Effect of Different Inclusion Rate of Pre-Treated BSFL on Physical and Nutrition Composition as Feed for *Macrobrachium rosenbergii* Larvae

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A thesis submitted in fulfillment of the requirements for the degree of Bachelor of Applied Science (Food Security) with Honours

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DECLARATION

I declare that the work embodied in this thesis is from my own research except for the content that has been cited and summarised I have made clear of the sources.

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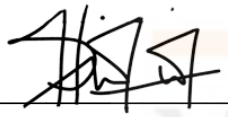

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ABSTRACT

In the current study, optimization of feed formulation using pre-treated-BSFL as feed for *Macrobrachium rosenbergii* larvae was carried out. In many places, the giant freshwater shrimp (*Macrobrachium rosenbergii*) is one of the most commercially important freshwater aquaculture species. The production of *Macrobrachium rosenbergii* has grown and increased every year. Along with the growth of production of aquaculture species, feed production also increased. *Artemia* nauplii are the main living feed, with egg custard serving as a substitute feed. Black Soldier Fly (BSFL) larvae are high in protein and can be used as feed for *M. rosenbergii* larvae. Meanwhile, treatment of egg custard using BSFL larvae is very important to increase the protein content of *M. rosenbergii* larvae. This study aims to produce feed that can help in producing fast-growing and healthy *M. rosenbergii* larvae. Different percentages of BSFL larvae consumption i.e. at 10%, 20%, 30%, and 40% were used for pre-treatment of egg custard with BSFL larvae while egg custard without BSFL would be used as a control diet. Findings from this study showed that egg custard without BSFL showed a higher protein decrease than egg custard with BSFL. In addition, egg custard with 40% BSFL had the highest crude protein content compared to egg custard with 10% BSFL which had the lowest crude protein content. Next, in terms of physical properties egg custard with BSFL is suitable for *M. rosebergii* larvae. This study provides evidence that egg custard formulated with BSFL has optimal nutrients that meet the requirements for *M. rosenbergii* larvae.

Keywords: Black Soldier Fly Larvae, Egg Custard, *Macrobrachium rosenbergii*, Protein Content, Feed Formulation

Kesan Kadar Pemasukan Berbeza BSFL Pra-rawatan Terhadap Komposisi Fizikal dan Nutrisi sebagai makanan untuk Larva Udang Galah (*Macrobrachium rosenbergii*)

ABSTRAK

Dalam kajian ini, Pengoptimuman Formulasi Makanan menggunakan Pra rawatan-BSFL sebagai Makanan untuk Larva *Macrobrachium rosenbergii* telah dijalankan. Di banyak tempat, udang air tawar gergasi (Macrobrachium rosenbergii) adalah salah satu spesies akuakultur air tawar yang paling penting secara komersial. Keluaran *Macrobrachium rosenbergii* telah berkembang dan meningkat pada setiap tahun. Seiring dengan pertumbuhan pengeluaran spesies akuakultur, pengeluaran makanan juga meningkat. Artemia nauplii ialah makanan hidup utama, dengan kastard telur berfungsi sebagai makanan gantian. Larva Black Soldier Fly (BSFL) mengandungi protein yang tinggi dan boleh digunakan sebagai makanan larva *M. rosenbergii*. Sementara itu, rawatan kastard telur dengan menggunakan BSFL adalah sangat penting untuk meningkatkan kadar protein bagi larva *M. rosenbergii*. Kajian ini bertujuan untuk menghasilkan makanan yang boleh membantu dalam menghasilkan larva *M. rosenbergii* yang cepat membesar dan sihat. Peratusan penggunaan BSFL yang berbeza iaitu pada 10%, 20%, 30% dan 40% digunakan untuk rawatan kastard telur dengan BSFL manakala kastard telur tanpa BSFL akan digunakan sebagai diet kawalan. Dapatan daripada kajian ini, menunjukkan bahawa kastard telur tanpa BSFL menunjukkan penurunan protein yang lebih tinggi berbanding kastard telur dengan BSFL. Di samping itu, kastard telur dengan 40% BSFL mempunyai kandungan protein kasar yang tertinggi berbanding kastard telur dengan 10% BSFL yang mempunyai kandungan protein kasar yang paling rendah. Seterusnya, dari segi ciri-ciri fizika kastard telur dengan BSFL sesuai untuk larva *M. rosebergii*. Kajian ini memberikan bukti bahawa kastard telur yang dirumus dengan BSFL mempunyai nutrien optimum yang memenuhi keperluan untuk larva *M. rosenbergii*.

Kata kunci: Larva Lalat Askar Hitam, Kastard Telur, *Macrobrachium Rosenbergii*, Kandungan Protein, Formulasi Makanan

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CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Giant freshwater prawns have become an important component of global aquaculture both in terms of quantity and value. It is one of the most important species for culture due to superior cultivable attributes such as a very fast growth rate, high market demand, hardiness, euryhaline nature, and its compatibility to grow with cultivable finfishes. Current data suggest that a digestible protein level of above 30% is required for maximum growth and protein efficiency. Ingredients such as mussel meat meal, squid meal, and shrimp meal serve as potential sources of protein

in formulated diets, but these materials are difficult to obtain in large quantities and have a difficult storage life. The livestock development of *M. rosenbergii* in Malaysia is limited due to several constraints. The most important is insufficient supply and poor quality post-production of *M. rosenbergii* larvae. Therefore, the strategy of increasing the post-larval production of *M. rosenbergii* is very important, especially in private hatcheries in Malaysia. The demand for *M. rosenbergii* post-larval offspring is increasing day by day but post-larval is not enough to meet demand, although there are several commercial hatcheries. Currently, many *M. rosenbergii* post-larval hatcheries in Malaysia do not operate due to a lack of proper scientific technology and are unable to produce quality post -larval (PL) systematically.

Along with the rapid growth of the aquaculture industry, there has been an increase in food production. *Artemia nauplii* and *Moina* are foods consumed at different larval levels. This type of food may be productive and reliable but expensive and this may be detrimental to small farmers. The utilization of locally available low-cost feed resources is the key to sustainable commercial production of aquaculture species including *M.rosenbergii*. The search for alternative protein sources has increased recently with the goal of producing low-cost, effective diets and high-protein feeds. Based on the mentioned problems the potential of BSFL as a protein substitute is very precise because it is well known that BSFL has a high protein content and is insect-free from disease. This study aims to improve the growth and survivability of *M. rosenbergii* larvae using BSFL.

1.2 PROBLEM STATEMENT

About 70% of the aquaculture cost of production is from feed cost. The problems faced by giant freshwater prawn producers are dependence on imported food for larval levels, low productivity levels, and outdated culture technology. This problem resulted in lower production volumes for the number of giant freshwater prawns. To ensure high prawn survival, good feeding practices and nutrition must begin at the larvae stage. The implementation of foods with better nutrient content can increase productivity levels and reduce dependence on high-cost imported foods. Healthy larval growth and survival throughout rearing will produce healthy post-larvae (PL).

1.3 HYPOTHESIS

H_0: Feed formulation using different inclusion rates of BSFL will not significant effects the growth and survivability of *M. rosenbergii* larvae

H_1: Feed formulation using different inclusion rates of BSFL will significantly affect the growth and survivability of *M. rosenbergii* larvae

1.4 OBJECTIVE

The main objective of the present study is to investigate the potential application of BSFL as feed for *M. rosenbergii* larvae. The specific objective is as follows:

1. To determine the effect on nutrition composition feed formulation for *M. rosenbergii* larvae.
2. To determine the effect on physical feed formulation for *M. rosenbergii* larvae.

1.5 SIGNIFICANCE OF STUDY

The outcome from this research project will provide data on nutrient composition and the quality of the feed formulated in this study. Potential application BSFL will be determined at the end of this. The application of BSFL as the main feed ingredient will significantly reduce the cost of feeding *M. rosenbergii* larvae.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of *M. rosenbergii*

The giant freshwater prawn scientifically known as *M. rosenbergii* is one of the freshwater prawns that can be found throughout the tropical and subtropical areas of the Indo-Pacific region, from India to Southeast Asia and Northern Australia. The giant freshwater prawn has also been introduced in Africa, Thailand, China, Japan, New

Zealand, the Americas, and the Caribbean (Lingyun, 2019). Table 2.1 shows the taxonomy of giant freshwater prawns.

Table 2.1 Taxonomy of Giant Freshwater Prawn (De Man, 1879)

Kingdom	**Animalia**
Phylum	Arthropoda
Class	Malacostraca
Order	Decapoda
Family	Palaemonidea
Genus	Macrobrachium
Species	*Macrobrachium rosenbergii*

In Malaysia, the giant freshwater prawn *M. rosenbergii* is becoming an increasingly important targeted species, as its culture, is considered to have the potential to raise income among impoverished farmers. Although the total production did not change significantly in 2019 compared to the previous year, the aquaculture production of *M. rosenbergii* increased from 213.42 tons in 2018 to 361.43 tons in 2019 (DOF,

2018)(DOF,2019). The number and production of food factories increased in 2019 compared to four years ago. Until recently, lack of post-larval breeding (PL) and stable food have been important barriers to the further development and expansion of *M. rosenbergii* cultures (Department of Fisheries, 2016) This paper reviews the current status of freshwater prawn culture in Malaysia and background history, and future prospects of freshwater prawn farming. It was concluded that freshwater prawn farming in Malaysia has a favorable scenario for expansion due to increasing demand and to prospects of an improved organization of the productive chain.

The minimum amount of protein estimated for juvenile *M. rosenbergii* is between 30 to 35% of the diet (Millikin, 1980). The basis of protein requirements is the essential amino acid requirements for growth and protein as a source of energy. The appropriate amount and type of dietary carbohydrates and lipids can reduce the use of dietary protein for energy. This condition allows the optimal dietary protein to be channeled into the tissues and reduces the amount of dietary protein for maximum growth. Age can affect dietary protein requirements. Effective food consumption depends on the protein/energy ratio of the diet because the amount of energy from non-protein sources affects the amount of consumption in the diet. Protein reduction as an energy source for juveniles *M. rosenbergii* occurs at a 1: 4 lipid to carbohydrate ratio. The highest growth and survival rates observed are at dietary protein: starch ratio of 1: 1 (Gomez Diaz, 2014)

During the early larval stage, the larvae of *M. rosenbergii* are very fragile, very small, and rely solely on their digestive system. Larvae of *M. rosenbergii* have an undeveloped intestine until they reach larval stage V to VI (D'Abramo and Kutty, 2010). Therefore, they should not be fed and digested artificial foods during the early

stages. During the first larval stage, *M. rosenbergii* is carnivorous and is fed with direct food such as zooplankton, to larval stages V to VI. Level VII larvae and beyond, larvae begin to become more omnivorous where they feed on a variety of foods including plant material and other invertebrates. Due to its highly fragile physique, *M. rosenbergii* larvae are not good hunters and will only catch nearby food. Live food will move strongly in the water so that it is not caught, which will cause the larvae to move quickly to chase the live food. Therefore, it can damage fragile larvae. In addition, the size of live or prey food is also an important feature of larval food. The size of the prey should not be larger than that of *M. rosenbergii* larvae (D'Abramo and Kutty, 2010).

