UNIVERSITI PUTRA MALAYSIA

THE EFFECT OF FEEDING BLACK SOLDIER FLY LARVAE ON GROWTH PERFORMANCE OF RED HYBRID TILAPIA (OREOCHROMIS SPP.)

SITI AISYAH BT MOHD HAFIZ NGOO

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SERDANG, SELANGOR

2020/2021

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SITI AISYAH BT MOHD HAFIZ NGOO

A project paper submitted to the
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CERTIFICATION

It is hereby certified that we have read this project paper entitled "The effect of feeding black soldier fly larvae (BSFL) on growth performance of Red Hybrid tilapia (*Oreochromis spp*.)", by Siti Aisyah Bt Mohd Hafiz Ngoo, and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

This project paper is dedicated to Allah S.W.T., who had created me and made all circumstances possible throughout this project,

To my family,

My father, Mohd Hafiz Ngoo bin Abdullah,

My mother, Siti Suri binti Arshad.

My siblings; Mohd Sofie, Siti Khairani, Saiful Daulah, Salahuddin,

My friends,

And to all my teachers who have committed themselves towards the noble cause of education. I sincerely thank you for your endless support and care.

May this will spark inspiration and enthusiasm for your future endeavours.

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Praise to Allah the Almighty for being granted physical and mental strength throughout my life, for desiring knowledge for self-improvement and contribution for my country in the economic and academic field to become more successful moving forward.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
ANOVA	Analysis of Variance
BSFL	Black soldier fly larvae
cm	Centimetre
DM	Dry matter
FCR	Feed conversion ratio
O. mossambicus	*Oreochromis mossambicus*
O. niloticus	*Oreochromis niloticus*
g	Gram
M	Mol
MLG	Mean body length gained
MWG	Mean body weight gained
ml	Millilitre
mol/L	Molar per litre
SEM	Standard error of mean
TBL	Total body length

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian keperluan kursus VPD4999 – Projek Tahun Akhir

KESAN PEMAKANAN LARVA LALAT ASKAR HITAM TERHADAP PRESTASI PERTUMBUHAN DALAM IKAN TILAPIA MERAH

(*Oreochromis spp.)*

Oleh

Siti Aisyah Bt Mohd Hafiz Ngoo

2020

Penyelia: Prof. Madya. Dr Hasliza Abu Hassim

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Dalam industri akuakultur, matlamat utama adalah untuk mengurangkan kos pengeluaran, terutamanya kos pemakanan, tanpa kesan sampingan yang ketara. Larva Lalat Askar Hitam (BSFL) merupakan sumber tempatan, mesra alam dan lestari yang tinggi dalam kandungan protein (42% DM) dan lemak (35% DM). Kajian ini dilaksanakan untuk mengenalpasti kesan prestasi pertumbuhan dan komposisi protein dan lemak jika BSFL diberi makan kepada ikan Tilapia Merah Hibrid. Sejumlah 120 ikan Tilapia Merah Hibrid telah dibahagikan kepada empat kumpulan (n=10) dengan tiga replikasi (Kumpulan 1: kawalan, tanpa kandungan

BSFL; Kumpulan 2: 90% diet komersial + 10% BSFL; Kumpulan 3: 80% diet komersial + 20% BSFL dan Kumpulan 4: 70% diet komersial + 30% BSFL). Analisis proksimat menunjukkan bahawa kandungan protein lebih tinggi kerana tahap kandungan BSFL lebih tinggi (Kumpulan 1: 33.92%, Kumpulan 2: 36.37%, Kumpulan 3: 35.72%, Kumpulan 4: 38.69%). Hasil kajian menunjukkan bahawa kemasukan BSFL ke dalam diet komersial adalah signifikan dalam kumpulan yang mempunyai kandungan BSFL yang tertinggi berbanding dengan kumpulan kawalan dari aspek berat badan, panjang badan dan komposisi protein dan lemak. Sememangnya, Kumpulan 4 yang mempunyai 30% BSFL mengandungi kandungan protein (80.30% DM) dan lemak (2.90% DM) yang tertinggi berbanding kumpulan lain. Oleh itu, Kumpulan 4 lebih cenderung untuk mempunyai prestasi pertumbuhan yang baik dan komposisi protein dan lemak yang lebih tinggi.

Kata Kunci: *Ikan Tilapia Merah Hibrid, Larva Askar Lalat Hitam, prestasi pertumbuhan, komposisi protein, komposisi lemak*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD4999 – Final Year Project

THE EFFECT OF FEEDING BLACK SOLDIER FLY LARVAE ON GROWTH PERFORMANCE AND NUTRITIONAL COMPOSITION OF RED HYBRID TILAPIA (*OREOCHROMIS SPP.*)

By

Siti Aisyah Bt Mohd Hafiz Ngoo

2020

Supervisor: Assoc. Prof. Dr Hasliza Abu Hassim

Co-Supervisor: Assoc. Prof. Dr Ina Salwany Md Yasin

In aquaculture industry, the crucial goal is to cut the production costs, especially feeding costs, without significant side effects. Black Soldier Fly larvae (BSFL) is a locally available, eco-friendly and sustainable source that is high in crude protein (42% DM) and fat (35% DM). This study was performed to determine the growth performance and composition of crude fat and protein in fingerlings of Red Hybrid tilapia fed on BSFL. A total of 120 fingerling Red Hybrid tilapia was divided into four groups (n=10) with three replicates (Group 1: without inclusion of BSFL, Group 2: 90% commercial fish feed + 10% BSFL, Group 3: 80% commercial fish feed + 20% BSFL, Group 4: 70% commercial fish feed + 30% BSFL). Proximate analysis of experimental diet showed that protein content is higher as the

level of BSFL inclusion is higher (Group 1: 33.92%, Group 2: 36.37%, Group 3: 35.72%, Group 4: 38.69%). Result shows that the inclusion of BSFL into commercial diet is significant in group with the highest BSFL percentage as compared to control group in relation to its body weight, total body length and protein and fat composition. Indeed, Group 4, which contain 30% BSFL inclusion contain the highest level of crude protein (80.30% DM) and fat (2.90% DM) as compared to other groups. Thus, Group 4 is concluded to have better growth performance and higher crude protein and fat composition.

Keywords: *Red Hybrid Tilapia, Black Soldier Fly Larvae, growth performance, protein composition, fat composition*

1.0 INTRODUCTION

1.1 Background

The geography of Malaysia shows the nation is surrounded by bodies of water. On the western coast of Peninsular Malaysia is the Straits of Malacca, on the north is the Andaman Sea, and between the eastern coast and Borneo is the South China Sea. Other than that, Malaysia also has many rivers and lakes. These ecosystems provide Malaysia with abundant aquatic resources for the population; hence, it is common to see aquatic products as part of the diet of the population. The population of Malaysia is expected to increase in the coming years and will in turn increases the consumption index; it is expected that the consumption index of fishes will increase from 53.1 kg in 2011 to 61.1 kg by 2020 (Yusoff, 2015), putting Malaysia as one of the highest fish consumers globally.

Commercial fish feed is an important component in commercial aquaculture as the feed was formulated for optimum growth of that particular species of fish. Commercial fish feed typically included fish meal and oil in livestock feed as it is well known to provide adequate amino acid and fatty acid profile. Nevertheless, the use of these ingredients is not simply limited to livestock but to humans as well. The competition with human consumption and limited stock increases the price of these ingredients, subsequently increases feeding costs and production costs as well. This problem leads to researchers and farmers alike to search for alternatives to fish meal and oil such as insect meal.

