Proximate Composition for Egg Custard as Alternative Feed for *Macrobrachium rosenbergii* Larvae

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled Proximate Composition for Egg Custard as Alternative Feed for *Macrobrachium rosenbergii* Larvae by Hong Shen Teng, matric number F15A0053 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandary Science) with Honours, Faculty of Agro Based Industry, Universiti Malaysia Kelantan.

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Date:

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LIST OF ABBREVIATIONS AND SYMBOLS

˚C	Degree celcius
%	Percentage
CO	Cobalt
g	Gram
g/L	Gram per litre
g^{-1}	Per gram
h	Hour
M	Molarity
mg	Milligram
Min	Minute
mL	Milliliter
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ppm	Parts per million
T	Temperature
UMK	University Malaysia Kelantan
$ZnCl_2$	Zinc chloride
µm	Micrometer

Proximate Composition for Egg Custard as Alternative Feed for *Macrobrachium rosenbergii* Larvae

ABSTRACT

Recently, the demand for aquaculture feed is increasing due to the expansion of small-scale hatcheries to large-scale or semi-intensive hatcheries. Giant freshwater prawn which also known as *M. rosenbergii* is an important source of income to farmers. However, the aquaculture development of this species is restricted to the unavailability and expensive feed formulation cost. An economical feed known as egg custard has been used extensively as the *M. rosenbergii* larvae feed to replace or partially replace the expensive live food such as *Artemia* nauplii. The present study comparing two different pretreatment methods prior for proximate analysis using freeze drying and oven drying on egg custard samples. Better pretreatment method obtained in the first experiment was then applied to another egg custard diet, which was egg custard + shrimp and squid (ss). Each sample was undergone proximate analysis including moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extracts. Oven-dried egg custard samples showed higher nutritional value compared freeze drying method. The proximate analysis on formulated egg custard diet showed that egg custard diet fit the nutritional requirements of freshwater prawn larvae better compared to egg custard + SS formulation.

Keywords**:** *Macrobrachium rosenbergii*, freeze drying, oven drying, egg custard, proximate analysis

Komposisi Proksimat Kustard Telur Sebagai Makanan Alternatif Untuk *Macrobrachium rosenbergii* Larva

ABSTRAK

Pada waktu ini, permintaan untuk makanan akuakultur semakin meningkat disebabkan oleh perkembangan pusat penetasan skala kecil ke hatcheri penternakan berskala besar atau separuh intensif. Hatcheri udang air tawar juga dikenali sebagai *Macrobrachium rosenbergii* merupakan sumber pendapatan yang penting kepada penternak. Walau bagaimanapun, pembangunan akuakultur adalah terhad disebabkan oleh ketiadaan dan kos penggubalan suapan yang mahal. Kustard telur digunakan secara meluas untuk larva udang air tawar. Kustard telur semakin digunakan untuk menggantikan atau sebahagiannya menggantikan makanan hidup yang mahal seperti *Artemia* nauplii.
Dalam kajian ini, dua kaedah pengeringan yang berbeza seperti pengeringan pembekuan dan pengeringan ketuhar digunakan dalam analisis proksimat. Hasilnya kemudian dibandingkan, kaedah pengeringan yang lebih baik digunakan untuk menjalankan analisis proksimat bagi kustard telur yang lain, dinamakan kustard telur + SS. Setiap sampel yang disediakan untuk menjalankan analisis komposisi proksimat seperti kelembapan, protein mentah, lemak mentah, serat mentah, abu dan estrak bebas nitrogen. Sample kering kustard telur menunjukkan nilai pemakanan yang lebih tinggi berbanding dengan pembekuan kering. Analisis proksimat menunjukkan bahawa formulasi kustard telur sesuai dengan keperluan pemakanan larva udang galah berbanding dengan kustard telur + SS.

Kata kunci: *Macrobrachium rosenbergii*, pengeringan pembekuan, pengeringan ketuhar, kustard telur, analisis proksimat

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Malaysian Government Economic Transformation Programme (ETP) has put some stress on aquaculture to stimulate country's economic activity so as to approach high income, sustainability and inclusiveness for the nation. Due to the availability and suitability of natural habitats in Malaysia such as ponds, rivers, as well as others water bodies for aquaculture activities, the expansion of aquaculture had contributed significantly not only in increasing the yield of food security but also generate more export revenues. In Malaysia, giant freshwater prawn is one of an important targeted species for aquaculture production. Department of Fisheries, Malaysia (DOF) give high priority to this commercial aquaculture species as foods for consumption as well as for export purpose. Supply of giant freshwater prawn to the local market never met the high demand from the local people, hence culture of this prawn in Malaysia has great potential for expansion.

There are several favourable factors that accelerate the evolution of freshwater prawn farming in Malaysia. Both large-scale and small-scale commercial rearing units is applicable and appropriate for giant freshwater prawn culture. Due to the fact that freshwater prawn culture is suitable for inclusion in monoculture, polyculture and integrated aquaculture systems, it surely will bring satisfactory production and profit to the farmer. With the

advanced technology, giant freshwater prawn can be cultured intensively.

The increased demand of giant freshwater prawn in the market leads to the tremendously expansion of small–scale hatcheries and large-scale as well as semi- intensive hatcheries of *M. rosenbergii*. There are potential and great export opportunities for giant freshwater prawn. However, the further expansion and development of *M. rosenbergii* had been restricted with the presence of some obstacles. The obstacles faced by the farmers includes unavailability or declining supply of quality broodstocks, lack of a stable nursery for Post Larvae (PL), shortage of PL poor productivity, inefficient culture technology and over-dependence on imported food for *M. rosenbergii* larval (Department of Fisheries, Malaysia, 2011). Until recently, these obstacles still remain unsolved.

Since sustainable production and expensive feed cost are among major concern in culture of *M. rosenbergii*, more attention on research and development (R&D) will help to intensify and sustain the development of food quality feed for giant freshwater prawn that is economically viable. The research on the feed for larval stages as well as the production of more ready availability of commercial feeds is important to reduce the dependence on import feed and expensive live food such as *Artemia nauplii*. Support from various sectors such as government, university and private sectors such as national farmer associations surely will enhance the development of giant freshwater prawn farming. The development in giant freshwater prawn culture not only will probably improve livelihoods, but also supply a cheap source of proteins. This situation will accelerate Malaysian economy due to the increasement in export values.

