UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SYNBIOTIC DIETARY SUPPLEMENTATION WITH QUORUM QUENCHING PROPERTIES ON Macrobrachium rosenbergii De Man JUVENILES

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By

WEE WEN CHEN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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December 2016

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Dietary supplementation of functional feed additives such as probiotics, prebiotics and synbiotic are widely studied in aquaculture to potentially enhance the growth of aquatic animals. Many pathogenic Gram-negative bacteria in fish and shrimp have been reported to use quorum sensing (QS) signal molecules to induce the production of virulence factors. The aim of this study was to examine the effects of probiotic, prebiotic and synbiotic on the growth and health performance in *Macrobrachium rosenbergii*. The first study was to isolate QS degrader from the prawn with prebiotic utilization activity. Two QS degrader strains, BP-MBRG/1b and BP-MBRH/1b were isolated from the gut and hepatopancreas of adult *Macrobrachium rosenbergii*. Isolation of QS degraders was based on the ability of microbial community to grow in a minimal medium which contains only the mixture of N-Acyl homoserine lactones (AHL). The QS degrader strain isolated from the gut showed strong inhibition of AHL and was identified as *Bacillus cereus* (BP-MBRG/1b). The results also showed that the degrader strain BP-MBRG/1b grew well in fructooligosaccharide (FOS) agar medium. Later, the second study was designed to investigate the effects of dietary prebiotic FOS at 0.1%, 0.4%, 1% and 2% to determine the optimum inclusion level of FOS required by *M. rosenbergii* post-larvae (PL) after 56 days of feeding. The specific growth rate (SGR) was significant ($p < 0.05$) highest in the 0.4% FOS fed PL. Furthermore, 0.4% FOS also significantly ($p < 0.05$) stimulated the highest intestinal short-chain fatty acids (SCFAs) production compared to the control treatment. After 56 days, the hepatopancreatic tubules of prawns in the 0.4% FOS treatment were more closely arranged with significantly ($p < 0.05$) more R- and E-cells. However, oxidative stress in prawns was increased with the increased of FOS concentration. Lastly, the third experiment was conducted to evaluate the effects of the probiotic and FOS in single or combined diets (synbiotic) supplementation in *M. rosenbergii* juveniles. After 28 days of feeding, results showed that the SGR was significant ($p < 0.05$) highest in the prawns fed with probiotic diet, accompanied by increased superoxide dismutase activity (SOD) and SCFAs. The prawns were then challenged with pathogenic

Aeromonas hydrophila AH-1N via static immersion. Prebiotic fed prawns had the highest survival although survival response was not significant ($p > 0.05$) different among the treatment. Both prebiotic and synbiotic diet significantly ($p < 0.05$) enhanced the SOD in prawns, suggesting the induction of reactive oxygen species (ROS) in order to kill pathogenic bacteria. Histological observations of hepatopancreas showed differences in severity and type of cell/tissue damage where synbiotic fed prawns showed the best hepatopancreas condition. In addition, synbiotic showed the greatest protection effects in juvenile prawns when *A. hydrophila* was present. Overall, this study showed that dietary FOS stimulated the growth in PL while probiotic diet enhanced the growth in juvenile prawns. In conclusion, dietary prebiotic supplementation stimulated the growth in PL while probiotic diet enhanced the growth in juvenile prawns. Synbiotic diet showed potential in protecting the prawn from *A. hydrophila* based on histological observation. The outcome of this study suggested addition of feed additives probiotic, prebiotic and synbiotic exert different beneficial effects in the prawns. Further optimization and study of additives inclusion dosage in diets are required as the present study showed probiotic, prebiotic and synbiotic exert different positive effects in *M. rosenbergii.*

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN SUPLEMEN DIET SINBIOTIK DENGAN PROPERTI DEGRADASI PENDERIAAN KUORUM TERHADAP JUVENIL *Macrobrachium rosenbergii* De Man

