UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF SELECTED MICROALGAE (Spirulina sp., Nannochloropsis sp. AND Chlorella sp.) AS FEED SUPPLEMENT FOR Macrobrachium rosenbergii (de Man) JUVENILES

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By

SUNIZA ANIS BINTI MOHAMAD SUKRI

Thesis Submitted to the School of Graduate Studes, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2017

DEDICATION

To my mom and dad who encourage me
TUAN YAH BINTI TUAN LONG
MOHAMAD SUKRI BIN MOHD ZAIN

Beloved Husband
JALAL IKRAM BIN MOHAMED NORI

Beloved sons
AHMAD FURQAN MAHDI BIN JALAL IKRAM
AHMAD FURQAN ZUHDI BIN JALAL IKRAM
AHMAD FIRMAN ZIKRI BIN JALAL IKRAM

Siblings
SAIFUDDIN BAKHTIAR BIN MOHAMAD SUKRI
SYURIYANA BINTI MOHAMAD SUKRI
SAIFULNIZAM BOKHARI BIN MOHAMAD SUKRI
SAIFULHISAM BOKHIRI BIN MOHAMAD SUKRI
NURSOFIAH ANIS BINTI MOHAMAD SUKRI
MUHAMMAD SYAFIQ BIN MOHAMAD SUKRI

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ASSESSMENT OF SELECTED MICROALGAE (*Spirulina* sp., *Nannochloropsis* sp. AND *Chlorella* sp.) AS FEED SUPPLEMENT FOR *Macrobrachium rosenbergii* (de Man) JUVENILES

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February 2017

Chairman : Associate Professor Che Roos Bin Saad, PhD
Faculty : Agriculture

A series of experiment were conducted to study the potential of feed formulation for freshwater prawn *Macrobrachium rosenbergii* juvenile. Study on the potential of selected microalgae (*Spirulina* sp., *Nannochloropsis* sp. and *Chlorella* sp.) as feed for *M. rosenbergii* juvenile was conducted with amino acid and fatty acid profile. From the results, methionine and tryptophan in amino acid were not detected in *Spirulina* sp., *Nannochloropsis* sp. and *Chlorella* sp. in all four different levels. The arginine, isoleucine, leucine, alanine, aspartic acid and glutamic acid level were high with increasing level of *Spirulina* sp. *Nannochloropsis* sp. and *Chlorella* sp. showed high percentage in leucine, lysine and arginine. For fatty acid analysis, only *Spirulina* showed a small portion of γ–linolenic acid (GLA). *Chlorella* showed no detection on Eicosapentaenoic acid (EPA) when compared to *Spirulina* and *Nannochloropsis*.

For second experiment, a series of experiments were conducted to study the potential of the feed formulations for freshwater prawn *Macrobrachium rosenbergii* juvenile based on three types of microalgae (*Spirulina* sp., *Nanochloropsis* sp. and *Chlorella* sp.). An experiment in triplicate with prawn (2.5-2.6 g) were fed until an apparent satiation with 32% crude protein diet containing three types of algae with different level (Diet 1= 0 %, Diet 2=2.5 %, Diet 3= 5 % and Diet 4= 10%) in varying algae levels ranging from 0, 2.5, 5 and 10% respectively for 56 days. At the end of the feeding trial, prawn given Diet 4 containing 10 % of *Spirulina* sp. gave the optimum growth which also contributed to the best feed efficiency. There was no significant difference in FCR in all treatments ($p<0.05$). There were significant differences ($p<0.05$) in growth rate among treatments and similarly prawns fed Diet 4 in *Spirulina* sp. had the highest weight gain (WG) and specific growth rate (SGR) 5.53 g and 2.05 %/day, respectively. The percentage survival of *M. rosenbergii* ranged from 63-83 %. This study also demonstrated that the nutrient from *Spirulina* sp. is more suitable to be

supplemented in prawn diet compared with *Nannochloropsis* sp. and *Chlorella* sp. The used of Cr_2O_3 marker is reliable for digestibility study.

Spirulina sp., *Nannochloropsis* sp. and *Chlorella* sp. were analyzed for antimicrobial activity to determine the potential of the algae against the selected pathogens. *Chlorella* sp. had successfully inhibited *Aeromonas hydrophilla* followed by *A. salmonicida* and *Vibrio alginolyticus*. The maximum inhibition zone by *Chlorella* sp. were 14.67 mm against *A. hydrophila*, followed by 11.33 mm against *V. alginolyticus* and 10.00 mm against *A. sobria*. Studies on the effect of *Spirulina* sp, *Nannochloropsis* sp. and *Chlorella* sp. on prawn challenged with *A. hydrophilla* for 48 hours showed there are no significant difference among the different treatments. Prawn's mortality was 100 % in control group.

