The Effects of Fish Meal Substitution with Black Soldier Larvae (Bsfl) And Earthworm on Growth and Physiological Properties of Experimental Diets in Betta Fish (*Betta Splendens*) Production

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DECLARATION

I hereby declare that the work embodied in here is the result of my own research except for the excerpt as cited in the references.

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The Effects of Fish Meal Substitution with Black Soldier Larvae (Bsfl) And Earthworm on Growth and Physiological Properties of Experimental Diets in Betta Fish (Betta Splendens) Production

ABSTRACT

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This study aims to identify the effects of fish meal substitution with Black Soldier Larvae (BSFL) and earthworm on growth and physiological properties of experimental diets in betta fish (*Betta Splendens*) production. The feed cost can rise by more than 70% of production because the soybean and fish meal are costly. The replacement of Black Soldier Fly Larvae (BSFL) and earthworm in feed ingredient can cut off the feed cost. Both BSFL and earthworm contain the nutrient composition that meets the requirement of fish feed. The excellent quality of feeds will determine the performance and survival rate of the fish. However, many factors can affect the development of the *Betta Splendens*, such as the substrate of nutrition, water quality, pathogen and stress. (R, N, & W., 2016). Two types of the feeding trial, BSFL and earthworm was used to determine the better survival rate and organoleptic of the water of Betta Fish (*Betta Splendens*).

Keywords: BSFL, earthworm, feed.

Kesan penggantian makanan ikan dengan larva askar hitam (bsfl) dan cacing tanah terhadap pertumbuhan dan sifat fisiologi diet eksperimen dalam pengeluaran ikan betta (Betta Splendens).

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti Kesan Pelbagai Makanan Ikan dan Persekitaran Terhadap Kadar Kemandirian dan Organoleptik Air Ikan Betta (*Betta Splendens*). Kos makanan boleh meningkat lebih daripada 70% daripada pengeluaran kerana kacang soya dan tepung ikan adalah mahal. Penggantian Black Soldier Fly Larva (BSFL) dan cacing tanah dalam bahan makanan boleh mengurangkan kos makanan. Kedua-dua BSFL dan cacing tanah mengandungi komposisi nutrien yang memenuhi keperluan makanan ikan. Kualiti makanan yang sangat baik akan menentukan perkembangan prestasi ikan. Walau bagaimanapun, banyak faktor boleh mempengaruhi perkembangan Betta Splendens, seperti substrat pemakanan, kualiti air, patogen dan tekanan. (R, N, & W., 2016). Dua jenis ujian pemakanan, BSFL dan cacing tanah digunakan untuk menentukan kadar kemandirian dan organoleptik air Ikan Betta (*Betta Splendens*) yang lebih baik.

Kata kunci: Larva Terbang Askar Hitam, cacing, makanan

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CHAPTER 1

INTRODUCTION

1.1 Research background

Betta fish (*Betta Splendens*) is a group of ornamental fish. The ornamental fish demand increases every year. It is because they are more attractive, colourful, and beautiful. However, many factors can affect the production of ornamental fish, especially betta fish. The feed cost can rise by more than 70% because the soybean and fish meal are very expensive. To cut off the cost of the production, we should replace the ingredients of the feed by doing innovative.

The quality and quantity of the feeds can be the significant factor affecting reproduction development. It is because we need to provide suitable feed for the fish to sustain and free from diseases. The excellent quality of feeds will determine the performance and reproduction development of the fish. Fish meal is the common ingredient in fish feed that contains a high level of protein, mineral and energy. According to Zinn (2009), the fish meal provides large quantities of energy and acts as a high-quality protein source and rich in digestible essential amino and fatty acids. Plus, the excellent quality of the feed contains 60% to 72% of crude protein (CP) by weight. (J. H. Cho I. H. Kim, 2010)

Meanwhile, The Black Soldier Fly Larvae (BSFL) *Hermetia illucens* and earthworm are alternative protein sources for animal feed. There is a type of premium protein that will be extracted from The Black Soldier Fly (BSFL), which is Hi. Protein. It contains a high crude protein content (50%) with a well-balanced amino acid profile and 35% of lipids. The role of amino acid is to improve the quality of the fish since they can break down the other protein in order to produce an energy. So, The Black Soldier Fly (BSFL) can replaced the fish meal because the nutrition contain is quite similar. There was research stated that earthworm is turn out to be a good source of protein. It is also an essential high protein component that used as a dietary supplement for fish species. This tropical earthworm species' high reproductive rate and biomass production make it ideally suited to worm meal production. The nutritional value of this earthworm is a significant prerequisite in serving as fishmeal substitution in feed production.

1.2 Problem statement

The global marketable size of ornamental fish is rising every year due to the high demand. There is a larger number of people like to buy ornamental fish as their pet or hobbies. The trending of luxury lifestyle is leading the growth of ornamental fish in market. The consumer like to buy a beautiful, unique and colourful species of the ornamental fish. The introduction of the advance strategy of breeding method can be one of the factors of the high demand of ornamental fish globally.

However, the ornamental fish will die easily if not cared properly because they are sensitive and fragile. The are many factors that can affecting the survival rate of the ornamental fish. The feed consumption or the feed content also can be the factor of the reproduction failure. It is because feed is the most important thing that need to focus on in order to produce the high quality of ornamental fish. the low quality of feed can lead to disease.

1.3 Hypothesis

Ho: There is no effect of different fish feed on organoleptic of the water of ornamental fish (betta fish).

HA: There is an effect of different fish feed on organoleptic of the water of ornamental fish (betta fish).

Ho: There is significance of different fish feed affecting growth development of fish.

HA: There is no significance of different fish feed affecting growth development of fish.

1.4 Scope of study

This study is highlight on making feed for ornamental fish by using The Black Soldier Fly (BSFL) and earthworm to replace fish meal as the main ingredient. The methods will be used to identify the effect of different fish feed on survival rate of fish and the organoleptic of water. This experiment will be conducted at Animal Laboratory University Malaysia Kelantan.

