Effect Of Different Inclusion Rate of Black Soldier Fly Larvae (BSFL) on Proximate Analysis in Macrobrachium Rosenbergii Juvenile Feed

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research except the excerpts and summaries that I have made a clear of the sources.

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Effect of Different Inclusion Rate of Black Soldier Fly Larvae (BSFL) on Proximate Analysis in *Macrobrachium rosenbergii* Juvenile feed

ABSTRACT

Giant freshwater prawn or scientifically known as *Macrobrachium rosenbergii* production well known for years due to its prominent size and good flesh quality. Although giant freshwater prawns' economic sector is popular among all sort of people, there's still problem risen from this production sector. The high-cost part in aquaculture production is the feed cost, comprises about 70% of total production cost. This has limits expansion of aquaculture industry especially for a small and medium scale farmer. In most studies, BSFL has proved to successfully replace commercial animal feed which BSFL has similar amino acid (AA) profile as fish meal (FM). Application of BSFL introduced as an alternative feed for *M. rosenbergii* might be potential. BSFL with other ingredients like rice bran, fish meal, corn meal, soybean meal and copra meal was mixed using feed formulation that was determined in Win feed software version 2.8. Different percentage of BSFL which are at 10%, 20%, 30% and 40% were used in feed formulation while formulation without BSFL will be used as control. Biochemical composition of all treatments were determined using proximate analysis on crude protein, crude fat, crude fibre, ash and moisture. For crude protein, treatment 4 (40%), (30.82 ±0.19) has the highest percentage compared to other treatments, Control, Treatment 1 (10% BSFL), Treatment 2 (20% BSFL) and Treatment 3 (30% BSFL), (23.01 ±0.13, 25.63 ±0.05, 28.29 ±0.5, 30.01 ±0.08). For crude fat, the highest percentage of crude fat is (12.77 ±0.38) for treatment 4 compared to other treatments , Control, Treatment 1 (10% BSFL), Treatment 2 (20% BSFL) and Treatment 3 (30% BSFL), (5.00 ±0.97, 6.08 ±0.66, 9.29 ±0.34, 12.52 ±1.22). For crude fibre, the highest treatment is treatment 2 (11.67 ±0.76) while for ash, the highest percentage is control (0.25 ±0.05). The treatment with high percentage of moisture is Treatment 4 with (26.66 ±0.37). The result shows that formulation with 40% of BSFL fulfil the nutrients requirement for *M. rosenbergii* juvenile. The other formulation did not fulfil the nutrients requirement in term of protein, fat, and fibre.

Keywords: Black Soldier Fly larvae (BSFL), *M. rosenbergii* juvenile, Feed Formulation

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Kesan Kadar Kemasukan Berbeza *Black Soldier Fly Larvae* (BSFL) ke Atas Analysis Proksimat Dalam Makanan *Macrobrachium rosenbergii* Juvenile

ABSTRAK

Udang air tawar gergasi atau dikenali secara saintifik sebagai pengeluaran Macrobrachium rosenbergii yang terkenal selama bertahun-tahun kerana saiznya yang menonjol dan kualiti daging yang baik. Walaupun sektor ekonomi udang air tawar gergasi popular di kalangan semua jenis orang, masih terdapat masalah yang timbul daripada sektor pengeluaran ini. Bahagian kos tinggi dalam pengeluaran akuakultur ialah kos makanan, merangkumi kira-kira 70% daripada jumlah kos pengeluaran. Ini telah menghadkan pengembangan industri akuakultur terutamanya untuk petani skala kecil dan sederhana. Dalam kebanyakan kajian, BSFL telah terbukti berjaya menggantikan makanan haiwan komersial yang mana BSFL mempunyai profil asid amino (AA) yang serupa dengan makanan ikan (FM). Penggunaan BSFL yang diperkenalkan sebagai suapan alternatif untuk M. rosenbergii mungkin berpotensi. BSFL dengan bahan-bahan lain seperti dedak padi, tepung ikan, tepung jagung, tepung kacang soya dan tepung kopra dicampur menggunakan formulasi suapan yang ditentukan dalam perisian suapan Win versi 2.8. Peratusan BSFL yang berbeza iaitu pada 10%, 20%, 30% dan 40% digunakan dalam formulasi makanan manakala formulasi tanpa BSFL akan digunakan sebagai kawalan. Komposisi biokimia semua rawatan ditentukan menggunakan analisis proksimat ke atas protein mentah, lemak mentah, gentian mentah, abu dan lembapan. Untuk protein kasar, rawatan 4 (40%), (30.82 ±0.19) mempunyai peratusan tertinggi berbanding rawatan lain, Kawalan, Rawatan 1 (10% BSFL), Rawatan 2 (20% BSFL) dan Rawatan 3 (30% BSFL) , (23.01 ±0.13, 25.63 ±0.05, 28.29 ±0.5, 30.01 ±0.08). Untuk lemak mentah, peratusan tertinggi lemak mentah ialah (12.77 ±0.38) untuk rawatan 4 berbanding rawatan lain , Kawalan, Rawatan 1 (10% BSFL), Rawatan 2 (20% BSFL) dan Rawatan 3 (30% BSFL), (5.00 ±0.97, 6.08 ±0.66, 9.29 ±0.34, 12.52 ±1.22). Bagi gentian mentah, rawatan tertinggi ialah rawatan 2 (11.67 ±0.76) manakala untuk abu, peratusan tertinggi ialah kawalan (0.25 ±0.05). Rawatan dengan peratusan lembapan yang tinggi ialah Rawatan 4 dengan (26.66 ±0.37). Keputusan menunjukkan bahawa formulasi dengan 40% BSFL memenuhi keperluan nutrien untuk juvana M. rosenbergii. Formulasi lain tidak memenuhi keperluan nutrien dari segi protein, lemak dan serat.

Kata kunci: *Black Soldier Fly Larvae* (BSFL), *Macrobrachium rosenbergii juvenile*, Formulasi Makanan

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LIST OF ABBREVIATIONS AND SYMBOLS

BSFL	Black Soldier Fly Larvae
M. rosenbergii	Macrobrachium Rosenbergii
%	Percentage
kg	Kilogram
g	Gram
mL	Millilitre
mg	Milligram
mm	Millimetre
min	Minute
h	Hour
°C	Degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Macrobrachium rosenbergii is locally known in Malaysia as "udang galah" and classified under crustacean subphylum. *M. rosenbergii* has become so popular among freshwater prawn breeder and seafood lovers because of its delicate, and sweet flavour. *M. rosenbergii* is economically important because grows faster among other freshwater prawn (Sarman, Vishal, Mahavadiya, & Sapra, 2018). *M. rosenbergii* is originally distributed from north-west India to Vietnam, Philippines, Papua New Guinea, and Northern Australia (Banu & Christianus, 2016) . In Malaysia, the first production of *M. rosenbergii* was recorded in 1970 according to FAO data (Banu & Christianus, 2016). Although giant freshwater prawns' economic sector contributed significantly to many producing countries, there are constraints that limit the expansion of this industry. The costliest part in aquaculture production is the feed cost, comprises about 70% of total production cost. Feed industry rely heavily on the imported feed material which higher in price. Besides price fluctuation is common due to commodity being traded in the market. This has limits expansion of aquaculture industry especially for a small and medium scale farmer. In recent years, some intensifications are devoted on the finding potential locally available feed materials that is cheap, safe, environmentally friendly and has ability for mass production. Black soldier fly

larvae (BSFL) is among insects that being studied for its great potential as partial or complete protein replacement in animal feed industry. BSFL has high protein composition which about 40% protein and 30% fat that can meet demands for protein requirement in many species. One of the most important dietary constituents among animal is protein source. Protein is important for growth and regeneration of tissue. Compared to poultry, protein requirement in most aquaculture species is higher to about 32-40% protein. This is because most of the protein source in Malaysia are quite cost and high dependency. Finding an alternative to the existing protein source will require a source that has high protein. Among all, many studies focusing on potential utilisation of insect such as BSFL. The high potential of BSFL as protein source is due to its ability to be produce at large scale and at low cost.