Composition of diet greatly affects the rearing of *M. rosenbergii* especially in the larval rearing stage. Nutritionally balanced diet is essential in the effort of reducing the mortality rate as well as enhancing the growth rate of *M. rosenbergii* larvae. There are various feed for larval available in the market that give optimum growth rate but these

feeds sources are expensive. Most hatcheries utilize expensive live food such as Artemia as feed for larval rearing of *M. rosenbergii*. In order to lower the production cost in larval rearing, experiments on alternative diets used in larvae rearing has to be carried out. Egg custard is a formulated diet with its nutrient content meet the basic requirements of giant freshwater prawns (Idris, 2014). Egg custard is suitable to be sued as an alternative feed due to its nutrition composition is highly digestible, economical and easy to be prepared and do not require expensive machine. The efficacy of this feed formulation is to accelerate the growth process and increase the vitality of giant freshwater prawns (Idris, 2014). There are many formulations of egg custard published but not all of their nutritional composition was revealed.

Thus, this study is designed to determine the nutrients composition of two different egg custard formulations. Outcomes form proximate analysis will helps to further improve the egg custard formulations to meet the optimum nutritional requirements of *M. rosenbergii* larvae.

1.2 PROBLEM STATEMENT

Diet composition is an essential component in larvae rearing of *M. rosenbergii* due to the dramatic impact it has on the survival and growth rate as well as larvae quality. Hence, it is essential to provide nutritionally balanced feed to the *M. rosenbergii* larvae with the main purposes are to reduce mortality rate and to improve its growth rate. An efficient and nutritional diet is known to have the ability to boost the immunity of the *M. rosenbergii* larvae. Various types of larval feeds are commercially available in the market. Many hatcheries, either the local or foreign country, use expensive live feed such as

Artemia nauplii in feeding *M. rosenbergii* larvae. In *M. rosenbergii* larvae culture, feeding *M. rosenbergii* larvae with egg custard formulation as an alternative to the *Artemia* nauplii is being practiced in most of the hatcheries. However, the nutrient composition of this formulation is undetermined and feeding regime is also not optimized.

In this study, two different formulation of egg custard were used to compare and determine its proximate composition. The findings will provide better information to determine the better egg custard formulation to be used as an alternative feed for *M. rosenbergii* larvae.

1.3 HYPOTHESIS

H null: The egg custard is not suitable to be used as an alternative feed for *Macrobrachium rosenbergii* larvae.

H one: The egg custard is suitable to be used as an alternative diet for *Macrobrachium rosenbergii* larvae.

1.4 OBJECTIVES

The main objective of this study is to determine the proximate composition for two different egg custard formulations as an alternative feed for *M. rosenbergii* larvae.

1.5 SCOPE OF STUDY

This study focused on the nutrition composition of egg custard. Egg custard was selected as alternative diet in the study is mainly due to the well establishment of egg custard performance as larval feed for *M. rosenbergii.* In the study, two different recipes of egg custard were used. Their proximate composition were analyzed and compared to determine which formulation is the best to meet the nutritional requirements of the *M. rosenbergii* larvae. Different drying methods were used for pre-treatments of the samples. freeze and oven drying were used to dry up the samples before proximate analysis can be conducted. The data from these two drying methods were collected to identify which pretreatment method provide the best results in the analysis of proximate composition for egg custard samples. T test was used to determine whether there is a significance difference between the mean of two different formulated egg custard recipe.

1.6 SIGNIFICANCE OF STUDY

Throughout this study, nutrient composition of egg custard as alternative feed for *M. rosenbergii* larvae was determined. Outcomes from this study could also assist in the feeding regime of *M. rosenbergii* larvae. Besides, a nutritionally balanced and economical feed is important as alternative feeds in the larval stages of *M. rosenbergii* to lower feeding expenses without compromise their nutrition requirement.

CHAPTER 2

LITERATURE REVIEW

2.1 GIANT FRESHWATER PRAWN

Giant freshwater prawn, commonly known as *Macrobrachium rosenbergii* is one of the most cultivated prawns in the world (Gabriel et al., 2011). It is one of the largest freshwater prawns in the world (Hiroshi and Kuronuma,1980). Its natural distribution is all over the tropical and subtropical areas of the Indo-Pacific region, which covers from India to Southeast Asia and Northern Australia (New, 2000). It could be found abundantly in freshwater bodies such as lakes, rivers, and ponds (Raman, 1967). To avoid stress and maintain stable conditions for freshwater prawn, parameters such as water temperature (25-34 °C) and salinity (0-20 ppt) should be monitored and controlled regularly. The largest males can achieve a total body length of 320 mm while the largest females can only attain 250mm (Holthuis,1980). Similar to all crustaceans, it owns a hard exoskeleton that will undergo molting routinely for growth to occur. After the completion of each molt, its body weight as well as body length will be increased.

Cultivation and farming of giant freshwater prawn have developed immensely since last decade. The giant freshwater prawn has been introduced and extensively cultivated in wide range of countries such as Japan, Thailand, Malaysia, and Africa (Sadiaet et al., 2015). The culture of *M. rosenbergii* involves three phrases which are

hatchery, nursery and pond grow-out. It is a conventional commercial species that being cultured popularly either to ensure food security as well as for export purpose. The continuous growth of human population indirectly increased the demand for *M. rosenbergii*, which mainly for the domestic consumption, and subsequently for the export markets. To intensify the commercial viability of freshwater prawn production, the provision of a nutritionally balance and high quality is inevitable.

M. rosenbergii is reported to have high resistance to the disease infection which is common in shrimp aquaculture. Despite that, there were still disease outbreaks reported from prawn farmers. Major outbreaks of the disease include White Tail Disease (WTD) occurred in India in the late 1990s and early 2000s (Australian Government Department of Agriculture, Fisheries and Forestry, 2007). Moreover, *M. rosenbergii* is considered to carry the white spot virus or resistant to it (Hameed et al., 2000). Therefore, it has the opportunities and probability to spread the disease to native shrimp species. *M. rosenbergii* larvae are generally more susceptible to infection which may cause mortality. A pathogen could enter a hatchery system through feed, broodstock, instruments, water as well as unhygienic practice of working staffs (Phatararpekar et al., 2002). The factors causing disease among *M. rosenbergii* is mainly due to inappropriate management practice such as improper water treatment, poor husbandry, overcrowding, poor sanitation, as well as inadequate quarantine procedures. The management practices mainly concentrate on feeding and water quality. To solve and avoid this problem, it is necessary to have good management practice in handling the *M. rosenbergii.*

For giant freshwater prawn, females normally begin to be reproductively mature at about 6 months of age (Holthuis,1980). Spawning will occur few hours after mating, the eggs that are fertilized by the sperm will be released, transferred and attached to the abdomen until hatching. The number of eggs produced per spawning depends on the size

of the female. Berried females refer to the female that carrying eggs. The hatching rate increases as water temperature increases. With the water temperature of 28 °C, the eggs will be hatched about 20-21 days after spawning (Holthuis,1980). The four phases in the life cycles of giant freshwater prawn known as eggs, larvae, PL and adults. In each stage or phase, the time length may be affected by environmental conditions especially temperature. The newly hatched freshwater prawn known as larvae and will be separated from the female to avoid cannibalism (Hiroshi and Kuronuma,1980). Larvae will then undergo metamorphosis becoming postlarvae (PL). In PL stage, the freshwater prawn will have body length of approximately 7-10 mm. At the last stage of larval stages, the larvae metamorphoses into juveniles. At juvenile stage, they sink down and stay at the bottom. Juveniles will continue in brackish water for 1-2 weeks and later, they will migrate upstream into water with lower salinity. They begin to be sexually mature in about nine months. The adult prawn's body showed colour changes from their natural transparency to bluish and brownish colour.

