



UNIVERSITI PUTRA MALAYSIA

***POTENTIAL OF BLACK SOLDIER FLY LARVAE AS
ALTERNATIVE PROTEIN SOURCE IN FISH DIET***

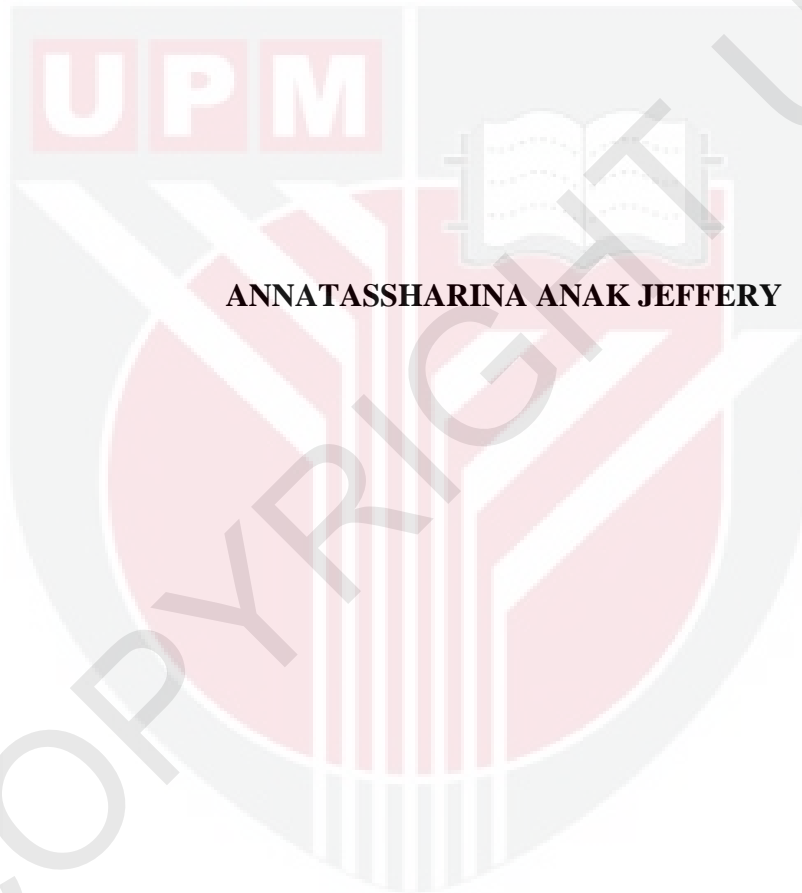
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2024

2023



**POTENTIAL OF BLACK SOLDIER FLY LARVAE AS ALTERNATIVE
PROTEIN SOURCE IN FISH DIET**

BY

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**A Project Report Submitted in Partial Fulfillment of the Requirement for Degree
of Bachelor of Science (Honors) Aquaculture in the Faculty of Agricultural Science
and Forestry University Putra Malaysia Bintulu Sarawak Campus.**

2024

ABSTRACT

Fish meal is a crucial component in aquaculture feed due to its high level of protein, Palatability, digestibility, and amino acid profile. However, the rising market prices and the unpredictable production drive up the production cost and lead to increasing shipping and tax costs, as well as the potential loss of the nutritional content during the shipment. Some insects are being investigated as the source of protein for animal feed due to their high content and rich amino acid profiles, such as the Black soldier fly. Black soldier fly larvae are valuable sources of protein for industrial research. This research is conducted to study growth and the suitability of the different replacements of Black soldier fly larvae in fishmeal. There are 4 diets and each treatment has 3 replications which are the control diet (0% BSFL), 10% BSFL, 20% BSFL, and 30% BSFL as a replacement to fishmeal for the 108 African catfish juveniles on growth performance. The growth performance is conducted by observing the Total Weight Gain (TWG), Total Length Gain (TLG), Condition Factor (K), Feed Efficiency Ratio (FER), Feed Intake, and Specific Growth Rate (SGR) for 30 days. After 30 days, the result showed that there was an increase in the growth performance of African catfish that are fed with diets of 20% BSFL. This resulted in the greatest performance of TWG (6.71 ± 3.87 g), SGR (3.57 ± 2.06), FER (192.76 ± 111.29), and K (3196 ± 1845.40). There is no significant difference between 30% BSFL and FM in terms of TWG, TLG, K, SGR, and FER. The poorest growth performance that is shown in the result is 10% BSFL which is TWG (3.06 ± 1.77 g), TLG (1.58 ± 0.91 cm), K (612.39 ± 353.56), SGR (2.38 ± 1.38) and FER (67.93 ± 39.22). This may indicate that 20% of BSFL might be better for African catfish and if the research is carried out over a long period, it may show a significant difference between the treatments.

Keywords: Black soldier fly larvae, Growth performance, African catfish



ABSTRAK

Makanan ikan merupakan komponen yang penting dalam pemakanan akuakultur. Hal ini kerana kandungan protein yang tinggi, amino asid, kebolehcernaan dan kesedapan. Walau bagaimanapun, kenaikan harga barang di pasaran dan kos pengeluaran yang tidak menentu meningkatkan kos penghantaran dan cukai, serta ianya mempunyai potensi untuk kehilangan kandung nutrisi semasa proses penghantaran. Seterusnya, terdapat pelbagai jenis serangga yang masih dianalisis sebagai sumber protein untuk makanan ikan. Hal ini kerana ianya mempunyai kandungan protein yang tinggi dan ianya juga kaya dengan amino asid, seperti lalat askar hitam. Larva lalat askar hitam ini adalah serangga yang mempunyai sumber protein yang berharga untuk penyelidikan industri. Penyelidikan ini dijalankan adalah untuk mengkaji pertumbuhan dan kesesuaian penggantian berbeza larva lalat askar hitam dengan baja ikan. Terdapat 4 jenis diet dan setiap rawatan tersebut itu mempunyai 3 replikasi iaitu 0%, 10% BSFL, 20% BSFL, 30% BSFL sebagai makanan tambahan untuk 108 ekor ikan keli afrika pada prestasi pertumbuhan anak ikan. Pertumbuhan anak ikan dijalankan dengan memerhatikan Jumlah Pertambahan Berat (TWG), Jumlah Pertambahan Panjang (TLG), Faktor Keadaan (K), Nisbah Kecekapan Makanan (FER), Pengambilan Makanan dan Kadar Pertumbuhan Spesifik (SGR) selama 30 hari. Selepas 30 hari, keputusan menunjukkan bahawa terdapat peningkatan pada tumbesaran ikan keli Afrika yang diberikan diet 20% BSFL. Ini menghasilkan prestasi terbaik TWG (6.71 ± 3.87 g), SGR (3.57 ± 2.06), FER (192.76 ± 111.29) dan K (3196 ± 1845.40). Terdapat tiada perbezaan yang begitu ketara diantara 30% BSFL dan FM dari segi TWG, TLG, K, SGR dan FER. Prestasi pertumbuhan terburuk yang dilihat dalam keputusan ialah 10% BSFL iaitu TWG (3.06 ± 1.77 g), TLG

(1.58±0.91 cm), K (612.39±353.56), SGR (2.38±1.38) dan FER (67.93±). 39.22). Oleh itu, berkemungkinan bahawa 20% daripada BSFL menunjukkan bahawa ianya lebih sesuai untuk keli Afrika dan jika penyelidikan dijalankan dalam jangka masa yang panjang berkemungkinan ianya dapat menunjukkan perbezaan yang ketara antara rawatan tersebut.

