REPLACEMENT OF SOYBEAN MEAL IN THE DIET OF NILE TILAPIA (*Oreochromis niloticus*) BY BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL AND THE COST IMPLICATIONS

BY

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MASENO UNIVERSITY

DECLARATION

Declaration by the candidate

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

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Supervisors' approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as the university supervisors.

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DEDICATION

This work is completely dedicated to my late mother, beloved wife and children who always were an inspiration in my academic journey.

ABSTRACT

Soybean meal (SBM) is a plant protein derivative, which is widely used in Nile tilapia (*Oreochromis niloticus*) feed formulation. However, as a major plant protein source, SBM creates a food-feed competition with a growing demand for both aquaculture and terrestrial animals. In addition, soybean is characterized by deficiency in methionine and cystine, low nutrient digestibility and lesser nutrient bio-availability that limits inclusion levels hence compromising optimal fish growth. It is the limitations of SBM that have prompted search for other alternative protein sources, for fish feed production. This study investigated the effect of replacing SBM with black soldier fly larvae meal (BSFLM) on the growth performance, feed utilization, carcass body composition and amino acid concentration of *O. niloticus*. Three isonitrogenous (30% crude protein) diets containing BSFLM in varying proportions of 0% ($BSFLM_0$), 50% ($BSFLM_{50}$) and 100% ($BSFLM_{100}$), were formulated to replace SBM. A commercial diet ($COMM_0$) sourced from the local market was used as a positive control. Male sex-reversed *O. niloticus* juveniles of mean weight 20.88± 0.16 g were stocked in 12 cages ($8m^3$) each at a density of 12.5 fish m^{-3}.The cages were suspended in earthen ponds (150 $m^{2)}$ with four cages per pond. Fish were hand fed at 5% (28 days), 3% (54 days) and 2.5% (84 days) of the body weight twice a day (1000hrs and 1600hrs). Significant differences ($P < 0.05$) were found in the final body weight, body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), survival rate and Fulton's condition factor (K). The best growth performance and feed utilization was recorded in fish fed on $BSFLM_{100.}$The diets had significant ($P < 0.05$) effects on body composition and amino acid profiles of the experimental fish. Fish fed on $BSFLM_{100}$ exhibited highest levels of crude protein of the fish carcasses with highest values for phenylalanine, threonine, Isoleucine, lysine, proline and glutamic acid amino acids. On enterprise budget analysis, replacing SBM with BSFLM at 50% and 100% reduced the cost of culturing *O. niloticus* by 8.8% and 12%, respectively compared to control diet $COMM_{0.}$ The study demonstrated that BSFLM is a cost-effective alternative to SBM in the diets of *O. niloticus* hence can replace soybean meal up to 100% without negative effect on growth and carcass body composition.

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ABBREVIATIONS AND ACRONYMS

ANOVA	One-Way Analysis of Variance
BM	Blood meal
BSF	Black soldier fly
BSFM	Black soldier fly meal
CSM	Cotton seed meal
DBSFM	Defatted Black soldier fly meal
DM	Dry matter
EAA	Essential Amino Acid
ESP	Economic Stimulus Programme
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed Conversion Ratio
FeM	Feather meal
FFEPP	Fish Farming Enterprise Productivity Program
MBM	Meat and Bone Meal
MWG	Mean Weight Gain
PAP	Processed Animal Proteins
PER	Protein Efficiency Ratio
SBM	Soya Bean Meal
SGR	Specific Growth Rate

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CHAPTER ONE

INTRODUCTION

1.1 Background

Aquaculture is critical to global food security and has the potential to meet human food demand in the future. The demand for fish and fish products is increasing and is expected to increase dramatically (Arru et al., 2019) as the world's population grows to 10 billion by 2050 (Béné et al., 2015). Globalization of trade, declines in capture fisheries, favorable economics of larger scale intensive farming, rising incomes and urbanization have all contributed to the growth of aquaculture (Bostock et al., 2010). Despite being the world's fastest growing food producing industry, it has experienced varying annual growth rates of 10.8% and 9.5% in the 1980s and 1990s, respectively, 5.8% from 2001 to 2010, and 4.5% from 2011 to 2018 (FAO, 2022). Regardless of global and regional growth, the aquaculture industry is facing sustainability challenges because productivity has been undermined by high feeding costs, which are estimated to account for 40%-70% of total production costs (Henry et al., 2015).

According to FAO (2020), aquaculture production has been constrained by over reliance on diminishing supplies of fishmeal and fish oil as the main protein source in fish feeds (Chang et al., 2016). This is because of its high-quality protein, well-balanced amino acid profile, and essential fatty acids near requirement levels for most cultivated aquatic organisms (Bruni et al., 2021). A steady decline in the capture fishery, as well as competition from animal feed manufacturers, has resulted in a rapid decrease in global supplies of fishmeal and fish oil, leading to high prices (De Roos et al., 2017). As a result, researchers are prompted to look for alternative protein sources (Luthada-Raswiswi et al., 2021).

Nile tilapia is the most preferred culture species in the tropical and subtropical regions in the world (El-Sayed, 2006), accounting for about 75% of production in Kenya (Munguti et al., 2014). This is due to its fast growth, prolific breeding, resistance to diseases and ability to grow in a wide range of culture systems. For a long time, soybean meal has been the key ingredient used in tilapia feeds following technological innovation in feed production and high incentives for seeking cost effective alternatives for fishmeal and fish oil (Chang et al., 2016). Despite soybean's pivotal role in animal production (Arriaga-Hernández et al. 2021), its use in tilapia feeds is limited by the competition from livestock feed industry, human food consumption, industrial production of ethanol and biodiesel (Lu et al., 2020), hence the need for alternatives.

Insects have been shown in studies to be a viable substitute for the global issues associated with the expensive, unsustainable protein sources used in tilapia feed (Huis et al., 2013). Among insect species, the black soldier fly (*Hermetia illucens*) is regarded as one of the most promising substitutes for other protein sources due to its widespread distribution in both tropical and subtropical climate zones (Zurbrügg et al. 2018), ease of culture, and ability to feed on various categories of organic waste and convert the wastes into high quality proteins, fat, vitamins, and minerals (Gasco et al., 2019). BSF can also easily reproduce, grow quickly, control bacteria and zoonotic disease transmission, and has a prebiotic effect on the fish (Gariglio et al., 2019).

The nutritive value of *H. illucens* larvae in aqua feeds has been successfully tested on several fish species including Nile tilapia (*O. niloticus*) (Devic et al., 2018; Agbohessou et al., 2021; Taufek, et al., 2021; Tippayadara et al., 2021), Pacific white shrimp (*Litopenaeus vannamei*) (Cummins et al., 2017), Atlantic salmon (*Salmo salar*) (Li et al., 2020), Jian carp (*Cyprinus carpio*) (Zhou et al., 2018), rainbow trout (*Oncorhynchus mykiss*) (Stadtlander et al., 2017) and Juvenile grass carp (*Ctenopharyngodon idella*) (Lu et al., 2020). However, most of the studies have focused on replacing fish meal protein with black soldier fly larvae meal (BSFLM) thus scarcity of information on the effects of replacing soybean meal with BSFLM in the diets of Nile tilapia. In addition, most of the studies are focused on a specific life stage of the fish in different culture systems especially at the larval rearing and juvenile stage hence discrepancies on the best BSFLM inclusion levels for grow out feed. Finally, there's limited documentation on the amino acid concentration of cultured Nile tilapia fed on black soldier fly meal as soybean replacement. In this regard the focus of the study was to evaluate the effects of partial and complete replacement of soybean meal with black soldier fly meal on weight gain, growth rate, feed conversion ratio, survival, proximate body composition and amino acid concentration of cultured Nile tilapia.

1.2 Statement of the problem

The extensive utilization of soybean meal (SBM) by farmers in tilapia feeds as an alternative to fishmeal is attributed to its high protein content, global availability and cost. Though a major plant protein in dietary foodstuffs and animal feed, soybean application in aqua feeds is limited due to the relatively high carbohydrate, low crude fat and the lower levels of sulphur-containing amino acids, compared to those found in fish meal. The major limiting sulfur amino acid is methionine which can be converted to cysteine, but animals cannot convert cysteine to methionine. The typical soya bean percentage is between 1% and 2%, which is significantly less than the 3-5% basic minimum requirements for human consumption (Nielsen, 1996).Enhancing protein level and amino acid profile entails technological innovations, however, due to the processing costs involved, the enhanced products are expensive hence not economical for large-scale utilization in aqua feeds. Similarly, though genetic engineering has achieved the same objective, bio-safety concerns associated with genetically modified products is still contentious.

The presence of anti-nutritional factors in the seeds is also a matter of concern, though these are normally destroyed in toasted soybean meal, in either way thermal treatment or processing of soya bean lowers protein quality in terms of inadequate activation of anti-nutritional factors with low heat and destruction and binding up of amino acids as indigestible compound using high heat. In this regard there’s need to find a high quality, cost efficient and sustainable alternative protein source for soybean meal. Black soldier fly meal is a potential solution to tilapia production regarding reduction of feeding costs however nutritional factors like essential body composition, amino acid composition and cost are critical and therefore should be determined through experimental study when utilized as a soybean replacement in the diets of Nile tilapia.

1.3 Objectives of the study

1.3.1 General Objective

To evaluate the utilization of black soldier fly larvae (*Hermetia illucens*) meal as soybean meal replacement in the diet of cultured Nile tilapia (*Oreochromis niloticus*) on growth parameters, body composition, amino acid concentration and cost implication.

1.3.2 Specific objectives

1. To determine the effects of partial and complete replacement of soybean meal with black soldier fly larvae meal on weight gain, growth rate, feed conversion ratio and survival of Nile tilapia.
2. To evaluate the effects of partial and complete replacement of soybean meal with black soldier fly meal on proximate body composition and amino acid profile of cultured Nile tilapia.

3. To evaluate the cost effectiveness of replacing soybean meal with black soldier fly meal in the diets of Nile tilapia.

1.4 Research hypothesis

H_{O1}: Replacement of soya bean meal with black soldier fly meal in the diets of Nile tilapia did not affect weight gain, growth rate, feed conversion ratio and survival.

H_{O2}: Replacement of soya bean meal with black soldier fly meal in the diets of Nile tilapia did not affect proximate body composition and amino acid profile of cultured Nile tilapia.

H_{O3}: Replacement of soya bean meal with black soldier fly meal in the diets of Nile tilapia was not cost effective.

1.5 Justification of the study

Studies on utilization of black soldier fly larvae meal as a replacement of soybean meal in the diet of Nile tilapia have demonstrated discrepancies on the best BSFL inclusion levels to guide fish farmers. This is mainly associated with different culture systems used, short period of fish life cycle and stage of larvae utilized. To cater for the discrepancies, the experimental design adopted a culture system and management practices that reflect those widely used by most fish farmers. The study assessed full implications of using BSFL as a substitute for soybean meal in terms of growth, condition of fish, economic and commercial viability since the experiment caters for all the stages of fish growth from juvenile to the grow-out. The findings from this study will make a contribution to the body of knowledge on BSFLM production and utilization hence inform strategies for on-farm and commercial fish feed formulation and processing in the aquaculture industry.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture production and dynamics

Mainstreaming aquaculture in global food production systems is a factor of increasing demand for fish and fish products. Aquaculture's contribution to global fish production has steadily increased over the last two decades. Aquaculture production accounted for 82 metric tonnes of the estimated 179 metric tonnes of fish produced globally, representing 46% in 2018, up from 25.7% in 2000 (FAO, 2022). Total fish output is expected to rise from 179 million tonnes in 2018 to 204 million tonnes by 2030. The aquaculture production is expected to total 109 million tonnes (FAO, 2022).

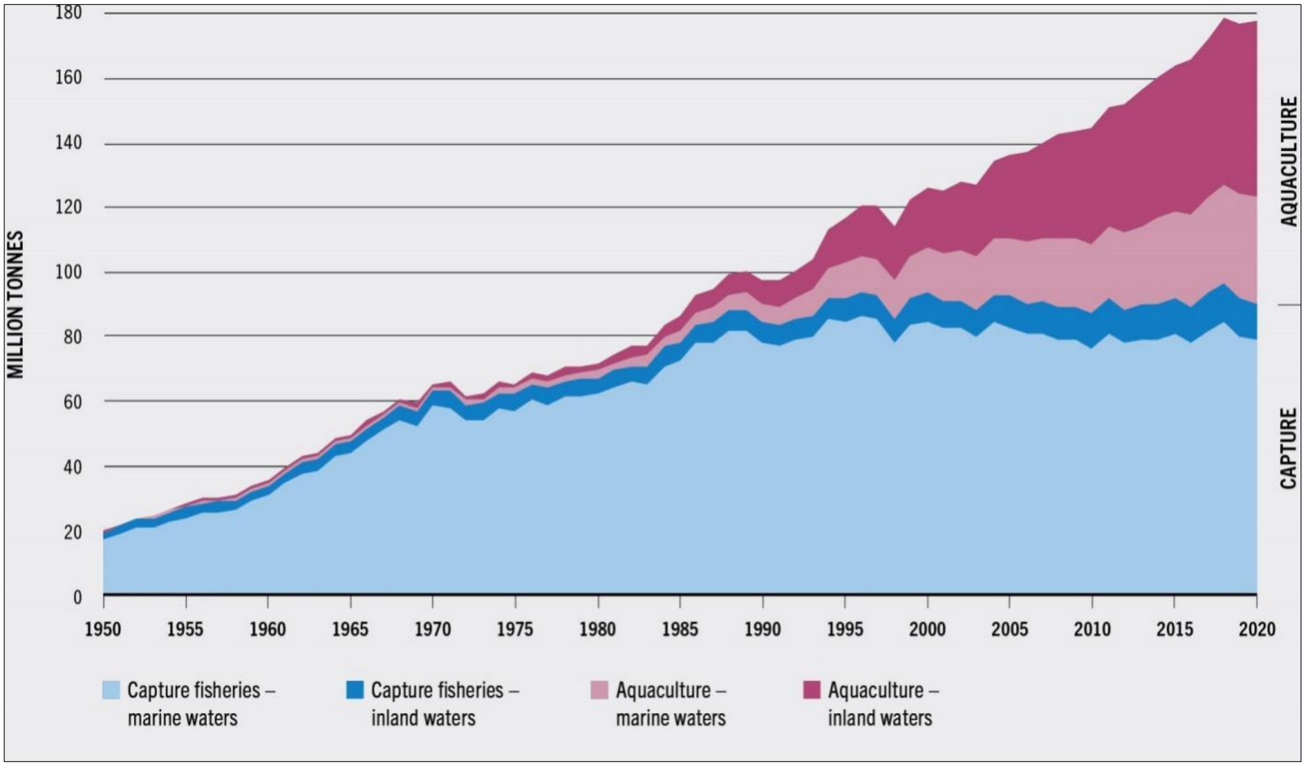

Figure 2.1: Global capture fisheries and aquaculture productivity, 1950– 2020 (FAO, 2022).