The growing awareness of the benefits of insect meal led the budding interest in using insect meal in animal feed. Some of the benefits are being a rich source of protein and fat, their consumption of decaying organic material, having a lower feed conversion ratio (FCR) and emit lesser greenhouse gasses (Oonincx *et al.*, 2010).

The black soldier fly, *Hermetia illucens*, had been studied to be able to provide high crude protein and fat content and feed on a wide variety of waste material as well. As reported by Newton *et al.* (1977) and Sheppard *et al.* (1994), BSFL contain about 42% protein and 35% fat.

There are various studies that had used BSFL in animal feed such as swine, poultry and aquaculture industry. In finishing pigs, BSFL may be included up to 4% of their diet (Yu *et al.*, 2019) while in Japanese quails (*Coturnix coturnix japonica*) BSFL may replace fish meal up to 50% (Widjastuti *et al.*, 2014). In aquaculture, BSFL can replace fish meal in the diets of Pacific white shrimp (*Litopenaeus vannamei*) up to 25% (Cummins Jr *et al.*, 2017), yellow catfish (*Pelteobagrus fulvidraco)* up to 48% (Xiao *et al.*, 2018), and juvenile barramundi (*Lates calcarifer*) and Nile tilapia (*Oreochromis niloticus*) up to 50% (Katya *et al.*, 2017; Muin *et al.*, 2017).

This research aimed to explore the possibility of using black soldier fly larvae as protein and fat resource in feed of Red Hybrid Tilapia as partial replacement to fish meal and oil, and to determine its effect on growth performance and protein composition on Red Hybrid Tilapia.

1.2 Justification

1. Black Soldier Fly Larvae (BSFL) is a locally available and sustainable source of protein and fat.
2. There is limited data about the use of BSFL in aquaculture in Malaysia, and no study about feeding BSFL to Red Hybrid tilapia have been carried out.

1.3 Objectives of study

The study aimed to determine and compare the effect of feeding BSFL at different inclusion levels on the growth performance and crude protein content of Red Hybrid Tilapia.

1.4 Hypothesis of study

H_0: use of BSFL at certain inclusion levels with commercial fish feed has no effect on growth performance and nutritional composition of Red Hybrid Tilapia.

H_A: use of BSFL at certain inclusion levels with commercial fish feed has an effect on growth performance and nutritional composition of Red Hybrid Tilapia.

2.0 LITERATURE REVIEW

2.1 Aquaculture Industry in Malaysia

Malaysia is recognized as one of the largest global producers of aquaculture. The growing population of Malaysians also increases the consumption index. The growing aquaculture industry supplies the national economy with employment, local and foreign demands for cheap protein, in addition to releasing pressure on capture fisheries industry that has plateaued for decades.

In 2012, the fisheries sector of both fish and non-food fish supplied RM11,440 million. The production of food fish sector consisted of marine capture, inland and aquaculture fisheries collectively amounted to 1,780,168 tonnes that contributed RM10,598 million. The marine capture fisheries are further divided to inshore and deep-sea fisheries, and are both major contributors that produced 1,472,240 tonnes (82.70%). The aquaculture fisheries produced 302,886 tonnes (17.01%) (Yusoff, 2015).

Non-food fish such as seaweed, aquatic plants ornamental fishes supplied RM843 million. The ornamental fishes were the biggest contributor in the subsector with a value of RM632 million, then seaweed at RM199 million, and aquatic plants at RM12.3 million (Yusoff, 2015).

In 2012, The freshwater and brackish water aquaculture contributed 163,757 tonnes (valued at RM992 million) and 139,129.51 tonnes (valued at 1,566.78 million) respectively. The main freshwater cultured species were freshwater catfish (*Clarias sp.*), black and red tilapia (*Oreochromis spp.*), riverine catfish (*Pangasius* *sp.*), and freshwater giant prawn (*Macrobrachium rosenbergii*). For brackish water, the main culture species were marine prawns (*Penaeus monodon* and *P. vannamei*), cockles (*Anadara granosa*), marine finfish, and mussels (*Perna viridis*) (Yusoff, 2015).

In the recent years, the consumption of freshwater fish has been on the rise due to government efforts to reduce capture fisheries. The inshore fisheries that have greatly contributed to the economy is generally accepted to be fully exploited, and even overfished to some extent. To further extent the capture fisheries, the production would need to come from offshore sub-sector towards the South China Sea (FAO, 2008).

In the 1940s, tilapia was first introduced from Java was the Mozambique tilapia, *Oreochromis mossambicus*, and raised in freshwater and brackish water farms. However, it was unpopular due to its poor growth performance. In the 1980s two new species of tilapia, which were the Nile tilapia (*Oreochromis niloticus*) from Thailand and Red Hybrid Tilapia (*Oreochromis mossambicus* x *O. niloticus*) from Taiwan, in order to encourage tilapia culture. Both the Nile and Red Hybrid Tilapia had largely replaced the Mozambique tilapia as they have better growth performances (De Silva, 1989). The dark coloured Nile tilapia did not bode well with the earthen ponds where the fishes were farmed, so the Red Hybrid tilapia with its more striking colour was more popular (FAO, 2008).

Animal feed usually relies on fish meal and oil that adequately provide complete amino acid and fatty acid profiles. But the rapid expansion of human population and aquatic industry as well as marine overexploitation has increased the costs of production of fish meal and oil, which subsequently also increases tilapia production costs. Thus, various studies on suitable alternative to partially or completely replace fish meal in commercial fish feed are carried out.

2.2 Red Hybrid Tilapia

Tilapia is a type of freshwater fish from the family of cichlids originated from Africa. Tilapia fishes have three genera: *Tilapia*, *Oreochromis* and *Sarotherodon*. The distinction between these three genera is primarily based on the reproductive differences. The *Tilapia* genus are substrate spawners, while *Oreochromis* and *Sarotherodon* are mouthbrooders. However, only female *Oreochromis* perform mouthbrooding, while both male and female *Sarotherodon* perform mouthbrooding (El-Sayed, 2006).

Figure 1: The Red Hybrid Tilapia (*O. mossambicus* x *O. niloticus*), originated from Taiwan. Source (Onyx Aqua Farm, n.d.).

Red Hybrid tilapia is a species derived from the crossing of *Oreochromis mossambicus* (*O. mossambicus*) and *Oreochromis niloticus* (*O. niloticus)*. It is popular among farmers due to its wide variety of diet, easy management and typically accepted protein source (Siddiqui & Al-Harbi, 1995), and even due to its attractive colour. This particular type of crossbred tilapia species is commonly farmed in Malaysia, contributing to around 90% of total tilapia production. The species is widely cultured in ponds, cages, tanks and pen culture system.

When it comes to farmed production, nutrition plays and important role to ensure the adequate growth and general health of the farmed species. In intensive farming, commercial feed is commonly used as they are formulated to suit the nutritional requirement of the fish at the current life stage. Floating pellets are typically preferred as the fishes can be observed during feeding.

Protein requirement differs according to their life stage. Fry tilapia requires 45% protein while fingerlings and advanced juveniles require 35% - 45% protein and adults require 30% protein for optimum growth (Abdel-Tawwab *et al.*, 2010). De Silva *et al.* (1991) and Chou & Shiau (1996) reported that lipid requirement is at minimum 5% and at maximum 12%. The increase of lipid from 6% to 18% increases protein sparing, but at 24% decreases protein sparing (De Silva *et al.*, 1991). Carbohydrate also have some protein sparing properties, but too much carbohydrate causes detrimental growth. Tilapia are able to utilize carbohydrates at 35 – 40% (Anderson *et al.*, 1984).

The estimated time to raise tilapia from egg to marketable size can vary, but it is generally accepted to be around 6 – 12 months. The variation in time is due to various factors such as water temperatures (25 – 30°C), sex, high protein supplemental feeding and stocking density (Chapman, 2000).