Kata kunci: Larva lalat askar hitam, Prestasi pertumbuhan, Ikan keli Afrika.



APPROVAL SHEET

I certify that this research project report entitled “**Potential of Black Soldier Fly Larvae as Alternative Protein Source in Fish Diet**” has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Science (Honors) Aquaculture in the Faculty of Agricultural Science and Forestry, University Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS/ NOTATIONS/ GLOSSARIES OF TERMS

FCR	Feed Conversion Ratio
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
BSFL	Black Soldier Fly Larvae
kg	Kilograms
g	Grams
cm	Centimeter
ml	Milliliter
CMC	Carboxymethyl
DM	Dry Matter
N	Nitrogen content
CF	Crude Fiber
EE	Ether Extract / Crude Protein
OM	Organic Matter
GE	Gross Energy
NDF	Natural Detergent Fiber
ADF	Acid Detergent Fiber
ADL	Acid Detergent Lignin
HPLC	High Performance of Liquid Chromatography
Vit. premix	Vitamin premix
Min. premix	Mineral premix
carbs	Carbohydrate
KJ/G	Kilojoule per gram
TWG	Total Weight Gain
TLG	Total Length Gain
K	Condition Factor
FER	Feed Efficiency Ratio
SGR	Specific Growth Rate
ANOVA	Analysis of Variance
SAS	Statistical Analysis System
STDERR	Standard Error
FM	Fish Meal
T1, T2, T3, T4	Treatment 1, Treatment 2, Treatment 3, Treatment 4
R1, R2, R3	Replicate 1, Replicate 2, Replicate 3
IntWt	Initial Weight
IntLgth	Initial Length
FinWt	Final Weight
FinLgth	Final Length
FedInt	Feed Intake

CHAPTER 1

INTRODUCTION

1.1 Research Background

Fish meal is a very important component in aquaculture feed because there is a variety of reasons such as the high protein content, the excellent amino acid profile, the good digestibility, and palatability. However, rising market prices for fish meal are a result of the product's unpredictable production and rising demand, which drives up production costs (Aanand, 2022.). According to Chun K. H (2007); Utama and Nuruddin (2005) state that the growth of aquaculture in Malaysia is being hampered by a lack of feed supplies. Additionally, it is anticipated that Malaysia will require the importation of 590000 metric tonnes of aquaculture food by the year 2010. The development of aquaculture in Sabah, which is the biggest contributor to the production of aquaculture in Malaysia, is also constrained by the same issue of a lack of feed supplies. (Department of Fisheries Malaysia, 2004; Utama and Nuruddin, 2005; Chun K.H, 2007).

According to Chun K.H (2007), the feed for aquaculture is in short supply, which drives up its price. Additionally, there are increased shipping and tax costs when feed is imported from other nations. In addition, the feed's nutritional content may be lost during shipment (Sim et al., 2005). The production cost will rise as a result of all these factors.

Insects are currently being investigated as a novel source of protein for animal feed (Premalatha et al., 2011; Aanand, 2022). Due to their high content and rich necessary amino acid profiles, insect meals provide a variety of alternatives to animal proteins (Van Huis et al., 2013; Ahmed et al., 2023). Insects can be harvested naturally or raised

economically at a low cost and with a fast production cycle (Oonincx and De Boer, 2012; Ahmed et al., 2023). It is possible to raise insects in regulated environments to maximize their nutritional value (Sealey et al., 2011). To lessen environmental contamination and turn waste into high-protein feed that could take the place of fish meal in aquafeed, some insect species can be cultivated organically. Therefore, from an ecological standpoint, insect cultures are viable and may be carried out in warehouses as they don't need as much space or water as crops need. Additionally, despite recycling waste, insect culture also has a low carbon footprint (Blonk et al., 2008).

Common Houseflies (*Musca domestica*), Yellow Mealworms (*Tenebrio molitor*), Black Soldier Flies (*Hermetia illucens*), and Blowflies (*Calliphora sp.*) are examples of insects that could be used as an alternative protein source in chicken diets (Čičková et al., 2015; Ahmed et al., 2023). According to Finke (2013) and Ahmed et al. (2023), insects are a good source of vitamins and minerals such as folic acid, riboflavin, thiamine, cyanocobalamin, and traces of retinol. Additionally, several peptides found in insects have antioxidant properties that are advantageous to animal health (Schiavone et al., 2017a; Ahmed et al., 2023). Water-soluble insect products have demonstrated superior antioxidant and free radical scavenging activities in comparison to other plant and animal-based protein hydrolysate products (Di Mattia et al., 2019; Ahmed et al., 2023). Due to their lack of energy requirements to sustain their high body temperatures, insects are the most effective food converters (Nijdam et al., 2012; Aanand, 2023). All bug species cannot be used as a feed ingredient, even when considering the insects and the meal as a feed component in aquaculture. Only a few insect species can be manufactured on a wide scale. Among those insects, black soldier fly larvae are a valuable source of protein that

can be used in aquafeed for industrial research.

1.2 OBJECTIVE OF THE STUDY

1. To study the growth and the suitability of the different replacements of black soldier fly larvae meal in fishmeal.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND OF AQUACULTURE INDUSTRY

Based on the statistics from the Department of Fishery, the freshwater aquaculture production in 2021 is 105.9000 tons while 2022 the aquaculture production is 97.2000 tons, brackish aquaculture production in 2021 is 311.3000 tons, and 302.9000 tons in 2022. An increase in fish production that reaches millions of tons has caused a very high production. The production of a large amount of food in fisheries will cause the price of the feed to become very high. It is also difficult for the fish farmers to get the feed source for their fish farms, with the existence of a group of researchers that offer an alternative feed based on the research that has been done, can help in reducing the increase of fish feed cost and it can support the income in the aquaculture farming.

Mohammed Suhaime A. (2017) states that since 2012, Malaysia has created yearly fish meal production of about 65,000 metric tons. 44,300 metric tons of bycatch would be used directly as marine fish feed out of the anticipated 216,000 metric tons of bycatch that were processed into fish meal per year using that amount of dry powdered form.

The majority of marine cage culture operators alternate feeding their fish with commercial pellets due to the high market value of marine fish. They adhere to the seabass culture trend, which has been entirely reliant on pelleted feed since 2013 either farm- or factory-made. According to estimates, Malaysian aquaculture feed contains 15% fish meal for marine species and 11% for freshwater species.

2.2 INTRODUCTION TO AFRICAN CATFISH

There is a large population of *Clarias gariepinus* in countries of Asia and Africa (Israel, Syria, and south of Turkey). Its primary habitats are placid lakes, rivers, and marshes in regions that occasionally flood (De Graaf and Janssen 1996, Winemiller and Kelso-Winemiller 1996; Vitule et al., 2006). As adaptations for living in still conditions or without water, *C. gariepinus* features lengthy bodies, pseudo-lungs, and a high mucus production rate. (1993; Vitule et al., 2006; Doneelly). It feeds on plant matter, plankton, arthropods, mollusks, fish, reptiles, and amphibians in its natural habitat (Yalin et al., 2001; Vitule et al., 2006). It reproduces seasonally, with gonadal maturation occurring during flood events. Although variations in water temperature and photoperiod have an impact on the maturation process, an increase in water levels is the primary determinant in their reproduction (Van der Waal, 1994; De Graaf et al., 1995; Yalin et al., 2001; Vitule et al., 2006).