World capture fisheries and aquaculture production reveal progressive growth from 1950 to 2000 with stagnation of production in marine capture till 2020 compensated by aquaculture expansion (FAO, 2022). Aquaculture growth has been driven by trade globalization, declines in the capture fisheries, favorable economics of larger scale intensive farming, rising incomes and urbanization (Bostock et al., 2010). Though fastest growing food producing industry in the world, it has experienced varying annual growth rates (FAO, 2022).

China continues to be the major producer of aquaculture in Asia, which has a variety of production methods and produced species. For instance, a sizable portion of production in 2018 came from Asia (34%) with China excluded, followed by America (14%) Europe (10%) Africa (2.7%) and Oceania (1%). (FAO, 2022). Aquaculture now accounts for 42% of Asian fish production (outside of China), up from 19.3% in 2000. (FAO, 2020). Numerous reasons, such as the region's long history of aquaculture, population and economic expansion, a supportive regulatory environment, and improved market mechanisms, have contributed to its rapid rise (Halwart, 2020).

Aquaculture development in Africa is still at infancy stage with 2.7 percent of world aquaculture production dominated by few countries (Halwart, 2020) although significantly increasing. The region recorded production increase from 110,200 to 2,196,000 tons from 1995 to 2018 with an annual growth rate of 15.55% (FAO, 2022). Tilapia and African catfish dominate production with 99% from the inland freshwater systems while mariculture only contributes 1% to the total production quantity. Irrespective of this, it is an emerging and promising subsector in addressing food insecurity, unemployment rates and economic development (Satia, 2011). Aquaculture centered research, policies and framework are critical in addressing constraints associated with species, breeding, cost effective feeds and dissemination of aqua technologies (Cocker, 2014).

Historically, fish farming in Kenya started last century and there was stagnation in production until 2007. Small-scale aquaculture in Kenya expanded in the 1960s though failed in 1970s mainly because of inadequate extension services, lack of quality fingerlings, feeds and insufficient trained extension workers (Ngugi & Manyala, 2004).

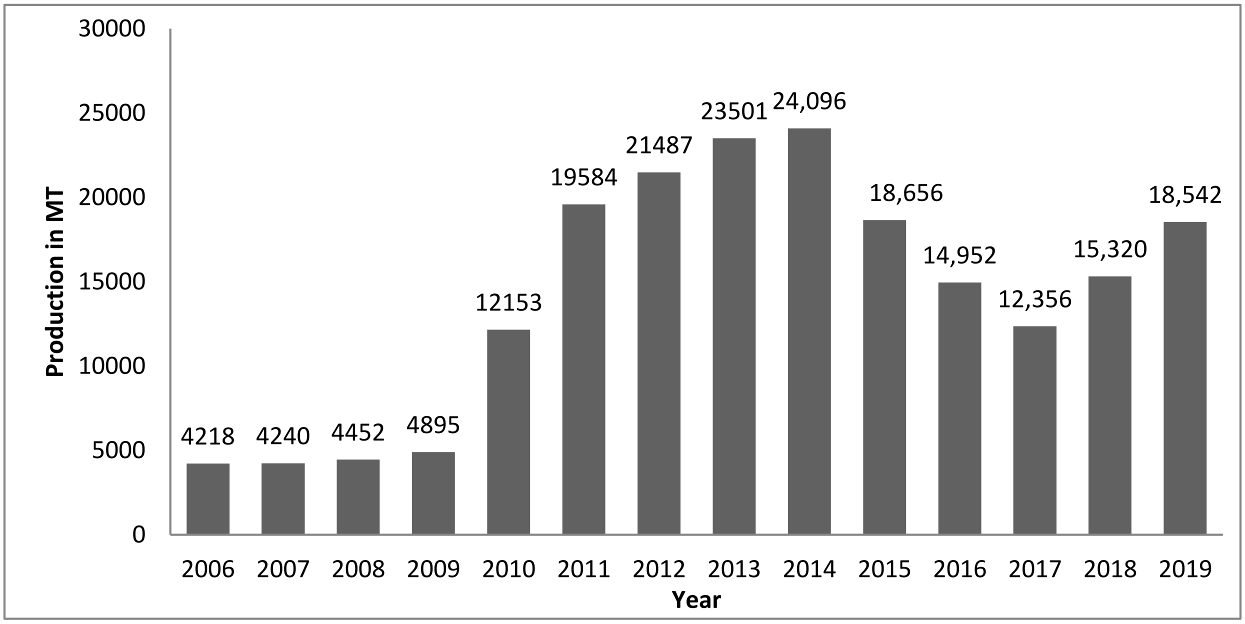

Figure 2.2: Aquaculture Production Trends in Kenya from 2006 to 2019 (Ogello et al., 2021).

With economic stimulus programme support, aquaculture production in Kenya increased markedly from 4,218 tons in 2006 to a peak in 2014 of around 24,096 tons dominated by Nile tilapia (75% of total production) and the African catfish (18%), followed by common carp (6%) and the rainbow trout (< 1%) (Opiyo et al., 2018). The initiative caused the demand for feed to increase from around 10,000 tonnes to about 50,000 tonnes per year (Munguti et al., 2014). A decrease in production to 12,356 tons was recorded in 2017 as a result of the program's termination.

2.2 Nile Tilapia culture

Nile tilapia is a worldwide important species in aquaculture (Lukistyowati et al., 2019) thus favored among aqua culturists. Nile tilapia has a versatile feeding behavior, characterized by generalist and opportunistic omnivorous feeding behavior (Canonico et al., 2015) and readily accepts complete pelleted feeds that contain plant and /or animal proteins.

Its diet composition may vary within a wide range of seasonal and spatial condition of the environments. The food composition may also vary depending on size of the fish, maturity, environmental condition and habitat types (Tesfahun & Temesgen., 2018). While adult tilapia need 20–30% dietary protein for optimal performance, juvenile tilapia have a protein demand of 30–40%.

Table 2.1: Protein requirement of Nile tilapia

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

Source: Mjoun et al., 2010

Tilapia can effectively utilize carbohydrate levels up to 30 to 40% in the diet, which is considerably more than most cultured fish. Jauncey, (2000) suggested that to maximize protein utilization, dietary fat concentration should be between 8 and 12% for juvenile tilapia and 6 to 8% for larger fish.

2.3 Alternative protein sources in aqua feeds native protein sources in aqua feeds

A wide range of unconventional protein sources, such as animal proteins, plant proteins, single-cell proteins, and industrial & agricultural wastes, have been assessed with regard to their applicability in farmed tilapia meals as substitutes for the widely used fishmeal (Montoya-

Camacho et al., 2019). Even though it's standard dietary practice to combine animal and plant protein to attain desired diet, some sources were discovered to be more cost effective than others.

2.3.1 Animal proteins

It's common practice to employ terrestrial processed animal proteins (PAP) in aquaculture diets, including blood meal (BM), meat and bone meal (MBM), feather meal, and poultry by-product meal (PBM) (Ogello et al., 2014). Blood meal, a byproduct of the meat industry, is utilized as a source of protein in fish diets. Blood meal is significantly higher in lysine and a better source of arginine, methionine, cystine, and leucine than fish meal or bone meal, but it is significantly lower in isoleucine and contains less glycine (Ogello et al., 2014). The meal can be included in diets to make up for the lack of lysine and methionine in diets based on vegetable proteins (Ayadi et al., 2012). Diets containing these ingredients have shown varying results.

The meal can be included in diets to make up for the lack of lysine and methionine in diets based on vegetable proteins (Ayadi et al., 2012). Blood-containing diets have reportedly produced a range of results. According to Kirimi et al. (2016), Nile tilapia showed decreased growth performance at greater levels of inclusion when fishmeal replacement with blood meal reached over 50%. Aladetohun et al. (2013) reported different results whereby blood meal can entirely replace fish meal without having any negative effects on growth. Bekibele et al. (2010) conducted research on the effects of local fish waste, blood meal, and imported fish meal on the growth and survival of young tilapia. The results showed that the fresh cattle blood-processed feed performed as well as the imported fish meal, boiled blood and local fish waste.

Meat meal and Meat bone meal are both produced by rendering the inedible or unsold by-products from slaughterhouse operations (Hicks et al., 2016). They hold several advantages, including a high protein content, with well-balanced amino acid profile; good source of digestible minerals, namely phosphorous and calcium; and lack of known anti-nutritional factors (Suloma et al., 2013). Meat and Meat bone meal has also good digestibility values, though great variability among fish species has been shown (Bureau et al., 2000). However, the high ash content, due to the presence of bone and other inorganic matter, is considered to be one of its major drawbacks and may limit its use in fish diets (Ogello et al., 2014). Numerous studies have been conducted globally to assess the possible usage of MBM in aquaculture species' diets. In a four-week experiment Siddika et al., (2012) indicated that up to 100% of FM protein can be replaced by MBM in diets for tilapia (Oreochromis niloticus) fry. Yilmaz et al., (2015) reported better utilization of SBM diet than MBM diet in Nile tilapia (*Oreochromis niloticus*) with low glycogen levels in muscle and liver tissues in fish fed with SBM diet than MBM diet.

Poultry by-product meal is a protein source produced from waste and by-products of processed chickens possibly including heads, feet, undeveloped eggs, gizzards and intestines (AAFCO, 2010) but excluding feathers. Lysine and methionine are deficient irrespective of high protein ranges from 51.6 to 81 (% DM) (Castillo-Lopez et al. 2016). Poultry by-product meal has been used successfully to replace FM at high levels of dietary inclusion for a number of finfish species. In juvenile Nile tilapia (*Oreochromis niloticus*), highest growth performance was achieved at 100% of the FM protein replacement with PBM (Yones et al., 2015). Dawood, et al (2020) observed that to obtain better growth performance and health condition the inclusion of PBM at 11.17–25.14% can be applied effectively in the diets of tilapia. Using poultry by- product meal- pet food grade (PBM) and porcine meal (PM) to determine apparent digestibility coefficients (ADC), Hernandez et al., (2010) showed statistical similarity in growth performance and feed utilization.

Feather meal is made from poultry feathers, and composed of keratins when raw that contain disulfide bounds that are only available on processing (Munguti et al., 2014). Feather Meal (FTM) has shown very promising findings when included in aquaculture feeds though deficient in Methionine, Lysine and Histidine (Ogello et al., 2014). An evaluation by Suloma et al., (2014) indicated that good-quality hydrolyzed feather meal (HFM) can successfully replace FM or SBM by up to 33 and 66 %, respectively, in Nile tilapia fry diets.

2.3.2 Plant protein derivatives

Among plant protein sources, soybean and cotton seed are the most frequently used among pea and lupin, corn gluten meal, simsim, peanut, as well as cereal concentrates, like maize and wheat (Munguti et al., 2014). Soybean is widely used as the most nutritive plant protein ingredient in pig, poultry and fish feed with a relatively high crude protein (CP) content (48% dry matter in standard meals) compared to FM that contains 50%–70% protein per kilo (Drew et al., 2007). However, validity of soy bean as a solution to the scarce and expensive fishmeal has been disputed following challenges associated with anticipated utilization in aquaculture species (Zhou, 2018). Soybean contain anti-nutritional factors such as trypsin inhibitor, antigens, lectins, saponins and oligosaccharides (Dersjant-Li, 2021) that adversely influence fish performance because they have high levels of non-soluble carbohydrates, such as fiber and starch, which have low nutrient digestibility, nutrient bioavailability, and palatability (Daniel,2018).Nutritional quality of soya bean meal can be enhanced by removing or inactivating the anti-nutritional factors either through thermal, mechanical, zymologic treatments or fermentation with microorganism (Li et al., 2015) or supplementation of essential amino acids.

The reduction in the feeding and growth with response to higher levels of dietary soybean proteins has been reported in several fish species. Studies have indicated that soy products may be incorporated in fish diets though with varying levels of inclusion. In a Nile tilapia trial whereby fish meal was partially substituted with methylated soy protein isolates, Othman (2020) observed increasing growth rate with increase with soy isolate up to 22% and inverse growth with increasing substitution of fishmeal. In Nile tilapia fed on a fishmeal and soybean diet, Sharda et al. (2017) showed similar outcomes. The amount of soybean meal in fish diets that exceeded 25% decreased development and growth parameters.

Amesa et al., (2018) observed body weight gain and body length gain of *O. niloticus* fed on soya bean meal substituted with fish offal meal, poultry litters and wheat bran. Dietary replacement of SBM with fish offal meal, poultry litters enhanced the growth of organic nutrients such as microbes, phytoplankton and zooplankton which would serve as source of protein for the fingerlings. Low growth was observed in fish fed with soya bean meal attributed to trypsin-inhibitor present in SBM. Godoy et al., (2019) achieved higher growth rates at around 45.0 g kg−1 of soybean inclusion oil to the finisher diet while using tilapia finisher diet with increasing levels of soybean oil 0,15,30,45 and 60 g kg^{-1}.

Ajani et al., (2016) demonstrated that soybean meal can either be used to partially replace fishmeal or completely replace it with methionine supplementation by feeding Nile tilapia fingerlings on diets with partial soybean meal inclusion, no soya bean meal, soybean only meal and soybean meal and methionine. Mean weight gain and increase in length were significantly high in fish fed with partial soya bean meal and least in fish fed with no soya bean.

However, total replacement caused a reduction in growth and feed utilization as highlighted by Dersjant-Li (2021), due to presence of approximately 15% of oligosaccharides (sucrose, raffinose and stachyose) which negatively affect nutrient utilization in fish. Ineffectiveness of replacing fishmeal with full-fat soybean in *Oreochromis niloticus* fingerlings was demonstrated by Abdel-

Warith, et al., (2019) by replacing fishmeal with 0%, 15%, 20%, and 25% soybean. Higher feed utilization was registered in control diet that had 0% of soybean with significant growth reduction with increasing soybean replacement that contributed to reduced availability of amino acids in diets than the minimum requirements of the fish.

Cottonseed meal is an important source of dietary protein for domestic animals, its use in commercial aquaculture feeds is limited because of low available lysine content and the presence of gossypol (a polyphenolic substrate) that is toxic hence associated with growth depression (Garcia-Abiado et al., 2004). In a 14- week experiment with diets supplemented with lysine El-Said et al., (2011) reported 75% of SBM replacement with CSM while Agbo et al., (2011) indicated 50% replacement in mono- sex male Nile tilapia fingerlings.