2.3 Black Soldier Fly Larvae

The black soldier fly (*Hermetia illucens*) is a species of fly belonging in the family *Stratiomyidae* that is native to the America. The adults appeared as large and conspicuous black flies that measured around 13 to 20 mm. In the United States, the black soldier fly is abundant in late spring and early autumn, and has three generations annually (Sheppard *et al.*, 1994; Tomberlin *et al.*, 2002). The fly is able to tolerate a wide range of temperature and are well adapted to the tropics and warmer regions. But in the recent times, the black soldier fly has spread all across the world.

Figure 2: An adult Black Soldier Fly (*Hermetia illucens*). Source (Dell, n.d.).

According to Tomberlin & Sheppard (2002), black soldier flies typically oviposit at temperatures more than 26°C. In a laboratory setting, adult flies begin to mate 2 days after hatching and oviposit 4 days after hatching. They found that the adult black soldier flies mate 2 days post-hatching, and oviposit 4 days post-hatching. The flies preferred to lay eggs at crevices in dry areas near to substrate for the larvae (Diener *et al.*, 2011), and A females can lay about 206 – 639 eggs in a single clutch.

During larval development, the larvae are voracious eaters. The larvae are able to consume a wide variety of decaying matter as well as manure (Newton *et al.*, 2005), but appeared to have preference for substrate high on fat and protein (Nguyen *et al.*, 2015). The adults on the other hand, do not require any food as they lack functioning mouthparts and never lay eggs on decaying organic material, thus the flies do not transmit diseases (van Huis *et al.*, 2013). Instead, the adult flies rely entirely on fat stores stocked up during larval stage. Studies carried out by Lalander *et al.* and Wang *et al.* (2013) showed that the bioconversion of animal manure and human faeces by larvae can potentially reduce harmful bacteria such as E. coli.

Figure 3: Larvae of Black Soldier Flies. Source (Solomko, n.d.)

The larvae of black soldier fly are also efficient waste managers. A study carried out by Newton *et al.* (1977) found that BSFL are able to convert cattle manure and urine slurry to 42% crude protein and 35% crude fat. A study carried out by Sheppard *et al.* (1994) by using chicken manure reported the same results as well, in addition to reducing manure accumulation by 50% and presence of house flies.

3.0 METHODOLOGY

3.1 Application for IACUC Approval

Six months prior to start of experiment, a request for IACUC (Institutional Animal Care and Use Committee) approval was requested. The approval was granted with IACUC reference code UPM/IACUC/AUP-U045/2020.

3.2 Experimental Design

Red Hybrid Tilapia measuring roughly two to three inches were bought from Balakong, Selangor and brought to Institute of Bioscience, UPM Serdang in transportation plastic bags. After one week of acclimatization, 120 fishes were selected and divided into four treatment groups (n=10) with three replicates in accordance to respective experimental formulation of feed. The groups were labelled as Group 1 (control group), Group 2, Group 3, and Group 4. Each group of fish were assigned to twelve tanks measuring 1 x 1 x 2 ft were filled with 10.5 US gallons (roughly 40 litres) of water.

The composition of the experimental diet is as shown in Table 1. The feeding trial was carried out for 21 days. The initial measurements of growth were taken at the beginning of the study at Day 0, and ongoing measurements of growth were taken at Day 7, Day 14 and Day 21 of the study course. Each treatment groups were given the same size of aquarium and the same source of water. The fishes were fed twice daily at 5% bodyweight, the water was changed daily and the tanks were cleaned once weekly. At the end of Day 21 of the study, the fishes were all sacrificed to determine its nutritional composition.

3.3 Experimental Diet

Different compositions of experimental feed containing specific materials were formulated using a feed formulation software and weighed accordingly and mixed well, as shown in Table 1. In the formulation, six ingredients were in fixed amount (fishmeal, fish oil, limestone, dicalcium phosphate, vitamins, minerals) whereas three ingredients were variable (corn grain, soybean meal and BSFL).

Table 1: Composition of experimental feed formulation containing different percentage of black soldier fly larvae meal fed to the Red Hybrid tilapia fishes.

Group / Ingredients (g)	BSFL level (%)			
	0	**10**	**20**	**30**
	Group 1	**Group 2**	**Group 3**	**Group 4**
Corn grain	15	16	13	10
Soybean meal	33	25	22	19
Fish meal	9.5	9.5	9.5	9.5
Fish oil	2.5	2.5	2.5	2.5
Limestone	2	2	2	2
Dicalcium phosphate	0.63	0.63	0.63	0.63
Vitamin	0.32	0.32	0.32	0.32
Mineral	0.32	0.32	0.32	0.32
BSFL meal	0	6.3	13	19

Group 1 was the control group, in which the fishes in this group were fed completely with commercial feed without any inclusion of BSFL. Group 2 was fed with 10% BSFL and 90% commercial feed, Group 3 was fed with 20% BSFL and 80% commercial feed, and Group 4 was fed with 30% BSFL and 70% commercial feed.

3.4 Analysis of Feed

3.4.1 Preparation of BSFL

The BSFL were purchased from a local supplier. The samples were grinded to a meal and kept at room temperature (26°C) in labelled airtight plastic bags prior to preparation of experimental diet.

3.4.2 Preparation of Formulated Feed

The formulation of feed was calculated using a feed formulation software to ensure that the protein and energy needed are sufficient. Each ingredient needed was weighed using an analytical balance (Sartorius BSA224S-CW, Germany) and mixed together by vigorous shaking. The amount of ingredient needed was tabulated in Table 1. The experimental diet was then kept at room temperature in an airtight container and used for proximate analysis of feed.

The mixture of ingredients was added with corn starch until a doughy consistency was achieved, then pelleted using a mini pelleting machine. The pellets were dried in an oven (Memmert Universal Oven UF110, Germany) at 40°C for 2 days.

3.4.3 Proximate Analysis of Feed

Proximate analysis is a series of analytical methods carried out on feed samples to estimate the nutritional composition of feed. Components analysed were dry matter, moisture, ash, crude fat, crude fibre and crude protein. All analyses carried out were in accordance to AOAC International protocol (Association of Official Agricultural Chemists, 2005).

3.4.3.1 Dry Matter (DM) and moisture content

Porcelain crucibles were labelled at dried in an oven (Memmert Universal Oven UF110, Germany) at 105°C for 30 minutes, then cooled in the desiccator for 20 minutes. The crucibles were then weighed. A total of three grams of experimental feed sample were weighed and put inside the crucibles according to their labels. The crucibles containing the samples were then dried at 105°C overnight. Afterwards, the crucibles were cooled in the desiccator and weighed once more. The dry matter and moisture content were calculated using the formula below:

$$\text{Moisture (\%)} = \frac{\text{Sample weight before drying} - \text{Sample weight after drying}}{\text{Sample weight before drying}} \times 100$$

$$\text{Dry matter \%} = 100\% - \text{Moisture \%}$$

3.4.3.2 Ash

The same crucibles used for dry matter previously were placed in the muffle furnace (Carbolite ELF 11/14B Furnace, United Kingdom) at 105°C for 4 hours. Next, the crucibles were cooled down in the desiccator for around 30 minutes, and later, weighed to determine the contents of ash. The ash content was calculated using the following formula:

$$\text{Ash \%} = \left(\frac{\text{Crucible weight after ashing} - \text{Empty crucible weight}}{\text{Sample weight}}\right) \times 100$$

3.4.3.3 Crude Fat

Round bottom flasks were labelled and dried inside the oven for an hour and cooled in the desiccator for 20 minutes after that. The empty flasks were later weighed. The sample were weighed three grams and then transferred into the extraction thimble, which then covered with fat free cotton wool. The thimbles were placed into the Soxhlet apparatus (Gerhardt Classic Soxhlet Apparatus, Germany). A total of 250ml of petroleum benzene were poured inside the flasks and fixed to the Soxhlet apparatus. Water was ensured to flow gently through the apparatus throughout the procedure. The heating plates were set to the lowest heat, and the temperature was gradually increased at intervals of 15 minutes until a stable boiling point was achieved. The procedure was run for 4 hours.

