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Effects of Differences in Physical Properties of The Earthworm and The Black Soldier Fly Larvae (BSFL) in Fish Feed Towards the Growth Rate and Performance of Betta Fish (*Betta Splendens*) with Commercial Feed as Control

Muhammad Syukri Amir Bin Zainuddin

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DECLARATION

I declare that this thesis entitled "Effects of Differences in Physical Properties of The Earthworm and The Black Soldier Fly Larvae (BSFL) in Fish Feed Towards the Growth Rate and Performance of Betta Fish (*Betta Splendens*) with Commercial Feed as Control" is the results of my own research except as cited in the references.

Signature : ____________________________

Student's Name : Muhammad Syukri Amir Bin Zainuddin

Date : 3 March 2022

Verified by:

ZULHISYAM ABDUL KARI@ABDULLAH
Lecturer
Faculty of Agro Based Industry,
Universiti Malaysia Kelantan,
Jeli Campus

Signature : ____________________________

Supervisor's Name : Dr. Zulhisyam Bin Abdul Kari@Abdullah

Stamp : ____________________________

Date : 3 March 2022

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ABSTRACT

Aquaculture industry had been affected with the skyrocketing prices of fish meal that eventually leads to high prices of commercial fish feed. Formulating and manufacturing good fish feeds requires high quality fish meal for efficient and fast growth. The increasing price of fish meal and commercial feeds has led to farmers experiencing difficulties in maintaining their farm and survival in the industry. Therefore, black soldier fly larvae and earthworm meal has been suggested as an alternative feed source in replacing the fish meal. BSFL and earthworm meal were chosen because of their good source of protein which is the most valuable component of fish feed. Both BSFL and earthworm meal were manually formulated according to the diet formulation which are the combination of wheat bran, rice bran, soybean meal, mustard oil cake, molasses and vitamin-mineral premix with targeted meal. These feeds will then be fed to the betta fish, *Betta splendens.* The growth rate and tendency of eating the feed were be measured by observing the activity of the fish during feeding, and the final weight of the fish once every week. The results were varied as different feeds were given to the fish, for instance, fish that were fed with BSFL and earthworm meal may have a greater size and active movement than the fish that were given commercial feed. This is due to the higher source of protein that helps the fish grows faster and healthier. The differences in physical properties of both experimental feeds, BSFL-based and earthworm-based pellet do give an effect to the growth performance of the betta fish, but not significantly high. This happened due to the handicapped pellet that was made manually. However, if the pellets were made by automated machine, the pellets might be produced perfectly and in large amount. Nonetheless, BSFL and earthworm-based pellets do help in improving the growth of the betta fish thus replacing the fishmeal in the aquaculture industry.

Keywords: Black soldier fly larvae, earthworm meal, fish meal.

Kesan Perbezaan Ciri Fizikal Cacing Dengan Lalat Terbang Askar Hitam Di Dalam Makanan Ikan Terhadap Kadar Tumbesaran Dan Prestasi Ikan Betta (*Betta Splendens*) Dengan Makanan Komersil Sebagai Kawalan

ABSTRAK

Industri akuakultur telah mendapat kesan negatif dengan lonjakan harga makanan ikan lantas membuatkan harga komersil ikan juga menaik. Pembuatan dan formulasi makanan ikan yang berkualiti tinggi memerlukan makanan ikan berkualiti untuk tumbesaran cepat dan efisien. Oleh hal yang demikian, kenaikan harga makanan ikan dan makanan komersil telah menimbulkan rasa khuatir dalam kalangan pengusaha akuakultur untuk terus menjaga dan menjalankan perusahaan mereka. Justeru, Lalat terbang askar hitam dan cacing telah dipersetujui bahawa kedua-dua sumber ini mampu menjadi makanan alternatif untuk menggantikan makanan ikan komersil. Hal ini demikian kerana, lalat terbang askar hitam dan cacing mempunyai kadar protein yang tinggi yang merupakan salah satu komponen yang amat penting dalam makanan ikan. Kedua-dua makanan ini telah diformulasi secara manual mengikut formulasi diet makanan ikan dengan kombinasi dedak gandum, dedak nasi, kacang soya, molasses, dan vitamin mineral pra-campuran. Makanan seterusnya akan diberikan kepada ikan betta, (*Betta Splendens*). Kadar tumbesaran dan kemahuan ikan untuk makan akan diukur dengan memerhatikan aktiviti ikan semasa makan dan berat akhir ikan tersebut. Keputusan akan berbeza kerana ikan-ikan tersebut akan diberi pelbagai makanan contohnya, ikan yang diberi makan lalat terbang askar hitam dan cacing akan mempunyai berat yang lebih tinggi danlebih aktif berbanding yang diberi makanan ikan komersil. Ini disebabkan oleh sumber protein yang lebih tinggi yang membantu ikan membesar dengan lebih cepat dan sihat. Perbezaan sifat fizikal kedua-dua makanan eksperimen, pelet berasaskan BSFL dan berasaskan cacing tanah memberi kesan kepada prestasi pertumbuhan ikan betta, tetapi tidak ketara . Hal ini berlaku kerana pelet cacat yang dibuat secara manual. Walau bagaimanapun, jika pelet dibuat oleh mesin automatik, pelet mungkin dihasilkan dengan sempurna dan dalam jumlah yang besar.

Kata kunci: Lalat terbang askar hitam, tepung cacing tanah, tepung ikan.

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LIST OF ABBREVIATIONS AND SYMBOLS

BSFL	**Black Soldier Fly Larvae**
BSF	**Black Soldier Fly**
EW	**Earthworm**
PDI	**Pellet Durability Index**
ER	**Expansion Ratio**
BD	**Bulk Density**
%	**Percentage**
FCR	**Feed conversion ratio**
SGR	**Specific growth rate**
BW	**Body weight**
±	**Plus minus mean and standard deviation**

CHAPTER 1

INTRODUCTION

1.1 Research Background

Good types of feeding choices and management are important aspects in aquaculture sector. Fish feeds for example, have many types of physical and chemical properties followed by nutritional value that can be chose according to the nutritional requirements of the fish species such as ornamental fishes. Moreover, good nutrition is the most important aspect in any animal farming sector including aquaculture. Approximately 50 percent of the production cost is covered by feed expenses. New and well-balanced diet in fish nutrition has been developed drastically in the past few years that effectively helps the fish farmer to provide proper diet for their aquaculture farming. For instance, black soldier fly larvae (BSFL) had been produced as fish feed due to its high content of protein that contributes to healthy fish diets. Other than that, the black soldier fly larvae can be manufactured according to the desired size. The feed can both be given either in live state or in a flour form to the fishes whichever necessary. Furthermore, this feed not only functions as an alternative source of protein, but also could reduce the production cost without affecting the quality for both the feed and fishes. Meanwhile, another alternative feed to be considered is earthworm meal. It is basically processed worms that are reared for vermicomposting which is a way of composting fruit, vegetable and animal wastes combined into organic soil amendments for agriculture. The byproduct of this process is the high-protein content earthworms. Red earthworms, however, promotes the most protein content for fish growth thus serving better performance and lower mortality. Therefore, the research is about comparing the effects of different physical properties of both feed in fish feed with commercial feed as control. Commercial fish feed which is fish meal will be used as a benchmark for both feeds which are BSFL and earthworm meal to determine which are going to exceed the preference of the fish's palatability. Fish meal is known to have high demand on market among fish farmers and mostly made of fish which is not suitable for human consumption. Furthermore, the feeds will be measured in 5 different properties which are expansion ratio, bulk density, pellet durability index, water stability and floatability. The purpose of the research is to determine the composition and properties in BSFL and earthworm meal. Both feeds are good sources of protein, but the preferences of the fish also depend on the physical properties of the feeds so that they can reach the fish's palatability. This research is important for fish farmer to choose their feed wisely so that they can give their fishes the best feed that helps in growth and health.