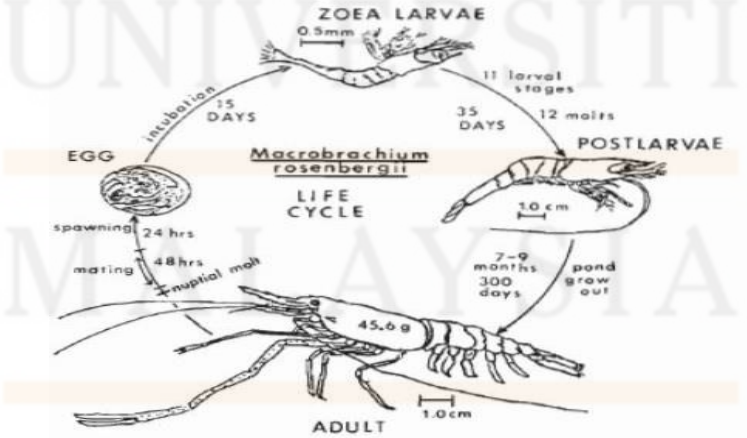

Figure 2.1: Life cycle of giant freshwater prawn *M. rosenbergii*

Source: Malecha (1984)

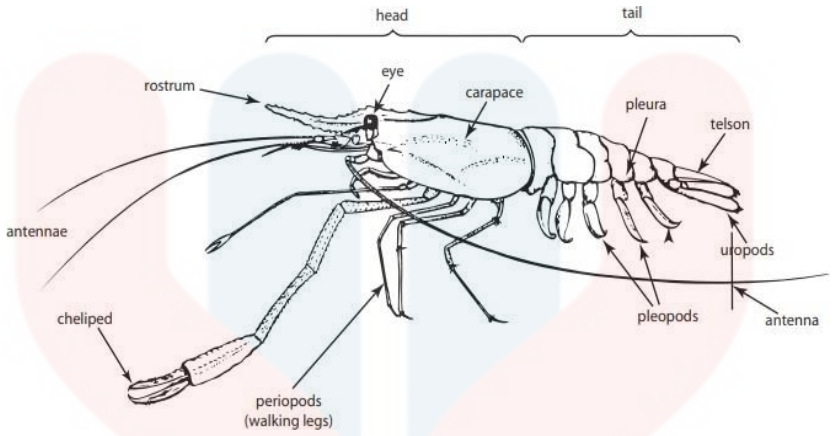

Figure 2.2: External anatomy of giant freshwater prawn *M.rosenbergii*

Source: Satya and Timothy

2.2 FRESHWATER PRAWN LARVAE AND POSTLARVAE

Newly hatched freshwater prawn larvae are planktonic and has to be transferred to the rearing tank with brackish water within 2 days or it will suffer fatality (Fisheries and Aquaculture Department, 2010). Larvae pass through 11 phases before undergo metamorphosis and turning into PL in approximately 35 days (Fisheries and Aquaculture Department, 2010). *M. rosenbergii* larvae necessitates some salinity to attain optimal growth (Malwine and Chaosu, 2009). Their growth and development, as well as breeding takes place in freshwater bodies. Metamorphosis of newly hatched larvae to PL reckon on portion and nature of food, temperature and various of water quality parameters (D'Abramo et al., 2003). The stocking and rearing of larvae can be carried out according to two- phase larval rearing system which utilize cylindro- conical tanks connectedly with

large tanks owning flat or U-shaped bottom. The system applicable forachieving better survival as well as production rate of post-larvae. During the first phase, from the stages I to V or VI, the larvae are reared in cylindro- conical tanks with high stocking density up to 500 to 700 larvae per litre. While in the second phase of rearing, from the stages of V or VI, larvae from the first phase will be collected and stocked in large tanks at the rate of 50 to 80 larvae per litre.

2.3 WATER QUALITY REQUIREMENTS OF FRESHWATER PRAWN LARVAE

To achieve both optimum survival rate and the production rate of *M. rosenbergii* larvae, it requires freshwater source which is clear and free from pollution. The marine water that collected has to be chlorinated with bleaching powder to kill the micro-flora and micro-fauna. Then, the water is left to settle for a week for the preparation of larval medium. Water temperature is key factor in the culture of prawn as it greatly influences their metabolism, oxygen consumption, growth, molting and survival (Soundarapandian et. al., 2008). It is recommended to keep the *M. rosenbergii* larvae salinity at 12ppt (Fisheries and Aquaculture Department, 2010). The ideal range of temperature for *M. rosenbergii* is about 28-31 °C (Fisheries and Aquaculture Department, 2010). Temperature below than 24-26 °C will significantly reduce the growth rate of *M. rosenbergii* larvae resulting in longer period taken to reach metamorphosis (Fisheries and Aquaculture Department, 2010). Turbidity influences the light penetration rate which has effect on photosynthesis and may cause algal growth. Stress can occur when dissolved

oxygen levels drop below around 4 ppm (Avault, 1987). Farmers generally maintain dissolved oxygen level at 6-8 ppm (New, 1990). In freshwater environment, dissolve oxygen levels can be altered notably, especially on the bottom of the growth out ponds (Cheng et al., 2003). Eutrophication refers to the process of accumulated organic substances in an ecosystem due to the raised of nutrient inputs such as nitrogen and phosphorus (Nixon, 1995). Human activities such as run-off from agricultural as well as aquaculture activities contributes to the occurrence of these nutrients (Anderson et al., 2002). Harmful algal blooms bring several of detrimental effects including oxygen depletion, alteration or change of habitat through shading of the benthos, contamination of the water sources and mortality of aquatic organisms. Management strategies need to be adopted to limit the ammonia and other nutrient content in the rearing water. The management practices including reduce the nutrient input by reduce feeding, use freshwater to dilute the nutrient concentrations, decrease stocking density to lower the frequency of nitrogenous excretion and reduce the pH level of the pond. It is crucial to control and maintain the optimum water quality and other parameters for *M. rosenbergii* larvae to avoid sudden change in these parameters which subsequently leading to stress and even death among the freshwater prawn.