2.2 Black Soldier Fly Hermetia illucens

The Black Soldier Fly or *Hermetia illucens* native to the tropical and hot climate zones of the American continent are insects proposed to serve as an alternative protein source that can be used for animal feed and aid in the disposal of organic waste and -products. It has spread to Europe, India, Asia, and Australia and originated in about 80% of the world between latitudes 46 ° N and 42 ° S as a result of climate change and human activities (Martínez-Sánchez, 2011).

There are four life stages for the Black Soldier Fly, which are eggs, larvae, pupae, and adults. Larvae hatch from eggs after 3-4 days of laying. It will turn into a prepupa within two weeks depending on food availability, humidity levels, and relative temperature. Puparization is a process that occurs when a prepupa turns into a pupa.

The process takes about two weeks in a dry medium where the prepupa will enter and embryonic development takes place in the puparium. During puparization, the prepupa will become stiff and immobile. The pupae will remain in the pores and hibernate for two weeks, embryonic development takes place in the exoskeletal casing which will eventually open and adult flies will emerge (Sheppard, 2002). The adult Black Soldier Fly is black and 15-20 mm long with a pair of black smoky wings. His body consists of his head, thorax, and abdomen. Adult black soldier flies will mate and breed in a lifespan of 5–12 days. The entire life cycle for the Black Soldier Fly to reach adulthood is around 40-43 days under optimal conservation conditions and most of the life cycle is spent at the larval and pupa stages (Diclaro, 2009).

2.3 Egg Custard

Nutritionally complete, formulated diets are seen as attractive and valuable alternatives to live food (Kovalenko *et al*., 2002). With the advanced technology and knowledge, egg custard has become a wet protein diet to feed *M. rosenbergii* larvae (Valenti & Daniels, 2000) to reduce the usage of *Artemia sp.* As a result, chicken eggs and skimmed milk become the main protein sources for *M. rosenbergii* larvae. In addition, the use of *Artemia sp.* alone might not fulfill the nutritional requirements of larvae and shrimp hatcheries (New, 1995; Nik Sin & Shapawi, 2016). Thus, New (1995) advised to use supplemental diets, such as egg custard and formulated feeds to replace live food (*Artemia sp.*) partially and completely (Nik Sin & Shapawi, 2016). There are

various types of egg custard with the respective formulation. General preparation of the egg custard is using chicken egg, the main ingredient of egg custard, and steam them until harden. Next, the egg custard passed through a test siever with the desired mesh size and weighs the particulate egg custard according to the desired quantity needed for feeding prawn and shrimp (Kungvankij *et al*., 1985).

2.4 Hardness

The hardness of feed pellets has a direct impact on their appearance and production properties. Controlling the hardness of feed pellets is thus a challenge for feed manufacturers. The feed ingredient grinding process, feed ingredient combining, water adding and steam modulating procedure, post-curing technology, and other elements all have an impact on hardness. The physical quality of pelleted feed may contribute to production efficiency and animal health, which is also an important factor in customer selection. Hence, we must measure the hardness to maintain and fulfill the customer demand. This measure should be possible to perform while the feed is being manufactured and should be indicative as to how the feed will appear when it arrives the animal. The hardness measurement is important to produce suitable pellets for target species and not too rough for them. Based on Cuperlovi. C et al (1973), hardness has been shown to influence the availability of nitrogenous components for intestinal absorption. Based on M. Thomas et al (1996), the hardness of a material is determined by employing equipment that measures the force required to fracture a pellet. In general, tension, compression, and impact-based devices may be distinguished, with the compression component being the most essential. Based on Winowiski. T (n.d.), pellet

hardness is tested one pellet at a time. Stokes and Pfizer created the first hardness tests. The Kahl Hardness Tester is now the most often used, however other instruments like the Acme Penetrometer can also be useful.

2.5 Bulk Density

Bulk density is defined as the weight of fiber per unit volume, often expressed as gmL−1, and is a good index of structural changes (Sreerama et al., 2009). Based on Heidarbeigi et al (2009), bulk and true densities are significant in the design of grain separating and cleaning procedures, as well as the size of grain hoppers and storage facilities. They can alter the rate of heat and mass transfer of moisture during the aeration and drying processes. Based on research carried out by Rotimi. D et. al (2017), they had been investigated some engineering characteristics of fish feed pellets of different sizes in Nigeria. It is due to a lack of information on the engineering properties of fish feed pellets, which provide an essential database and unique understanding for engineers, food scientists, and processors to design equipment for handling operations, conveying, packaging, floatability, separation, drying, storage, and process for optimum efficiency. It was shown that the average bulk and true densities of the fish feed pellets studied were 494±12.63 kg m-3, 501±17.92 kg m-3 570±19.3 and 586±14.93 and 785±21.47, 858±29.39, 894±16.28, 963±21.38 for 2.0 mm, 3.0 mm, 4.0 mm and 6.0 mm respectively. This indicates that increasing pellet size leads to an increase in bulk and actual densities for all four pellet sizes.

2.6 Sinking Velocity

Based on the study of Leah R. F. et al (2008), the effects of food type and concentration on fecal pellet characteristics of the calanoid copepod *Acartia tonsa* were examined in the laboratory. The resultant faecal pellets were measured in length, width, and density. Based on these factors, sinking rates were computed using a semi-empirical model. Diets that produced big pellets produced the least dense pellets in general. In this study, the coefficient of variation of pellet sinking rates across all diets was over 40%. If the diet is not accounted for, this demonstrates the uncertainty in anticipated sinking rates. They define an L-ratio, which is the proportion of pellet degradation per unit length of sinking, by combining the sinking rates from this investigation with published diet-specific faecal pellet degradation rates. The L-ratio might be useful in forecasting the degree of pellet recycling inside the mixed layer.

Next, the research by Obirikorang. K. et. al (2015) had been carried out to investigate the effects of the inclusion of three oilseed by-products (soybean, copra, and palm kernel meals) on some physical characteristics of pelletized feeds as well as on voluntary feed intake and faecal matter production by the Nile tilapia, *Oreochromis niloticus*. The results had shown that sinking velocities of the pellets were positively correlated ($p < 0.05$; r2= 0.979) with feed bulk densities. The pellets of the soybean meal diet recorded the highest mean sinking velocity of 10.75 ± 0.99 cm s^{-1} which was significantly higher ($p < 0.05$) than the pellet sinking velocities of the other three diets. The palm kernel meal diet and the control diets recorded similar mean sinking velocities of 7.70 ± 0.84 and 7.80 ± 0.95 cm s^{-1} respectively. The 30% inclusion of copra meal to the diet reduced the mean sinking velocity of the resulting pellets to 7.13 ± 1.06 cm s^{-1} which was significantly lower ($p < 0.05$) than the other feed types. Since the sinking velocities of all the various feed pellets were well-suited to the eating habits of Nile

tilapia, it is doubtful that the feed pellets used in this study will greatly flesh out the nutrients intended for the target species before ingestion.

Moreover, the other study of Vassallo. P. et. al (2005) had been carried out the determination of physical properties of feed pellets for Mediterranean aquaculture. Even though some data on the physical properties of feed pellets have been published in the context of salmonid rearing, there is a complete lack of information related to the Mediterranean Sea in terms of typical temperature, salinity, and feed composition for Gilthead Sea Bream (*Sparus aurata L.*) and Sea Bass (*Dicentrarchus labrax L.*). They measured the diameters, water adsorption characteristics, floating durations, and sinking velocities of a typical developing sequence of pellets for the above species in a laboratory setting that replicated Mediterranean Seawater. The results show that the sinking velocity rises with pellet size, from 0.087 ms^{-1} for the smallest (3mm) pellet to 0.144 ms^{-1} for the largest (5mm) particle. The largest pellet extruded (6 mm) descends at a slower rate (0.088 ms^{-1}). The floating time before the pellet falls is discovered to be a crucial factor in influencing sinking velocity. According to the linear Stokes' Law, a particle falls in seawater with a sinking velocity depending upon its dimensions, density, and viscosity of the medium. The sinking velocity of the feed pellets is non-Stokessian, due to the higher Reynolds number of the flow and the form factor of the pellets as mentioned by both Chen et al. (1999) and Elberizon & Kelly (1998).

CHAPTER 3

METHODOLOGY

3.1 Material and Apparatus

Material and apparatus used in this study were skimmed milk powder, grade A chicken egg, Black Soldier Fly Larvae (BSFL), hand mixer, a steamer set, distilled water, weighing scale, analytical balance, spatula, 300-micron test siever, bowl, aluminium foil, Kjeldahl tablets (contain Copper (II) Sulphate (CuSO4), Sodium Hydroxide (NaOH), Hydrochloric Acid (HCl), Sodium Dihydrogen Phosphate (NaH2PO4), Boric Acid (H3BO3), Potassium Sulphate (K2SO4), Sulphuric Acid (H2SO4), Potassium Dihydrogen Phosphate (KH2PO4), Bradford reagent, Petroleum Ether, Celite 545, 95% Ethanol, cotton wool, thimble, filter paper (ø 180 mm and ø 90 mm), burette, retort stand, crucible with lid, media bottle (2 L and 250ml), beaker (1 L, 500 mL, 250 mL, and 50 mL), conical flask, round bottom flask (500 mL), measuring

cylinder (100 mL, 50 mL, 10 mL, and 5 mL) and micropipette with a pipette tip (1 mL and 100 μL).

3.2 Sampling location

The experiment will be carried out near the aquaculture lab of University Malaysia Kelantan (UMK), Jeli Campus. A total of 2 kg of live Black soldier fly larvae (BSFL) were purchased from a local seller in Kota Bharu Kelantan. After being brought to University Malaysia Kelantan, the selection process was done to select the pulpa stage only. Then, the larvae selected will be through a drying process. The drying process is done to make it easier for the larvae to be powdered. Dried larvae are ground using a blender and stored in an airtight container.