1.2 Problem Statement

Black soldier fly larvae (BSFL) and earthworm meal are both feeds that has similar prices in market and good sources of protein. In fact**,** aquaculture farmers surely would prefer the best quality feed for their fishes. However, the farmers also should know the preferences of their fishes' palatability regarding the physical properties of the feeds. If the feed only has high quality content but not preferable for the fish due to its physical properties, then the fish would not feed on them and the feed will be wasted. Ornamental fishes which is betta fish (*Betta Splendens*) species will be the constant variable during the research. Meanwhile, the feeds which are Black Soldier Fly Larvae (BSFL) and earthworm meal will be the key factors of the study with fish meal acting as control. The research will prove which feed is going to be the preference for the betta fish according to their growth rate and palatability.

1.3 Research Objectives

1) To investigate which fish feed between Black Soldier Fly Larvae (BSFL) and earthworm meal will give the most advantages towards betta fishes according to their differences in physical properties.

2) To determine how far the BSFL and earthworm-based pellets affect the growth rate of fish instead of commercial feed.
3) Helps the fish farmer to prevent excessive feed waste and manage the feed production cost wisely thus harvesting a good quality fish.

1.4 Hypothesis

H°: The differences of physical properties in both feeds affect the growth performance of betta fish during feeding trial.

H^{1}: The differences of physical properties in both feeds does not affect the growth performance of betta fish during feeding trial.

1.5 Research Questions

1) Will the fish feed on both feeds that was formulated using earthworm powder and Black Soldier Fly Larvae (BSFL) meal?
2) Which feed will meet the betta fish's palatability regarding the physical properties of the feed?
3) Can earthworm meal or Black Soldier Fly Larvae (BSFL) meal compete with fish meal in terms of nutritional content?
4) Are there any obvious differences in betta fish's growth rate after feeding on different formulated feeds?

1.6 Scope Of Study

Betta fish are omnivores when in wild environment, thus they eat variation of diet including insect larvae mainly, water-bound insects and small amount of plant matter. However, they still need a protein-based diet to be healthy survive. Generally, protein content of 35% was shown to have the best outcome of growth and reproduction in betta fish. Other than that, betta fish do not have "true" or acidic stomach, thus making it to hard to digest lots of animal-based protein, or more carnivorous diets, and carbohydrates, fiber for instance. Hence, limited dietary fiber is suitable for betta fish, including other fish species mostly.

1.7 Significance Of Study

This research potentially could help every aquaculture farmer to learn the importance of formulating better quality fish feed to produce a good generation of fish to be distributed to other farmers. Knowing all these feeds' properties that could meet every species of fish's palatability, the farmer is guaranteed can save a lot of production cost in formulating or buying the feed. Furthermore, physical properties of every pellet made could be improved more in the future thus achieving every fish's palatability and producing good quality aquaculture-based producs.

CHAPTER 2

LITERATURE REVIEW

2.1 Ornamental fish

A fish anatomy and physiology are well compared with the better-known mammalian model. However, the anatomical and physiological in survival with mammals are always assumed to share the same solutions, but often, they do not as stated by Roberts et al. (2011). Ornamental fish has wide variety of shapes, sizes and colors that is usually reared as pets and exhibition purposes. Rearing this fish has been a part of millennials' interest as luxury lifestyle at global level that is expected to be a key factor for the industry growth. Besides, studies show that the presence of ornamental fishes in a household aquarium tank can bring psychological benefits to the millennials as it is good in reducing stress after a daily hectic routine, thus providing a feeling of good experience.

2.1.1 Betta fish (*Betta Splendens*)

Betta splendens is well-known as the Siamese fighting fish or said as betta. This type of fish can be found in Malaysia, Thailand and Indonesia. The famous behavioral characteristic of Betta is fighting where male Betta, more commonly than females, instinctively fight with each other to guard their territory. Fighting fish has been bred and produced for competitive fighting for centuries. Furthermore, wild fighting fish rarely keep up their fights for more than 15 minutes, not like the cultivated varieties which are considered weak if they fight for less than an hour.

2.2 Fish meal making

Fish meal is a well-known component in animal feed due to its high source of digestible protein, essential vitamins and minerals, long chain omega-3 fatty acids (EPA and DHA) (IFOMA et al., 2001). Fish meal was used globally for past 50 years and it was produced from species of fish that was not used directly for human consumption or from the production of by-products of seafood processing. However, a lot of research has been done to find alternatives of fish meal that can be more effective than it (Naylor et al., 2000). The others that was not a part of the research in search of an alternative for the fish meal had embrace the issue for a couple of reason in the aquaculture industry and the awareness had been expended over the segment of the scientific community that need to face the issues every day (Naylor et al.,2000). Other than that, fish meal can be obtained through brief steps such as cooking, processing, drying and milling fresh raw fish or fish trimming (IFFOO et al., 2006). In cooking process, the fish at first were cooked and the degraded protein coagulated, some of the water and the oil will be run off or can be removed by pressing.

Meanwhile, Rome et al. (2020) stated that the water could be reduced by 70% or 50% of the water content during the pressing process. Meanwhile, the oil can be reduced to 4%. Moreover, in the drying process, it is important to provide a suitable drying condition to prevent the meal from effected by mold and bacteria. There are two main types of dryer which are direct and indirect. The temperature of the direct dryer is up to 500˚C that will be passed over to material or it will tumble in the cylindrical drum. The temperature of hot air will not be the same as the meal due to the rapid evaporation of the water from every particle of fish caused by cooling. Generally, the temperature of the product will remain about 100˚C and the unpleasant odor of the fish meal was from the dryer.

In terms of protein content, fishmeal is one of the best animal protein supplements in the diets of farm animals. On dry matter basis, the general inclusion rate of fishmeal in terrestrial livestock diets is usually 5% or less. Meanwhile, for lipids, it can be found in the fish and can be made into both types that are liquid fish oils and solid fats. Most of the obtained oil was generally extracted during the processing of the fishmeal, however, the leftover lipid usually represents between 6% and 10% by weight, and also can range from 4% to 20% as defined by Cho et al. (2011).

2.2.1 Nutrient composition of fish meal

The contribution of fish meals in fish feed diets can help to improve the feed efficiency and growth for better feed palatability and at the same time enhance nutrient consumption, absorption, and digestion. Other than that, Young et al. (1994) stated that the fish meal consists of a well-balanced amino acid composition that can provide a synergistic impact with the other animal and the vegetable protein that present in the diet that can promote powerful growth. Furthermore, with the addition of fish meal into the aquatic animal's diet, it also can help to reduce the pollution due to the wastewater effluent by offering a better nutrient digestibility. On the other hand, high-quality fish meal usually consists more than 66% of crude protein, about 8 to 11% of fat, and usually less than 12% of ash. Kaushik et al. (2010) proved that other fishery by-products generally will consist of fish protein concentrate with a high level of protein which is more than 70%. The fish meal not only has high protein content but also in other nutrients like essential fatty acid, cholesterol, phospholipids minerals, and certain vitamins (Tacon et al., 2009).