1.5 Significance of study

This study will be able to provide information on suitable feed ingredient to replace fish meal in order to reduce the feed cost. It will also help the farmer select good nutrients that meet the requirement diet of the fish to produce a better growth performance. BSFL and earthworm are the best protein replacement that can improve the growth performance of ornamental fish.

1.6 Objective of the proposal

- To determine the effect of different fish feed towards survival rate of fish and its organoleptic of feed and water.
- To identify the factor that affecting growth development.

1.7 Limitation of study

There were several limitations during the study, there were lack of the information and previous resources about the replacement of fish meal by earthworm in animal feed especially for the Betta fish. Besides that, there was a ton of study on BSFL as replacement of fish meal for animal feed but mostly they were using BSFL more focusing on poultry, so lacking resources and samples on other types of feed for using earthworm and BSFL for fish feed.

There were also lack of information regarding betta fish management and performance data especially researches that being conducted in Malaysia besides lack of effectiveness to conduct study in Laboratory UMK due to lack of equipment.

CHAPTER 2

LITERATURE REVIEW

2.1 Ornamental fish

Ornamental fish is an aquarium fish that is trending as hobbies, aesthetic display, pet companionship and educational purposes due to their beautiful and colourful morphology. In the trade industry, ornamental fish is the essential profit of multimillion-dollar (Jun Yu Chen1, 2019). These ornamental fish one of the crucial sectors worldwide, especially in the marine aquarium trade. There are more than 2500 species involved in the marine ornamental species trade, consisting of various species of fish and invertebrate species (Palmtag,2017). Srikrishnan et al. reported that freshwater ornamental fish, including betta fish, guppy and molly fish, are more attractive because they are more colourful and striking. So, ornamental fish became popular among consumer especially aqua culturists, and business owners.

For centuries, Betta Splendens also known as a Siamese fighting fish. Betta fish very familiar with aggressive behaviour, including tail biting, chasing and biting other fish, frontal and lateral displays and other aggressive behaviour (Chadwick V Tillberg,2018).

It was observed by J.L. Snekser et al. that the male betta fish, already known as the aggressive fish and had been used for fighting purpose. Both male and female are aggressive toward each other.

2.1.1 Nutritional Requirements of ornamental fish

One of the most significant and vital aspects of effective aquarium and pond maintenance is ornamental fish diet. Most of the energy in ornamental fish comes from dietary fat. To avoid hepatic lipidosis, fat should not account for more than 15% of daily calories. Protein breaks down into poisonous ammonia and nitrite, while fat breaks down into water and carbon dioxide (nontoxic in the aquarium or pond) (Lewbart, 1998). Fats also important for their growth and survival rate because fats are essential in the flavour and texture of fish feed (Sales, 2003).

Furthermore, ornamental fish required more protein from dry matter that obtain from feed fish. In confinement, ornamental fish must maximize the utilization of their feed protein, as the breakdown products of protein metabolism (mostly ammonia) would directly damage their living habitat. (Sales, 2003).

2.2 Fish meal

The aquaculture industry has used fish meal as a commercial diet. It is about 46% of the fish meal production per year. The use of fish meal as commercial feed has increased the feeding cost and operating expenses. It is because the high quality of fish meal more expensive than others feedstuff. According to Chapman (2006), fish meal meets the dietary requirement for energy, essential amino acids, fatty acids, and other nutrients of aquaculture species. The replacement of fish meal with another feed formulation that meets the nutritional and energy requirement of aquaculture species can reduce the feed costs. The objectives of feed formulation are to improve the growth and reproduction and increase physical performance by increasing the dietary nutrient density and digestibility.

The process of produce fish meal including cooking, drying and grinding. We need to prepare a mixture of oil, water, and soluble protein to remove liquor during the cooking process. According to Chapman (2006), it is an essential step to remove the oil before storage. An antioxidant was added to stabilize the oil and avoid it exposed to air, heat or light (Chapman, 2006).

2.2.1 Nutrient composition fish meal

In the 19th century, the fish meal was used as feedstuff in Northern Europe before being introduced worldwide. K. E. Zinn et al. stated that fish meal contains a high concentration of crude protein compared to other protein sources from the animal by-product. As we have known, fish meal is the main ingredient in animal feed that provides the high protein value. We can determine a good quality of fish meal by observing the level of crude protein (66% and above), fat (8%) and, ash (below 12%) (Kaushik,2010).

Fish meal is the animal protein supplement in the diet. Usually, the diet for fish contains about 32% up to 45% total protein by weight. Protein is made of amino acids. A recent study stated that animal needs more amino acids for better digestibility. The benefit of fish meal is providing amino acids to animals through protein. The amino acids in the fish meal as a protein supplement make the feed more attractive. Fish meal is the essential ingredient in fish feed because it is palatable and reducing nutrient leaching.

Fish meal commonly made from oily fish. Seafood processing industries processed it. Based on several kinds of research (Gaines et al., 2005) and (Maldjian et al., 2005)., sound reproduction and growth development came from essential amino acids, phospholipids and fatty acids in fish meal. There are a few methods to process fish meal using the basic principle of separating solid from oil and water. Besides, fish meal contains omega-3 fatty acids and multivitamins, including vitamin B12, vitamin A, D and E fish that provides energy and minerals for animals (J. H. Cho I. H. Kim, 2010). The function of vitamin A is to help in bone growth, improve reproduction and importance for maintenance epithelial cells. Vitamin

D provides calcium that is good for stimulating the synthesis of calcium-binding protein that absorbs the diet. Meanwhile, the role of vitamin E in protecting the cells and tissues from damage (McDowell, L. 2000).

2.3 Black Soldier Fly Larvae (BSFL)

Black Soldier Fly Larvae, Hermetia illucens (BSFL) is an insect species known as another source of protein for animal feed (Stame, 2015). BSFL is the replacement of expensive protein sources in animal feed. (Liu X, 2017). According to Newton et al. (2005), The black soldier fly is often associated with the outdoors and animals and prefers to congregate near rotting organic matter such as animal waste or plant matter. Due to their ability to eat rotting matter, black soldier fly larvae minimize animal manure in commercial swine and poultry operations. BSFL can convert the organic waste into compost, and the larval biomass produced could be harvested for its protein and fatty acid content as well (Ewusie,2019).