2.4 FOOD AND FEEDING HABIT OF FRESHWATER PRAWN

Freshwater prawn is omnivorous and coprophagous (Gapa, Mukhopadhyay and Chattopadhyay, 2005). It has been shown its preference in utilizing natural food as compared to artificial feeds (Gapa, Mukhopadhyay and Chattopadhyay, 2005). Nowadays, many commercial hatcheries utilize Artemia nauplii in combination with a

wet diet such as egg custard or dried supplement during the larval rearing of freshwater prawn (Correia et al., 2000; Valenti and Daniels, 2000; Lavens et al., 2000). There is limited knowledge and lack of evidence regarding the feeding behaviour and nutrients requirements of *M. rosenbergii* larvae (Valenti and Daniels, 2000). Some authors advocated that for each larval stage, the inert food particle size is specific (Aquacop, 1977; Corbin et al., 1983; New and Shingholka, 1985; Daniels et al., 1992; New, 1995) while at the same time, some authors verified the utilization of several particle size for the eleven larval stages of *M. rosenbergii* (Sick and Millikin, 1983; Barros, 1996).

Supplying suitable and appropriate physical characteristics of the feed for each larval stage of *M. rosenbergii,* feeding efficiency could be optimized due to enhanced palatability (Valenti and Daniels, 2000). The factors that need to be considered in the selection of feedstuffs including availability, cost, and avoidance of conflict with consumption. In the first two stages of zoea, there are no-feeding required as the larvae survive with the remains of yolk from the egg. Newly hatched *Atermia nauplii* served as primary food source *for M. rosenbergii* larvae in the early life cycle stage (stages II to VI). The larvae normally showed omnivorous feeding habits from the third stage zoea. In the shortage of live food, *M. rosenbergii* larvae will feeding on small particles organic matter (Ling, 1969; New and Singholka, 1985). Deshimaru and Shigeno (1972) claimed that animal origin food is preferred over plant material.

A wide range of fresh feeds such as shrimp and clam meats will be given to post larvae and juvenile at regular intervals. The feed has to be sieved through a mesh to obtain suitable particle size. Suitable feed to be given to the larvae including living aquatic animals such as small aquatic worms, *Acetes sp*. Such as small clams as well as plant materials such as tapioca. Formulated pellets are suggested at the rate of 20% of post larval body weight. The survival rate is believed to reach about 75-80% with proper

feeding regime.

High feed concentration is encouraged with the aim to get maximum survival and this is accompanying with careful monitoring of the water quality. The frequency of feeding fish has adverse effect on larval survival rate. A low feeding frequency probably will cause mortality due to cannibalism. Excess feeding or over-feeding should be avoided as it not only affects the water quality but also has detrimental effect on larval health. Overfeeding probably will cause the building up of organic matter that may results in the reinforcement of bacteria while underfeeding lead to poor growth rate (Valenti and Daniels, 2000). The intake of feed in freshwater prawn larvae highly depends on the water temperature.

2.5 NUTRITIONAL REQUIREMENTS OF FRESHWATER PRAWN

M. rosenbergii is capable of digesting both of plant and animal origin food in a wide range (Gapa, Mukhopadhyay & Chattopadhyay, 2005). It requires ten essential amino acids similar to others aquaculture species, however, the quantitative requirements are still not yet identified. Diets with about 35-40% protein are verified needed for development of *M. rosenbergii* (Gapa, Mukhopadhyay and Chattopadhyay, 2005). *M. rosenbergii* are known to utilize about 30 % dietary fiber (Gapa, Mukhopadhyay and Chattopadhyay, 2005). The amount of lipid in prawn diets can be 5 % (Gomez, Nakagawa and Kasahara, 1988). *M. rosenbergii* requires 60-150mg vitamin C in one kilogramme diet (Gapa, Mukhopadhyay and Chattopadhyay, 2005).

Carbohydrates serves as a reservoir of dietary energy in the synthesis of chitin, steroids and fatty acids. Cellulose, a dietary carbohydrate, helps in facilitating the

movement of passage of food. Cellulose in diets may enrich the prawns' nutrition (Briggs 1991). Dietary glucosamine enhances molting which maylead to increased growth (Mitra *et al*., 2005). The dietary mineral requirement and information regarding quantitative and qualitative requirements for *M. rosenbergii* is not yet clarified (Kanazawa, Teshima and Sasaki, 1984). The significance of dietary minerals has been proved by the ability of prawn to absorb specific types of minerals such as calcium and magnesium. Both calcium and magnesium are essential in comprising a significant portion of *M. rosenbergii* exoskeleton (Mukhopadhyay, Rangacharyulu, Mitra and Jana, 2003). These elements were being absorbed in large quantities during premolt period. Calcium and magnesium are macro- elements, which are important to keep exoskeletons of *M. rosenbergii* strong. Both calcium and magnesium are inorganic components found in the carapace and exoskeleton of prawns (Fieber and Lutz 1985; Greenaway 1993). While in the low water hardness, *M. rosenbergii* requires longer time for postmolt hardening of the exoskeleton (Adhikari et al. 2007). Calcium and magnesium are critical for its molting frequency (Wilder et al. 2009) and larvae survival. Water bodies with low- alkalinity water generally have low calcium concentration and calcium carbonate usually supplied to solve this problem. Performance of the freshwater giant prawns were better when 3% calcium was provided in soft water (Gapa, Mukhopadhyay and Chattopadhyay, 2005).