2.3 Black soldier fly larvae (BSFL) life cycle

Black soldier fly larvae or known as *Hermetia illucens Linnaeus* 1758 was used as fish feed in aquaculture due to the excessive of adulterated and low quality fish feed that causes environmental hazards and profitability reduction in fish farming. Wild BSF usually lay their eggs in such places like rotten kitchen wastes, mustard oil cake and wheat which then lead to emergence of larvae. BSF culture as fish feed is one of the techniques that bring additional advantages towards organic wastes utilization (Diener et al., 2009) plus providing guaranteed solution for safe manure management. For the BSF production, rotten wheat is more attractable than rotten vegetables and mustard oilcake for them to lay eggs which makes the production of BSF in rotten wheat is 4 times higher than rotten vegetables and mustard oilcake (Rana, Salam et al., 2015). This result may be the cause of differences in putrescent odour and nutritional quality of the waste material.

In the study of 65 days feeding trials in yellow catfish, BSFL meal protein has shown great potential in replacing fish meal partially protein in the diet of the yellow catfish. The highest growth performances in the feeding trial was shown when 25% of the fishmeal protein was replaced by BSFL meal protein (Xiao, Jin et al. 2018) Meanwhile, in another study, pellet with inclusion of BSFL shows that the expansion ratio, sinking velocity and water stability of the pellets were decreasing when the inclusion of BSFL meal increased (Lei, Kim et al., 2019).

2.4 Physical properties of feed.

The physical properties of the feed should be high and durable to uphold handling, transportation and pneumatic conveying, without leaving large amount of dust and particles. However, in general, there were no standards established in terms of hardness or durability. Furthermore, there is also lack of standardization of equipment and measurements in recognizing the physical properties of extruded fish feed (Khater, Bahnasawy et al., 2014).

On the other hand, a study was conducted where fermented soy pulp (FSP) is used as coating material in fish feed pellet to test the loading potential of probiotics on fish feed pellet and growth state of African catfish (*Clarias gariepinus).* The FSP coated diets were altogether observed for amino acids and bacterial quantification by a machine called High-Performance Liquid chromatography (HPLC) and bacterial plate culture techniques. The physical properties of the diets are obtained through measurement of the feed diameter, expansion rate, bulk density, pellet durability index, floatability and water stability. At first, the fermented soy pulp was obtained and was put through fermentation process by combining *Lactobacillus* spp with molasses. Then, the FSP was left dried under bright sunlight until less than 10% moisture and the powder of FSP was used in the coating of the pellet (Kari, Kabir et al., 2021).

2.5 Earthworm meal

Earthworm meal is proved to be a good supplement as addition in the fish diet as it gives the best result for the fishes' development in mean weight gain, growth rate and protein conversion ratio (ABD RAHMAN, Razak et al., 2012). Cheaper formulation production can also be achieved if the earthworm meal is used than focusing only on the production of fish meal.

In this case, earthworm meal and BSFL has the same potential of giving a good result for fishes' performance and growth rate, however, these feeds should have a good palatability requirement for the fishes to feed on. This will determine which feed will be the primary choice in aquaculture farming. In recent studies, fish meal was partially replaced with earthworm meal in African catfish feed. The diets has shown significant effect on growth performance and feed utilization where the feed with 25% of earthworm meal content has the highest in final weight, weight gain, daily weight gain, and specific growth rate meanwhile the diets exceeding 25% for earthworm has shown decrement in growth (Dedeke, Owa et al., 2013).

2.6 Physical characteristics of commercial feed

Prices of commercial feed varies and significantly designed for only one species according to its nutrient requirements. Hence, aquaculture farmers should pay attention to the feeds as it will affect the growth and survival of the target species. One finding was that fish do have definite preferences among feeds and attack the preferred feeds with greater gusto (Tamaru et al., 2009). This research also proved that more palatable feed should yield greater growth with equal nutrition. Commercial feed was obliged to be tested extensively to guarantee maximum performance and feed conversion ratio. According to (Khater, Bahnasawy et al., 2014), in order to improve digestibility, extruder procedures has been done where it can improves feed conversion ratio, control the pellet density, greater feed stability in water, improved production efficiency and versatility. Extrusion cooking resulted in various reactions such as thermal treatment, gelatinization, protein denaturation, hydration, alteration of texture and destruction of microorganisms. Furthermore, extrusion is the predominant process in the manufacturing of fish feed compound. Extrusion of microbial load decreases the level of thermolabile antinutrients present in plant material thus improving the digestibility of the dietary compounds (Sørensen et al., 2012). Other than that, quality of physical properties of fish feed has become extremely important due to increment of pneumatic feed delivery systems. However, (Khater, Bahnasawy et al., 2014) claimed that the effect of physical pellet quality on the biological performance of the fish has not been researched in detail.

2.7 Fish nutrition

It is necessary to give good nutrition in animal production systems especially in economical production to harvest good quality product including aquaculture farming. Specific diet formulations are slowly advancing throughout the year in order to satisfy the demands for reasonable, secure and good-quality fish and aquaculture product. For example, protein is the most important but expensive aspect in fish nutrition. Fishes at early stages need higher protein source in order to grow but the requirement decreases as the fish grow larger. Commercial fish diets are formulated as either extruded which is floating or pressure-pelleted feeds which sinks. Both types of feed can provide satisfactory growth. However, some species prefer floating, while others sinking.

2.8 Growth and survival of ornamental fish

Ornamental fishes, hardly live and feed on natural feed and should be reared in high density indoor system. Most importantly, they should be provided with a complete diets which contain all the ingredients needed by the fish for growth and health. In general, complete diet formulated with organic and inorganic components constitute the following proportions, protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash

(<8.5%), phosphorus (<1.5%), water (<10%), and trace amounts of vitamins, and minerals, (Altaff et al., 2015). According to (Budiardi, Nursyams et al., 2005), the larval of fighting fish (*Betta splendens Regan*) requires live foods for their growth and survival. In the research conducted, the larvae were fed on either *Paramecium* with *Artemia*, *Paramecium*, *Artemia* with *Tubifex*, *Paramecium* with *Moina* or *Paramecium*, *Moina* with *Tubifex*. Furthermore, the fish were fed Paramecium from 2nd day until 7th day after hatching. Then, the live food was changed according to the treatments until 28th day. Finally, the result showed that fish fed on Paramecium with Artemia significantly gave the highest total length than other treatments. However, survival rate of fish had no significant differences affected by the treatments.

2.9 Nutritional content of BSFL and earthworm

The amino acid content in fish feed formulation in both BSFL and earthworm flour ratios resulted that the values of the amino acids when 100% BSFL and 100% earthworm treatment rationed was the highest (Hodar, Vasava et al., 2020). Meanwhile, when 50% of BSFL and 50% earthworm combined, the amino acid content was at the lowest. This proves that when two different types of natural food sources combined, the nutrient balance was affected, thus reducing the amino acid profile in the feed. However, the protein content and amino acid content of earthworm are better than the fish meal and soybean milk. In terms of dry matter, earthworm contains 60-70% protein, 2-3% minerals, 6-11% fat, 5-21% carbohydrates, and a range of vitamins including niacin. An earthworm is known to be higher in essential amino acids such as methionine, lysine, and either meat or fish meat (Edwards et al., 1985). The earthworm is also high in the essential long chain of fatty acids.