2.3.3 Insect-based meals

Insects are a natural food source for marine and fresh water fish species thus insect meal has stood out as an alternative ingredient in animal feeds (Van Huis et al., 2013). Black soldier fly (BSF), *Hermetia illucens* among common housefly (HF), *Musca domestica*; yellow mealworm (MW), *Tenebrio molitor*; lesser mealworm, *Alphitobius diaperinus*; house cricket, *Acheta domesticus;* banded cricket, *Gryllodes sigillatus*; field cricket, *Gryllusas similis* has been approved and tried in aquaculture feeds (EU, 2017).

2.4 The Black Soldier Fly

2.4.1 Ecology

The Black Soldier Fly (*Hermetia illucens*), a dipteran, inhabits both tropical and subtropical areas between the latitudes of 40°S and 45°N (Dortmans et al., 2017). BSF has several advantages over other insect species. The species is polyphagous and its gut extracts have high amylase, lipase and protease activities (Kim et al., 2011) hence the capability to feed on various categories of wastes and convert the wastes into high quality proteins (Li, 2016). They reproduce easily, grow fast, can control bacteria favoring low risk of zoonotic disease transmission (Van Huis et al., 2013) with great potential chemical to produce biodiesel (Barragan-Fonseca et al., 2017).

2.4.2 Lifecycle

BSF has five stages in its lifecycle: egg, larvae, prepupal, pupae and adult (Alvarez et al., 2012).

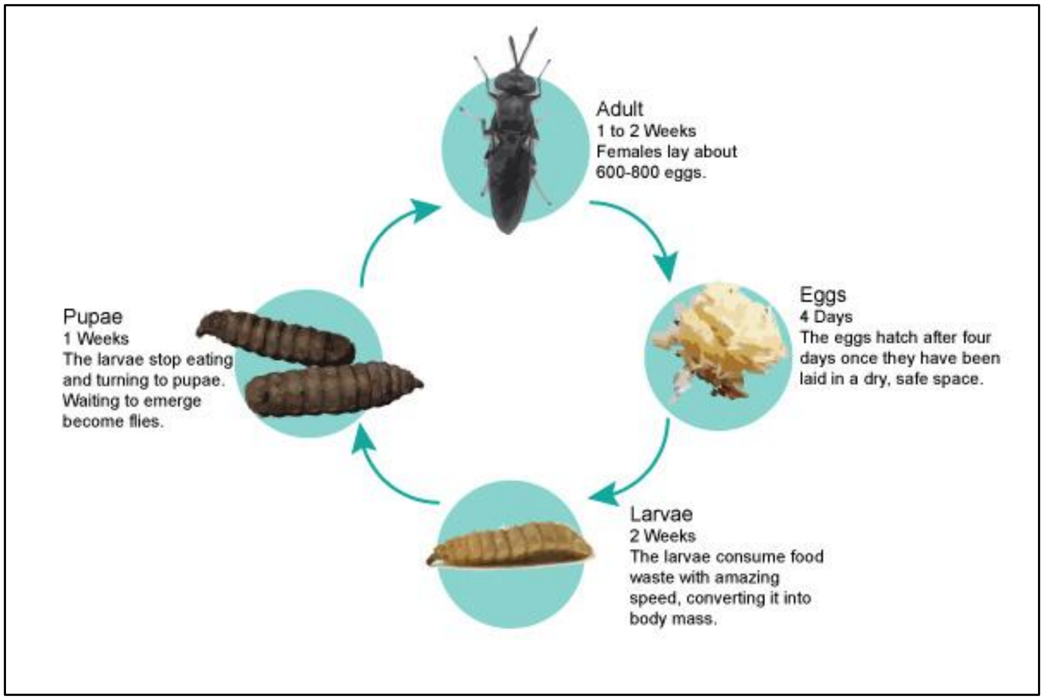

Figure 2.3: Black soldier fly lifecycle (Adapted from Dortmans et al., 2017)

The life cycle from egg to adult lasts 40 days but this length depends on the environmental conditions present and the rearing diet. Adult females lay a package of 400 to 800 eggs close to decomposing organic matter, into small, dry, sheltered cavities. The eggs hatch into larvae after 4 days, feed voraciously on the decomposing organic matter for period of 14-16 days to store enough fat reserves and protein to allow the larvae to undergo pupation (Dortmans et al., 2017). Adults emerge after 10-14 days, survive on the fat stored in their larval stage and feed on nothing except water (Nakamura et al., 2016), copulate and lay eggs before dying (Dortmans et al., 2017). Body composition of BSF larvae varies among substrates not only in protein content (ranging from 37 to 63% dry matter; DM) but also fat content, which has the most variation (ranging from 7 to 39% DM) (Barragan-Fonseca et al.,2017).

2.5 Utilization of Black Soldier Fly Larvae Meal (BSFLM) in aqua feeds

2.5.1 Effect of BSFLM on fish growth performance

Sustainability value attached to BSF meal is mainly attributed to the ability of BSF to be reared on organic waste with high feed conversion efficiency. Research on BSF meal inclusion in aqua feeds has been intensified and variations in reported studies are influenced by meal preparation methods (Gasco et al., 2018), substitution levels, stage of fish development and trial duration. In a 32-day Nile tilapia experiment by Devic et al., (2017), substitution of fish meal, fish oil and soybean meal with black soldier fly at 0, 30, 50 and 80 g/kg showed no difference in final weight, weight gain and SGR, feed utilization efficiency (Feed conversion ratio, protein efficiency ratio and feed intake. The results indicated that inclusions of up to 80 g/kg black soldier fly meal had no effect on feed quality for advanced nursing tilapia. Similarly, Dietz et al., (2018) reported a replacement of soya protein concentrate with partly defatted black soldier fly meal up to 50%, had no negative effect on growth performance and improved the dietary protein quality of feeds.

The trial was conducted on Nile tilapia in a semi-closed in-door water re-circulating system with 25, 50 or 100% replacement of Soya protein concentrate. However, they concluded that a higher inclusion rate of more than 50% HM tended to impair growth associated with indigestion of chitin. In another study, Wenqing et al., (2019) established that the replacement of soybean meal by *Hermetia illucens* larvae culture residue meal at 0%, 10%, 20%, 30%, 40% and 50% increased the weight gain rate and specific growth rate of tilapia though highest at 10% replacement. The study reported no significant difference in feed coefficient and survival rate of each group.

Yildirim-Aksoy et al., (2020) evaluated effects of diets containing frass at levels of 0, 5, 10, 20, and 30% as partial replacements of a combination of soybean meal, wheat short from black soldier fly larvae, *Hermetia illucens* in hybrid tilapia (Nile × Mozambique, *Oreochromis niloticus* × *O.mossambicusm*). They observed that final weight gain was significantly higher in fish fed with diet including the highest level of frass (30%). Fish fed diets containing frass (5% to 30%) had significantly higher protein efficiency than the group fed diet without frass (control diet). Feed intake and feed utilization efficiency were not significantly affected by dietary treatments. Analysis by Toriz-Roldan et al., (2019) of final body weight, weight gain, specific growth rate, mean biomass, total biomass, and survival, feed utilization of sex-reversed juvenile Nile tilapia (*Oreochromis niloticus*) fed on varying dietary supplementation levels 3, 6, and 9% of black soldier fly, *Hemertia illucens* larvae pre-pupae meal did not differ with commercial diet. The 49 days for the study were limited to detect any significant changes in growth though they concluded that larger protein efficiency ratio was at 6% black soldier fly meal supplementation level.

Zebra fish (*Danio rerio*), fed on black soldier fly-based diets (25 and 50% fish meal substitution) for six months, Zarantoniello et al., (2019) observed a negative fish growth with increasing percentage of BSF meal in the diets compared to the control due to unbalanced fatty acid composition in the diets. Gut histological analysis on intestine samples did not show signs of inflammation and both stress markers and immune response markers did not show significant differences among the experimental groups. In juvenile *Lotus japonicus*, replacement of FM with 0 %, 16 %, 32 %, 48 % and 64 % showed that growth performance, somatic indexes, hepatic, intestinal histo-morphology, intestinal antioxidant and immunity indexes of fish were not affected by dietary treatments and suggested supplementation of defatted black soldier fly larvae meal up to 192 g/kg (Wang et al.,2019). Contrary results were reported by Adeniyi et al., (2015) in *Clarias gariepinus* fingerlings where BSF prepupae meal incorporated at 0, 25, 50, 75 and 100% to replace fish meal had a positive correlation up to 50%. However, the reduced growth performance observed at 75 % and 100 % BSF inclusion levels could be due to lack of chitinase activity to digest high chitin in the meal, low feed intake and poor acceptability of the diet by the fish, hence resulting in reduced protein and energy intake required for African catfish.

In sea-water Atlantic salmon (Salmo salar) fed diets with increasing substitution of fish meal with black soldier fly meal at 33%, 66%, and 100%, feed intake, daily growth gain, and feed conversion ratio were also unchanged. This study demonstrated that it was possible to completely replace fish meal in the diets of sea-water Atlantic salmon with black soldier fly larvae meal without having any negative effects on growth performance, feed utilization, nutrient digestibility, liver traits, or the sensory qualities of the fillet (Belghit et al., 2019).

In Yellow Catfish (*Pelteobagrus fulvidraco*) involving diets that had an inclusion of 0 (control group), 10 %, 15 % ,20 %, 25 % and 30 % BSF showed that 20 % of the FM in the control diet could be replaced with BSF without significantly reducing weight gain, feed conversion ratio, or whole body and muscle proximate composition (Hu et al., 2017).

2.5.2 Effect of BSFLM on feed digestibility

According to Fontes et al., (2019), knowing digestibility of novel dietary ingredients is the basis for determining their bioavailability and hence their sustainability for inclusion in fish diets. Therefore, apparent digestion coefficient (ADC) offers details about how much a fish has digested and retained a feed. Zhou & Yue (2012) determined the apparent digestibility coefficient (ADCs) of dry matter, crude protein, crude lipid, gross energy, phosphorus and amino acids for different diets in juvenile hybrid tilapia. The results indicated that the ADCs of dry matter for juvenile hybrid tilapia ranged between 71.88 and 89.53% for animal products and 65.89 and 79.98% for plant products. For crude protein, apparent digestibility coefficients of protein (ADCp) exceeding 90% were observed for fermented soybean meal, soybean meal and Peruvian fish meal, and ADCp of meat and bone meal was the lowest among all the treatments.

Apparent digestibility coefficients of lipid in all the treatments were above 90%; the results indicate that lipids from both animal and plant sources were well digested by hybrid tilapia. The ADCs of phosphorus of local fishmeal, Peruvian fish meal, poultry by-product meal, meat and bone meal, tilapia by-product meal, ranged between 58.04-74.44 % while for fermented soybean meal, soybean meal, peanut meal, canola meal and cottonseed meal ranged between 52.65 and 64.23 % respectively.

An evaluation done by Fontes et al., (2019) on the nutritional value and energy apparent digestibility coefficient (ADC) of five insects namely cinereous cockroach (*Nauphoeta cinerea*) meal (Blattodea), *Zophobas morio* larvae meal (ZMM) (Coleptera), *Gromphadorhina portentosa* meal (GPM) (Blattodea), *Gryllus assimilis* meal (GAM) (Orthoptera) and *Tenebrio molitor* larvae meal (TMM) (Coleptera) in Nile tilapia male fingerlings. The fish diets comprised 20% test ingredient (insect meals) and 80% control diet (commercial diet with 32% crude protein.

The results indicated that using diets with chitin levels of 0 %, 2 %, 5 % and 10 %, found that the highest level of supplementation was accompanied by a lower level of dry matter ADC for Nile tilapia. In the opinion of Longvah et al., (2011), protein digestibility reduction is a result of chitin, which impedes dietary utilization of protein. A linear relationship was also shown in Nile tilapia in an experiment where commercial feed was substituted at 0 %, 12 %, 14 %, 16 % and 18 % with maggot flour (Kurniawan et al., 2018). Highest protein content gained at 18% with 17.267 % while the lowest was 16.344 % at 0 % substitution level.

In a related study, Teye-Gaga (2017) compared fishmeal and soya bean meal to BSF larval meal at inclusion levels of 25, 50, and 75% in formulated diets for O. niloticus fingerlings. The components in BSF meal, fishmeal, and soybean meal have high apparent digestibility coefficients (ADCs) of nutrients (> 52%), indicating good use of feed for tissue formation and metabolic processes. Mohamad-Zulkifli et al. (2019) measured the apparent digestibility coefficient of dry matter, protein, and lipid for three different types of black soldier fly (Hermetia illucens) larvae (BSFL) in juvenile hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*). Fishmeal and soybean meal were used as the control diet. The various BSFL meal types had a considerable impact on the ADC values. Fish fed with BSFL showed improved growth, feed efficiency, and survival rates.

The various BSFL meal types had a considerable impact on the ADC values. When compared to fish fed a control diet, fish fed BSFL meal-based diets did not significantly vary in terms of growth performance, feed efficiency, or survival. The results indicated that BSFL meal is highly digested by fish and may be utilized as a great replacement for fishmeal in practical diets without affecting hybrid grouper (*E. fuscoguttatus E. lanceolatus)* growth, feed efficiency, or survival.

In Atlantic salmon, apparent digestibility coefficient (ADC) of protein and lipid, protein efficiency ratio and lipid retention were reduced linearly with increasing inclusion level of BSFL meal and 12.5 % BSFL meal replacement was recommended as the best replacement level (Bruni et al., 2020). Low apparent digestibility coefficient for organic matter, crude protein, crude lipid, and gross energy were reported in juvenile turbot (*Psetta maxima*) attributed to chitin that affected feed intake, availability and digestibility Kroeckel et al., (2012) whereas in juvenile European sea bass (*Dicentrarchus labrax*) , high apparent digestibility coefficients (ADC) of protein, lipids, dry matter, organic matter, and energy were reported with high ADC of arginine, histidine, and valine diets with BSFLM (Magalhães et al.,2017).

2.5.3 Effect of BSFLM on fish body composition

Protein quality in relation to human requirements is measured by amino acid profiles and digestibility Van Huis et al.,(2013), consequently protein assimilation and retention in fish flesh is a factor of the amount of protein in the feed which influence feed utilization. A positive correlation was shown in Nile tilapia by Kurniawan et al., (2018) in an experiment where commercial feed was substituted at 0%, 12%, 14%, 16% and 18% with maggot flour. Highest protein content gained at 18% with the lowest 16.34% at 0% substitution level. There was an increasing protein content in Nile tilapia along with the increase of the maggot flour addition in each treatment.

