environmental Entomology*, 13: 421-423.
- Bullock, N., Chapin, E., Evans, A., Elder, B., Givens, M., Jeffay, N., Robinson, W. (2013). The Black Soldier Fly How-to-Guide. UNC Institute for the Environment, 1–12.
- Bureau, D. P., Cho, C. Y. (1999). Measuring Digestibility in Fish. UG/OMNR Fish Nutrition Research Laboratory 1 Technical Document, University of Guelph, Ontario, Canada., 1–9.
- Bwanika, G.N., Murie, D.J., & Chapman, L.J. (2007). Comparative age and growth of Nile tilapia (*Oreochromis niloticus* L.) in lakes Nabugabo and Wamala, Uganda. *Hydrobiologia*, 589, 287-301
- Cobbina, R. (2010). Aquaculture in Ghana: Economic Perspectives of Ghanaian Aquaculture for Policy Development. *United Nations University*, 1–47.
- Cooper, C., Packer, N., & Williams, K. (2001). Amino acids protocols. *Humana Press* 1 - 265.
- Craig, S., & Helfrich, L. A. (2004). Understanding Fish Nutrition, Feeds, and Feeding. *Virginia Cooperative Extension*, 1–18.
- Dandie, C. (2011). Poultry manure biochar application to Ferralsol: Short-term effects on N cycling processes. *CSIRO* pp, 14.
- Datta, S. N., Kaur, V. I., Dhawan, A., & Jassal, G. (2013). Estimation of length-weight relationship and condition factor of spotted snakehead *Channa punctata* (Bloch) under different feeding regimes. *SpringerPlus*, 2, 436. <https://doi.org/10.1186/2193-1801-2-436>
- Davis, D., Nguyen, T., Li, M., Gatlin, D. M., & O’Keefe, T. (2009). Advances in aquaculture nutrition: catfish, tilapia and carp nutrition. In *New Technologies in Aquaculture* (pp. 440–458). <https://doi.org/10.1533/9781845696474.3.440>
- Diclaro II, J. W., & Phillip E. K. (2009). Black Soldier Fly - *Hermetia Illucens*." Black Soldier Fly - *Hermetia Illucens*. University of Florida.
- Diclaro, J. W., Kaufman, P. E., & Ii, J. W. D. (2012). Black soldier fly *Hermetia illucens* Linnaeus (Insecta : Diptera : Stratiomyidae). *Eeny* 461, 5. <http://entnemdept.ifas.ufl.edu/creatures/.2>.
- Diener S, Zurbrugg C&Tockner K (2009) Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Waste Management & Research* 27,

603–610.

- Diener, S., Solano, N. M. S., Gutierrez, F. R., Zurbrugg, C., & Tockner, K. (2011). Biological treatment of municipal organic waste using black soldier fly larvae. *Waste and Biomass Valorization*, 2(4), 357–363. <https://doi.org/10.1007/s12649-011-9079-1>
- El-Sayed, A. F. M. (2004). Protein nutrition of farmed tilapia: searching for unconventional sources. *New Dimensions in Farmed Tilapia: Proceedings of the Sixth International Symposium on Tilapia Aquaculture*, (January 2004), 364–378. <https://doi.org/10.1080/10454438.2011.627780>
- El-Sayed, A. F. M. (2006). Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials. *VIII Simposium Internacional de Nutrición*, 95–106. Retrieved from <http://www.nutricionacuicola.uanl.mx/numeros/8/7ElSayed.pdf>
- El-Sayed, A. F. M. (2013). Tilapia feed management practices in sub-Saharan Africa. *M.R. Hasan and M.B. New, Eds. On-Farm Feeding and Feed Management in Aquaculture. FAO Fisheries and Aquaculture Technical Paper No. 583. Rome, FAO. Pp. 377–405, 377–405.*
- Ekman, J. (2014). Production of fish feed from vegetable waste. *Applied Horticultural Research*. (pp 74)
- FAO. (2012). The State of World Fisheries and Aquaculture (SOFIA) 2012. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, FAO, Rome, Italy, 209pp. <https://doi.org/10.5860/CHOICE.50-5350>.
- FAO. (2013). *Edible insects*. Future prospects for food and feed security. *Food and Agriculture Organization of the United Nations* (Vol. 171).
- FAO. (2014). The State of World Fisheries and Aquaculture 2014. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, FAO, Rome, Italy, 223.
- FAO. (2016). The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, FAO, Rome, Italy, 200pp. <https://doi.org/92-5-105177>
- FAO. (2016). Fisheries and Aquaculture Country Profiles; Ghana. Part I Overview and main indicators Country brief.
- FAO. (2017). Food and Agriculture Organization of the United Nations. *Regional review on status and trends in aquaculture development in sub-Saharan Africa – 2015. FAO Fisheries and Aquaculture Circular, Rome, Italy* (Vol. 1135). Retrieved from <http://www.fao.org/3/a-i6873e.pdf>