Table 2.2: Nutrient requirements of freshwater prawn based on laboratory trials

Nutrients	**Growth Stages**	**Requirement**
Protein (%)	Broodstocks	38-40
	Juveniles	35-37
	Adults	28-30
Carbohydrate (%)	For all stages	25-35
Lipid (%)	For all stages	3-7
Cholesterol (%)	For all stages	0.5-0.6
Vitamin C (mg/kg)	Grow out	100
Calcium/ Phosphorus		1.5-2.01
Zn (mg/kg)		90
Other minerals		Quantitative requirements not yet known
Energy	Broodstocks	3.7-4.0 kcal/g feed
	Other stages	2.9-3.2 kcal/g feed

Source: Gapa, Mukhopadhyay and Chattopadhyay (2005)

Table 2.3: Summary of proximate analysis of feed ingredients for Freshwater prawn

Feed Ingredients	Moisture	Crude Protein	Crude Fat	Crude Fiber	Ash	Nitrogen Free Extracts
Cassava leaf meal	3.83	27.56	7.66	11.73	7.4	45.7
Cotton seed cake	7	37	6.7	13	1	35.3
Rice bran	10.1	12.6	11.3	19.3	10.2	36.5
Blood meal	11	76.7	1.1	1.2	4	5.96
Meat and bone meal	5.36	48.4	9.67	1.78	33.56	7.36

Source: Gerpacio and Castillo, 1979; Yamazaki, Lopez and Kaku (1988)

2.6 GROWTH AND SURVIVAL RATE OF FRESHWATER PRAWN

There are several growth parameters available to determine the growth performance and development of freshwater prawn, which are length (cm) and weight (g) gain, specific growth rate (%), survival rate (%), FCR and Net Production (kg/decimal). The sampling will be collected randomly by using a cast net for the determination of their total body weight and length. The diet containing crude protein(31.50 %), lipid of (6.79 %), ash of (4.91 %) and moisture of (8.98 %) are recommended to be a suitable supplementary feed for growth of freshwater prawn (Prianka and Anisur Rahman, 2016). It was proved to contribute the enhanced growth performance in terms of the growth rate, feed conversion ratio (FCR), SGR, survival rate and production.

2.7 EGG CUSTARD

Egg custard is used widely as the primary food source for the larviculture of most of the aquatic species, including *M. rosenbergii* (Idris, 2014). This scenario happened due to the fluctuation in the manufacturing of Artemia cysts which results from the changes in environmental conditions which may affect their availability as well as their nutritional quality (Lavens et al., 2000; Dhont et al., 2010). The remarkable alterations in production of the Artemia nauplii affect the market price of the cysts. By considering this situation, an effective and economical alternative diet known as egg custard was introduced to replace Artemia. A study found that *M. rosenbergii* larvae accept egg custard as inert diet from stage IV onwards and shows a markedpreference for this food from stage VII onwards (Araujo and Valenti, 2007).

Nowadays, the egg custard used in the prawn hatcheries usually contained ingredients such as milk powder, and chicken egg yolk (New 1990; Alam et al. 1993; Hien et al. 2005). The ingredients of egg custard can be adjusted in order to determine the combinations of ingredients that could produce the diet which capable enhance the survival rate as well as the growth rate of *M. rosenbergii* larvae. In this study, different egg custard recipes with the use of locally available ingredients, were tested with proximate analysis to determine which recipes most suitable to meet the nutritional requirements of freshwater prawn larvae. The egg custard that meet the freshwater prawn nutrients requirements is considered capable to reduce the rearing period and improve its growth rate. Egg custard will be made into particles size smallerthan 1mm before supply to the *M. rosenbergii* larvae. The amount of egg custard provided to the *M. rosenbergii* larvae will be determined by observing whether there is residue at the bottom of the tanks in night.

Table 2.4: Basic composition of egg custard for freshwater prawn larvae

Ingredients	Inclusion Levels
Egg	1 egg
Spirulina powder	1 g
Baker's yeast	1 g
Artemia flakes	2 g
Corn flour	3 g
Milk powder	3 g
Cod liver oil	1 ml

Source: Jiwan et al. (2007)

2.8 PROXIMATE ANALYSIS

Feed is a major component which deciding the aquaculture viability and profitability. Development of a prawn feed involves proximate analysis of feed components, digestibility, performance efficiency as well as feeding cost (Gapa, Mukhopadhyay and Chattopadhyay, 2005). Proximate analysis is a feed analysis designed to measure the major nutrients in livestock feeds. It is developed in Germany by Henneberg and Stohman the late 1860s. In proximate analysis, there are few categories including moisture, ash, crude protein, crude fat and crude fiber that be measured based on chemical properties. Proximate analysis is the analysis of macronutrients including protein, fat, moisture, carbohydrates, amino acids and ash. They were identified in accordance to AOAC methods (2005).

In the study, the egg custard was sieved into powder form with uniform size with the use of sieve tube. The egg custard was then immediately processed for analysis of proximate composition to determine its nutrients content. The proximate composition of *M. rosenbergii* larval diet not only provide information of its nutritional contents but also useful for product development, quality control (QC) as well as regulatory purposes. The proper sample handling and collection is essential to ensure analysis of a homogenous sample in order to obtain accurate yet reliable results. At 100 ^{o}C, the loss in weight that results from removal of water or drying of a known weight of food is known as moisture. The ash content is determined by ignition of a known weight of the food at 550 ^{o}C till the removal of all carbon. Even the ash is considered the inorganic constituents of the food, it may contain organic origin material such as Sulphur and phosphorus from protein. Hence, the ash is not an accurate representative of the inorganic material.

A modification of a technique originally devised by Kjeldahl was introduced to calculate the crude protein (CP) over 100 years ago. An estimation of protein value is determined by the multiplying the nitrogen percentage by 6.25. The calculation for crude protein is based on the assumption that the nitrogen derived from protein containing 16 % nitrogen. Since the crude protein value is determined by others sources such as free amino acids and nucleic acids besides protein, it is not a representative for true protein. The Ether Extract (EE) can be determined through the continuous extraction of the sample with petroleum ether for a definite period. Crude fat is investigated by the continuous extraction with petroleum ether in a defined period. Both Crude Fiber (CF) and the nitrogen-free extractives (NFE) contain carbohydrates. CF was analyzed by treating the residues from ether extraction by boiling acid and alkali of defined concentration, the organic residue is known as crude fiber. Nitrogen-free extractives (NFE) is calculated by subtracting the sum of percentage of others proximate analysis factors from 100.

Nitrogen-free extractives, such as starch and sugars are heterogenous mixtures which are not categorized in the other fractions.

2.9 OVEN DRYING AND FREEZE DRYING

Drying is widely used food preservation method with the aim to reduce the moisture content or water activity which consequently contributes to physicochemical and biological stabilization (Warren, Julius and Peter, 2005). Proper drying process is necessary as quality of food products may be partially or totally affected by drying processes. Physical, chemical or biological changes not only influence the characteristics of food products during drying process but also affect its physical properties such as colour, texture and flavour but also bring about unfavourable biochemical reactions such as deterioration of food products or degradation of nutritional substances (Ratti, 2001).

Type of oven used, and the time and temperature of drying are factors that decide the amount of moisture. The time for moisture analysis may be from a few minutes to over 24 h. Particle size distribution, sample sizes as well as its surface area has effect on the rate and efficiency of moisture removal. The advantages of oven drying including it could maximize the retention of nutrients and minimize the risk of oxidation with little air present. While the disadvantages of oven drying are it has low production rate.