2.3.1 Nutrient composition of BSFL

The Black Soldier Fly Larvae Hermetia illucens L. (BSFL) have an amino acid profile like fishmeal and contain up to 50% crude protein (CP), up to 35% lipids, and up to 35% lipids in their dry weight. They are known and used as alternative protein sources for chickens, goats, and many types of fish and shrimp feed (Marwa et al., 2019). The insect is rich in energy, essential amino acids, fatty acids, and micronutrients such as copper, iron, and zinc (Spranghers, 2017). A recent study has found that BSFL is good in supporting the performance growth of the animals.

2.3.2 The usage of bsfl in fish feed industry

The recent study stated that bsfl are good for replacement of fish meal in aquafeed because bsfl was rich in protein. Many insect species have higher protein levels than fish meal or soy meal (Sánchez-Muros et al., 2016). Plus, the use of black soldier flies as a substitute had no effect on the protein digestibility of fish. In carp species, aquafeeds containing BSFL pulp have been demonstrated to have improved nutritional content, and the nutritional content of BSFL pulp has recently been compared to full-fat BSFL meal for Atlantic salmon feed (English et al., 2021). According to Abdel-Tawwab et al., replacing 50% of dietary FM with BSFL meal (148 g/kg inclusion) resulted in a 15.6 percent savings in feed cost due to the cheaper BSFL meal while retaining a similar FCR.

2.4 Earthworm

The earthworm is commonly used as fish bait and feed for chicken. Researchers have shown that red earthworm meal can substitute natural feeds at a 50% replacement level of fishmeal in semi-intensive Indian carp farming (Musyoka, 2019). Based on a previous study, red earthworms can improve growth efficiency, reproduction, feed digestibility, stress reduction, longevity, lower feed conversions, and feed utilization of fish production.

In addition, earthworms are the most suitable meal to replace commercial feed since they are the source of protein but low in cost. Their growth rates increase too fast, and they can adapt to a variety of organic materials and transform biodegradable matter up to five times (Musyoka, 2019)

2.4.2 Nutrient composition of earthworm

The earthworm is an animal protein supplement that can replace the fish meal in feed ingredient because it is high in protein. Earthworm contains much nutrient that helps in breakdown the food which is iron and amino acid (Baitz, 2016). They are also known as the source of calcium. According to Jacob (2013), Earthworms are composed of 60% - 70% protein, 6% - 11% percent fat, 5% -21% carbohydrates, and 2% - 3% percent minerals and vitamins, like niacin.

Furthermore, Earthworms contain more essential amino acids like lysine and methionine than beef or fish meal. Earthworm meal has a high concentration of essential long-chain fatty acids. The recent research stated that earthworm is suitable for fish feed because their nutritional content meets the requirements of most fish.

2.4.3 The usage of earthworm in fish feed industry

According to recent research, there are the use of earthworms as a source of protein for fish. Earthworms are being use in the transformation of organic waste to animal feed. It is because earthworm contain a high level of protein sources that meet the dietary requirement of fish feed (Parolini et al., 2020).

Next, earthworms are widely used for aquaculture farming to improve the production. For example, earthworm production techniques on a small scale have long been utilized to boost fish harvests in semi-intensive agricultural systems. Plus, In India, an integrated vermiculture/fish culture system provides earthworm biomass as well as vertices as an organic fertilizer in semi intensive catfish ponds (The red earthworm as an alternative protein source in aquafeeds - Responsible Seafood Advocate, 2019). While supporting fish growth, earthworm meal can effectively and sustainably replace a variety of conventional animal and plant protein sources.

2.5 Factors affecting growth development

Many factors are affecting the survival rate of fish (B. Splendens). The Betta splendens can be affected due to the substrate of nutrition, water quality, pathogen and stress. (R, N, & W., 2016). According to Degani and Yehuda (1996), One of the most important influencing factors for growth productivity and breeding of animals is the quantity and quality of feeds. It is because feed gives the energy to fish to spawn (James, 2004). For fish culture and water quality, water hardness is essential (Chanua, 2010).

The hardness level of water can lead to high mortality in the larvae of the fish. Due to the discomfort caused by an imbalance of calcium in the physiology, new-borns cannot withstand harmful environmental conditions such as very rough water. Betta splendens grows and reproduces well in soft water (Cavalcanti, 2009).

2.6 Organoleptic

The aspects of food, water, or other substances that a person perceives through their senses are referred to as organoleptic properties while sensory evaluation is a scientific practice that uses the senses of sight, smell, touch, taste, and hearing to elicit, measure, evaluate, and interpret reactions to things (Baron, 2021).

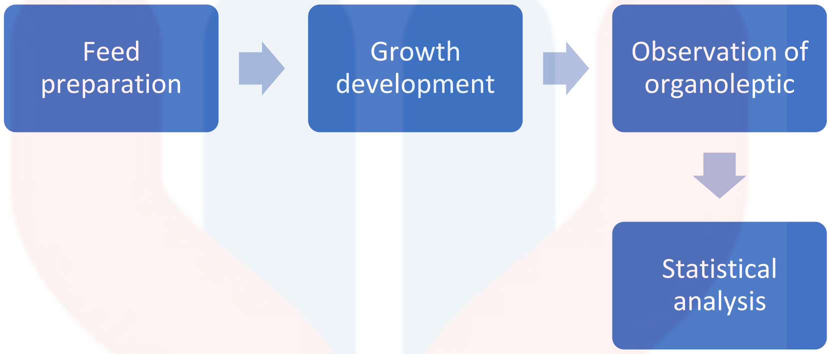
CHAPTER 3

Methodology

3.1 Experimental Design

Black soldier fly larvae meal (*Hermetia illucens*) and earthworm were used as the main ingredient to replace fish meal in this experiment. There were five formulated feed that contain different BSFL inclusion and five formulated feed that contain different earthworm inclusion which are 0%, 25%, 50%, 75% and 100% in betta fish diets on organoleptic and survival rate parameter. The organoleptic of pellet for both BSFL and earthworm was observed. Proximate analysis was performed to determine the nutrient composition in fish feed. 60 betta fish were tested and six betta fish for each treatment. Each treatment had different percent of BSFL meal and earthworm. This experiment was carried out at animal analysis laboratory at UMK. There were three tanks allocated in the same size to separate each treatment. The betta fishes were observed two times a day including feeding routine on morning and evening. The organoleptic of water was observed and the water was changed every two days. At the end of experiment, the survival rate of betta fish was calculated.