- Fitzsimmons, K. (1997). Introduction to tilapia nutrition. In: Fitzsimmons, K., (Ed.) *Tilapia Aquaculture: Proceedings of the Fourth International Symposium on Tilapia in Aquaculture*, pp 9-12. Ithaca, N. Y.: Northeast Regional Agricultural Engineering
- Fitzsimmons, K. (2005). Tilapia culture. pp. 563-590. In A. Kelly and J. Silverstein (eds), *Aquaculture in the 21 Century*. American Fisheries Society, Maryland. 643.
- Fitzsimmons, K (2013). Latest trends in tilapia production and market worldwide Tilapia. University of Arizona.
- Frimpong, Samuel Kwadwo., Adwani, A. (2015). The Challenges and Prospects of Fish Farming in Ghana : A Project Management Perspective, *International Journal of ICT and Managemen*, Vol III (2), 29–34.
- Furukawa, H. and Tsukahara, H. (1966). On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bull Jpn Soc Sci Fish* 32(6):502-50.
- Ghana Statistical Service (GSS). (2014). Statistics for Development and Progress. Gross Domestic Product 2014, (April), 3. Retrieved from http://www.statsghana.gov.gh/docfiles/GDP/GDP_2014.pdf
- Glencross, B. D., Booth, M., & Allan, G. L. (2007). A feed is only as good as its ingredients - A review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition*, 13(1), 17–34. <https://doi.org/10.1111/j.1365-2095.2007.00450.x>
- Gobbi, P., Martínez-Sánchez, A., & Rojo, S. (2013). The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *European Journal of Entomology*, 110(3).
- Godoy, A.C., Fries, E., Corrêia, A.F., Melo, I.W.A., Rodrigues, R.B., & Boscolo, W.R. (2016). Apparent Digestibility of Fish Meat and Bone Meal in Nile Tilapia. *Arch. Zootech.* 65(25), 341-348.
- Guimarães, I. G., Pezzato, L. E., & Barros, M. M. (2008). Amino acid availability and protein digestibility of several protein sources for Nile tilapia, (*Oreochromis niloticus*). *Aquaculture Nutrition*, 14(5), 396–404. <https://doi.org/10.1111/j.1365-2095.2007.00540.x>
- Hagbayan, S., & Mehrgan, M. S. (2015). The Effect of Replacing Fish Meal in the Diet with Enzyme-Treated Soybean Meal (HP310) on Growth and Body Composition of Rainbow Trout Fry. *Molecules*, 21058–21066. <https://doi.org/10.3390/molecules201219751>
- Hale, O.M. 1973. Dried *Hermetia illucens* larvae (Stratiomyidae) as a feed additive for poultry. *Journal of the Georgia Entomological Society*, 8: 16–20.
- Hiheglo, P. K. (2008). Aquaculture in Ghana: Prospects, Challenges, Antidotes and Future Perspectives. MSc Thesis. University of Tromsø. Retrieved from <http://munin.uit.no/handle/10037/1431>