In a 56 days study using (BSFM) with replacement levels at 0%, 25%, 50%, 75% and 100% for fishmeal in Nile tilapia, large variations in the lipid content were observed though the body protein content was not affected by diet composition (Muin et al., 2017). Cummins et al., (2017) found reduced whole-body protein and lipid in shrimp associated with deficiencies in essential amino acid (EAA) as well as increasing imbalances of EAA/nonessential amino acids (NEAA) contrary to findings by Kroeckel et al., (2012) in juvenile turbot (*Psetta maxima*) of high protein retention that decreased significantly with increasing HM supplementation. There was no alteration of whole-body protein content though whole-body lipid decreased with increasing BSFM inclusion level.

2.5.4 Effect of BSFLM on fish body amino acid profile

Fish eat protein in order to get amino acids. Free amino acids are produced during protein digestion, which are then absorbed from the intestine and utilized by different tissues to produce new proteins. The limited information that is currently available regarding the amino acid composition of protein in tilapia carcasses or edible portions has shown that changes in the amino acid contents depend on the amount of protein consumed. The value of the amino acids content per 100 g of protein can be used to describe how much the amino acids pattern of Nile tilapia protein is impacted. Increased amounts of essential amino acids, such as arginine, histidine, lysine, and methionine in L. calcarifer have been shown by Katya et al. (2017), while Jian carp's amino acid profiles were very variable when fed with BSFL-incorporated diets (Li et al., 2017; Zhou et al., 2018). According to Belghit et al. (2019), the amino acid profiles of S. salar were unaltered.

2.6 Black soldier fly legislation and safety aspects

Insect utilization in feed and food is a novel industry that lack regulations in many countries, especially developing countries to address safety concerns. This is contrary to western countries where majority of them are either in the process of developing, reviewing and /or implementing their regulations (Ravi et al., 2020). For instance, in European union there is legislation on rearing BSF whereby substrates are regulated while utilization of insects feed ingredients is subjected to Novel Food Regulation (EU) 2015/2283 (EU, 2015), Federal Agency for the Safety of the Food Chain, 2019; International Platform of Insects for Food and Feed, 2020 and a veterinary assessment according to Regulation 2019/2007 of imports (European Commission, 2019).

Also Regulation No 2017/893 permits the use of whole living insects and insect-derived from seven insect species including BSF and covers the maximum level of undesired substances such as heavy metals (HMs), pesticides and mycotoxin in feed used for the production of insects as well as feedstuff containing insects (Belluco et al., 2013).

In East Africa legislation on use of insects in animal feed was introduced in 2017 and customized to specific country bureau of standards. In Kenya, The Kenya Bureau of Standards (KEBS) has been mandated to practically ensure that food standards meet the international standards of the International Organization for Standardization and the Codex Alimentarius Commission, following the philosophy of WTO/SPS. The KEBS, in consultation with other relevant agencies through an established technical committee, has issued standards for insects used as food and feed. These standards provide specific requirements for the edible insect as a whole insect, an insect product and powdered insect products either sourced from domesticated farms or wild. The KEBS (via Kenya Standard [KS] 2922-1:2020 and KS 2922-2:2020) requires that edible insect products meet a set of general and specific requirements and specifications of additional ingredients. The legislation focuses on the end product and ensures there are no heavy metals, microbial or mycotoxin contaminants. Although BSFL are at a risk of carrying biological and/or chemical food safety hazard as a function of feeding substrate (Diener et al., 2015), pre-requisite treatment such as targeted thermal processing could eliminate biological hazards, such as pathogenic biota (macro and micro). Also, careful choice of feed substrate is critical in minimizing contamination (Ravi et al., 2020).

2.7 Cost effectiveness of BSFLM

In aquaculture enterprises, optimal profit is mainly related to the prices of feed price, feeding rate, stocking density, fish size, fish yield, and fish sales (Limbu et al., 2022). In the utilization of alternative feeds, economic efficiency is critical in ensuring they meet the economic balance on the market by price or positive effects on animal production in terms of feed efficiency or animal health. In sea bass partial replacement of dietary FM protein with DBSFLM protein showed a positive economic outcome and reduced the feeding cost by 15.6% (Abdel-Warith et al., 2019). Economic profitability due to improved fish growth performance and feed utilization parameters as a result of BSFLM introduction in Siberian sturgeon diets has been reported (Rawski et al., 2022). Limbu et al., (2022) demonstrated that feeding tilapia fry black soldier fly reduced feed cost by 30% leading to higher economic returns of 4%.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The experiment was conducted at Kenya Marine and Fisheries Research Institute, (KMFRI) Sagana -Aquaculture Centre (0^{o}19´S and 37^{o}12´E) for 168 days.

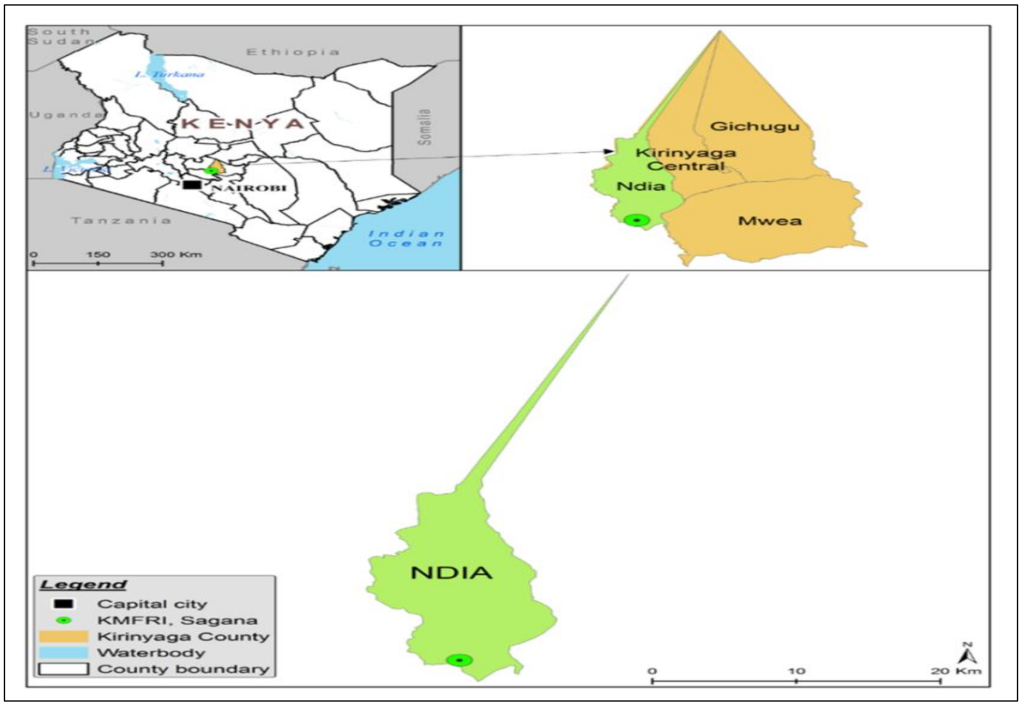

Figure 3.1: Map showing study site (Kenya Marine and Fisheries Research Institute) – Sagana fish farm (Courtesy Google maps)

3.2 Experimental diets

3.2.1 Black soldier fly larvae culture and processing

Black soldier fly larvae were reared for 21 days in a greenhouse (27 ± 1 °C; 65 ± 5% relative humidity) following the procedures used by Dortmans et al, 2017 on a growth substrate consisting of 75% chicken droppings and 25% (w/w) kitchen waste with 70% final moisture after adding distilled water. The rearing density was 0.3/cm^2 and the feeding rate per larva was 100 mg/day. The growth substrate was replaced once a week until the prepupal stage was reached. Harvesting was done after 15 days of feeding and re-feeding, at which time separation of larvae from the unutilized organic residues took place using a vibration gauge machine. The larvae were frozen at −20 °C for 24 h after sifting and washing by immersing them in a basket of cool water and then draining. They were oven dried at 65 °C for 24 h in a conduction oven in trays with a maximum thickness of larvae layer ≤ 1 cm. The dried samples were stored at room temperature and deffatted mechanically.

Dried larvae were extracted using a screw-press expeller (InVIA, Barcelona, Spain) with a heating jacket to allow the drainage of the oil. The press was pre-heated at the minimum temperature (136.0 ± 13.1 °C), and the temperature of the press head was continuously monitored during pressing, reaching a mean maximum value of 140.4 ± 10 °C and a mean minimum value of 126.6 ± 11.9 °C. The collected crude fat was centrifuged (Multifuge 3SR+ centrifuge, Thermo Scientific, Waltham, MA, USA) at 3400× g for 10 min to remove co-extracted solids. The sterilized and dried larvae were then ground into powder for the subsequent fish diets formulation.

3.2.2 Soy bean meal processing

Whole soybeans were cracked, dried, heated (steamed) and fed to a mechanical press (screw press).The resulting flakes were passed through a screw press to extract the oil, dried , ground and refined into soybean flour.

3.2.3 Experimental diets formulation

Three diets were formulated following standard protocols to meet the nutrient requirements of Nile tilapia. Proximate analysis of test ingredients was conducted to guide formulation of three isonitrogenous (30% crude protein) experimental diets. The insect meal was used to replace soybean meal partially and completely at various inclusion levels: $BSFLM_0$ (control) (100% SBM + 0% BSFLM); $BSFLM_{50}$ (50% SBM + 50% BSFLM), $BSFLM_{100}$ (0% SBM + 100% BSFLM).A commercial diet (COMM$_0$), sourced from local feed manufacturer in Nairobi was used as a positive control. In the production of diets: BSFLM$_0$, BSFLM$_{50}$ and BSFLM$_{100,}$ the ingredients were mixed thoroughly with water to make homogeneous dough and pelletized using 2-4 mm diet commercial pelletizing machine into floating pellets. The pellets were sun-dried, packed and stored in clean, dry and cool environment.

Table 3.1: Ingredients of the experimental diets

Ingredient inclusion (%)	**BSFLM$_0$**	**BSFLM$_{50}$**	**BSFLM$_{100}$**
Wheat bran	28	28	28
Soybean meal	30	15	0
Black soldier fly larvae meal	0	15	30
Maize germ	18	18	18
Freshwater shrimp	12	12	12
Sunflower seed cake	9	9	9
Mono-calcium phosphate	1	1	1
Vitamin premix	1	1	1
Soybean oil	1	1	1

3.3. Proximate analysis of the experimental diets and fish carcass evaluation

The analysis of experimental diets and final fish carcass composition was carried out in triplicates following standard methods by Association of Official Analytical Chemists (AOAC, 1995). Dry matter (DM) was determined gravimetrically by reweighing 2 grams of sample that had been oven dried for six hours to constant weight at 105^{o}C. Crude protein (CP) was analyzed by Copper Catalyst Kjeldahl Method. Analysis was done by taking 1g of each sample and 2 tablets of catalyst (Kjeltabs) which were digested in 15 mL concentrated sulfuric acid at 420°C. The samples were cooled and automatically distilled in Kjeldahl equipment with 40% NaOH and ammonia gas trapped in 4% Boric acid was reverse titrated using 0.2 N HCl. The nitrogen content was determined and converted to crude protein content using a nitrogen factor for the crude protein calculation of 6.25. Ash was determined by expressing the weight of 2 g of the ground sample burnt at 600°C for 3 hours in a muffle furnace as a percentage of the un-burnt sample weight. Crude fat was extracted by heating 3 g of the sample in Diethyl ether under reflux at 105°C for 30 min in a VELP Solvent Extraction unit. Ether extract was calculated as the difference between the original sample and the ether extract residue. Crude fibre (CF) was determined gravimetrically by chemical digestion and solubilization, and quantified by: CF (%) = dried sample (g) – ashed sample (g) / initial sample weight × 100). Nitrogen free extract (NFE) was determined by the difference between the original weight of the sample and sum of the weights of its moisture, crude protein (CP), crude fat (CF), ash and crude fibre as: N.F.E = 100 - (% moisture + % protein + % lipid + % fiber +% ash);

Amino acids were analyzed using ion exchange liquid chromatography via continuous flow chromatography. Samples of 100 mg were transferred into a 5 ml micro- reaction vial into which 2ml of 6N HCl was added and closed after introduction of nitrogen gas. The samples were hydrolyzed for 24 h at 110 °C. For tryptophan analysis, 100 mg from each of the samples were separately transferred into a 5 ml micro-reaction vial into which 2 ml of 6N NaOH were added and then capped after introduction of nitrogen gas. The samples were hydrolyzed for 24 h at 110°C. After the hydrolysis, the mixtures were evaporated to dryness under vacuum. The hydrolysates were reconstituted in 1 ml 90:10 water: acetonitrile, vortexed for 30 seconds, sonicated for 30 minutes, and then centrifuged at 14,000 rpm and the supernatant analysed using LC-MS. The compounds were identified and quantified using authentic sample mixture (amino acid standard solution (AAS 18) purchased from Sigma-Aldrich (Chemie GmbH, Munich, Germany).

3.4. Experimental design

3.4.1. Sex reversal of fish

A hormone treated feed was prepared as described by Killian and Kohler (1991). Gonadally undifferentiated Tilapia niloticus fry of mean total length of 10 mm were stocked in aquaria, each measuring 50 x 30 x 30 cm length, width and depth, respectively at a rate of 200 fry per aquarium. Room temperature was maintained at 25^0C while water temperature ranged from 28 - 31^0C. The fry were fed with methyl testosterone at the dosage of 50 mg/kg of dry diet for a period of 21 days.

3.4.2. Experimental layout and design

Twelve cages of size 4 m^3(2m by 2m by 1m) were constructed using plastic pipes into a cage frame with an inner netting (1 inch) and outer netting (2.0 inches) were securely fixed in and out of the cages.

