



University of Natural Resources
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Growth performance and survival rate of Nile tilapia (*Oreochromis niloticus* L.) reared on diets containing Black soldier fly (*Hermetia illucens* L.) larvae meal

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by

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DECLARATIONS

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I Rita Nkirote Nairuti declare that this thesis is my original work and has not been presented for award of a degree in any university or institution.

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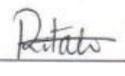
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DEDICATION

To my dearly beloved parents, Mr Robert Kabithi Daniel and Mrs Veronica Nthiori.

My siblings Fridah Makena Nairuti and Lewis Kinoti Nairuti.

You will forever be my heroes.

“The most important thing in life is to have a great aim and the determination to attain it”

(Johann Wolfgang von Goethe, 1748)

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ABSTRACT

Aquaculture, as a food industry continues to be an important source of high-quality protein for the global population. However, following the rapid growth and development of the industry, there is increasing demand for feeds rich in high protein. Fish meal (FM) continues to be an important source of protein in fish feeds, but its sustainability has been questioned following the overexploitation, leading to its scarcity and high prices. To evaluate a promising alternative source of protein for fish meal, a 72-day study was conducted at an experimental station, KMFRI-Sagana, Kenya, to assess the effects of replacing fish meal (FM) with different proportions of black soldier fly larvae (BSFLM) in the complete feed on the growth performance and survival rates of Nile tilapia. Four test diets were formulated with substitutions of FM by BSFLM made at 0%, 33.3%, 66.7% and 100%. 240 male Nile tilapia ($52.3 \pm 0.29\text{g}$ mean weight) were divided into 4 groups (4 replicates/group) and placed in 16 hapa nets (15 fish/hapa), mounted in an 800m² earthen pond. Fish were fed twice per day at 5% of body weight. Sampling of the fish took place every two weeks; mortalities were recorded daily while the physico-chemical parameters were monitored weekly. The growth performance and survival rates were not significantly different ($p > 0.05$) between treatments. In conclusion, the present study indicates that even full fat BSFLM can replace up to 100% of the FM without negative effects on the growth performance and survival rates of Nile tilapia. To improve the protein amount in the larvae, defatting is recommended before utilising it for fish feeds. Similarly, studies should be done to determine the contribution of the culture system to the substitution and utilization of the larvae. Additionally, economic viability should be examined before BSFLM is used commercially so as to determine the profit margins. Overall, *Hermetia illucens* protein could be a promising option to make *O. niloticus* feed formulation more economical and sustainable in the long term.

Keywords: *Hermetia illucens*, Nile tilapia, Fish meal, Growth performance, Survival rate, Dietary protein source, Insect meal, Defatting.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADF	Acid detergent fibre
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association.
BOD	Biological oxygen demand
BSFLM	Black soldier fly larvae meal
BWG	Body weight gain
CF	Crude fibre
CM	Chicken manure
CP	Crude protein
D1	Diet 1
D2	Diet 2
D3	Diet 3
D4	Diet 4
DO	Dissolved oxygen
DM	Dry matter
ESP-FFEPP	Economic Stimulus Programme - Fish Farming Enterprise Productivity Programme
EAA	Essential amino acids
EE	Ether extracts
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
FM	Fish meal
FWS	Freshwater shrimp
H ₂ SO ₄	Sulfuric acid
KW	Kitchen waste

ME	Metabolizable energy
MT	Metric tonnes
GHG	Greenhouse gas emission
HCL	Hydrochloric acid
KMFRI	Kenya Marine and Fisheries Research Institute
MS	Microsoft excel
NaOH	Sodium hydroxide
NDF	Neutral detergent fibre
NFE	Nitrogen Free Extracts
PER	Protein efficiency ratio
SG	Spent grain
SGR	Specific growth rate
SPSS	Statistical Package for the Social Sciences
SR	Survival rate

CHAPTER ONE

INTRODUCTION

1.1 Background information

The global population is rising, and projections have shown that by 2050, approximately 9.7 billion people will be inhabiting the globe (Bene et al., 2015; Shumo et al., 2019). This increase in population is directly impacting pressure on the global food basket. To meet the food demands of the increasing population, 70% more food needs to be produced by the world (FAO, 2009; Schiavone et al., 2017; Shumo et al., 2019). World bank projections show that the developing countries will account for 91% of the total fish consumption (Anderson et al., 2001).

Both capture fisheries and aquaculture sectors have been geared into providing fish products to the increasing population and have been rising by a factor of 8 since 1950 (Tidwell and Allan, 2001). However, the capture fisheries have been over exploited leading to massive reduction in the yields. FAO (2010) and Barroso et al. (2014) reported a 3% decline in the capture fisheries yields between 2004 and 2009. This led to more focus being put in aquaculture to increase the yields to bridge the gap. Currently, aquaculture is one of the fastest growing food sectors (Bene et al., 2015). A growth rate of 5.6 % per year (FAO, 2010) is a strong growth indicator. Additionally, a report by FAO (2016), indicated that aquaculture production accounts for half the fish products consumed globally.

Aquaculture production is highly dependent on quality protein feeds and hence the fish meal (FM) alongside fish oils are the main drivers of success of the industry. Due to the high protein, lipid and amino acid profile, fish meal has been highly utilised as a feed ingredient with reports indicating that in 2011, 75% of all the total world fish meal was used in aquaculture production (Shepherd and Jackson, 2013). However, the product is becoming scarce day in day out spelling doom for the aquaculture industry. The scarcity may be attributed to the elnino effects, overexploitation, excessive use of pesticides and forest clearances (Rana and Hasan, 2009). The scarcity of the resource is directly impacting on the price of the commodity hence increasing the production cost in fish farming (Ayoola, 2010).

Scarcity and high cost of fish meal has prompted researchers to seek for high quality, less expensive and sustainable alternative protein sources for fish feed formulation (Schiavone et al., 2017). Fish nutritionists have used plant and animal-based products for the substitution of FM; however, the plant-based products have antinutritional compounds that hinder them

from successfully replacing the FM. Further, most of the animal-based products are utilised in the manufacture of other animal feeds and as human food and hence could be conflicting with the main goal of human nutrition hence a need to find an alternative source of protein for FM.

Insect meals from raised insects have shown potential success in the replacement of fish meal (Tacon et al., 1983). The African catfish (*Clarias gariepinus*) has shown positive growth performance when reared with housefly maggots at 25-30% inclusion levels (Adewolu et al., 2010). Consequentially, high survival rates and feed conversion ratios have been reported in catfish fed with housefly maggots at 30% and 20% inclusion, respectively (Idowu et al., 2013). Black soldier fly larvae (BSFL) meal has also shown to be a promising fish meal replacement in the diets of the pacific white shrimp at < 25% inclusions level (Cummins et al., 2017). This study focuses on assessing the growth performance and survival rates of Nile tilapia (*Oreochromis niloticus*) fed on different proportions of BSFL meal as an alternative dietary protein source for FM.

1.2 Problem statement

Fish feeds contribute to over 60% of the total operational costs in a fish farm (Munguti et al., 2012). Protein is a key component needed in the fish feeds. However, it is the most expensive dietary component in fish feeds (El-Sayed, 1999; Munguti et al., 2012). Fish meal has been an important component in fish diets due to its high digestibility and acceptance alongside its high protein, essential amino acids and fatty acids profiles (Tacon and Metian, 2009; Yildirim, 2009). Recent studies have shown that the fish meal stocks are declining, and this scarcity is leading to increased prices of the product hence increasing the fish production costs (Ayoola, 2010; Barroso et al., 2014; Muin et al., 2017). There is an urgent need to find a high quality, cost efficient and sustainable alternative protein source for FM (Schiaivone et al., 2017) to be used in the formulation for the Nile tilapia (*Oreochromis niloticus*) diets that will enhance optimum growth and development of the species and further allow aquaculture production to remain economically and environmentally viable over generations (Ayoola, 2010).

1.3 Justification

By the year 2030, the Kenyan population is expected to reach 67 million (World Population Prospects, 2017). The current consumption rate of fish in Kenya is at 4.5 kilograms per person per year (Opiyo et al., 2018) and is argued by Rothuis et al. (2011) to be lower than the Food and Agriculture Organization (FAO) average recommended consumption rate of 20

kg/person/year. The capture fisheries showed a decline in the yields from 170,000 tonnes in 2014 to slightly lower than 120,000 tonnes in 2017 (FAO, 2018). Further, the fish imports also declined from 36,000 tonnes to 23,000 tonnes in 2006 and 2016, respectively (FAO, 2018). To feed the growing population and maintain the per capita consumption rate (4.5 kg/yr), approximately 270,000 tonnes of fish is needed to be produced by 2030. If the capture fisheries are revived and stabilized at 120,000 tonnes, this would still mean that aquaculture is to contribute additional 150,000 tonnes to meet the 270,000 tonnes fish demand by 2030 (FAO, 2018).

Aquaculture is among the fastest growing food industry in Kenya. The industry is focused on providing quality, affordable and reliable protein source to improve the livelihoods, curb malnutrition and reduce food insecurity. At an annual growth rate of about 11.5%, Nile tilapia is the most preferred culture species in the tropical and subtropical regions in the world (El-Sayed, 1999), accounting for about 75% of production (Munguti and Ogello, 2014). Successful fish farming ventures require the use of protein rich feeds. Although various animal and plant-based products have been utilised for the rearing of *Oreochromis niloticus* (El-Sayed, 1999) FM has still been on the lead as the main source of protein in the fish feeds. However, studies show that the supply is dramatically decreasing, leading to its high prices. BSFL meal is an insect-based meal that has shown potential of replacing the FM due to its availability, low cost of production and its high-quality protein content.

1.4 Objectives

1.4.1 General Objective

To assess the nutritive value of black soldier fly larvae meal and the growth performance and survival rates of Nile tilapia (*Oreochromis niloticus*) fed on this alternative source of protein in Kenya.

1.4.2 Specific objectives

1. To assess the background information of the black soldier fly larvae production in Kenya
2. To compare the nutritive composition of locally produced black soldier fly larvae and fish meal used in the formulation of Nile tilapia diets.
3. To determine the growth performance and survival rates of Nile tilapia fed on diets containing different proportions of locally produced black soldier fly larvae meal.

1.5 Research questions

1. What is the proximate composition of locally produced black soldier fly larvae meal and fish meal used in the formulation of the Nile tilapia diet?
2. What is the growth performance and survival rates of Nile tilapia raised on different proportions of locally produced black soldier fly larvae meal as dietary replacement for fish meal?
3. What is the optimal inclusion rate of locally produced black soldier fly larvae meal in Nile tilapia diets?

1.6 Hypothesis

1. The proximate composition of locally produced black soldier fly larvae is similar to that of fish meal, but it may be lacking a few essential amino acids.
2. At 33.3% inclusions rate, the growth and survival parameters of the Nile tilapia will be optimum, a further increase in substitution will have a negative effect on the growth and survival of the fish.