Oleh

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Disember 2016

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Diet makanan tambahan seperti probiotik, prebiotik dan sinbiotik dikaji secara meluas dalam akuakultur kerana potensinya meningkatkan pertumbuhan haiwan akuatik. Kebanyakan bakteria patogenik Gram-negatif dalam ikan dan udang menggunakan penderiaan kuorum (QS) untuk penghasilan faktor virulen. Tujuan kajian ini adalah untuk mengkaji kesan probiotik, prebiotik dan synbiotik dalam prestasi pertumbuhan dan kesihatan *M. rosenbergii*. Kajian pertama adalah untuk mengasingkan kuorum sensing degrader dari udang dengan aktiviti penggunaan prebiotik. Dalam kajian ini, dua strain bakteria yang mampu mendegradasi QS telah diasingkan daripada usus dan hepatopankreas *M. rosenbergii* dewasa. Pengasingan bakteria degradasi QS adalah berdasarkan keupayaan komuniti mikrob untuk hidup dalam medium minimum yang hanya mengandungi campuran AHL. Strain degradasi QS yang diasingkan daripada usus menunjukkan perencatan AHL yang terkuat telah dikenal pasti sebagai *Bacilus cereus* (BP-MGRG/1b). Keputusan menunjukkan bahawa strain degradasi BP-MBRG/1b tumbuh dengan baik dalam medium agar fruktooligosakarida (FOS). Pemakanan prebiotik FOS pada 0.1, 0.4, 1 and 2% kemudiannya telah dirumuskan untuk menentukan kepekatan optimum pada pasca larva (PL) *M. rosenbergii* selepas 56 hari pemberian makanan. Kadar pertumbuhan tertentu (SGR) adalah tertinggi ($p < 0.05$) dalam udang yang memakan 0.4% FOS. Sementara itu, 0.4% FOS juga merangsangkan pengeluaran asid lemak rantaian pendek serta meningkatkan sel-R dan sel E dalam hepatopankreas udang. Walau bagaimanapun, tekanan oksidatif dalam udang didapati meningkat dengan peningkatan kepekatan FOS. Akhir sekali, kajian ketiga telah dijalankan untuk menilai kesan probiotik dan FOS secara tunggal atau digabungkan diet (sinbiotik) suplemen dalam juvenil *M. rosenbergii*. Selepas 28 hari pemberian makanan, SGR adalah paling tinggi ($p < 0.05$) dalam udang yang diberi makan diet probiotik, diiringi dengan peningkatan pengeluaran asid lemak rantaian pendek dan aktiviti antioksidan superoxide dismutase (SOD). Kemudian, udang dicabar dengan patogen AH-1N secara rendaman static. Selepas cabaran patogen, diet prebiotik memberikan hidup yang tertinggi kepada udang walaupun tidak ketara ($p > 0.05$) berbanding dengan kumpulan diet yang lain. Suplemen pemakanan FOS secara tunggal atau gabungan meningkatkan SOD dalam udang dengan ketara ($p < 0.05$). Ini menunjukkan spesies oksigen reaktif (ROS)

diinduksi untuk membunuh bakteria patogenik. Pemerhatian histologi hepatopankreas menunjukkan tahap perbezaan dari segi kerosakan tisu dan sel. Di samping itu, sinbiotik menunjukkan kesan perlindungan yang terbaik dalam udang juvenile yang dicabar dengan *A. hydrophila*. Kesimpulannya, pemakanan prebiotik merangsangkan pertumbuhan pasca larva manakala diet probiotik meningkatkan pertumbuhan udang juvenil. Selain itu, pemakan sinbiotik juga menunjukkan potensi dalam melindungi udang daripada *A. hydrophila* berdasarkan permehatian histologi. Hasil kajian ini menunjukkan makanan tambahan probiotik, prebiotik dan sinbiotik memberikan kesan yang berbeza dalam udang. Pengoptimuman lanjut dan kajian dos tambahan diperlukan kerana kajian ini menunjukkan potensi probiotik, prebiotik dan sinbiotik dalam memberikan kesan positif yang berbeza dalam *M. rosenbergii*.

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I wish every one of you a bright future with a lot of happiness. Never give up.

I certify that a Thesis Examination Committee has met on 14 December 2016 to conduct the final examination of Wee Wen Chen on her thesis entitled "Effects of Synbiotic Dietary Supplementation with Quorum Quenching Properties on *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 Master of Science.

