# EFFECTS OF A PROBIOTICS ON THE GROWTH AND SURVIVAL OF WHITE LEG SHRIMP POST LARVAE (*Litopenaeus vannamei*) IN RECIRCULATION WATER SYSTEM

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# KESAN PROBIOTIK TERHADAP PERTUMBUHAN DAN KEMANDIRIAN PASCA LARVA UDANG PUTIH (*Litopenaeus vannamei*) DALAM SISTEM AIR KITAR SEMULA

AFFENDY BIN DERISA

TESIS INI DIKEMUKAKAN UNTUK MEMENUHI SEBAHAGIAN DARIPADA SYARAT MEMPEROLEHI IJAZAH SARJANA MUDA SAINS DENGAN KEPUJIAN

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## ABSTRACT

The growth and survival of Whiteleg shrimp, *Litopenaeus vannamei* post larvae were evaluated in four different treatment tank assigned as Treatment Bio-Aqua, Treatment Bio-Block, Control-N and Control tank by using four 7-ℓ aquaria. There were 30 tails of *L. vannamei* post larvae reared in each tank. In Treatment Bio-Aqua and Bio-Block tank, each was treated with commercial probiotics, B-Aqua and B-Block, respectively with the addition of nitrifying bacteria by immersion technique, Control-N tank treated with nitrifying bacteria and control tank had no addition of the commercial probiotics and nitrifying bacteria. Each of the treatment was conducted in duplicate. Feeding was given according to shrimp body weight. After a 54-day feeding trial with probiotics supplied through water immersion and additional 9 days of challenging with pathogenic bacteria, *Vibrio harveyii* by immersion technique, post larvae exhibited significant difference ($P<0.05$) in term of growth, whereas Treatment Bio-Aqua, Treatment Bio-Block and Control-N had significant ($P<0.05$) higher growth than control tank. For survival, *L. vannamei* was not significantly affected ($P>0.05$) by the treatments. But, Treatment Bio-Aqua, Bio-Block and Control-N have the higher survival rate compared to the control tank. After 62 days of feeding, cultured with commercial probiotics and challenge test with pathogenic bacteria, *V. harveyii*, the used of commercial probiotics may view as one of the solutions which is available to promote growth and survival besides the use of conventional methods such as anti-biotic and chemicals.

# *ABSTRAK*

Pertumbuhan dan kemandirian pasca larva udang putih, *Litopenaeus vannamei* telah dikaji di dalam empat rawatan yang berbeza iaitu Bio-Akua, rawatan Bio-Blok, Kawalan-N dan kawalan dengan menggunakan akuarium 7- ℓ. Sejumlah 30 ekor pasca larva udang putih telah diternak di dalam setiap tangki rawatan. Untuk tangki Bio-Akua dan Bio-Blok, setiap satu telah dirawat dengan probiotik komersial iaitu B-Aqua dan B-Blok masing-masing, dan penambahan bakteria nitrifikasi. Kawalan-N hanya ditambah dengan bakteria nitrifikasi dan tangki kawalan tidak ditambah dengan probiotik mahupun bakteria nitrifikasi. Setiap jenis rawatan telah dilaksanakan dalam duplikasi akuarium. Pemberian makanan adalah berdasarkan kepada peratusan daripada jumlah berat badan. Selepas 52 hari dikultur menggunakan probiotik yang dibekalkan dalam air, dan tambahan selama 9 hari untuk ujian ketahanan terhadap bakteria patogenik, *Vibrio harveyii* juga melalui teknik rendaman, pasca larva udang putih ini mempunyai berat yang sinifikan ($p<0.05$) di mana rawatan Bio-Akua, Bio-Blok dan Kawalan-N, mempunyai berat badan yang signifikan ($p<0.05$) lebih tinggi berbanding dengan udang di tangki kawalan. Untuk kemandirian, udang putih ini tidak menunjukkan keputusan yang signifikan ($p>0.05$) untuk setiap rawatan. Akan tetapi, rawatan Bio-Akua, Bio-Blok dan Kawalan-N mempunyai tahap kemandirian yang tinggi berbanding dengan tangki kawalan. Selepas 62 hari dikultur dengan menggunakan probiotik komersial, dan juga ujian ketahanan terhadap bakteria patogenik, *V. harveyii,* probiotik komersial dilihat sebagai salah satu daripada jalan penyelesaian yang tersedia untuk menggalakkan pertumbuhan dan kemandirian udang di samping penggunaan teknik konvesional seperti penggunaan antibiotik dan bahan kimia.