The non-native fish species *C. gariepinus* was brought to Malaysia through aquaculture from Thailand between 1986 and 1989. The first record of *C. gariepinus* culture in Malaysia dates to 1987 with a yearly yield of 6.46 metric tonnes, and it has steadily increased since then, according to Csavas I (1995) and Dauda et al. (2018). When it surpassed red tilapia, which had previously been the most widely cultivated finfish, in 2008, the industry had expanded dramatically over the previous two decades to become the highest-produced finfish cultured in either fresh or brackish water.

The largest freshwater clariid fish is *Clarias gariepinus*, sometimes known as the African catfish, according to Arun Prasad Baidya et al. (2004). It reaches a length of more than 170 cm and a weight of 60 kg (Robins et al., 1991). In the nations where it is sold,

this African catfish is known by a variety of regional names. As a result, it is known as "Keli Africa" in Malaysia and "Hige-Namazu" in Japan (Frimodt, 19945; Arun Prasad Baidya et al., 2004). Due to its numerous benefits, including its rapid growth rate, great disease resistance, and omnivorous feeding habits, *C. gariepinus* is a well-liked species in the Southeast Asian region (Huisman and Richter, 1987; Arun Prasad Baidya et al., 2004).

Table. 1 Nutrient Recommended for Catfish Grow-out Feeds

Nutrient	Recommended Level
Protein (%)	26-32
Essential Amino acids (% of protein)	
1. Arginine	4.3
2. Histidine	1.5
3. Isoleucine	2.6
4. Leucine	3.5
5. Lysine	5.1
6. Methionine	2.3
7. Phenylalanine	5.0
8. Threonine	2.0
9. Tryptophan	0.5
10. Valine	3.0
Digestible energy (kcal/g protein)	8.5-9.5
Lipid (%)	4-6
Carbohydrate (%)	25-35

Vitamins:	
1. A	1,000 IU/lb
2. D3	500 IU/lb
3. E	30 ppm
4. K	4.4 ppm
5. Thiamin	2.5 ppm
6. Riboflavin	6 ppm
7. Pyridoxine	5 ppm
8. Pantothenic acid	15 ppm
9. Nicotinic acid	None
10. Biotin	None
11. Folic acid	2.2 ppm
12. B-12	0.01 ppm
13. Choline	None
14. Inositol	None
15. Ascorbic acid	50 ^b ppm
Minerals:	
1. Calcium	None
2. Phosphorus, available	0.3 % - 0.35%
3. Magnesium	None
4. Sodium, potassium and chloride	None
5. Sulfur	None
6. Cobalt	0.05 ppm

7. Iodine	2.4 ppm
8. Zinc	200 ppm
9. Selenium	0.1 ppm
10. Manganese	25 ppm
11. Iron	30 ppm
12. Copper	5 ppm

Source: Robinson, E., Li, M. H., & Hogue, C. D. (2006). *P2412 Catfish Nutrition: Nutrient Requirements*.

2.3 FEEDING MANAGEMENT

2.3.1 FEEDING PHASES OF FISH

Ebenezzar et al. (2023) state that several types of feed may be used for starting, frying, fingerling, grow-out, and broodstock. Generally speaking, mash feed, microencapsulated diets, micro-bound diets, micro-coated diets, flakes, and crumble foods make up the majority of larval stages or larval feed.

According to Ebenezzar et al. (2023), the starting feed is given to the larvae that eat first when their internal food source runs out. Starter feed, which often takes the shape of crumbles, flakes, or paste, is extremely important. This is generally in dry or semi-moist. The started feed is different in terms of composition and type depending on the nutritional requirements and the size of the fish at the first feeding. This feed should be nutritionally complete, easily digestible, water stable with the appropriate particle size. This particle size of the feed can vary from 50 μm – 70 μm and it is in the form of fine crumbles or flakes. The microencapsulation is the most effective technique to prepare the starter

The next feeding phase is called fry feed, and it is reserved for fish that are in the fry stage and weigh between 0.5 and 0.75 g. The fry feed is often in the form of a flake or pellet. The fry feed size is in the 0.8–1.2 mm range. Generally speaking, this diet has a lot of protein. The reason for this is that throughout the early phases of a fish's existence, a substantially larger amount of protein and energy are needed per unit. With higher nutritional quality in the fry feed at a higher feed cost, the fry stages yield the highest relative

growth. Additionally, it's critical to make sure that the culture system's development phases recognize the entire growth potential. (Ebenezar et al., 2023)

Also, fingerling phases are described as the period between metamorphoses and growth, ranging from 1.2 g to 10–20 g. Depending on the type and size of the fish, the food for fingerlings can vary from crumble to pellets ranging in size from 1.2 to 2.4 mm. Compared to fry and starter feed, fingerling feed typically has a lower protein content (10–15%) and lower calorie content. However, the growth rate of the stages is faster than that of the other feed types. Therefore, it is crucial to ensure that the fish's growth is being acknowledged by giving them a diet that is marginally overboosted with limiting amino acids and vitamins. (Ebenezar et al., 2023)

The grow-out phase diet is designed to encourage the fish's economical and efficient development to market size, according to Ebenezar et al. (n.d.). Generally speaking, adult fish that have reached maturity are fed the grow-out diet. The weight will rise throughout the stages at a generally consistent pace; it will only slightly decrease as the fish grows and its weight increases and it seems to have quite constant nutritional needs. The culture system's biomass will rise significantly, which will raise the total amount of feed that is required to reach optimal efficiency. Since there are slight variations in the feed cost and the feed conversion ratio (FCR), these are important variables to take into account when choosing the grower diets. It significantly affects a fish farm's

capacity to make money. As a result, the culture can save the most feed at this point. This feed has a diameter of more than 4 mm.

Finally, the broodstock meal is only intended for the broodstock and helps the fish develop their gonadal organs and reach sexual maturity. It is well recognized that the quality of the nutrition at this time influences the quality of the progeny. As a result, the broodstock feed should be designed to meet the fish's nutritional requirements. High levels of protein, energy, and certain vitamins linked to the conversion of maternal tissue nutrients to egg nutrients are often found in the formulation. Additionally, unique and necessary nutrients like cholesterol, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EFA) are included in broodstock feed to support gonadal development and excellent egg quality. (Ebenezar et al., 2023)

2.3.2 IMPORTANCE OF FEEDING PHASES FOR FISH

Feeding phases are important in fish farming because the quality and quantity of feed available significantly affect the growth and survival of fish. If the fish are not fed enough, they may grow, lose weight, or even die from food deficiency. According to Assan et al., 2021 it is essential for the optimization of growth and body composition. One of the main goals of intensive fish farming is to increase food conversion efficiency and reduce nutritional losses, which may be achieved by optimizing food intake. This will improve fish development and body composition.

Next, according to FAO, the feeding phases will be essential for adapting to the life stages of the fish. Fish have different dietary needs based on their size, species, and stage of life. More food is needed by smaller fish than by larger ones, and in warmer water than in colder water. FAO also mentions that the feeding phases are essential for the feeding frequency. The growth and feed conversion are impacted by the frequency of feeding. The larger fish need less frequent feeding, whereas the smaller fish need to be fed almost constantly.

Moreover, FAO mentioned that the feeding phases could avoid food deficiency. Because there is a shortage of both quantity and quality of nutrition, the fish may not be able to grow, may even lose weight, or may even perish. Only once the requirements for upkeep are met will there be growth.

FAO also mentioned that feeding phases are suitable for adjusting for environmental conditions. The temperature of the water and the quality of the meal that the fish are fed will both affect how active the fish are. It also lessens waste and pollution since, to get the greatest results, feeding must be adjusted throughout the production cycle to prevent overfeeding, which should be avoided to prevent more waste on expensive feed, water pollution, and other undesirable effects.