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LIST OF ABBREVIATIONS

ACH	Alternative Complement Activity
ADS	Appendage Deformity Syndrome
AHl	N-*acyl homoserine lactones*
AI-2	Autoinducer 2
AXOS	Arabinoxylan oligosaccharides
BHL	N-butanoyl-L-homoserine lacton
BLAST	Basic local alignment search tool
CAT	Catalase
cfu	Colony forming unit
DOFM	Department of Fishery Malaysia
EC	Enrichment Culture
EED	Exuvia Entrapment Disease
EER	Energy Efficiency Ratio
EPV	Energy Productive Value
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
FER	Feed Conversion Ratio
FOS	Fructooligosaccharide
GI	Gastrointestinal
H&E	Hematoxylin and Eosin
HHL	N-hexanoyl-L-homoserine lactone
HIS	Hepatosomatic Index
HSP	Heat Shock Protein
Ig	Immunoglobulin
IMN	Idiopathic Muscle Necrosis
IMO	Isomalto-oligosaccharide
LAB	Lactic Acid Bacteria
LBA	Luria-Bertani
LER	Lipid Efficiency Ratio
LPV	Lipid Productive Value
MC	Microbial Community
MCD	Mid cycle disease
MDA	Malondialdehyde
MDS	Moult Death Syndrome
MHPV	*Macrobachium* Hepatopancreatic Parvovirus
MMV	*Macrobrachium* Muscle Virus
MOS	Mannanoligosaccharide
MrNV	*M. rosenbergii* nodavirus
PEMBA	Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base
PER	Protein Efficiency Ratio
PL	Post-larvae
PO	phenoloxidase
PHB	Poly-b-hydroxybutyrate
PPV	Protein Productive Value
proPO	Prophenoloxidase

QS	Quorum Sensing
QSI	Quorum Sensing Inhibition
ROS	Reactive Oxygen Species
SCFAs	Short-chain fatty acids
scFOS	short-chain Fructooligosaccharide
SE	Standard Error
SGR	Specific Growth Rate
SOD	Superoxide Dismutase
spp.	species
TOS	Transgalacto-oligosaccharide
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
TVC	Total Viable Count
VSI	Vicerosomatic Index
WG	Weight Gain
WHO	World Health Organization
WSS	White Spot Syndrome
WSSV	White Spot Syndrome Virus
WST	*Water-Soluble Tetrazolium*
WTD	White Tail Disease
XOS	Xylooligosaccharide
XSV	Extra Small Virus

CHAPTER 1

INTRODUCTION

Aquaculture is one of the rapidest food producing sectors in the world due to the huge demand for aquaculture products in human consumption. Aquaculture not only provide food source and nutrition, but also the main source of livelihood and wealth (FAO, 2014). Fishery products remain as one of the highly traded food commodities in the world. The most recent statistic of FAO Fishery Statistical Collection (2016) shows that global aquaculture production had increased from 68 million tonnes in 2008 to 101 million tonnes in 2014. If sustainably is practiced and developed, aquaculture may have the potential in providing lifelong benefits for global food security and economic expansion (FAO, 2014).

The Giant Freshwater Prawn, *Macrobrachium rosenbergii*, is one example of commercial crustacean species with high market value and demand. In many countries, this freshwater prawn is a valuable aquaculture farming due to its commercial value (New, 2002). Likewise, in Malaysia, *M. rosenbergii* is one of the important freshwater aquaculture species for domestic and export market (FAO, 2016). However, according to Department of Fisheries Malaysia (DOFM) the production of *M. rosenbergii* fell from 398 tonnes in 2014 to 268 tonnes in 2015 (DOFM, 2015). The shortage of healthy and quality seedlings are among major obstacles in the rapid development of *M. rosenbergii* hatchery production farming.

Intensification and commercialization of aquaculture often leads to disease outbreaks. Similar to marine farmed shrimp, disease outbreak is one of the major constraints in the expansion of *M. rosenbergii* prawn farming (Nhan et al., 2010). *Aeromonas* spp. and *Vibrio* spp. are among the crucial pathogenic bacteria that are responsible for disease related problem in freshwater prawn culture (Hoa et al., 2002). Therefore, it is important to prevent and enhance disease resistance of cultured organisms that in turn will improve the growth performance and feed efficiency particularly in the intensive aquaculture production.