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# LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| °C | Degree Celsius |
| ± | Plus or minus |
| $\mu$ | Micron |
| % | Percentage |
| < | Less than |
| > | More than |
| ~ | Approximately |
| (w/v) | weight per volume |
| ANOVA | Analysis of Variance |
| BMRI | Borneo Marine Research Institute |
| CFU | Colony Forming Unit |
| DO | Dissolved oxygen |
| FAO | Food and Agriculture of Organization |
| g | Gram |
| *L* | *Litopenaeus* |
| ℓ | Liter |
| mℓ | milliliter |
| mt | metric tonne |
| P | *Penaeus* |
| *P* | Probability |
| PL | Post-larvae |
| ppm | Parts per million |
| SST | School of Science and Technology |
| SD | Standard Deviation |
| TSB | Tryptic Soy Broth |
| TSA | Trptic Soy Agar |
| TCBS | Thiosulphate Citrate Bile Salt Agar |
| UMS | University Malaysia Sabah |

# CHAPTER 1

# INTRODUCTION

## 1.1 The Present and Future Status of Shrimp Farming

During the last 20 years, global shrimp productions in terms of values and volumes have rise significantly. With this rapid growth, there has been a concurrent and increasing demand for improved sustainability of shrimp aquaculture, social acceptability, and improved quality and safety product produced by the sector (FAO, 2004).

Shrimp farming had become an important economic activity in many countries, particularly in the tropics. But, the industry often resulted in serious economic loss which is due to the nature of the operation. In large scale production facilities, an aquatic animal, which is shrimp are exposed to stressful condition, which relates to disease problems and deterioration of environmental condition (Balcazar *et al.*, 2006). The intensification of aquaculture activities has increased the occurrence of disease (Shariff *et al.*, 2001).

## 1.2 White Leg Shrimp *( Litopenaeus vannamei)* Culture Status

During the last decades, total world shrimp production rose by 107% from 869, 576 mt in 1994 to 1,803,895 mt in 2003 due to its expansion in new countries such as India, Vietnam and Indonesia as well as the refinement of culture techniques. Recently the growth had slowed due to the effects of 2004 tsunami, white spot disease outbreaks and shortages of healthy brood stock, especially tiger shrimp, *Penaeus monodon* (Kongkeo, 2006).

As a result, countries particularly in Southeast Asia, mostly had shifted to the culture and farming of *Litopenaeus vannamei. L. vannamei* broodstock can be easily domesticated, and commercially developed as specific pathogen-free, in addition of their tolerance to a wider range of salinity, temperature and poor bottom condition. (Kongkeo, 2006).

*L. vannamei* was introduced into Asia experimentally from 1978-79, but commercially only since 1996 into the mainland China and Taiwan Province of China, and then followed by most of the other coastal Asian countries in 2000-01 (FAO, 2004). In year 2000, Asia region only contributed 1.5 % of *L. vannamei* aquaculture production, which at the same time Central America dominates 98.5 % of *L.vannamei* production. However, the following years, the production increased each every year and in year 2003, Asia contributed 75, 80, 82 and 85 percent of the total production in year 2004, 2005 and 2006 respectively. It showed an increased of 99.87 % in 7 years as shown in Figure 1.1.