- Holmes, L. a., Vanlaerhoven, S. L., & Tomberlin, J. K. (2012). Relative Humidity Effects on the Life History of *Hermetia illucens* (Diptera: Stratiomyidae). *Environmental Entomology*, 41(4), 971–978. <https://doi.org/10.1603/EN12054>
- Hossain, M. A., Johan, P., Kikuchi, K. (2000). Nutrient digestibility coefficients of diets with varying energy to protein ratio for Japanese flounder, *Paralichthys olivaceus*. *Bangladesh Journal of Fisheries. Research*, 4(2), 105-112
- Huis, A. Van. (2013). Potential of Insects as Food and Feed in Assuring Food Security. *Annual Review of Entomology* . <https://doi.org/10.1146/annurev-ento-120811-153704>
- Huis, A. van (2015). Edible insects contributing to food security? *Agriculture & Food Security*, 4(1), 20. <https://doi.org/10.1186/s40066-015-0041-5>
- Hussain, S. M., Afzal, M., Salim, M., Javid, A., Khichi, T. A. A., Hussain, M., & Raza, S. A. (2011). Apparent digestibility of fish meal, blood meal and meat meal for Labeo rohita fingerlings. *Journal of Animal and Plant Sciences*, 21(4), 807–811.
- IFIF (2012). Insects as animal feed. *International Feed Industry Federation*, 7, 89–97.
- Jauncey, K. (2000). Nutritional requirements. pp 327-375. In M.C.M. Beveridge and B.J. McAndrew (eds.) *Tilapias: Biology and Exploitation*. Kluwer Academic Publishers, Great Britain.
- Jimoh, A. W., Awodele, O. A., Okemakin, F. Y., Ayeloja, A. A., Abdulsalami, S. A., & Azeez, F. A. (2015). Apparent Digestibility Experiment with Nile Tilapia (*Oreochromis Niloticus*) Fed Diets Containing Citrullus Lanatus Seedmeal. *Annals Biology*, XVIII(2), 159–168.
- Karapanagiotidis, I. T., Daskalopoulou, E., Vogiatzis, I., Rumbos, C., Mente, E., & Athanassiou, C. G. (2014). Substitution of Fishmeal by Fly *Hermetia illucens* Prepupae Meal in the Diet of Gilthead Seabream (*Sparus aurata*). *HydroMedit 2014*, (September 2015), 110–114.
- Kassam, L. (2014). Nutrition project Aquaculture and food security , poverty alleviation and nutrition in Ghana : Case study prepared for the Aquaculture for Food Security , Poverty Alleviation and Nutrition project, WorldFish, Penang, Malaysia. Project Report: 2014-48. 48. Retrieved from <https://cgspace.cgiar.org/handle/10568/56967>
- Kenis, M., Koné, N., Chrysostome, C. A. A. M., Devic, E., Koko, G. K. D., Clottey, V. A., Mensah, G. A. (2014). Insects used for animal feed in West Africa. *Entomologia*, 2(2), 107–114. <https://doi.org/10.4081/entomologia.2014.218>
- Köprücü, K., & Özdemir, Y. (2005). Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 250(1–2), 308–316. <https://doi.org/10.1016/j.aquaculture.2004.12.003>
- Kroeckel, S., Harjes, A.-G.E., Roth, I., Wuertz, S., Susenbeth, A., Schulz, C., & Kiel, C.

- (2012). When a turbot catches a fly : Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute. *Aquaculture*, 364–365(April 2015). <https://doi.org/10.1016/j.aquaculture.2012.08.041>
- Kurbanov, A. R., Milusheva, R. Y., Rashidova, S. S., & Kamilov, B. G. (2015). Effect of replacement of fish meal with silkworm (*Bombyx mori*) pupa protein on the growth of *Clarias gariepinus* fingerling. *International Journal of Fisheries and Aquaculture*. 2(6), 25–27.
- Kushwaha, M. P. (2013). Replacement of fish meal by soybean (*Glycine max*) in the formulation of fish feed ingredients essential for Immunostimulation and growth performance of carps. *International Journal of Fauna and Biological Studies* 1 (2), 35-38.
- Lee, S. M. (2002). Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). *Aquaculture*, 207(1–2), 79–95. [https://doi.org/10.1016/S0044-8486\(01\)00751-7](https://doi.org/10.1016/S0044-8486(01)00751-7)
- Li, Q., Zheng, L., Cai, H., Garza, E., Yu, Z., & Zhou, S. (2011). From organic waste to biodiesel: Black soldier fly, *Hermetia illucens*, makes it feasible. *Fuel*, 90(4), 1545–1548. <https://doi.org/10.1016/j.fuel.2010.11.016>
- Lim, C., & Webster, C. (2008). Tilapia: Biology, Culture and Nutrition. *African Journal of Aquatic Science*, 33(1), 103–103. <https://doi.org/10.2989/AJAS.2008.33.1.14.415>.
- Liu, Q., Tomberlin, J. K., Brady, J. A., & Sanford, M. R. (2008). Black Soldier Fly (*Diptera*: *Stratiomyidae*) Larvae Reduce *Escherichia coli* in Dairy Manure. *Environmental Entomology* 37(6) 1525–1530.
- Lock, E. R., Arsiwalla, R., & Waagbo, R., (2013). Insect larvae meal as an alternative source of nutrients in diets of Atlantic salmon (*Salmon salar*) postsmolt. *Aquaculture Nutrition*. (31 pp). doi 10.111/anu.1243.
- Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 197, 1–33. <https://doi.org/10.1016/j.anifeedsci.2014.07.008>
- Migiro, K. E., Ochieng, E., & Munguti, J. M. (2014). The Length-Weight Relationship and Condition Factor of Nile Tilapia (*Oreochromis niloticus* L .) Broodstock at Kegati Aquaculture Research Station , Kisii , Kenya. *International Journal of Advanced Research*, 2(5), 777–782.
- Mjoun Kamal, Rosentrater, Kurt A., & Brown, Michael L. (2010). Tilapia : Environmental Biology and Nutritional Requirements. *Fact Sheets*. 963-02, (1975), 7. Retrieved from http://pubstorage.sdstate.edu/AgBio_Publications/articles/FS963-02.pdf
- Miller, J.W. (1976). Fertilization and feeding practises in warm water pond fish culture in Africa. Symposium on Aquaculture in Africa, Accra, Ghana, 30 September - 2 October 1975. CIFA Technical Paper 4 (supplement 1). 512-541. Rome: FAO.
- MOFAD. (2015). Ministry of Fisheries and Aquaculture Development, 2015 Annual