After 4 hours, the heating plates were switched off and the flasks were detached from the Soxhlet apparatus. Solvents within the apparatus were drained. The flasks were then dried in the oven at 80°C overnight to ensure petroleum within the flasks were completely evaporated. Then, the flasks were cooled in the desiccator and weighed. The crude fat content was calculated using the formula below:

$$\text{Crude fat (\%)} = \left(\frac{\text{Dried RBF weight after evaporation} - \text{empty RBF weight}}{\text{Sample weight}}\right) \times 100$$

3.4.3.4 Crude Fibre

Empty Fibrebags were labelled, including the blanks, and dried in the oven at 105°C for an hour. The dried Fibrebags were cooled in the desiccator for 30 minutes, and later weighed.

Empty porcelain crucibles were labelled, including the blanks as well, and incinerated in the muffle furnace for 600°C for 30 minutes. Next, the crucibles were placed in the oven for three minutes at 105°C, then cooled in the desiccator for 30 minutes. The empty crucibles were weighed.

Glass spacers were placed inside the dried Fibrebags, and samples weighing one gram were put inside the bags. The Fibrebags were attached to the carousel and placed into the beaker, subsequently 360ml of 0.13 mol/L sulphuric acid were poured into the beaker. The samples within the bag and the acid were mixed by rotating the carousel for a minute. The beaker is then placed onto the preheated heating plate of the Fibrebag system apparatus (FIBREBAG® Gerhardt, Germany); the heating plate was set to full for around three minutes in order to boil the mixture, before being reduced to gain a gentle simmer for 30 minutes. The beaker was removed from the apparatus, the carousel was taken out and the solution within the beaker was discarded. The carousel was rinsed with boiling hot water prepared beforehand for several times.

Following that, 360ml of 0.313 mol/L sodium hydroxide was poured into the beaker and the carousel with the Fibrebags attached were once more inserted into the beaker. The procedure of heating the mixture with the Gerhardt Fibrebag system apparatus was repeated yet again.

After the procedure was completed, the Fibrebags were rinsed with running tap water and dried in the oven at 105°C overnight. After drying, the bags were cooled in the desiccator for half an hour, and then put inside the incinerated crucibles prepared earlier. The crucibles containing the dried bags were weighed and incinerated inside the muffle furnace for 4 hours at 600°C. Then, the crucibles were dried again in the oven at 105°C for 30 minutes and cooled in the desiccator for half an hour. The porcelain crucible, now containing ash, were weighed. The crude fibre content was calculated using the following formula:

$$\text{Crude fibre (\%)} = \left[\frac{(A - B) - [C - (D - E)]}{F}\right] \times 100$$

Where,

A = Crucible and sample weight before incineration

B = Empty Fibrebag weight

C = Crucible and sample weight after incineration

D = Blank crucible and empty Fibrebag weight after incineration

E = Blank crucible weight

F = Sample weight

3.4.3.5 Crude Protein

Sample weighing 1g was put inside Kjeldahl's flask and one Kjeldahl catalyser tablet was put inside the flask. A total of 20 ml of sulphuric acid (3.5 g K_2SO_4 + 0.4 g $CuSO_4$ x $5H_2O$) was poured into the flask and shaken gently for a minute. The flasks were fixed to the Kjeldahl digestion set (Gerhardt Classic Digestion Set, Germany) and heated gradually until maximum heat was achieved. The samples were heated until the solution turned bluish or greenish in colour. The chemical reaction occurring during the digestion is as follows:

$$\text{Sample} = H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2$$

After the process had completed, the flasks were left to cool before proceeding to distillation process using (Gerhardt Vapodest® VAP20s, Malaysia).

Meanwhile, 75ml of 2% boric acid were poured into Erlenmeyer flasks, and eight drops of methyl red and bromocresol green indicator were dropped into each flask. About 25ml of distilled water was poured slowly into Kjeldahl flask containing the digested solution and the remaining 25ml were used to rinse the Kjeldahl flask of any remaining digested solution, then transferred into the distillation tube. The distillation tube and Erlenmeyer flask were fixed onto distillation and distillate platforms respectively. The distillation process took roughly 3 minutes. The process involved releasing the entrapped sulphate salts of ammonium, thus producing ammonia. The chemical reaction that occurred during the distillation is as follows:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO + 2NH_3 + H_2O$$

Succeeding that, the ammonia was collected by the 2% boric acid at the distillation set, as shown by the chemical reaction below:

$$2NH_3 + H_2O \rightarrow NH_4OH \text{ (collected by boric acid)}$$

The mixture in the Erlenmeyer flask was titrated with 0.1M hydrochloric acid in order to determine the nitrogen content, as shown by the formula below:

$$\text{Nitrogen (\%)} = \left[\frac{(\text{Titrant volume} - \text{Blank value}) \times \text{Acid normality} \times 1.4007}{\text{Sample weight}}\right] \times 100$$

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Where,

Blank value = 0.2ml

Acid normality = 0.1 ml

The value of nitrogen is then further multiplied by protein factor, which is 6.25, to obtain the crude protein value.

3.5 Measurement of Growth

The growth of the fishes was measured by determining its body weight and total body length. The body weight was determined by weighing the fish on an electronic balance (Imperial Houseware, unknown country). Total body length was measured using a metal ruler on a flat surface.

3.6 Meat Analysis of Red Hybrid Tilapia

At the end of the 21st day, all fishes from different groups were sacrificed to harvest their flesh and subjected to proximate analysis. The head, fins, skin and organs were all removed. The bones are partially removed due to difficulty deboning a small sized fish (three to six cm). All the flesh collected were separated according to their treatment groups.

Before carrying out the analysis, the flesh was dried in the oven for a day at 60°C. The next day, the sample was grinded using a grinder (Shen Lian SL-150, China) and duplicate test was done to all four treatment groups.

3.7 Analysis of data

For the statistical analysis, all data obtained were tabulated first in Microsoft Excel 2016 and the data were all expressed as mean ± standard error of mean (SEM). The mean body length gained (MLG) and mean body weight gained (MWG) were calculated using the following formula:

$$\text{Mean body length gained (MLG)} = \text{mean final length} - \text{mean initial length}$$

$$\text{Mean body weight gained (MWG)} = \text{mean final weight} - \text{mean initial weight}$$

Data was then analysed using IBM SPSS version 25. One-way analysis of variance (ANOVA) was performed for normally distributed data and Kruskal-Wallis Test for non-normally distributed data. Dunnett’s test was carried out to test significance between control and treatment groups at $p<0.05$.

4.0 RESULTS

4.1 Proximate Analysis

4.1.1 Nutritional Composition of Experimental Feed

The results of the proximate analyses of the four experimental feed were tabulated in Appendix 1. The value used was mean ± standard error of mean for triplicate of each group. Figure 4 and 5 shows the nutritional composition of each groups. The results showed the feed formulation of Group 4 with the highest concentration of BSFL (30%) has the highest percentage in every element except for ash content, while Group 2 has the highest content in ash. Statistical analysis showed that there are significant differences ($p<0.05$) in crude protein and fat content between control and treatment groups.

Figure 4: The dry matter content of experimental feed between groups. There were no significant differences across groups ($p>0.05$).