Figure 3.2: Photo showing layout of cages in the ponds at study site (Sagana fish farm)

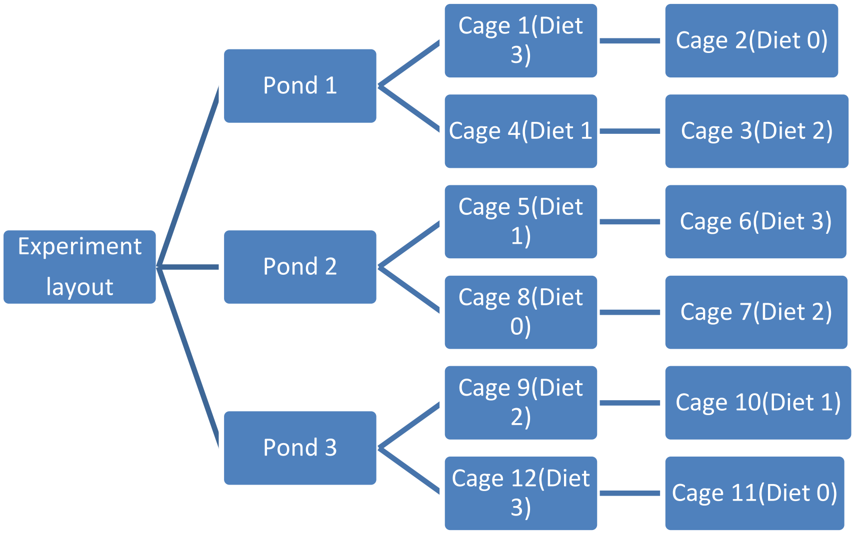

Figure 3.3: Experimental layout and design

3.4.3 Fish stocking

A total of six hundred sex reversed male *O. niloticus* juveniles of 20.88 ± 0.16 g mean weight were sourced from Kenya marine and fisheries research institute and acclimatized for seven days prior to the experiment. The experimental fish were randomly stocked in 12 cages each at a density of 12.5 fish /m^3 with four cages suspended in 150 m^2 earthen ponds.

3.5. Determination of growth performance

The experimental fish were hand fed twice a day (1000hrs and 1600hrs) at 5 % of body weight for 28 days, 3 % of body weight for 54 days and 2.5% of body weight for 84 days. The fish were randomly assigned to the four experimental diets containing BSFLM in varying proportions of 0% (BSFLM$_0$), 50% (BSFLM$_{50}$), 100% BSFLM$_{100}$) and a commercial diet (COMM$_0$) in triplicates. The experimental fish were hand fed twice a day (1000hrs and 1600hrs) at 5 % of body weight for 28 days, 3 % of body weight for 54 days and 2.5% of body weight for 84 days. The initial standard and total lengths of the fishes were measured to the nearest ± 0.1 cm using a fish measuring board.

Their various weights were also taken to the nearest ±1.0 g using a weighing balance for each treatment before stocking.

Fish sampling was done every 28 days. The amounts of feed in respective feed types were determined through the sampling that was carried throughout the culture period to monitor growth performance. At least 50 fish in each cage were randomly sampled by partially lifting the cage netting and removing fish with a dip net. The cage nets were cleaned on monthly basis.

The individual length (cm) and weight (g) of the fish were determined using electronic weighing scale (model EHB-3000, China) and a measuring board respectively. To evaluate the growth and feed efficiency the following standard formulas were used:

Specific growth rate (SGR, %) = 100 × [(Ln BW final (g) – Ln BW initial (g)) / days of experiment]

Body weight gain (BWG, g) = Final weight (g) - Initial weight (g)

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

Fulton's condition factor (K) = $100(W/L^3)$

Length- weight relationship was determined by the formula=$W = aL^b$, where W = weight (g) and L = total length (cm), a and b are the regression slope and intercept (regression coefficient) a

Percentage Survival (%) = 100 × (final number of fish)/ (initial number of fish)

3.6. Evaluation of carcass composition and amino profile

Forty eight pieces of Nile tilapia of age 168 days representing 4 diets in triplicate were sampled. The fish samples were cleaned, descaled, eviscerated and filleted manually using sterile plastic knife. The muscles of the dried samples were prepared into powdered form and labeled for the proximate composition analyses

3.7. Water quality monitoring

Dissolved oxygen, water temperature, pH, conductivity and total dissolved solids were monitored weekly at 1000hrs using a multi parameter water quality meter (YSI industries, yellow springs, OH, USA). Water samples were collected using 250 milliliter (mL) plastic bottles after every two weeks for ammonia, nitrites, nitrates and phosphorus analysis in the laboratory following the procedures by APHA (1995).

3.8. Enterprise budget analysis

Partial enterprise budget was used to evaluate the economic implications of substituting SBM with BSFLM in the diets of Nile tilapia. Variable costs included the cost of labor, feeds and fingerlings. The cost of formulated diets ($BSFLM_0$, $BSFLM_{50,}$ and $BSFLM_{100}$) was calculated based on the cost of the ingredients in each diet and the cost of feed production while the price of commercial diet was based on feed company retail prices. Labor costs were based on the prevailing market prices within the experiment site. The US dollar exchange rate against Kenya shillings was pegged at KES 107.15.

The study assumed that all other costs of production were constant for all dietary treatments and thus not considered. Fish yield was the total quantity in kilogrammes of fish harvested per treatment. Fish harvested per treatment was sold at US$ 3.73Kg^{-1}, which is the market price of Nile tilapia. Further, to gauge on the profitability of the alternative the break-even price was calculated by adding the total fixed cost to Variable Cost per unit. While, Break-even point = (Total fixed costs)/ [(Revenue per unit) - (variable costs per unit)]

Table 3.2: Partial enterprise budget input expenditures

Item	**Units**	**Quantity**	**Unit cost (US$)**	**Total (US$)**
Nile tilapia fingerlings	Pcs/cage	50	0.09	4.5
$BSFLM_0$	Kgs/ cage	12.57	1.06	13.32
$BSFLM_{50}$	Kgs/ cage	12.68	0.95	12.05
$BSFLM_{100}$	Kgs/ cage	13.74	0.86	11.82
$COMM_0$	Kgs/ cage	13.31	0.94	12.51
Labor (feeding & pond management)	US$/cage/Month	6	0.4	2.4
Cost of packaging fish	US$/Kg	-	0.009	-
Cost of transporting fish	US$/Kg	-	0.027	-
Tax on sale of fish	US$/Diet		0.47	
Total yield $BSFLM_0$	Kgs	17.77		
Total yield $BSFLM_{50}$	Kgs	16.08		
Total yield $BSFLM_{100}$	Kgs	20.54		
Total yield $COMM_0$	Kgs	20.81		

1US$=KES 107.15

3.9. Data analysis

The obtained data was cleaned and subjected to the Shapiro–Wilk test of normality. Statistical analyses were performed using MS excel and Statistical Package for the Social Sciences (SPSS version 23). Comparison was done by analysis of variance (one-way ANOVA) followed by Tukey HSD post hoc test to determine the pairwise differences among the diets. Results were presented as means ± SE (standard error of mean) with $P < 0.05$ considered significant. Percentage data were arcsine transformed prior to analysis to normalize data.

CHAPTER FOUR

RESULTS.

4.1. Water quality parameters

The mean values for water quality parameters of the pond water were stable with minimal variations during the experiment period as shown in Table 4.1. However, they were within the recommended optimal levels for Nile tilapia.

Table 4.1: The physicochemical water-quality parameters measured in the experimental ponds for 168 days. Values are mean ± standard deviation

Parameter	Mean	Min	Max	Optimal levels
Temperature (^{0}C)	25.31±1.89	20.40	27.60	20-30
D.O(Mgl^1)	5.47±1.47	1.56	8.45	>3
Conductivity(µScm^1)	104.59±4.59	60.50	155.00	150 & 500
TDS (Mgl^1)	67.04±7.04	39.80	97.97	0.13
Salinity (Mgl^1)	0.05±0.04	0.03	0.07	< 0.19
pH (Mgl^1)	8.35±1.35	7.51	9.91	6.5-9
PO4 Conc. (Mgl^1)	0.003±0.00	0.0013	0.0056	<0.05
NO2 Conc. (Mgl^1)	0.002±0.00	0.0002	0.0040	<0.05
NO3 Conc. (Mgl^1)	0.004±0.00	0.0001	0.0142	<0.05
NH4 Conc. (Mgl^1)	0.002±0.00	0.0007	0.0034	<0.05

DO; Dissolved oxygen, TDS; Total dissolved solids, PO_4; Phosphates, NO_2; Nitrites, NO_3; Nitrates, NH_4; Ammonium.

4.2 Proximate composition of the analysed ingredients and experimental diets.

4.2.1 Proximate composition of the analysed ingredients

Table 4.2: Proximate composition of the analysed ingredients used in diet formulation (%)

Parameter	Unit	SBM	BSFLM	Wheat bran	Cottonseed cake	Sunflower cake	Maize germ
DM	g/kg	937	958	894	905	920	894
Crude protein	g/kg	621	253	322	273	423	322
Crude fibre	g/kg	99	80	95	287	179	95
Ether extracts	g/kg	124	273	114	86	99	114
Ash	g/kg	300	147	32	53	53	32
NFE	g/kg	218	105	331	205	166	331

Table 4.3: Calculated essential amino acid (EAA) content (g/kg feed) of the SBM AND BSFLM used in diet formulation (%)

	SBM		**BSFLM**	
Essential amino acids	**g/kg feed**	**g/kg protein**	**g/kg feed**	**g/kg protein**
Histidine	13.6	21.8	6.5	25.7
Threonine	22.8	36.7	11.1	44.0
Arginine	33.4	53.8	13.7	54.2
Valine	28.8	46.4	16.3	64.4
Isoleucine	25.4	40.8	12.0	47.5
Phenylanine	23.8	38.3	11.7	46.2
Leucine	44.1	71.1	19.1	75.6
Lysine	2.6	8.6	16.7	66.2
Methionine	1.3	2.2	14.5	17.6
Tryptophan	5.5	8.8	3.4	13.5
Non-essential amino acids				
Alanine	41.5	66.8	18.7	73.9
Tyrosine	18.8	30.2	18.0	71.3
Glycine	37.6	60.5	13.9	55.1
Asparagine	51.5	83	25.8	101.8
Glutamate	92.6	149.1	34.5	136.2
Serine	20.1	32.4	11.8	46.8
Cysteine	3.5	5.6	2.5	9.8

4.2.2. Proximate analysis of experimental diets.

Proximate analysis of the dietary treatments are shown in Table 4.4. The analyzed protein content in the diets was in the range of (30.20-30.50 %) as the approximated 30% CP level, with minimal fluctuations between diets. The lipid level did fluctuate more from the approximated 7%, where the BSMF$_{50}$ diet had lowest value (6.31%) but COMM$_0$ the highest value (6.8%).

The analyzed lipid level was within the recommended levels. The ash content was varying between diets, in the range of (7.3-8.23 %). The calculated content of Nitrogen free extracts (fibers + other carbohydrates) was high due to inclusion of wheat bran and in the range of (42.0-48.1 %) with COMM$_0$ exhibiting the highest NFE value.

Table 4.4: Composition and results from proximate analysis of test diets fed to Nile tilapia for 168 days containing different levels of black soldier fly larvae meal (BSFLM) as a replacement for soybean meal (SBM).

Proximate composition (%)	**$BSFLM_0$**	**$BSFLM_{50}$**	**$BSFLM_{100}$**	**$COMM_0$**
Crude protein	30.2 ±0.32	30.28 ±0.61	30.34 ±0.15	30.5 ±0.22
Crude fat	6.35 ±0.03	6.31 ±0.02	6.46 ±0.00	6.8 ±0.00
Ash (dry)	7.75 ±0.26	7.99 ±0.12	8.23 ±0.42	7.3 ±0.16
Nitrogen free extract (NFE)	42.00	45.40	45.90	48.10
Essential amino acids				
Histidine	6.9	8.4	9.7	9.2
Threonine	13.2	14.9	16.5	17.1
Arginine	29.2	28.4	30.1	29.9
Valine	15.1	17.7	19.3	19.7
Isoleucine	14.7	14.5	16.2	15.1
Phenylanine	14.6	16.7	18.2	18.3
Leucine	22.1	26	28.1	28.2
Lysine	20.3	22.9	25.6	24.7
Methionine	1.1	9.2	13.8	10.5
Tryptophan	3.1	3.5	4.7	2.7

$BSFLM_{100}$ showed higher concentrations (g/kg feed) of essential amino acids (Table 4.4). On the other hand, concentrations of essential amino acids (in g/kg protein were comparable in $BSFLM_0$ with an exception for lysine and methionine.

4.3. Determination of growth performance

The results for the growth performance of Nile tilapia fed on diets with different levels of BSFLM are shown in Table 4.5. The BSFLM replacement level had a significant effect on the growth of the fish ($P < 0.05$).

The final mean body weight of fish fed on $BSFLM_{100}$ was significantly higher than $BSFLM_0$ and $BSFLM_{50}$. Fish body weight increased by 89% in fish fed on $BSFLM_{100}$ and $COMM_{0,}$ while an increase of 87% was observed in fish fed on $BSFLM_0$ and $BSFLM_{50.}$

Fish fed on $BSFLM_{100}$ had significantly higher BWG and SGR ($P < 0.05$) and the lowest FCR compared to those fed on $BSFLM_0$ and $BSFLM_{50}$. Fish fed on $BSFLM_0$ presented significantly lower BWG, SGR and the highest FCR. All the diets recorded a positive correlation co-efficient ((R^2) of 0.98 that were insignificantly different among the diets ($P > 0.05$). The values of Fulton's condition factor recorded for all the fish ranged between 1.86 and 1.89. There were significant differences ($P < 0.05$) in the Fulton's condition factor (K) between the $BSFLM_0$, $BSFLM_{50}$, $BSFLM_{100}$, and $COMM_0$ with control diet ($COMM_0$) having the highest Fulton's condition factor. Survival rates were relatively higher in all the treatments with significant variations ($P < 0.05$) among the diets. The highest survival rates were recorded in diet $BSFLM_0$ (95.33%) while the lowest in $BSFLM_{100}$ (92.67%)

Table 4.5: Growth performance parameters of *O. niloticus* fed on diets with different levels of defatted black soldier fly larvae meal and commercial diet

Parameter	**$BSFLM_0$**	**$BSFLM_{50}$**	**$BSFLM_{100}$**	**$COMM_0$**
Initial length (cm)	10.57±0.07	10.54±0.06	10.57± 0.06	10.56± 0.05
Initial weight (g)	20.83±0.33	20.86±0.33	20.85±0.33	20.88±0.30
Final length (cm)	$21.04±0.13^a$	$21.35±0.13^a$	$21.67±0.12^b$	$21.53±0.17^b$
Final weight (g)	$166.19±3.18^a$	$169.03±2.91^a$	$193.35±3.69^b$	$188.56±5.46^b$
SGR (% day $^{-1}$)	$1.23±0.02^a$	$1.24±0.01^a$	$1.32±0.013^b$	$1.29±0.02^b$
BWG (g)	$145.36±3.22^a$	$148.17±2.94^a$	$172.40±3.66^b$	$167.68±5.46^b$
FCR	$1.87±0.04^a$	$1.86±0.046^b$	$1.79±0.03^a$	$1.82±0.05^a$
Condition factor (K)	$1.86±0.01^a$	$1.86±0.01^a$	$1.88±0.01^{ab}$	$1.89±0.01^b$
SR (%)	$95.33±0.10^a$	$94.67±0.10^b$	$92.67±0.20^c$	$93.33±0.26^c$