1.2 Problem Statement

The main problem that had been facing by most of the farmers is the cost production of the feed. The use of fishmeal, shrimp meal, soybean meal and others as main protein source are a bit costly. The source of protein is important in *M. rosenbergii* growth to produce a good quality of Juvenile *M. rosenbergii*. The use of BSFL is not commercially use as protein replacement in *M. rosenbergii* feed. BSFL can be used as protein source because of its high rich protein source and cheap compared to others protein source.

1.3 Significance of Study

Feed formulation using BSFL as protein source give benefits in terms of growth and quality to *M. rosenbergii* juvenile. From this study, data on its nutrients composition the feed industry was revealed. Finding form this study will eventually determine the optimum formulation for *M. rosenbergii* juvenile feed. Indirectly, it will reduce cost of production and reduce the dependant on imported feed materials, and these will help to grow the industry.

1.4 Objective of the Study

The main objective of the present study is to investigate the potential application of BSFL as feed ingredient to partially replace protein requirement for *M. rosenbergii* juvenile. The specific objectives are as follows:

i. To formulate the feed for M. rosenbergii juvenile using BSFL
ii. To determine the chemical composition of newly formulated feed using BSFL for M. rosenbergii juvenile

1.5 Hypothesis

- H_0 : Different inclusion levels of BSFL has no significant effect on the chemical composition of *M. rosenbergii* juvenile feed
- H_1 : Different inclusion levels of BSFL has significant effect on the chemical composition of *M. rosenbergii* juvenile feed

CHAPTER 2

LITERATURE REVIEW

2.1 General Descriptions of M. rosenbergii

Giant freshwater prawns, *M. rosenbergii* has been raised and fetched excellently in Asia market seafood (Beijnen, 2019). *M. rosenbergii* has been introduced to more than 40 countries and become one of the most cultivated freshwater prawns in the world (Iketani, et al., 2010). Nowadays, several countries actively cultivated *M. rosenbergii* as human food sources. *M. rosenbergii* is usually found in tropical and subtropical areas. Most of this species were found at inland areas like swamps, rivers, lakes, and ponds and also this species also found in estuarine areas as well. *M. rosenbergii* species mostly require the brackish water to initiate their initial stage of life cycle (Semwal, Arya, Yadav, & Upreti, 2021). The research reported that Giant freshwater prawn is one of the biggest freshwater prawns in the world. *M. rosenbergii* actually can grow up to 320 mm and their weight can reach over 200g. During the juvenile phase, the standard size of *M. rosenbergii* is about 7 to 10 mm long and 6 to 9 mg for weight The characteristics that will help when identifying *M. rosenbergii* are *M. rosenbergii* has long rostrum, which is 8-10 ventral teeth and 11-14 dorsal teeth. The second is adult male of *M. rosenbergii* has long chelipeds (all the segments

have blunt spines and elongate). The reproductive structure of giant freshwater prawn is in the cephalothorax. The reproductive structure of male consists of pair of testes, which located dorsally in cephalothorax. The simple tubes that end with terinal ampule known as paired of vasa differentia that contain spermatophores. The reproductive structure of female consists of pair of ovaries that located at stomach and hepatopancreas. When the ovaries of female are matured, they will experience pre-mating moult that only occur at night. This mating stages consists of six stages (Kai-Hsiang, Jui-Pin, Shu-Yin, Da-Ji, & Hon-Cheng, 2010). The first step is female *M. rosenbergii* will approaches male *M. rosenbergii* and the female will climb onto male telson. Female will allocate its body until female between his chelae. After that, *M. rosenbergii* female will face the male and making contact. The male will start its action rubbing on female reproductive parts and lastly the male will let go its spermatophores. Larvae stages of *M. rosenbergii* is going through 11 stages before metamorphosis stage. The life cycle of *M. rosenbergii* starts with eggs and then becomes the post-larvae then becomes juvenile and lastly becomes adult. Giant freshwater prawn moults like other crustaceans as well. The duration of moults and number of moults are not justified because it is depending on environment, the existence of food and temperature. During mating, the male will depose spermatophores on female's underside of thorax which located between walking legs. Then, the female will release its eggs a days after it. The fertilized eggs will move to brood chamber which located underside the female's abdominal region and then the eggs will incubate for 21 days before its hatch. Female *M. rosenbergii* lay 10,000 – 50,000 eggs up to five times per year. The time may vary but is generally less than three weeks (Grave, Shy, Wowor, & Page, 2013)

2.2 Nutrients requirement of *M. rosenbergii* juvenile

Every species of animals has their owns diet requirements. All the type of animals requires a balanced diet and good nutrients sources like nutrients, fluids, minerals, and vitamins. It is because the good nutrients source will make them reproduce, develop a strong immunity to fight off infections and. vigour to grow. All these advantages lead to more profitable and sustainable agriculture. The nutrients composition that generally stated in diet requirements are protein, fat, vitamins, and carbohydrate. The diet requirements for giant freshwater prawns, *M. rosenbergii* have not been systematized. However, during the last decade, expertise that study on diet requirements for giant freshwater prawns, *M. rosenbergii* has been experimentally conducted for practical applications (KM & Paturi, 2018). Every diet requirements have their owns important and specifications in terms of total intake. Table 1 shows the dietary nutrient required by giant freshwater prawn.

Table 0.1 dietary nutrient required by giant freshwater prawn

Proximate composition	Growth Stages				
	Broodstock	Juveniles (2^{nd} – 4^{th} month)	Adults (5^{th} – 6^{th} month)	Published Diets %	For All stages
Protein (%)	38 - 40	35 – 37	28 - 30	24 - 33	-
Carbohydrate (%)	-	-	-	30 – 42	25 – 35
Lipid, including phospholipids (%)	-	-	-	6 – 11	3 – 7
Cholesterol (%)	-	-	-	-	0.5 – 0.6
Vitamin C (mg/kg)	-	-	-	-	100
Ash (%)	-	-	-	10 – 20	-
Fibre (%)	-	-	-	5 – 9	-
Moisture (%)	-	-	-	<11	-
Calcium; Phosphorus	-	-	-	-	1.5 – 2.1
Calcium (mg/kg)	-	-	-	2	-
Phosphorus (mg/kg)	-	-	-	1.35	-
Gross energy (kj/g)	-	-	-	16 – 17	-
Zinc (mg/kg)	-	-	-	-	90
Energy (kcal/g feed)	3.7 – 4.0	-	-	4	2.9 – 3.2

Source: FAO (2013)

The importance of protein in *M. rosenbergii* is in their growth, body repair, formation of body components, and production of antibodies, enzymes, and hormones (KM & Paturi, 2018). The choice of protein plays a vital role in prawn growth and the acid of a profile should be identical to that of *M. rosenbergii* (KM & Paturi, 2018). The amino acid composition is used to give the guidance values in feed formulation (Mitra, Chattopadhyay, & Mukhopadhyay, 2005). Carbohydrate digestibility in shrimp differed according to flour type, botanical origin of starch, and inclusion stage. The best results were obtained with regular wheat starch, which is widely used as the primary starch source in shrimp feeds. The incorporation of phospholipids (lecithin) into the diet has a better growth-promoting effect in shrimp (Lena, Kolandhasamy, Chandran, & P. Saravan, 2009). From the past, soya bean

meal is the most commercially important source of lecithin, which is commonly used in shrimp diets (Gupta, Sehgal, & Sehgal, 2007).

2.3 Common ingredients and feed properties for M. rosenbergii juvenile feed

M. rosenbergii is a prawn that can digest both plant and animal based. Usually, *M. rosenbergii* will eat all type of foods as long as from animal or plant source. *M. rosenbergii* often eat natural feed like zooplankton and oligochaete worms for nutrition and formulated feed that contains complete nutrients that require by the *M. rosenbergii* (Mitra, Chattopadhyay, & Mukhopadhyay, 2005). The common ingredients that used in *M. rosenbergii* are fishmeal, bone meal, meat meal, rice bran, and soybean meal. The reason why these ingredients are the regularly used in *M. rosenbergii* feed because the protein contains of each ingredients fulfil the requirements and give good impacts to *M. rosenbergii.* There are six types of feed according to size of prawn. Mostly the shape of feed for size prawn between 0.01 - 5 g is in crumbles, while for the prawn 5 g and above, the shape of feed is in pellet (New, 2005).