Furthermore, BSFL are known for the richness in an extend of supplements such as protein, fat, calcium, and phosphorus. For the 100 grams of dried BSFL, it comprises of up to 50% protein, 6% calcium, 35% of fat, 1% Magnesium, 1.2% phosphorus, and 0.3% of sodium (Schmitt, Belghit et al., 2019). The fat that was discovered in BSFL, contains an amino corrosive composition that is comparative to the angle supper. In addition, BSFL is additionally known and utilized as an elective protein source for the pigs, poultry, angle, and shrimp species. On the other hand, the BSFL is encouraged with diverse sources, it can affect the supplement composition of the BSFL hatchlings. Furthermore, when the BSFL is bolstered with fertilizer, the sum of protein will be higher whereby the protein substance within the fly hatchlings can be diverse from 31.2% to 45.7%. BSFL contains around 80 times more than total of calcium compared to other commonly eaten insects. The BSFL is generally rich and wealthy in fat whereby the fat is more qualitative than the other conventional nourish fats. This is often due to the 53% of fat contained within the BSFL that is known as lauric acid which may be a great substance that offers assistance to the pets to absorb the nutrients easier.

3.0 Life cycle and metabolism of Black Soldier Fly

According to (Liu, Chen et al., 2017), the black soldier fly larvae (BSFL), *Hermetia illucens* L., develops on organic wastes, decreasing the ecological pollution and changing waste biomass into protein and fat rich insect biomass. The fly's life cycle will survive approximately 5 to 6 weeks. After female dies, the oviposition process will start, producing 400 to 800 eggs. The first larvae will hatch after 4 days and will be a few millimeters long and voracious. They will continue to develop for another 12 to 14 days if given proper environmental conditions and good organic substrate. However, the fate of nutritional spectra in BSF during its life cycle phases is still weakly understood. A study from (Liu, Chen et al., 2017) about metabolic changes in nutrition composition of BSF from egg to adult claimed that rapid increase in crude fat content happened since the development of 4-14 days of larvae with the maximum level reaching 28.4% dry mass while the crude protein resulted in continuous decreasing trend in the same development phases with minimum level of 38% at larval phase which is 12 days and reach level of 46.2% at early pupa stage. Moreover, a rapid drop in crude fat was observed form early prepupae to late pupae (24.2% and 8.2% respectively). Nonetheless, maximum value of 57.6% in crude protein has been shown at postmortem adult stage with 21.6% fat level. On the other hand, fatty acids, amino acids, minerals and vitamins composition in different development stages of BSF were presented and compared. In conclusion, the results in this research could bring podium to food and feed industry for formulating an ingredient for specific molecular nutritional component intake into the diets of human, aquaculture and animals. Moreover, it is also proven that BSFL is the most valuable insect that can be applied to fight the food scarcity of countries that suffering micronutrient deficiency thus contributing to advance exploring for developmental and metabolic biology of this edible insect (Liu, Chen et al., 2017).

3.1 Life cycle and morphology of earthworm

The earthworm generally is segmented, displaying indeterminate growth after sexual maturity, bilaterally symmetrical invertebrates, and their length can reach up to approximately 30 cm. The earthworms have a digestive tract that consists of a mouth, a crop and gizzard, an intestine, and an anus. Furthermore, the earthworm circulatory system consists of a blood vessel and several pair of hearts which is called 5 Lombricids. Other than that, they do not have any lungs thus the respiratory exchange will occur through the skin. The earthworms are also known as hermaphrodites where the earthworm has both female and male sexual organ.

In addition, both sexual organs have to mature and bred in order for mating to occur. They must move closer to each other and deliver their sperm through the clitellum which is the whitish part of the earthworm. Then, the sperm will be stored in a sac and will begin to form cocoons to house the fertilized eggs. Thus, the eggs will then be transferred to the cocoons, where they will be fertilized with sperm obtained from another earthworm. However, if the earthworm runs out of sperm, the earthworm will continue to produce eggs and sperm. After that, the cocoons would be released to the ground and the cocoons will hatch in approximately 2 to 3 weeks where each cocoon will release one to five young earthworms. The cocoons will be inactive for a long time, waiting for the right conditions. The young worms are only an inch to a half-inch long when they are born. The young worms are translucent and white. Edwards and Lofty et al. (1972) also said that the embryos grow inside the cocoon, and the incubation period can last anywhere from 5 to 20 weeks, depending on the species and the temperature where they live

CHAPTER 3

METHODOLOGY

3.1 Material

3.1.1 Equipment

The equipment used are Tumbling box tester, 15 cm ruler, external calliper, 100 ml beaker, 2mm siever and stopwatch.

3.1.2 Raw Materials

The raw materials used in this study are earthworm and black soldier fly larvae (BSFL) which is the main ingredient and focus of the fish feed that was made.

3.2 Methods

3.2.1 Feed Preparation

For this research, two types of fish feed were formulated which are earthworm based feed and Black soldier fly larvae (BSFL) based feed. Earthworm based feed were made from earthworm obtained from Masjid UMK Jeli that was then bred using an aquarium with the origin soil of the earthworm. The feed then were mixed with other various ingredients determined from the formula, and binded with an amount of molasses. 100% fish meal or known as 0% BSFL and 0% earthworm functions as control feed. BSFL and earthworm feed with inclusion of 25%, 50%, 75% and 100% were experimented in the fish feed.

3.2.2 Proximate analysis

Proximate composition analyses of the experimental diets were performed according to AOAC (1997). The composition of the diets ingredients and proximate composition that are given. All experimental diets were analyzed for amino acids and other compositions using HPLC (High Performance Liquid Chromatography) system (Breeze, Water Corporation, Milford, MA, USA) according to the manufacturer instructions.

Table 3 1 shows the formulation of earthworm-based pellet

Ingredients (g/kg)	Feeds				
	0% earthworm (EW 1)	25% earthworm (EW 2)	50% earthworm (EW 3)	75% earthworm (EW 4)	100% earthworm (EW 5)
Fish meal	26	19.5	13	6.5	0
Earthworm	0	6.5	13	19.5	26
Soybean meal	30	30	30	30	30
Wheat flour	10	10	10	10	10
Vitamin mineral premix	2	2	2	2	2
Molasses (binder)	4	4	4	4	4
Rice bran	28	28	28	28	28
Total	100	100	100	100	100

Table 3.2 shows the formulation of BSFL-based pellets

Ingredients (g/kg)	Feeds				
	0% Black Soldier Fly Larvae (BSFL 1)	25% Black Soldier Fly Larvae (BSFL 2)	50% Black Soldier Fly Larvae (BSFL 3)	75% Black Soldier Fly Larvae (BSFL 4)	100% Black Soldier Fly Larvae (BSFL 5)
Fish meal	26	19.5	13	6.5	0
Earthworm	0	6.5	13	19.5	26
Soybean meal	30	30	30	30	30
Wheat flour	10	10	10	10	10
Vitamin mineral premix	2	2	2	2	2
Molasses (binder)	4	4	4	4	4
Rice bran	28	28	28	28	28
Total	100	100	100	100	100

3.3 Location and study design

The research was carried out at Animal Lab in Universiti Malaysia Kelantan, Jeli Campus, Kelantan for four weeks. The pellet made was first measured to determine the physical properties for each inclusion for both type of feed.