Figure 1: Shows the flowchart of the study



3.2 Materials

Betta Splendens (Male and Female), breeding tanks, substrates for breeding, betta fish feeds (control feed/BSFL formulated feed/earthworm formulated feed), aquatic plants, lightning, Oxygen.

3.2.1 Chemicals and Reagents

Sodium hydroxide solution (NaOH), *Kjeltab tablet*, *Boric acid* (H3BO3), concentrated *sulphuric acid* (H2SO4), concentrated *sulphuric acid* (H2SO4), distilled water, receiver solution, standard 0.1 M HCl, *Petroleum ether*, *celite*.

3.2.2 Apparatus

Multiparameter, Oven, Blender, Precision balance, Air-circulation oven, Aluminium foil, Zipper bag, Petri dish, Spatula, Container, Muffle Furnace, Desiccator, Porcelain, Crucibles, Gerhardt Kjeldatherm, Titration flask, Burrete, Glove, Digestion rack, Digestion tubes, Soxhlet extraction system, Receiving flask, Round bottom flask, Measuring cylinder and, Fume chamber, Extraction beaker with boiling stones, Tripod, Filter funnel, and extraction thimbles necessary standard laboratory instrument.

3.3 Preparation of tank for Betta Fish

The aim of the experiment is to study The Effects of Fish Meal Substitution with Black Soldier Larvae (Bsfl) And Earthworm on Growth and Physiological Properties of Experimental Diets in Betta Fish (*Betta Splendens*) Production. For tanks, thirty small plastics aquaria were used to put the fish. There were ten treatment in total and three replicates for each treatment with label. In each tank, the fish were paired randomly. To do the observation of feeding trial on betta fish, we need to set up the permanent Betta tanks. Betta fish cannot be kept in a tiny or tank because they can quickly become sick.

A gentle filter is needed to reduce the number of harmful bacteria that can affect the fish's reproduction development. It is better to use a filter with a GPH 4 times greater than the tank's size. Besides, we need to add gravel to the tank to set up the plants and decorations. Betta fish love to swim in a tank that contains the plant and a comfortable place to hide and sleep. Is recommended to use live plant since it can help to clean the water.

3.4 Feed preparation

The preparation BSFL meal and earthworm inclusion in betta fish has these following steps: weighing, grinding, mixing, moulding and drying. The ingredients used will be weighed according to the ration formulae calculated based on ration formulation. Each feed was made using the different percentage of BSFL and earthworm from 0%,25%,50%, 75% and 100%. There were 5 treatment for each different feed. BSFL meal was bought from supplier while the earthworm was collected at UMK areas. The earthworms have been dried using air circulating oven 48 hours at 70°C. The earthworm was finely ground in a blender to make it simpler to mix with the molasses and binder. By hand, the components will be well combined.

3.5 Proximate analysis

Proximate analysis was used to determine the nutritional values of the feed to ensure whether it was safe for animal consumption. The proximate analysis was carried out to analyse the amount of compound in the sample that contain fats, protein, fibre, carbohydrate. The experimental diets were conducted to AOAC-recommended proximate composition analyses (1997).

Table 3.1: Proximate composition of Earthworm experimental diets

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	4	4	4	4	4
Rice bran	28	28	28	28	28
Total	100	100	100	100	100
Proximate analysis (%)					
Crude fibre	1.59	1.78	2.03	1.82	2.1
Crude fat	2.67	2.87	3.57	3.00	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 3.2:Proximate composition of Black Soldier Fly Larvae (BSFL) experimental diets

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	4	4	4	4	4
Rice bran	28	28	28	28	28
Total	100	100	100	100	100
Proximate analysis (%)					
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

3.6 Determination of growth development

Survival rate was a measure of the number of surviving fish. Calculate survival rate by (number of surviving fish / total number of fish at the start of experiment) *100.

3.7 Determination of Organoleptic

The organoleptic test was determined specifically the colour and odour of each feed treatment, through visual observation of the experimental feeds. The test was observed 3 times a week.

3.8 Statistical analysis

The statistical analysis was carried out using the software SPSS (Version 26.0), and the importance of the results was determined. Analysis of variance (ANOVA) methods was applied to calculate the proximate analysis, organoleptic and growth development of the fish. The Duncan New Multiple Range Test was used to analyse the difference in mean values. The significance of different treatments was determined using one-way analysis of variance on *B. splendens* feeding parameters. The variations were found important at $p<0.05$ when the results were expressed as mean standard deviation.

CHAPTER 4

Result & discussion

4.1 Proximate Analysis

The qualities of ingredients used in the formulation of experimental diets were based on proximate analysis of dry materials with a ready to keep up the protein, fat, and carbohydrate contents in all types of experimental fish feed pellets at various levels. The recent study stated that the protein requirements for betta splendens was discovered that fish fed a diet containing 35% crude protein had higher growth rates and feeding efficiency (Lúcia Helena Sipaúba-Tavares et al., 2016). It was because Bettas were carnivorous fish that require a high protein diet (Robert, 2019). Based on proximate analysis of both experimental diets, (earthworm and bsfl), it was stated that earthworm has a high level of protein than bsfl. However, the amount of protein consumed by fish in did not change as a function of the protein content of the experimental diets.

The findings were not supported with recent research that the level of crude protein and fats content in earthworm was about 10% and 1.6% (Dr. J. 2011). Meanwhile, the range of crude protein in earthworm diets for this experiment was about 37% and 3%.