2.4 Black soldier fly larvae, BSFL

Black soldier fly larvae or *Hermetia illucens* sp. is a widespread fly from the stratiomyidae family. Though originally native to the Americas, it now occurs worldwide in

tropical and temperate regions (Čičková, Newton, Lacy, & Kozánek, 2014). The life cycle of BSFL are divided into five main stages which are the first stage is egg, the second stage is larva, the third stage is prepupal, the fourth stage is pupal and the last stage is adult. The longest cycle phase is at the larval and pupal stage while the shortest cycle phase is at the egg and adult stages. Usually, the females can lay about 500 to 900 eggs. The eggs commonly hatch in four days if the eggs incubated in region, and temperature. Their larval growth period of more than three weeks is longer than that of flies like house and carrion flies (5 days), implying that a single larva can eat more substrate and grow larger pupae (Čičková, Newton, Lacy, & Kozánek, 2014). Furthermore, when BSFL are in the pre-pupa stage, they will instinctively leave the substrate and shift to a strong, clean place, a behaviour known as "self-harvesting," which eliminates an otherwise labour-intensive phase in their farming their larvae are 42 percent crude protein and 29 percent fat, with a higher saturated fat content than most insects. Adults consume nothing but water, do not approach humans, do not bite or sting, and do not vector or disseminate any specific diseases (Yu-Shiang & Matan, 2017). The adults of BSFL measure about 16 mm (5⁄8 in) long (Chul-Hwan, et al., Use of Black Soldier Fly Larvae for Food Waste Treatment and, 2021). These medium-sized flies have a black body, with metallic reflections ranging from blue to green on the thorax and sometimes with a reddish end of the abdomen. BSFL can easily produce with rotting organic matter such as animal waste or decaying crops and from food waste. There are a lot of advantages of BSFL like the operation to produce BSFL is simple. They can be grown and harvested without dedicated facilities and are not pestiferous. Their most significant benefit over other insects is their ability to turn waste into food, generating demand and closing nutrient loops while reducing emissions and costs. This general benefit

is perhaps their biggest drawback when social stigmas and legal bans against consuming animals that consume garbage are applied to the still existing taboos against eating insects (Yu-Shiang & Matan, 2017). Table shows the percentage of crude protein and crude fat from various sources of BSFL.

Table 0.2 The percentage of Crude Protein and Crude Fat from various sources of BSFL

Sources of BSFL	% Crude protein	% Crude fat	References
By-products (Rotten mussels)	32.2 ±0.3	29.7 ±0.3	(Ewald, et al., 2020)
Fruits and Vegetables	38.5	6.63	(Nguyen, Tomberlin, & Vanlaerhoven, 2015)
Chicken manure	40.1 ±2.5	27.9 ±8.3	(Xiaopeng, et al., 2018)
Chicken feed	47.9 ±37.1	14.6 ±4.4	(Nguyen, Tomberlin, & Vanlaerhoven, 2015)
Cattle manure	42.1	34.8 ±29.9	(Qing, et al., 2011)
Restaurant waste	-	39.2	(Chul-Hwan, et al., Use of Black Soldier Fly Larvae for Food Waste Treatment and, 2021)

2.5 Potential application of BSFL in industry

BSFL has big potential application to the industries like agriculture and medical industry. BSFL can be used as biofertilizer. Some of organic matter like manure, food waste and market waste can produce the biogas that can harm our biodiversity. The use of BSFL

is to lower the percentage of biogas. BSFL larvae has potential to convert organic waste and each of larvae can ingest food waste up to 200 mg per day. Due to that potential, it can remove some toxic substances from waste. Recent study of BSFL focus on antimicrobial natural product from BSFL (Soon-IK, Byung Soo, & Sung Moon, 2014). The study identified the cecropins and defensins from larval extraction (Soon-IK, Byung Soo, & Sung Moon, 2014). The cecropin-like peptide 1 (CLP1) exhibits better efficacy than the antibiotic ampicillin towards the Gram-negative bacteria such as *Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* with minimum inhibitory concentration (MIC) values. BSFL can use as a protein source in animal feed because BSFL contains a high rich in protein. *Hermetia illucens,* which is capable of efficiently converting a wide variety of organic materials, from food waste to manure, into insect biomass. Pesticides or mycotoxins are not concentrated they are now cultivated and recommended for use as animal feed, but there are regional legal constraints on how this is accomplished. Larvae may theoretically be milled and processed into a textured protein with a good flavour for consumer use in human foods.

CHAPTER 3

METHODOLOGY

3.1 Sample preparation and processing

3.1.1 Defatting process of Black Soldier Fly Larvae (BSFL)

A total of dried 3 kg of black soldier fly larvae (BSFL) powder was purchased from local producer. Then, the seller was posted to Universiti Malaysia Kelantan Jeli Campus (UMK). About 20 g of BSFL powder was putted into a piece of filter paper. After that, the BSFL powder was deposited in a thimble cellulose. The process of extracting fat from the BSFL powder takes about 5 to 6 h by using Soxhlet method. About 300ml of 95% of ethanol was used as an extractor to extract the BSFL powder. After that, the defatted BSFL powder were taken out and air dried in the open air for more than 48 hours to remove moisture and ethanol.

3.2 Feed formulation

3.2.1 Formulate feed formulation

Win feed software version 2.8 was used to formulate the feed formulation for this project. Chemical composition of raw materials like BSFL, rice bran, fish meal, corn meal, soybean meal and copra meal were determined using proximate analysis. Firstly, select the animal

requirement before starts the formulation. To start the new formulation, the nutrient composition and the limits nutrients for every ingredient was filled manually in the main window. About 5 formulations need to be formulated. For treatment 1, the percentage of defatted BSFL that was used is 10% whereas for treatment 2 fed the percentage of defatted BSFL that was used is 20%. Treatment 3, the percentage of defatted BSFL that was used is 30% and the last treatment which treatment 4 is 40%. The other ingredients that were used in this project is fish meal, soybean meal, copra meal, tapioca, rice bran, corn meal, mineral premix and vitamin. For control group, there were no defatted BSFL used. Table 3.1 shows each formulation.

Table 0.3 Feed Formulation for Each Treatment

INGREDIENTS (g)	Control	10%	20%	30%	40%
Palm oil	1	1	1	1	1
Mineral Premix	1	1	1	1	1
Tapioca	15	15	15	15	15
Copra meal	15	15	15	15	15
Defatted BSFL	0	10	20	30	40
Rice bran	17	14.5	12	9.5	7
Corn meal	17	14.5	12	9.5	7
Soybean meal	17	14.5	12	9.5	7
Fish meal	17	14.5	12	9.5	7
Total	100	100	100	100	100

3.2.2 Production of pellet

The weight of each ingredient was weighed according to the formulation that was formulated. The defatted BSFL, fish meal, soybean meal, copra meal, tapioca, rice bran, corn

meal, mineral premix and vitamin was mixed slowly using mixer for about 1 h to make sure the ingredients mixed properly. Then, the mixture of ingredients was crushed properly into a powder using dried blender. To make pellet, the amount of appropriate water was added into the powder to ensure it bond nicely. The water was added slowly to avoid from over water.

$$\frac{[(\mathrm{mf})(\mathrm{wi}) - (\mathrm{mi})(\mathrm{wi})]}{100 - \mathrm{mf}} \qquad 0.1)$$

Where, mf = final moisture (30%)

wi = sample weight (g)

mi = initial moisture (%)

After the water was added, the mixture was pelletized into small pellet using pelletizer according to the standard size for *M. rosenbergii* juvenile which is 0.9 to 1.4 mm. The pellet was dried inside the oven for a night at 40 °C.