SGR - Specific growth rate, BWG - Body weight gain, FCR - Feed conversion ratio, SR - Survival rate

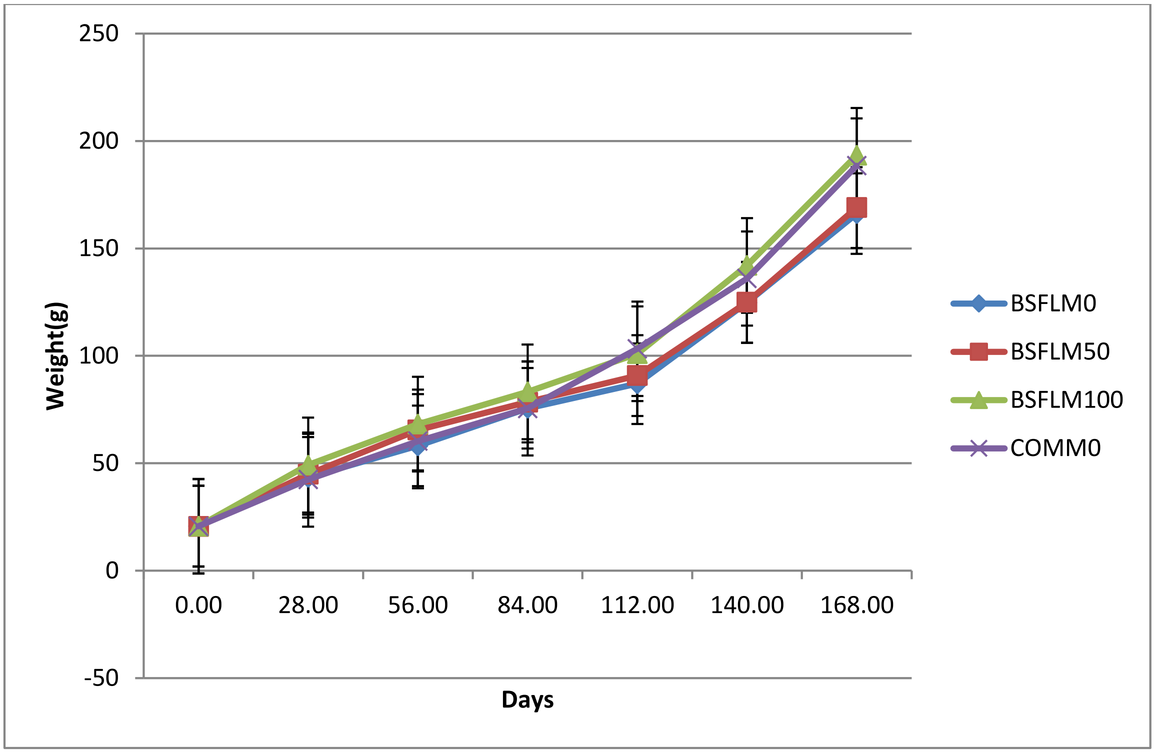

Figure 4.1: Growth curves for O. niloticus fed on the test diets with varying levels of BSFL meal during the 168 days culture period.

Figure 4.1 shows changes in weight of fish fed with different experimental diets. All the groups showed a gradual growth in the first, second and third month with convergence of diet $BSFLM_0$, $BSFLM_{50}$ and $COMM_0$ in the third month. $COMM_0$ showed dramatic growth in the fourth month with an overlap with $BSFLM_{100}$ at the end of the fifth month. $BSFLM_{100}$ recorded the highest weight gain followed by $COMM_0$, $BSFLM_{50}$ and finally $BSFLM_{0.}$

4.4 Body composition analysis

Partial replacement of SBM with BSFLM affected the whole-body composition in terms of protein, fat and NFE (Table 4.6). After 168 days of experiment, the carcass ash content was recorded the highest (7.2 ± 0.39%) in the fingerlings fed on diet $BSFLM_{50}$. The least carcass ash content (6.8± 0.15) was recorded in the fingerlings fed on diet BSFLM100.There was no significant difference (P>0.05) in the carcass ash content of the fingerlings fed on diets BSFLM0, BSFLM50 . $BSFLM_{100}$ and control diet $COMM_{0.}$

The carcass crude protein was recorded the highest (75.7 ± 1.03%) in the fingerlings fed on diet $COMM_0$. The least carcass crude protein (72.4 ± 0.81%) was recorded in the fingerlings fed on diet $BSFLM_{0.}$ There was no significant difference (P>0.05) in the carcass crude protein of the fingerlings fed on control diet $COMM_0$ and diet $BSFLM_{100}$ .The carcass crude protein of the fingerlings fed on diet $BSFLM_0$ and $BSFLM_{50}$ was significantly lower (P<0.05) as compared to the carcass crude protein of the fingerlings diet $COMM_{0.}$The carcass protein content increased significantly (P<0.05) with the increase in BSFLM. The least carcass crude fat content (3.3 ± 0.01) was recorded in the fingerlings fed on diet $BSFLM_{50}$.There was significant difference in the carcass crude fat content of the fingerlings fed on diets $BSFLM_{0,}BSFLM_{50\,,}$ $BSFLM_{100}$ and control diet $COMM_{0.}$The carcass nitrogen free extract content exhibited inverse relationship with the carcass crude fat and also with the crude protein dietary lipid level.

Table 4.6: Body carcass composition of Nile tilapia fed on diets with different levels of defatted black soldier fly larvae meal and commercial diet

Parameter (%)	**$BSFLM_0$**	**$BSFLM_{50}$**	**$BSFLM_{100}$**	**$COMM_0$**
Ash	7.0 ± 0.59^a	7.2 ± 0.39^a	6.8± 0.15^a	6.9 ± 0.49^a
Crude protein	72.4 ± 0.81^a	73.8 ± 0.46^a	75.3 ± 0.96^b	75.7 ± 1.03^b
Crude fat	3.3 ± 0.01^a	2.9 ± 0.29^b	5.0 ± 0.31^c	8.3 ± 0.26^d
NFE	5.5^a	6.5^b	2.3^c	1.13^d

The whole body essential amino acid pattern of *O. niloticus* is presented in Table 4.7. Out of 20 amino acids, a total of 16 (10 essential and 6 non-essential) were identified. For essential amino acids, fish fed on $BSFLM_{100}$ exhibited significantly ($P < 0.05$) higher values for phenylalanine, threonine, isoleucine, lysine, histidine and leucine.

The non-essential amino acids: glutamic acid, cysteine, proline were higher in fish fed in BSFLM$_{100}$ as compared to control diets (COMM$_0$ and BSFLM$_0$). Fish fed on COMM$_0$ registered highest mean values for arginine, glycine, methionine, tyrosine, tryptophan and tyrosine although, they were statistically similar to fish fed on BSFLM$_{100}$. The lowest values for glycine, alanine, lysine, cysteine, glutamic acid, methionine, and isoleucine were registered in fish fed on BSFLM$_{50}$ while arginine, histidine, tryptophan, leucine threonine, proline were lowest in fish fed on BSFLM$_0$ (control).

Table 4.7: Amino acids profile of O. niloticus fed on diets with different levels of defatted black soldier fly larvae meal and commercial diet.

Amino acids (%)	**BSFLM$_0$**	**BSFLM$_{50}$**	**BSFLM$_{100}$**	**COMM$_0$**
Essential amino acids				
Phenylalanine	24.7 ± 2.30^a	23.3 ± 2.90^b	67.8 ± 5.40^c	64.8 ± 4.00^d
Threonine	3.7 ± 0.10^a	9.7 ± 0.70^b	19.8 ± 1.60^c	17.5 ± 1.10^d
Arginine	0.4 ± 0.10^a	0.6 ± 0.30^a	1.5 ± 0.50^b	1.7 ± 0.60^b
Valine	7.9 ± 0.60^a	5.7 ± 1.10^b	15.9 ± 1.00^c	14.0 ± 1.00^d
Isoleucine	4.8 ± 0.40^a	3.2 ± 0.50^b	10.1 ± 0.80^c	9.4 ± 0.60^d
Methionine	2.9 ± 0.20^a	1.7 ± 0.10^b	5.0 ± 0.70^c	6.6 ± 1.10^d
Tryptophan	9.0 ± 0.40^a	27.0 ± 1.90^b	44.6 ± 2.70^c	50.2 ± 5.40^d
Lysine	21.1 ± 1.20^a	17.4 ± 1.30^b	56.3 ± 3.60^c	51.0 ± 3.70^d
Histidine	2.4 ± 0.10^a	2.0 ± 0.50^a	4.9 ± 0.30^b	4.9 ± 0.30^b
Leucine	1.2 ± 0.10^a	1.2 ± 0.40^a	2.7 ± 0.40^b	2.5 ± 0.30^b
Non-essential amino acids				
Alanine	3.7 ± 0.20^a	3.6 ± 1.10^a	8.9 ± 0.70^b	8.6 ± 0.80^b
Tyrosine	1.5 ± 0.10^a	1.6 ± 0.60^a	2.9 ± 0.20^a	3.1 ± 0.20^a
Glycine	30.4 ± 1.60^a	23.0 ± 1.00^b	70.3 ± 8.70^c	73.0 ± 4.10^d
Glutamic Acid	4.4 ± 0.30^a	3.8 ± 1.10^b	9.1 ± 0.60^c	7.5 ± 0.70^d
Cysteine	3.5 ± 0.30^a	2.8 ± 0.70^a	8.0 ± 0.90^b	6.4 ± 0.40^c
Proline	7.2 ± 0.60^a	4.7 ± 0.60^b	14.7 ± 0.90^c	12.4 ± 1.20^d

4.6. Partial enterprise budget analysis

The mean yield in kilograms was higher in the fish fed on COMM$_0$ and BSFL$_{100}$ followed BSFLM$_0$ and BSFLM$_{50}$ (Table 4.8). All the experimental diets registered significantly positive net return above total costs ($P < 0.05$).

The total cost and variable cost decreased gradually as SBM was replaced with BSFLM with lowest values exhibited from BSFLM$_{100}$. Break even price (Total fixed cost/Production unit volume) + Variable Cost per unit were significantly different with lowest values (US $ 0.35±0.01) being recorded in BSFLM$_{100}$ ($P < 0.05$). An indicator of a higher value for the yield to cater for the operational costs that corresponds to a lower break-even point by the diet.

Table 4.8: Partial enterprise budget for the dietary treatments with different levels of defatted black soldier fly larvae meal and commercial diet.

Parameters	**Units**	**Treatments**			
		BSFLM$_{0}$	BSFLM$_{50}$	BSFLM$_{100}$	COMM$_{0}$
Mean yield per cage	Kg	5.92±0.01^{a}	5.36±0.01^{b}	6.84±0.01^{c}	6.93±0.02^{c}
Total yield	Kg	17.77±0.01^{a}	16.08±0.01^{b}	20.54±0.01	20.81±0.02^{d}
Gross revenue	US $	66.28±0.01^{a}	59.98±0.01^{b}	76.61±0.01^{c}	77.6±0.35^{d}
Variable costs	US $	59.5±0.12^{a}	57.42±0.01^{b}	47.93±0.00^{c}	58.97± 0.02^{d}
Fixed costs	US $	0.47± 0.02^{a}	0.47± 0.00^{a}	0.47± 0.00^{a}	0.47± 0.00^{a}
Total costs	US $	59.97±0.01^{a}	57.88±0.01^{b}	48.4±0.23^{c}	59.44±0.01^{d}
Net return above total costs	US $	6.32±0.01^{a}	2.09±0.00^{b}	28.21±0.01^{c}	18.16±0.02^{d}
Unit selling price	US $	3.73±0.01^{a}	3.73±0.02^{a}	3.73±0.00^{a}	3.73±0.01^{a}
Breakeven price (total costs)	US $	0.41±0.01^{a}	0.43±0.01^{b}	0.35±0.01^{c}	0.41±0.01^{a}
Breakeven yield	Kg	0.07±0.02 ^{a}	0.18±0.01^{b}	0.02±0.01^{c}	0.03±0.01^{c}

CHAPTER FIVE

DISCUSSION

5.1 Overview

The present study was conducted to evaluate the utilization of black soldier fly larvae (*Hermetia illucens*) meal as soybean meal replacement in the diet of cultured Nile tilapia (*Oreochromis niloticus*) on growth parameters, body composition, amino acid concentration and cost implication.

5.2 Water quality parameters

In this study, dissolved oxygen, water temperature, pH, conductivity and total dissolved solids (Table 4.1) were within the optimum range for rearing Nile tilapia (Boyd et al., 2014). Thus, any changes in growth performance parameters is associated with experimental diets.

5.3 Growth performance and nutrient utilization

All the diets registered gradual increase in the mean weight (Figure 4.1). This can be attributed to the similarity in the initial crude protein and fat content of the extruded experimental diets coupled with the well-balanced and formulated diets. Fish fed on $BSFLM_{100}$ registered statistically similar final weight to control diet $COMM_0$ though significantly higher than diets $BSFLM_0$ and $BSFLM_{50.}$ Similar findings have been reported in juvenile grass carp (*Cyprinus carpio*) by Lu et al., (2020) but contrary to (Dietz & Liebert, 2018) in Nile tilapia that registered lowest weight with highest soybean concentrate replacement with BSFLM.

Fish body weight increased by 89% in fish fed on $BSFLM_{100}$ and $COMM_0$ and by 87% in fish fed on $BSFLM_0$ and $BSFLM_{50}$. Previous studies on Nile tilapia have reported contrasting results to this study, for instance, a lower body weight increase of 30% (Tippayadara et al., 2021), 64% (Devic et al., 2018), 73% (Muin et al., 2017) and higher body weight gain of 96% (Rana et al.,

2009) have been reported in diets containing 100% BSFLM. The differences in the increase of the body weight between the present and previous trials may have been mainly associated with varying fish development stages, culture systems and study periods of between 32 and 96 days compared to the present study that lasted for a period of 168 days.

The lowest BWG and SGR were recorded in fish fed on $BSFLM_0$ and $BSFLM_{50}$ with 100 and 50 % soybean inclusion levels respectively (Table 4.5). Similar results have been reported by Lu et al. (2020) in juvenile grass carp but contrary to Dietz et al. (2018) in Nile tilapia. Previous studies have also reported reduced feeding and growth as a result of higher levels of dietary soybean proteins and recommended different optimal inclusion levels of 25% (Sharda et al., 2017 & Abdel-Warith, et al., 2019), 4% (Amesa et al., 2018), 4.5% (Ajani et al., 2016 and Godoy et al., 2019) in Nile tilapia.