4.2 Growth performance

There were no significant variations in fish growth or survival (P > 0.05) related to the introduction of earthworms in fish meals (Table 4.1). The final weight, weight gain %, and specific growth rate of fish fed varied earthworm diets showed no significant changes (P > 0.05). Fish fed the experimental diets ingested them daily without rejection or loss, indicating that the inclusion of earthworms in fish diets had no effect on their palatability. In this case, it was discovered that feed intake was roughly the same across all treatments, ranging from 0.1 g feed/fish twice a day. Similarly, FCR values were similar across treatments with no significant variations, and their range was 2.19-5.00. Table 3 shows that there were no significant variations in specific growth rate among all fish groups (P > 0.05). Hence, fish survival was lower in 25% earthworm feed diets (33.33%), while the highest percent survival (100%) was recorded in 75% earthworm feed diets.

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Table 4.1: Growth performance of *Betta Splendens* with experimental diets (Earthworm) for 5 weeks. Data expressed as mean ± standard deviation (SD)

Parameters	Diets (%)					
	0	**25**	**50**	**75**	**100**	**P-value**
Initial weight (g)	1.22±0.17	1.06±0.16	1.16±0.20	1.17±0.24	0.99±0.24	0.32
Final weight (g)	1.29±0.18	1.25±0.15	1.37±0.30	1.33±0.29	1.06±0.27	0.22
Weight gain (%)	6.49±2.99	19.67±26.37	19.33±16.83	12.85±8.12	6.23±5.29	0.34
Survival rate (%)	66.67±28.87	33.33±1.73	66.67±28.87	100.00±0.00	83.33±0.00	0.09
Feed conversion rate (%)	2.19±1.97	13.71±20.79	1.02±1.05	1.14±1.00	5.00±6.02	0.16
Specific growth weight (%)	0.002±0.001	0.005±0.007	0.006±0.006	0.004±0.003	0.001±0.001	0.23

The growth performance of B. Splendens fed different levels of BSFL inclusion on experimental diets was shown in Table 4.2. The experimental meals have no significant (p 0.05) differences in terms of final weight, weight gain (%), specific growth rate (%), or feed conservation rate. In comparison to other experimental diets, the 75% of BSFL inclusion fish gained the most weight and had the highest SGR (0.005 %). Similarly, as compared to other experimental diets. However, *B. Splendens* in the 75% of BSFL in feed diets had the lowest no significantly ($p > 0.05$) feed conservation rate. The overall survival rate, on the other hand, was 100% of the *B. Splendens* with no significant ($p > 0.05$) changes in mean values within the several diets tested. Hence, fish survival was lower in 100% BSFL feed diets (83.33%), while the highest percent survival (100%) was recorded in others BSFL feed diets.

Table 3.2: Growth performance of *Betta Splendens* with experimental diets (Bsfl) for 5 weeks. Data expressed as mean ± standard deviation (SD)

Parameters	Diets (%)					
	0	25	50	75	100	P-value
Initial weight (g)	1.22±0.17	1.15±0.25	1.11±0.13	1.13±0.19	1.16±0.03	0.37
Final weight (g)	1.29±0.18	1.22±0.30	1.21±0.82	1.30±0.21	1.23±0.07	0.89
Weight gain (%)	6.49±2.99	5.34±8.25	9.81±11.93	14.25±4.28	8.85±7.70	0.33
Survival rate (%)	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	83.33±28.87	0.45
Feed conversion rate (%)	2.19±1.97	7.66±8.99	4.88±6.49	0.67±0.18	4.34±6.25	0.30
Specific growth rate (%)	0.002±0.001	0.001±0.003	0.002±0.003	0.005±0.001	0.003±0.002	0.83

In this study, the replacement of fish meal with earthworm and BSFL showed no significant differences (P<0.05) in means values showing that earthworm and BSFL not give a big effect on growth and development of ornamental fish. Earthworms were also discovered to have a higher protein content than BSFL. BSFL meal has a high level of protein (30–58% protein, dry weight), research has indicated that black soldier fly larvae (BSFL, *Hermetia illucens*) was a suitable nutritional source for aquafeed inclusion due to their high digestibility in feed trials (English et al., 2021). Meanwhile, several studies have shown that the earthworm powder content ranges from 48 and 71% protein more than protein content in BSFL meal (Aly, 2020).

The range of the protein content in both types, earthworm and BSFL for this study were 35-37% and 32-38%. This finding was supported by previous study stated that typically the range protein requirement for ornamental fish was 25%-50% depending on their size, age, feeding rate, genetic and protein to energy ratio (Yanong, 1999). Even though the results obtained from the protein content within the range of the nutritional requirement of ornamental fish, there was still no significant differences values $p<0.05$. It was vital to understand that proximate analysis was not a nutrient analysis; rather, it was a classification system that divides nutrients and non-nutrients into groups based on chemical similarities (Analytical Techniques in Aquaculture Research, 2022). Although the betta fish were the same age, they were not the same size or weight. Even though the BSFL feed had the smallest weight, it had the best growth results.

The weight gain and Specific Growth Rate (SGR) were highest in 50% of earthworm inclusion and 75% of BSFL inclusion. This implies that utilising earthworm and BSFL on fish feed pellets increases *B. Splendens* growth. The weight difference between the fish fed earthworm and those fed with BSFL inclusion indicated that supplementary feeding was vital in growth development, however the weight difference was not statistically significant (Yaqub, 2022).

Over and above that, the survival rate of the fish for BSFL inclusion was greater in than fish fed earthworm inclusion. Disease, competition, cannibalism, old age, predation, pollution, temperature or any other natural event that causes fish death could all be contributing factors to the declining survival rate for earthworm and BSFL inclusion at the end of the experiment. Previous studied Wedemyer & Mcleay, 1981; Hur & Habibi, 2007; Kang et al., 2007 proved that abnormalities in water temperature cause fast stress, resulting in immunity loss, increased sickness, physiological changes, and even mass mortality, all of which have a direct impact on productivity and economic loss.

4.3 Organoleptic

Table 4.3 shows the organoleptic observations treated with test diets. The results demonstrate that the colour and odour of the feed varied depending on the feeding treatment. The colour and odour of the feed were darker in yellowish black, and the flavour was greater as the proportion of earthworm content in the fish feed pellet increased. Furthermore, the water colour of the 100% earthworm inclusion was turbid, but the water colour of the control was bright yellowish turbid.