3.3 Proximate analysis

3.3.1 Crude protein

This test was using Kjeldahl test. The digestion block was turned on and heated to 420 °C. The 1 Kjeltabs Cu catalyst tablets and 12 ml of Sulphuric acid were added inside the digestion tube. A sample was placed inside the Kjeldahl tube. The heat side shields were attached to digestion tube. The digestion process takes about 60 min. When the sample finished up the digestion process, the digestion block was turned off and the rack of digestion tubes were removed to let it cool for 20 min. After the sample were cooled down, distilled water was added followed by NaOH. The distillation unit was steamed to clean it up the unit. The digestion tube was attached to distillation unit. On the other side, the receiver was placed on receiving platform. After settles the distillation process, the receiver flask was taken out to undergoes titration process. After slowly added HCL inside the receiver flask, the receiver colour was changed into pink. The amount of HCL used was recorded. The percentage of crude protein were calculated by using following formula:

$$N\ \% = \frac{(-\mathrm{Vb}) \times 0.1 \times 14.007}{w\ (mg)} \times 100$$

0.2)

$$Crude\ protein\ \% = N\ \% \times 6.25$$

Where, V = final volume,

Vb = initial volume,

w = sample weight

3.3.2 Crude fat

Before starts the process, the aluminium cups were heated at 105 °C for 30 min. Then, the aluminium cups were placed inside desiccator for 20 min. The weight of empty aluminium cups was recorded. The weight of sample was weighed according to recommended sample weight based on previous study. The samples were placed inside the thimble and the thimbles were moved to the thimble stand by using the thimble handler. The layer of defatted cotton was placed on top of the sample and the thimbles were moved to the extraction unit using the magnet to attach it. The aluminium cups were filled with solvent (petroleum ether) about 80 ml and the aluminium cups were placed inside extraction unit using cup holder. The RUN button was pressed, and the extraction of fat was started. The Soxtec machine were run the sample for one h. After the one h, the excess fat was got, and the sample were put inside the

oven for 30 min at 105 °C. After that, the sample and the excess fat were let cool down for 20 min before weighing. The dried sample were transfer to small plastic bag and were used later for crude fibre test. The weight of the excess fat was recorded, and the percentage of crude fat were calculated by using following formula:

$$CF\ \% = \frac{B-A}{C} \times 100 \qquad 0.3)$$

Where, A = weight of empty cup (g),

B = weight of cup with fat (g),

C = sample weight (g)

3.3.3 Crude fibre

The samples that have undergo crude fat test were used for crude fibre test. Thus, the weight of the samples will be slightly decreased, for example, from 1 g to 0.8 g. Firstly, the fibre crucible was placed inside the oven for 15 min at 105 °C to make sure there is no water inside the fibre crucible. After 15 min, the crucible was placed inside desiccator to let it cool for 20 min. The sample was placed inside the fibre crucible. Then, the celite was added into the fibre crucible that contains sample before the fibre crucible placed inside the crude fibre test machine. The fibre crucible was labelled according to each sample. NaOH and sulphuric acid was placed inside the solution holder inside the machine. The machine was run for about

2 h. After 2 h, the fibre crucible was placed carefully inside the oven for 2 h at 130 °C followed by cooling process for 20 min in desiccator. After it cool, the fibre crucible with sample was weighed, and the weight was recorded. After weighing, the fibre crucible with sample was placed inside the furnace for 3 h at 525 °C. The weight of the fibre crucible will be taken on the next day. The weight of the fibre crucible with sample after furnace was recorded and the percentage of crude fibre was calculated by using following formula:

$$CF\ \% = \frac{A-B}{C} \times 100 \qquad (0.4)$$

Where, A = weight after oven (g),

B = weight after furnace (g),

C = sample weight (g)

3.3.4 Ash

The crucibles were labelled at bottom of crucible using pencil. After that, the crucible was placed inside the oven for 15 min at 105 °C to make sure there is no water inside the crucible. After 15 min, the crucible was placed inside desiccator to let it cool for 20 min before weighing. The weight of empty, clean, and dry crucible with lid was recorded. About 1 g of sample were placed inside the crucible. Then the crucible that contain sample were placed

inside the furnace for 5 h at 550 °C. After taking out the crucible with sample from furnace, the crucible was let cooled in desiccator for 20 min before weighing. The weight of the sample and crucible with lid were recorded and the percentage of ash were calculated by using following formula:

$$\text{Ash } \% = \frac{W3-W1}{W2} \times 100 \quad (0.5)$$

Where, W1 = weight of empty crucible with lid (g),

W2 = weight of sample (g),

W3 = weight of crucible and ash (g)

3.3.5 Moisture

The aluminium foil was folded until it shapes become a cup that can hold the Sample. The sample was placed inside the folded aluminium foil and the weight of the sample and aluminium foil were recorded. The sample then was placed inside the oven for overnight or 24 h at 105 °C. Then the sample were cooled down inside desiccator for 20 min before weighing. The weight after the oven process were recorded and the percentage of moisture were calculated by using following formula:

$$\text{Moisture } \% = \frac{A-B}{C} \times 100$$

0.6)

Where, A = weight of sample with Al foil (g),

B = weight after oven (g),

C = sample weight (g)

3.4 Statistical analysis

All the collected data was analysed using one-way ANOVA available from Statistical Package for the Social Science (SPSS version 25) to find the significant different between treatment group and followed by Tukey post hoc test at the level of significance 5% ($P<0.05$). Data was presented as mean ± SEM.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Biochemical Composition of different inclusion level of BSFL in *Macrobrachium rosenbergii* feed formulation

The biochemical composition of treated diets and formulated egg custard is reported in Table 4.1.

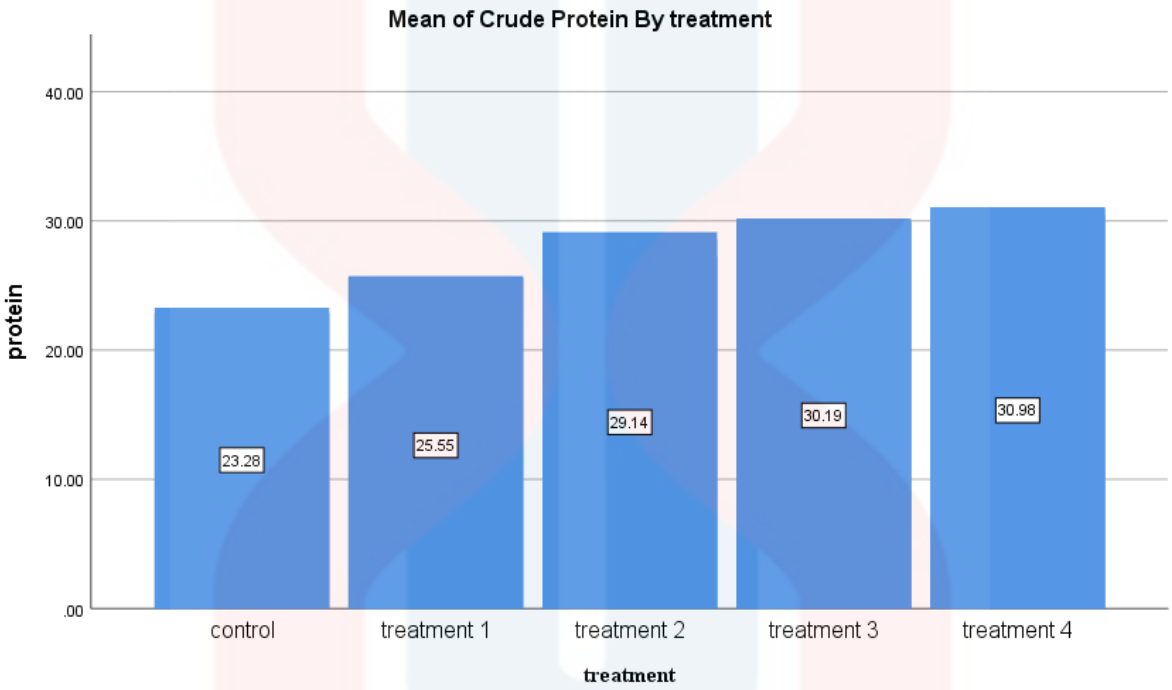
Table 0.4 Biochemical composition of treated diets