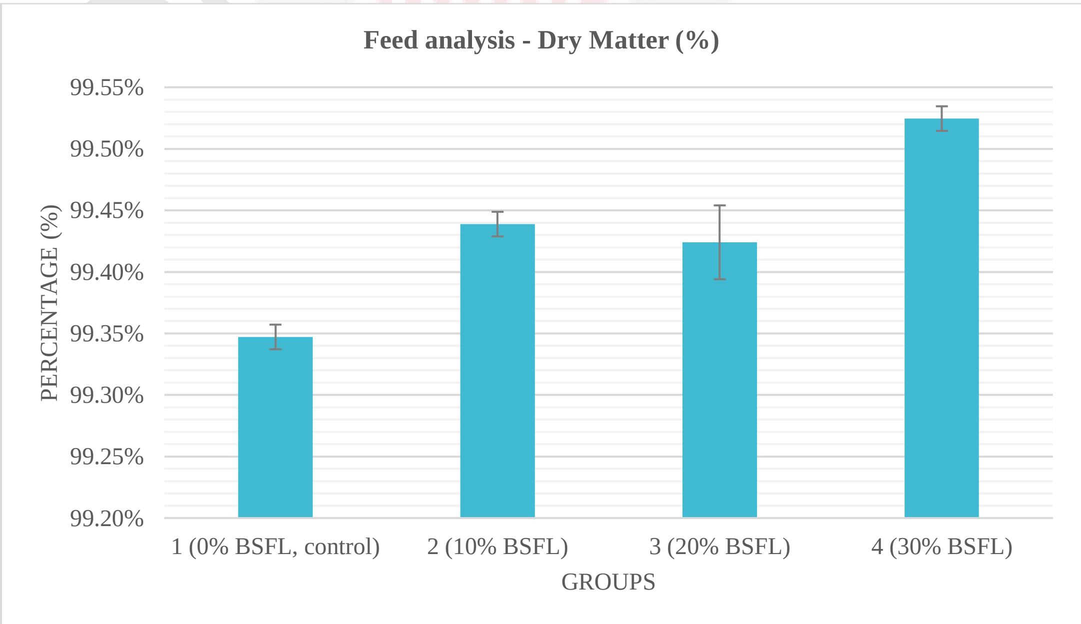
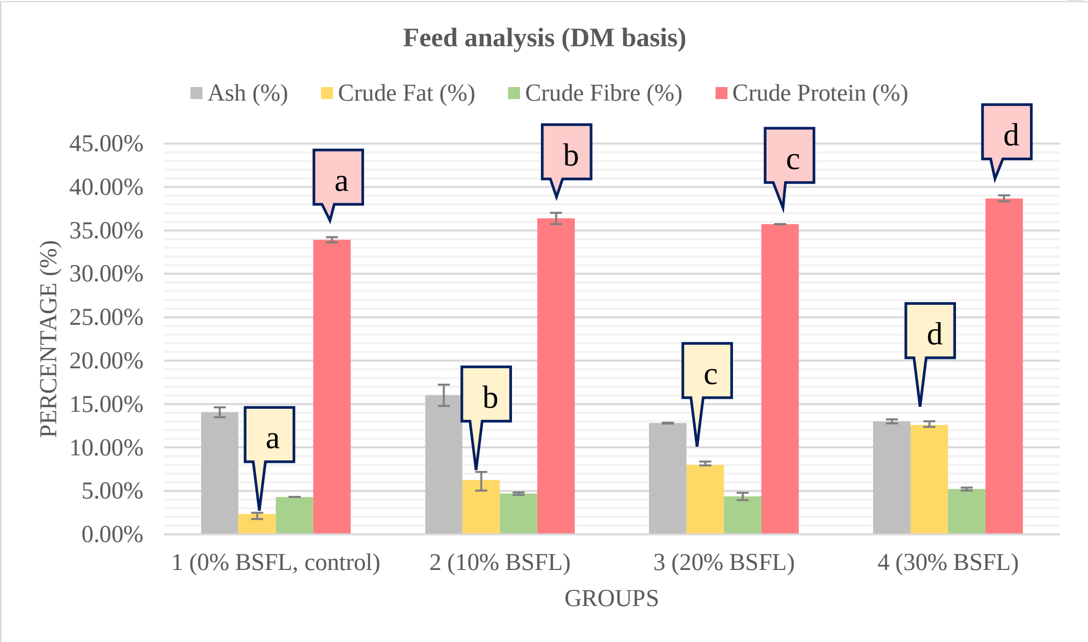


Figure 5: The nutritional content of experimental feed between groups. Groups with different superscripts were significantly different at p<0.05.



4.1.2 Nutritional Composition of Fish

The results of the proximate analyses of meat of each group were tabulated in Appendix 7. Figure 6 showed the fishes of Group 4 had the highest crude protein content at 79.21 - 81.39%, followed by Group 3 with crude protein content at 71.29 - 76.89%. As for Group 1 (control group) and Group 2, the crude protein content at 63.78 - 67.00% and 62.67 - 67.63%, respectively were recorded. Statistical analyses showed that there were significant differences (p<0.05) for crude protein between Group 1, 3 and 4.

Figure 6: The percentage of crude protein in fish according to experimental groups. Groups with different superscripts are significantly different at p<0.05

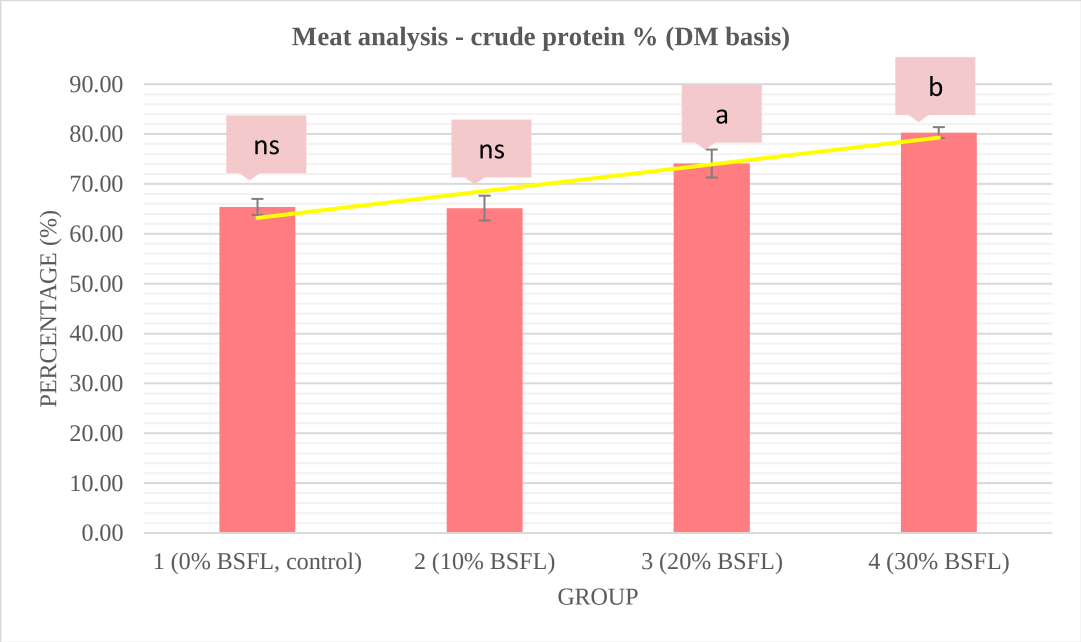
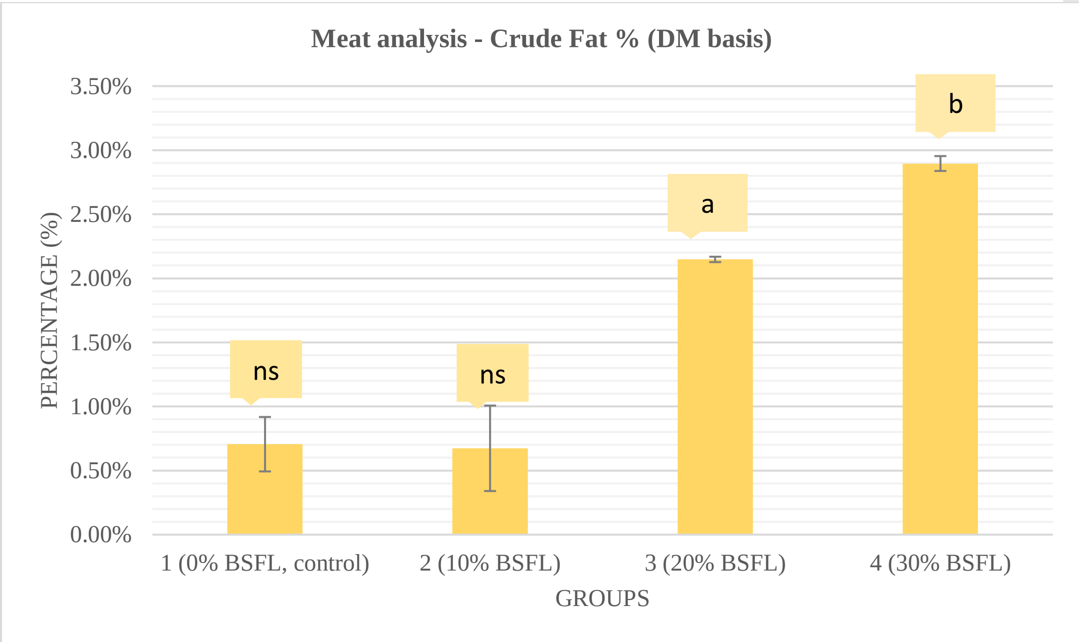