Spirulina sp. with 10% algae level showed a good potential to be as a feed supplement for *M. rosenbergii* juveniles. *Spirulina* sp. contained a variety of amino acids and fatty acids to support good growth performance, digestibility and disease resistance as compared to *Nannochloropsis* sp. and *Chlorella* sp.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN MIKROALGAL TERPILIH (*Spirulina* sp., *Nannochloropsis* sp. DAN *Chlorella* sp.) UNTUK MAKANAN SUPLEMEN JUVENIL *Macrobrachium rosenbergii* (de Man)

Oleh

SUNIZA ANIS BINTI MOHAMAD SUKRI

Februari 2017

Pengerusi : Profesor Madya Che Roos Bin Saad, PhD
Fakulti : Pertanian

Satu siri kajian telah dijalankan untuk mengkaji potensi makanan rumusan pilihan untuk juvenile udang galah *Macrobrachium rosenbergii*. Potensi mikroalgae pilihan (*Spirulina* sp., *Nannochloropsis* sp. dan *Chlorella* sp.) telah dikaji sebagai makanan untuk juvenil *M. rosenbergii* dengan mengkaji profil asid amino dan asid lemak. Daripada keputusan, metionina dan triptofan dalam asid amino tidak dikesan dalam *Spirulina* sp., *Nanochloropsis* sp. and *Chlorella* sp. dalam keempat-empat tahap yang berbeza. Arginina, isoleusina, leusina, alanina, asid aspartik dan glutamik asid mempunyai paras yang tinggi terhadap peningkatan tahap dalam *Spirulina* sp. *Nannochloropsis* sp. dan *Chlorella* sp. menunjukkan peratusan yang tinggi dalam leusina, lisina dan arginina. Untuk analisa asid lemak, hanya *Spirulina* sp. menunjukkan sebahagian kecil asid γ–linoletik (GLA). *Chlorella* sp. tidak menunjukkan kehadiran asid eicosapentaenoik (EPA) berbanding *Spirulina* sp. dan *Nannochloropsis* sp.

Untuk kajian kedua, satu siri kajian telah dijalankan bagi mengkaji potensi makanan rumusan untuk juvenil udang galah *M. rosenbergii* berdasarkan tiga jenis mikroalga (*Spirulina* sp., *Nannochloropsis* sp. dan *Chlorella* sp.). Satu kajian dijalankan dalam tiga replikasi, dengan menggunakan udang (2.5-2.6 g) yang diberi makan sehingga kenyang dengan 32 % diet protein mentah yang mengandungi tiga jenis peringkat alga (Diet 1 = 0 %, Diet 2 = 2.5 %, Diet 3 = 5 % dan Diet 4 = 10 %) dalam pelbagai peringkat alga dari 0, 2.5, 5 dan10 % masing-masing selama 56 hari. Pada akhir kajian, makanan udang yang diberikan Diet 4 mengandungi *Spirulina* 10% memberi pertumbuhan yang optimum yang juga menyumbang kepada kecekapan makanan terbaik. Tidak ada perbezaan yang signifikan dalam FCR dalam semua rawatan ($p<0.05$). Terdapat perbezaan yang signifikan ($p<0.05$) dalam kadar pertumbuhan antara rawatan dan begitu juga udang diberi makan *Spirulina* sp. Diet 4 mempunyai berat badan (WG) yang tertinggi dan kadar tertentu pertumbuhan (SGR) masing-

masing 5.53 g dan 2.05 %/hari. Peratusan kadar hidup *M. rosenbergii* adalah di antara 63-83 % Kajian ini juga menunjukkan bahawa nutrien dari *Spirulina* sp. lebih sesuai ditambah dalam diet udang berbanding *Nannochloropsis* sp. dan *Chlorella* sp. Penanda Cr_2O_3 yang digunakan adalah teknik yang boleh dipercayai untuk kajian penghadaman.

Spirulina sp., *Nannochloropsis* sp. dan *Chlorella* sp. telah dianalisa untuk aktiviti antimikrob bagi memeriksa potensi alga terhadap patogen yang dipilih. *Chlorella* sp. telah berjaya merencatkan *Aeromonas hydrophilla* diikuti oleh *A. salmonicida* dan *Vibrio alginolyticus*. Zon perencatan maksimum *Chlorella* sp. terhadap *A. hydrophila* adalah 14.67 mm, diikuti oleh *V. alginolyticus* 11.33 mm dan *A. sobria* 10.00 mm. Kajian cabaran udang galah yang diberi makan diet yang mengandungi *Spirulina* sp, *Nannochloropsis* sp. dan *Chlorella* sp. ke atas udang dengan *A. hydrophilla* selama 48 jam tidak menunjukkan terdapat perbezaan yang signifikan dalam kesemua rawatan tersebut. Kadar kematian 100% diperhatikan dalam rawatan kawalan.