The *Betta splendens* was purchased from a local Betta breeder from Kelantan at the age of 3 months. The earthworm used in the feed was obtained from the vicinity of Masjid UMK Jeli that was then bred using an aquarium together with their own origin soil. The earthworm was bred for 3 months before the research. On the other hand, the Black Soldier Fly Larvae (BSFL) that was used in another feed was obtained from a local supplier meanwhile the fish meal was obtain from UMK Jeli.

3.4 Fish tanks preparation

A total of 81 betta fishes (*betta splendens*) were divided equally into 27 tanks which has the same basic needs prepared in the tanks such as light source and water control equipment (pH meter, thermometer, electric balance, dissolved oxygen meter and oxygen pump). The betta fishes also must be from same species, sizes and ages. The fishes were left for adaptation period in 1-2 weeks to let them feel comfortable and relaxed in the new environment. Before starting the feeding trial, the fishes were not fed to make sure they are ready to consume a new type of feed.

3.5 Preparations and measurement of feeds in terms of physical properties

The fish feeds were processed before the arrival of the fishes to prepare early for the fish to be fed. Before feeding, the physical properties of the feeds were observed and measured. The expansion ratio, bulk density, pellet durability, diameter and floatability are measured to determine the feed's physical properties. Pellet durability index (PDI) is the weight of pellets remaining on the sieve divided by the total weight of pellets before tumbling and multiplied by 100. It can be determined by employing a Tumbling box tester (Seedburo, Chicago IL, USA) for tumbling. Three samples of 100 g pellets are taken and weighed into analytical instrument and for tumbling for over 10 minutes at speed 50 rpm. Then, the samples are sieved to get rid the dust according to standard sieve sizes which is 2 mm. Meanwhile, the expansion ratio is calculated by measuring the diameter of each pellet made by vernier calliper. Then, the expansion ratio in percent, can be determined by using the formula mentioned in the next method. Other than that, the bulk density is measured by calculating feed mass per unit volume of space the feed occupies. Floatability of the feeds can be calculated using 100 ml beakers. The end condition and time taken of both feeds whether they float or sunk are noted in 20 minutes. Both tanks will be given the feeds and the growth rate, performance and palatability of the fishes will also be measured every week.

3.6 Methods for measuring expansion ratio

For expansion ratio, approximately 50 pieces of feed pellets were picked for vertical and horizontal diameter measurement by using an electronical caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan). For each feed, the measurement were done three times and the mean value is determined. The following equation is a calculation for expansion ratio:

$$Expansion\ ratio\ (\%) = (mean\ value\ (target\ feed)\ \ pellet\ diameter - mean\ value\ of\ commercial\ feed\ diameter) \times (mean\ value\ of\ commercial\ feed\ diameter - 1) \times 100$$

3.7 Observations on growth rate and performances of the fish

At the end of the feeding trial, the fishes' growth from both aquarium tanks were be measured and observed. Growth measurement was done by collecting the fishes in the tanks and the dry weight of the fishes can be calculated by drying the fish gently before weighing. Therefore, growth can be determined by subtracting the new dry weight with initial dry weight. Mean body weight of the fish is calculated by dividing the total wet fish weight in the aquarium by the number of fish in the aquarium.

3.8 Statistical analysis

For analyzing the data, the analysis will be carried out using the software SPSS (Version 16.0), and the importance of the results was determined. In this case, one-way ANOVA is used to find the significant effects of different physical properties of feed types and growth rate of the betta fish. Moreover, Duncan's multiple range test was used to analyse the difference in means. The significance of different treatments was determined using one-way analysis of variance on *B. splendens* feeding parameters. All the data was analyzed with triplicate and the significant difference ($P<0.05$).

3.8.1 Data collection and analysis

Data of the growth performance of the betta fish was recorded and the weight was determined using an electronic balance of model. The weight gain (WG), percentage weight gain (PWG), feed conversion ratio (FCR), specific growth rate (SGR) were determined using the specific formula.

i. FCR = Total feed intake (g) / Total wet weight gain (g)

ii. PWG (%) = 100 × (Final mean body weight (g)-initial mean body weight (g))/ (Initial mean body weight (g))

iii. SGR = Final body weight of fish - Initial body weight of fish / No. of days reared

iv. BW (body weight gain) = Final weight of fish - Initial weight of fish

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physical properties of BSFL-based and earthworm-based pellets.

Measurements of physical quality of pelleted feed is extremely important to correlate well with the product quality, found in the trading line. Physical behaviour of animal feed pellets, or in this case, fish pellet, is generally affected by the treatment during manufacturing or mixing. Furthermore, feed pellets have been known as brittle pellets where the feed are described as three-phased materials composed of solid, liquid and gas particles (Salas-Bringas, Plassen et al., 2007). One of the physical properties measured from this research is the expansion ratio which defined as the ratio of diameter of the pellet to diameter of pellet die. The density of the pellets is inversely proportional where higher expansion ratio will cause low density (Tumuluru., 2015). In this case, the highest mean for feed with earthworm meal is 25% which is 10.78% meanwhile for the lowest is 100% earthworm meal with 6.59% based on the Table 4.2.1. On the other hand, BSFL meal with 100% has the highest mean, while the lowest mean is 0% BSFL. Next, pellet durability index (PDI) is a method to test the ability of the pellet to resist attenuation during transport and storage. Although there are numerous pellet durability methods have been used to tell how good pellets will withstand in harsh conditions, but in this research, the pellets were tested with Tumbling box tester. For floatability, all the pellets were tested in a beaker with 100 ml water and the time taken for the pellets to reach the bottom of the beaker was recorded. However, both pellets which are from BSFL based and earthworm based took a really short time to reach the bottom. The mean bulk density in earthworm-based pellet ranged from 0.357 to 1.797 g/ml while for BSFL-based pellet ranged from 0.906 to 1.297 g/ml. Moreover, BSFL-based pellet has the highest mean bulk density among both types of pellets which is 75% BSFL meal inclusion.

Table 4 1 shows the measurement of physical properties of BSFL-based pellets

Parameter	**Diets**				
	0%	**25%**	**50%**	**75%**	**100%**
ER (%)	5.34±0.13	7.51±0.85	5.58±0.63	6.53±0.51	8.17±0.47
PDI (%)	97.39±0.48	99.5±0.09	99.17±0.38	99.84±0.06	99.44±0.60
Floatability (s)	0.98±0.05	0.85±0.10	1.14±0.05	0.87±0.05	0.70±0.02
BD (%)	1.28±0.05	0.49±0.11	0.57±0.03	1.79±0.09	0.36±0.04
Diameter (cm)	0.31±0.04	0.27±0.00	0.30±0.00	0.30±0.00	0.26±0.00

Table 4.2 shows the measurement of physical properties of earthworm-based pellets

