UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SELECTED PROBIOTICS ON THE GROWTH, SURVIVAL AND GASTRO-INTESTINAL BACTERIAL POPULATION OF JUVENILE GIANT FRESHWATER PRAWN (Macrobrachium rosenbergii, de Man)

SARA RAVAN RAMZANI

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By

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for degree of Masters of Science

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Chairman: Associate Professor Che Roos Bin Saad, PhD

Faculty: Agriculture

Two experiments were carried out in this project. The first study was done to determine the effectiveness of putative bacteria as a probiotics for growth enhancement and survival of juvenile *Macrobrachium rosenbergii* and protection against *Aeromonas hydrophila* challenge. A total of 360 juveniles *M. rosenbergii* were individually weighed and kept in twenty one 60 L aquaria. Four treatments were conducted as follows; T_1 (Control group), T_2 (*B. subtilis),* T_3 (*Lactobacillus acidophilus*) and T_4 (Commercial probiotic bacteria). The final concentration of the probiotic bacteria in the treated feeds was set at 8×10^9 CFU/g feed. All prawn treated with probiotic-supplemented diets showed better growth performances and feed utilization as compared to those in the control fed with basal diet. The higher growth parameter and survival rate of the prawns fed with *B. subtilis*–added diets over the control feed indicated the probiotic-ability of the *B. subtilis.* There was a significant different ($P<0.05$) in weight gain, specific growth

rate, feed intake and FCR between the treated and control tanks. The prawns fed with the control diet with no addition of bacteria, recorded the lowest feed intake. Tthe best FCR value (2.56 ± 0.09) was derived from the juvenile fed diet containing *B. subtilis.* After 60 days of comparison among putative *B. subtilis, Lactobacillus acidophilus,* commercial probiotic bacteria and probiotic free diets in juvenile *M. rosenbergii,* there was no significant difference ($P > 0.05$) in biochemical composition between groups treated putative *B. subtilis* and *Lactobacillus acidophilus* and commercial probiotic treated groups. There was significant difference between groups receiving putative *B. subtilis, Lactobacillus acidophilus* and commercial probiotic bacteria diets in growth enhancement of juvenile *M. rosenbergii.* This study indicated the increase in the growth and survival of the treated prawns, with reduced mortality as compared to the control group.

The second study was conducted to determine the effects of periodical application of probiotics and pathogenic effects of *A. hydrophila* on the survival and growth enhancement of juvenile *M. rosenbergii* with a total of 840 *M. rosenbergii* post-larvae procured from Center of Marine Science (COMAS), Port Dickson, UPM. These prawns were individually weighed and kept in twenty one 60 L aquaria. Two trials were carried out. The first trial had seven feeding regimes designed as: T_1 (Control group); T_2 (*B. subtilis* bacteria once every day), T_3 (*B. subtilis* once every 5 day), T_4 (*B. subtilis* once every 10 days), T_5 (Commercial Probiotic (CP) once every day), T_6 (CP once every 5 days) and T_7 (CP once every 10 days). The final concentration of the probiotic bacteria in the feeds was determined at 8×10^9 CFU/g feed. After 60 days of feeding, the prawns in each treatment were measured for growth performance. Then the prawns in each

treatment were challenged with *A. hydrophila* at 10^7 CFU/mL for 21 days, and measurements for growth and survival rates were recorded at end of the challenge experiment. Parameters such as histopathology, bacteriological study, growth measurement and water quality were taken. Significant differences ($P< 0.05$) in weight gain, specific growth rate, feed intake and FCR between the treated *B. subtilis* and control groups were observed. Results showed a significant difference between control and treatments in the log of *Bacillus* count (Control: Mean = 1.40 ± 0.03, CPF; Mean= 4.10 ± 0.064, PF; Mean = 3.46 ± 0.084) and the log of gram negative count (Control; Mean= 3.29 ± 0.08, CPF; Mean = 1.91 ± 0.045, PF; Mean = 2.22 ± 0.059) ($P < 0.001$). Appropriate probiotic applications have been shown to improve intestinal microbial balance, thus leading to improved food digestion, and reduced pathogenic bacteria multiplication problems in the gastrointestinal tract.

Abstrak tesis dipersembahkan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

KESAN PROBIOTIK TERPILIH KE ATAS PERTUMBUHAN, KEMANDIRIAN DAN POPULASI BAKTERIA GASTROINTESTIN BAGI JUVANA UDANG GALAH (*Macrobrachium rosenbergii,* de Man)