Figure 7 showed the crude fat composition of each group is shown in Figure 5. The same pattern in crude protein content followed for crude fat content. Group 4 achieved the highest crude fat content at 2.84 – 2.95%, followed by Group 3 with crude fat content at 2.13 – 2.17%. As for Group 1 (control group) and Group 2, the crude fat content at 0.49 – 0.92% and 0.34 – 1.01%, respectively were recorded. Statistical analyses showed that there were significant differences (p<0.05) for fat content between Group 1, 3 and 4.

Figure 7: The percentage of crude fat in fish according to experimental groups. Groups with different superscripts are significantly different at p<0.05.

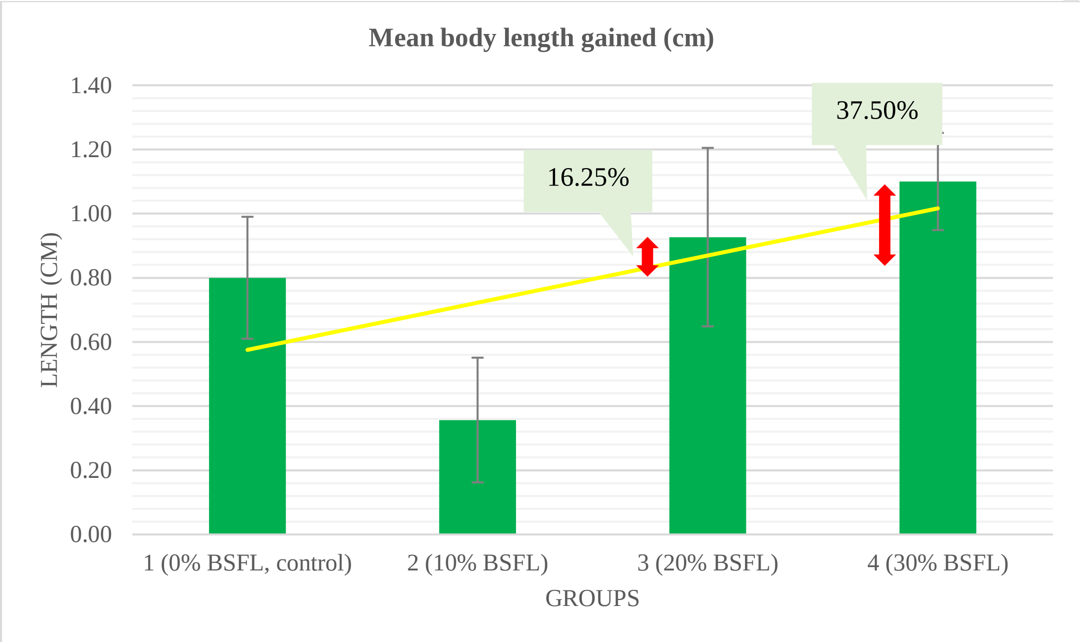


4.2 Growth performance

4.2.1 Result of Body Length Gained

The results of mean body length and weight gained were tabulated in Appendix 6. The graph shown in Figure 8 shows the mean body length gained in respective groups. Group 4 showed the highest increase in body length at 1.10 cm, followed by Group 3 at 0.93 cm, then Group 1 at 0.80 cm and lastly Group 2 at 0.36 cm. The increment in body length for Group 3 and 4 were 16.25% and 37.05%, respectively as compared to the control group. However, statistical analysis showed no significant differences between all groups at p<0.05.

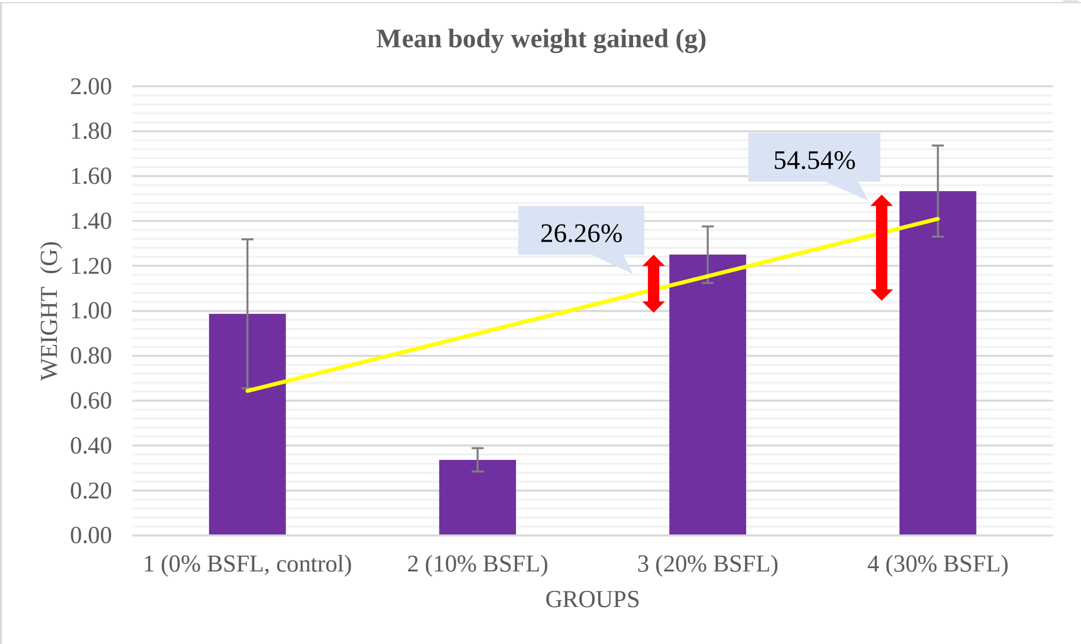
Figure 8: The mean body length gained for each experimental group. There were no significant differences between control and treatment groups (p>0.05)



4.2.2 Result of Body Weight Gained

The graph shown in Figure 9 shows the mean weight length gained in respective groups. Group 4 showed the highest increase in body length at 1.53 g, followed by Group 3 at 1.25 g, then Group 1 at 0.43 g and lastly Group 2 at 0.99 g. The increment in body weight for Group 3 and 4 were 26.26% and 54.54%, respectively as compared to the control group. However, statistical analysis showed no significant differences between all groups at $p<0.05$.