Parameter	Test Diet				
	Control	Treatment 1 (10 % of BSFL)	Treatment 2 (20 % of BSFL)	Treatment 3 (30 % of BSFL)	Treatment 4 (40 % of BSFL)
Crude Protein	23.01 ±0.13[a]	25.63 ±0.05[b]	28.29 ±0.56[c]	30.01 ±0.08[d]	30.82 ±0.19[d]
Crude Fat	5.00 ±0.97[a]	6.08 ±0.66[b]	9.29 ±0.34[b]	12.52 ±1.22[c]	12.77 ±0.38[c]
Crude fibre	7.37 ±0.32[a]	10.60 ±0.51[a]	11.67 ±0.76[a]	11.25 ±1.33[b]	9.92 ±0.32[a]
Ash	0.25 ±0.05[a]	0.21 ±0.03[a]	0.16 ±0.19[a]	0.13 ±0.03[a]	0.17 ±0.04[a]
Moisture	26.10 ±0.06[a]	26.48 ±0.72[a]	26.37 ±0.34[a]	24.91 ±0.22[a]	26.66 ±0.37[a]

[abcd] means with different superscripts in a row is significantly difference (P>0.05)

Table 4.1 shows the proximate analysis for crude protein. The crude protein shown that statistically significant (P>0.05) to all treatments with 0.000. The mean of crude protein for Treatment 4 (30.82 ±0.19) which is contain 40% of defatted BSFL is the highest compared to the mean of crude protein for Control (23.01 ±0.13) did not contain any of defatted BSFL, so the percentage of crude protein is the lowest one compared to the other treatments. This is due to the different percentage of defatted BSFL in formulation. The mean of crude protein for Treatment 1 (25.63 ±0.05) which is contain only 10% of defatted BSFL is the second one lowest compared to other treatments. The means of crude protein in Treatment 2 (28.29 ±0.56) and Treatment 3 (30.01 ±0.08). The best crude protein percentage for *Macrobrachium rosenbergii* juveniles that suggested by Food and Agriculture Organization of the United Nations (FAO), 2013 is around 35 – 37%. The reason why juveniles stages need more protein is for their growth development. The best percent of crude protein from this study just about (30.82 ±0.19) from Treatment 4. The main reason why this study did not achieve the standard is due to the technical error that occur during Kjeldahl process. The distillation machine was not purely clean. There are a various type of sample using this machine and the chance of this machine did not clean properly is high and it affects the result because during distillation process, the nitrogen was transferred inside the receiver. Then, the samples should grinded and mixing uniformly to obtain accurate result (Campbell and Plank, 1992). It is because the digestion stage will easily process if the sample in small particles (Sáez-Plaza, P., Navas, M. J., Wybraniec, S., Michałowski, T., & Asuero, A. G. (2013). The blender that used is not suitable to grind the sample. The lack of facilities including for grinded and mixing affecting the final result.

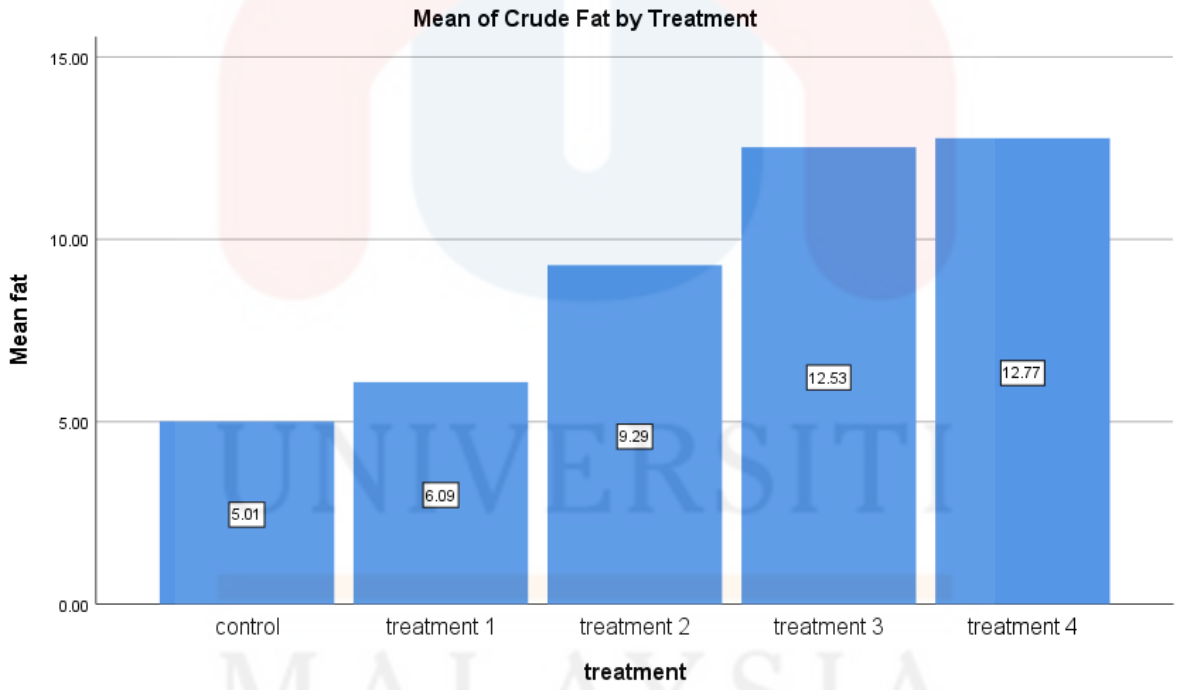
Figure 1 Graph Mean of Crude Protein by Treatments



The crude fat shown that statistically significant (P>0.05) to all treatments with 0.000. As shows in Table 4.1, the crude fat for Treatment 4 (12.77 ±0.38) which is contain 40% of defatted BSFL is the highest compared to Control (5.00 ±0.97) which is did not contain any of defatted BSFL. The differences of crude fat percentage in every diet are due to the different percentage of defatted BSFL in formulation. Treatment 1 (6.08 ±0.66) contain only 10% of defatted BSFL, so the percentage of crude fat is the second lowest one compared to the other treatments. The amount of crude fat in Treatment 2 (9.29 ±0.34) and Treatment 3 (12.52 ±1.22) is not the as much as Treatment 4 because contains only 20% of defatted BSFL for Treatment 2 and 30% of defatted BSFL for Treatment 3. The crude fat content is increase with the addition

percentage of defatted BSFL in formulation. This is because the source of fat is from BSFL (defatted BSFL crude fat 13 – 14 %). The other study also showed that formulation with 9.3% of crude fat was the best for feed composition (Fahrur, M., Asaad, A. I. J., & Fahmi, M. R., 2021).

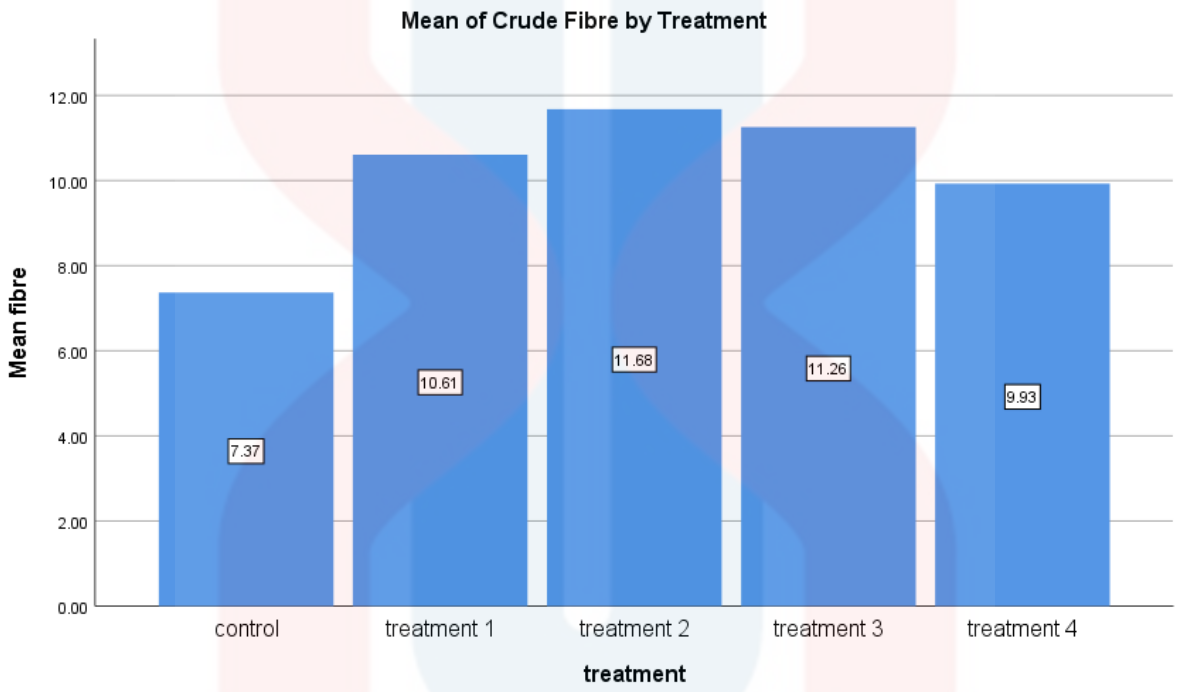
Figure 2 Graph Mean of Crude Fat by Treatments