Soybean meal has been reported to have growth-inhibitory effect which has been linked to anti-nutritional factors (Lu et al., 2020) associated with phytate. In general soybean meal contain 1.0–1.5% phytate that is heat-resistant and not as easily degraded with boiling. Phytate integrate with cation groups on protein, amino acids, starch and lipids in feedstuff reducing the digestibility of these nutrients in fish. Also phytate strongly chelate with cations such as calcium, magnesium, zinc, copper, iron and potassium to form insoluble salts. This adversely affects the absorption and digestion of these minerals in fish (Papatryphon et al., 1999) and lastly lack of phytase enzyme in tilapia to hydrolyze phytates in soybean that causes unavailability of amino acids.

Better BWG and SGR were recorded in fish fed on $BSFLM_{100.}$ However, this is a factor of the BSFLM utilized in the present study which had a high ash content rich in calcium and phosphorus. Besides $BSFLM_{100}$ exhibited a better amino acid profile with higher levels of glycine, alanine, isoleucine, leucine and phenylalanine compared to control $BSFLM_0$, hence contributing to better BWG and SGR (Barroso et al., 2014; El-Hack et al., 2020). Contrary to the findings of this study, lower SGR and BWG in diets containing high inclusion levels of BSFLM have been reported by Li et al. (2017) and Devic et al. (2018) in Nile tilapia (*Oreochromis niloticus*) and Lu et al. (2020) in grass carp (*Ctenopharyngodon idellus*). They linked the diminishing performance with increasing BSFLM in the test diet to imbalances in amino acids and inadequate chitinase activity. Similar growth patterns of fish fed on diets with 100% BSFLM and the commercial diets indicated that the diets had similar nutritional values to support the growth of fish.

Food conversion ratio (FCR) serves as an indicator of feed utilization (Devic et al., 2018), fish fed on $BSFLM_{100}$ registered lowest FCR of 1.79 with better feed utilization. The FCR values in the present study were lower than those reported by Muin et al. 2017 (3.31) and Tippayadara et al. 2021 (2.1) when BSFLM was included in the diets of tilapia. On the other hand, Xiao et al. 2018 reported lower values (1.19) of FCR in yellow catfish (*Pelteobagrus fulvidraco)* as compared to the present study when conventional protein sources were replaced at 100% with BSFLM. Low FCR in fish fed $BSFLM_{100}$ may have been a factor of utilization of defatted BSFLM. Observations by Basto et al. (2020) indicated that defatting insect meals improved digestibility and utilization of nutrients though the chitin content remained similar between defatted and non-defatted insect meals. Additionally, the heat and pressure that BSFL is exposed to during processing can play an important role in their digestibility and utilization. This process increases free amino acids in the muscle tissues that can directly be used for protein synthesis, resulting in improved feed utilization and growth rates (Choi et al., 2017).

The high values of FCR recorded in fish fed on $BSFLM_0$ and $BSFLM_{50}$ indicates that high content of SBM negatively influenced feed utilization.

This could be due to the unavailability of macro and micronutrients in SBM that compromises feed utilization. Specifically, phytic acid chelation diminishes bioavailability of calcium, phosphorus, magnesium, manganese, zinc and iron. Soybean processing by thermal heating destroy heat-labile antinutritional factors however the process decrease the concentration and availability of heat-sensitive amino acids, particularly lysine that influence protein, carbohydrate and moisture on milliard reactions (Ghosh & Ray, 2017).

The survival rates from the present study were above 92% in all the dietary treatments (Table 4.5). The survival rates recorded in this study agrees with results of Tippayadara et al. (2021) in Nile tilapia, Xiao et al. (2018) in yellow catfish (*Pelteobagrus fulvidraco*), Stadtlander et al. (2017) in rainbow trout (*Oncorhynchus mykiss*), Katya et al. (2017) in juvenile barramundi, (*Lates calcarifer*), Li et al. (2017) in juvenile Jian carp (*Cyprinus carpio*, Lu et al. (2020) and Rawski et al. (2020) in Siberian sturgeon (*Acipenser baerii*) fed on black soldier fly larvae meal. As reflected in the Fulton's condition factor, acclimatization of fish prior to stocking, consistent and appropriate feeding regimes in an optimal environment in favor of fish physiological processes played a major role in high survival rates. This suggests good physiological conditions of fish in relation to its welfare associated with optimal physico-chemical and biological qualities of water in fish ponds.

5.4 Body composition analysis

The body carcass composition of fish was influenced by different dietary treatments and showed significant fluctuations in crude protein, crude fibre and total lipids contents in the fish body (Table 4.6). This is related to changes in the synthesis, muscle deposition rate and/or differential growth rate (Abdel-Warith, et al., 2019). $BSFLM_{100}$ had the highest protein deposition compared to control $COMM_0$ and $BSFLM_0$, due to improved dietary protein utilization for body protein synthesis and lipids for energy purposes. This could also be due to different absorption kinetics of free and protein-bound amino acids that create asynchrony in post absorptive availability of individual amino acids thus increase in carcass protein content with the increase in dietary protein. Lower protein deposition in diets $BSFLM_0$ and $BSFLM_{50}$ is due to the decrease in protein utilization and digestibility above 50% dietary soybean level. Contrary results by Huda et al. (2020) associated low protein retention results to chitin content in the pure black soldier fly carcass meal, interfering with the digestive ability of fish. Additionally, Cummins et al. (2017) found reduced protein and lipid levels in Pacific white shrimp (*Litopenaeus vannamei*) associated with deficiencies in essential amino acid (EAA) as well as increasing imbalances of essential and nonessential amino acids. This also parallels findings by Kroeckel et al. (2012) in juvenile turbot (*Psetta maxima*) of high protein retention that decreased significantly with increasing BSFLM supplementation. In grass carp, replacement of soybean meal with BSFLM at inclusion levels of 25%, 50%, 75% and 100% for dietary did not affect the contents of crude protein, crude lipid, moisture and crude ash in the muscle (Lu et al., 2020).

The variations in crude fat in the carcass can be explained by protein-sparing and growth promoting effect that entails partially replacing dietary protein with lipid and/or carbohydrate to meet fish growth demands (Zhou et al., 2019).In trout, Renna et al., (2017) reported increased ether extract contents of dorsal fillet, at highest BSF inclusion level above 40% with exacerbated muscle lipids health indexes. On another study, Sealey et al., (2011) reported significant alteration in muscle moisture, lauric acid content and lipid composition that is, increased muscle moisture and lower lipid in rainbow trout. Similar results have been observed by Muin et al., (2017) of increased lipid content though the body protein content was not affected by diet composition.

5.5 Amino acid profile

Protein quality is linked to amino acids content. The dietary essential amino acids (EAA) in high quality proteins are readily digested and present in amounts that meet human requirements (Wu et al., 2014). In the current study amino acids concentration in the fish fillet increased with increasing dietary BSFLM. Fish fed on $BSFLM_{100}$ and $COMM_0$ had enhanced and satisfactory amino acid profile compared to fish fed on $BSFLM_0$ and $BSFLM_{50}$.

Fish fed on $BSFLM_{100}$ had significantly higher values for six out of the ten identified essential amino acids associated with amino acid balance in the feed coupled with better physiological utilization after digestion, absorption and oxidation (Wu et al., 2014). This is also reflected in low FCR. The amino acids profile of the fish fed on $BSFLM_{100}$ and the fish fed on $COMM_0$ were comparable which means the quality of the feed with 100% BSFM led to the same effect on the amino acid profile of the fish fed on commercial diet ($COMM_0$).

5.6 Cost benefit analysis

Feed constitutes a major expense in aquaculture species hence profitability is a factor of cost minimization (Luthada-Raswiswi et al., 2021). In the current study feed costs were 46.33% ($BSFLM_{100}$), 48.37 % ($BSFLM_{50}$,) 50.79% ($BSFLM_0$) to 53.04 % ($COMM_0$) of the total variable costs (Table 4.8). Replacing SBM with BSFLM at 50% and 100% reduced the cost by 8.8% and 12% respectively compared to control diet $COMM_0$.This was contributed by lower BSFLM price of 0.80 USD/kg against SBM of 0.9 USD/kg at the time of the experiment. Although $COMM_0$ had the highest total yield and gross revenue attributed to good growth performance, the diet registered lower net return above total costs in comparison to $BSFLM_{100}$. Similar results of cost of feed reduction and increased net returns have been reported by Abdel-Tawwab et al. (2020) in European sea bass, Wachira et al. (2021) in Nile tilapia and Rawski et al. (2021) in Siberian Sturgeon when conventional sources of proteins were replaced with BSFLM. In addition, non-utilization of basal ingredients, mainly SBM and oil reduced the costs of formulating BSFLM_{100} hence contributing to its cost effectiveness. Lower net return above total costs values were recorded in fish fed diet BSFLM_{0} and BSFLM_{50} mainly contributed by high amounts of soybean included in these diets to supplement on limiting macro and micro elements.

This is also compounded by low utilization of the feeds that contributed to low yields. As evidenced by low values for breakeven price (total costs) and break-even yield, replacing SBM with 100% BSFLM is economically viable.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study demonstrated that when BSLFM substituted 50% or 100% of soybean meal in the diets for Nile tilapia, there was enhanced growth performances, carcass traits and amino acids concentration. Fish fed on $BSFLM_{100}$ exhibited better growth performance in terms of body weight gain, specific growth rate and feed conversion ratio compared to the commercial diet.

Similarly, amino acids deposition in fish fed on $BSFLM_{100}$ was relatively better than $COMM_0$ suggesting enhanced and comparative nutritive effects of $BSFLM_{100}$ and $COMM_0$.

The study also demonstrated BSFLM as a cost-effective alternative source of protein to the SBM in the diets of Nile tilapia.

6.2 Recommendation

In this study the author recommends the ideal diet formulation for O. *niloticus* diet to be $BSFLM_{100}$ for best growth, feed efficiency and amino acids profile.

6.3 Further research

Further research is necessary:

- To explore alternatives to the conventional mechanical defatting of H. illucens, that allow production of bioactive extracts from this insect for other value-added applications and the nutritive value of BSF exoskeleton.
- To explore the ideal approach to maximize the nutritive value of plant-based diet through hydrolysis of indigestible phytate by use of exogenous phytase enzyme.

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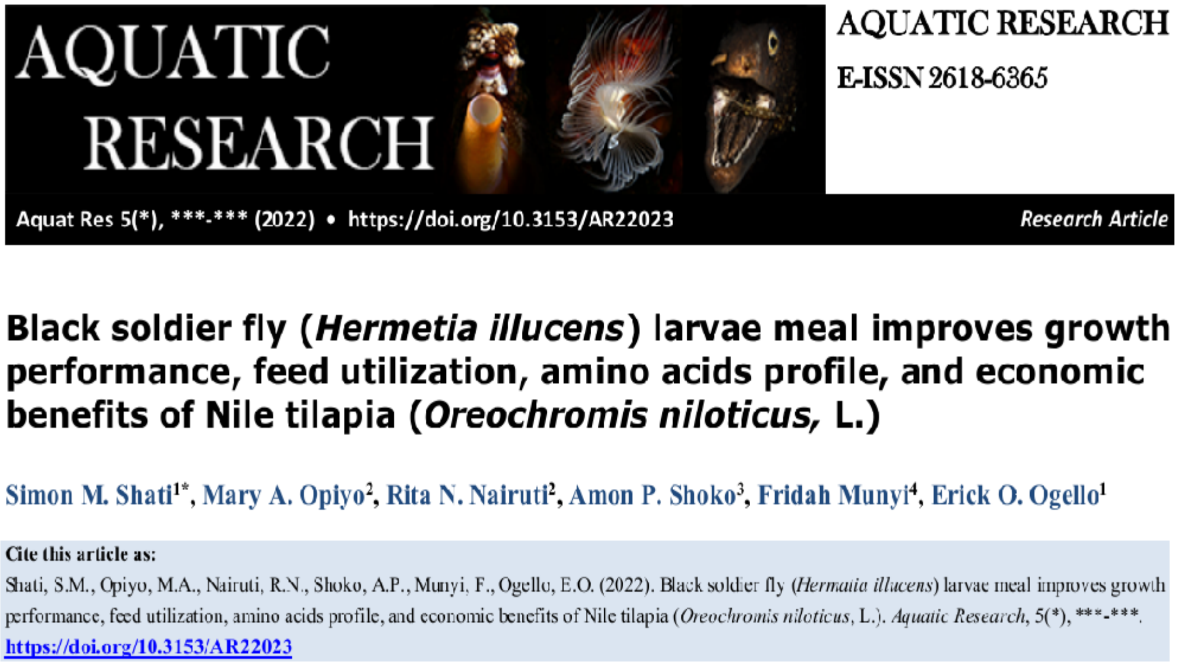
APPENDICES

APPENDIX 1: PUBLICATION



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Research Article

Black soldier fly (*Hermetia illucens*) larvae meal improves growth performance, feed utilization, amino acids profile, and economic benefits of Nile tilapia (*Oreochromis niloticus,* L.)