Figure 9: The mean body weight gained for each experimental group. There were no significant differences between control an d treatment groups (p>0.05)



5.0 DISCUSSION

The nutritional composition of the experimental feed was higher than what was obtained by other studies carried out such as by Muin *et al*. (2017) and Teye-Gaga (2017). The differences may be contributed to difference in source of BSFL obtained. The proximate composition of BSFL is also influenced by the substrate the larvae fed on (Newton *et al.*, 2005; Nguyen *et al.*, 2013). Studies by Nguyen *et al*. (2013) and Spranghers *et al*. (2017) found that larvae that were fed on fruits and vegetables have lower crude protein and fat content than those fed on manure.

The growth of fish can be measured by body length and body weight. In this study, total body length (TBL) was used to measure body length, starting from the very end of the mouth of fish to the end of caudal fin. Group 4 (contained 30% BSFL) followed by Group 3 (contained 20% BSFL) show a good increment in growth as compared to Group 1 (0% BSFL) and Group 2 (10% BSFL). This may be due to high protein and fat content in the Group 3 and 4 as compared to Group 1 and 2. These components play a vital role in enhancing the growth of Red Hybrid Tilapia. Although statistical analyses showed that the increase in growth were insignificant at $p<0.05$, the results does prove that the growth performance achieved by fishes fed BSFL diet were slightly better. The inclusion of BSFL up to 30% in commercial diet did not compromise the growth performance. Thus, the substitution of BSFL as an alternative protein source could potentially replace fish meal and reduce feeding cost.

The nutritional composition of meat for crude protein and fat content in this study showed some significant differences between control and treatment groups at p<0.05. This finding was in contrast with other studies that reported protein and fat content in meat were unaffected by diet composition (Siddiqui *et al*., 1998; Teye-Gaga, 2017). Nevertheless, studies by Muin *et al*., 2017 and Devic *et al.*, 2018 were in line with the current finding which reported the protein and meat content were affected by diet composition. These various findings were perhaps due to differences in proximate composition of BSFL due to different rearing substrate.

6.0 CONCLUSION

To conclude this study, the inclusion of BSFL in commercial diet did enhance the growth performance and nutritional composition of Red Hybrid Tilapia, thus accepting the alternative hypothesis. Fishes that were fed with BSFL diet has quality that is roughly equal or slightly equal to fishes fed with commercial fish feed.

7.0 RECOMMENDATIONS

For future study, the feed formulation may be tested to Red Hybrid tilapia of different life stages such as juveniles and adults, or perhaps to other species of fish due to similar biological and physiological function. Additionally, further meat analysis may be conducted such as texture and taste analysis. A larger sample size with larger tank to accommodate the fishes can also be used to minimize statistical error. In addition, cost analysis could be carried out to compare the difference in feeding cost of fishes fed with commercial fish feed and BSFL inclusion diet.

REFERENCES

Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A., & Shalaby, A. M. (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, Oreochromis niloticus (L.). *Aquaculture*, *298*(3-4), 267-274.

Anderson, J., Jackson, A. J., Matty, A. J., & Capper, B. S. (1984). Effects of dietary carbohydrate and fibre on the tilapia Oreochromis niloticus (Linn.). *Aquaculture*, *37*(4), 303-314.

AOAC International. (2005). Official methods of analysis of AOAC International. AOAC international.

Chapman, F. A. (2000). *Culture of hybrid tilapia: A reference profile*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Chou, B. S., & Shiau, S. Y. (1996). Optimal dietary lipid level for growth of juvenile hybrid tilapia, Oreochromis niloticus x Oreochromis aureus. *Aquaculture*, *143*(2), 185-195.

Cummins Jr, V. C., Rawles, S. D., Thompson, K. R., Velasquez, A., Kobayashi, Y., Hager, J., & Webster, C. D. (2017). Evaluation of black soldier fly (Hermetia illucens) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (Litopenaeus vannamei). *Aquaculture*, *473*, 337-344.

Dell, J. N. (n.d.). *Adult Black Soldier Fly* [Photograph]. Insect Images. https://www.insectimages.org/browse/detail.cfm?imgnum=5348010

De Silva, S. S. (1989). *Exotic aquatic organisms in Asia: proceedings of a workshop on introduction of exotic aquatic organisms in Asia*. Asian Fisheries Society, Manila, PH.

De Silva, S. S., Gunasekera, R. M., & Shim, K. F. (1991). Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. *Aquaculture*, *95*(3-4), 305-318.

Devic, E., Leschen, W., Murray, F., & Little, D. C. (2018). Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (Oreochromis niloticus) fed diets containing Black Soldier Fly (Hermetia illucens) larvae meal. *Aquaculture nutrition*, *24*(1), 416-423.

Diener, S., Solano, N. M. S., Gutiérrez, F. R., Zurbrügg, C., & Tockner, K. (2011). Biological treatment of municipal organic waste using black soldier fly larvae. *Waste and Biomass Valorization*, *2*(4), 357-363.

El-Sayed, A. (2006). *Tilapia culture*. CABI Publishing.

Food and Agricultural Organization of the United Nations [FAO] (2008). National Aquaculture Sector Overview. Malaysia. *National Aquaculture Sector Overview Fact Sheets. FAO Fisheries and Aquaculture Department. Available online from www. fao. org/fishery/countrysector/naso_malaysia/en*.

Katya, K., Borsra, M. Z. S., Ganesan, D., Kuppusamy, G., Herriman, M., Salter, A., & Ali, S. A. (2017). Efficacy of insect larval meal to replace fish meal in juvenile barramundi, Lates calcarifer reared in freshwater. *International Aquatic Research*, *9*(4), 303-312.

Lalander, C., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A., & Vinnerås, B. (2013). Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens)—From a hygiene aspect. *Science of the Total Environment*, *458*, 312-318.

Muin, H., Taufek, N. M., Kamarudin, M. S., & Razak, S. A. (2017). Growth performance, feed utilization and body composition of Nile tilapia, Oreochromis niloticus (Linnaeus, 1758) fed with different levels of black soldier fly, Hermetia illucens (Linnaeus, 1758) maggot meal diet. *Iranian Journal of Fisheries Sciences*, *16*(2), 567-577.

Newton, G. L., Booram, C. V., Barker, R. W., & Hale, O. M. (1977). Dried Hermetia illucens larvae meal as a supplement for swine. *Journal of Animal Science*, *44*(3), 395-400.

Newton, L., Sheppard, C., Watson, D., Burtle, G., & Dove, R. (2005). Using the Black Soldier Fly, Hermetia Illucens, as a Value-Added Tool for the Management of Swine Manure.*North Carolina State University*.

Nguyen, T. T., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. *Environmental entomology*, *44*(2), 406-410.

Onyx Aqua Farm. (n.d.). *The Red Hybrid Tilapia* [Photograph]. Importance of Tilapia Farming in Aquaculture. https://onyxaqua.com/importance-of-tilapia-farming-in-aquaculture/

Oonincx, D. G., Van Itterbeeck, J., Heetkamp, M. J., Van Den Brand, H., Van Loon, J. J., & Van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PloS one*, *5*(12), e14445.