Parameter	Diets				
	0%	**25%**	**50%**	**75%**	**100%**
ER (%)	7.33±0.56	10.78±0.22	9.63±0.26	9.55±0.19	6.59±0.06
PDI (%)	98.44±0.32	97.45±0.43	94.68±0.35	99.3±0.47	99.5±0.66
Floatability (s)	1.11±0.05	0.91±0.03	0.8±0.04	0.83±0.04	1.17±0.08
BD (%)	1.26±0.09	0.91±0.02	0.72±0.09	0.91±0.01	1.29±0.03
Diameter (cm)	0.31±0.04	0.22±0.04	0.24±0.02	0.24±0.02	0.29±0.02

From the tables above, shows the parameters of both experimental diets regarding the physical properties of the pellets made. As it can be seen, the expansion ratio in BSFL-based diet mostly has low mean measurement than the expansion ratio for earthworm-based diet. Expansion ratio which is the increase in the pellet cross-sectional area compared to the die cross-sectional area was calculated using the formula mentioned in the methodology (Abubakar, Momoh et al., 2016). Moreover, 100% BSFL diet has the highest mean expansion ratio than the other BSFL inclusions which is 8.17%. However, in recent experiment, the expansion of the pellets when the inclusion of BSFL meal and paste was increased has shown lower measurement

(Weththasinghe, Hansen et al., 2021). In this case, the expansion ratio has shown increment when the inclusion of BSFL increased. This is due to the limitation of using an automatic machine during the making of the pellets where they had to be made manually. There was also no machine available to process the feed that is too small in sizes. Meanwhile, in 25% earthworm-based pellet, its expansion ratio peaked the highest, 10.78% among the other earthworm meal inclusions. The lowest expansion ratio in the earthworm-based pellets is the inclusion of 100% which is 6.59%. According to (Hakim, Wullandari et al., 2000), expansion ratio is an important factor during floating feed production for fish because it affects the unit density, floatability and hardness of the pellets produced. Other than that, the volume expansion on fish pellet is created by gelatinization process of the raw material formulation in an extruder.

In terms of Pellet Durability Index (PDI), the pellets were measured by using a Tumbling Box Tester obtained from the university lab. However, due to the size of the pellets which is small and limited, only a few amounts of the pellets were tested. All of the inclusions in both treatments showed that all of the pellets' durability are in good levels which is less than 90%. Earthworm-based diet with 50% inclusion has the lowest durability index from all the diets tested which is 94.68%. On the other hand, the PDI for BSFL diets with 50,75 and 100% inclusions are not significantly different meanwhile the PDI for 25, 50, 75 and 100% inclusions for earthworm pellet are significantly different from each other. The quality of pellets generally affected by the raw materials depending on their "binding properties" and its proportion in the formulation (Engineering., 2022).

Floatability for both pellets were measured by recording time taken for 1 g of pellet to reach the bottom of a 100 ml beaker. Both experimental pellets had shown a slight significant measurement which is less than 2 seconds. 100% inclusion of earthworm diet recorded the longest time taken to reach the bottom which was 1.17 seconds while BSFL diet with 50% inclusion comes in second with 1.14 seconds. These results had proved that the floatability aspect in both experimental diets are not in good condition. This is because according to Fenn (2015), in order to get food, betta fish will swim quickly towards the pellets located on the surface of the water. In this case, the floatability of both experimental diets were so low that it will distract the eating behaviour of the betta fish thus affecting the weight gain that will be discussed later in subsection 4.3.

Next, bulk density of the pellets were obtained by measuring the rise of water level displaced in 100 ml beaker using the Archimedes principle according to Changmai and Purkait (2021). The mean value in BSFL diets and earthworm diets were significantly different in every inclusion. 75% inclusion of BSFL has the highest bulk density which is 1.79 while 100% inclusion of earthworm diet with 1.29. Furthermore, 25%, 50% and 100% inclusion of BSFL diets are significantly different from each other while in earthworm diets, 25%, 50% and 75% inclusions were not significantly different. The bulk density in both experimental diets also shows that the feed are not perfectly made that resulted in very low bulk density. As stated by Huang et al. (2009), smaller fibre particles tend to have a higher bulk density and may lower the ability of the fibre to absorb water and oil.

4.2 Proximate analysis

Table 4.3 shows the result of proximate analysis in BSFL-based pellet

Ingredients (g/kg)	Feeds				
	0% Black Soldier Fly Larvae (BSFL 1)	25% Black Soldier Fly Larvae (BSFL 2)	50% Black Soldier Fly Larvae (BSFL 3)	75% Black Soldier Fly Larvae (BSFL 4)	100% Black Soldier Fly Larvae (BSFL 5)
Crude fibre	2.1	2	1.9	2	2.07
Crude fat	3.63	3.17	5.03	5	8.67
Crude protein	32.73	32.47	33.27	33.7	33.5
Moisture	4.43	8.27	6.77	6.83	7.77
Ash	6.67	6.4	7.1	7.1	6.97
Total carbohydrate	58.4	51.13	58.77	61.77	61.03
Energy value (kcal/100g)	361.42	327.03	358.44	389.58	416.66

Table 4.4 shows the result of proximate analysis for earthworm-based pellet

Ingredients (g/kg)	Feeds				
	0% earthworm (EW 1)	25% earthworm (EW 2)	50% earthworm (EW 3)	75% earthworm (EW 4)	100% earthworm (EW 5)
Crude fibre	1.59	1.78	2.03	1.82	2.1
Crude fat	2.67	2.87	3.57	3	2.63
Crude protein	35.17	36.47	37.4	37.17	32.7
Moisture	4.62	7.53	7.47	8.1	10.57
Ash	6.63	9.97	7.73	7.5	11.33
Total carbohydrate	60.33	58.1	58.87	61.4	55.57
Energy value (kcal/100g)	367.88	363.59	372.69	380.38	350.96

Table 4 and 5 showed the results of proximate analysis of both experimental diets, BSFL-based pellet and earthworm-based pellet with 0, 25, 50, 75 and 100% inclusions. Earthworm-based pellets mostly has high crude protein content than all BSFL-based pellets where 50% and 75% earthworm inclusions showed the highest percentage which is 37.4% and 37.17%. Meanwhile, the highest crude protein content in BSFL-pellets is 33.7% with 75% inclusion. However, all the inclusions from both experimental diets have higher crude proteins than control diet which is fish meal.

Moisture value for 100% earthworm inclusion is the highest among all of other inclusions which is 10.57% while the lowest moisture content other than 0% inclusion is in 50% BSFL inclusion which is 6.77%. However, 0% inclusion from both pellets give the lowest moisture content than other inclusions. Furthermore, 25% and 50% inclusions of both experimental diets have no significant difference of moisture content while 75% and 100% inclusions from both diets have significant difference. This shows that once every inclusion increased, will increase the moisture content which is perfect for betta fish as it needs only 6-10% of moisture content of the pellet. Moisture in food decides the rate of food absorption and assimilation within the consumer. It also determines the keeping quality of food which means that the determined values indicated that both experimental diets except control diet may not be stored at room temperature for a long period of time (Sodamade, Bolaji et al., 2013).

Ash on food indicates the extent of mineral matters largely that is likely to be found on food substance. Ash content in both experimental diets have no significant difference except for 25% and 100% earthworm inclusion which are 9.97% and 11.3% respectively. Moreover, the trend also has shown that with the increase of inclusion, the ash content will also increase. High content of ash will reduce digestibility thus leaving more fish waste in water. In this case, most of the ash content do not exceed the maximum standard ash content for fish pellet which is 8%.