Thus, in fish farming, feeding stages are particularly important for ensuring the fish's best possible development, survival, and general health. It is more important to comprehend and effectively manage the various feeding stages in fish farming as good feeding techniques will help to enhance production efficiency and reduce environmental consequences.

2.3.3 FEEDING PERIOD OF FISH

Fish size has a role in both feeding rates and feeding frequencies, according to Steven Craig (2017). It is necessary to regularly provide a meal high in protein to both the fry and the tiny larval fish. Due to their high energy needs, the little fish required hourly feedings and constant food intake. About the volume of water in the culture system, just a minimal amount of feed is needed to feed the small fish.

According to Steven Craig (2017), feeding rates, frequency, and feed protein content will decrease as fish develop. Reduced feeding enables the grower to maintain the same protein intake during the grow-out phase. As a result, it will make feed inventory and storage simpler. Fish at labor-intensive facilities, such as huge catfish farms with several ponds, often only need to be fed once a day, whereas smaller farms require feeding twice a day. Generally speaking, the frequency of feeding correlates with an increase in growth and feed conversion. To enhance development at the ideal temperature in an indoor culture, fish must be fed five times a day.

The feeding rates of fish can be influenced by several factors, including their life stage, time of day, season, water temperature, dissolved oxygen content, and other variables related to water quality. For instance, it is not a good idea to feed grown fish in ponds early in the morning because the ponds have the lowest levels of dissolved oxygen. However, a recirculating aquaculture system could assist the farmer in feeding the fish early in the morning because it continuously supplies the fish with oxygen. (Craig, Steven (2017)

2.4 CONVENTIONAL FEED PROTEIN INGREDIENT

Grain that has been genetically altered to accept the maximum concentrations of pesticides and herbicides is used in conventional feed. The least expensive feed that is readily available is conventional feed. Fishmeal is the primary conventional feed that farmers utilize. Fishmeal is the primary element in many fish species' professionally produced diets, according to Goda et al. (2007). due to the rapidly expanding fish and shellfish aquaculture industry. According to Liu et al. (2021), fishmeal has been employed as a high-quality protein source for aquafeed because of its abundance of vitamins and minerals, good digestibility, and well-balanced amino acid profile. Furthermore, due to the continuous expansion in demand, fishmeal prices have increased dramatically over the past few years and are projected to do so going forward (Hardy and Tacon, 2002; Goda et al., 2007).

Regarding the rising costs of fishmeal, new forms of conventional feed protein have been developed to take its place. One of the best substitutes for fishmeal is soybean meal. Among plant protein feedstuffs, soybean meal offers the best protein quality, is inexpensive, easily accessible, and high in protein (Lovell 1988; Rumsey et al., 1993; Goda et al., 2007).

2.5 BLACK SOLDIER FLY

2.5.1 INTRODUCTION TO BLACK SOLDIER FLY

According to Aanand (2021) states that the Black Soldier Fly is native to the Neotropics. These insects are 16 mm long adults with a black body, it has blue to green metallic reflection on the thorax, and on rare occasions, it has a red-ended abdomen. The translucent regions are located in the second abdominal tergite. The antennae are twice as long as its head, and it has a large head and well-developed eye. The wings are membranous, folded horizontally over the belly, and overlaps the legs which are black with pale tarsi.

Originating in America, the black soldier fly has spread to temperate and tropical regions from Argentina to Central America (Sheppard et al., 1994; Ahmed et al., 2023b). Through human activities, it gets transported to other parts of the planet, such as Europe, Asia, Australia, and Africa. According to Martnez-Sánchez et al. (2011), Gujarathi and Pejaver (2013), and Ahmed et al. (2023b), the Black Soldier Fly can tolerate a wide range of climatic conditions, which accounts for its vast distribution throughout diverse locations. The polyphagous black soldier insect has activity for protease, amylase, and lipase in its gastrointestinal excretions (Kim et al., 2011; Ahmed et al., 2023b).

These characteristics make it a viable choice for the recycling of animal leftovers (Myers et al., 2014; Ahmed et al., 2023b), feces (Oonincx et al., 2015a), and other organic leftovers (Ahmed et al., 2023b) (Gurahathi and Pejaver, 2013; Ahmed et al., 2023b). With these traits, it can transform animal feed into an additional source of protein for chicken diets (Veldkamp et al., 2012; Ahmed et al., 2023b). Due to the lack of pest behavior, Black

Soldier Fly production differs from others and doesn't need particular care (Liu et al., 2008; Ahmed et al., 2023b). According to Müller et al. (2017) and Ahmed et al. (2023b), the larvae of the Black Soldier Fly include dipterocins, atacins, cecropins, and defensins, among other antimicrobial peptides.

The composition of the larval black soldier fly depends on the substrate, according to Spranghers et al. (2017). According to Tomberlin et al. (2002) and Liu et al. (2017), It is made up of lipids and amino acids in proportions of 30 to 35 percent, followed by 50 to 60 percent crude protein. The only factor influencing the black soldier fly larvae's body composition is the type of food they consume (Nguyen et al., 2015). The dry matter (DM) content of black soldier fly larvae gradually rises from the day-old larval stage to the prepupal stage, where it averages 35 to 45 percent. This value stays the same throughout the pupal stage and subsequently drops to 20–25 percent as the larvae mature. (Liu et al., 2017, Newton et al., 2005, Gangadhar et al., 2018).

The greatest percentage of crude protein (CP) content, which is extraordinarily variable throughout the life cycle and stays at around 40% during the larval stage, is reported by Li et al. (2015) and Gangadhar et al. (2018). Additionally, the amount of The larvae's body crude fat (CF) fluctuates based on the kind of food they eat. (Gangadhar et al., 2018). However, from larvae that are a few days old to the prepupal stage, the crude fat value will stay at an average of 24-28 percent (Li et al., 2015).

Additionally, there are plenty of sources of amino acids for the larvae, Significant differences in their body composition have been seen according to the culture medium. According to St. Hilaire et al. (2007)a, Al-Qazzaz and Ismail (2016), and Kim et al. (2021), cattle excrement, which has been proven to contain a significant number of amino

acids, is used to feed black soldier fly. Examples of amino acids found in black soldier fly larvae include alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and tyrosine (Makkar et al., 2014; Shumo et al., 2019).

In general, the larvae of the black soldier fly contain 19%–40% mono and polyunsaturated fatty acids (PUFAs) and 58–72% saturated fatty acids, according to Kroeckel et al. (2012). According to Lalander et al. (2019), the nutritional value of BSF biomass is greatly influenced by the growth substrate selection. Using an organic substrate with the right proportions of PUFAs has been shown in numerous experiments to increase the prepupae biomass of Black Soldier Larvae (Barroso et al., 2017; Marco et al., 2021). Additionally, according to Bosch et al. (2014) and Maurer et al. (2015), chitin content gradually rises with age and significantly increases throughout the prepupal phases.

Insect nutrition and growth were typically supported by the application of minerals. According to Ortiz et al. (2016) and Spranghers et al. (2017), black soldier fly larvae are the main source of minerals and calcium, making black soldier fly the finest food source. Black soldier fly larvae are known to possess a significant number of critical elements, including iron, zinc, calcium, phosphorus, copper, and magnesium, when compared to other insect larvae, according to Dierenfeld and King (2008). The levels of calcium and phosphorus are noticeably higher in larval stages than in adults.