Report. April 2016. Accra, Ghana.

- Myers, H. M., Tomberlin, J. K., Lambert, B. D., & Kattes, D., (2008). Development of the Black Soldier Fly (Diptera: Stratiomyidae) Larvae Fed Dairy Manure. *Environmental Entomology*, 37, 11–15
- Newton, G.L., Booram, C.V., Barker, R.W., & Hale, O.M. (1977). Dried *Hermetia illucens* larvae meal as a supplement for swine. *Jour. Anim Sci.*, 44, 395–400.
- Newton, G.L., Sheppard, D.C., Watson, D.W., Burtle, G.J., Dove, C.R., Tomberlin, J.K. & Thelen, E.E. (2004). The Black Soldier Fly, *Hermetia Illucens*, as a Manure Management/Resource Recovery Tool. (2004): 1-5. Abstract. (n.d.): n. pag. Print.
- Newton L, Sheppard C, Watson DW, Burtle G, Dove, R. (June 2005). Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Waste Management Programs. North Carolina State University. http://www.cals.ncsu.edu/waste_mgt/smithfield_projects/phase2report05/cd,web%20files/A2.p
- Newton, B. G. L., Sheppard, D. C., & Burtle, G. J. (2008). Research Briefs: Black Soldier Fly Prepupae - A Compelling Alternative to Fish Meal and Fish Oil. *Annual Meeting of the Regional Research Committee, S-1032 “Animal Manure and Waste Utilization, Treatment and Nuisance Avoidance for a Sustainable Agriculture”*. Retrieved from <http://www.extension.org/pages/15054/research-summary>.
- Newton, R. (n.d.). Process manual for the establishment of a Black Soldier Fly (*Hermetia illucens*) production system.
- Ng, W. K., & Romano, N. (2013). A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture*. <https://doi.org/10.1111/raq.12014>
- Nguyen, T. N., Davis, D. A., & Saoud, I. P. (2009). Evaluation of alternative protein sources to replace fish meal in practical diets for juvenile tilapia, *Oreochromis spp.* *Journal of the World Aquaculture Society*, 40(1), 113–121. <https://doi.org/10.1111/j.1749-7345.2008.00230.x>
- Nguyen, T. T. X., Tomberlin, J. K., & Vanlaerhoven, S. (2013). Influence of resources on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. *Journal of Medical Entomology*, 50(4), 898–906. <https://doi.org/10.1603/ME12260>
- NRC. (1993). Nutrient requirement of fish. National Research Council, Academy of Sciences, National Academy Press, Washington D.C. (114 pp).
- Nunes, C., Silva, A., Soares, E., Ganas, K., Nunes, C., Silva, A., Soares, E. (2011). The Use of Hepatic and Somatic Indices and Histological Information to Characterize the Reproductive Dynamics of Atlantic Sardine *Sardina pilchardus* from the Portuguese Coast. Special Section: Fisheries Reproductive Biology, 5120. <https://doi.org/10.1080/19425120.2011.556911>