CHAPTER TWO

LITERATURE REVIEW

2.1 Status, trends, challenges and future outlook of aquaculture in Kenya

According to Ngugi et al., (2007) aquaculture in Kenya took root in the early 1900s through introduction of trout in rivers for sport fishing. Later, in the 1920s the sport fishing advanced to static pond culture which involved the culturing of tilapia, common carp and catfish species. Sagana and Kiganjo trout fish farms were the pioneers of small-scale rural fish farming. The farms focused on rearing the warm water and cold-water species respectively (Ngugi and Manyala, 2004; Ngugi et al., 2007). By the year 1960, aquaculture had become popular in most regions around the country with a dramatic growth of the sector being observed over the last decade. Currently, aquaculture is majorly practised in Kakamega, Bungoma, Busia, Kisii, Meru, Nyeri, Kisumu, Muranga and Embu counties with less practice in Kitui, Lamu and Elgeyo Marakwet (Opiyo et al., 2018).

Approximately 1.4 million hectares of land is suitable for aquaculture production in the country, however of this, only 0.014% is currently being utilised with 95% of the practice being small scale (Otieno, 2011). In 2003, thanks to the “Eat More Fish Campaigns” championed by the government, the production rose from 1000 to 4000 Metric tonnes (MT) (Opiyo et al., 2018). By 2007, approximately 722.4 hectares of land was being used by about 7500 farmers for production of fish from approximately 7,477 production units (Nyonje et al., 2011; Munguti et al., 2014). From these units, the mean yield was estimated to be approximately 5.84 MT/yr which accounted for only 3% of the total fish production (Munguti et al., 2014).

In an attempt to alleviate poverty, curb malnutrition, enhance economic development, promote economic recovery and trigger regional development in Kenya (Nyonje et al., 2011) the government channelled Kes 22 billion into key sectors, the major beneficiary being the aquaculture sector. From this, the ambitious Economic Stimulus Programme-Fish Farming Enterprise Productivity Programme (ESP - FFEPP) was born in 2009. “The programme aimed to increase production of farmed fish from 4,000 MT to over 20,000 MT in the medium term and to more than 100,000 MT in the long term” (Charo-Karisa and Gichuri, 2010).

The first year of the programme foresaw the construction of over 200 new fishponds. The fishpond area increased from 220 hectares in 2008 to 468 hectares in 2009 while the total gross land for aquaculture rose to 825 hectares (2009) from 728 hectares (2008) (Charo-Karisa and Gichuri, 2010; Musa et al., 2012; Opiyo et al., 2018). With these, the yields rose

from 4895 MT to 12,153MT/yr (Nyonje et al., 2011; Opiyo et al., 2018) and placed Kenya 4th in the aquaculture production in Africa (Nyonje et al., 2011).

Positive growth in fish production in the country was seen following the ESP - FFEPP. In 2014 a peak production was experienced at 24,096MT. However, in 2015 the production dropped to 18,656MT and further to 14,952 MT in 2016 (Opiyo et al., 2018). The reduction in the number of operational fishponds from 69,194 (2013) to 60,277 (2015) and the shrinking of the operational area from 2,105 to 1,873 hectares in 2013 and in 2015 respectively was attributed to by various factors among them; poor extension services to the beneficiaries, inadequate capacity building, poor husbandry practices by the farmers, inadequate quality and quantity of fish farm inputs like feeds, poor marketing infrastructure and dependency syndrome on government and donor support. The major blow was felt when the aquaculture sector was devolved from the national government into the county government operations. Counties that lacked sufficient support programmes for fish recorded a reduction in aquaculture practices (Opiyo et al., 2018).

2.2 Cultured fish species in Kenya

The culture of *Oreochromis niloticus* dominates the warm water aquaculture in Kenya followed by *Clarias gariepinus* that make up to 75% and 18% of the total production respectively (Opiyo et al., 2018). Tilapia occurs in most culture systems and this is due to its adaptability and prolific breeding. Additionally, they have a high consumer preference both in the local and regional markets. To control the breeding and populations of tilapia in the ponds, sex reversal of tilapia alongside polyculture of the African catfish and tilapia is adopted.

Farm Africa (2016) listed the common carp - *Cyprinus carpio* (6%), rainbow trout - *Oncorhynchus mykiss* (1%), koi carp - *Cyprinus carpio carpio*, goldfish - *Carassius auratus* and largemouth bass as the exotic species cultured. African Carps (*Labeo victorianus*), Ngege (*O. esculentus*), Victoria tilapia (*O. variabilis*), Tilapia jipe and Lungfish are the indigenous species cultured in the country (Charo-Karisa and Maithya, 2010; Maithya et al., 2017; Opiyo et al., 2018; Orina et al., 2018).

2.3 Nile Tilapia (*Oreochromis niloticus*)

The culturing of tilapia dates back to over 4,000 years ago. Ornamental fishponds were used to culture the species in the prehistoric Egyptian times (Balarin and Hatton, 1979; FAO, 2007). Tilapia belong to the family Cichlidae and is a warm water fish species reared in fresh

water (El-Sayed, 2006). Worldwide distribution of Mozambique tilapia (*Tilapia mossambicus*) was in early 1930s while the worldwide distribution of Nile tilapia occurred in the 1960s to 1980s (FAO, 2006; Russell et al., 2012). Apart from the Nile tilapia and *Tilapia mossambicus*, Norman and Bjørndal, (2010) stated that there are over 70 tilapia species but only 10 are farmed globally. Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*O. mossambica*), Blue tilapia (*O. aureus*), Mango tilapia (*Sarotherodon galilaeus galilaeus*), Black chin tilapia (*S. melanotheron*), Longfin tilapia (*O. macrochir macrochir*), Redbelly tilapia (*Tilapia zilli*), Redbreast tilapia (*Tilapia rendalli*), Sabaki tilapia (*O. spirulus spirulus*) and Three spotted tilapia (*O. andersonii*) are the species listed (FAO, 2006).

Nile tilapia is the most cultured species in Africa with Popma and Masser (1999) and Lorenzen et al. (2001) indicating that 90% of the cultured species in Africa is of *Oreochromis niloticus* and this could be due to their rapid growth rates, adaptability to a wide range of environmental conditions, resistance to stress hence allow for relatively high stocking densities, high resistance to diseases in poor environmental conditions, ability to grow and reproduce in captivity and feed on low trophic levels (El-Sayed, 2006; Ngugi et al., 2007). Reports by Chervinski (1982) indicated that Nile tilapia is native to Africa and Israel. In Africa, the species is absent only in the Northern Atlas and South West Africa. However, Pillay (1990) noted that in the 20th century the species was introduced into the tropical, subtropical and temperate regions of the world.

2.4 Factors that affect the growth and survival of Nile tilapia

Nile tilapia exhibit excellent growth rates at water temperatures between 22 and 29°C, preferably 28°C (Morgan, 1972). Temperatures below 20°C inhibit spawning of the fish while temperatures below 16°C leads to limited growth rate (Morgan, 1972). Reduced feeding activities of the fish is observed at 15°C and any further decrease in the temperatures (< 12°C) is noted to lead to absolute mortalities of the fish if no intervention is made (Yashouv, 1960; El-Sayed, 2006; Ngugi et al., 2007).

When temperature and pH are kept favourable for the fish, tilapia is able to survive levels of dissolved oxygen lower than 3mg/L (Ngugi et al., 2007). Amounts of organic manures applied in the ponds for fertilization should be well monitored since the manures can lead to algal blooms in the ponds, consequentially reducing the oxygen amounts especially during the night. Death of algal blooms can also lead to oxygen depletion in the ponds due to the high biological oxygen demand (BOD) during the organic matter breakdown.

According to Bowen (1982) and El-Sayed (2006), *O. niloticus* is able to withstand a wide range of salinity hence are cultured successfully in brackish waters. Ngugi et al. (2007) noted that *O. niloticus* is able to grow and spawn effectively in salinity levels of up to 19 mg/L. A study by Fineman-Kalio (1988) observed the development of the gonads and spawning of *O. niloticus* at salinities between 17 - 29 ‰.

Nile tilapia thrives in pH ranges between 6.5 and 9; exposure of the fish to extreme pH conditions leads to epithelial cell damage (El-Sayed, 2006; Ngugi et al., 2007). The unionized ammonia is lethal to fish, most of the ammonia is from the fish excreta and feed remains. Ammonia levels in the ponds should be maintained at less than 0.01 mg/L (El-Shiafey, 1998). Ionized ammonium is not toxic to fish however toxicity is found to increase with decreasing levels of dissolved oxygen (DO) while an increase in carbon dioxide leads to decrease in its toxicity (Chervinski, 1982).

2.5 Nutritional requirements of Nile tilapia

Nile tilapia are omnivorous (Ngugi et al., 2007). They exhibit ontogenetic feeding niche shifts and diel feeding pattern (FAO, 2019) whereby the food is ingested during the day and digested at night (Trewavas, 1983). Tilapia fry feed on live animal feeds (zooplankton) and later in their adult stages they filter large amounts of plant material from the water body (phytoplankton, bacteria, macrophytes and detritus) (Moriarty, 1973; Getachew 1987; Getachew and Fernando 1989; Diana et al, 1991; Dempster et al., 1993; Munguti et al., 2007). The pH of 2 in the stomach facilitates the lysis of the algal cells. The possession of a long gut (6 times the body length of the fish) allows for sufficient digestion and absorption of nutrients from the algae fed on (Opuszynski and Shireman, 1995).

To increase the production in a pond, farmers supplement the live feeds with artificial commercial feeds that have high protein, quality carbohydrates and fats, vitamins and minerals (Steven et al., 1984). The protein requirements for tilapia decreases with increase in size of the fish (Munguti et al., 2012). Tilapia juveniles require protein levels between 30 to 40%, while the mature tilapia require 20–30% dietary protein amounts in their diets for optimum growth (Liti et al., 2006).

2.6 Status of fish feeds in Kenya

High cost and unavailability of quality fish feeds for the fry, fingerlings, juveniles and adult fish remain to be a major challenge in the aquaculture industry in Kenya. The challenge was worsened by the high demand for fish feeds following the government programmes. The stocking of ponds by the government under the ESP - FFEPP with approximately 28 million certified fingerlings prompted a demand of over 14,000 MT of formulated fish feeds (Musa et al., 2012). Since then, demand has increased dramatically to about 50,000MT/ year (Munguti et al., 2014). This saw different unqualified fish feed dealers penetrating the industry and provided fish feeds that had been compromised in quality (Munguti et al., 2014). Further, the frequent closure of the Lake Victoria fisheries also created temporary shortages in the availability of fish meal (Munguti et al., 2009). To date, the government alongside other donors and researchers are looking for alternatives to reduce the fish feed challenge in the country.

Fish feed types available in Kenya include mash and powdered feeds for the fry and fingerlings, on farm pelleted fish feeds, pressed pellets made by cottage industries and extruded floating pellets that are mainly imported from Netherlands, Norway, Denmark, Israel and Ghana. (Munguti et al., 2014, Opiyo et al., 2018). Floating pellets are best since one can easily get to know if all the feed is eaten by the fish and make adjustments to the feed rations, further the floating pellets are less harmful to the water quality in the ponds, easy to store and less susceptible to nutrient oxidations (Landau, 1992; Pillay and Kutty, 2005).

Feeds are administered depending on the body weight of the fish (Munguti et al., 2009) and the feeding of the fish can be done through the use of automatic feeders, use of demand feeders and use of hand to feed the fish to satiation (Pillay and Kutty, 2005). Researchers recommend feeding fry and fingerlings at 5 to 8% of their body weight while the adults are to be fed 3% of their body weight. Since temperature and DO influence the metabolism of fish, feeding should be done when the temperatures are favourable and the dissolved oxygen is high, preferably in the morning and evening (Pillay and Kutty, 2005). Pillay and Kutty (2005) further noted that the fish can be fed anywhere from 2 times to 6 times a day, depending on the weather and life stage of the fish.