2.5.2 LIFE CYCLE OF BLACK SOLDIER FLY

Five phases make up the life cycle of the Black Soldier Fly, according to Alvarez (2012). The egg is the initial stage, and it will hatch in four days or so. The second stage is the larvae stages, which have five instars and last for a duration of between 13 and 18 days. Next comes the prepupa stage, which is the sixth and last instar and lasts for around seven days. Pupa, the fourth stage, can take anywhere between 10 and 30 days to develop into an adult, depending on the environment. The black soldier fly typically survives between five and eight days in its adult phases. The division of the larval stages into instars occurs when the exoskeleton molts repeatedly. Different fly species have varying numbers of instars. Additionally, as the larva molts from each instar, it feeds to its fullest potential while storing fat for use in the adult stage. The adult black soldier flies just needs to mate, lay eggs, and hatch them while eating the body's fat reserves. After the bodily fat is gone, the adult black soldier flies will die. Ahmed and colleagues (2023; Myers et al., 2014).

Nguyen (2010) claims that the larvae may devour a variety of things, including spoiled fruits, vegetables, and meat as well as leftovers from restaurants and kitchens. According to Ahmed et al. (2023), the black soldier fly larvae's entire life cycle depends on variables like nutrition accessibility and environmental parameters. Depending on the component, the overall life period of the larva is around 40 days (Diener et al., 2009). Because the female black soldier flies will lay her eggs close to the food sources, the nymphs will look for nearby feed and begin eating on it. If the feed has a moisture level of between 60 and 90% and is at an ideal temperature of between 27 and 30 °C, the maggots will consume it continually (Sheppard et al., 2002). Before consuming the waste products, the maggots will release a certain type of enzyme secretion like amylase or

maltase (Gold et al., 2018). For maggots, the amount of moisture is vital because it directly affects their life cycle (Fatchurochim et al., 1989; Ahmed et al., 2023).

2.5.3 APPLICATION OF BLACK SOLDIER FLY

According to Mohan et al. (2022), in the black soldier fly, many domains have made use of *H. illucens*, particularly for nutritional components and amino acid profiles. Furthermore, the best source of protein is the black army fly pupae and pre-pupae, which are used to make pig feed and poultry (Zhang et al., 2014). (Makkar et al., 2014). It is also fed to other creatures, such as the gigantic ditch frog and the American alligator (Bodri and Cole, 2007). (Dierenfeld and King, 2008).

Besides, according to Belghit et al. (2019), several research studies have been conducted about the use of black soldier fly larvae as food for aquatic creatures. Salmon tartare, *Oreochromis niloticus* (Yildirim-Aksoy et al., 2020a. 2020b), and many more. According to Bruni et al. (2020b), Jahan and Haque, (2021), and Melenchón et al. (2021), these black soldier fly larval meals had a beneficial effect on the health of the fish in the aquaculture industry.

Black soldier fly meal is a promising fishmeal substitute that can be used in the diets of Atlantic salmon, turbot, rainbow trout, Jian carp, and Pacific white shrimp. When given up to 50% of their diet as defatted black army fly larval meal, juvenile Jian carps (*Cyprinus carpio var. Jian*) exhibit good development, carcass composition, antioxidant enzyme activities, and digestive enzyme activities (Senlin et al., 2017; Aanand, 2022). However, a 75 % replacement has negative consequences for the fish, including nutritional

stress and intestinal histological damage. Black soldier fly pupae raised on dairy cattle manure and trout offal can substitute up to 50% of the fishmeal and fish oil in the diet of rainbow trout (*Oncorhynchus mykiss*) to encourage healthy growth (Wendy et al., 2011; Aanand, 2022). Additionally, black soldier fly larvae food can be utilized for the culture of ornamental fish, particularly for guppy (*Poecilia reticulata*), where it can substitute fish meal up to 25–50%. (Sanjaya et al., 2020; Aanand, 2022). Along with fin fish, it is benefiting shellfish, especially the Pacific White Shrimp species (*Litopenaeus vannamei*), which has good weight gain, a specific growth rate, and food conversion with a replacement of less than 25% for fishmeal diet (Vaun et al., 2017; Aanand, 2022).

Table 2: Fish species, inclusion level of Black Soldier Fly Larvae meal, and result

Species	Form	Inclusion level	Result	Citation
Barramundi (<i>Lates calcarifer</i>)	BSFL	30% defatted BSFL and extracted oil	<ul style="list-style-type: none"> - Bactericidal activity and mucin cells increase. - Serum lysozyme activity is still the same - Up-regulated immune-relevant gene expression. 	Hender et al., 2021
Dusky Kob (<i>Argyrosomus japonicus</i>)	BSFL	50 g/kg, 100g/kg, and 200 g/kg	<ul style="list-style-type: none"> - Feed conversion ratio and specific growth rate increased (200 g/kg) - All blood parameters fell 	Madibana et al., 2020

			within the normal range	
Nile Tilapia <i>(Oreochromis niloticus)</i>	BSFL	10%, 20%, 40%, 60% 80% and 100%	- There were no appreciable changes to the survival rate, feed intake, growth indexes, or feed utilization efficiency indices. - Lysozyme and peroxidase activity in skin mucus increased.	Tippayadara et al., 2021
Japanese eel <i>(Anguilla japonica)</i>	BSFL	75% of BSFL	- Didn't cause any adverse effect on growth performance.	Kuo et al., 2022
Siamese Fighting Fish <i>(Betta splendens)</i>	BSFL	13% of BSFL	- Enhance growth performance, blood hematology, and liver and gut morphology	Kari et al., 2023

<p>Yellow Catfish <i>(Pelteobagrus fulvidraco)</i></p>	<p>BSFL</p>	<p>0%, 13%, 25%, 37%, 48%, 65%, 85% and 100%</p>	<ul style="list-style-type: none"> - Growth performance and immune indexes increased (48%) - Feed conversion ratio decreased (25%) - No significant differences in survival rate, body indexes, or composition. 	<p>(Xiao et al., 2018a)</p>
<p>African Catfish <i>(Clarias gariepinus)</i></p>	<p>BSFL</p>	<p>25%, 50% and 75%</p>	<ul style="list-style-type: none"> - The increase of the ultimate body weight increase, body protein, protein productive value, feed conversion ratio, specific growth rate, and protein efficiency ratio. 	<p>Fawole et al., 2020</p>

			<p>(25 and 50% of the total)</p> <ul style="list-style-type: none"> - Liver function enzyme decrease (50%) - The activity of the enzyme's catalase and superoxide dismutase rise (50%)
	0%, 25%, 50% and 100%		<ul style="list-style-type: none"> - Nutrient consumption and growth performance indicators did not significantly differ from one another (25% and 50%) - Growth, feed intake, and protein

			<p>efficiency ratio decreased (100%)</p> <ul style="list-style-type: none"> - There was no discernible change in the haematological parameters. 	Adeoye et al., 2020
Salmonidae	BSFL	300 g kg ⁻¹	<ul style="list-style-type: none"> - No adverse effects on the physiological condition, growth, or protein consumption - Increasing the digestibility of the protein 	Melenchón et al., 2021

			- Omega-3 fatty acid in the fillet decreased	
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CHAPTER 3

MATERIALS AND METHOD

3.1 DURATION OF STUDY

The experiment was conducted for 30 days (1 month) to set up the propagation of black soldier fly larvae meal over 108 catfish juveniles.

3.2 MATERIALS

The materials that are used in this experiment are a grinder, electric weighing balance, pelletizer, zip bag, beaker, stainless steel ladle, fishmeal, sundried black soldier fly larvae, wheat flour, fish oil, carboxymethyl cellulose (CMC), α - cellulose, choline chloride, vitamin premix, and mineral premix.