The crude fibre shown that statistically significant (P>0.05) to all treatments with 0.017. In Table 4.1, the mean of crude fibre for Treatment 2 (11.67 ±0.76) which is contain 20% of

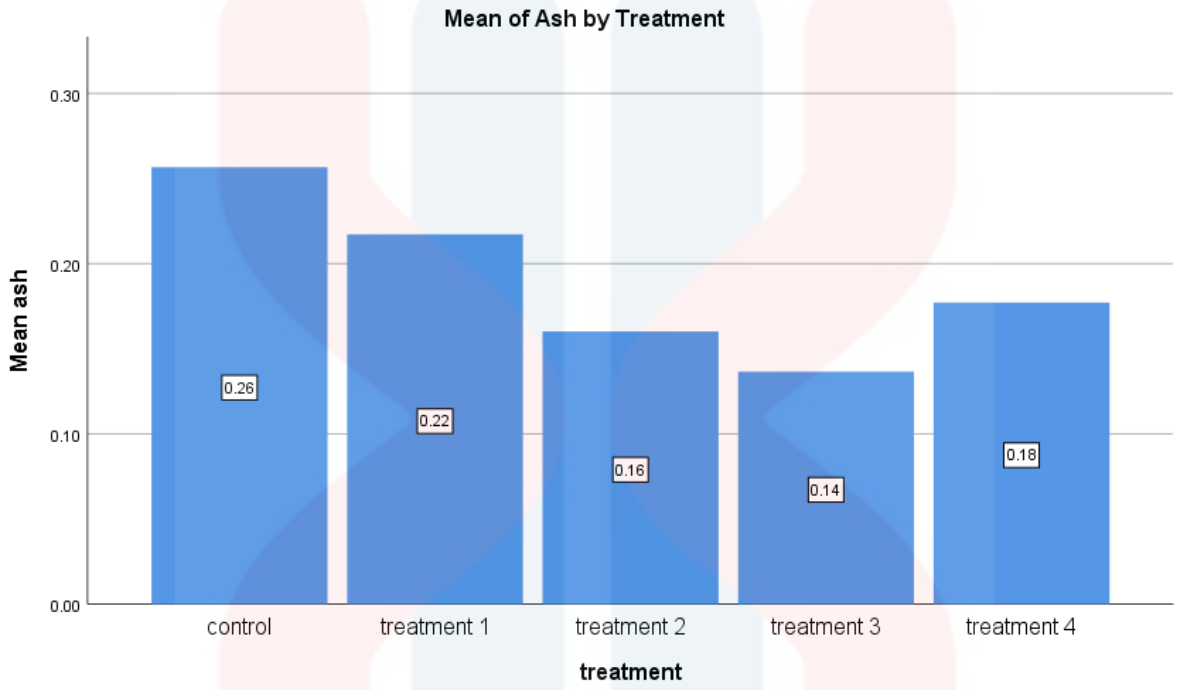
defatted BSFL is the highest compared to Control (7.37 ±0.32) which is did not contain any of defatted BSFL. Treatment 4 (9.92 ±0.32) which is contain 40% of defatted BSFL, show the second lowest one compared to the other treatments. The amount of crude fibre in Treatment 1 (10.60 ±0.51) and Treatment 3 (11.25 ±1.33) is not as much compared to Treatment 4 because contains only 10% of defatted BSFL for Treatment 1 and 30% of defatted BSFL for Treatment 3. The best crude fibre percentage for *Macrobrachium rosenbergii* juveniles that suggested by Food and Agriculture Organization of the United Nations (FAO), 2013 is around 5 - 10%. Based on the suggested crude fibre by FAO, Control (7.37 ±0.32) and Treatment 4 (9.92 ±0.32) are included in the standard percentage crude fibre suggested by FAO. The proximate composition of BSFL for crude fibre is 14.6%. The study shows that BSFL contains chitin, that is an insoluble fibre which resulted percentage of crude fibre increase. The treatment to decrease the percentage of crude fibre in BSFL is pre-treating BSFL with salt. The salt dissolution occurs will breakdown the fibre content (Chen *et al.*, 2014).

Figure 3 Graph Mean of Crude Fibre by Treatments



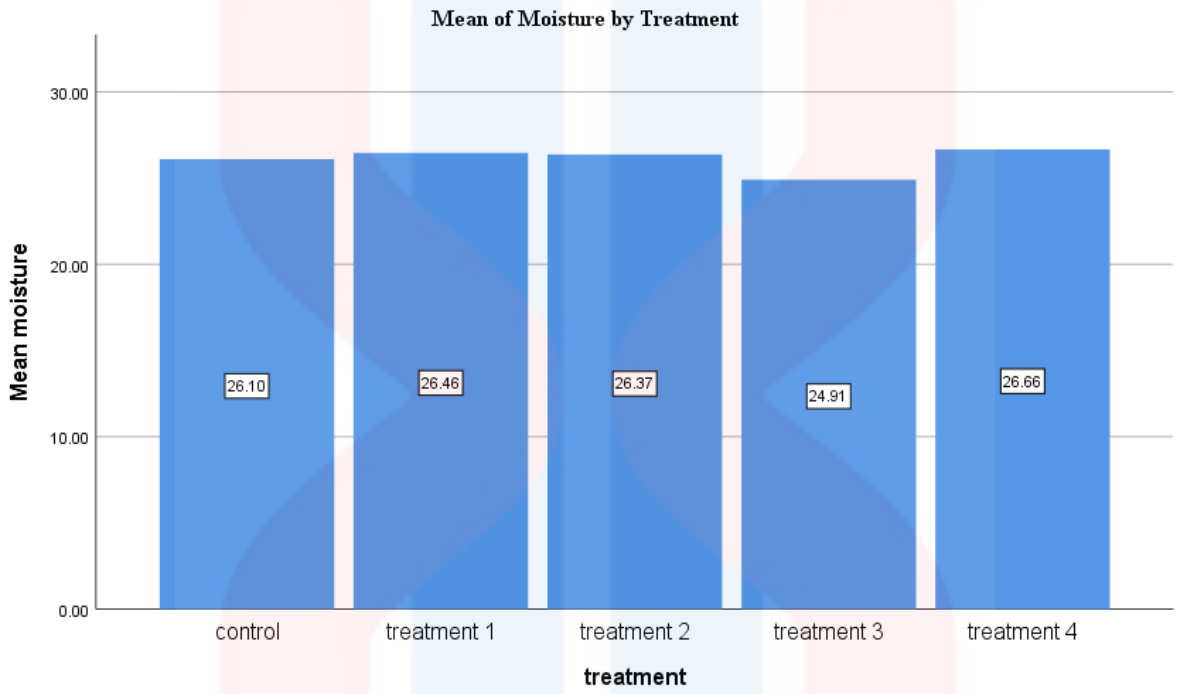
The ash shown that not statistically significant (P>0.05) to all treatments with 0.2670. Based on the result obtained from table 4.1, mean ash score for Control (0.25 ±0.05) was the highest one compared to Treatment 3 (0.13 ±0.03). Treatment 2 (0.16 ±0.19) contain about 20% of defatted, the percentage of ash is the second lowest one compared to the other treatments. The amount of ash in Treatment 1 (0.21 ±0.03) and Treatment 4 (0.17 ±0.04) is not as much compared to other treatment. The best ash percentage for *Macrobrachium rosenbergii* juveniles that suggested by Food and Agriculture Organization of the United Nations (FAO), 2013 is around 10 – 20 %.