- Obirikorang, K. A., Amisah, S., Fialor, S. C., & Skov, P. V. (2015a). Local agro-industrial by-products with potential use in Ghanaian aquaculture: a review. *Aquaculture International*. <https://doi.org/10.1007/s10499-014-9831-1>
- Obirikorang, K. A., Amisah, S., Agbo, N. W., Adjei-boateng, D., Adjei, N. G., & Skov, P. V. (2015b). Evaluation of Locally-available Agro- industrial Byproducts as Partial Replacements to Fishmeal in Diets for Nile Tilapia (*Oreochromis niloticus*) Production in Ghana. *iMedPub Journals*, Vol. 1 No. 1:2, 1–9
- Offem, B. O., Omoniyi, I. T., Campus, O., & State, C. R. (2009). Length-weight Relationship, Condition Factor and Sex Ratio of Forty Six Important Fishes in a Tropical Flood River. *Research Journal of Fisheries and Hydrobiology*, 4(2): 65-72
- Ogello, E. O., Munguti, J. M., Sakakura, Y., & Hagiwara, A. (2014). Complete Replacement of Fish Meal in the Diet of Nile Tilapia (*Oreochromis niloticus* L.) Grow-out with Alternative Protein Sources . A review. *International Journal of Advanced Research*, 2(8), 962–978.
- Ogunji, J., Summan Toor, R. U. A., Schulz, C., & Kloas, W. (2008). Growth performance, nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. *Turkish Journal of Fisheries and Aquatic Sciences*, (1), 141–147.
- Olaniyi, C. O., & Salau, B. R. (2013). Utilization of maggot meal in the nutrition of African cat fish. *African Journal of Agricultural Research*, 8(37), 4604–4607. <https://doi.org/10.5897/AJAR12013.7154>.
- Orire, A. M., & Ozoadibe, T. N. (2015). Evaluation of Growth Performance and Body Composition of *Clarias gariepinus* for Graded Level Inclusion of Soybean Waste. *Journal of Fisheries and Aquatic Science*, 10(5), 384–391. <https://doi.org/10.3923/jfas.2015.384.391>
- Park, H. H., (2015). Black Soldier Fly Larvae Manual. *Student Showcase*. Paper 14. 13pp. http://scholarworks.umass.edu/sustainableumass_studentshowcase/14
- Pimentel, D., Berger, B., Filiberto, D., Newton, M., Wolfe, B., Karabinakis, E., Clark, S., Poon, E., Abbett, E. & Nandagopal, S. (2004). Water resources: agricultural and environmental issues. *BioScience*, 54: 909–918.
- Popma, T., & Masser, M. (1999). Tilapia Life History and Biology. *South Regional Aquaculture Center*, (283), 1–4. <https://doi.org/10.1111/j.1365-2095.2004.00329.x>
- Rana, K. M. S., Salam, M. A., Hashem, S., & Islam, A. (2015). Development of Black Soldier Fly Larvae Production Technique as an Alternate Fish Feed. *International Journal of Research in Fisheries and Aquaculture*, 5(1), 41–47.
- Rao, D., Perrino, E. S., & Barreras, E. (2012). The Sustainability of Tilapia Fish Farming in Ghana. *Unpublished*.