Spirulina sp. dengan peringkat alga 10% berpotensi sebagai makanan tambahan yang sesuai untuk makanan juvenil *M. rosenbergii*. *Spirulina* sp. mengandungi asid amino dan asid lemak yang sesuai untuk menyokong pertumbuhan dan daya tahan penyakit berbanding *Nannochloropsis* sp. dan *Chlorella* sp.

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I certify that a Thesis Examination Committee has met on 8 February 2017 to conduct the final examination of Suniza Anis binti Mohamad Sukri on her thesis entitled "Assessment of Selected Microalgae (*Spirulina* sp., *Nannochloropsis* sp. and *Chlorella* sp.) as Feed Supplement for *Macrobrachium rosenbergii* (de Man) Juveniles" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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Name of Chairman of Supervisory Committee: Associate Professor Dr. Che Roos Bin Saad

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Name of Member of Supervisory Committee: Professor Dr. Mohd Salleh Bin Kamarudin

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Name of Member of Supervisory Committee: Associate Professor Dr. Ina Salwany Binti Md Yasin

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ADC	Apparent Digestibility Coefficients
ALA	Alanine
ANOVA	Analysis of Variance
ARA	Arachidonic acid
CFU	Colony-Forming Unit
DHA	Decosahexaenoic acid
DO	Dissolved Oxygen
DW	Dry Weight
EAA	Essential Amino Acid
EPA	Ecosapentaenoic acid
FAME	Fatty Acid Methyl Esters
FCR	Feed Conversion Ratio
FW	Final Weight
GC	Gas Chromatography
GLA	γ–linolenic acid
HPA	Heneicosapentaenoic acid
HPLC	High Performance Liquid Chromatography
HUFA	Highly Unsaturated Fatty Acids
IW	Initial Weight
mt	metric tonnes
%	Percentage
PUFA	Polyunsaturated Fatty Acids
SD	Standard deviation

SGR	Specific Growth Rate
TSA	Tryptic Soy Agar
WG	Weight Gain

CHAPTER 1

INTRODUCTION

1.1 Prawn nutrition

Microalgae is a single-celled algae or phytoplankton represent the natural nutritional base and primary source of nutrient in aquatic food chain and play as nutritional role to marine animals and also in aquaculture (Catarina and □avier, 2012). Microalgae are an indispensable food sources in the commercial rearing of many cultivated species, including all growth stage of bivalve molluscs (clams, oyster, scallops), larval stages of some crustacean species, gastropods (abalone, conch) and very early growth stage of some fish species such as cod, halibut and tilapia (Coutteau, 1996□Catarina and □avier, 2012□Becker, 2013). The algae si□e ranging from 5 to 25 microns are ideally good for early stages of aquatic animals. They can easily and rapidly grow, stable in diverse environmental conditions, and rich in good nutrient compositions. Algae such as Spirulina sp., Chlorella sp., Tetraselmis sp. and Chaetoceros sp. have been used in aquaculture mostly for nutritional purposes (Muller-Feuga, 2000). Microalgae can be given directly as feed or mixed in formulated diets for early larval stages. This leads to better results than a diet contained of single microalgae (Yamayugi, 1997□Becker, 2013).

Nutritional composition of algae is well documented and mostly contains protein, carbohydrate, lipids and trace nutrients such as vitamins, antioxidants and trace elements (Yaacob et al., 2014). Microalgae with good nutritional value also contains high PUFA, EPA and DHA content, that can provide a high quality nutritional package for different stages of aquatic animals. Combinations of suitable algal species provide a well-balanced diet, which facilitates the larval development and they are source of essential vitamins such as A, B, C, E, folic acid and panthothenic acid (Nakagawa and Montgomery, 2007□Priyadarshani and Rath, 2012□Shanmugapriya and Ramanathan, 2012). Furthermore, they are also rich source of essential amino acid (protein), minerals, carbohydrates and essential fatty acid such as linolenic acid (Quoc and Pascaud, 1996).

The production of live algae will remain a critically important aspect of successful hatchery management into the foreseeable future. Microalgae have been widely used as an aquaculture food as first feeding of fish larvae (Reitan et al., 1997□Spolaore et al., 2006□Cai et al., 2007, Catarina and □avier, 2012) and as green water for Macrobrachium rosenbergii larvae (Cohen et al., 1976). The use of microalgae during the fish feeding process, either directly or through improving the nutritional value of the rotifer can improve the nutritional conditions of the fish larvae (Reitan et al., 1997□ Th□pot et al., 2016). The cultivation of micro-algae is an inherent part of aquaculture and phytoplankton comprises the base of the food chain in the marine and freshwater environment and also provided energy and nutrient essential for growth and development of marine organism (Cai et al., 2007).