Figure 4 Graph Mean of Ash by Treatments



The ash shown that not statistically significant (P>0.05) to all treatments with 0.081. From Table 4.1, mean moisture score for Treatment 4 (26.66 ±0.37) which is contain 40% of defatted BSFL is the highest compared to other treatments. The second highest moisture content is treatment 1 with (26.48 ±0.72) which is contain only 10% of defatted BSFL. The third highest moisture content is treatment 2 with (26.37 ±0.34) and the moisture content for control and treatment 3 are (26.10 ±0.06) and (24.91 ±0.22). The highest percentage of moisture content due to the addition of water in feed formulation to strongly bind the pellet.

Figure 5 Graph Mean of Moisture by Treatments



CHAPTER 5

CONCLUSION

5.1 Conclusion

The present study demonstrated that formulation feed for *M. rosenbergii* juvenile using BSFL can be applied in industry. Most of the problem that had been facing by the farmers is the cost production of the feed. So, the objectives of this study which is to determine the chemical composition of newly formulated feed using BSFL for M. rosenbergii juvenile was achieved. Thus, different inclusion levels of BSFL have significant effect on the chemical composition of *M. rosenbergii* juvenile feed. The best treatment in this study is Treatment 4 because required the nutrient composition of for *M. rosenbergii* juvenile. Although the biochemical composition was identified. Feeding trial is recommended to future determine suitable feed for *M. rosenbergii* juvenile with the study of the acceptance level of *M. rosenbergii* juvenile.

5.2 Recommendation

Creating specific feed for *M. rosenbergii* juvenile by feeding with specific food on BSFL will get different nutrient composition can be achieved with determination on their

biochemical composition. Removal of fish meal for ingredient is recommended due to higher cost and low resource availability and sustainability to reduce the financial support for small-scale aquaculture farmers.

Protein analysis can be done to future identity the content in detail. Acid Amino content of experimental feed able to determine by using high-performance liquid chromatography (HPLC) to improve protein digestibility. Besides, the determination of chitin content of experimental feed can be to enhance growth of *M. rosenbergii* juvenile. Determination of mineral and vitamin content of experimental feed can be test with ultimate analysis.

In the future, feeding trial on the experimental feed needs to be carried out on *M. rosenbergii* juvenile. Feeding trial help to determine the acceptance of the feed for *M. rosenbergii* juvenile since the assumption on replacing BSFL as juvenile feed can be achieved from analysis on their biochemical composition. During feeding trial, the growth performance and survival rate of *M. rosenbergii* juvenile can be monitored. At the same time, effect on water quality of pond such as pH value, dissolved oxygen and salinity after can be monitored and observed.

Storage condition on experimental feed can be testing for determination effect of nutrient content and physical properties. It can be conducted with feeding trial to determine the effect on the growth performance of *M. rosenbergii* juvenile by feeding with different storing conditions of feed from vacuum packaging, chiller and refrigerator. Besides, total plate count (TPC) analysis and bacteria identification on experimental feed help to analyse the existence of the microbe on feed.

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APPENDIXES

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
protein	control	3	23.0125	.23150	.13366	22.4374	23.5876	22.84	23.28
	treatment 1	3	25.6375	.08750	.05052	25.4201	25.8549	25.55	25.73
	treatment 2	3	28.2917	.98348	.56781	25.8486	30.7348	27.21	29.14
	treatment 3	3	30.0125	.15155	.08750	29.6360	30.3890	29.93	30.19
	treatment 4	3	30.8292	.33127	.19126	30.0062	31.6521	30.45	31.06
	Total	15	27.5567	3.01380	.77816	25.8877	29.2257	22.84	31.06
fat	control	3	5.0091	1.69133	.97649	.8076	9.2106	3.53	6.85
	treatment 1	3	6.0859	1.15563	.66720	3.2151	8.9566	5.10	7.36
	treatment 2	3	9.2925	.59188	.34172	7.8222	10.7629	8.64	9.79
	treatment 3	3	12.5271	2.12274	1.22557	7.2539	17.8003	10.38	14.62
	treatment 4	3	12.7745	.66446	.38363	11.1238	14.4251	12.05	13.36
	Total	15	9.1378	3.50794	.90575	7.1952	11.0804	3.53	14.62
fibre	control	3	7.3700	.56666	.32716	5.9623	8.7777	6.72	7.76
	treatment 1	3	10.6067	.88625	.51167	8.4051	12.8082	10.09	11.63
	treatment 2	3	11.6767	1.31668	.76018	8.4059	14.9475	10.40	13.03
	treatment 3	3	11.2567	2.31656	1.33746	5.5020	17.0113	9.58	13.90
	treatment 4	3	9.9267	.56501	.32621	8.5231	11.3302	9.41	10.53
	Total	15	10.1673	1.92120	.49605	9.1034	11.2313	6.72	13.90
ash	control	3	.2566	.09846	.05685	.0120	.5012	.15	.34
	treatment 1	3	.2172	.05397	.03116	.0831	.3513	.16	.27
	treatment 2	3	.1602	.03321	.01917	.0777	.2427	.14	.20
	treatment 3	3	.1365	.05360	.03095	.0034	.2697	.09	.19
	treatment 4	3	.1772	.07578	.04375	-.0111	.3654	.12	.26
	Total	15	.1895	.07167	.01851	.1498	.2292	.09	.34
moisture	control	3	26.1033	.10504	.06064	25.8424	26.3643	26.00	26.21
	treatment 1	3	26.4633	1.26057	.72779	23.3319	29.5948	25.50	27.89
	treatment 2	3	26.3700	.60225	.34771	24.8739	27.8661	25.80	27.00
	treatment 3	3	24.9100	.38354	.22143	23.9572	25.8628	24.50	25.26
	treatment 4	3	26.6633	.65577	.37861	25.0343	28.2924	25.99	27.30
	Total	15	26.1020	.88216	.22777	25.6135	26.5905	24.50	27.89