- Rumpold, B. A., & Schlüter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition and Food Research*, 57(5), 802–823. <https://doi.org/10.1002/mnfr.201200735>.
- Sakita, G. Z., Meira, D. C., Neto, H. B., & Abdalla, A. L. (2015). Chromium oxide ($^{51}\text{Cr}_2\text{O}_3$) used as biological marker was not absorbed by fish. *Arq. Bras. Med. Vet. Zootec.*, 67(3), 755–762.
- Sánchez-muros, M., Barroso, F. G., & Manzano-agugliaro, F. (2014). Insect meal as renewable source of food for animal feeding: a review. *Journal of Cleaner Production*, 65, 16–27. <https://doi.org/10.1016/j.jclepro.2013.11.068>
- Shiau, S.Y. (2002). Tilapia, *Oreochromis* spp. pp. 273-292. In C.D. Webster and C.E. Lim (eds.) *Nutrient Requirements and Feeding of Finfish for Aquaculture*. CABI Publishing, Oxfordshire, 418.
- Sheppard, D. C., Newton, G. L., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource Technology*. [https://doi.org/10.1016/0960-8524\(94\)90102-3](https://doi.org/10.1016/0960-8524(94)90102-3)
- Sheppard, D. C., Tomberlin, J. K., Joyce, J. A, Kiser, B. C., & Sumner, S. M. (2002). Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, 39(4), 695–698. <https://doi.org/10.1603/0022-2585-39.4.695>
- Sheppard, C., Newton, G. L., & Burtle, G.,(2007). Black Soldier Fly Prepupae. A Compelling Alternative to Fishmeal and Fish Oil. University of Georgia, Tifton.
- Sklan, D., Prag, T., & Lupatsch, I. (2004). Apparent digestibility coefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus* x *Oreochromis aureus* (Teleostei, Cichlidae). *Aquaculture Research*, 35(4), 358–364. <https://doi.org/10.1111/j.1365-2109.2004.01021.x>
- Sprangers, T., Ottoboni, M., Klootwijk, C., Olyn, A., Deboosere, S., De Meulenaer, B., ... De Smet, S. (2016). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*. <https://doi.org/10.1002/jsfa.8081>
- St-Hilaire, S., Sheppard, C., Tomberlin, J. K., Irving, S., Newton, L., McGuire, M. a., ... Sealey, W. (2007a). Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society*, 38(1), 59–67. <https://doi.org/10.1111/j.1749-7345.2006.00073.x>
- Stamer, A., Wesselss, S., Neidigk, R., & Hoerstgen-Schwark, G. (2014). Black Soldier Fly (*Hermetia illucens*) larvae-meal as an example for a new feed ingredients class in aquaculture diets. Rahmann, G.& Aksoy, U. (Eds.) (2014) *Proceedings of the 4th ISOFAR Scientific Conference. "Building Organic Bridges", at the Organic World Congress 2014, 13-15 Oct., Istanbul, Turkey (Eprint ID 24223)*, 13–15.
- Stankus, A. (2013). Integrating Biosystems to foster Sustainable Aquaculture : MSc

Thesis. University of Hawaii, Manoa.

- Steiner, C., Das, K.C., Melear, N., Lakly, D., 2010. Reducing nitrogen loss during poultry litter composting using biochar. *J Environ Qual.* 39, 1236-1242.
- Tacon, A. G. J., & Metian, M. (2009). Fishing for Aquaculture: Non-Food Use of Small Pelagic Forage Fish—A Global Perspective. *Reviews in Fisheries Science*, 17(3), 305–317. <https://doi.org/10.1080/10641260802677074>
- Tomberlin, J. K., Sheppard, D. C., & Joyce, J. A. (2002). Selected Life-History Traits of Black Soldier Flies (Diptera: Stratiomyidae) Reared on Three Artificial Diets. *Annals of the Entomological Society of America*, 95(3), 379–386. [https://doi.org/10.1603/0013-8746\(2002\)095\[0379:SLHTOB\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2002)095[0379:SLHTOB]2.0.CO;2)
- Tomberlin, J. K., Adler, P. H., & Myers, H. M. (2009). Development of the black soldier fly (Diptera: Stratiomyidae) in relation to temperature. *Environmental Entomology*, 38(3), 930–934. <https://doi.org/10.1603/022.038.0347>.
- Veldkamp, T., & Bosch, G. (2015). Insects : a protein-rich feed ingredient in pig and poultry diets, *Animal Frontiers*, 5(2), 45–50. <https://doi.org/10.2527/af.2015-0019>
- Vidal, L. V. O., Xavier, T. O., Michelato, M., Martins, E. N., Pezzato, L. E., & Furuya, W. M. (2015). Apparent Protein and Energy Digestibility and Amino Acid Availability of Corn and Co-products in Extruded Diets for Nile Tilapia, *Oreochromis niloticus*. *Journal of the World Aquaculture Society*, 46(2), 183–190. <https://doi.org/10.1111/jwas.12184>
- Vincke, M.M. (1969) Compte-rendu d'activite annee. Madagascar: Division des Recherches Piscicoles, Centre Technique Forestier Tropical, (30 pp).
- Vrij, M., (2013). Insects as alternative raw material for use in fish feeds. *Aquacultuur*, 1–8.
- Weiss, W., & Buchinger, J. (2004). Establishment of a production, sales and consulting infrastructure for solar thermal plants in Zimbabwe (www.aee-intec.at). *Institute for Sustainable Technologies*, 110. Retrieved from <http://www.aee-intec.at/0uploads/dateien553.pdf>
- Zhang, J. B., Huang, L., He, J., Tomberlin, J. K., Li, J. H., Lei, C. L., Yu, Z. N. (2010). An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. *Journal of Insect Science*, 10(202), 202. <https://doi.org/10.1673/031.010.20201>.
- Zhou, Q.-C., Tan, B.-P., Mai, K.-S., & Liu, Y.-J. (2004). Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. *Aquaculture*, 241(1–4), 441–451. <https://doi.org/10.1016/j.aquaculture.2004.08.044>