Sheppard, D. C., Newton, G. L., Thompson, S. A., & Savage, S. (1994). A value added manure management system using the black soldier fly. *Bioresource technology*, *50*(3), 275-279.

Siddiqui, A. Q., & Al-Harbi, A. H. (1995). Evaluation of three species of tilapia, red tilapia and a hybrid tilapia as culture species in Saudi Arabia. *Aquaculture*, *138*(1-4), 145-157.

Solomko, M. D. (n.d.). *Larvae of Black Soldier Fly* [Photograph]. Shutterstock. https://www.shutterstock.com/image-photo/larvae-hermetia-illucens-black-soldier-fly-623060345

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., ... & De Smet, S. (2017). Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture, 97*(8), 2594-2600.

Teye-Gaga, C. (2017). *Evaluation of Larval Meal Diet of Black Soldier Fly (Hermetia Illucens: L. 175) On Fingerlings Culture of Nile Tilapia (Oreochromis Niloticus: L.)* [Doctoral dissertation, University of Ghana, Legon]. UGSpace Digital Collections.

Tomberlin, J. K., & Sheppard, D. C. (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. *Journal of Entomological Science, 37*(4), 345-352.

Tomberlin, J. K., Sheppard, D. C., & Joyce, J. A. (2002). Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. *Annals of the Entomological Society of America, 95*(3), 379-386.

Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects: future prospects for food and feed security* (No. 171). Food and Agriculture Organization of the United Nations.

Wang, H., Zhang, Z., Czapar, G. F., Winkler, M. K., & Zheng, J. (2013). A full-scale house fly (Diptera: Muscidae) larvae bioconversion system for value-added swine manure reduction. *Waste Management & Research, 31*(2), 223-231.

Widjastuti, T., Wiradimadja, R., & Rusmana, D. (2014). The effect of substitution of fish meal by Black Soldier Fly (Hermetia illucens) maggot meal in the diet on production performance of quail (Coturnix coturnix japonica). *Animal Science, 57*, 125-129.

Yusoff, A. (2015). Status of resource management and aquaculture in Malaysia. In *Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia: Challenges in Responsible Production of Aquatic Species: Proceedings of the International Workshop on Resource Enhancement and Sustainable Aquaculture Practices in Southeast Asia 2014 (RESA)* (pp. 53-65). Aquaculture Department, Southeast Asian Fisheries Development Center.

Yu, M., Li, Z., Chen, W., Rong, T., Wang, G., Li, J., & Ma, X. (2019). Use of Hermetia illucens larvae as a dietary protein source: Effects on growth performance, carcass traits, and meat quality in finishing pigs. *Meat science, 158*, 107837.

Xiao, X., Jin, P., Zheng, L., Cai, M., Yu, Z., Yu, J., & Zhang, J. (2018). Effects of black soldier fly (Hermetia illucens) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (Pelteobagrus fulvidraco). *Aquaculture research, 49*(4), 1569-1577.

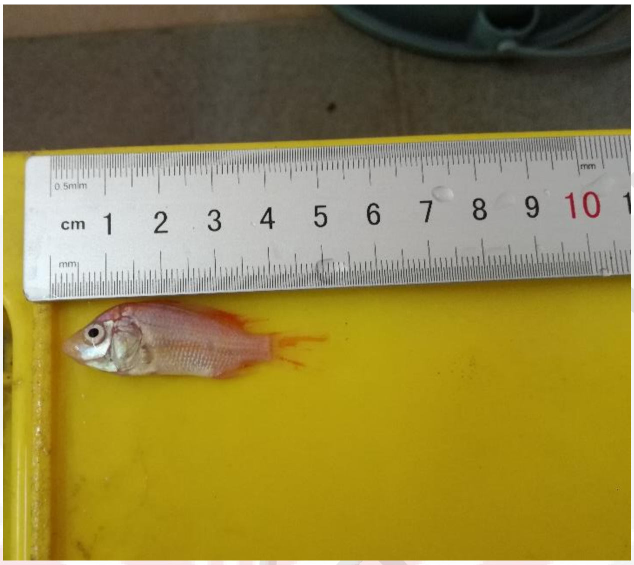
APPENDICES

Appendix 1. Proximate analysis of the four experimental diet feed with varying concentrations of BSFL

Nutritional component (%)	Groups			
	1	2	3	4
Dry Matter ^{ns}	99.35 ± 0.01	99.44 ± 0.01	99.42 ± 0.03	99.52 ± 0.01
Ash ^{ns}	14.06 ± 0.58	16.01 ± 1.23	12.81 ± 0.05	13.01 ± 0.23
Crude Fat	2.33 ± 0.15 ^{a}	6.26 ± 0.93 ^{b}	7.99 ± 0.40 ^{c}	12.60 ± 0.42 ^{d}
Crude Fibre ^{ns}	4.30 ± 0.01	4.68 ± 0.15	4.37 ± 0.43	5.21 ± 0.17
Crude Protein	33.92 ± 0.31 ^{a}	35.63 ± 0.66 ^{b}	36.37± 0.01 ^{c}	38.69 ± 0.35 ^{d}

Values are means ± standard error for triplicate experimental groups. ^{ns} Not statistically significant at $p<0.05$. ^{abcd} Values with different superscripts at the same row differed significantly at $p<0.05$. Group 1: no BSFL inclusion, Group 2: 90% commercial fish feed + 10% BSFL, Group 3: 80% commercial fish feed + 20% BSFL, Group 4: 70% commercial fish feed + 30% BSFL.

Appendix 2. The length of fishes was measured by placing the fish on a flat surface and measure using a ruler.



Appendix 3. The weight of fishes was measured using a weighing balance

Appendix 4. Crude fat content obtained by using Soxhlet fat analysis method. The samples were added with petroleum benzene to extract the fat.

Appendix 5. Crude Protein content obtained by using the Kjeldahl method. The samples were digested, distilled and then titrated.

Appendix 6. Growth performance of fishes of each group in terms of length and weight gained throughout the duration of the study.

Parameters	**Group**			
	1	**2**	**3**	**4**
Mean body length gained (BLG) ^{ns}	0.80 ± 0.19	0.36 ± 0.19	0.93 ± 0.28	1.10 ± 0.15
Mean body weight gained (BWG) ^{ns}	0.99 ± 0.33	0.34 ± 0.05	1.25 ± 0.13	1.53 ± 0.20

Values are means ± standard error for triplicate experimental groups. ^{ns} Not statistically significant at $p<0.05$. ^{abcd} Values with different superscripts at the same row differed significantly at $p<0.05$. Group 1: no BSFL inclusion, Group 2: 90% commercial fish feed + 10% BSFL, Group 3: 80% commercial fish feed + 20% BSFL, Group 4: 70% commercial fish feed + 30% BSFL.

Appendix 7. Proximate analyses of meat of each group in terms of crude protein and fat.

Nutritional component (%)	Groups			
	1	2	3	4
Crude Fat	0.71 ± 0.30 ^{ns}	0.67 ± 0.47 ^{ns}	2.15 ± 0.03 ^{a}	2.90 ± 0.08 ^{b}
Crude Protein	65.39 ± 1.61 ^{ns}	65.15 ± 3.51 ^{ns}	74.09 ± 3.96 ^{a}	80.30 ± 1.54 ^{b}

Values are means ± standard error for triplicate experimental groups. ^{ns} Not statistically significant at $p<0.05$. ^{ab} Values with different superscripts at the same row differed significantly at $p<0.05$. Group 1: no BSFL inclusion, Group 2: 90% commercial fish feed + 10% BSFL, Group 3: 80% commercial fish feed + 20% BSFL, Group 4: 70% commercial fish feed + 30% BSFL.