Crude fat functions as a beacon for amount of energy available in the pellet (Sodamade, Bolaji et al., 2013). For earthworm-based pellet, 50% inclusion has the highest crude fat but does not significantly different among other inclusions. Meanwhile, for BSFL-based pellet, 50%, 75% and 100% inclusions give large significant difference than 0% and 25%. Moreover, betta fish need only a minimum of 7% crude fat for its nutrition. Thus, in this case, only 100% BSFL-based pellet reached the required amount of fat for the betta fish. Meanwhile, crude fibre only needed for maximum of 7% for the betta fish as their nutrient. From the table above, all the inclusions from both diets reached the required amount of crude fibre for the betta fish's nutrition. Both BSFL and earthworm diets do not have significant difference in all of the inclusions. Furthermore, the highest crude fibre in both experimental diets are 0% BSFL and 100% earthworm while the lowest are 50% BSFL and 0% earthworm.

The protein and energy content of a diet are extremely related and there are few reports on the effect of both factors on growth and feed usage of betta fish. When the dietary energy content of a diet is not enough to meet the energy demand of a fish, the amino acids which compound of the protein will be consumed as source of energy thus excreting high amount of nitrogen by deamination of the amino acids in excess. Other than that, a low energy to protein ratio could reduce the fish growth rates due to the increment of metabolic energy demand to excrete nitrogen and reduces the water quality which is the most important aspect of ornamental fish in aquaria. On the other hand, large energy to protein ratio could lead to lipid accumulation deposition on body carcass and reduce the nutrient intake leading to lack of nutrients and reduced disease resistance of fish (Lemos, Arantes et al., 2014). Overall, the energy value and carbohydrate in BSFL-based pellet mostly has high readings instead of earthworm-based pellet.

4.3 Growth performance of the fish

Table 4.5 shows the growth performance of betta fish fed with BSFL-based pellet with control

Parameters	**0%**	**25%**	**50%**	**75%**	**100%**
Weight gain (g)	8.608±5.657	5.463±8.436	9.757±11.578	13.255±4.855	12.607±8.519
Feed conversion rate (%)	1.506±1.10	12.476±21.249	3.756±4.358	0.742±0.211	2.55±4.394
Specific growth rate (%)	0.311±0.226	0.381±0.487	0.279±0.295	0.409±0.104	0.389±0.271

Table 4.6 shows the growth performance of betta fish fed with earthworm-based pellet with control

Parameters	0%	25%	50%	75%	100%
Weight gain (g)	8.608±5.657	7.741±12.893	20.16±18.02	14.73±8.54	10.67±10.45
Feed conversion rate (%)	1.506±1.100	6.947±8.117	0.921±0.806	0.875±0.598	3.511±4.161
Specific growth rate (%)	0.309±0.226	0.489±0.657	0.652±0.600	0.492±0.325	0.264±0.222

From the result above, the feed conversion ratio (FCR) was significantly higher for 25% inclusion of BSFL whereas the specific growth rate (SGR) was not significantly different in both groups. The highest SGR the pellet could reach is in 75% earthworm inclusion. Hence, this shows that the difference in physical properties of the pellets do not give any effect on the SGR as it gave little differences in all the inclusions. However, most of the inclusions from BSFL and earthworm experimental diets do give an effect on boosting the SGR of the betta fish. Similar result has been reported by one of the researcher (Ahmed, Eissa et al., 2020) for earthworm diets, but unlikely result for BSFL diets, Sinansari and Fahmi (2020) where it was claimed that BSFL diets greatly produced significant effect on the growth of the experimental fish.

On the other hand, the feed conversion ratio (FCR) percentage from both groups indicates that only with 25% inclusions, could increase the significant difference, however, increasing the inclusion more than 25% could lower the FCR. Nonetheless, this might be affected by the physical properties of the pellets that caused the fish to not be able to feed on the pellets properly. Other than that, for earthworm-based pellet and BSFL-based pellet, 75% inclusion has lowered the FCR exceeding the control feed but not significantly different. Moreover, when feed has low FCR value, it will take less feed to grow bigger but need higher feed if the FCR is high. Low FCR is a good characteristic of high quality feed. In BSFL pellets, 75% of inclusion produces a good FCR for the betta fish which is 0.742% with weight gain of 13.255 g. Meanwhile, for earthworm pellets, 50% inclusion gives the most suitable FCR which is 0.921% with weight of 20.16 g.

In terms of weight gain, 50% inclusion of earthworm-based pellet has the highest significant than the other inclusions which is 20.16%. Meanwhile in BSFL-based pellet, the highest weight gain recorded was in 75% inclusion which is 13.225%. from this result, it shows that earthworm diets provide more nutrients to the fish due to its high crude protein content. Similar result from other researcher (Hodar, Vasava et al., 2020), claiming that fishmeal partially replaced by earthworm meal and enriched with 5% sardine oil resulted in the better growth of fish.

4.4 Physical properties of experimental pellets towards growth performance

The weight gain for fish fed with BSFL diets has shown significant effect in 75% inclusion whereas for fish fed with earthworm diets, the most significant result is shown in 50% inclusion. Therefore, the expansion ratio with the range of 6-9% and 0.26-0.3 cm in diameter could produce BSFL-based pellets with high weight gain for betta fish. Meanwhile, for earthworm-based pellet, approximately 9% of expansion ratio can function as good weight gain supplement. Although the range of expansion ratio in both pellets to produce a good weight gain contributor are not significantly different, the weight gain in 50% inclusion earthworm gives higher percentage than 75% inclusion of BSFL due to the high protein content.

However, FCR in 25% BSFL diets showed higher significant of FCR than all other inclusions while 25% inclusion of earthworm diet ended up the highest in the earthworm diet group. This might depend on the behaviour of the fish in every treatment where the floatability of all the pellets were low that make it hard for the fish to feed on themselves. In terms of bulk density, earthworm diets showed no significant difference in the measurement but the highest in the group is in 100% inclusion but contributes very low on boosting the FCR of the fish. This means that increasing the bulk density does not give a massive change on the fish's FCR. In addition, BSFL diets also shows a similar result where the highest bulk density is on 75% inclusion but does not give any effect on the FCR. Specific growth rate in in BSFL diets has no significant difference even the changes in physical properties of both pellets are drastically changed. On the other hand, earthworm diets also resulted in no massive changes with every inclusion.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, the differences in physical properties of both experimental feeds, BSFL-based and earthworm-based pellet do give an effect to the growth performance of the betta fish, but not significantly high. This happened due to the handicapped pellet that was made manually. However, if the pellets were made by automated machine, the pellets might be produced perfectly and in large amount. Nonetheless, BSFL and earthworm-based pellets do help in improving the growth of the betta fish thus replacing the fishmeal in the aquaculture industry.

Overall, earthworm-based pellet is proven to give higher nutrition for the betta fish in terms of growth performance than BSFL pellets. Not to mention, the physical properties of the earthworm pellets made are contributes more on reaching the palatability of the betta fish. This will help aquaculture farmers to save cost on purchasing fish feed such as fishmeal that is now sold in higher price. Earthworm is an easy source to find and rear rather than finding or making other high price feed.

5.2 Recommendations

A few recommendations can be made in order to improve the research for future studies. The animal laboratory in Universiti Malaysia Kelantan should have one automated pellet machine to produce small pellets for ornamental fish. The presence of the machine may help the students to produce mass production of fish pellets ranging from small to bigger sizes thus could determine the physical properties properly.