APPENDICES

Appendix I. Protein requirements (%) for Nile Tilapia

Life stage	Weight (g)	Protein Requirement (%)
First feeding		45-50
Fry	0.02 - 1.0	40
Fingerlings	1.0- 10.0	35 - 40
Juveniles	10.0 - 25.0	30 - 35
Adults	25 - 200	30 - 32
	>200	28 - 30
Broodstock		40 - 45

(Shaiu, 2002; El-Sayed, 2004; Ng and Romano, 2013)

Appendix II. The essential amino acid (% of protein) for Nile Tilapia

Essential amino acid	Dietary Protein (%)
Arginine	4.20
Histidine	1.72
Isoleucine	3.11
Leucine	3.39
Lysine	5.12
Methionine	2.68
Phenylalanine	3.75
Threonine	3.75
Tryptophan	1.00
Valine	2.80

Source:<http://www.fao.org/fishery/affris/species-profiles/nile-tilapia/nutritionalrequirements/en/>. (Shaiu, 2002; El-Sayed, 2004; Ng and Romano, 2013)

Appendix III. Recommended Amounts of Vitamin and Mineral Mix in Tilapia Diet

Vitamins	Amount (mg/kg)	I. Units
Thiamin	11	
Folic acid	5	
Riboflavin	20	
Vitamin B12	0.01	
Pyridoxine	11	
Choline	275	
Panthenic acid	35	
Nicotinic	88	
Ascorbic acid (C)	375	
Vitamin K	4.4	
Vitamin A		4400
Vitamin D3		2200
Vitamin E		66
Minerals	g/kg	
Calcium (Ca)	3.0	
Phosphorus (P)	7.0	
Magnesium (Mg)	0.5	
Iron (Fe)	0.15	
Zinc (Zn)	0.20	
Copper (Cu)	0.003	
Manganese (Mn)	0.013	
Selenium (Se)	0.0004	
Iodine (I)	0.001	

Source: (Shaiu, 2002; El-Sayed, 2004; Ng and Romano, 2013)

<http://www.fao.org/fishery/affris/species-profiles/nile-tilapia/nutritional-requirements/en/>.

Appendix IV. Effect of Nutritional Deficiencies in Nile tilapia.

Nutrient	Deficiency Signs/ Syndrome
Essential amino acid	
Lysine	Dorsal/caudal fin erosion, retarded growth, increased mortality
Methionine	Retarded growth, cataract
Tryptophan	Retarded growth, scoliosis, lordosis, caudal fin erosion
Essential fatty acid	Retarded growth, swollen pale liver, fatty liver
Minerals	
Phosphorus	Lordosis, poor growth
Calcium	Reduced growth, poor feed conversion, and bone mineralisation
Potassium	Reduced growth and feed efficiency, anorexia, convulsions
Magnesium	Reduced growth/whole-body hypercalcinosis
Iron	Microcytic, homochronic anaemia
Zinc	Reduced growth and appetite, cataracts, high mortality, erosion of fins and skin, short body dwarfism, fin erosion.
Manganese	Reduced growth and skeletal abnormalities, anorexia, loss of equilibrium
Copper	Reduced growth, cataracts
Selenium	Increased mortality, muscular dystrophy, reduced growth, cataracts, anaemia
Iodine	Thyroid hyperplasia (goitre)
Vitamin	
Vitamin E	Hemorrhages, lordosis, exophthalmia

Source: (NRC, 1993; Jauncey, 2000).

<http://www.fao.org/fishery/affris/species-profiles/nile-tilapia/nutritional-requirements/en/>.