To lower the production cost and make the fish farming venture profitable, the farmers in Kenya narrow down into the utilization of on farm feed formulation using the locally available ingredients (Munguti et al., 2014). The ingredients are of both animal and plant-based origins. Though the feeds formulated sustain the fish, they are most times lacking the minimum recommended protein levels and are lacking in most of the essential amino acids. Farmers also

opt to fertilize their ponds using organic manure from livestock and encourage the growth of plankton that serves as live feeds for the fish (Munguti et al., 2014). White (2013) stated that most of the farmers unfortunately focus on reducing the cost of production unaware of the nutrient requirements of their farmed fish species.

Plant-based ingredients are from the products and by products of agriculture while freshwater shrimp (*Caridina nilotica*) and FM - Omena (*Rastrineobola argentea*) are the main animal-based ingredients used in the formulation of fish feeds. The locally available ingredients used by fish farmers in Kenya for the formulation of on farm fish feeds are; grasses, leaves (e.g. cassava and arrowroot) and seeds of leguminous shrubs; aquatic plants like the *Lemna*, water hyacinth and water lettuce; by-catch fish from the lakes; rice (broken, bran, hulls); wheat (germ, bran); maize (bran, germ); seed cakes (groundnut, cotton, sunflower, soybean); brewers waste; slaughterhouse wastes and blood-meals (Liti et al., 2006; Munguti et al., 2012; Munguti et al., 2014).

The main challenge is that these ingredients despite their high protein profiles they are lacking in one or more essential amino acids (EAA) profiles (Munguti, 2007), have antinutritional components, multiple uses and contribute to an increase in the Greenhouse gas emission (GHG) during the production process (Mertenat et al., 2019). Table 1 provides a summary of the fish feed suppliers in Kenya.

Table 1. Fish feed suppliers in Kenya. Adapted from (Munguti et al., 2014; Opiyo et al., 2018).

Company Name	Type	Location	Type of Feed
<i>Commercial manufacturers</i>			
Sigma Feeds Ltd	Local	Rongai, Kajiado County	Floating pellets
Lenalia Fish feeds	Local	Limuru, Kiambu County	Floating and sinking pellets
Maisha Bora Fish Feeds Ltd	Local	Kikuyu, Kiambu County	Sinking pellets
Kwality Fish Feeds Limited	Local	Ruiru, Kiambu County	Sinking pellets
Sare Millers Ltd	Local	Kisumu County	Floating and sinking pellets
Jewlet Fish Farm Enterprises	Local	Kendubay, Homabay County	Floating and sinking pellets
Unga Feeds Ltd-Nairobi	Local	Industrial Area Nairobi	Floating pellets
Ugachick Fish Feeds	Imported	Uganda	Floating pellets
Raanan Fish Feeds	Imported	Nairobi County	Floating pellets
Nile Aqua	Imported	Uganda	Floating pellets
Skretting Fish Feeds	Imported	Nairobi County	Floating pellets
Aller Aqua fish Feeds	Imported	Nairobi County	Floating pellets
LFL Riche Terre	Imported	Nairobi County	Floating pellets
Food Tech Africa	Local	Nairobi County	Floating pellets
<i>Cottage feed industries</i>			
Othaya Fish Feeders S.H.G	Local	Othaya, Nyeri County	Sinking pellets
Chumara Fish Feeds	Local	Chuka, Meru County	Sinking pellets
Mabro Fish Farm Enterprises	Local	Usigu, Siaya County	Sinking pellets
Bidii Fish Farmers S.H.G	Local	Luanda- Emuhaya	Floating and Sinking pellets
Osifeeds Ltd.	Local	Kajiado County	Sinking pellets
Zibag Fish producers & Processors	Local	Nyandarua County	Sinking pellets
Hesao Integrated Fish Farming Organization	Local	Nyalenda B, Kisumu County	Sinking pellets
Dominion Fish Feed limited	Local	Siaya County	Sinking pellets
Nyawara Animal Feed Plant	Local	Gem, Siaya County	Sinking pellets
Kenya Marine and Fisheries Research Institute	Local	Sangoro, Kisumu County	Sinking pellets

2.7 The Black soldier fly

The black soldier fly (*Hermetia illucens*) belongs to the order Diptera and in the Stratiomyidae family (Zheng et al., 2013). *H. illucens* occurs in the tropical and sub-tropical regions of the world, between the latitudes 40°S and 45°N (Dortmans et al., 2017).

The *H. illucens* possess holometabolous metamorphosis (Rumpold and Schlüter, 2013). Their life cycle starts from an egg which also marks the end of the previous life stage (Dortmans et al., 2017). On laying the eggs, the female fly dies. A female fly lays 400 to 800 eggs in tiny, dry and well sheltered cavities and in close proximity to a food source, preferably decomposing organic matter. These moves protect the eggs from predators and prevent dehydration of the egg packages by direct sunlight alongside increasing the chances of the survival of the larvae on hatching (Dortmans et al., 2017).

On hatching, the cream - like larvae take up large amounts of decomposing organic matter and increase from few millimetres to approximately 2.5 cm in length and 0.5 cm in width (Dortmans et al., 2017). The voracious feeding allows for storage of enough fats and proteins to aid in the process of pupation, fly emergence, mating and oviposition. Larval development takes a period of 14 to 16 days.



Figure 1. Black soldier fly larvae. Adapted from icipe.org

Transformation of the larvae into a pupa is marked by change in colour from cream like to charcoal grey and modification of the mouth parts into a hook-shaped structure that enable the pupa to self-harvest into a dry, warm, shaded and safe environment secure from predators (Sheppard et al., 1994; Dortmans et al., 2017; Wang and Shelomi, 2017). Around two to three weeks the imago emerges from the pupa and flies out. The life span for the adult fly is one week, within this time the fly does not feed but drinks water and uses the protein and fats accumulated during the larval stage (Wang and Shelomi, 2017).



Figure 2. Black soldier fly. Adapted from Tony Kiragu, (2017)

2.8 Optimal environmental conditions and food sources for the BSFL

Ideal temperature: temperature influences the survival of the fly (Shumo et al., 2019). The black soldier fly larvae grow and reproduce optimally at temperature ranges between 24 and 30°C. Temperatures above 30°C force the larvae to move away from the substrate in search of cooler areas, this leads to reduced growth of the larvae since much of the energy is used in the movements. Metabolism and growth are also reduced when the temperatures drop lower than 24°C (Dortmans et al., 2017).

Shaded environment: larvae are seen to have a high preference of areas that are shaded.

Water content of the food: the larvae prefer food substrates with moisture content between 60% and 90%. Water is also seen to increase the life span of the adult fly (Wang and Shelomi, 2017).

Nutrient requirements of the food: the larvae are the only stage in the life cycle of the BSF that feeds. Successful larval growth and performance is highly influenced by the dietary composition in the substrates. Substrates rich in protein and easily available carbohydrates result in good larval growth (Dortmans et al., 2017). Bacterial or fungal decomposition aids in further breakdown of organic matter making it easier for the larvae to access the nutrients (Dortmans et al., 2017).

H. illucens often referred to as the loo maggots due to their close association with faecal matter are native to Kenya. They are found majorly in and around faecal waste piles of livestock, poultry, swine and humans. Further, they are found colonising wastes from processing plants. *H. illucens* exhibit phenotypic elasticity allowing them to adapt to any environmental variability (Shumo et al., 2019). Various researchers have demonstrated utilization of the black soldier fly meals as feeds in the aquaculture and livestock sectors (Newton et al., 2005). Further the residue products from the post treatment unit are rich in nitrogen and calcium hence can be used as organic manure for crop production (Dortmans et al., 2017; Lohri et al., 2016).

2.9 Nutritive composition of Black soldier fly larvae

Black soldier fly larvae (BSFL) to be used as feeds can either be partially or highly defatted. Defatting increases the amount of crude protein in the meal up to 60% but significantly lowers the lipid amount (Schiavone et al., 2017; Wang and Shelomi, 2017). BSFL contains approximately high crude protein (CP) and up to 35% lipids and have an amino acid profile that is similar to that of fish meal (Elwert et al., 2010; Barroso et al., 2014; Muin et al., 2017; Van Huis, 2018). The defatted BSFL meal has a relatively higher crude protein than soybean meal and other plant - based products like the maize germ, sunflower seed cake and cotton seed cake.

The mineral content in the BSFL is important. It is influenced by the processing methods and the type of substrates on which the BSFL grow on (Fasakin et al., 2003). The larvae accumulate calcium and manganese but not sodium or sulfur (Wang and Shelomi, 2017). A study by Ushakova et al. (2018) recorded high haemoglobin and glucose in the blood of *Tilapia mossambicus* fed on BSFL meal that had high manganese levels than in the control. However, black soldier fly larvae have been reported to have low polyunsaturated fats and to be limiting in methionine. (Toriz-Roldan et al., 2019). Table 2 gives a summary of both the essential and non-essential amino acids contained in the black soldier fly larvae.

Table 2. The relative composition of amino acids (g/100g protein) of black soldier fly larvae. Adapted from Toriz-Roldan et al. (2019).

Essential amino acids	g/100g protein
Lysine	5.14
Arginine	5.46
Histidine	6.08
Isoleucine	4.38
Leucine	6.64
Valine	4.04
Methionine	2.83
Phenylalanine	6.46
Threonine	14.71
Non-essential amino acids	g/100g protein
Aspartate	5.38
Glutamate	8.37
Serine	3.81
Glycine	2.68
Alanine	5.62
Tyrosine	6.16

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The experiment was conducted from October 2019 to January 2020 at Sagana fish farm of the Kenya Marine and Fisheries Research Institute (KMFRI) where the feed formulation, proximate analyses, feed trials alongside data collection were done.

KMFRI, Sagana was established in 1948 (Government of Kenya, 1992) under the colonial British Government rule with the primary objectives of providing extension services and the production of both market size and fish seeds for the local community and fish farmers.

The farm is located approximately 2 km from Sagana town in Kirinyaga county, roughly 105 km Northeast of Nairobi City. It lies at coordinates 0°19'S and 37°12'E (Figure 3) and at 1230 meters above the sea level.

The mean annual rainfall in the area ranges between 1166 and 1612mm/year (Veverica et al., 1999). The area experiences short rains from October to December while the long rains start in March and end in May. Temperature ranges between 17 and 26°C (Veverica et al., 1999). River Ragati supplies the farm with water by gravity through a 1.5 km long canal (Munguti et al., 2014). There is a total of 109 fishponds among which 72 are used for research and the rest are used for the culturing of tilapia, catfish and ornamental fish.

About 80% of the black cotton soils found in the area is made up of clay hence providing a suitable location for fish farming. Additionally, the gentle sloping land, availability of water all year round and favourable climatic conditions make KMFRI, Sagana a suitable area for the culturing of warm water fish species like Nile tilapia.