Table 4.3: Organoleptic of Earthworm experimental diets

Parameters	Diets (%)				
	0%	**25%**	**50%**	**75%**	**100%**
Feed colour	Ash	Turbid yellowish black	Brown yellowish black	Deep yellowish black	Deeper yellowish black
Feed odour	Fishy odour	Characteristics flavour	Powerful flavour	Strong flavour	Stronger flavour
Water colour	Strong yellowish turbid	More yellowish turbid	Yellowish turbid	Brownish turbid	Turbid

The organoleptic findings treated with test diets are shown in Table 4.4. The results show that the feed colour and odour differed depending on the feeding method. As the amount of earthworm content in the fish feed pellet increased, the colour and odour of the feed became darker in yellowish black, and the flavour became stronger.

Furthermore, the water colour of the control was strong yellowish turbid, same with the water colour of the 100% bsfl inclusion.

Table 4.4: Organoleptic of Black Soldier Fly Larvae (BSFL) experimental diets

Parameters	Diets (%)				
	0	**25**	**50**	**75**	**100**
Feed colour	Ash	Brown yellowish black	Turbid yellowish black	Deep yellowish black	Deeper yellowish black
Feed odour	Fishy odour	Characteristics flavour	Powerful flavour	Strong flavour	Stronger flavour
Water colour	Strong yellowish turbid	Yellowish turbid	Brownish turbid	More yellowish turbid	Strong yellowish turbid

According to the findings, there was a lot of diversity in feed colour and odour between feeding treatments in this study. When the colour of fish meals changes into brown or deep brown, it usually means there was microbial contamination (Hossen et al., 2011). Within two different feeding treatment, the inclusion of earthworm and bsfl showed the same result in feed colour and odour, and water colour changed. As the amount of earthworm and bsfl content in the fish feed pellet increased, the colour and odour of the feed became darker in yellowish black, and the flavour became stronger. The finding was supported by recent study stated that microbial growth by-products have been reported to be the primary cause of odour patterns in aquaculture environments (Mahmoud and Buettner).

Meanwhile, the colour of the water may be affected by the colour of fish feed. Mahmoud and Buettner (2017) stated that there was relationship between the feed odour and water quality.

CHAPTER 5

Conclusion and recommendation

To sum up, the results of this studied present that using 50% earthworm inclusion and 75% of BSFL inclusion levels as replacement of fish feed pellets for *B. Splendens* were perform better in growth and development. Earthworm and BSFL meal, could be used as partial replacement for fishmeal. Due to a lack of equipment to pelletize the feed fish into a suitable size for B. Splendens, the data may significantly impact growth performance. Apart of that, the availability of laboratory equipment should be expanded since there was no pelletizer for the smallest fish. On top of that, the further research in growth development of ornamental fish was highly recommended to improve the experiments by increasing the numbers of fish for observation.

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Appendix A

Descriptive analysis and ANOVA of growth and development of Betta Splendens of BSFL inclusion via SPSS

```
ONEWAY weight BY BSFL
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

weight

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
1.00	6	6.487846	2.9913820	1.2212266	3.348583	9.627109	3.9192	11.4434
2.00	6	5.340235	8.2532439	3.3693727	-3.321014	14.001483	.4032	21.8787
3.00	6	9.812364	11.9277254	4.8694735	-2.705016	22.329744	.5232	32.6814
4.00	6	14.247406	4.2750813	1.7452946	9.760983	18.733828	9.9535	19.9227
5.00	6	8.381776	7.7044139	3.1453138	.296490	16.467063	.5200	20.4525
Total	30	8.853925	7.8210587	1.4279234	5.933494	11.774357	.4032	32.6814

ANOVA

weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	289.053	4	72.263	1.217	.329
Within Groups	1484.847	25	59.394		
Total	1773.900	29			

```
ONEWAY FCR_BSFL SGR_BSFL Initial_BSFL BY BSFL
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
FCR_BSFL	0%	6	2.193517	1.9651980	.8022887	.131168	4.255865	.6468	6.0205
	25%	6	7.660840	8.9899618	3.6701366	-1.773546	17.095226	.3421	25.0000
	50%	6	4.876196	6.4916613	2.6502096	-1.936385	11.688776	.3400	16.3934
	75%	6	.673845	.1816180	.0741452	.483249	.864442	.3802	.9166
	100%	6	4.340408	6.2514845	2.5521578	-2.220122	10.900939	.4338	16.3934
	Total	30	3.948961	5.8759583	1.0727983	1.754842	6.143080	.3400	25.0000
SGR_BSFL	0%	6	.002097	.0013805	.0005636	.000648	.003546	.0005	.0044
	25%	6	.001898	.0031912	.0013028	-.001451	.005247	.0001	.0084
	50%	6	.002801	.0030422	.0012420	-.000391	.005994	.0002	.0084
	75%	6	.004574	.0015505	.0006330	.002947	.006201	.0031	.0075
	100%	6	.002737	.0024853	.0010146	.000129	.005345	.0002	.0066
	Total	30	.002821	.0024662	.0004503	.001901	.003742	.0001	.0084
Initial_BSFL	0%	6	1.221083	.1651351	.0674161	1.047785	1.394382	.9972	1.3510
	25%	6	1.153050	.2490313	.1016666	.891708	1.414392	.9722	1.5789
	50%	6	1.109050	.1331298	.0543500	.969339	1.248761	.8999	1.2591
	75%	6	1.134133	.1858496	.0758728	.939096	1.329171	.8771	1.3201
	100%	6	1.161000	.0298998	.0122066	1.129622	1.192378	1.1270	1.2110
	Total	30	1.155663	.1612374	.0294378	1.095456	1.215870	.8771	1.5789

```
ONEWAY Final_BSFL BY BSFL
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

Final_BSFL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
0%	6	1.294835	.1808890	.0738476	1.105004	1.484666	1.0410	1.5056
25%	6	1.219467	.3036459	.1239629	.900810	1.538124	.9891	1.6283
50%	6	1.207100	.0820178	.0334836	1.121028	1.293172	1.0756	1.3110
75%	6	1.294233	.2109497	.0861199	1.072855	1.515612	1.0219	1.5831
100%	6	1.256800	.0661765	.0270164	1.187352	1.326248	1.1791	1.3575
Total	30	1.254487	.1803014	.0329184	1.187161	1.321813	.9891	1.6283

ANOVA

Final_BSFL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.040	4	.010	.278	.890
Within Groups	.903	25	.036		
Total	.943	29			