APPENDIX V. Cost of Ingredients and Feed (based on current market price) used for Growth Trial

Feed item	Cost/kg (GHS)
Fishmeal	3.6
BSF Larval Meal	2.38
Soybean Meal	2.2
Wheat bran	0.6
Maize (white)	1.2
Cassava Flour	3.3
Common Salt	2
ARDEC	3.19
RAANAN	4.6

Appendix VI. Amount (kg) and cost (GHS) of various feed items fed to *O. niloticus* fingerlings.

Feed item	A (ARDEC FEED)		B (RAANAN FEED)		C (BSF 25)		D (BSF 50)		E (BSF 75)	
	Kg	Cost	kg	cost	kg	cost	kg	cost	kg	cost
Fishmeal	-	-	-	-	6.83222	24.59597	4.5279	16.30044	2.19609	7.905924
BSFLM	-	-	-	-	2.27741	5.078613	4.5279	10.09722	6.58827	14.69184
Soybean	-	-	-	-	0.91096	2.004116	2.26395	5.388201	3.952962	8.696516
Wheat bran	-	-	-	-	1.67010	1.002058	2.26395	1.35837	1.551904	0.931142
Maize(white)	-	-	-	-	1.71565	2.058774	0.75465	0.90558	0.146406	0.175687
Cassava flour	-	-	-	-	1.62455	5.361011	0.60372	1.992276	0.058562	0.193256
Common Salt	-	-	-	-	0.15183	0.303654	0.15093	0.30186	0.146406	0.292812
ARDEC	15.1164	43.39918	-	-	-	-	-	-	-	-
RAANAN	-	-	14.5938	67.1315	-	-	-	-	-	-
		4.339918		0	-	4.04042		3.634394		3.288718
Total	15.1164	47.7391	14.5938	67.13148	15.18270	44.44462	15.093	39.97834	14.6406	36.1759"

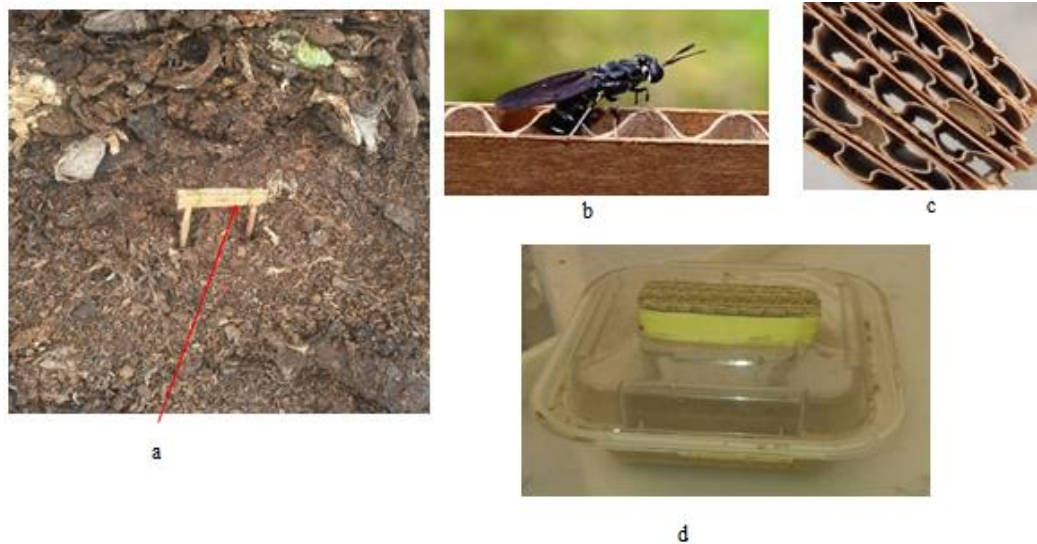


Plate 1: Obtaining BSF eggs. (a) Strips of corrugated cardboard (b) Female BSF laying eggs (c) clutches of egg (d) Nursery box



Plate 2: Indoor breeding of BSF



Plate 3: Inoculation, sampling and drying of BSF larvae. (a) Plastic bins containing food wastes and larvae (b) Removal of larvae from feed treatment (c) Drying of larvae (d) Greenhouse solar dryer.



Plate 4: Dietary treatments for growth trial



Plate 5: Experimental pond setup



Plate 6: Culture management practices. (a) Mounting of hapas. (b) Stocking of fingerlings. (c) Administering feed. (d) Sampling.



Plate 7: Experimental tanks setup for digestibility test.