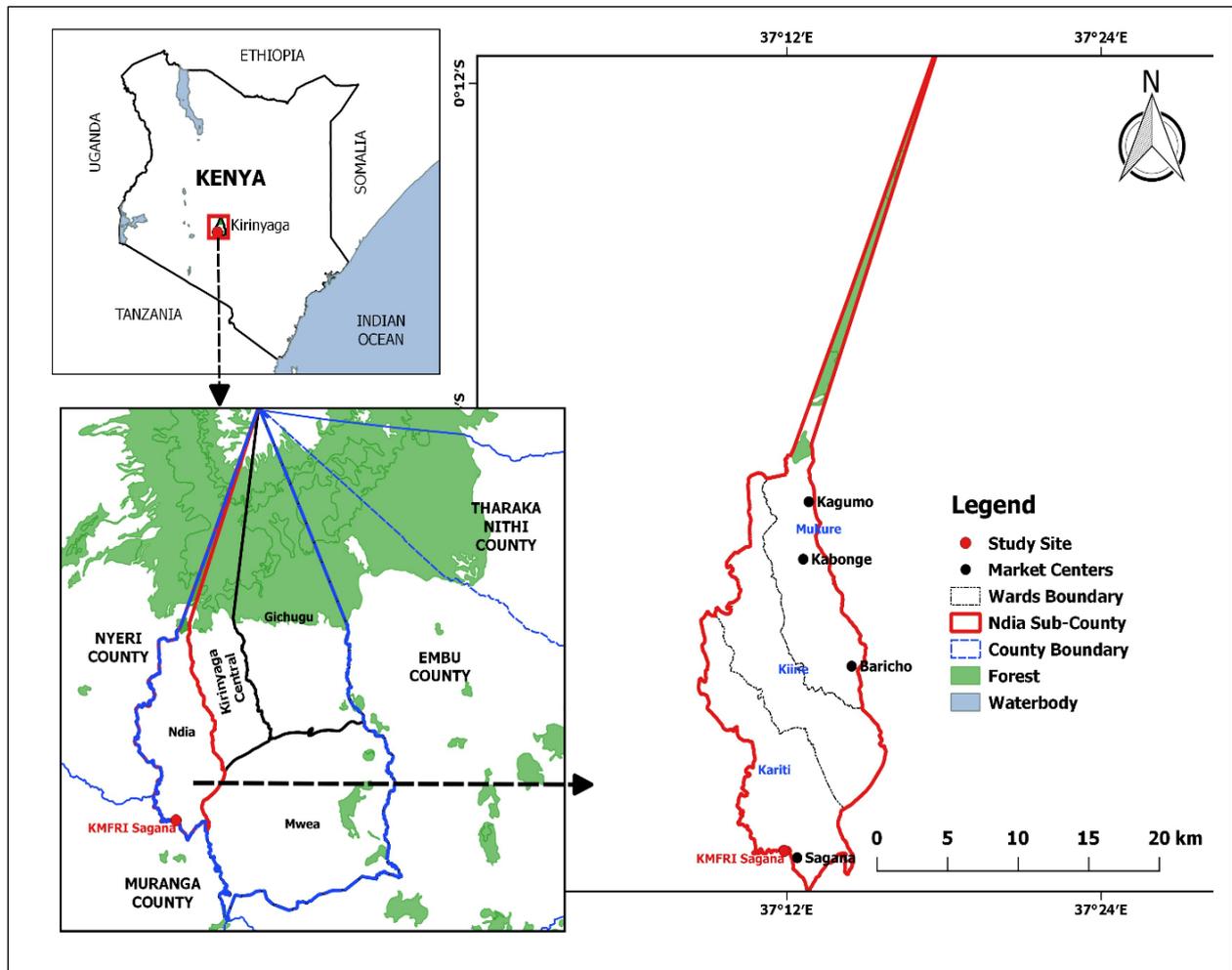


Figure 3. A map of Kenya showing the location of Sagana fish farm. Map prepared by Hassan Saeed (Egerton University).

3.2 Sampling design

3.2.1 Ingredients collection

The black soldier fly larvae (*Hermetia illucens*), Fish meal-Omena (*Rastrineobola argentea*), Freshwater shrimp (*Macrobrachium rosenbergii*), cotton seed cake (*Gossypium* spp), sunflower seed cake (*Helianthus annuus*) and maize germ (*Zea mays*) were sourced around local animal feed outlets in the Thika, Kiambu county. Ingredients were transported to the farm in air-tight containers.



Figure 4. Some feed ingredients used for the formulation of the test diets for the study. A - Black soldier fly larvae B - Fish meal; Omena (*Rastrineobola argentea*), C - Freshwater shrimp, D - Sunflower seed cake, E - Cotton seed cake, F - Maize germ.

3.2.2 Proximate analysis of individual ingredients and the formulated test diet

The ingredients and test diet's dry matter, crude protein (CP), crude fibre, ether extracts (EE) and ash determination were done at KMFRI – Sagana centre, generally following the procedure by Association of Official Analytical Chemists (AOAC) (1995) and given in Table 3 and 4 respectively. The Amino acids (AA) for the black soldier fly larvae and fish meal were analysed in triplicates.

Dry matter (DM) was determined by drying 5 grams of sample placed in crucibles in an oven for six hours to constant weight at 105°C. The samples were cooled in a desiccator at room temperature and weighed. Ash was determined by heating the samples in a muffle furnace set at 600 °C for three hours.

$$\text{DM (\%)} = [(\text{weight of initial sample (g)} - \text{weight of dried sample (g)}) / \text{initial sample weight}] \times 100$$

CP was quantified by the standard micro-Kjeldahl Nitrogen method as described in AOAC (1995). Using a sample size of 0.4 g, a Behroset InKje M digestion apparatus and a Behr S 1 steam distillation apparatus (both: Labor-Technik GmbH, Düsseldorf, Germany). The ammonia distillate was trapped in 4 % boric acid solution prior to titration with 0.1N HCl. Crude protein was estimated by:

$$\% \text{ Nitrogen} = [(\text{ml standard acid} - \text{ml blank}) \times \text{N of acid} \times 1.4007] / \text{weight of sample in grams. CP} = (\% \text{ total nitrogen} \times 6.25)$$

Ether extracts were analysed using a sample size of 2 grams in a Soxhlet extractor with petroleum ether at a boiling point of 40 – 60°C. To calculate for EE, the formulae below was used.

$$\text{Ether extract (\%)} = [(\text{weight of dry flask} - \text{weight of empty flask}) / \text{weight of the sample}] \times 100$$

Crude fibre (CF) was determined by boiling 1 g of sample in a standard solution of 3.13 % H₂SO₄ for 10 minutes. The remaining sample was rinsed with hot water followed by boiling in 3.13 % NaOH for another 10 minutes. Thereafter the remaining sample was rinsed repeatedly with water followed by acetone. The residue was then oven dried at 60°C for 4 hours, cooled in a desiccator and weighed. The residue was ashed at 550°C in a muffle furnace overnight. CF was quantified by:

$$\text{CF (\%)} = \text{dried sample (g)} - \text{ashed sample (g)} / \text{initial sample weight} \times 100$$

Amino acid analysis was conducted at the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria laboratory using chromatographical methods according to EU standard methods (Commission regulation (EC) No 152/, 2009). Nitrogen Free Extracts (NFE) were estimated from the differences in (DM - CP - EE - CF - ash).

Table 3. Proximate composition of the ingredients used in diet formulation (% as feed basis); DM = Dry matter, NFE = Nitrogen Free Extracts, ME = Metabolizable energy. ^A FWS, Freshwater shrimp, ^B FM; Fish meal = Omena (*Rastrineobola argentea*), ^C BSFLM, Black soldier fly larvae meal. Means \pm SD of the ingredients used in the formulation of the experimental diets. (n = 3).

Component	FWS ^A	FM ^B	BSFLM ^C	Cottonseed cake	Sunflower seed cake	Maize germ
DM (%)	90.6 \pm 0.80	93.7 \pm 0.45	95.8 \pm 1.06	90.5 \pm 1.17	92.0 \pm 1.01	89.4 \pm 1.46
Crude protein (%)	66.6 \pm 2.03	62.1 \pm 0.86	25.3 \pm 0.68	27.3 \pm 0.98	42.3 \pm 2.07	32.2 \pm 1.57
Crude fibre (%)	8.0 \pm 0.84	5.9 \pm 0.28	8.0 \pm 0.65	28.7 \pm 1.23	17.9 \pm 0.87	9.5 \pm 0.55
Ether extracts (%)	9.6 \pm 0.92	12.4 \pm 0.27	27.3 \pm 0.55	8.6 \pm 0.74	9.9 \pm 0.31	11.4 \pm 1.35
Ash (%)	28.7 \pm 0.78	30.0 \pm 2.68	14.7 \pm 0.79	5.3 \pm 0.61	5.3 \pm 1.33	3.2 \pm 0.93
NFE (%)	-	-	20.5 \pm 2.19	20.5 \pm 0.82	16.6 \pm 2.12	33.1 \pm 1.67
ME (kcal/kg)	2367 \pm 50.65	2643 \pm 104.03	3924 \pm 54.42	2407 \pm 20.02	2901 \pm 38.51	3255 \pm 0.83

3.2.3 Feeds formulation

Black soldier fly larvae were fed on brewer's grain waste, frozen and later dried at 50°C for 2 hrs before being ground, mixed with other ingredients and formulated to a meal. Boiling water was used during the mixing of the feed ingredients to allow for starch gelatinization and inhibit heat labile anti-nutritional factors. Fish oil is rich in long chain poly-unsaturated fatty acid hence was added in the formulation. Since fish are not able to synthesis vitamins, premix was added in the formulation to promote fish growth and development.

Fish meal-Omena, Freshwater shrimp, cotton seed cake, sunflower seed cake and maize germ were sun-dried and ground to coarse particles using a blender liquidizer (model A989, Hampshire, UK). They were further ground into finer particles using an electric grinder with a 1 mm sieve (Thomas-Wiley intermediate mill, 3348-L10 series, USA) and dried in an oven to a constant weight at 60°C. The nutrients of all the ingredients were analysed to assist in final feed formulation. The formulated diets were analysed to confirm whether the target nutrients were attained.

Four experimental diets were formulated to meet the commercial fish feed standards for tilapia fingerlings (Appendix; Table A1) (Munguti et al., 2014; KEBS, 2015). Substitution of FM using BSFL was made at 0% (control - D1), 33.3% (D2), 66.7% (D3) and 100% (D4) as shown in Table 4. Diet 1 (D1) was the control diet, since it did not contain BSFL meal. Freshwater shrimp was used as the basal ingredient while maize germ was used as binding agent for all diets. The formulation of the complete diet involved thoroughly mixing of the ingredients in specific proportions following the pearsons-square method. To attain a consistency for pelleting and make a soft dough of the powdered mixture, tap water was added. To obtain a homogenous diet the feeds were minced severally, and pellets of 4mm were made using a pelletizer. Later the pellets were sun-dried for 24 hrs and finally placed in large gunny bags and stored in a cool and dry room.

Table 4. Formulations and proximate composition of the four test diets fed to Nile tilapia for 72 days containing different levels of black soldier fly larvae (BSFL) meal as a replacement for fish meal (FM). DM = Dry matter, NFE = Nitrogen Free Extracts, ME = Metabolizable energy. ^A D1, Diet 1, ^B D2, Diet 2, ^C D3, Diet 3, ^D D4, Diet 4. Means \pm SD of the test diets.