3.3 Methodology

3.3.1 Soxhlet (Defatting of Black Soldier Fly Larvae (BSFL))

The milled BSFL larvae were then sieved using a 300-micron test siever to obtain the desired texture. A total of 20 g of BSFL powder was filled in a folded piece of filter

paper and inserted into a cellulose thimble. By using the Soxhlet method in which fat from BSFL powder is extracted for 5 to 6 hours along with 95% ethanol and after 6 hours the accumulated oil is discarded. After the defatting process, the defatting BSFL powder was dried in an open area for over 48 hours to remove moisture and ethanol.

3.3.2 Feed formulation

Eggs and skimmed milk were used to create the egg custard. There are a total of 5 egg custard formulations were prepared (0%, 10%, 20%, 30%, and 40% of pre-treated BSFL). All ingredients (Table 3.1) are mixed using a hand mixer until well blended and steamed for 30 minutes. Make sure the steaming water is properly boiling before placing the egg custard. Then refrigerate the egg custard for a while before covering it with plastic and storing it in the refrigerator overnight. After cooling overnight, egg custard was sifted using a 300 microl-test siever into small particles suitable for feeding *M. rosenbergii* larvae.

Table 3.1 Treatment formulation

Treatment	Egg	Skimmed milk (g)	BSFL (g)
Control EC	1	70	0
EC (10% BSFL)	1	63	7
EC (20% BSFL)	1	56	14
EC (30% BSFL)	1	49	21
EC (40% BSFL)	1	42	28

3.3.3 Proximate analysis

3.3.3.1 Crude protein

Crude proteins were determined using the Kjeldahl method. 1 gram of each sample was weighed and poured into a test tube. One tablet of Kjeltabs (CuSO4 and K2SO4) was added (increasing the boiling point and accelerating the reaction rate) and followed by 12 mL of concentrated sulfuric acid into each digestion tube. The digestion process is carried out at a temperature of 400 ° C for 1 hour 30 minutes or more until the sample turns a bright green color and after the digestion process, the digestion tube is cooled for 20 minutes before the next step.

For the distillation process, 80 mL of distilled water was put into a digestion tube before adding 50ml of 40 % NaOH. The sample will change its shape from green to blue. While waiting for the digestion process to be done, 4% boric acid was prepared and added with green bromocresol and methyl red. Thereafter, 30 mL of solution was poured into a conical flask. For the distillation process, the sample is first placed in a distillation machine and followed by a conical flask containing a solution of boric acid. The distillation process was carried out for about 3 minutes until the red solution of boric acid turned into a green solution. Then the titration process, the solution is titrated with HCl so that it returns to a red solution. The step is repeated at each treatment. The percentage of crude protein was calculated using the following formula:

$$\% \text{ Nitrogen Content} = \frac{\text{V x N x } 14.007}{\text{Weight of Sample in mg}} \text{ X } 100\ \%$$

Where,

V = Volume of solution using during titration, mL

N = Normality of titrant

$$\% \text{ Crude Protein} = \% \text{ Nitrogen Content x } 6.25$$

3.3.3.2 Crude fat

The empty aluminium cup was heated for 20 minutes at 130 ° C and after that, the empty cup was cooled in the desiccator for 15 minutes. Next, the empty aluminium cup is weighed and its weight recorded. While the cup was heated, 1 g of sample was put into a thimble and placed in a Soxtec machine. Thereafter, an empty aluminium cup was filled with 80 mL of petroleum ether and placed at the bottom of the sample. The Soxtec machine runs for over an hour. After 1 h, the cup was filled with excess fat, and the sample was placed in the oven for 20 min at 130 ° C. Afterwards, the samples and cups containing the fat were allowed to cool for 20 minutes in a desiccator before being weighed. The dried samples were put into small plastic bags and used later for fibre tests. The weight of the cup containing fat was recorded, and the percentage of crude fat was computed using the formula:

$$\% \text{ Crude Fat} = \frac{B - A}{C} \text{ X } 100\ \%$$

Where,

A = weight of empty aluminium cup, g

B = weight of aluminium cup with extraction, g

C = weight of sample in g

3.3.3.3 Crude fibre

The crude fiber test was performed on samples that had passed the crude fat test previously. However, the weight of the previous sample was slightly reduced and had to be added up to 1 gram. After that, the sample is placed in a fiber container. Before the fiber bowl was inserted into the fiber test equipment, 1 g of celite was added to the fiber bowl containing the samples. 1 L 1.25 percent NaOH and 1.25 percent sulfuric acid were put into the machine solution container. The machine is turned on for about 2 hours. After 2 h, the fiber container was placed in the oven for 2 h at a temperature of 130 ° C, followed by a cooling procedure for 20 min in a desiccator. After cooling, the fiber cups were weighed and the weight recorded. After weighing, the fiber crucible was put in a 525 ° C furnace for 3 hours. The weight of the fiber cup was weighed the next day. The percentage of crude fiber were calculated by using the following formula:

$$\% \text{ Crude Fibre} = \frac{A - B}{C} \text{ X } 100 \%$$

Where,

A = weight of sample with cup after oven, g

B = weight of sample with cup after furnace, g

C = weight of sample in g

3.3.3.4 Ash

The crucible with lid is heated at 105 ° C for 30 minutes to remove moisture. Next, the crucible with the lid is cooled for 20 minutes in a desiccator. After cooling, the crucible is weighed with a lid. About 1 g of sample was placed inside the crucible and the weight of the crucible with the sample was recorded. Then the incandescent containing the sample was placed in a furnace for 5 hours at a temperature of 550 ° C. After the furnace, the crucible and the sample were cooled in a desiccator for 20 min before being weighed. The weight of the sample and the crucible with the lid were recorded and the percentage of ash was calculated using the following formula:

$$\% \text{ Ash Content} = \frac{C - A}{B - A} \text{ X } 100 \%$$

Where,

A = weight of empty crucible with lid (g)

B = weight of crucible with lid and sample (g)

C = weight after furnace (g)

3.3.3.5 Moisture

The aluminium foil was folded until it formed a cup that could accommodate the sample. 1 g of sample was placed inside the folded aluminium foil, and the weight of the sample and foil were both recorded. The sample was then heated in an oven for 24 hours or overnight at 105 °C. The sample was then chilled in a desiccator for 20 minutes before being weighed. The weight after the oven process was recorded, and the moisture % was determined using the following formula:

$$\% \text{ Moisture Content} = \frac{W1 - W2}{W3} \text{ X } 100\ \%$$

Where,

W1 = weight of sample with aluminium foil before drying (g)

W2 = weight of sample with aluminium foil after drying (g)

W3 = weight of sample in gram

3.3.4 Physical Test

3.3.4.1 Texture Analysis

The hardness was determined using a Texture Analyzer. A needle shape probe (TA39) was used to test the hardness of the formulated egg custard (control EC, EC

10% BSFL, EC 20% BSFL, EC 30% BSFL and EC 40% BSFL). The cube samples were put on the platform and pressed twice with the needle shape probe. The hardness of the sample was detected, and the result was displayed after the pressing process. The hardness data and results were recorded.

3.3.4.2 Colour Analysis

For colour analysis, a chromameter was utilized. The chromameter sensor was put directly on the samples, and the measurement button was pressed twice to obtain the result. The outcome is presented on the chromameter's LCD. There were three types of data obtained: lightness (L), redness (a), and yellowness (Y) (b). The procedures were done twice for each sample, and the data were recorded.

3.3.4.3 Bulk Density

The bulk density test was carried out using a 5 mL measuring cylinder. The empty measuring container was weighed first, and the weight was recorded. The sample was then poured into the measuring cylinder until it reached 5 mL. After that, 2 tabs or more were necessary to lower the sample until it became consistent. As an end, the sample volume was slightly less than 5 mL, and the new volume was recorded. Thereafter, the measuring cylinder containing the sample was re-weighed. The bulk density was calculated by using the following formula:

$$\text{Bulk density } (g/cm^3) = \frac{B - A}{V}$$

Where,

A = weight of measuring cylinder (g)

B = weight of measuring cylinder with sample (g)

V = volume of measuring cylinder occupied by sample (cm^3)

3.3.4.4 Sinking Velocity

50 mL of distilled water were added to a 50 mL measuring cylinder. The height of the distilled water was then measured. A pinch of the sample was dropped into the water, and the stopwatch start as soon as the sample began to sink. When the first two particles reached the bottom of the measuring cylinder, the stopwatch paused. The amount of time spent was recorded. The sinking velocity was determined using the formula:

$$\text{Sinking Velocity } (cm/sec) = \frac{H}{T}$$

Where,

H = Height of water (cm)

T = Time taken for each piece to reach the bottom (sec)

3.3.4.5 Statistic Analysis

All collected data were analyzed using Statistical Package for the Social Science (SPSS) with one-way ANOVA and Tukey post hoc test to find significant differences between all treatments with significant differences at the 0.05 level ($P \leq 0.05$).

CHAPTER 4

RESULT AND DISCUSSIONS

4.1 Proximate Composition of Feed Treatment

The biochemical composition of egg custard and formulated egg custard plus BSFL is reported in Table 4.1. Biochemical compositions (crude protein, crude fat, crude fibre, ash, and moisture) showed significantly different ($P < 0.05$) in all formulated egg custard.