Freeze drying, a dehydration process which used to freeze the product at low temperature, then the ice will be removed by sublimation (Ratti and Cristina, 2008). This method is conventional in evaporating water by using heat. Freeze drying usually will produce high quality product due to the use of low temperature in processing (Ratti and Cristina, 2008). The original shape and quality of the dehydrated product can be

maintained with the use of freeze-drying method. There are various ways to freeze a product, freezing can take place in a freezer, shell freezer, or on a shelf in the freeze dryer. For sublimation to occur, the material will be cooled below its triple point. The second phase of freeze drying is a slow process which also known as sublimation, an estimation of 95 % water was removed in this phase. The final phase of freeze drying is adsorption in which the ionically-bound water molecules will be eliminated. Most of the materials will have the residual moisture of approximately 1-5 %. The advantages of freeze drying including it enabled the products to last for months or even years (Bruso, 2011) while its disadvantages is it is a slow process and involves the use of expensive equipment.

Table 2.5: Proximate composition in the feed

Proximate Composition		Substances in Respective Composition	
Moisture		Water, volatile substances	
Dry Matter	Organic Matter	Crude Protein	Pure protein, amino acids, non-protein compound
		Crude Fat	Fat, complex lipid, sterols, fatty acids, fat- soluble dyes
		Crude Fiber	Cellulose, hemicellulose, lignin
		Nitrogen Free Extracts	Organic acids, water soluble dyes
	Inorganic Matter	Ash	Pure ash, organic residue, soil

Source : Jiwan et al. (2007)

CHAPTER 3

MATERIALS AND METHODS

3.0 EXPERIMENTAL SETUP AND DESIGN

This study focused on the proximate composition of the two different egg custard recipes formulations, which were egg custard and egg custard + SS as an alternative feed for *M. rosenbergii* larvae. Each of the proximate analysis carried on the two formulations which has three replications.

3.1 PREPARATION OF EGG CUSTARD

For egg custard formulation (recipe 1), all the ingredients, starting with the egg was milled by using mixer for about 1 minute until it become softer. One bag sunlac milk powder were added gradually to make sure it was homogenised in the blender for about 3 minutes. Then, the mixture was steamed for about one hour in a steaming pot. The egg custard was cooled at room temperature before shredded on fine nets in order to produce uniform particle size egg custard. The egg custard was airtight and stored in a refrigerator for one day. Then, the egg custard was dried under the fan for it to become hard. The egg custard was grated with the use of blender. The egg custard was then placed into grater and grate according to seed size.

All the ingredients (80 g milk powder, 80 g squid, 80 g shrimp, 5 chicken eggs, 5 g multivitamin and 5 g mineral salt), starting with the egg was milled for one minute until it was thoroughly mixed. The squid and the prawn were milled for 3 min until it is homogenized with the eggs. Mineral salt, vitamin and milk powder were added to the homogenized mixture gradually. The mixture was milled for another 2 minutes and steamed for 45 min. The egg custard was grated into uniform particle size.

Table 3.1: Egg custard ingredient

Ingredients	Quantity
Milk Powder	500 g
Eggs	6 piece

Source: Jabatan Negeri Perak (2017)

Table 3.2: Egg custard + shrimp squid (SS) ingredient

Ingredients	Quantity
Milk Powder	80 g
Squid	80 g
Shrimp	80 g
Chicken egg	5 piece
Multivitamin	5 g
Mineral salt	5 g

Source: Idris Ahmad (2014)

3.2 PRETREATMENT FOR EGG CUSTARD PRIOR TO PROXIMATE ANALYSIS

Before the proximate analysis of egg custard was carried out, egg custard sample was pre-treated for drying process using oven-dried and freeze-dried methods. The data of the proximate composition of egg custard from the oven- dried and freeze-dried was then compared to determine which method is more effective in reducing the moisture content of egg custard. Then, the more effective method was used in identifying the proximate analysis of the egg custard + SS formulation.

3.3 ANALYSIS OF PROXIMATE COMPOSITION OF EGG CUSTARD

For proximate analysis, all the experiments were replicated three times to obtain a more accurate and reliable results. In the analysis of the proximate composition of the egg custard formulation, the proximate analysis parameters were determined.

3.3.1 WATER ANALYSIS

All the crucible will be dried in oven for 30 min. After drying, the crucible was cooled in desiccator to avoid moisture absorption. The sample was grounded to ensure the uniformity of heat penetration. The cooled dried crucible was weighed. About 5 g of ground sample will be placed in the crucible and it will be dried at the temperature of 105 °C for one whole day. The crucible with the samples will then be cooled in the desiccator

and reweighed.

Percentage of dried matter and moisture content will be calculated as formula bellow:

$$\text{Dry Matter (\%)} = \frac{\text{Weight of dried matter}}{\text{Weight of sample}} \times 100\% \quad (3.1)$$

$$\text{Moisture (\%)} = 100 - \text{\% of dried matter} \quad (3.2)$$

3.3.2 CRUDE PROTEIN (CP) ANALYSIS

About 10 g of sample was weighed and inserted into each digestion tubes. Each tube is also was added with 2 pieces tablets of Kjeltabs catalyst. 12 mL of concentrated H_2SO_4 was added into each tube inside the fume chamber and slowly shaked. The tubes were connected to the digester. After 5 minutes, the system was stopped when acid vapour appeared at exhaust system. The tubes were cooled vertically for about 10 to 20 min. 75 ml distilled water was poured into cold tube and continue distillation process. For preparing receiving solution, 25 ml of 4% Boric acid with 10 drops of Green Bromocresol indicator will be filled into 250ml conical flask. Receiving solution was placed to distillation unit. 50ml of 40 % of NaOH automatically flow into tube. Distillation process will be operated 4 minutes until light green solution formed. The product from distillation process was titrated with 0.1 N HCl until the colour turn into grey. Titration volume will be recorded. Percentage of crude protein (CP) will be calculated by using formula below:

Denominations:

P (%) = Percentage protein content of samples (%)

V = Volume of 0.2 N HCl used in sample titration (mL) N = Normality of HCl (N)

W = Weight of samples (g)

14.007 = Molecular mass of nitrogen

6.25 Conversation factor of protein-nitrogen

$$\text{Crude protein (\%)} = \frac{\text{Titration of volume sample x N x 14.007 x6.25 x100\%}}{\text{Weight of sample (g)}} \quad (3.3)$$

