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

FP 2011 55
EFFECTS OF SELECTED PROBIOTICS ON THE GROWTH, SURVIVAL AND GASTRO-INTESTINAL BACTERIAL POPULATION OF JUVENILE GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii*, de Man)

By

SARA RAVAN RAMZANI

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

December 2011
Effects of selected probiotics on the growth, survival and gastro-intestinal bacterial population of juvenile giant freshwater prawn (Macrobrachium rosenbergii, de Man)

By

SARA RAVAN RAMZANI

December 2011

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; T₁ (Control group), T₂ (B. subtilis), T₃ (Lactobacillus acidophilus) and T₄ (Commercial probiotic bacteria). The final concentration of the probiotic bacteria in the treated feeds was set at $8 \times 10^9$ 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. The 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: T₁ (Control group); T₂ (*B. subtilis* bacteria once every day), T₃ (*B. subtilis* once every 5 day), T₄ (*B. subtilis* once every 10 days), T₅ (Commercial Probiotic (CP) once every day), T₆ (CP once every 5 days) and T₇ (CP once every 10 days). The final concentration of the probiotic bacteria in the feeds was determined at 8 × 10⁹ 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 10⁹ 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 perbandingan selama 60 hari di antara *B. subtilis*, *Lactobacillus acidophilus*, probiotik komersial dan diet tanpa probiotik dengan juvana *M. rosenbergii*, tiada perbezaan ketara antara diet makanan tersebut. Namun, terdapat perbezaan ketara antara *B. subtilis*, *Lactobacillus acidophilus* dan probiotik komersial yang mempengaruhi pertumbuhan juvana *M. rosenbergii*. Kajian ini menunjukkan peningkatan pertumbuhan dan kemandirianan 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 diaghkan 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 × 10⁹ CFU/g makanan. Selepas 60 hari pemberian makanan mengikut setiap rawatan, kesemua udang tersebut telah diukur bagi prestasi pertumbuhan. Kemudian semua udang telah diuji ketahanannya dengan *A. hydrophila* pada tahap 10⁷ 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 gastrointestinal.
ACKNOWLEDGEMENTS

In the name of Allah, The Beneficent, The Merciful

First and most important, I would like to offer my earnest appreciation to the Assoc. Professor Dr. Che Roos Saad, chairman of my advisory committee, for providing me with a great opportunity to finish my Master studies under his excellent supervision. Apart from the provision of the required funding, this work would not have been possible without his patience, never-ending inspiration, advice and knowledge. On account of recurrent meeting and his open door policy, Assoc. Prof Dr. Che Roos Saad made a great contribution to this dissertation and my educational development, together with my professional and individual life.

My deepest gratitude is also extended to Assoc. Prof Dr. Kamaruzaman Sijam who is a member of my supervisory committee and provided me some equipment for laboratory and assisted throughout the course of the study. Furthermore, I would like to extend my most heartfelt gratefulness and thanks to Assoc. Prof Dr. Hassan Mohd Daud, for helpful advice and guidance during the study time. I am also thankful for their desire to serve on my committee, provide me support whenever essential, participation in my qualifying examination, and for assessing this dissertation.
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 Gastrino-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:

**Dr Aziz Arshad, PhD**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Mohd Salleh Kamarudin, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Siti Shapor Siraj, PhD**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Ng Wing Keong, PhD**  
Professor  
School of Biological Sciences  
Universiti Sains Malaysia  
(Independent Examiner)

---

**Seow Heng Fong, PhD**  
Professor  
and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia  
Date
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:

Che Roos Saad, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Hassan Mohd Daud, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Kamaruzaman Sijam, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

-----------------------------
BUJANG BIN KIM HUAT, PhD
Professor and Dean,
School of Graduate Studies
Universiti Putra Malaysia

Date:
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
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 INTRODUCTION