3.3 METHODOLOGY

3.3.1 PREPARATION OF BLACK SOLDIER FLY LARVAE

The sundried black soldier fly larvae will grind using the grinder machine. After the black soldier fly larvae have become powder. The fishmeal, wheat flour, fish oil, carboxymethyl cellulose (CMC), α - cellulose, choline chloride, vitamin premix, and mineral premix were weighed using an electric weighing balance for 2kg for the entire treatment. Then, the dry ingredients are mixed in the container, 200 ml of water is added

to the mixture of the dry ingredients and slowly the fish oil is poured into the mixture. The mixture is stirred until it is combined. Then, the mixture is poured into the palletizer machine. After that, the pallet from each treatment is placed on the tray and ready to be put in the oven for 24 hours at a temperature of 40°C. After 24 hours, the pallet is filtered using a sieve and it is placed into a large size of zip bag and labeled using marker. The pallet is placed into the refrigerator to maintain the feed at an optimum temperature and to extend its life.

3.3.2 EXPERIMENTAL DESIGN

The experiment has 4 treatments and each of the treatments has 3 replications. In treatment 1 (T1) the tank are supplied with a normal diet (0% of black soldier fly larvae meal) as the supplementary feed for the juveniles. Next, in treatment 2 (T2) the tank will be supplied with fishmeal and 10% of black soldier fly larvae meal as the supplementary feed for the juveniles. For treatment 3 (T3) the tank will be supplied with fishmeal and 20% of black soldier fly larvae meal and lastly, for treatment 4 (T4) the tank will be supplied with fishmeal and 30% of black soldier fly larvae meal.

Table 3: Formulation of experimental diets (dry matter basis) (%)

RAW MATERIALS	Diet 1 FM	Diet 2 BSFL (10)	Diet 3 BSFL (20)	Diet 4 BSFL (30)
FISH MEAL	58.33	52.50	46.66	40.83
BSF	0.00	6.25	12.50	18.75
WHEAT FLOUR	29.70	29.15	29.00	29.10
FISH OIL	7.63	7.50	7.38	7.25
CMC	1.00	1.00	1.00	1.00
α-CELLULOSE	1.14	1.40	1.26	0.87
CHOLINE CHLORIDE	0.70	0.70	0.70	0.70
VIT.PREMIX	0.75	0.75	0.75	0.75
MIN.PREMIX	0.75	0.75	0.75	0.75
TOTAL	100.00	100.00	100.00	100.00

COMPOSITION				
CRUDE PROTEIN (%)	35.00	35.00	35.00	35.00
CRUDE FAT (%)	12.00	12.00	12.00	12.00
TOTAL CARBOHYDRATE	31.84	32.09	32.35	32.60
CARBS (Energy)	30.70	30.69	31.09	31.73
GE (KJ/G)	18.00	18.00	18.00	18.00

3.3.3 SURVIVAL GROWTH, THE PRODUCTION OF FISH AND COLLECTION

DATA

The fish samples were collected to estimate the growth in length (cm) and weight (g) and to check the health of the African catfish juveniles. The growth will be conducted by observing the Total Weight Gain (TWG), Total Length Gain (TLG), Condition Factor (K), Feed Efficiency Ratio (FER), Feed Intake, and Specific Growth Rate (SGR).

$$\text{Total Weight Gain (TWG)} = \text{Final Weight (g)} - \text{Initial weight (g)}$$

$$\text{Total Length Gain (TLG)} = \text{Final Length (cm)} - \text{Initial Length (cm)}$$

$$\text{Condition Factor (K)} = [\text{Weight (g)} / \text{Length (cm)} * 3] * 100$$

$$\text{Feed Efficiency Ratio (FER)} = [\text{Weight gain (g)} / \text{food intake (g)}] * 100$$

$$\text{Feed Intake} = [\text{average weight of fish} * (5/100)] * \text{total of fish}$$

$$\text{Specific Growth Rate (SGR)} = [\ln (\text{Final weight}) - \ln (\text{Initial weight}) / \text{days}] * 100$$

3.3.4 STATISTICAL ANALYSIS

All data were subjected to 1-way ANOVA using the Statistical Analysis System (SAS) and the average of the data was calculated with Microsoft Excel. The mean values of the fish meal and black soldier fly larvae meal were separated by Duncan's multiple range test, whereby fishmeal will serve as the control group. The result was considered statistically significant at $p < 0.05$. Data were reported as Mean \pm STDERR (Standard Error)

CHAPTER 4

RESULT

4.1 DATA THAT ARE COLLECTED WITHIN 30 DAYS

Table 4: Growth Parameter (Mean \pm STDERR) after 30 days of feeding with control (100% FM) and FM that are replaced with 10%, 20% and 30% of BSFL meal on African Catfish juvenile (*Clarias gariepinus*)

	FM	10% BSFL	20% BSFL	30%BSFL
IntWt (g)	3.43 \pm 1.98 ^a	3.14 \pm 1.8 ^a	3.13 \pm 1.81 ^a	3.13 \pm 1.81 ^a
IntLgth (cm)	8.13 \pm 4.69 ^a	8.02 \pm 4.63 ^a	7.92 \pm 4.57 ^a	8.01 \pm 4.62 ^a
FinWt (g)	8.83 \pm 5.10 ^a	6.20 \pm 3.58 ^a	9.83 \pm 5.68 ^a	7.57 \pm 4.37 ^a
FinLgth (cm)	10.23 \pm 5.19 ^a	9.60 \pm 5.54 ^a	9.53 \pm 5.50 ^a	9.77 \pm 5.64 ^a
TWG (g)	5.41 \pm 3.12 ^a	3.06 \pm 1.77 ^a	6.71 \pm 3.87 ^a	4.43 \pm 2.56 ^a
TLG (cm)	2.11 \pm 1.22 ^a	1.58 \pm 0.91 ^a	1.61 \pm 0.93 ^a	1.76 \pm 1.02 ^a
K	1045.66 \pm 603.71 ^a	612.39 \pm 353.56 ^a	3196.33 \pm 1845.40 ^a	769.17 \pm 444.08 ^a
SGR (%)	2.85 \pm 1.64 ^a	2.38 \pm 1.38 ^a	3.57 \pm 2.06 ^a	3.03 \pm 1.75 ^a
FER	143.05 \pm 82.59 ^a	67.93 \pm 39.22 ^a	192.76 \pm 111.29 ^a	94.46 \pm 54.54 ^a

IntWt, Initial Weight, IntLgth, Initial Length, FinWt, Final Weight, FinLgth, Final Length, TWG, Total weight gain, TLG, Total length gain, K, Condition factor, SGR, Significant growth ratio, FER, Feed efficiency ratio. The result is presented as Mean \pm STDERR. Parameters show no significant difference. (P<0.05)

The performance of African catfish in all of the treatments is shown from Figure 4.1 to Figure 4.8. The result indicates that there is no significant difference between the formulated diet and the control diet (FM). The initial average length and weight were statistically similar in all treatments except treatment 3 in the case of length (7.92 ± 4.57 cm). At the time of harvest, the highest final average weight was 20% BSFL (9.83 ± 5.68 g) except for the length (9.53 ± 5.54 cm) was found after 30 days of rearing the fish in the tray. On the other hand, TWG, SGR, and FER of African catfish that are fed with 20% BSFL are higher than the two treatments (10% BSFL and 30% BSFL) as well as the control diet (FM). That means 20% of Fishmeal replaced by the BSFL could affect the growth of the fish.