Ingredient (g/kg)	Diet (replacement level)			
	D1 ^A (0%)	D2 ^B (33.3%)	D3 ^C (66.7%)	D4 ^D (100%)
Freshwater shrimp	30.0	30.0	30.0	31.5
Fish meal	22.0	14.8	7.4	0.0
Black soldier fly larvae	0.0	9.8	19.5	29.3
Cotton seed meal	20.0	20.0	21.0	20.0
Sunflower seed meal	4.0	4.0	4.0	4.0
Maize germ	17.7	15.4	16.7	7.3
Starch	1.0	1.0	0.0	5.0
Fish oil	5.2	4.9	1.3	2.8
Premix	0.1	0.1	0.1	0.1
Total	100	100	100	100
Proximate composition				
DM (%)	91.5 \pm 0.82	91.1 \pm 0.61	91.2 \pm 0.96	92.7 \pm 1.15
Crude protein (%)	30.9 \pm 0.06	31.3 \pm 0.61	30.3 \pm 0.90	30.3 \pm 1.37
Ether extracts (%)	14.0 \pm 0.92	11.6 \pm 0.39	11.5 \pm 0.43	15.3 \pm 0.70
Crude fibre (%)	9.8 \pm 0.10	9.8 \pm 0.76	8.1 \pm 0.30	16.4 \pm 0.87
Ash (%)	14.3 \pm 0.65	11.3 \pm 0.65	9.3 \pm 1.18	25.5 \pm 1.23
NFE	22.5 \pm 0.76	27.1 \pm 1.93	32.0 \pm 2.19	5.2 \pm 2.91
ME (kcal/kg)	3059 \pm 50.71	3030 \pm 31.69	3160 \pm 27.04	2543 \pm 143.63

3.2.4 Experimental fish and rearing conditions

All male Nile tilapia fish were sourced from KMFRI Sagana centre. The fish were placed in a single hapa net (4 x 3 m) that was mounted in an 800m² earthen pond and acclimatised for 1 week prior to the starting of the experiment. During the acclimatization period, the fish were fed at 1000 and 1600hrs with the commercial floating feeds.

3.2.5 Experimental design and protocol

A total of 240 all male *O. niloticus* ($52.3 \pm 0.29\text{g}$ initial body weight, mean \pm SE) were randomly assigned to 4 groups (4 replicates for each diet) and distributed into 16 hapa nets ($2 \times 2 \times 1$ m) mounted in an 800 m² earthen pond with 15 fish per hapa. The four test diets (D1, D2, D3 and D4) were randomly assigned to the hapa nets by using a table of random numbers. Cover nets were placed on each hapa net to control predators.

Fish were hand-fed twice a day at 1000 and 1600 hrs at 5% of the wet body weight and the quantity of feed was recorded throughout the experimental period of 72 days. Sampling of the fish was done after every two weeks to monitor the growth, mortality, feed efficiency and in addition to adjusting the feeds ration.

Physico-chemical water quality parameters (dissolved oxygen, water temperature, pH, conductivity, salinity and total dissolved solids) were monitored weekly at 1000hrs using YSI industries, Yellow springs, OH, USA, multiparameter water quality meter while water samples were collected for analysis of ammonia, nitrites and nitrates in the laboratory following the procedures by (APHA 1995).

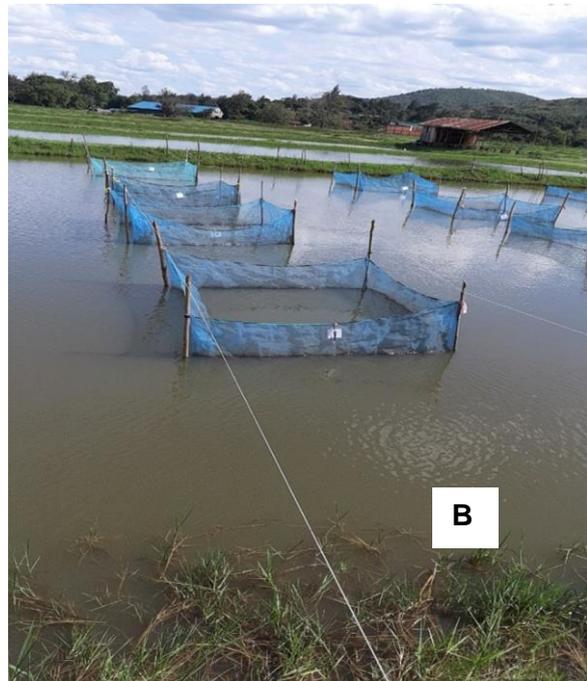




Figure 5. A - Mounting of the hapa nets in the earthen pond, B - Hapa nets mounted in the earthen pond, C - Feeding of the fish with the test diets, D - Fish sampling (measuring the weight of the fish).

3.2.6 Calculated parameters

To evaluate the growth and feed efficiency the following standard formulas were used:

Body weight gain (BWG, g) = final weight (g) - initial weight (g).

Specific growth rate (SGR, %) = $100 \times [(\ln \text{BW}_{\text{final}} (\text{g}) - \ln \text{BW}_{\text{initial}} (\text{g})) / \text{days of culture}]$.

Feed conversion ratio (FCR) = feed provided/live weight gain (g).

Survival rate (SR, %)= $100 \times (\text{final number of fish}) / (\text{initial number of fish})$.

Protein efficiency ratio (PER) = live weight gain (g)/total protein intake (g).

3.2.7 Statistical analysis

The collected data were subjected to the Shapiro–Wilk test of normality followed by One-way ANOVA that tested on the growth response and survival rates of the stocked fish fed on different diets. To determine the pair-wise differences among the diets, the Tukey-HSD post hoc test was employed. All the statistical analyses were performed using MS Excel and SPSS statistics (version 21). Results were interpreted to be significant at $p \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Water quality

Water quality parameters (Table 5) varied ($p < 0.05$) during the culture period. The DO values ranged between 5.9 – 8.2mg/ L (mean = 6.8 ± 0.52 mg/L). Water temperature ranged from 23.5 to 27.8°C (mean = 25.8 ± 1.06 °C). The pH values ranged from 7.4 to 9.0. Conductivity was quite variable. It ranged from 51.1 – 102.6 μ S/cm (mean = 71.6 ± 16.87 μ S/cm). TDS ranged from 33.8 – 63.1mg/ L (mean = 45.8 ± 9.78 mg/ L). Salinity levels were relatively stable and ranged from 0.02 – 0.05mg/ L (mean = 0.03 ± 0.01 mg/ L). Water quality parameters were generally within the recommended values for tilapia culture (Appendix; Table A3).

Table 5: Physico-chemical parameters of the water during the experimental period and recommended values. DO = Dissolved oxygen, TDS = Total dissolved solids, PO₄ = Phosphates, NO₂ = Nitrites, NO₃ = Nitrates, NH₄ = Ammonium. Values represent mean \pm SD, n = 3.

Parameter	Unit	Mean	Min	Max	Recommended values for tilapia culture
Temperature	(°C)	25.8 ± 1.1	23.5	27.8	22 - 29 (Morgan, 1972)
DO	(mg/L)	6.8 ± 0.5	5.9	8.2	> 3 (Ngugi et al., 2007)
Conductivity	(μ S/cm)	71.6 ± 16.9	51.1	102.6	
TDS	(mg/L)	45.8 ± 9.8	33.8	63.1	500 - 1200
Salinity	(mg/L)	0.03 ± 0.01	0.02	0.05	< 0.019 (El-Sayed)
pH			7.4	9.0	6.5 – 9.0 (Ngugi et al., 2007)
PO ₄	(mg/L)	0.002 ± 0.0004	0.002	0.003	< 0.05 (El- Shiafey, 1998)
NO ₂	(mg/L)	0.001 ± 0.0002	0	0.001	< 0.05 (El- Shiafey, 1998)
NO ₃	(mg/L)	0.001 ± 0.0002	0.001	0.002	< 0.05 (El- Shiafey, 1998)
NH ₄	(mg/L)	0.01 ± 0.001	0.01	0.01	< 0.05 (El- Shiafey, 1998)

4.2 Background information of the black soldier fly larvae in Kenya

Production of the larvae is being carried out at both small scale and large scale. Further, large institutions are putting in place production units to increase the seed provisioning for the farmers. The local prices of the individual ingredients used to formulate the 4 test diets are shown in the Table 6. All the ingredients were procured from local animal feed outlets in Thika. The exchange rate for the Euro (€) to Kenya shillings (KES) was pegged at 111.50. FM and *Caridina nilotica* were retailing at KES 140 and 100/kg, respectively. The BSFL and the plant-based ingredients were retailing at prices below KES 100. Of our 2 protein sources of interest the BSFL (KES 80/kg) was retailing at a lower price than fish meal (KES 140/kg).

Table 6. Cost of ingredients used in formulating the test diets.

Ingredient	Price * (KES) per kg
Fish meal ^A	140
Freshwater shrimp	100
Black soldier fly larvae	80
Cotton seed cake	40
Sunflower seed cake	40
Maize germ	28

Note: ^A Fish meal = Omena (*Rastrineobola argentea*).

*Prices of feed ingredients at the prevailing market in Kenya (2019).

1 Euro = 111.50 Kes.

4.3 Proximate analysis of the individual ingredients and the test diets

Table 3 provides a summary of the nutritional contents of the ingredients used in the formulation of the experimental diets. Maize germ had the least dry matter content (89.4%), followed by cotton seed cake (90.5%), FWS (90.6%), sunflower seed cake (92.0%) and the highest DM content was in fish meal (FM) (93.7%).

The CP contents ranged between 25.3% - 66.6%, with the animal origin products having higher CP contents than the plant origin. The lowest crude protein (CP) was in BSFL meal (25.3%). Among the plant origin ingredients, sunflower seed cake, maize germ and cotton seed cake had CP of 42.3%, 32.2% and 27.3% respectively. On the other hand, freshwater shrimp and FM had crude protein values of 66.6% and 62.1% respectively. Similar to CP the animal-based products had higher ash contents than the plant-based products. Maize germ had

the least ash content (3.2%), followed by sunflower seed cake (5.3%), cotton seed cake (5.3%), BSFL meal (14.7), FWS (28.7%) and the highest ash content was recorded in FM (30.0%).

The plant origin products had higher crude fibre (CF) contents than the animal-based products. FM had the least CF content (5.9%) followed by FWS and BSFL meal that have similar contents (8.0%), maize germ (9.5%), sunflower seed cake (17.9%) whereas cotton seed cake had the highest CF content (28.7%).

Cotton seed cake had the least ether extracts (EE) contents (8.6%) followed by FWS (9.6%), sunflower seed cake (9.9%), maize germ (11.4), FM (12.4%) while BSFL meal recorded the highest EE content (27.3%). Sunflower seed cake had the least NFE (16.6%) followed by black soldier fly larvae meal (20.5%), cotton seed cake (20.5%) while maize germ had the highest NFE content (33.1%).

FWS had the least metabolizable energy (ME) (2367kcal/kg), followed by cotton seed cake (2407kcal/kg), FM (2643 kcal/kg), sunflower seed cake (2901kcal/kg), maize germ (3255 kcal/kg) and the highest was in BSFLM (3924 kcal/kg).

The 4 test diets (Table 4) met the nutritional requirements for tilapia commercial feeds (KEBS, 2015) with an exception of diet 4 that had higher than recommended contents of CF and ash.