```
ONEWAY SURVIVAL BY BSFL
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

SURVIVAL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
0%	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	100.00
25%	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	100.00
50%	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	100.00
75%	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	100.00
100%	3	83.3333	28.86751	16.66667	11.6225	155.0442	50.00	100.00
Total	15	96.6667	12.90994	3.33333	89.5174	103.8160	50.00	100.00

ANOVA

SURVIVAL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	666.667	4	166.667	1.000	.452
Within Groups	1666.667	10	166.667		
Total	2333.333	14			

Descriptive analysis and ANOVA of growth and development of Betta Splendens of Earthworm inclusion via SPSS

```
GET
  FILE='C:\Users\candy\Desktop\FYP\Final weight Earthwr
om bsfl.sav'.
DATASET NAME DataSet1 WINDOW=FRONT.
DATASET ACTIVATE DataSet0.
DATASET CLOSE DataSet1.
ONEWAY Weight_gain BY Earthworm
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

Weight_gain

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0%	6	6.487846	2.9913820	1.2212266	3.348583	9.627109	3.9192	11.4434
25%	6	19.674685	26.3671016	10.7643242	-7.995892	47.345261	.1838	66.1660
50%	6	19.329193	16.8305090	6.8710265	1.666657	36.991729	2.5517	45.2404
75%	6	12.853831	8.1215050	3.3155905	4.330835	21.376828	2.6587	22.8184
100%	6	6.215906	5.2947117	2.1615570	.659447	11.772365	.6709	14.9934
Total	30	12.912292	14.9081172	2.7218374	7.345510	18.479075	.1838	66.1660

ANOVA

Weight_gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1038.150	4	259.538	1.200	.335
Within Groups	5407.156	25	216.286		
Total	6445.307	29			

```
ONEWAY Final_Weight BY Earthworm
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

Final_Weight

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0%	6	1.294835	.1808890	.0738476	1.105004	1.484666	1.0410	1.5056
25%	6	1.247750	.1458348	.0595368	1.094706	1.400794	1.0359	1.4493
50%	6	1.375900	.2956256	.1206886	1.065660	1.686140	1.0931	1.9400
75%	6	1.326933	.2893062	.1181088	1.023325	1.630542	.7772	1.5710
100%	6	1.055000	.2721471	.1111036	.769399	1.340601	.7952	1.3997
Total	30	1.260084	.2534952	.0462817	1.165427	1.354740	.7772	1.9400

ANOVA

Final_Weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.368	4	.092	1.537	.222
Within Groups	1.496	25	.060		
Total	1.864	29			

```
ONEWAY FCR Initial_weight SGR BY Earthworm
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
FCR	0%	6	2.193517	1.9651980	.8022887	.131168	4.255865	.6468	6.0205
	25%	6	13.706423	20.7925843	8.4885370	-8.114056	35.526902	.1733	52.6316
	50%	6	1.016261	1.0542958	.4304144	-.090154	2.122677	.2003	3.0488
	75%	6	1.142508	.9968823	.4069755	.096344	2.188672	.3499	3.0030
	100%	6	4.999454	6.0178432	2.4567742	-1.315885	11.314793	.5479	15.6250
	Total	30	4.611633	10.2631129	1.8737795	.779323	8.443942	.1733	52.6316
Initial_weight	0%	6	1.221083	.1651351	.0674161	1.047785	1.394382	.9972	1.3510
	25%	6	1.064717	.1632327	.0666395	.893414	1.236019	.8722	1.2814
	50%	6	1.156867	.1991636	.0813082	.947857	1.365876	.9381	1.4407
	75%	6	1.174750	.2365496	.0965710	.926506	1.422994	.6990	1.3364
	100%	6	.991350	.2374727	.0969478	.742138	1.240562	.7400	1.3279
	Total	30	1.121753	.2063347	.0376714	1.044707	1.198800	.6990	1.4407
SGR	0%	6	.002097	.0013805	.0005636	.000648	.003546	.0005	.0044
	25%	6	.005186	.0066619	.0027197	-.001805	.012177	.0001	.0165
	50%	6	.006258	.0055229	.0022547	.000462	.012054	.0009	.0143
	75%	6	.004348	.0030986	.0012650	.001096	.007600	.0010	.0082
	100%	6	.001255	.0007830	.0003197	.000433	.002076	.0002	.0018
	Total	30	.003829	.0043185	.0007885	.002216	.005441	.0001	.0165

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FCR	Between Groups	682.047	4	170.512	1.797	.161
	Within Groups	2372.567	25	94.903		
	Total	3054.613	29			
Initial_weight	Between Groups	.205	4	.051	1.244	.318
	Within Groups	1.030	25	.041		
	Total	1.235	29			
SGR	Between Groups	.000	4	.000	1.520	.227
	Within Groups	.000	25	.000		
	Total	.001	29			

```
DATASET ACTIVATE DataSet0.
ONEWAY survival_rate BY Earthworm
  /STATISTICS DESCRIPTIVES
  /MISSING ANALYSIS
  /POSTHOC=TUKEY DUNCAN ALPHA(0.05).
```

Oneway

[DataSet0]

Descriptives

survival_rate

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0%	3	66.6667	28.86751	16.66667	-5.0442	138.3775	50.00	100.00
25%	3	33.3333	28.86751	16.66667	-38.3775	105.0442	.00	50.00
50%	3	66.6667	28.86751	16.66667	-5.0442	138.3775	50.00	100.00
75%	3	100.0000	.00000	.00000	100.0000	100.0000	100.00	Double-click to activate
100%	3	83.3333	28.86751	16.66667	11.6225	155.0442	50.00	
Total	15	70.0000	31.62278	8.16497	52.4879	87.5121	.00	100.00

ANOVA

survival_rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7333.333	4	1833.333	2.750	.089
Within Groups	6666.667	10	666.667		
Total	14000.000	14			

Descriptive analysis and Test of Homogeneity of variances of organoleptic of Betta Splendens via SPSS