Table 4.1: Mean Score for Biochemical Composition of treatment and BSFL formulation with Crude Protein, Crude Fat, Crude Fibre, Moisture and Ash

	Treatment and BSFL Formulate				
	Control EC	EC, BSFL 10%	EC BSFL 20%	EC, BSFL 30%	EC, BSFL 40%
Crude Protein	2.71±0.09[a]	22.19±0.13[b]	26.18±0.53[c]	25.34±0.48[c]	31.30±0.13[d]
Crude Fat	1.45±0.01[a]	2.17±0.27[ab]	3.05±0.23[ab]	4.44±0.40[ab]	6.08±1.57[b]
Crude Fibre	1.76±0.60[a]	2.61±0.20[a]	3.59±0.57[a]	4.13±1.20[a]	4.92±0.51[a]
Moisture	35.64±1.01[a]	28.4±1.44[a]	30.81±1.8[a]	26.49±4.15[a]	25.23±0.68[a]
Ash	0.07±0.00[a]	0.09±0.00[ab]	0.09±0.00[ab]	0.10±0.00[b]	0.10±0.00[b]

Mean within the column with a different letter(s) indicate a significant difference between treatments by Tukey's HSD test at $P \leq 0.05$. Column represent the mean values ± standard error

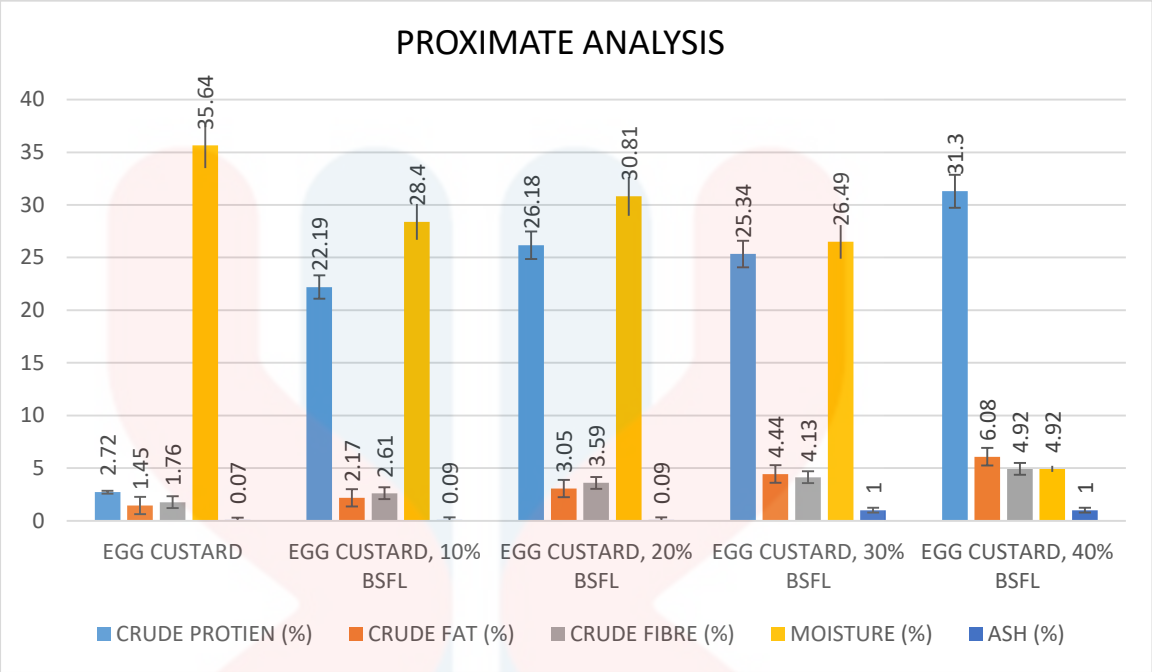

Figure 4.1: Percentage of crude protein, crude fat, crude fibre, moisture, and ash for Treatment and Egg Custard Formulation

In Table 4.1, the mean score of crude protein, egg custard with BSFL was higher than that of egg custard without BSFL in the treated diet. Where crude protein for egg custard (2.71 ± 0.09 %) while the mean score of crude protein for egg custard 40 % BSFL is the highest (31.30 ± 0.13 %) among egg custard formulated with BSFL (22.19 ± 0.13 %, 26.818 ± 0.53 % and 25.34 ± 0.48 %). Egg custard 10% BSFL has lower crude protein than other BSFL co -formulations but not as significantly. The amount of BSFL added was directly proportional to the percentage of crude protein content in the egg custard formulated with a total BSFL of 10%, 20%30%, and 40%. This indicates that the higher the percentage of BSFL used the higher the crude protein content. This indicates that BSFL has a higher protein content than skim milk powder as a protein source for *M. rosenbergii larvae* because the dry weight of BSFL contains up to 50 %

crude protein (Shumo *et al*., 2019) compared to 34 % crude protein found in milk powder skim (APDI, 2021).

The average percentage of treatment crude fat scores using BSFL was higher than that of egg custard not using BSFL in the treated diet. That is, crude fat for egg custard treatment is only (1.45 ± 0.01 %). while the mean crude fat score for the treatment using BSFL was highest was egg custard 40 %BSFL (6.08 ± 1.57 %) and among BSFL formulated with egg custard (2.17 ± 0.27 %, 3.05 ± 0.23 %, and 4.44 ± 0.40 %) shown in Table 4.1. egg custard 40% BSFL has the highest fat content compared to formulations using other BSFLs because the crude fat content of egg custard formulated with BSFL increased with the reduction of skim milk and the addition of BSFL. Coupled with a source of fat from chicken eggs (fat content: 8.7 to 11.2 per 100 g of whole eggs) (Réhault-Godbert *et al*., 2019). Moreover, the protein found in powdered milk binds with the fat from chicken eggs and the protein absorbs to retain the fat to form an emulsion with lipid interaction (Zayas, 1997) after mixing. According to Mitra *et al*. (2015), low lipid requirements with adequate EFA levels (0.075 % of n-3 and n-6 HUFA) provided optimal growth for *M. rosenbergii* larval. Due to that, BSFL is best for reducing lipids in the treatment, this is because BSFL does not form an emulsion after being mixed with chicken eggs.

The mean crude fiber score of BSFL formulated with egg custard was higher than that of egg custard without BSFL (1.76 ± 0.60 %) in the treated diet while the mean crude fiber score of 40 %BSFL egg custard was the highest (4.92 ± 0.51 %) between in egg custard formulated with BSFL (1.14 ± 0.35 %, 2.94 ± 0.14 %, 5.84 ± 0.01 %and 11.30 ± 0.13 %). BSFL has chitin, an insoluble fiber that results in a gradual increase in the percentage of crude fiber against the formulated egg custard. This indicates an increase in BSFL of 10%, 20%, 30%, and 40% responding to powdered

milk and chicken eggs. Milk powder and chicken eggs have less than 1% crude fiber (SN Permadi, S Mulyani, A Hintono, 2012)

From Table 4.1, the mean moisture score of unformulated egg custard with BSFL was higher (35.64 ± 1.01) in the treated diet while the mean moisture score of egg custard with BSFL was highest (30.81 ± 1.8 %) and among co-formulated egg custard with BSFL (28.4 ± 01.44 %, 26.49 ± 4.15 %, and 25.32 ± 0.68 %). This shows that the content that uses BSFL does not absorb moisture and this shows that BSFL is not soluble in chicken egg liquid compared to powdered milk. Furthermore, milk powder is easily soluble and absorbs water content (Augustin & Margetts, 2003) from chicken eggs in formulated egg custard. Therefore, the moisture content of the treatment formulated with BSFL increased gradually as the BSFL content increased. Based on a study from De Barros and Valenti (2003), the use of wet diet and dry diet together according to different *M. rosenbergii* larval stages is recommended and this indicates the moisture content does not affect the growth rate of *M. rosenbergii* larvae.

Based on the results obtained from Table 4.1, the mean score of egg custard ash without BSFL was lower (0.07 ± 0.00 %) than BSFL in the treated diet. While the mean scores ash of egg custard 30% BSFL and egg custard 40% BSFL were the highest (0.10 ± 0.00) among egg custard formulated with low egg custard was 10% and 20% BSFL (0.09 ± 0.00%). Milk powder and chicken eggs did not affect the ash with BSFL, where the ash was directly proportional to the amount of BSFL used in the formulated egg custard. This is because the more amount of BSFL is used, the higher the ash produced.

4.2 Physical Properties of Experimental Feed

4.2.1 Texture Analysis

The Hardness tests were conducted for egg custard and egg custard formulated with BSFL. The hardness in all samples showed statistically significant ($P < 0.05$). Table 4.3 shows the hardness in all samples.

Table 4.2: Texture analysis of hardness of egg custard and egg custard with BSFL

Parameter	Treatment and BSFL Formulate				
	Control EC	EC, BSFL 10%	EC BSFL 20%	EC, BSFL 30%	EC, BSFL 40%
TPA (Hardness)	328.00 ± 4.00^{ab}	$458.50 \pm 57.50a^{b}$	272.00 ± 15.00^{a}	270.00 ± 27.00^{a}	513.50 ± 40.50^{b}

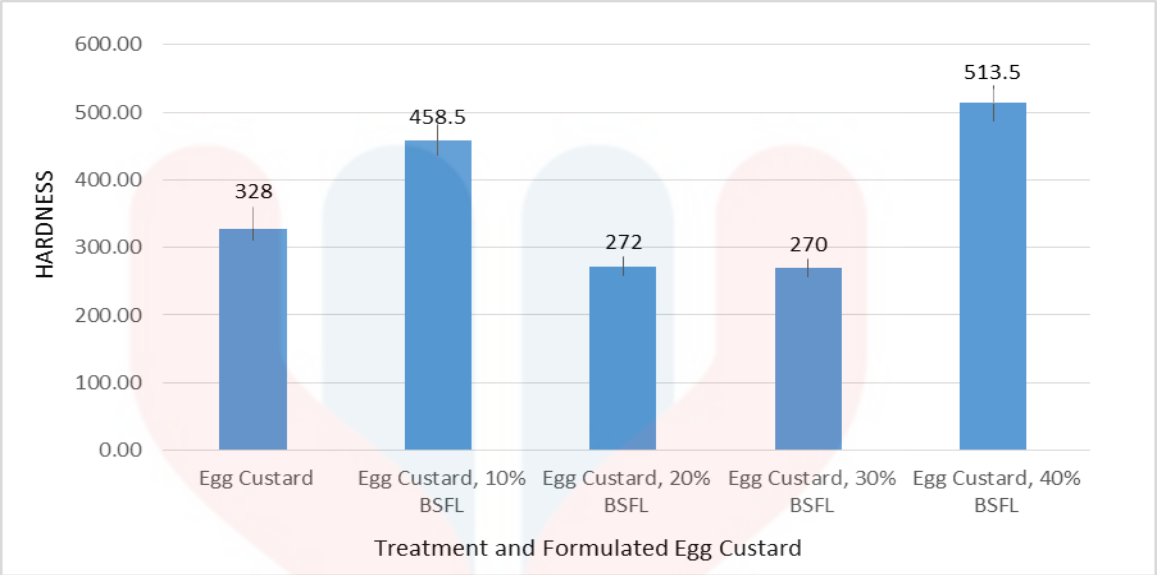

Figure 4.2: TPA hardness of Treatment and Egg Custard Formulation

Hardness showed statistically significant ($P < 0.05$) in all formulated egg custards. Based on Table 3.2, egg custard with 40% BSFL has the hardest texture (513.50 ± 40.50) followed by egg custard with 10% BSFL second hardest (458.50 ± 81.32) while egg custard with 20% and 30% BSFL has the softest texture that is (272.0 ± 15.0 and 270.0 ± 27.0). Although the texture of egg custard with BSFL became harder as BSFL increased, egg custard without BSFL also had a hardness that was almost the same as egg custard formulated with BSFL. Egg custard cubes with 40% BSFL start to disintegrate from the second compression, this texture may be due to skim milk powder acting as a binding reagent. However, this texture feature was not used for *M. rosenbergii* larvae because all treatments had to be sieved to a size of 300 µm to obtain the most suitable small particles for larval use.