1.1 Background of Study 1
1.2 Problem Statement 4
1.3 Significant of the Study 5
1.4 Objectives 6

### 2 LITERATURE REVIEW

2.1 General Morphology 7
2.1.1 Taxonomy and Life history of *M. rosenbergii* 8
2.1.2 Prawn species 9
2.1.3 Feeding behavior and Environmental factors of *M. rosenbergii* 11
2.1.4 Growth 15
2.1.5 Breeding 15
2.1.6 Seed Supply 17
2.2 Larval Culture 17
2.3 Nursery phase 18
2.3.1 Effect of stocking different Size- Grade of Juvenile Prawns 20
2.4 Stocking of Juveniles 20
2.5 General Review on Probiotics 21
2.5.1 Definition of Probiotics 21
2.5.2 Studies on Probiotic Quality control in Aquaculture 23
2.5.3 Modes of Action and Competitive Exclusion of Probiotics 24
2.5.4 Digestion Enhancement of Probiotics 26
   2.5.4.1 Immune Response Enhancement 27
2.5.4.2 Water Quality Improvement 27
2.5.4.3 Colonization 29

3  GENERAL METHODOLOGY 31
3.1 Location of the Study 31
3.2 Experimental Set-up 31
3.3 Bacterial Enumeration 32
3.4 Growth and Feeding Efficiency 33
3.5 Proximate analysis 33
   3.5.1 Crude Protein 34
   3.5.2 Lipids 34
   3.5.3 Crude Fiber 35
   3.5.4 Moisture Determination 36
   3.5.5 Ash Determination 37
3.6 Water Quality Management 37
3.7 Staining Method 38
3.8 Histopathology 38
   3.8.1 Haematoxylin & Eosin staining 39
3.9 Statistical Analysis 39

4  EFFICIENCY OF *Bacillus subtilis*, *Lactobacillus acidophilus* AND COMMERCIAL PROBIOTIC BACTERIA ON THE GROWTH AND SURVIVAL OF JUVENILE *Macrobrachium rosenbergii* 40
4.1 Introduction 40
4.2 Material and Methods 42
   4.2.1 Bacterial strain 43
   4.2.2 The preparation of the Diets 44
   4.2.3 Feeding 45
   4.2.4 Growth and Feeding Efficiency 45
   4.2.5 Proximate Analyses 46
   4.2.6 Water Quality Parameters 47
   4.2.7 Bacteriological Study 47
   4.2.8 Statistical Analysis 47
4.3 Results 48
4.4 Discussion 54
### 5 RESPONSE OF JUVENILE *Macrobrachium rosenbergii* TO DIFFERENT DIETARY LEVELS OF PROBIOTICS IN FEED AND CHALLENGE TEST WITH *Aeromonas hydrophila*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Introduction</td>
<td>58</td>
</tr>
<tr>
<td>5.2 Material and Methods</td>
<td>60</td>
</tr>
<tr>
<td>5.2.1 Bacterial strain</td>
<td>61</td>
</tr>
<tr>
<td>5.2.2 The preparation of the Diets</td>
<td>62</td>
</tr>
<tr>
<td>5.2.3 Feeding</td>
<td>63</td>
</tr>
<tr>
<td>5.2.4 Pathogen Challenge Trial</td>
<td>64</td>
</tr>
<tr>
<td>5.2.5 Bacteriological Study</td>
<td>64</td>
</tr>
<tr>
<td>5.2.6 Histopathological examination</td>
<td>65</td>
</tr>
<tr>
<td>5.2.7 Growth Measurement</td>
<td>65</td>
</tr>
<tr>
<td>5.2.8 Water Quality Parameters</td>
<td>65</td>
</tr>
<tr>
<td>5.2.9 Statistical Analysis</td>
<td>66</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>66</td>
</tr>
<tr>
<td>5.4 Discussion</td>
<td>80</td>
</tr>
</tbody>
</table>

### 6 GENERAL DISCUSSION CONCLUSION AND RECOMMENDATIONS

REFERENCES | 86
APPENDICES | 106
BIODATA OF STUDENT | 116