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
protein	Between Groups	124.840	4	31.210	134.387	.000
	Within Groups	2.322	10	.232		
	Total	127.162	14			
fat	Between Groups	153.291	4	38.323	20.183	.000
	Within Groups	18.988	10	1.899		
	Total	172.279	14			
fibre	Between Groups	34.622	4	8.656	5.076	.017
	Within Groups	17.052	10	1.705		
	Total	51.674	14			
ash	Between Groups	.027	4	.007	1.526	.267
	Within Groups	.045	10	.004		
	Total	.072	14			
moisture	Between Groups	5.815	4	1.454	2.862	.081
	Within Groups	5.080	10	.508		
	Total	10.895	14			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Protein	control	treatment 1	-2.62500*	.39348	.000	-3.9200	-1.3300
		treatment 2	-5.27917*	.39348	.000	-6.5741	-3.9842
		treatment 3	-7.00000*	.39348	.000	-8.2950	-5.7050
		treatment 4	-7.81667*	.39348	.000	-9.1116	-6.5217
	treatment 1	Control	2.62500*	.39348	.000	1.3300	3.9200
		treatment 2	-2.65417*	.39348	.000	-3.9491	-1.3592
		treatment 3	-4.37500*	.39348	.000	-5.6700	-3.0800
		treatment 4	-5.19167*	.39348	.000	-6.4866	-3.8967
	treatment 2	Control	5.27917*	.39348	.000	3.9842	6.5741
		treatment 1	2.65417*	.39348	.000	1.3592	3.9491
		treatment 3	-1.72083*	.39348	.009	-3.0158	-.4259
		treatment 4	-2.53750*	.39348	.001	-3.8325	-1.2425
	treatment 3	Control	7.00000*	.39348	.000	5.7050	8.2950
		treatment 1	4.37500*	.39348	.000	3.0800	5.6700
		treatment 2	1.72083*	.39348	.009	.4259	3.0158
		treatment 4	-.81667	.39348	.301	-2.1116	.4783
	treatment 4	Control	7.81667*	.39348	.000	6.5217	9.1116
		treatment 1	5.19167*	.39348	.000	3.8967	6.4866
		treatment 2	2.53750*	.39348	.001	1.2425	3.8325
		treatment 3	.81667	.39348	.301	-.4783	2.1116
fat	control	treatment 1	-1.07677	1.12511	.868	-4.7796	2.6260
		treatment 2	-4.28343*	1.12511	.022	-7.9862	-.5806
		treatment 3	-7.51800*	1.12511	.000	-11.2208	-3.8152
		treatment 4	-7.76537*	1.12511	.000	-11.4682	-4.0626
	treatment 1	Control	1.07677	1.12511	.868	-2.6260	4.7796
		treatment 2	-3.20667	1.12511	.099	-6.9095	.4961
		treatment 3	-6.44123*	1.12511	.001	-10.1440	-2.7384
		treatment 4	-6.68860*	1.12511	.001	-10.3914	-2.9858
	treatment 2	Control	4.28343*	1.12511	.022	.5806	7.9862
		treatment 1	3.20667	1.12511	.099	-.4961	6.9095
		treatment 3	-3.23457	1.12511	.095	-6.9374	.4682
		treatment 4	-3.48193	1.12511	.068	-7.1847	.2209
	treatment 3	Control	7.51800*	1.12511	.000	3.8152	11.2208
		treatment 1	6.44123*	1.12511	.001	2.7384	10.1440
		treatment 2	3.23457	1.12511	.095	-.4682	6.9374
		treatment 4	-.24737	1.12511	.999	-3.9502	3.4554
	treatment 4	Control	7.76537*	1.12511	.000	4.0626	11.4682

		treatment 1	6.68860*	1.12511	.001	2.9858	10.3914
		treatment 2	3.48193	1.12511	.068	-.2209	7.1847
		treatment 3	.24737	1.12511	.999	-3.4554	3.9502
fibre	control	treatment 1	-3.23667	1.06620	.074	-6.7456	.2723
		treatment 2	-4.30667*	1.06620	.016	-7.8156	-.7977
		treatment 3	-3.88667*	1.06620	.029	-7.3956	-.3777
		treatment 4	-2.55667	1.06620	.193	-6.0656	.9523
	treatment 1	control	3.23667	1.06620	.074	-.2723	6.7456
		treatment 2	-1.07000	1.06620	.848	-4.5789	2.4389
		treatment 3	-.65000	1.06620	.970	-4.1589	2.8589
		treatment 4	.68000	1.06620	.965	-2.8289	4.1889
	treatment 2	control	4.30667*	1.06620	.016	.7977	7.8156
		treatment 1	1.07000	1.06620	.848	-2.4389	4.5789
		treatment 3	.42000	1.06620	.994	-3.0889	3.9289
		treatment 4	1.75000	1.06620	.506	-1.7589	5.2589
	treatment 3	control	3.88667*	1.06620	.029	.3777	7.3956
		treatment 1	.65000	1.06620	.970	-2.8589	4.1589
		treatment 2	-.42000	1.06620	.994	-3.9289	3.0889
		treatment 4	1.33000	1.06620	.726	-2.1789	4.8389
	treatment 4	control	2.55667	1.06620	.193	-.9523	6.0656
		treatment 1	-.68000	1.06620	.965	-4.1889	2.8289
		treatment 2	-1.75000	1.06620	.506	-5.2589	1.7589
		treatment 3	-1.33000	1.06620	.726	-4.8389	2.1789
ash	control	treatment 1	.03940	.05456	.946	-.1402	.2190
		treatment 2	.09643	.05456	.440	-.0831	.2760
		treatment 3	.12007	.05456	.254	-.0595	.2996
		treatment 4	.07943	.05456	.609	-.1001	.2590
	treatment 1	control	-.03940	.05456	.946	-.2190	.1402
		treatment 2	.05703	.05456	.829	-.1225	.2366
		treatment 3	.08067	.05456	.597	-.0989	.2602
		treatment 4	.04003	.05456	.944	-.1395	.2196
	treatment 2	control	-.09643	.05456	.440	-.2760	.0831
		treatment 1	-.05703	.05456	.829	-.2366	.1225
		treatment 3	.02363	.05456	.992	-.1559	.2032
		treatment 4	-.01700	.05456	.998	-.1966	.1626
	treatment 3	control	-.12007	.05456	.254	-.2996	.0595
		treatment 1	-.08067	.05456	.597	-.2602	.0989
		treatment 2	-.02363	.05456	.992	-.2032	.1559
		treatment 4	-.04063	.05456	.941	-.2202	.1389
	treatment 4	control	-.07943	.05456	.609	-.2590	.1001

		treatment 1	-.04003	.05456	.944	-.2196	.1395
		treatment 2	.01700	.05456	.998	-.1626	.1966
		treatment 3	.04063	.05456	.941	-.1389	.2202
moisture	control	treatment 1	-.36000	.58194	.969	-2.2752	1.5552
		treatment 2	-.26667	.58194	.990	-2.1819	1.6485
		treatment 3	1.19333	.58194	.311	-.7219	3.1085
		treatment 4	-.56000	.58194	.866	-2.4752	1.3552
	treatment 1	control	.36000	.58194	.969	-1.5552	2.2752
		treatment 2	.09333	.58194	1.000	-1.8219	2.0085
		treatment 3	1.55333	.58194	.130	-.3619	3.4685
		treatment 4	-.20000	.58194	.996	-2.1152	1.7152
	treatment 2	control	.26667	.58194	.990	-1.6485	2.1819
		treatment 1	-.09333	.58194	1.000	-2.0085	1.8219
		treatment 3	1.46000	.58194	.164	-.4552	3.3752
		treatment 4	-.29333	.58194	.985	-2.2085	1.6219
	treatment 3	control	-1.19333	.58194	.311	-3.1085	.7219
		treatment 1	-1.55333	.58194	.130	-3.4685	.3619
		treatment 2	-1.46000	.58194	.164	-3.3752	.4552
		treatment 4	-1.75333	.58194	.077	-3.6685	.1619
	treatment 4	control	.56000	.58194	.866	-1.3552	2.4752
		treatment 1	.20000	.58194	.996	-1.7152	2.1152
		treatment 2	.29333	.58194	.985	-1.6219	2.2085
		treatment 3	1.75333	.58194	.077	-.1619	3.6685

*. The mean difference is significant at the 0.05 level.

protein

Tukey HSD[a]

treatment	N	Subset for alpha = 0.05			
		1	2	3	4
control	3	23.0125			
treatment 1	3		25.6375		
treatment 2	3			28.2917	
treatment 3	3				30.0125
treatment 4	3				30.8292
Sig.		1.000	1.000	1.000	.301

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

fat

Tukey HSD[a]

treatment	N	Subset for alpha = 0.05		
		1	2	3
control	3	5.0091		
treatment 1	3	6.0859	6.0859	
treatment 2	3		9.2925	9.2925
treatment 3	3			12.5271
treatment 4	3			12.7745
Sig.		.868	.099	.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

fibre

Tukey HSD[a]

treatment	N	Subset for alpha = 0.05	
		1	2
control	3	7.3700	

treatment 4	3	9.9267	9.9267
treatment 1	3	10.6067	10.6067
treatment 3	3		11.2567
treatment 2	3		11.6767
Sig.		.074	.506

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ash

Tukey HSD[a]

		Subset for alpha = 0.05
treatment	N	1
treatment 3	3	.1365
treatment 2	3	.1602
treatment 4	3	.1772
treatment 1	3	.2172
control	3	.2566
Sig.		.254

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

moisture

Tukey HSD[a]

		Subset for alpha = 0.05
treatment	N	1
treatment 3	3	24.9100
control	3	26.1033
treatment 2	3	26.3700
treatment 1	3	26.4633
treatment 4	3	26.6633
Sig.		.077

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

huhu

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