4.2.2 Colour Analysis, Bulk Density and Sinking Velocity Analysis

Table 4.3: Colour Characteristics of Treated Diet and Formulated with *L* a* b** scale

Parameter	**Treatment and Formulation**				
	Control (egg custard)	Egg custard + 10% BSFL	Egg custard + 20% BSFL	Egg custard + 30% BSFL	Egg custard + 40% BSFL
L* **scale (lightness)**	71.22 ± 0.69^{c}	44.61 ± 2.45^{b}	45.06 ± 0.57^{b}	29.96 ± 3.11^{a}	27.18±0.32^{a}
a* **scale (redness)**	8.22 ± 0.14^{d}	5.44 ± 0.55^{c}	4.70 ± 0.18^{bc}	2.64 ± 0.34^{a}	3.11± 0.01^{ab}
b* **scale (yellowness)**	28.32 ± 1.65^{c}	16.95 ± 0.90^{b}	15.10 ± 0.26^{b}	8.08 ± 1.23^{a}	7.82 ± 0.06^{a}
Bulk Density	0.22 ± 0.02^{a}	0.28 ± 0.00^{b}	0.29 ± 0.02^{c}	0.28 ± 0.00^{c}	0.33 ± 0.00^{d}
Sinking Velocity	0.24 ± 0.03^{a}	0.67 ± 0.04^{ab}	1.14 ± 0.19^{ab}	1.36 ± 0.54^{ab}	1.94 ± 0.32^{b}

Mean with a different superscript in a row is significantly different (P<0.05)

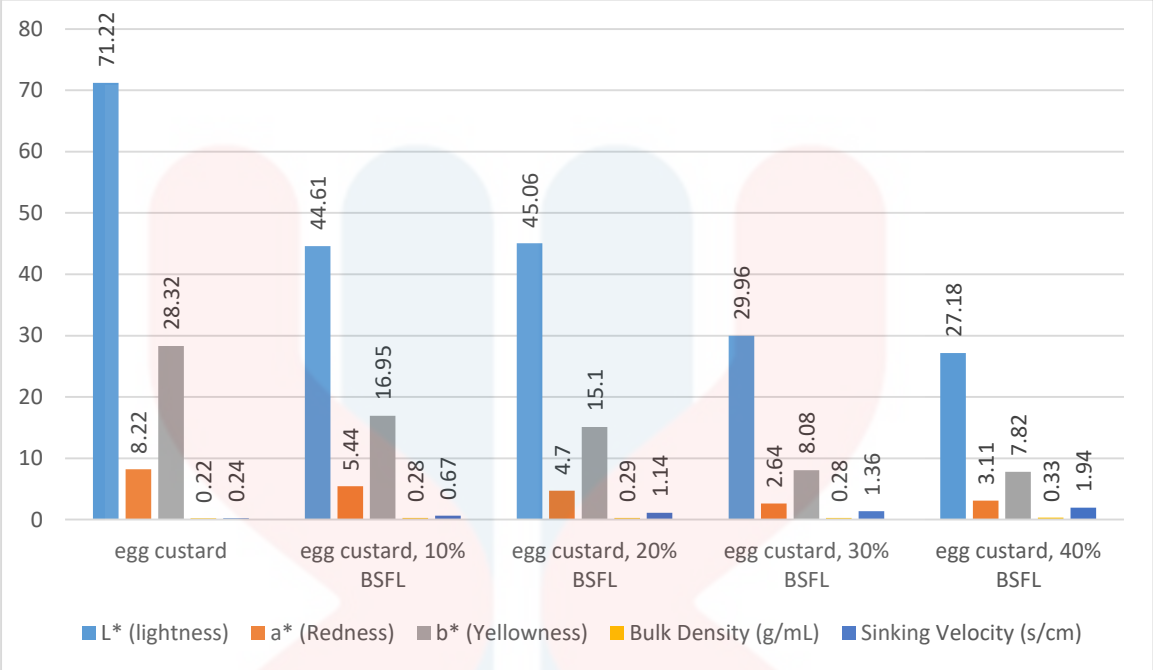

Figure 4.3: Colour analysis, Bulk Density and Sinking Velocity of Treatment and Egg Custard Formulation

Colour characteristics (*L** (brightness value), *a** (red/green value), and scale *b** (yellow/blue value) showed statistically significant (P <0.05) in all treated diets and egg custard formulated with BSFL. From Table 4.3, there was no difference in colour characteristics in the formulated BSFL but there was only a slight decrease. Egg custard 10% BSFL was the highest (*L**: 44.64 ± 2.45, *a**: 5.44 ± 0.55, *b**: 16.95 ± 0.90) and the lowest egg custard 40% BSFL (*L**: 27.18 ± 0.32, *a**: 3.11 ± 0.01, *b**: 7.82 ± 0.06) .However, the scale of *L**(lightness) (44.61 ± 2.45, 45.61 ± 0.57, 29.96 ± 3.11, and 27.18 ± 0.23), scale *a** (redness) (5.44 ± 0.55, 4.70 ± 0.18, 2.64 ± 0.34 and 3.11 ± 0.01) and *b** (yellowness) (16.95 ± 0.90, 15.10 ± 0.26, 8.08 ± 1.23 , and 7.82 ± 0.06) decreased in formulations formulated with BSFL. This is because the percentage of BSFL added increased from 10 %, 20 %, 30 %, and 40 % in the formulated egg custard.

According to the study of *M. rosenbergii* larvae receive light-colored foods better than darker foods (Kawamura et al., 2017). Moreover, the colour choices of *M. rosenbergii* larvae were blue and white because blue-dyed egg custard showed a higher body weight than yellow egg custard (Kawamura *et al*., 2017). Therefore, feeding testing requires a relatively long time identification of the feeding behavior of *M. rosenbergii* larvae.

Bulk density tests were conducted for BSFL -formulated egg custard and egg custard samples. As shown in table 4.3, the bulk density of egg custard and egg custard treatment with BSFL showed significantly ($P < 0.05$) the highest egg custard with 40% BSFL i.e. 0.34 ± 0.00 g/mL and followed by egg custard with 20% BSFL (0.29 ± 0.02). g/mL), egg custard with 10% and 30% BSFL with 0.28 ± 0.01 g/mL and 0.28 ± 0.00 g/mL, respectively. While egg custard without BSFL had the lowest density of 0.22 ± 0.02 g/mL. This indicates that high-density values have greater compaction with smaller pores, while the lowest density has less compaction and more pores. Usually, the density can vary due to different particle sizes, compaction, or water content (Herman, 2001). The bulk density of egg custard without BSFL was the lowest probably due to the absence of BSFL, as BSFL had a larger particle size than the particle size of egg custard.

Sinking velocity tests were performed for egg custard and all egg custard formulated with BSFL. This test is to determine the time taken for the sample to sink and reach the bottom. The sinking velocity showed significance ($P < 0.05$) for all samples. Based on Table 4.3, egg custard with BSFL had a significantly higher sinking velocity rate ($P < 0.05$) of 1.94 ± 0.32 s/cm, and egg custard without BSFL had a lower sinking velocity rate of 0.24 ± 0.03 s/cm. The higher the sinking velocity rate indicates that the sample takes longer to sink while the lower the sinking velocity indicates that the sample takes a shorter time to sink. The rate of sinking velocity of BSFL is

influenced by the chitin component present in it. Chitin takes a relatively long time to sink and is insoluble to some solvents due to its hydrogen bonding and crystal structure (Sudha *et*. Al., 2014). The higher the sinking velocity rate indicates that the sample is suitable for *M. rosenbergii* larvae because, during the larval stage, the larvae are mainly located around the water surface area. Therefore, samples that have a high sinking velocity rate or take a relatively long time to sink are suitable for *M. rosenbergii* larvae.

CHAPTER 5

CONCLUSION

5.1 CONCLUSION

This study shows that egg custard formulated with BSFL has the ability to replace *M. rosenbergii* larval feed, egg custard formulated with BSFL to be a potential feed for *M. rosenbergii* larvae because it has shown high protein content of egg custard formulated with 31.30% content crude protein after feed formulation. The crude protein of egg custard formulated with BSFL is much higher than that of regular egg custard. Thus, using BSFL to improve egg custard formulations showed high potential for use as feed for *M. rosenbergii* larvae, and egg custard formulated with BSFL had the

slowest sinking velocity, 1.94 cm/s suitable for feeding *M. rosenbergii* at the larval stage. Also, due to the current economic downturn, the price of raw materials for making egg custard is quite expensive. Therefore, reducing the use of skim milk powder by replacing BSFL can reduce the cost of raw materials. Nutritional experiments are recommended to determine suitable food for *M. rosenbergii* larvae in the future with a study of the level of acceptance of *M. rosenbergii* larvae and growth of *M. rosenbergii* larvae.

5.2 RECOMMENDATION

As for recommendations, In the future, a feeding trial of the die test formulated of egg custard with BSFL should be conducted to obtain information on the reception and effect on the growth and survival of *M. rosenbergii* larvae. This will be able to create a special food for the larvae of *M. rosenbergi* that can be marketed. Reduction of skim milk powder and chicken eggs is also recommended because in this way the cost can be reduced, especially for small-scale aquaculture farmers.

In addition, larval growth should be monitored by running a larval stage index. The larval stage index was carried out by randomly selecting larvae from the tank in treatment and observing them under a microscope. Observations using a microscope give an idea of the current stage the larvae are in. Therefore, the larval stage in each treatment can be compared to determine its growth. Monitoring the growth stage of the larvae provides information on the performance of food on the larvae and provides information on the growth of the larvae.