Oleh

SARA RAVAN RAMZANI

Disember 2011

Pengerusi : Prof. Madya Che Roos Bin Saad, PhD

Fakulti : Pertanian

Dua kajian telah dilakukan dalam projek ini. Kajian pertama telah dijalankan untuk menentukan keberkesanan bakteria yang dianggap sebagai probiotik bagi meningkatkan pertumbuhan dan kemandirian juvana *M. rosenbergii* dan perlindungan daripada cabaran bakterium *Aeromonas hydrophila.* Sejumlah 360 juvana *M. rosenbergii* telah ditimbang secara berasingan dan dimasukkan ke dalam 21 tangki akuarium berkapasiti 60L. Empat rawatan telah dijalankan seperti berikut; T1 (Tiada penambahan *B. subtilis*), T2 (Penambahan *B. subtilis*), T3 (*Lactobacillus acidophilus*), dan T4 (Komersial bakteria probiotik). Kepekatan akhir bakteria probiotik dalam makanan yang dirawat telah ditetapkan pada tahap 8 x 10^9 CFU/g makanan. Kesemua udang yang mendapat probiotik tambahan menunjukkan prestasi pertumbuhan dan penggunaan makanan yang lebih baik berbanding udang yang memakan diet kawalan. Terdapat perbezaan yang bererti ($P<0.05$) untuk kadar pertumbuhan dan kemandirian hidup yang lebih tinggi bagi

udang yang diberi makan dengan diet yang ditambah *B. subtilis* berbanding dengan udang kawalan. Ini menunjukkan bahawa keupayaan probiotik dimiliki oleh *B. subtilis*. Nilai FCR terbaik (2.56 ± 0.09) diperolehi dari pada juvana dengan diet yang mengandungi *B.subtilis*. Selepas perbandingnan selama 60 hari di antara *B. subtilis*, *Lactobacillus acidophilus,* probiotik komersial dan diet tanpa probiotik dengan juvana *M. rosenbergii*, tiada perbeazaan ketara antara diet makanan tersebut. Namun, terdapat perbezaan ketara antara *B. subtilis, Lactobacillus acidophilus dan* probitik komersial yang mempengaruhi pertumbuhan juvana *M. rosenbergii*. Kajian ini menunjukkan peningkatan pertumbuhan dan kemandiriaan udang yang dirawat, dengan pengurangan dalam kadar kematian berbanding kumpulan kawalan.

Kajian kedua telah dijalankan bagi menentukan kesan patogenik oleh *A. hydrophila* diatas pemberian probiotik secara berkala dengan menggunakan 840 ekor pasca larva *M. rosenbergii* yang diperolehi dari Pusat Sains Marin, UPM Port Dickson (COMAS). Kesemua udang telah ditimbang secara berasingan dan diagihkan kedalam 21 tangki akuarium berkapasiti 60 L. Dua percubaan telah dijalankan. Percubaan pertama telah direka bentuk dengan tujuh rejim pemberian makanan: T1 (Kawalan), T2 (*B. subtilis* setiap hari sekali), T3 (*B. subtilis* setiap 5 hari sekali), T4 (*B. subtilis* setiap 10 hari sekali), T5 (Commercial Probiotik (CP) setiap hari sekali), T6 (CP setiap 5 hari sekali), dan T7 (CP setiap 10 hari sekali). Kepekatan akhir bakteria probiotik di dalam makanan telah ditentukan pada tahap 8×10^9 CFU/g makanan. Selepas 60 hari pemberian makanan mengikut setiap rawatan, kesemua udang tersebut telah diukur bagi prestasi pertumbuhan. Kemuudian semua udang telah diuji ketahanannya dengan *A. hydrophila* pada tahap 10^7 CFU/mL selama 21 hari dan ukuran berangka untuk kadar pertumbuhan

dan kemandirian setiap rawatan ditentukan pada akhir kajian ini. Parameter seperti histopatologi, kajian bakteriologi, pengukuran pertumbuhan, dan kualiti air telah diambil. Perbezaan bererti ($P<0.05$) dalam pertambahan berat badan, kadar pertumbuhan spesifik, pengambilan makanan, dan nisbah pertukaran makanan (FCR) di antara kumpulan yang dirawat *B. subtilis* dengan kumpulan kawalan telah diamati. Hasil kajian menunjukkan terdapat perbezaan bererti antara kumpulan kawalan dan rawatan bagi kiraan log *Bacillus* (Kawalan: Min = 1.40± 0.03, CPF; Min= 4.10 ± 0.064, PF; Min = 3.46 ± 0.084) dan kiraan log gram negatif (Kawalan: Min= 3.29 ± 0.08, CPF; Min = 1.91 ± 0.045, PF; Min = 2.22 ± 0.059) ($P < 0.001$). Aplikasi probiotik yang sesuai menunjukkan peningkatan keseimbangan mikrob usus, sekali gus membawa kepada pencernaan makanan yang lebih baik dan mengurangkan masalah pembiakan bakteria patogen di dalam saluran gastrousus.

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I certify that an Examination Committee met on 16 December 2011 of viva to conduct the final examination of Sara Ravan Ramzani on his Master of Science thesis entitled "Effects of Selected Probiotics on the Growth, Survival and Gastrno-Intestinal Bacterial population of Juvenile Giant Freshwater Prawn (*Macrobrachium rosenbergii*)" in accordance with University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106 15 March 1998. The Committee recommends that the candidate be awarded the Masters of Science.

Member of the Thesis Examination Committee are as follows:

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This thesis was submitted to the Senate of University Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that this thesis is my original work except for quotations and citation except as cited in references, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

SARA RAVAN RAMZANI

Date: 16 December 2011

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