The most common method to control bacterial disease relies on the use of antibiotic and chemotherapeutics (Kumar et al., 2006). However, these compounds have been criticized for the spread of antibiotic resistant bacteria in aquaculture environment. There is also the transmission risk of these antibiotic-resistant bacteria to human (Cabello, 2006). Furthermore, due to the rise of awareness on the food quality and safety issues, the application of antibiotic has started to be withdrawn in many countries.

Various solutions have been proposed for the development of alternative strategies in disease control. Several sustainable options of feed additives are available that include immunostimulants, probiotics, prebiotics or synbiotics (combination of probiotic and

prebiotic) which have been found to enhance immune status, feed efficiency, nutrients utilization and disease resistance (Gatlin III et al., 2006). Meanwhile, probiotic through the disruption of QS mechanism has been suggested as an alternative technique to control bacterial virulence expression. The QS system in which bacteria communicate with each other using small signal molecules such as AHL control the expression of virulence factor productions of various aquatic pathogens such as *A. hydrophila* and *Vibrio harveyi* (Defoirdt et al., 2004). Bacteria that are able to degrade QS molecule without growth interference of the pathogen could be a potential biocontrol agents in aquaculture (Whitehead et al., 2001). Applications of synbiotics are promising alternatives to antibiotics against diseases through manipulation of host-microbe interaction in aquaculture. Many studies found that diet composition such as prebiotic affect the host by stimulating the intestinal microflora of the host (Ringø & Gatesoupe, 1998; Ringø & Birkbeck, 1999).

Furthermore, information on dietary supplementation of prebiotic, probiotic and synbiotic to enhance the growth and health in freshwater prawn are limited. To the best of our knowledge, there is no study of dietary supplementation of probiotic quorum quenching (QQ) bacteria, prebiotic FOS and the combination of both (synbiotic) on *M. rosenbergii*. The goal of this study was to improve the growth and health condition of *M. rosenbergii* through dietary supplementation. It is hypothesized that supplementation of probiotic, prebiotic and synbiotic will improve the growth and health condition in prawns.

Hence, the objectives of this study are as follows:

1) To isolate potential probiotic QS degraders with fructooligosaccharide utilization activities from adult *M. rosenbergii.*

2) To explore the administration of formulated prebiotic fructooligosaccharide diet on the growth performance and health condition *M. rosenbergii* post-larvae.

3) To investigate the effects of synbiotic diet supplementation on growth performance of *M. rosenbergii* juvenile challenged with *A. hydrophila*.

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LIST OF PUBLICATIONS

Journal

Noorashikin Md. Noor, **Wee Wen Chen**, Nur Izzati Ahmad, Farha Fazidah, Ina Salwany, Murni Karim, Sharr Azni Harmin and F.M.I. Natrah (2014) Selection of Potential Bacterial Bioremediator for Tiger Grouper (*Epinephelus fuscoguttatus*) Juveniles Culture. Journal of Pure and Applied Microbiology 8(Special edn.): 477-482. IF: 0.073

Wee, W.C., Romano, N., Ebrahimi, M., Natrah, I. (2016). Optimal inclusion level of dietary fructooligosaccharide increased the growth, intestinal short chain fatty acids and hepatopancreatic condition of the giant freshwater prawn (*Macrobrachium rosenbergii*) post-larvae. *Aquaculture*, *469*, 95-101.

Wee, W.C., Lai, K.S, Kong, C.L., Yap, W.S. (2016).Effects of within -row plant spacing and fixed fruit setting on yield and quality of rockmelon. *Scientia Agricola.* (Submitted)

Proceedings

Wee Wen Chen, Chon Cheng Yong, Nicholas Romano, F.M.I Natrah (2015). Screening of Bacterial Quorum Sensing Degrader with Fructo-Oligosaccharide Utilization Activities from Freshwater Prawn, *Macrobrachium rosenbergii* Adult. National *Seminar* on Advances in *Fish Health* (*NSAFE). Selangor. 4-5 February 2-15.(Poster presentation)*

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