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Figure A.1: Selection of BSFL larvae to obtain pupae stage

Figure A.2: Grind the material to be fine

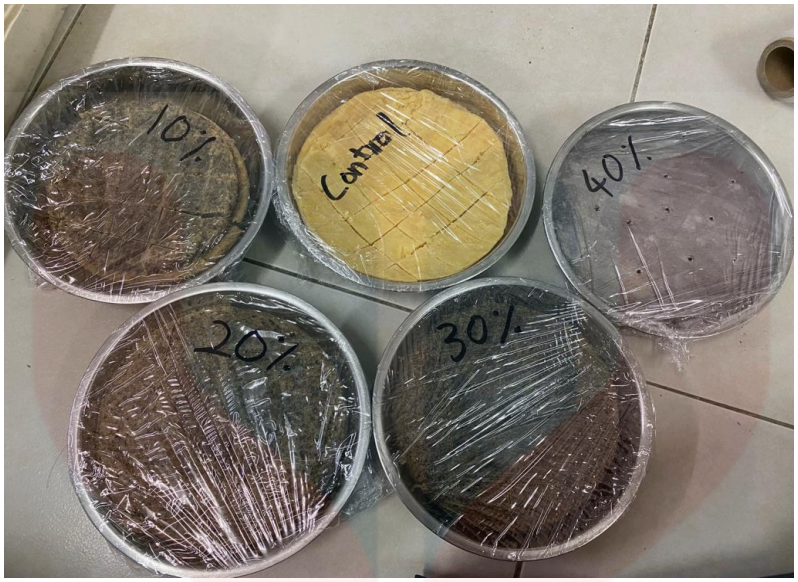

Figure A.3: All treatment after steaming

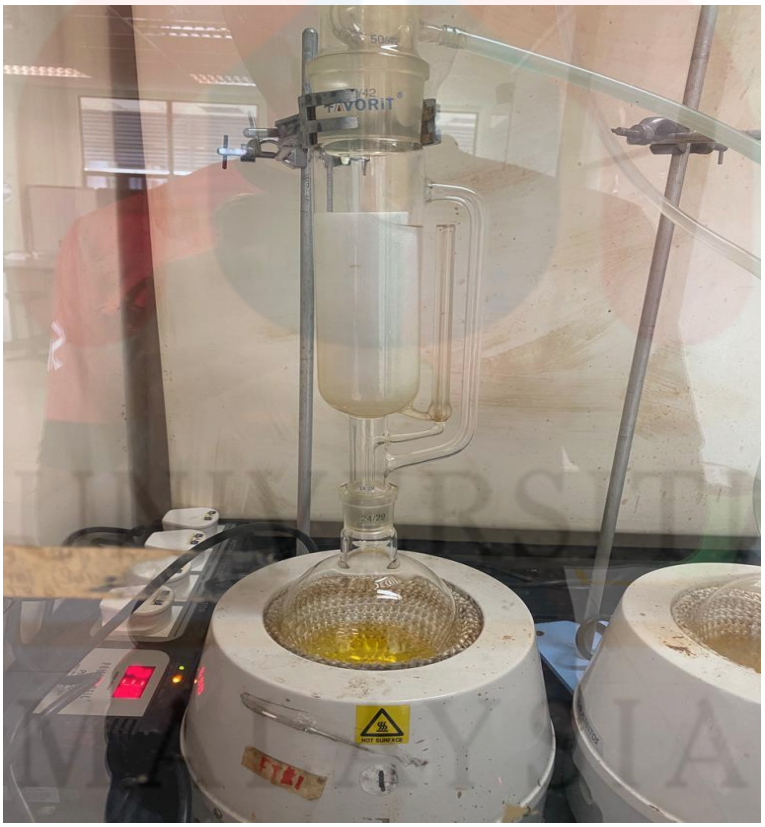

Figure A.4: Defatting process used Soxhlet extraction to remove fat content from BSFL

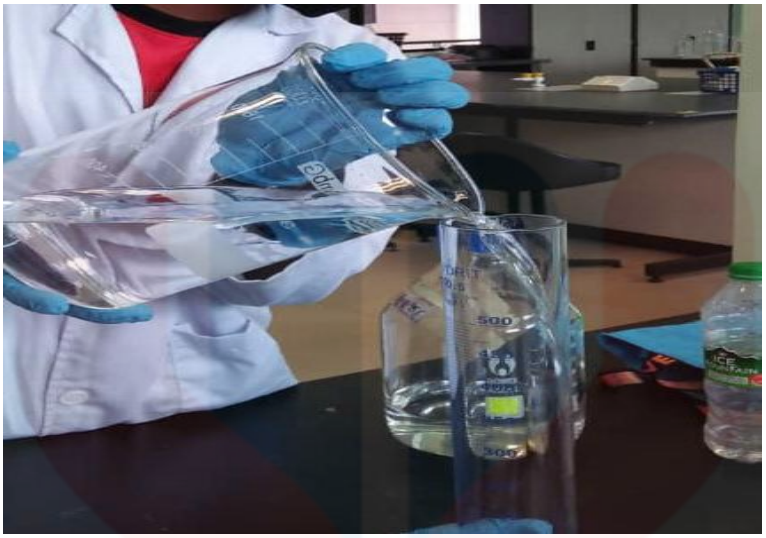

Figure A.5: Prepare distilled water for Soxhlet extraction

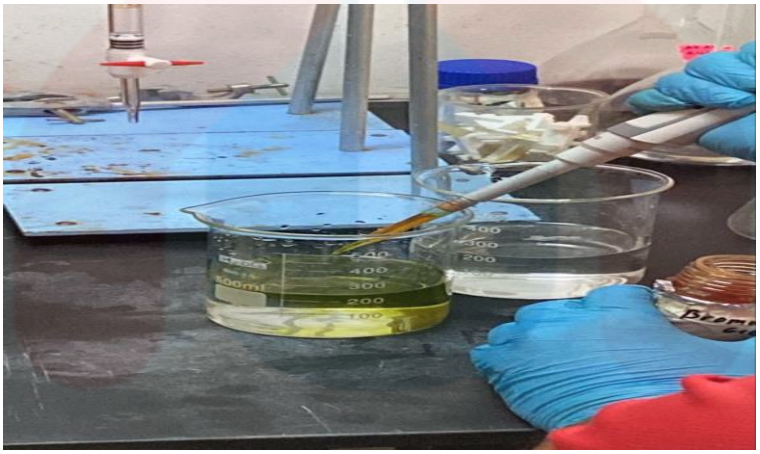

Figure A.6: Add bromocresol green into distilled water for digestion

Figure A.7: FOSS analytical ST 255 Soxtec™ used to extract fat in the sample

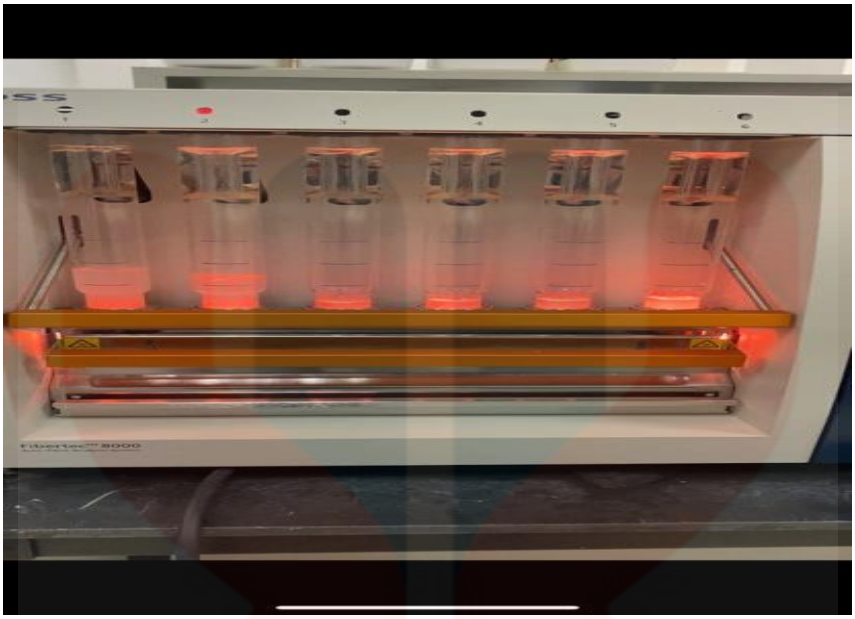

Figure A.8: FOSS Fibertec™ 8000 machines used to identify crude fat in the sample

Figure A.9: last step for fibre analysis, put the sample into the furnace

Figure A.10: Colour characteristics of the sample were tested and L, a, b scale were displayed at CR-400 Chroma Meter

Figure A.11: All apparatus for sinking velocity

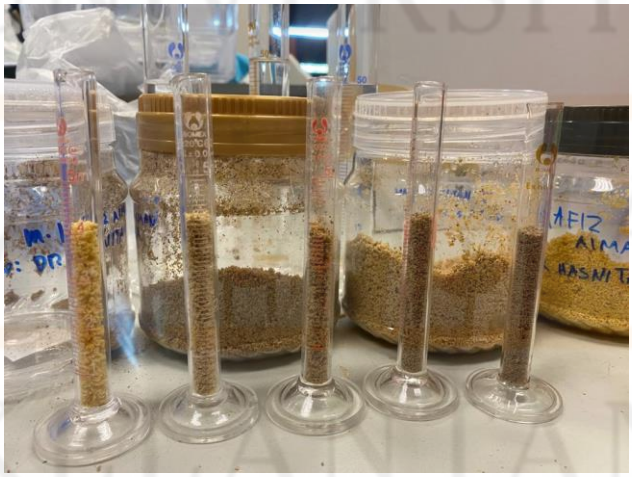

Figure A.12: All apparatus for bulk density

Table A.1: Descriptive for one way ANOVA (Crude Protein)

Descriptives

Protein

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	2.7139	.12381	.08755	1.6014	3.8263	2.63	2.80
10%	2	22.1923	.18576	.13135	20.5234	23.8613	22.06	22.32
20%	2	26.1756	.74282	.52525	19.5016	32.8495	25.65	26.70
30%	2	25.3439	.68094	.48150	19.2259	31.4619	24.86	25.83
40%	2	31.2969	.18569	.13130	29.6286	32.9652	31.17	31.43
Total	10	21.5445	10.39823	3.28821	14.1061	28.9830	2.63	31.43

Table A.2: Descriptive for one way ANOVA (Crude Fat)

Descriptives

fat

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	1.4500	.01414	.01000	1.3229	1.5771	1.44	1.46
10%	2	2.1700	.38184	.27000	-1.2607	5.6007	1.90	2.44
20%	2	3.0500	.32527	.23000	.1276	5.9724	2.82	3.28
30%	2	4.4400	.56569	.40000	-.6425	9.5225	4.04	4.84
40%	2	6.0800	2.22032	1.57000	-13.8687	26.0287	4.51	7.65
Total	10	3.4380	1.91169	.60453	2.0705	4.8055	1.44	7.65

Table A.3: Descriptive for one way ANOVA (Crude Fibre)