However, FER, TWG, TLG, and SGR that are fed with 10% BSFL have the lowest value compared to the other treatments. The survival of the fish in the culture tray is affected by the different dietary treatments during the entire experimental duration. The feed intake for 20% BSFL has the lowest value compared to FM and it has an average value of TLG among the other treatments. In terms of the condition factor, there are no significant differences among all the groups but the value was less for the group that is fed with 10% BSFL and 30% BSFL compared to the other groups. In contrast, the replacement of BSFLs that exceed 30% and less than 10% of BSFL is relatively poor compared with the control diet (FM) in terms of growth performance. Thus, although there is no significant difference observed, the growth was shown higher by 20% compared to the other treatment. This may indicate that 20% of BSFL might be better for African catfish and if the research is carried out over a long period, it may show a significant difference between the treatments.

CHAPTER 5

DISCUSSION

According to Xiao et al. (2018), several studies indicate that animal protein sources, especially the black soldier fly are usable alternatives to fish meal in the aquaculture industry. This study indicates that 20% fish meal protein replaced by the black soldier fly meal resulted in the best in terms of FinWt (9.83 ± 5.68^a g), TWG (6.71 ± 3.87^a g), SGR (3.57 ± 2.06^a) and FER (192.76 ± 111.29^a) compared to the fish meal (control diet) FinWt (8.83 ± 5.10^a g), TWG (5.41 ± 3.12^a g), SGR (2.85 ± 1.64^a) and FER (143.05 ± 82.59^a) and the replacement of 10% BSFL with the FinWt of (6.20 ± 3.58^a g), TWG (3.06 ± 1.77^a g), SGR (2.38 ± 1.38^a) and FER (67.93 ± 39.22^a). As for the 30% BSFL, the FinWt is (7.57 ± 4.37^a g), TWG (4.43 ± 2.56^a g), SGR (3.03 ± 1.75^a) and FER (94.46 ± 54.54^a). The results are consistent with a previous study that suggested black soldier fly larvae meal could replace fishmeal protein in the diet of Nile Tilapia (*Oreochromis niloticus*) by 20%. It also demonstrated that there was no discernible difference between the control diet and the black soldier fly replacement for fish meal in terms of total feed intake, rate of fish intake, fish conversion ratio, feed efficiency, and total digestibility of the feed. (Tippayadara et al., 2021). In the Japanese eel research, defatted black soldier flies may substitute 30% of the fishmeal without negatively affecting the growth performance, fillet texture, serum biochemical markers, or intestinal histomorphology (Kuo et al., 2022). Furthermore, research suggests that providing young barramundi diets designed to replace fish meal with 100% processed black army fly larval meal may lower their FCR, SGR, and WG (*Lates calcarifer*) (Katya et al., 2017). Furthermore, the inclusion of black soldier larvae meal up to 30 percent indicated that there were no appreciable differences between

the SGR and FCR. For rainbow trout, it is thus advised to use FCR values that are less than 30% of black soldier larvae meal inclusion (*O. mykiss*) (Terova et al., 2019).

The chemical, physical, and biological properties of water that typically preserve it for a certain purpose are referred to as its quality. The fish typically have difficulty detecting the food while it is being observed at feeding time. This is because the feed sinks to the bottom of the pond, making it crucial to utilize the right materials when making the feed. For instance, Sumardiono & Siqihny (2019) state that tapioca flour may be used as a binding agent to increase the hardness of the pellet and the proportion of feed that floats in addition to providing carbs.

Additionally, the water tends to get muddy and filthy quickly around feeding time. Using a beaker filled with water and left for half an hour, the solubility of the feed in the water was analyzed. The pH and ammonia levels of the water were measured using the API test kit, and after 30 minutes, the pH of the water was found to be in the neutral range (7) and the ammonia level had climbed to 0.25 ppm. Fish exposed to ammonia may experience stress and illness, including bacterial illnesses, according to Roy (2018). The fish in the tank will exhibit signs such as surface breathing, appetite loss, and dull, sluggish behavior. These symptoms indicate that the amount of ammonia in the water is rising. The fish will suffer tissue damage and develop wounds on its body and fins as a result of the rising ammonia levels. After feeding the fish, the feed waste will either be drained out of the tank or the water percentage will be increased from 25% to 50% to stop the ammonia buildup. It is crucial to check the ammonia level in the fish tank since even at a concentration of 1 ppm, the fish will get agitated and may even die.

Moreover, cannibalism among the juveniles occurred during the research, resulting in some damage to the fish as well as their eventual mortality. Solomon et al. (2011) define cannibalism as the behavior of a species wherein its members eat the body parts of other members of the same species. Cannibalism occurs naturally in a range of animals and is not limited to situations involving artificial or scarce food sources. The presence of food in the catfish culture is one of the elements that lead to cannibalism. Solomon et al. (2011) state that studies on environmental factors have demonstrated that territorial invasion is a source of various behavioral patterns and that it may be managed by varying the availability of feed. The next factor that might lead to cannibalism in fish is size variation. Solomon et al. (2011) state that the main factors that contribute to cannibalism in terms of size variation include eating habits, high population stocking densities, and distribution sizes that can kill other fish. Therefore, during the investigation, it is crucial to ensure that the fish have access to an adequate diet.

Lastly, there are a few catfish fries that have injuries on their body that might kill the young fish. The fish have lost their appetite and grown nervous due to the sores on their bodies. Injuries to the body of the fish also lead to the development of fungi, which infect other fish. The fish may potentially perish as a result of this. Isnar Sumatarno et al. (2017) state that if the fish has bodily damage, it will be susceptible to fungal infection. Fish with fungal infections have been seen to exhibit several traits, one of which is the growth of fine hairs surrounding the lesion—the *saprolegniasis*-growing fungus. The fungus *Saprolegnia* sp. is the cause of *saprolegniasis*, also known as *saprolegniosis*. It is a prevalent illness in freshwater fish and certain estuary species in warm, tropical climates, making it one of the most significant fish diseases. Stress at high temperatures and

immunological suppression are also frequently linked to these illnesses. Thus, it's critical to find methods to maintain the fish's health, such as an adequate diet, preventing overpopulation, and maintaining high-quality water. Choudhury, Gon, and others (2014).



CHAPTER 6

CONCLUSION

It could be concluded that, in terms of African catfish juvenile development performance, black soldier fly larvae (BSFL) have the potential to be an effective alternative meal. When African catfish are given fishmeal as the control group and substitute 10%, 20%, and 30% of the protein in fish meal with protein from black soldier flies, the growth performance of the fish is comparable. When compared to the tested diets for 30 days, the food substitute including 20% black soldier fly yields the greatest values for TWG, SGR, FER, and K. Based on the results obtained, black soldier fly larval meal may substitute 20% of fish meal in African catfish diets with or without impairing fish health, growth performance, or feed efficiency.