```
ONEWAY colour odour BY BSFL_pellet
  /STATISTICS DESCRIPTIVES HOMOGENEITY
  /MISSING ANALYSIS
  /POSTHOC=TUKEY ALPHA(0.05).
```

Oneway

[DataSet0]

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
colour	0%	3	1.0000	.00000	.00000	1.0000	1.0000	1.00	1.00
	25%	3	1.6667	.57735	.33333	.2324	3.1009	1.00	2.00
	50%	3	3.3333	.57735	.33333	1.8991	4.7676	3.00	4.00
	75%	3	3.6667	.57735	.33333	2.2324	5.1009	3.00	4.00
	100%	3	5.0000	.00000	.00000	5.0000	5.0000	5.00	5.00
	Total	15	2.9333	1.53375	.39601	2.0840	3.7827	1.00	5.00
odour	0%	3	1.3333	.57735	.33333	-.1009	2.7676	1.00	2.00
	25%	3	2.3333	.57735	.33333	.8991	3.7676	2.00	3.00
	50%	3	3.3333	.57735	.33333	1.8991	4.7676	3.00	4.00
	75%	3	3.6667	.57735	.33333	2.2324	5.1009	3.00	4.00
	100%	3	4.6667	.57735	.33333	3.2324	6.1009	4.00	5.00
	Total	15	3.0667	1.27988	.33046	2.3579	3.7754	1.00	5.00

```
ONEWAY Colour BY BSFL_Water
  /STATISTICS DESCRIPTIVES HOMOGENEITY
  /MISSING ANALYSIS
  /POSTHOC=TUKEY ALPHA(0.05).
```

Oneway

[DataSet1] C:\Users\candy\Documents\SPSS\BSFL WATER COLOUR.sav

Descriptives

Colour

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
T1	3	1.6667	1.15470	.66667	-1.2018	4.5351	1.00	3.00
T2	3	4.3333	1.15470	.66667	1.4649	7.2018	3.00	5.00
T3	3	3.6667	.57735	.33333	2.2324	5.1009	3.00	4.00
T4	3	2.3333	.57735	.33333	.8991	3.7676	2.00	3.00
T5	3	2.0000	1.00000	.57735	-.4841	4.4841	1.00	3.00
Total	15	2.8000	1.32017	.34087	2.0689	3.5311	1.00	5.00

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Colour	Based on Mean	1.053	4	10	.428
	Based on Median	.136	4	10	.965
	Based on Median and with adjusted df	.136	4	6.914	.964
	Based on trimmed mean	.911	4	10	.494

```
GET
  FILE='C:\Users\candy\Documents\SPSS\organoleptic (pellet).sav'.
DATASET NAME DataSet1 WINDOW=FRONT.
ONEWAY Colour Odour BY Earthworm_pellet
  /STATISTICS DESCRIPTIVES HOMOGENEITY
  /MISSING ANALYSIS.
```

Oneway

[DataSet1] C:\Users\candy\Documents\SPSS\organoleptic (pellet).sav

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Colour	0%	3	1.6667	1.15470	.66667	-1.2018	4.5351	1.00	3.00
	25%	3	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
	50%	3	2.0000	.00000	.00000	2.0000	2.0000	2.00	2.00
	75%	3	4.0000	.00000	.00000	4.0000	4.0000	4.00	4.00
	100%	3	5.0000	.00000	.00000	5.0000	5.0000	5.00	5.00
	Total	15	3.1333	1.35576	.35006	2.3825	3.8841	1.00	5.00
Odour	0%	3	1.3333	.57735	.33333	-.1009	2.7676	1.00	2.00
	25%	3	2.0000	.00000	.00000	2.0000	2.0000	2.00	2.00
	50%	3	3.0000	.00000	.00000	3.0000	3.0000	3.00	3.00
	75%	3	4.0000	.00000	.00000	4.0000	4.0000	4.00	4.00
	100%	3	5.0000	.00000	.00000	5.0000	5.0000	5.00	5.00
	Total	15	3.0667	1.38701	.35813	2.2986	3.8348	1.00	5.00

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Colour	Based on Mean	16.000	4	10	.000
	Based on Median	1.000	4	10	.452
	Based on Median and with adjusted df	1.000	4	2.000	.556
	Based on trimmed mean	12.603	4	10	.001
Odour	Based on Mean	16.000	4	10	.000
	Based on Median	1.000	4	10	.452
	Based on Median and with adjusted df	1.000	4	2.000	.556
	Based on trimmed mean	12.603	4	10	.001

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Colour	Between Groups	23.067	4	5.767	21.625	.000
	Within Groups	2.667	10	.267		
	Total	25.733	14			
Odour	Between Groups	26.267	4	6.567	98.500	.000
	Within Groups	.667	10	.067		
	Total	26.933	14			

```
ONEWAY Colour BY EARTHWORM
  /STATISTICS DESCRIPTIVES HOMOGENEITY
  /MISSING ANALYSIS
  /POSTHOC=TUKEY ALPHA(0.05).
```

Oneway

[DataSet0]

Descriptives

Colour

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
T1	3	1.6667	1.15470	.66667	-1.2018	4.5351	1.00	3.00
T2	3	2.6667	1.15470	.66667	-.2018	5.5351	2.00	4.00
T3	3	3.3333	.57735	.33333	1.8991	4.7676	3.00	4.00
T4	3	3.6667	.57735	.33333	2.2324	5.1009	3.00	4.00
T5	3	4.6667	.57735	.33333	3.2324	6.1009	4.00	5.00
Total	15	3.2000	1.26491	.32660	2.4995	3.9005	1.00	5.00

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
Colour	Based on Mean	2.182	4	10	.145
	Based on Median	.136	4	10	.965
	Based on Median and with adjusted df	.136	4	6.914	.964
	Based on trimmed mean	1.719	4	10	.222

Appendix C

Dead fish

Appendix D

The rearing of earthworms