Descriptives

fibre

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	1.7600	.84853	.60000	-5.8637	9.3837	1.16	2.36
10%	2	2.6100	.28284	.20000	.0688	5.1512	2.41	2.81
20%	2	3.5850	.79903	.56500	-3.5940	10.7640	3.02	4.15
30%	2	4.1300	1.69706	1.20000	-11.1174	19.3774	2.93	5.33
40%	2	4.9250	.71418	.50500	-1.4916	11.3416	4.42	5.43
Total	10	3.4020	1.38409	.43769	2.4119	4.3921	1.16	5.43

Table A.4: Descriptive for one way ANOVA (Moisture)

Descriptives

moisture

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	35.6350	1.42128	1.00500	22.8653	48.4047	34.63	36.64
10%	2	28.4000	1.44250	1.02000	15.4397	41.3603	27.38	29.42
20%	2	30.8150	1.80312	1.27500	14.6146	47.0154	29.54	32.09
30%	2	26.4850	5.86192	4.14500	-26.1822	79.1522	22.34	30.63
40%	2	25.2250	.95459	.67500	16.6483	33.8017	24.55	25.90
Total	10	29.3120	4.44920	1.40696	26.1292	32.4948	22.34	36.64

Table A.5: Descriptive for one way ANOVA (Ash)

Descriptives

ash

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	.0732	.00537	.00380	.0249	.1215	.07	.08
10%	2	.0903	.00983	.00695	.0019	.1786	.08	.10
20%	2	.0908	.00184	.00130	.0743	.1073	.09	.09
30%	2	.1046	.00375	.00265	.0710	.1383	.10	.11
40%	2	.0967	.00240	.00170	.0751	.1183	.10	.10
Total	10	.0911	.01165	.00368	.0828	.0995	.07	.11

Table A.5: Descriptive for one way ANOVA (Hardness)

Descriptives

hardness

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	328.0000	5.65685	4.00000	277.1752	378.8248	324.00	332.00
10%	2	458.5000	81.31728	57.50000	-272.1068	1189.1068	401.00	516.00
20%	2	272.0000	21.21320	15.00000	81.4069	462.5931	257.00	287.00
30%	2	270.0000	38.18377	27.00000	-73.0675	613.0675	243.00	297.00
40%	2	513.5000	57.27565	40.50000	-1.1013	1028.1013	473.00	554.00
Total	10	368.4000	111.25167	35.18087	288.8154	447.9846	243.00	554.00

Table A.6: Descriptive for one way ANOVA (Colour Analysis)

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
lightness	control	2	71.2200	.97581	.69000	62.4527	79.9873	70.53	71.91
	10%	2	44.6050	3.45775	2.44500	13.5383	75.6717	42.16	47.05
	20%	2	45.0550	.79903	.56500	37.8760	52.2340	44.49	45.62
	30%	2	29.9550	4.39113	3.10500	-9.4978	69.4078	26.85	33.06
	40%	2	27.1800	.45255	.32000	23.1140	31.2460	26.86	27.50
	Total	10	43.6030	16.58855	5.24576	31.7363	55.4697	26.85	71.91
redness	control	2	8.2150	.19092	.13500	6.4997	9.9303	8.08	8.35
	10%	2	5.4400	.77782	.55000	-1.5484	12.4284	4.89	5.99
	20%	2	4.7000	.25456	.18000	2.4129	6.9871	4.52	4.88
	30%	2	2.6400	.48083	.34000	-1.6801	6.9601	2.30	2.98
	40%	2	3.1050	.00707	.00500	3.0415	3.1685	3.10	3.11
	Total	10	4.8200	2.11278	.66812	3.3086	6.3314	2.30	8.35
yellowness	control	2	28.3150	2.32638	1.64500	7.4133	49.2167	26.67	29.96
	10%	2	16.9500	1.27279	.90000	5.5144	28.3856	16.05	17.85
	20%	2	15.0950	.36062	.25500	11.8549	18.3351	14.84	15.35
	30%	2	8.0800	1.73948	1.23000	-7.5486	23.7086	6.85	9.31
	40%	2	7.8200	.08485	.06000	7.0576	8.5824	7.76	7.88
	Total	10	15.2520	7.96268	2.51802	9.5558	20.9482	6.85	29.96

Table A.6: Descriptive for one way ANOVA (Bulk Density)

Descriptives

bulk

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	.2222	.00311	.00220	.1942	.2502	.22	.22
10%	2	.2810	.00134	.00095	.2689	.2930	.28	.28
20%	2	.2977	.00318	.00225	.2692	.3263	.30	.30
30%	2	.2797	.00049	.00035	.2752	.2841	.28	.28
40%	2	.3396	.00057	.00040	.3345	.3447	.34	.34
Total	10	.2840	.03983	.01260	.2555	.3125	.22	.34

Table A.7: Descriptive for one way ANOVA (Sinking Velocity)

Descriptives

sinking

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	2	.2496	.03571	.02525	-.0713	.5704	.22	.27
10%	2	.6763	.05127	.03625	.2157	1.1368	.64	.71
20%	2	1.1350	.26276	.18580	-1.2258	3.4958	.95	1.32
30%	2	1.3357	.76615	.54175	-5.5479	8.2192	.79	1.88
40%	2	1.9419	.45106	.31895	-2.1108	5.9945	1.62	2.26
Total	10	1.0677	.68228	.21576	.5796	1.5557	.22	2.26

Table A.8: Post Hoc Test (Protein)

Multiple Comparisons

Dependent Variable: protein

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	-19.47850*	.46899	.000	-21.3599	-17.5971
	20%	-23.46170*	.46899	.000	-25.3431	-21.5803
	30%	-22.63005*	.46899	.000	-24.5114	-20.7487
	40%	-28.58305*	.46899	.000	-30.4644	-26.7017
10%	control	19.47850*	.46899	.000	17.5971	21.3599
	20%	-3.98320*	.46899	.002	-5.8646	-2.1018
	30%	-3.15155*	.46899	.006	-5.0329	-1.2702
	40%	-9.10455*	.46899	.000	-10.9859	-7.2232
20%	control	23.46170*	.46899	.000	21.5803	25.3431
	10%	3.98320*	.46899	.002	2.1018	5.8646
	30%	.83165	.46899	.473	-1.0497	2.7130
	40%	-5.12135*	.46899	.001	-7.0027	-3.2400
30%	control	22.63005*	.46899	.000	20.7487	24.5114
	10%	3.15155*	.46899	.006	1.2702	5.0329
	20%	-.83165	.46899	.473	-2.7130	1.0497
	40%	-5.95300*	.46899	.000	-7.8344	-4.0716
40%	control	28.58305*	.46899	.000	26.7017	30.4644
	10%	9.10455*	.46899	.000	7.2232	10.9859
	20%	5.12135*	.46899	.001	3.2400	7.0027
	30%	5.95300*	.46899	.000	4.0716	7.8344

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

Table A.9: Post Hoc Test (Fat)

Multiple Comparisons

Dependent Variable: fat

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	-.72000	1.04896	.951	-4.9279	3.4879
	20%	-1.60000	1.04896	.590	-5.8079	2.6079
	30%	-2.99000	1.04896	.158	-7.1979	1.2179
	40%	-4.63000*	1.04896	.035	-8.8379	-.4221
10%	control	.72000	1.04896	.951	-3.4879	4.9279
	20%	-.88000	1.04896	.907	-5.0879	3.3279
	30%	-2.27000	1.04896	.322	-6.4779	1.9379
	40%	-3.91000	1.04896	.065	-8.1179	.2979
20%	control	1.60000	1.04896	.590	-2.6079	5.8079
	10%	.88000	1.04896	.907	-3.3279	5.0879
	30%	-1.39000	1.04896	.691	-5.5979	2.8179
	40%	-3.03000	1.04896	.152	-7.2379	1.1779
30%	control	2.99000	1.04896	.158	-1.2179	7.1979
	10%	2.27000	1.04896	.322	-1.9379	6.4779
	20%	1.39000	1.04896	.691	-2.8179	5.5979
	40%	-1.64000	1.04896	.571	-5.8479	2.5679
40%	control	4.63000*	1.04896	.035	.4221	8.8379
	10%	3.91000	1.04896	.065	-.2979	8.1179
	20%	3.03000	1.04896	.152	-1.1779	7.2379
	30%	1.64000	1.04896	.571	-2.5679	5.8479

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

Table A.10: Post Hoc Test (Fibre)

Multiple Comparisons

Dependent Variable: fibre

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	-.85000	.98270	.898	-4.7921	3.0921
	20%	-1.82500	.98270	.437	-5.7671	2.1171
	30%	-2.37000	.98270	.250	-6.3121	1.5721
	40%	-3.16500	.98270	.108	-7.1071	.7771
10%	control	.85000	.98270	.898	-3.0921	4.7921
	20%	-.97500	.98270	.849	-4.9171	2.9671
	30%	-1.52000	.98270	.579	-5.4621	2.4221
	40%	-2.31500	.98270	.265	-6.2571	1.6271
20%	control	1.82500	.98270	.437	-2.1171	5.7671
	10%	.97500	.98270	.849	-2.9671	4.9171
	30%	-.54500	.98270	.977	-4.4871	3.3971
	40%	-1.34000	.98270	.671	-5.2821	2.6021
30%	control	2.37000	.98270	.250	-1.5721	6.3121
	10%	1.52000	.98270	.579	-2.4221	5.4621
	20%	.54500	.98270	.977	-3.3971	4.4871
	40%	-.79500	.98270	.917	-4.7371	3.1471
40%	control	3.16500	.98270	.108	-.7771	7.1071
	10%	2.31500	.98270	.265	-1.6271	6.2571
	20%	1.34000	.98270	.671	-2.6021	5.2821
	30%	.79500	.98270	.917	-3.1471	4.7371

Table A.11: Post Hoc Test (Moisture)

Multiple Comparisons

Dependent Variable: moisture

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	7.23500	2.91977	.233	-4.4777	18.9477
	20%	4.82000	2.91977	.529	-6.8927	16.5327
	30%	9.15000	2.91977	.118	-2.5627	20.8627
	40%	10.41000	2.91977	.077	-1.3027	22.1227
10%	control	-7.23500	2.91977	.233	-18.9477	4.4777
	20%	-2.41500	2.91977	.911	-14.1277	9.2977
	30%	1.91500	2.91977	.958	-9.7977	13.6277
	40%	3.17500	2.91977	.807	-8.5377	14.8877
20%	control	-4.82000	2.91977	.529	-16.5327	6.8927
	10%	2.41500	2.91977	.911	-9.2977	14.1277
	30%	4.33000	2.91977	.611	-7.3827	16.0427
	40%	5.59000	2.91977	.413	-6.1227	17.3027
30%	control	-9.15000	2.91977	.118	-20.8627	2.5627
	10%	-1.91500	2.91977	.958	-13.6277	9.7977
	20%	-4.33000	2.91977	.611	-16.0427	7.3827
	40%	1.26000	2.91977	.991	-10.4527	12.9727
40%	control	-10.41000	2.91977	.077	-22.1227	1.3027
	10%	-3.17500	2.91977	.807	-14.8877	8.5377
	20%	-5.59000	2.91977	.413	-17.3027	6.1227
	30%	-1.26000	2.91977	.991	-12.9727	10.4527