The two protein sources of interest, FM and BSFL meal had different nutritional composition (Table 3). FM had higher ash (30.0%) and crude protein (62.1%) contents than the BSFL meal (14.7% and 25.3%) respectively. On the other hand, BSFL meal exhibited higher contents of DM, crude fibre, EE, NFE and ME in comparison to FM.

Table 7. Amino acid composition (g/kg feed; g/kg protein) of fish meal and black soldier fly larvae meal.

	FMA ^A		BSFLM ^B		% differences ^C	<i>O. niloticus</i>
	g/kg feed	g/kg protein	g/kg feed	g/kg protein		
Essential amino acids						
Histidine	13.6	21.8	9.9	39.1	-27.2	17.2
Threonine	22.8	36.7	17.0	67.0	-25.7	37.5
Arginine	33.4	53.8	20.9	82.6	-37.4	42.0
Valine	28.8	46.4	24.8	98.1	-13.9	28.0
Isoleucine	25.4	40.8	18.3	72.3	-27.9	31.1
Phenylalanine	23.8	38.3	17.8	70.3	-25.2	37.5
Leucine	44.1	71.1	29.1	115.1	-34.1	33.9
Lysine	42.6	68.6	25.5	100.9	-40.1	51.2
Methionine	16.3	26.2	6.8	26.8	-58.2	26.8
Tryptophan	5.5	8.8	5.2	20.5	-5.0	10.0
Non-essential amino acids						
Alanine	41.5	66.8	28.5	112.6		
Tyrosine	18.8	30.2	27.5	108.6		
Glycine	37.6	60.5	21.2	84.0		
Asparagine	51.5	83.0	39.2	155.0		
Glutamate	92.6	149.1	52.5	207.4		
Serine	20.1	32.4	18.0	71.3		
Cysteine	3.5	5.6	3.8	15.0		

^A - Fish meal - Omena (*Rastrineobola argentea*), ^B - Black soldier fly larvae meal, ^C - calculated as $(AA_{BSFLM} - AA_{FM}) / AA_{FM} * 100$, AA is the amino acid in the ingredient (g/kg feed) (Cummins et al., 2017). The negative values represent the potential degree of limitation of essential amino acids in the case of substitution of FM by BSFLM. ^D - g/kg protein (Santiago and Lovell, 1988).

The AA composition of black soldier fly larvae meal and fish meal is presented in Table 7. FM had higher amounts of AA (g/kg feed) than BSFL meal with an exception of cysteine and tyrosine. However, when the amount in protein (g/kg CP) was computed, the BSFL meal protein recorded higher contents of AA than fish meal protein.

Leucine and lysine were the most abundant essential amino acids in both FM (44.1 g/kg feed, 42.6g/kg feed) and BSFL meal (29.1 g/kg feed, 25.5 g/kg feed), respectively. Tryptophan and histidine were the least abundant essential amino acids in FM (5.5 g/kg feed, 13.6 g/kg feed) while tryptophan and methionine were the least abundant in BSFL meal (6.8 g/kg feed, 5.2 g/kg feed).

Glutamate was the most abundant non-essential amino acid in both FM and BSFL meal (92.6 g/kg feed, 52.5 g/kg feed) while the least abundant was cysteine (3.5 g/kg FM, 3.8 g/kg BSFL meal).

In the present study, methionine had the highest potential degree of limitation if FM would be substituted by BSFL meal, followed by lysine, arginine, leucine, isoleucine, histidine, threonine, phenylalanine, valine, while tryptophan was least limiting, based on the percent difference (Cummins et al., 2017).

4.4 Performance parameters

The growth performance and feed utilization parameters of *O. niloticus* fed on the test diets, reflecting partial or total replacement of fish meal with BSFL meal are presented in Table 8. The average survival rate of the fish during the experiment was 99.95 ± 0.03 . No significant differences ($p > 0.05$) were found in the survival rates (SR), body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) between the diets.

Table 8. The average growth performance in hapas per treatment for *O. niloticus* during the 72-day culture-period. BW - Body weight; BWG - Body weight gain; SGR - Specific growth rate, SR - Survival rate; FCR - Feed conversion ratio; PER - Protein efficiency ratio. Diets represent: Diet 1- control (without black soldier fly larvae meal inclusion), Diet 2 (33.3% substitution rate), Diet 3 (66.7% substitution rate) and Diet 4 (100% substitution rate, i.e. maximum BSFLM inclusion). Means \pm SE of four groups of fish.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4
Initial BW (g)	51.6 \pm 0.53	52.2 \pm 0.60	53.0 \pm 0.42	52.2 \pm 0.29
Final BW (g)	120.7 \pm 6.98	124.6 \pm 6.00	118.4 \pm 7.16	112.8 \pm 2.62
BWG (g)	69.1 \pm 7.33	72.3 \pm 6.36	65.4 \pm 7.45	60.5 \pm 2.11
SGR	1.2 \pm 0.09	1.2 \pm 0.08	1.1 \pm 0.09	1.1 \pm 0.02
SR (%)	99.9 \pm 0.10	100.0 \pm 0.00	100.0 \pm 0.00	99.9 \pm 0.07
FCR	1.0 \pm 0.06	1.0 \pm 0.06	1.0 \pm 0.07	1.1 \pm 0.01
PER	3.3 \pm 0.20	3.4 \pm 0.18	3.3 \pm 0.25	3.1 \pm 0.03

During the 72-day experimental period, the fish grew from an average initial weight of $52.3 \pm 0.29\text{g}$ to an average final weight of $119.1 \pm 2.89\text{g}$. The final weight among the diets ranged from $112.8 \pm 2.62\text{g}$ (D4) to $124.6 \pm 6.00\text{g}$ (D2). The mean BWG of all the fish was $66.8 \pm 3.00\text{g}$ and ranged between 60.5 ± 2.11 (D4) to $72.3 \pm 6.36\text{g}$ (D2) among the diets. The average SGR of the fish was $1.1 \pm 0.04\%$ and ranged between 1.1 ± 0.02 (D4) to $1.2 \pm 0.09\%$ (D1) among the diets. Concerning the feed utilization parameters, FCR ranged from 1.0 ± 0.06 to 1.1 ± 0.01 while the PER among the diets recorded values between 3.1 ± 0.03 (D4) and 3.4 ± 0.18 (D2).

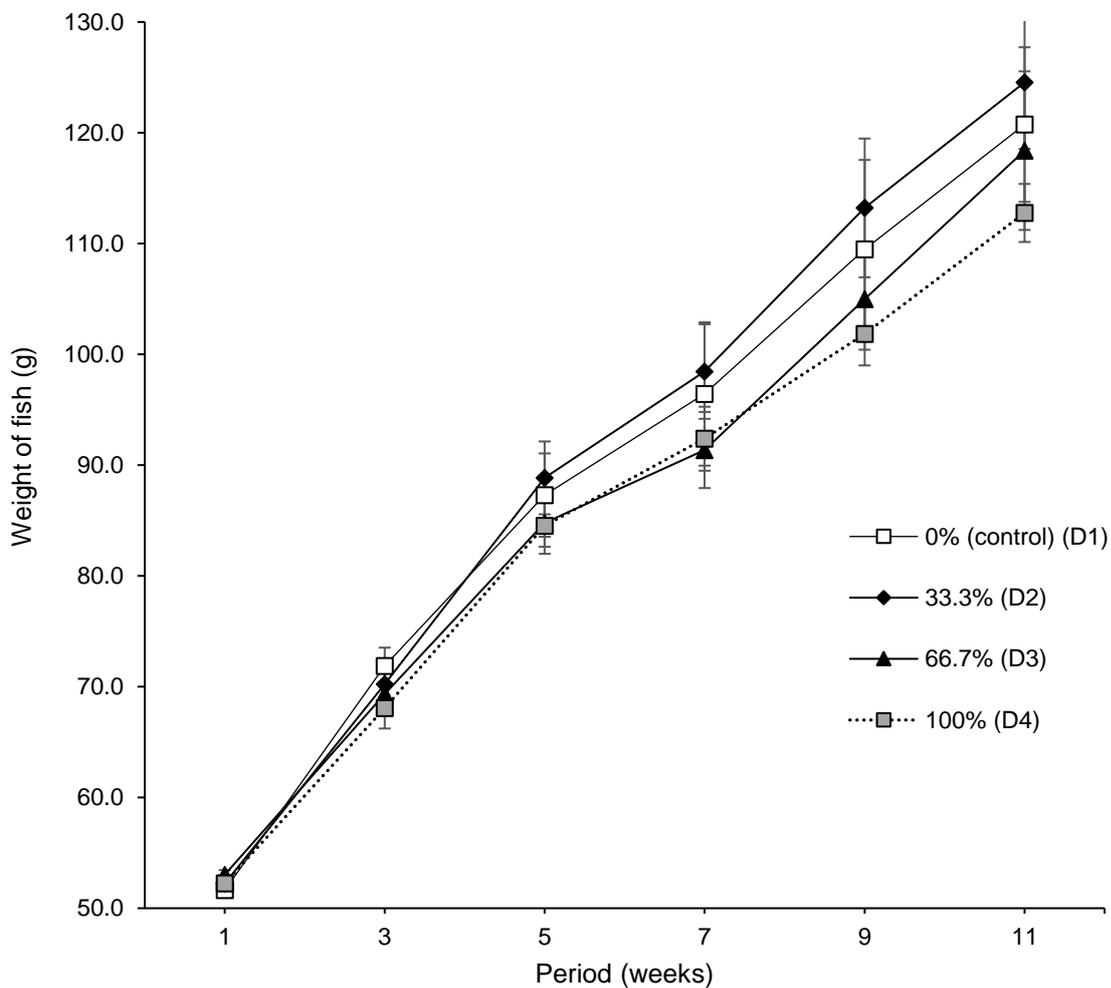


Figure 6. Growth curves for *O. niloticus* fed on the test diets with varying levels of BSFL meal during the 72 days culture period.

All fish groups showed a steady increase in weight following exposure to their respective test diets (Figure 6) through the 72-day culture period. Across all feeding regimes,

exponential growth curves were displayed as the weight gain increased gradually from the start of the experiment.

During week 1 and 3, all the curves displayed some overlap; by the 5th week the overlaps were between D1 and D2, and D3 and D4. Nevertheless, by the 9th week there was a separation of the curves between the diets till the end of the experimental period. By the end of the experiment, the growth curves in the diet 2 registered the highest weight followed by diet, D3 while D4 (100% BSFLM inclusion) resulted in the lowest weight.

CHAPTER FIVE

DISCUSSION

Aquaculture plays a major role in supplying high quality protein, reducing hunger and further curbing malnutrition across the globe. Fish meal has been an important source of protein in fish feeds for the longest time. However, studies have shown that overexploitation of fish meal has led to increase in prices of the product leading to questions on its sustainability in the aquaculture industry. Musyoka et al. (2019) noted that a sustainable and quality fish feed ingredient should have all the nutrient contents and be easily accepted by the fish. Additionally, the ingredient should improve disease resistance of the fish alongside maintaining good water quality in the culture system. Therefore, it is key for fish nutritionists and farmers to obtain quality feed at affordable prices so as to produce quality fish and realise economic success.

The aim of this study was to determine the nutritive composition of the locally produced black soldier fly larvae meal and compare it to that of FM. Further, the growth performance and survival rates of *O. niloticus* fed on test diets containing different proportions of the meal should be assessed. Based on this, the results should be utilized to establish suggestions on the optimal inclusion rates of black soldier fly larvae meal in Nile tilapia diets.