Fish meal is the most expensive ingredient in fish feeding. According to Tacon and Metian (2008), in year 2006, aquaculture was consumed 3.7 million tonnes of fish meal. Many researchers tried to solve the problem and tried to decrease the use of fish meal in aquafeed. They tried to find alternative protein source to replace protein source from fish meal (Grabner, 1985 Lemos et al., 2009) and tried to check the potential of oyster processing by-product to replace fish meal for tilapia fry (Serrano et al., 2016). Another study by Gan on-Naret (2014a) reported that, 10% of Moringa oleifera leaf meals as plant protein to replace fish meal in Lates calcarifer diet showed an effect on growth performance and health welfare of the fish.

The researchers also tried to find alternative protein source to replace protein from soybean. Study by Maisashvili et al. (2015), was used Chlorella pyrenoidosa to replace protein soybean meal in aquaculture. A study by Gan on-Naret (2014b), mung bean seed was used to replace soybean meal protein in the seabass diet. Mung bean seed contain 20 to 25% of protein and amino acid except methionine, cycteine, and tryptophan. El-Saidy and Amal (2011) reported that, soybean meal was replaced with cottonseed meal and showed good growth performance of Nile tilapia. Cottonseed meal also used to replace soybean for growth, feed utili ation and blood physiological of juvenile black carp (Hu et al., 2015).

The freshwater prawn, Macrobrachium rosenbergii has been popular as a diet for Asia and the Pacific region since the 1960s and the farming of this species has spread throughout the world such as Bangladesh, China, India, Malaysia, Taiwan, Thailand, Vietnam (New, 2002). The freshwater prawn is in high demand in national and international markets (Soundarapandian et al., 2009). According to FAO (2009), five major countries that producing freshwater prawn such as China (56.3%), Thailand (12.5%), India (12.3%), Bangladesh (11%) and Taiwan (4.5%) in year 2006 with total production of 3.2 million tonnes of M. rosenbergii. The production of the freshwater prawn in Malaysia reached RM106.9 million (DoF, 2008) to RM139.3 million in 2013 (DoF, 2013).

Giant freshwater prawn can survive in a wide range of salinity between 0 to 18 ppt but this species has some inherent problem such as uneven growth and low harvest yields (FAO, 2004). This current study try to use microalgae such as Spirulina sp. Nannochloropsis sp. and Chlorella sp. to solve the culture problem and tried to increase production of M. rosenbergii.

1.2 Objectives

Objectives of this study were described as follow:

1. **To determine the profile of amino acid and fatty acid of feed and Macrobrachium rosenbergii carcass fed with different microalgae supplemented diets.**
2. **To determine the growth performance and survival of Macrobrachium. rosenbergii fed with microalgae supplemented diets.**

3. **To measure the digestibility of the microalgae supplemented feed in Macrobrachium rosenbergii juvenile.**
4. **To examine the antimicrobial activity and efficacy of feed containing microalgae (Spirulina sp., Nannochloropsis sp. and Chlorella sp.) and following Aeromonas hydrophilla immersion challenge.**

1.3 Justification

Microalga of different species are known as having very high nutritional value (high in energy, amino acid, vitamins, pigments, sterols, PUFAs). The successes of the research will provide new information in the field of fish nutrition. The use of microalgae during the fish-feeding process either directly or through improving the zooplankton nutrition can improve the nutritional conditions of the larvae and also can provide energy and nutrient essential for the growth and development. This research was conducted in order to study the amino acid as well as the fatty acid profiles in various species of algae (Spirulina sp., Nannochloropsis sp. and Chlorella sp.). Growth performance, digestibility, antimicrobial activity and bacterial challenge on these algae also were carried out. These algae species are believed to provide a good balance nutrient as they contain higher protein especially Essential Amino acid (EAA) and unsaturated fatty acids composite of Ecosapentaenoic acid (EPA), Decosahexaenoic acid (DHA) and Arachidonic acid (ARA) for fish growth as compared to single algae species.

Hypothesis:

H_0: Selected microalgae (Spirulina sp., Nannochloropsis sp. and Chlorella sp.) did not have any effect on feed for Macrobrachium rosenbergii juveniles.

H_A: Selected microalgae (Spirulina sp., Nannochloropsis sp. and Chlorella sp.) have positive effect on feed for Macrobrachium rosenbergii juveniles.

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