Table A.12: Post Hoc Test (Ash)

Multiple Comparisons

Dependent Variable: ash

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	-.01705	.00545	.119	-.0389	.0048
	20%	-.01760	.00545	.107	-.0395	.0043
	30%	-.03145*	.00545	.011	-.0533	-.0096
	40%	-.02350*	.00545	.038	-.0454	-.0016
10%	control	.01705	.00545	.119	-.0048	.0389
	20%	-.00055	.00545	1.000	-.0224	.0213
	30%	-.01440	.00545	.197	-.0363	.0075
	40%	-.00645	.00545	.762	-.0283	.0154
20%	control	.01760	.00545	.107	-.0043	.0395
	10%	.00055	.00545	1.000	-.0213	.0224
	30%	-.01385	.00545	.218	-.0357	.0080
	40%	-.00590	.00545	.810	-.0278	.0160
30%	control	.03145*	.00545	.011	.0096	.0533
	10%	.01440	.00545	.197	-.0075	.0363
	20%	.01385	.00545	.218	-.0080	.0357
	40%	.00795	.00545	.624	-.0139	.0298
40%	control	.02350*	.00545	.038	.0016	.0454
	10%	.00645	.00545	.762	-.0154	.0283
	20%	.00590	.00545	.810	-.0160	.0278
	30%	-.00795	.00545	.624	-.0298	.0139

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

Table A.13: Post Hoc Test (Hardness)

Multiple Comparisons

Dependent Variable: hardness

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	-130.50000	48.64771	.188	-325.6505	64.6505
	20%	56.00000	48.64771	.777	-139.1505	251.1505
	30%	58.00000	48.64771	.757	-137.1505	253.1505
	40%	-185.50000	48.64771	.060	-380.6505	9.6505
10%	control	130.50000	48.64771	.188	-64.6505	325.6505
	20%	186.50000	48.64771	.059	-8.6505	381.6505
	30%	188.50000	48.64771	.057	-6.6505	383.6505
	40%	-55.00000	48.64771	.787	-250.1505	140.1505
20%	control	-56.00000	48.64771	.777	-251.1505	139.1505
	10%	-186.50000	48.64771	.059	-381.6505	8.6505
	30%	2.00000	48.64771	1.000	-193.1505	197.1505
	40%	-241.50000*	48.64771	.022	-436.6505	-46.3495
30%	control	-58.00000	48.64771	.757	-253.1505	137.1505
	10%	-188.50000	48.64771	.057	-383.6505	6.6505
	20%	-2.00000	48.64771	1.000	-197.1505	193.1505
	40%	-243.50000*	48.64771	.021	-438.6505	-48.3495
40%	control	185.50000	48.64771	.060	-9.6505	380.6505
	10%	55.00000	48.64771	.787	-140.1505	250.1505
	20%	241.50000*	48.64771	.022	46.3495	436.6505
	30%	243.50000*	48.64771	.021	48.3495	438.6505

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

Table A.14: Post Hoc Test (Colour)

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
lightness	control	10%	26.61500*	2.57035	.001	16.3040	36.9260
		20%	26.16500*	2.57035	.001	15.8540	36.4760
		30%	41.26500*	2.57035	.000	30.9540	51.5760
		40%	44.04000*	2.57035	.000	33.7290	54.3510
	10%	control	-26.61500*	2.57035	.001	-36.9260	-16.3040
		20%	-.45000	2.57035	1.000	-10.7610	9.8610
		30%	14.65000*	2.57035	.012	4.3390	24.9610
		40%	17.42500*	2.57035	.006	7.1140	27.7360
	20%	control	-26.16500*	2.57035	.001	-36.4760	-15.8540
		10%	.45000	2.57035	1.000	-9.8610	10.7610
		30%	15.10000*	2.57035	.011	4.7890	25.4110
		40%	17.87500*	2.57035	.005	7.5640	28.1860
	30%	control	-41.26500*	2.57035	.000	-51.5760	-30.9540
		10%	-14.65000*	2.57035	.012	-24.9610	-4.3390
		20%	-15.10000*	2.57035	.011	-25.4110	-4.7890
		40%	2.77500	2.57035	.811	-7.5360	13.0860
	40%	control	-44.04000*	2.57035	.000	-54.3510	-33.7290
		10%	-17.42500*	2.57035	.006	-27.7360	-7.1140
		20%	-17.87500*	2.57035	.005	-28.1860	-7.5640
		30%	-2.77500	2.57035	.811	-13.0860	7.5360
redness	control	10%	2.77500*	.43301	.007	1.0380	4.5120
		20%	3.51500*	.43301	.002	1.7780	5.2520
		30%	5.57500*	.43301	.000	3.8380	7.3120
		40%	5.11000*	.43301	.000	3.3730	6.8470
	10%	control	-2.77500*	.43301	.007	-4.5120	-1.0380
		20%	.74000	.43301	.502	-.9970	2.4770
		30%	2.80000*	.43301	.007	1.0630	4.5370
		40%	2.33500*	.43301	.015	.5980	4.0720
	20%	control	-3.51500*	.43301	.002	-5.2520	-1.7780
		10%	-.74000	.43301	.502	-2.4770	.9970
		30%	2.06000*	.43301	.026	.3230	3.7970
		40%	1.59500	.43301	.068	-.1420	3.3320
	30%	control	-5.57500*	.43301	.000	-7.3120	-3.8380
		10%	-2.80000*	.43301	.007	-4.5370	-1.0630

		20%	-2.06000*	.43301	.026	-3.7970	-.3230
		40%	-.46500	.43301	.813	-2.2020	1.2720
		control	-5.11000*	.43301	.000	-6.8470	-3.3730
	40%	10%	-2.33500*	.43301	.015	-4.0720	-.5980
		20%	-1.59500	.43301	.068	-3.3320	.1420
		30%	.46500	.43301	.813	-1.2720	2.2020
		10%	11.36500*	1.42794	.003	5.6368	17.0932
	control	20%	13.22000*	1.42794	.001	7.4918	18.9482
		30%	20.23500*	1.42794	.000	14.5068	25.9632
		40%	20.49500*	1.42794	.000	14.7668	26.2232
		control	-11.36500*	1.42794	.003	-17.0932	-5.6368
	10%	20%	1.85500	1.42794	.704	-3.8732	7.5832
		30%	8.87000*	1.42794	.008	3.1418	14.5982
		40%	9.13000*	1.42794	.007	3.4018	14.8582
		control	-13.22000*	1.42794	.001	-18.9482	-7.4918
	20%	10%	-1.85500	1.42794	.704	-7.5832	3.8732
yellowness		30%	7.01500*	1.42794	.023	1.2868	12.7432
		40%	7.27500*	1.42794	.019	1.5468	13.0032
		control	-20.23500*	1.42794	.000	-25.9632	-14.5068
	30%	10%	-8.87000*	1.42794	.008	-14.5982	-3.1418
		20%	-7.01500*	1.42794	.023	-12.7432	-1.2868
		40%	.26000	1.42794	1.000	-5.4682	5.9882
		control	-20.49500*	1.42794	.000	-26.2232	-14.7668
	40%	10%	-9.13000*	1.42794	.007	-14.8582	-3.4018
		20%	-7.27500*	1.42794	.019	-13.0032	-1.5468
		30%	-.26000	1.42794	1.000	-5.9882	5.4682

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

Table A.15: Post Hoc Test (Bulk Density)

Multiple Comparisons

Dependent Variable: bulk

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	-.05875*	.00211	.000	-.0672	-.0503
	20%	-.07555*	.00211	.000	-.0840	-.0671
	30%	-.05745*	.00211	.000	-.0659	-.0490
	40%	-.11740*	.00211	.000	-.1258	-.1090
10%	control	.05875*	.00211	.000	.0503	.0672
	20%	-.01680*	.00211	.003	-.0252	-.0084
	30%	.00130	.00211	.966	-.0071	.0097
	40%	-.05865*	.00211	.000	-.0671	-.0502
20%	control	.07555*	.00211	.000	.0671	.0840
	10%	.01680*	.00211	.003	.0084	.0252
	30%	.01810*	.00211	.002	.0097	.0265
	40%	-.04185*	.00211	.000	-.0503	-.0334
30%	control	.05745*	.00211	.000	.0490	.0659
	10%	-.00130	.00211	.966	-.0097	.0071
	20%	-.01810*	.00211	.002	-.0265	-.0097
	40%	-.05995*	.00211	.000	-.0684	-.0515
40%	control	.11740*	.00211	.000	.1090	.1258
	10%	.05865*	.00211	.000	.0502	.0671
	20%	.04185*	.00211	.000	.0334	.0503
	30%	.05995*	.00211	.000	.0515	.0684

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

Table A.16: Post Hoc Test (Sinking Velocity)

Multiple Comparisons

Dependent Variable: sinking

Tukey HSD

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	10%	-.42670	.41555	.834	-2.0937	1.2403
	20%	-.88545	.41555	.333	-2.5524	.7815
	30%	-1.08610	.41555	.202	-2.7531	.5809
	40%	-1.69230*	.41555	.047	-3.3593	-.0253
10%	control	.42670	.41555	.834	-1.2403	2.0937
	20%	-.45875	.41555	.799	-2.1257	1.2082
	30%	-.65940	.41555	.560	-2.3264	1.0076
	40%	-1.26560	.41555	.129	-2.9326	.4014
20%	control	.88545	.41555	.333	-.7815	2.5524
	10%	.45875	.41555	.799	-1.2082	2.1257
	30%	-.20065	.41555	.986	-1.8676	1.4663
	40%	-.80685	.41555	.403	-2.4738	.8601
30%	control	1.08610	.41555	.202	-.5809	2.7531
	10%	.65940	.41555	.560	-1.0076	2.3264
	20%	.20065	.41555	.986	-1.4663	1.8676
	40%	-.60620	.41555	.623	-2.2732	1.0608
40%	control	1.69230*	.41555	.047	.0253	3.3593
	10%	1.26560	.41555	.129	-.4014	2.9326
	20%	.80685	.41555	.403	-.8601	2.4738
	30%	.60620	.41555	.623	-1.0608	2.2732

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