3.3.3 CRUDE FAT ANALYSIS

All the glass apparatus was rinsed by petroleum ether and dry at 102 °C in the oven and will be cooled in the desiccator. About 5 g of dried sample was weighed and placed in the extraction thimble. A 150 ml round bottle flask was taken and was filled with 300 ml of petroleum ether. The whole setting was placed on heating mantle and allowed the petroleum ether to boil at boiling temperature. The extraction process was continued almost 6 h. The condensing unit was removed from extraction unit and allowed the sample to cool down. Almost all the solvent was collected after distillation. The round bottle flask was placed in the oven and dried at 102 °C for 1-2 h until constant weight reach. The round bottle flask was cooled in desiccator and weighed. Percentage of Crude fat was calculated by using formula below:

$$\text{Crude fat (\%)} = \frac{\text{Weight of flask with extract}}{\text{Weight empty flask}} \times \frac{100\%}{\text{weight of sample}} \quad (3.4)$$

3.3.4 CRUDE FIBER (CF) ANALYSIS

The grinded sample was weighed about 1 g. Then, 1.25 % sulfuric acid was added up to 150 ml notch after preheating by the hot plate in order to reduce the boiling time. About 3-5 drops of octanol which act as antifoam agent was added. Then it was boiled for 30 min. After boiling, it was connected to vacuum for draining sulfuric acid. It was washed with 30 ml of hot deionized water for three times, each time was connected to compressed air for stirring the content. 150 ml of preheated potassium hydroxide 1.25 % and 3-5 drops of antifoam were added. After 30 min of boiling processed, the sample was filtered and washed. Cold deionized water was used for last washing, aimed to cool the crucible. The crucible content was washed for 3 times with 25 ml of acetone, stirring each time. The crucible was removed, and the dry weight was determined after drying in an oven at 105 °C for an hour or up to constant weight.

Percentage of Crude Fiber (CF) will be calculated by using formula below:

$$\text{Crude fiber (\%)} = \frac{(\text{Weight of crucible + dried sample}) - \text{Weight of sample}}{\text{Weight of sample}} \times 100\ \% \quad (3.5)$$

3.3.5 ASH ANALYSIS

The samples were grinded and weighed about 5 g. The dried crucible was weighed prior to sample loading. The samples in the crucible was placed into the furnace at 550 °C for 24 h. Next day, the samples were taken out from the furnace

and the residue was calculated.

Percentage of ash will be calculated by using formula below:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Initial weight (g)}} \times 100\% \qquad (3.6)$$

3.3.6 NITROGEN FREE EXTRACT (NFE)

Nitrogen free extract can be deliberated by using 100 to subtract the percentage of water, crude protein, ether extract, crude fiber and ash.

3.4 STATISTICAL ANALYSIS

The results were analyzed statistically by using t test (XLSTAT, Microsoft Excel 2010) and the significant difference was 95 confident level. An independent t test, which also known as unpaired sample t test was commonly used in comparing the means among groups. P value or probability value (P=0.05) is a quantitative measure to determine the result of statistical hypothesis testing

CHAPTER 4

RESULT AND DISCUSSION

4.1 DIFFERENT PRETREATMENT METHOD ON EGG CUSTARD PRIOR FOR PROXIMATE ANALYSIS

The experiment showed that the moisture content in the freeze-dried egg custard was higher than that of the oven-dried egg custard, with the average reading of 9.3603 % and 9.7130 % (Table 4.1). The high moisture content in the egg custard diet showed that they are more prone to the deterioration, will have a shorter shelf life since the food or feed with high moisture content are prone to deterioration due to the favourable condition for bacterial activity (Fennema and Tannenbaum, 1996). The higher the moisture content in a food, the higher is the water activity.

Table 4.1: Proximate analysis of the oven-dried and freeze-died egg custard samples

Egg custard sample	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Nitrogen Free Extracts (NFE)
Oven-dried egg custard	9.3603	32.232	5.8437	5.5824	7.7058	39.276
Freeze-dried egg custard	9.7130	30.994	4.3726	5.2458	7.9378	41.737
P value	0.3368	0.0006	0.0077	0.2200	0.3419	0.0016

The experiment showed that the crude protein content in the oven-dried egg custard was higher than that of the freeze-dried egg custard, with the average reading of 32.2319 % and 30.9944 % respectively. Similar to other crustaceans, the optimum crude protein level for freshwater prawn larvae ranges from 30-45 % (Rangacharyulu, 1999). Hence, it can be concluded that oven-dried egg custard able to meet the protein requirements of freshwater prawn larvae.

The experiment showed that the crude fat content in the oven-dried egg custard was higher than that of the freeze-dried egg custard, with the average reading of 5.8437 % and 4.3726 % respectively. The experiment showed that the crude fat content in the oven-dried egg custard was higher than that of the freeze-dried egg custard, with the average reading of 5.5824 % and 5.2458 % respectively.

The experiment showed that the ash content in the freeze-dried egg custard was higher than that of the oven-dried egg custard, with the average reading of 7.9378 % and 7.7058 % respectively. The experiment showed that the nitrogen free extracts content in

the freeze-dried egg custard was higher than that of the oven-dried egg custard, with the average reading of 41.737 % and 39.276 % respectively.

Verification of drying method (oven-dried and freeze-dried) for egg custard performed through the analysis on its proximate composition. The moisture (%) of the egg custard used as an indicator that determine either oven drying or freeze drying is better in determining the proximate composition of egg custard. Since the p value for the moisture is 0.3368 ($p>0.05$), it can be concluded that there is no significant difference between the moisture content between two different egg custard samples. The oven-dried and freeze-dried egg custard have different result in proximate analysis as the nutrient composition of two different egg custard formulations were greatly affected by different drying methods.

4.2 COMPARISON OF THE PROXIMATE COMPOSITION OF EGG CUSTARD RECIPES

According to the proximate analysis results (Table 4.2), there is no significant difference between the oven-dried egg custard (first recipe), freeze-dried egg custard (first recipe) and oven-dried egg custard + SS (second recipe).