Other than that, in the future, another research about the effect of differences in the physical properties of pellets towards growth rate should be done widely so that this research could be improved more on the making of the pellet. Furthermore, studies about finding another alternative to replace fishmeal other than BSFL and earthworm also should be ongoing to improve the pellet quality for aquaculture farming.

Furthermore, every fish's way of eating also should be observed more in order to determine the exact physical properties of future pellets so that the growth and performance of the betta fish will improve more thus helping the aquaculture farmers to manage their cost of making or purchasing the fish feed conveniently.

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APPENDIX A

Figure A 1 shows the earthworm during searching around Masjid UMK

Figure A 2 shows the earthworm after being collected and put into a container

Figure A 3 shows the betta fishes reared in a plastic tanks with label for each treatment

Figure A 4 shows the earthworm-based pellet made

Figure A 5 shows the BSFL-based pellet made

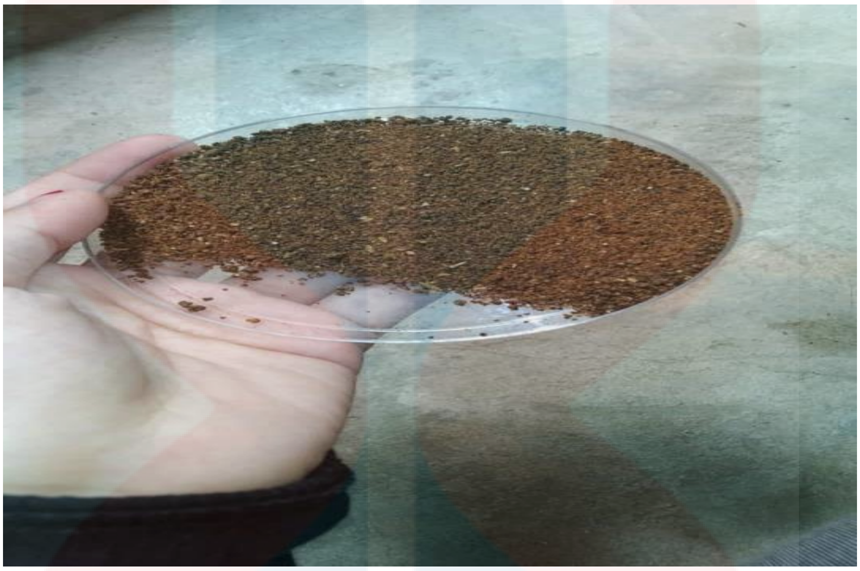

Figure A 6 shows on of betta fish swimming in the plastic tank.

Figure A 7 shows every tanks with BSFL and earthworm treatment with 3 replicates.

APPENDIX B

Table B 1 shows the expansion ratio for BSFL and earthworm 0% diets

TREATMENT	DIAMETER (CM)				AVERAGE	EXPANSION RATIO (%)
	0.2	0.3	0.3	0.267		
BSFL	0.3	0.3	0.4	0.333	0.311	5.34
	0.3	0.4	0.3	0.333		
	0.3	0.2	0.3	0.267		
EW	0.3	0.4	0.3	0.333	0.278	7.32
	0.2	0.2	0.3	0.233		

Table B 2 shows the expansion ratio of 25% BSFL and earthworm diets

TREATMENT	DIAMETER (CM)				AVERAGE	EXPANSION RATIO (%)
	0.3	0.2	0.3	0.267		
BSFL	0.2	0.3	0.3	0.267	0.267	7.98
	0.2	0.3	0.3	0.267		
	0.2	0.2	0.2	0.20		
EW	0.2	0.2	0.2	0.20	0.222	10.58
	0.3	0.2	0.3	0.267		

Table B 3 shows the expansion ratio of 50% BSFL and earthworm diets

TREATMENT	DIAMETER (CM)				AVERAGE	EXPANSION RATIO (%)
BSFL	0.3	0.3	0.3			
	0.3	0.3	0.3	0.300	0.300	6
	0.3	0.3	0.3			
EW	0.3	0.2	0.3	0.267		
	0.2	0.2	0.3	0.233	0.244	9.36
	0.2	0.2	0.3	0.233		

Table B 4 shows the expansion ratio for 75%BSFL and earthworm diets

TREATMENT	DIAMETER (CM)				AVERAGE	EXPANSION RATIO (%)
BSFL	0.3	0.3	0.3			
	0.3	0.3	0.3	0.300	0.300	6
	0.3	0.3	0.3			
EW	0.2	0.2	0.3	0.233		
	0.2	0.2	0.3	0.233	0.244	9.36
	0.2	0.3	0.3	0.267		

Table B 5 shows the expansion ratio for 100% BSFL and earthworm diets

TREATMENT	DIAMETER (CM)				AVERAGE	EXPANSION RATIO (%)
BSFL	0.2	0.3	0.2	0.233	0.256	8.54
	0.3	0.2	0.3	0.267		
	0.3	0.3	0.2	0.267		
EW	0.3	0.3	0.3	0.300	0.289	6.56
	0.3	0.3	0.3	0.300		
	0.3	0.3	0.2	0.267		

Table B 6 shows the PDI of BSFL diets

BSFL

WEIGHT (g)	(%)				
	0	25	50	75	100
BEFORE TUMBLING	0.776	0.706	0.727	1.127	0.827
AFTER TUMBLING	0.759	0.702	0.721	1.235	0.824
PDI	**97.85**	**99.45**	**99.26**	**99.83**	**99.67**

Table B 7 shows the PDI for earthworm diets

WEIGHT (g)	(%)				
	0	25	50	75	100
BEFORE TUMBLING	0.709	0.501	0.519	2.706	0.975
AFTER TUMBLING	0.696	0.489	0.489	2.701	0.975
PDI	**98.07**	**97.74**	**94.35**	**99.81**	**99.96**

Table B 8 shows the floatability of BSFL diets

BSFL (100ML)				
%	TIME (SECONDS)			AVERAGE
0	1.28	0.72	0.8	0.933
25	0.88	0.87	0.89	0.880
50	1.33	0.85	1.23	1.137
75	0.89	0.7	0.88	0.823
100	0.7	0.68	0.75	0.710

Table B 9 show the floatability of earthworm diets

EW (100ML)

%	TIME (SECONDS)			AVERAGE
0	1.35	0.89	1.22	1.153
25	1.05	0.76	0.88	0.897
50	0.76	0.79	0.82	0.790
75	0.97	0.78	0.76	0.837
100	1.84	0.78	0.88	1.167

Table B 10 shows the bulk density of BSFL diets

BSFL (1.5 g)

Diet (%)	Volume of increasing water (ml)			Average	Bulk density
0	1	1.5	1	1.167	1.285
25	5	4	2	3.667	0.409
50	3	4	1	2.667	0.562
75	1	1	0.5	0.833	1.801
100	5	6	3	4.667	0.321

Table B 11 shows the bulk density of earthworm diets

	EW (1.5g)				
Diet (%)	Volume of increasing water (ml)			Average	Bulk density
0	1	1.5	1	1.167	1.285
25	5	3	4	4.000	0.375
50	1	3	2	2.000	0.750
75	1	3	1	1.667	0.899
100	0	1	0.5	0.500	1.285