The results gathered have captured this aim very well. A background check on the locally produced BSFL meal in Kenya revealed that the larvae is retailing at lower prices than fish meal (prevailing market prices as of 2019; see Table 6).

BSFLM has lower protein content than FM but higher ether extracts than FM. The AA profile of BSFLM and FM are similar, however when BSFLM substitutes FM methionine has a high potential of being limiting. No significant differences ($p > 0.05$) were reported in the survival rates (SR), body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) between the diets (Table 8).

5.1 Water quality

The physico-chemical water quality parameters analysed showed significant differences ($p < 0.05$) during the culture period (Table 5). However, they were within the recommended ranges for culturing of *O. niloticus* (Appendix; Table A1). Similar to Devic et al. (2018), the water quality parameters varied during the trial period ($p < 0.05$; Table 5).

However, the parameters were within the recommended values for the culturing of tilapia (Appendix; Table A3). Water quality parameters in a culture system enables fish farmers to understand the feeding regimes of the fish and implement good pond management practices to increase the fish yields.

5.2 Background information of the black soldier fly larvae in Kenya

5.2.1 Production of the black soldier fly larvae

The production of the larvae is widespread in Kenya with the rearing being done in most of the counties around the country. The production is mainly aimed at providing a cheap source of protein for the on-farm animal feed formulation and sale to commercial animal feed producers (Nyakeri et al., 2017). Shumo et al. (2019) highlights Kenya as one of countries that has several organic waste streams that can be used for the rearing of the black soldier fly larvae.

Shumo et al. (2019) further state that the larvae's contents of crude protein (CP), ether extracts (EE), minerals, amino acids, acid detergent fibre (ADF) and neutral detergent fibre (NDF), but not of vitamins are dependent on the substrate used. Studies by Shumo et al. (2019) showed higher CP levels in the BSFL reared on brewers' spent grain (SG) as compared to those reared on chicken manure (CM) and kitchen waste (KW) (Appendix D). Additionally, Nyakeri et al. (2017) noted that the flies' highest production was observed when the plant-based products and not the animal-based products were used as substrates.

ICIPE is an international governmental organisation in Kenya that is focused on the research of the BSF. It offers initial training, extension services and seeds to the farmers venturing into the BSF production (icipe.org).

Sanergy is a company located in Machakos county and was established in 2011. It is involved in mass production of the BSF on human faecal matter from the Nairobi slums. The farm produces 7 tonnes/ month of the dried insect meal and aims to increase the yields to 300 tonnes/month. Further, after the larvae have self-harvested, they leave behind 400 tonnes of organic fertilizer that is rich in nitrogen and calcium contents (sanergy.com).

5.2.2 Availability of the black soldier fly larvae in Kenya

Black soldier fly occurs in many areas in the country and Nyakeri et al. (2017) found the insects to be native to Bondo area, western Kenya. Ruiru and Kikuyu areas in Kiambu county, also recorded BSF production by small scale farmers (Mugo, 2018). BSF prefer temperatures between 24 and 30°C. In areas where the temperature is not within the range, the producers use greenhouses to increase the temperatures to provide an environment suitable for

BSF production. Reports show that poor environmental conditions (temperature and relative humidity) and poor diet lengthens the duration of the life cycle of BSF by several months (Veldkamp et al., 2012; Nyakeri et al., 2017).

5.2.3 Economics of the black soldier fly larvae in Kenya

BSFL have lower prices than other animal and plant-based products (Table 6). These low costs may be due to the fact that the larvae self-harvest and feed on low value organic wastes (Diener and Trockner, 2009; van Huis, 2013).

5.3 Proximate analysis

The nutrients (CP, EE, carbohydrates, minerals and ash) in fish feed ingredients play a specific role in the growth and development of the fish (Pillay, 1990; Steven et al., 2009). FM and BSFL meal (Table 3) used in the present study had different nutritional composition. The black soldier fly larvae were higher in ether extracts and lower in crude protein and ash compared to the fish meal in the present study. These results are similar to those of (St-Hilaire et al., 2007).

Additionally, the BSFLM contained higher amounts of DM, crude fiber (CF), metabolizable energy (ME) and NFE than fish meal. FM had a negative value for NFE, and this may have been due to the fact that FM contains extremely low amounts of non-fibrous carbohydrates (Landau, 1992). The ash content in the BSFLM was within ranges reported by previous studies (Finke, 2013; Makkar et al., 2014)

The nutrient contents of FM used in the present study were similar to those reported by Cummins et al. (2017). The crude protein of the BSFL meal was lower than values reported by Sheppard (2002) (37.8%), Arango et al. (2004) (37%), Kroeckel et al. (2012) (47.6%), Barroso et al. (2014) (36.7%) and Muin et al. (2017) (41.74%), but comparable to those reported by Tschirner and Simon (2015) (23.8%).

Processing of the larvae has been argued to be the cause of differences in the nutrient compositions reported for black soldier fly larvae meal. When Kroeckel et al. (2014) defatted the larvae, the resulting EE content was lower than in the present study. Based on Kroeckel et al. (2014), the low amount of CP and high EE content in the BSFL meal used in our study can be attributed to the lack of defatting of the larvae. Defatting of the larvae is important as it leads to an increase in CP and lowers EE, further reducing the risk of lipid oxidation which may be toxic and can also destroy some of the vitamins (Castell, 1986; Landau., 1992; Reena et al., 2017; Dumas et al., 2018). Further, the oil from this process can be utilised for biofuel

(Makkar et al., 2014; Henry et al., 2015). Barragan et al. (2017) also reported that the CP content in BSFL decreases with increasing age and that larvae fed on fresh manure have more protein than those fed on aged manure.

Protein is an important component in the fish diet as it provides both nitrogen and amino acids (Pillay, 1990), essential for the formation of and build-up of muscles in fish. Wilson (2003) noted that protein makes up to 65-75% of fish dry weight. To compensate for the relatively low CP in BSFL meal, freshwater shrimp was added to the formulation.

EE of the BSF larvae meal in the present study was similar to that reported by Devic et al. (2018) and Toriz-Roldan et al. (2019), but lower than that reported by Hilaire et al. (2017). The difference in the EE of the larvae may have been due to the type of substrate used for rearing the larvae. A study by Shumo et al. (2019) reported higher EE content for larvae reared on kitchen waste in comparison to brewers' waste and chicken manure (Appendix; Table A4).

The ash and DM contents in the BSFLM in the present study were lower than the values reported by Toriz-Roldan et al. (2019), but higher than those in Tschirner and Simon (2015). BSFLM in the present study has similar contents of fiber and ash to those reported by Devic et al. (2018) and Shumo et al. (2019).

Ojewola et al. (2005), Henry et al. (2015) and Mérida et al. (2019) argued that the variability in the nutrient contents of BSFL can be attributed to factors such as type of substrate used to rear the larvae, stage of harvesting, methods of processing and duration of drying. Further, Shumo et al. (2019) reported varying proximate compositions of BSFLM raised on 3 different substrates (kitchen waste, chicken waste and spent grain) (Appendix; Table A4).

The FM and BSFLM used in the present study displayed different AA profiles (Table 7). When the amount of AA in protein (g/kg crude protein) was computed, values for BSFLM were found to be higher than for FM. These results go a long way into indicating that the BSFLM contains high quality protein. Additionally, Tschirner and Simon. (2015) noted that despite the limiting effect of methionine in BSFLM, when compared to FM it displayed a good overall protein quality. This is in line with the results presented herein (Table 7).

Cummins et al. (2017) and Schiavone et al. (2017) reported similar abundant, least abundant EAA and similar abundant and least abundant non – EAA as those in the present study for both FM and BSFLM. Although the latter and present study display similar patterns for the abundances in AA, the present study's AA abundances were lower than those in Schiavone et al. (2017) who utilised the highly defatted BSFL meal. These differences in the

AA abundances may have been attributed to the defatting process.

It has been argued that defatting of the larvae increases the CP and decreases the lipid value in the insect and that the AA concentration is directly proportional to the CP content in the larvae (Schiavone et al., 2017). Schiavone et al. (2017) further confirmed that the AA of defatted BSF meals were higher than those for full-fat BSFL one.

5.4 Growth performance and nutrient utilization

All the fish appeared healthy and with no disease outbreaks throughout the 72-day trial period. Mortality was extremely low, only 3 fish died during the early part of the experiment (Appendix; Table A2). The mortalities were not diet-related but may have been due to the stress caused during fish handling at the time of sampling (Xiao et al., 2018). Further, all the diets were well accepted by the fish, paralleling reports by Adewolu et al. (2010). No significant differences ($p > 0.05$) were found in the SR among all the test diets (Table 8). The survival percentage in our study ranged between 99.9 – 100%. This range was higher than in the study by Toriz- Roldan et al. (2019) (87- 90.0%).

Additionally, the high mean survival percentage (99.8%) agreed with a previous study that recorded a mean SR of >98% using black soldier fly larvae meal as replacement of FM in test diets for yellow catfish (Xiao et al., 2019). Feed acceptance by the fish, improved nutritional value of the diets due to pellet extrusion and good water quality parameters that were well within the recommended ranges (Appendix; Table A3) during the culture period may have contributed to the high mean SR.

The SR in the present study are similar to those reported by Molina-Poveda and Morales (2004), Roy et al. (2009), Morris et al. (2011) and Ye et al. (2011) under green water culture systems. When fish were reared in clear water culture systems, the SR were somewhat lower than our present study results (Izquierdo et al., 2006; Liu et al., 2012; Ye et al., 2012; Bulbul et al., 2013; Cummins et al., 2017). Cummins et al. (2017) argued that the green water culture systems provided continuous supplementary nutrients (natural food) to the cultured species, hence increasing the chances of survival. The good survival in the present study is an indicator of, among others, the diet's acceptance (Muin et al., 2015).

Fish body weight increased by a factor of two during the 72-day growth period (Table 8 and Figure 6). On the other hand, Devic et al. (2018) reported a factor of 3 on Nile tilapia reared on diets containing varying proportions (0, 30, 50, 80%) of the BSFL meal as FM replacement.

No significant differences ($p > 0.05$) were reported in the BWG and SGR (Table 8) between the test diets in the present study. These results are in agreement with those of Toriz-Roldan et al. (2019) and Devic et al. (2018), when *O. niloticus* were reared on diets containing varying proportions of BSFL meal. Similarly, a partial or total dietary replacement of FM with BSFLM did not lead to differences in the BWG and SGR of juvenile Japanese bass and rainbow trout, respectively (Belghit et al., 2019; Reena et al., 2017; Wang et al., 2019).

The results of the present study suggest that it is possible to substitute up to 100% fish meal with black soldier fly larvae meal in diets for Nile tilapia without negative effects on growth performance. On the other hand, Muin et al. (2015) reported significant differences in the growth parameters and substitution of FM by BSFLM of >50% led to a decrease in the BWG and SGR of the *O. niloticus*. These differences in the substitution levels of FM and BSFLM of the present and previous studies may have been attributed to the culture systems used.

Munguti et al. (2009) realised a higher BWG and SGR in *O. niloticus* when reared in earthen ponds as opposed to aquaria. Earthen ponds have the ability to support the growth of natural food and hence offer extra nutrition and eventually supplement the deficient essential amino acids in the test diets for the fish (Munguti et al., 2009). Muin et al. (2015) conducted their study in plastic tanks, while the present study was conducted in an earthen pond where development of natural food was possible which might have contributed to a sufficient supply with deficient EAA.