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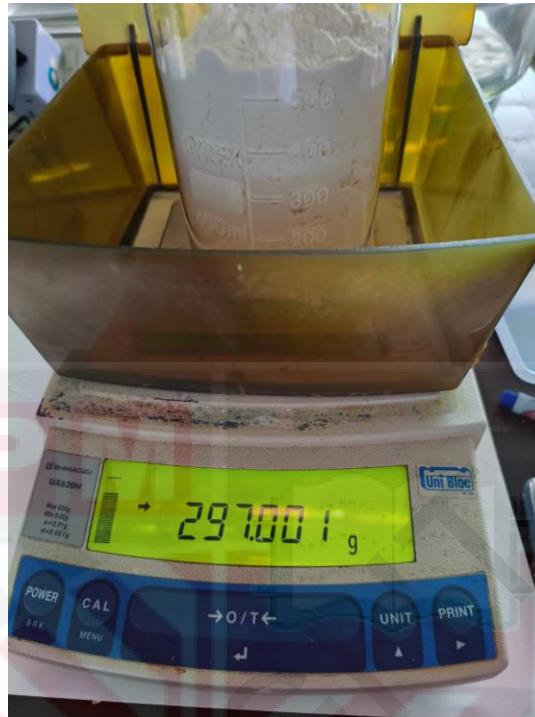
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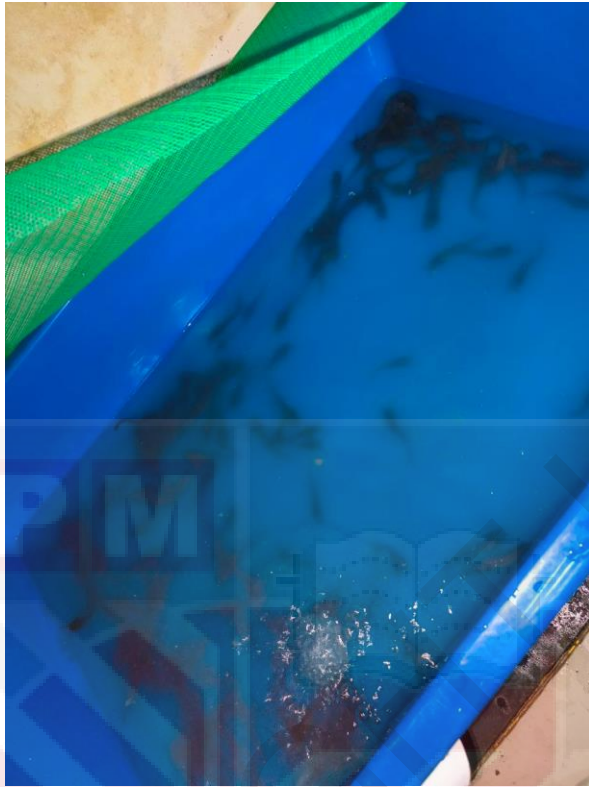
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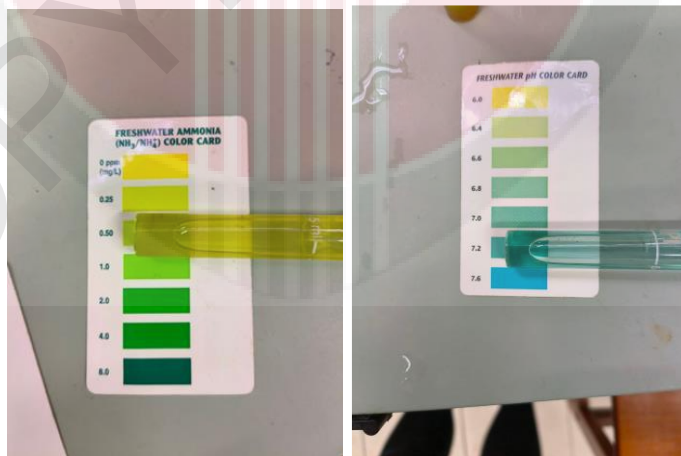
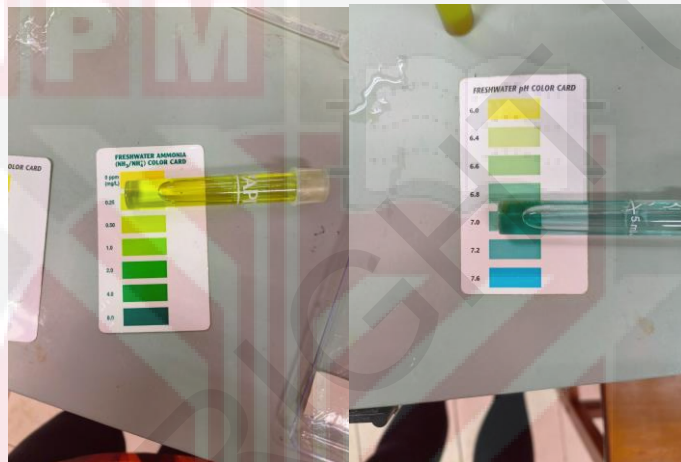
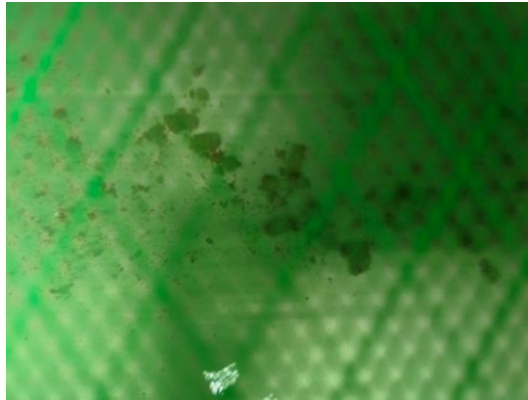
APPENDICES





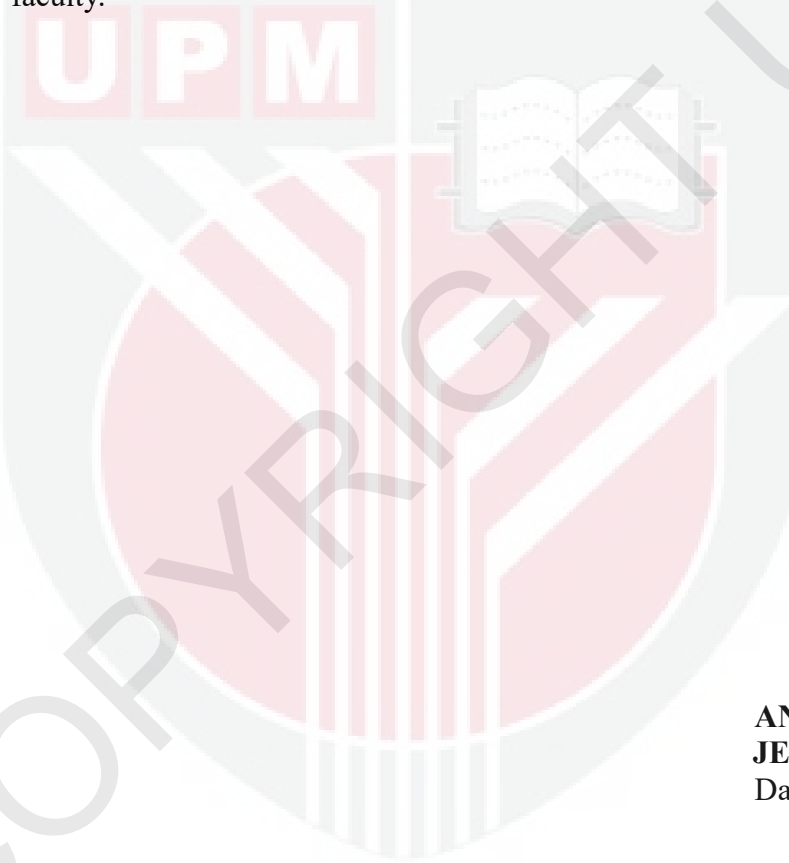






PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled “**Potential of Black Soldier Fly Larvae as Alternative Protein Source in Fish Diet**” by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agricultural Science and Forestry, University Putra Malaysia Bintulu Sarawak Campus, and published in the form approved by the faculty.



**ANNATASSHARINA
JEFFRY**
Date: **06/01/2024**