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APPENDIX 2: SPSS DATA SHEETS

Case Processing Summary

	DATA (Month)	Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
DATA (Length)	0	360	100.0%	0	0.0%	360	100.0%
	1	359	100.0%	0	0.0%	359	100.0%
	2	360	100.0%	0	0.0%	360	100.0%
	3	360	100.0%	0	0.0%	360	100.0%
	4	360	100.0%	0	0.0%	360	100.0%
	5	360	100.0%	0	0.0%	360	100.0%
	6	360	100.0%	0	0.0%	360	100.0%
	7	360	100.0%	0	0.0%	360	100.0%
DATA (Weight)	0	360	100.0%	0	0.0%	360	100.0%
	1	359	100.0%	0	0.0%	359	100.0%
	2	360	100.0%	0	0.0%	360	100.0%
	3	360	100.0%	0	0.0%	360	100.0%
	4	360	100.0%	0	0.0%	360	100.0%
	5	360	100.0%	0	0.0%	360	100.0%
	6	360	100.0%	0	0.0%	360	100.0%
	7	360	100.0%	0	0.0%	360	100.0%

Descriptives

	DATA (Month)			Statistic	Std. Error
DATA (Length)	0	Mean		10.521	.0306
		95% Confidence Interval for Mean	Lower Bound	10.461	
			Upper Bound	10.581	
		5% Trimmed Mean		10.518	
		Median		10.500	
		Variance		.338	
		Std. Deviation		.5810	
		Minimum		9.0	
		Maximum		12.0	
		Range		3.0	

	Interquartile Range		1.0	
	Skewness		.111	.129
	Kurtosis		-.508	.256
1	Mean		13.129	.0465
	95% Confidence Interval for Mean	Lower Bound	13.037	
		Upper Bound	13.220	
	5% Trimmed Mean		13.135	
	Median		13.100	
	Variance		.777	
	Std. Deviation		.8817	
	Minimum		10.6	
	Maximum		15.2	
	Range		4.6	
	Interquartile Range		1.3	
	Skewness		-.080	.129
	Kurtosis		-.421	.257
2	Mean		14.677	.0603
	95% Confidence Interval for Mean	Lower Bound	14.558	
		Upper Bound	14.795	
	5% Trimmed Mean		14.689	
	Median		14.700	
	Variance		1.307	
	Std. Deviation		1.1432	
	Minimum		11.5	
	Maximum		17.2	
	Range		5.7	
	Interquartile Range		1.6	
	Skewness		-.166	.129
	Kurtosis		-.441	.256
3	Mean		15.930	.0710
	95% Confidence Interval for Mean	Lower Bound	15.791	
		Upper Bound	16.070	
	5% Trimmed Mean		15.949	
	Median		16.000	
	Variance		1.816	
	Std. Deviation		1.3478	

	Minimum		12.5	
	Maximum		19.1	
	Range		6.6	
	Interquartile Range		2.0	
	Skewness		-.188	.129
	Kurtosis		-.487	.256
4	Mean		17.169	.0791
	95% Confidence Interval for Mean	Lower Bound	17.014	
		Upper Bound	17.325	
	5% Trimmed Mean		17.194	
	Median		17.300	
	Variance		2.255	
	Std. Deviation		1.5017	
	Minimum		12.8	
	Maximum		21.0	
	Range		8.2	
	Interquartile Range		2.3	
	Skewness		-.269	.129
	Kurtosis		-.396	.256
5	Mean		18.276	.0943
	95% Confidence Interval for Mean	Lower Bound	18.091	
		Upper Bound	18.462	
	5% Trimmed Mean		18.311	
	Median		18.500	
	Variance		3.202	
	Std. Deviation		1.7894	
	Minimum		10.1	
	Maximum		22.8	
	Range		12.7	
	Interquartile Range		2.5	
	Skewness		-.558	.129
	Kurtosis		1.151	.256
6	Mean		19.028	.1073
	95% Confidence Interval for Mean	Lower Bound	18.817	
		Upper Bound	19.239	
	5% Trimmed Mean		19.045	

		Median		19.150	
		Variance		4.148	
		Std. Deviation		2.0368	
		Minimum		11.7	
		Maximum		23.8	
		Range		12.1	
		Interquartile Range		2.9	
		Skewness		-.213	.129
		Kurtosis		-.269	.256
		Mean		20.531	.0883
		95% Confidence Interval for Mean	Lower Bound	20.358	
			Upper Bound	20.705	
		5% Trimmed Mean		20.526	
		Median		20.600	
		Variance		2.805	
	7	Std. Deviation		1.6749	
		Minimum		10.0	
		Maximum		25.6	
		Range		15.6	
		Interquartile Range		2.0	
		Skewness		-.523	.129
		Kurtosis		3.875	.256
		Mean		20.5617	.16500
		95% Confidence Interval for Mean	Lower Bound	20.2372	
			Upper Bound	20.8862	
		5% Trimmed Mean		20.5250	
		Median		20.4500	
		Variance		9.801	
DATA (Weight)	0	Std. Deviation		3.13062	
		Minimum		14.50	
		Maximum		27.80	
		Range		13.30	
		Interquartile Range		4.88	
		Skewness		.159	.129
		Kurtosis		-.736	.256
	1	Mean		44.9615	.46993

	95% Confidence Interval for Mean	Lower Bound	44.0374	
		Upper Bound	45.8857	
	5% Trimmed Mean		44.8560	
	Median		44.0000	
	Variance		79.280	
	Std. Deviation		8.90396	
	Minimum		21.70	
	Maximum		68.05	
	Range		46.35	
	Interquartile Range		13.25	
	Skewness		.203	.129
	Kurtosis		-.461	.257
2	Mean		63.0534	.75372
	95% Confidence Interval for Mean	Lower Bound	61.5711	
		Upper Bound	64.5356	
	5% Trimmed Mean		62.9053	
	Median		62.0000	
	Variance		204.512	
	Std. Deviation		14.30076	
	Minimum		30.85	
	Maximum		102.00	
	Range		71.15	
	Interquartile Range		21.87	
	Skewness		.161	.129
	Kurtosis		-.453	.256
3	Mean		78.2119	.98423
	95% Confidence Interval for Mean	Lower Bound	76.2763	
		Upper Bound	80.1474	
	5% Trimmed Mean		77.9310	
	Median		77.0500	
	Variance		348.734	
	Std. Deviation		18.67443	
	Minimum		31.20	
	Maximum		133.35	
	Range		102.15	
	Interquartile Range		27.10	

		Skewness		.219	.129
		Kurtosis		-.347	.256
	4	Mean		95.5553	1.35043
		95% Confidence Interval for Mean	Lower Bound	92.8995	
			Upper Bound	98.2110	
		5% Trimmed Mean		94.9392	
		Median		93.7500	
		Variance		656.522	
		Std. Deviation		25.62269	
		Minimum		11.50	
		Maximum		187.00	
		Range		175.50	
		Interquartile Range		34.38	
		Skewness		.368	.129
		Kurtosis		.230	.256
	5	Mean		116.8024	1.72250
		95% Confidence Interval for Mean	Lower Bound	113.4149	
			Upper Bound	120.1898	
		5% Trimmed Mean		115.6184	
		Median		114.4500	
		Variance		1068.123	
		Std. Deviation		32.68214	
		Minimum		46.20	
		Maximum		250.15	
		Range		203.95	
		Interquartile Range		45.44	
		Skewness		.520	.129
		Kurtosis		.559	.256
	6	Mean		129.1332	2.15849
		95% Confidence Interval for Mean	Lower Bound	124.8884	
			Upper Bound	133.3781	
		5% Trimmed Mean		127.2748	
		Median		126.9250	
		Variance		1677.263	
		Std. Deviation		40.95440	
		Minimum		45.20	

	Maximum		277.80	
	Range		232.60	
	Interquartile Range		57.28	
	Skewness		.622	.129
	Kurtosis		.479	.256
	Mean		161.5314	2.11016
	95% Confidence Interval for Mean	Lower Bound	157.3816	
		Upper Bound	165.6812	
	5% Trimmed Mean		159.2262	
	Median		158.2500	
	Variance		1602.996	
7	Std. Deviation		40.03743	
	Minimum		93.50	
	Maximum		333.50	
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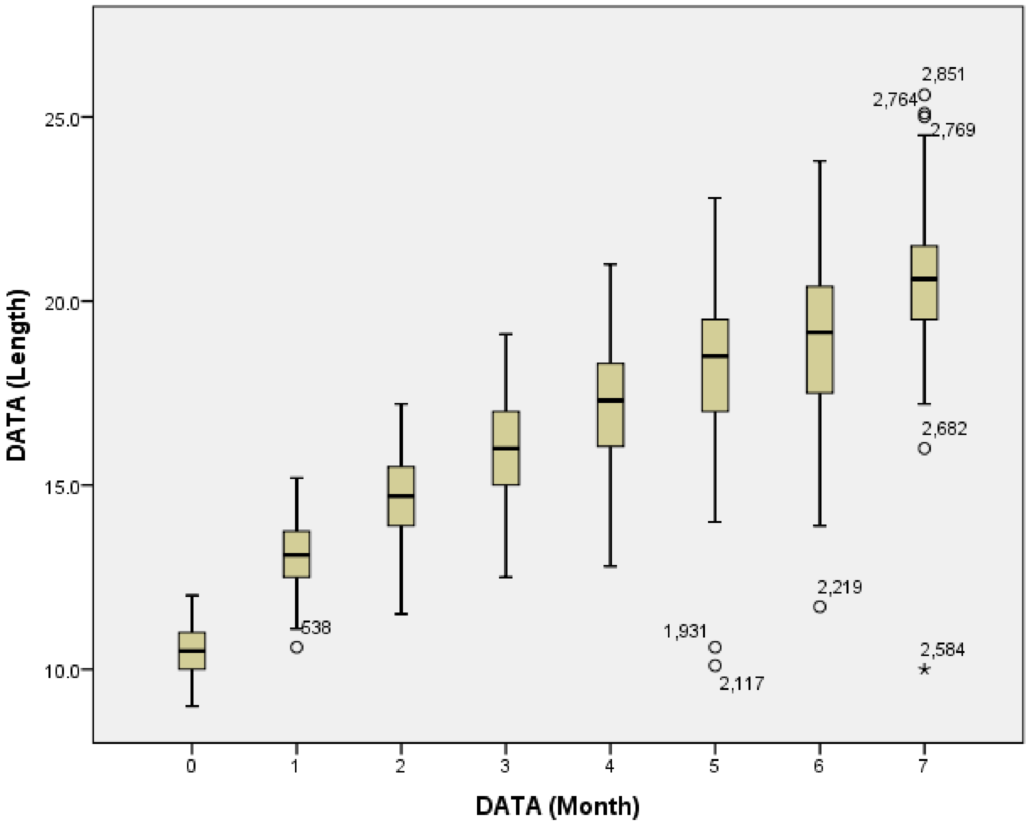
	Interquartile Range		49.50	
	Skewness		.947	.129
	Kurtosis		1.493	.256



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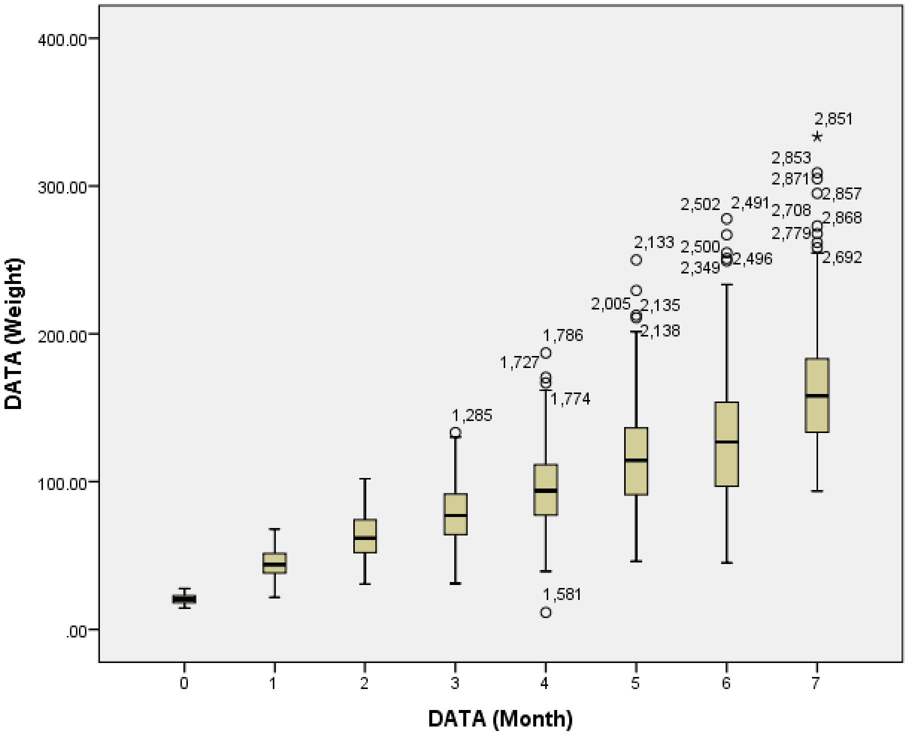
6
7
DATA (Month)



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2,500
2,496
2,349
2,692
2,005
2,135
2,138
1,786
1,727
1,774
1,285
1,581
0
1
2
3
4
5
6
7
DATA (Month)

One way

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
BWG	0	90	130.9422	30.74825	3.24115	124.5021	137.3823	78.20	228.80
	1	90	122.2889	27.69220	2.91901	116.4889	128.0889	73.10	231.70
	2	90	157.7067	34.46275	3.63269	150.4886	164.9247	109.50	245.40
	3	90	152.9411	51.69213	5.44883	142.1144	163.7678	68.30	312.30
	Total	360	140.9697	40.00282	2.10833	136.8235	145.1160	68.30	312.30
SGR	0	90	1.0665	.14699	.01549	1.0357	1.0972	.76	1.38
	1	90	1.0232	.12906	.01360	.9962	1.0502	.74	1.35
	2	90	1.1437	.11595	.01222	1.1194	1.1680	.91	1.38
	3	90	1.1156	.17097	.01802	1.0798	1.1514	.68	1.47
	Total	360	1.0872	.14900	.00785	1.0718	1.1027	.68	1.47
SR	0	90	95.33	.948	.100	95.13	95.53	94	96
	1	90	86.67	.948	.100	86.47	86.87	86	88
	2	90	90.00	3.284	.346	89.31	90.69	86	94
	3	90	96.00	1.642	.173	95.66	96.34	94	98
	Total	360	92.00	4.327	.228	91.55	92.45	86	98
FCR	0	90	.8848	.21222	.02237	.8403	.9292	.48	1.40
	1	90	.9377	.22125	.02332	.8914	.9840	.47	1.49
	2	90	.7941	.16265	.01715	.7600	.8282	.49	1.09
	3	90	.8488	.29606	.03121	.7868	.9108	.37	1.70
	Total	360	.8663	.23310	.01229	.8422	.8905	.37	1.70

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BWG	Between Groups	78566.738	3	26188.913	18.800	.000
	Within Groups	495914.242	356	1393.018		
	Total	574480.980	359			
SGR	Between Groups	.767	3	.256	12.639	.000
	Within Groups	7.203	356	.020		
	Total	7.971	359			
SR	Between Groups	5360.000	3	1786.667	467.686	.000
	Within Groups	1360.000	356	3.820		
	Total	6720.000	359			
FCR	Between Groups	.986	3	.329	6.319	.000
	Within Groups	18.521	356	.052		
	Total	19.507	359			

BWG

Tukey B

Diet	N	Subset for alpha = 0.05	
		1	2
1	90	122.2889	
0	90	130.9422	
3	90		152.9411
2	90		157.7067

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 90.000.

SGR

Tukey B

Diet	N	Subset for alpha = 0.05	
		1	2
1	90	1.0232	
0	90	1.0665	
3	90		1.1156
2	90		1.1437

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 90.000.

SR

Tukey B

Diet	N	Subset for alpha = 0.05			
		1	2	3	4
1	90	86.67			
2	90		90.00		
0	90			95.33	
3	90				96.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 90.000.

FCR

Tukey B

Diet	N	Subset for alpha = 0.05		
		1	2	3
2	90	.7941		
3	90	.8488	.8488	
0	90		.8848	.8848
1	90			.9377

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 90.000.

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Condition factor (K)	2879	.18	15.62	1.8827	.44122
Valid N (listwise)	2879				