Table 4.2: Mean ± SD reading of the proximate composition of the egg custard and egg custard + SS formulation

Egg custard sample	**Moisture (%)**	**Crude Protein (%)**	**Crude Fat (%)**	**Crude Fiber (%)**	**Ash (%)**	**Nitrogen Free Extracts (NFE)**
Oven-dried egg custard (first recipe)	9.3603 ± 0.4870	32.232 ± 0.1287	5.8437 ± 0.2743	5.5824 ± 0.3368	7.7058 ± 0.1332	39.276 ± 0.4152
Oven- dried egg custard + SS (second recipe	9.7130 ± 0.7743	30.994 ± 0.3360	4.3726 ± 0.1758	5.2458 ± 0.6619	7.9378 ± 0.4882	41.737 ± 0.3247
P value	0.0265	3.8406	9.4862	0.0014	0.0097	0.0016

The results showed that the moisture level in oven-dried egg custard (second recipe) is the highest and oven-dried egg custard (first recipe) is the lowest with the moisture level of 11.173 ± 0.7743 ($p<0.05$) and 9.3603 ± 0.4870 ($p<0.05$) respectively. Hence, the oven-dried egg custard + SS (second recipe) has the shortest shelf life while

the oven-dried egg custard (first recipe) has the longest shelf life. There is significant difference between the moisture content of the two different formulated egg custard diet.

The crude protein content in the oven-dried egg custard + SS (first recipe) was higher which was 32.232 ± 0.1287 ($p>0.05$) while that of oven-dried egg custard + SS (second recipe) was 4.952 ± 0.036 ($p>0.05$). Protein is important for normal functioning, growth and development as well as the maintenance of the body tissues. Proteins are made up of chains of amino acids which determine its cell structures, and also serves as the key components as well as energy source. Several meals such as fish meal, shrimp meal and meat meal are provided as protein sources for freshwater prawn larvae to improve growth rate, moulting frequency as well as survival rate (Leena et al. 1997). These meals are more expensive in ingredients as compared to the egg custard diet. The high protein content in the formulated egg custard can replace other expensive animal protein source in feed manufacturing.

The contents of the crude fat as an indicator of the palatability of the egg custard diet as the dietary fats plays vital role in increasing food palatability by fascinating and retaining the flavour. The higher the palatability of a diet, the higher the nutrient could be absorbed by an animal body. Lipids are efficient energy source as it contains more than twice the energy of carbohydrates and proteins (Okuzumi and Fugii, 2000). This is due to the fact that 1g of lipid can give 9 kcal energy (Okuzumi and Fugii, 2000). Despite as an energy sources, dietary fats provide essential fatty acids that required for normal growth and maintenance, and proper functioning of physiological processes (Kanazawa et al., 1977; Corbin et al., 1983). The results showed that crude fat level in oven-dried egg custard + SS (second recipe) is higher as compared to that of oven-dried egg custard (first recipe), which is 11.138 ± 0.1758 ($p>0.05$) and 5.8437± 0.2743($p>0.05$) respectively. According to Aqua feeds (2005), the nutritional requirements of giant freshwater prawn

for lipids, including phospholipids is in the range of 3-7 %. Hence, the first recipe of oven-dried egg custard is more suitable to be used as an alternative feed for *M.rosenbergii* larvae. New (1980) reported that dietary lipid level more than 10 % was not able to be tolerated by freshwater prawn. Hence, the second recipe of egg custard is not suitable to be the alternative feed for freshwater prawn larvae unless its ingredients is modified to less fat content.

The ash contents of oven dried egg custard + SS (second recipe) is higher, which were 9.0634 ± 0.4882 ($p<0.05$) and 7.7058 ± 0.1332 ($p<0.05$) respectively. The higher the ash contents, the higher is the mineral contents in the diet. There is limited information regarding the quantitative mineral requirement of freshwater prawn. However, dietary supply of calcium has been proved have the potential in improving the freshwater prawn growth rate (Aqua feeds, 2005). Macro-elements are required in considerable quantities as they play vital role in healthy growth and development of freshwater prawn (New, 1976). Besides that, micro-elements which also known as trace minerals responsible for various body functions. It has significant impact on immune functions and disease resistance of shrimp and fish. Performance of the giant freshwater prawn shown better when calcium with 5 ppm concentration was provided (Aqua feeds, 2005).

The higher percentage of crude fiber in the egg custard diet makes them a suitable and appropriate diet for M. rosenbergii larvae as fiber facilitates the movement of food through the digestive tract. In the study, the crude fiber contents of oven dried egg custard + SS (second recipe) is higher than that of oven-dried egg custard (first recipe) which is 8.9408 ± 0.6619 ($p<0.05$) and 5.5824 ± 0.3368 ($p<0.05$) respectively. The high crude fiber content makes the egg custard a favorable diet for freshwater prawn larvae as high fiber content food aids in digestion (Saldanha, 1995; WHO., 2005).

In the present study, the nitrogen free extracts contents of oven dried egg custard

+ SS (second recipe) was higher than oven-dried egg custard (first recipe), which were 54.760 ± 0.7005 ($p>0.05$) and 39.276 ± 0.4152 ($p>0.05$) respectively. The higher the contents of NFE, the higher the carbohydrates content in the diet.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The results of the study revealed the proximate composition of each egg custard samples through the conduct of proximate analysis. Parameters such as moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extracts were calculated and recorded as the indicator of the nutritional value of the egg custard. To conclude, the oven-dried egg custard + SS sample (second recipe) contains higher contents of crude fat, crude fiber, ash, and moisture as compared to oven-dried egg custard (first recipe). However, the proximate composition showed no significant result except moisture, crude fiber and ash, it can be concluded that both of the formulated egg custard diet (first recipe of egg custard) and second recipe most meets the nutritional requirements of the freshwater prawn larvae, the diet was considered and recommended suitable as the alternative feed for *M. rosenbergii* larvae.

5.2 RECOMMENDATION

Present study suggests that the formulated egg custard diet (first recipe) can be recommended as an alternative feed for *M. rosenbergii* larvae due to the presence of good nutrients source such as protein, fiber and minerals However, more advanced research such as feeding trial need to be carried out to access the performance of *M. rosenbergii* larvae which fed with the formulated diet. Throughout the feeding trial, the growth and survival rate of freshwater prawn larvae should be monitored and recorded to investigate whether their performance show improvement with the provision of formulated egg custard diet as alternative feed. Feeding trial will give more accurate and direct results in determining whether a formulated diet is suitable for an animal as compared to proximate analysis. This is due to the fact that through feeding trial, the acceptability of the diet was evaluated based on the observations of the feeding behavior of the freshwater prawn larvae. The growth and survival rate can be determined during the termination of the feeding trial.

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APPENDICES

(a) (b) (c)

Figure A.1: The egg custard was prepared (a) The egg custard was steamed and allow it to be cooled. (b) The egg custard was started to grate for uniform size. (c) The egg custard sample with uniform size was produced for drying.

(a) (b)

Figure A.2: The egg custard samples were prepared for proximate analysis (a) The egg custard samples were placed in the Soxhlet extractor for crude fat analysis. (b) The egg custard samples were placed in the kjeldahl distillation apparatus for crude protein analysis.