There are about 20 amino acids, of these only 10 are indispensable since the fish are not able to synthesise them, hence these need to be provided by the diet. Essential amino acids are argued to promote growth and reproduction rates alongside improving the disease resistance in *O. niloticus* (Andersen et al., 2016; Musyoka et al., 2019). In the present study, as compared to FM, BSFLM probably supplied too little methionine (Table 7), therefore the natural food in the pond may have compensated for it and may have promoted a better balanced amino acid profile in all the diets, consequentially promoting the growth of the fish and allowing for the high substitution levels.

Additionally, combining of two or three animal-based protein sources has been documented as a good way to promote a balanced amino acid profile and also compensating for the high EE in the larvae (Newton et al., 1977; Phonekhampheng, 2008). Inclusion of the FWS in the present study alongside the natural food may have led to a well-balanced AA profile among the diets BSFL contain the dietary structural polysaccharide-chitin, the b1,4-

bond has been reported to be indigestible for several fish species due to different chitinase activities among the fish (Rust, 2002). Chitin is said to decrease the lipid digestibility and inhibit nutrient absorption from the intestinal tract (Razdan and Pettersson, 1994; Han et al., 1999; Tharanathan and Kittur, 2003; Kroeckel et al., 2012). In addition to this, Lindsay et al. (1984) noted that chitin digestibility in turbot and rainbow trout is generally very low or completely absent.

When Kroeckel et al. (2012) reared juvenile turbot on diets containing different proportions of BSFLM, substitution levels of FM by BSFLM >33% led to a decrease in the BWG and SGR. The authors speculate that chitin may have been the dietary factor behind the decrease in the growth performance parameters allowing for lower substitution levels than the present study. In addition, studies by Shiau and Yu (1999), Gopalakannan and Arul (2006) and Olsen et al. (2006) have noted that any increase in the inclusion rate of chitin (> 1%) in the diets of tilapia (*Oreochromis niloticus* × *O. aureus*) led to a decrease in feed intake and growth.

No significant differences ($p > 0.05$) were found in the FCR between the test diets containing varying proportions of BSFLM (Table 8). FCR is a measure of how efficient the feed is converted into flesh by the fish (Arori et al. (2019) and is inversely related to feed efficiency (Cottle and Pitchford, 2014). The FCR in the present study ranged from 1.0 to 1.1 (Table 8), meaning that for the fish to attain 1kg of flesh it will consume 1.0 to 1.1kg of the diet. Hepher (1989) and Lovell (1989) noted that the optimum feed ration is one that promotes the best FCR alongside the growth of the fish.

A low FCR (≤ 1) is the best and is an indication of a high-quality feed (USAID, 2011; Arori et al., 2019). Similar to the present study, no significant differences were reported in FCR when BSFLM was used to replace FM in the diets of *O. niloticus* (Muin et al., 2015; Devic et al., 2018; Toriz et al., 2019). Additionally, when juvenile Japanese bass were reared with different proportions of BSFLM, no significant difference in FCR was found (Wang et al., 2019).

The present study's FCR (no significant differences, $p > 0.05$) between the treatments suggests that replacement of FM by BSFLM of up to 100% is possible, without any negative effect on the FCR. On the contrary, Adewolu et al. (2010) and Kroeckel et al. (2012) reported significant differences in the FCR between the diets containing BSFL meal and the control diet fed on juvenile turbot. The authors speculate that chitin may have been the dietary factor behind the depressed and significant differences in the FCR among the test diets.

Chitin is said to inhibit the absorption of nutrients from the intestinal tract and lead to reduced feed utilization efficiencies (Razdan and Pettersson, 1994). Low apparent digestibility coefficients of AA, lipid, energy, dry matter and protein were reported in tilapia fed on diets containing high contents of ash and chitin (Köprücü and Özdemir, 2005). Observations from the present study suggest that *O. niloticus* might have a high efficacy in digesting chitin or may be tolerable to certain amounts. El-Sayed (1998) found out that fish meal could be replaced by shrimp meal (rich in chitin) without any negative effects on the red tilapia, *O. niloticus* and *O. hornorum*.

On the contrary, when chitin was added to the diets of other tilapia species, a decrease in the growth was observed (Shiau and Yu, 1999). Though, observations from the present study suggest, *O. niloticus* might have had a high efficiency to digest chitin, thereby insect meal.

Deitz et al. (2018) reported higher FCR, but Kroeckel et al. (2012) reported lower FCR values than those of the present study. The differences may have been due to the processing of the larvae. Sheppard et al. (2008) noted that the processing of the BSF larvae e.g. drying, defatting and cuticle removal led to increased nutrient availability, digestibility and acceptability of the larvae. The present study utilized full fat larvae, while prior to feed formulation, Kroeckel et al. (2012) mechanically defatted the larvae, consequentially improving the larvae's nutrient availability and digestibility and improving FCR.

There was no significant difference ($p > 0.05$) in the PER between the treatments (Table 8). These results are in agreement with Adeniyi et al. (2015) and Devic et al. (2018) when varying proportions of BSFL meal were used to replace fish meal in the diets of *Clarias gariepinus* and *O. niloticus*, respectively. PER in the present study (3.1 – 3.3) were higher than those reported by Muin et al. (2015), but comparable to those of Toriz et al. (2019).

Muin et al. (2015) argued that PER indicates the quality of protein in feeds and that feeds are better utilized at high PER. Additionally, Akiyama et al. (1992) noted that the protein availability and utilization can be influenced by the chemical composition of the ingredient, freshness of the raw material, method of processing and storage, and length of storage.

In the present study the PER suggests that a replacement of FM by BSFLM up to 100% is possible, without any negative effect on this feed utilization parameter. On the other hand, Xiao et al. 2018 reported significantly lower PER when inclusion of BSFLM in the diets of *O. niloticus* were made up to 48%. Similarly, Yigit et al. (2006) reported significantly lower

PER between diets that had > 75% substitution of FM by poultry by products. Increased inclusion of poultry by-product meal may have led to amino acid imbalances in the diets, consequentially reducing the protein retention. Similar amino acid imbalances were not present in the current study owing to the similar AA profile of FM protein and the BSFL meal protein (Table 7).

CONCLUSION & RECOMMENDATIONS

The production of BSFL is widespread in Kenya and the prices are lower than those for fish meal. Although black soldier fly larvae meal has lower contents of crude protein and higher ether extracts in comparison to fish meal, the larvae have been found to contain high quality protein. To improve the protein amount in the larvae, defatting is recommended before utilising it for fish feeds. The present study indicates that even full fat BSFLM can replace up to 100% of the FM without negative effects on the growth performance and feed utilization of Nile tilapia. However, further studies on the chitin amounts in the larvae and chitinase activity of tilapia species should be conducted to strengthen the findings of the present study. Further, studies should be done to determine the contribution of the culture system to the utilization and substitution of FM by the larvae. Additionally, both technical and economic viability should be studied before applying the supplement commercially so as to determine the profit margins. Overall, the present study suggests that insect protein derived from *H. illucens* could be an option to make *O. niloticus* feed formulation more economically and environmentally viable over generations.

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APPENDICES

Table A1. Requirements for tilapia fingerlings/ growers' commercial feeds. Specification adapted from Kenya Bureau of Standard 2289- 1:2016

S/No		Tilapia fingerlings: (Starter diet)	Tilapia growers' (grower diet)*	Finisher	Brooder diet
i)	Weight in grammes	1-49	50-200	200-market size	≥50 females ≥80 males
ii)	Crude protein % (min)	35	30	24	32
iii)	Energy (DE) Kcal/kg (min)	2500	2750	2900	2800
iv)	Amino acid levels				
	a) Lysine, g/kg (min)	2.1	1.7	1.7	1.7
	b) Methionine, g/kg (min)	0.9	0.8	0.8	0.8
	c) Methionine + cysteine, g/kg (min)	1.4	1.1	1.1	1.0
	d) Threonine, g/kg (min)	1.3	1.0	1.0	0.4
	e) Tryptophan, g/kg (min)	0.5	0.4	0.4	
v)	Crude fibre % (max)	4	10	10	10
vi)	Ether extracts % (max)	5-12	5-15	5-15	5-15
vii)	Ash (max)	10	20	20	20
viii)	Pellet size (mm)	2 (max)	2-5	4-6	2-5
ix)	Pellets should float on water for (minutes)	2	2	2	2
x)	Moisture content of pellets (% max)	10	10	10	10
xi)	Calcium %	0.4-0.6	0.4-0.6	0.4-0.6	0.45-0.65
xii)	Phosphorus %	0.5-0.7	0.5-0.7	0.5-0.7	0.6-0.7

Table A2. The mortality of the *O. niloticus* during the study period (72 days) per treatment.

Treatment diet	Initial stocking	Final stock	Mortality
D1	60	58	2
D2	60	60	0
D3	60	60	0
D4	60	59	1

Table A3. Limits and optima of water quality parameters for tilapia. Adapted from Mjoun, Rosentrater and Brown, 2010.

Parameter	Range	Optimum for growth	Reference
Salinity, parts per thousand	Up to 36	Up 19	El-Sayed (2006)
Dissolved oxygen, mg/L	Down to 0.1	> 3	Magid and Babiker (1975); Ross (2000)
Temperature, C°	8–42	22–29	Sarig (1969); Morgan (1972); Mires (1995)
pH	3.7–11	7–9	Ross (2000)
Ammonia, mg/L	Up to 7.1	< 0.05	El-Shafey(1998); Redner and Stickney, (1979)

Table A4. Means (\pm standard error) of proximate composition (in % dry matter) of black soldier fly larvae reared on three different rearing substrates. *Means ($n = 2$) in the same row followed with different superscripts are significantly different at $p < 0.05$; DM, Dry Matter; OM, Organic Matter; CP, Crude Protein; NDF, Neutral Detergent Fibre; ADF, Acid Detergent Fibre; EE, Ether Extract; CM, Chicken Manure; KW, Kitchen Waste; SG, Spent Grain; BSFL, black soldier fly larvae. Adapted from Shumo et al.,2019.

Parameters	CM fed BSFL	KW fed BSFL	SG fed BSFL	p-values
DM	80.7 ^{a*} \pm 1.2	87.7 ^b \pm 1.0	83.1 ^a \pm 1.6	0.0050
Ash	9.3 \pm 1.8	9.6 \pm 1.6	11.6 \pm 0.5	0.4818
OM	59.8 ^a \pm 0.4	90.4 ^b \pm 1.6	88.4 ^b \pm 0.5	<0.0001
CP	41.1 ^a \pm 0.3	33.0 ^b \pm 1.0	41.3 ^a \pm 0.5	<0.0001
NDF	21.9 ^a \pm 0.6	20.4 ^a \pm 0.6	28.6 ^b \pm 1.0	<0.0001
ADF	12.6 ^a \pm 0.3	13.2 ^a \pm 0.1	15.0 ^b \pm 0.8	0.0101
EE	30.1 ^a \pm 0.4	34.3 ^b \pm 0.4	31 ^a \pm 0.4	<0.0001

Figure A1. Length weight relationship of *O. niloticus* used in the study (Fulton's condition factor)