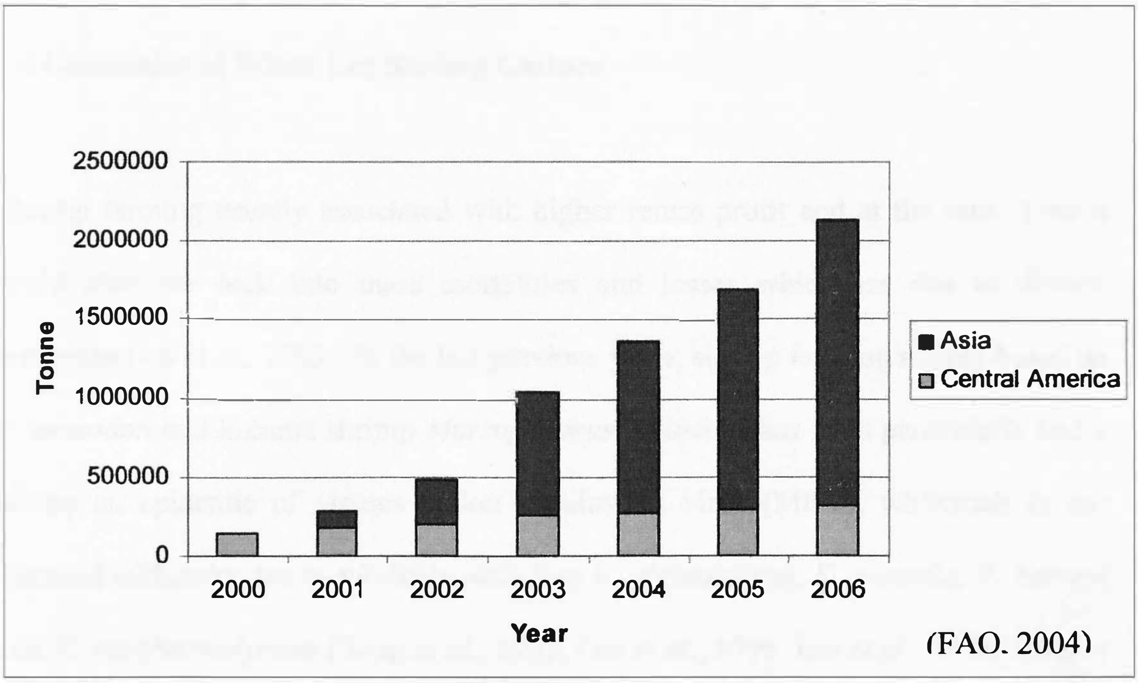


**Figure 1.1** Comparison of Aquaculture Production of White Legs Pacific Shrimp, *L. vannamei* from year 2000 – 2006 between Asia and Central America

## 1.3 Status of White Leg Shrimp Culture in Malaysia

In Malaysia, the culture of *L. vannamei* was introduced illegally since the year 2001 (FAO, 2004). Despite being a prohibited species, illegal production during year 2003 to 2005 was estimated between 5,000 to 7,000 mt. considering of the yet unsolved disease problem in *P. monodon*, and the total country shrimp production due to the lower cost production competitors of other export countries such as India, Vietnam and Indonesia, government finally took a stand legalize its culture effective as April 2004. Nevertheless, in a step to contain disease transmission there is still a control on fry and brood stock entry into the country (Fariduddin, 2006)

## 1.4 Constraint of White Leg Shrimp Culture

Shrimp farming usually associated with higher return profit and at the same time it could alter the luck into mass mortalities and losses which are due to disease outbreaks (Lo *et al.,* 2003). In the last previous years, shrimp farming mainly based on *P. monodon* and kuruma shrimp *Marsupenaeus japonicus* has been particularly badly hit by an epidemic of viruses nodon baculoviros virus (MBV), whitesuch as mo diseased outbreaks due to vibriosis including *V. alginolyticus, V. damsela, V. harveyi and V. parahaemolyticus* (Song *et al.*, 1993, Lee *et al.*, 1996, Liu *et al.*, 1996, Sung *et al.*, 2001). These diseases have been reported to be associated with increases of *Vibrio* populations of cultured pond waters (Sung *et al*, 2001).

Another problem for most shrimp producers is the well publicized European Unions restrictions related to the detection of banned antibiotics residues in shrimp and the USA which has also introduced much stricter controls over testing for these banned antibiotics (chloramphenicol and nitrofurans). With the introduction of technology capable of detecting 0.1 ppb levels of these substances, the testing for and enforcement of these levels on future shrimps will inevitably lead to problems for major exporting countries (FAO, 2004).

The search for technological solutions to problems related to high density aquaculture practices has resulted in a proliferation of often unproven and potentially dangerous solution. Products and procedures such as chlorination, antibiotics, and even toxic insecticides are touted as cures for problems in hatcheries and farms. Although some of these products can improve the culture environment or exclude disease carries, misuse has been shown to compounds existing problems (Balcazar *et al.*, 2007).

The solution lies in the field of microbial ecology, not in the field of pharmacology which developing new antibiotics or vaccines (Moriarty, 1999). The use of effective microorganisms such as probiotics to displace pathogenic bacteria by competitive process is a better remedy than administering antibiotics.

In Malaysia, shrimp pond is synonym with mangrove swamp area. However it is slowly make way to less critical and better area such as costal land, abandoned coconut estates or paddy field which is close to infrastructure and facilities. Water source is supplied by mean of pump or connected by canals. Commercial farms integrate reservoir and sediment ponds to cater for their operation in ensuring good quality water supply. Pond structures and design are of several types and the most common one are earthen pond (Fariduddin, 2006). These are the general characteristics of traditional shrimp aquaculture production, whereby a large quantity of water is required to fill a pond and an equivalent volume also is required to compensate for evaporation and seepage during the year.

In the past of recent years, the increase of oil prices has increase the cost of the conventional method of culturing shrimp resulting in high cost of operation, including labor and maintenance cost. One of the suggested solutions is by using recirculation water system, which may offer an alternative for aquaculture practices technology. Through water treatment (by means of bacteria) and reuse, recirculation system uses a fraction of the water required by ponds to produce similar yields (Losordo *et al.*, 1998).

## 1.5 Significant of Study

The significant of this study lies on the basis of diverse knowledge background and absolutely contributes to the wide application to the real industry. This study is important for these two main reasons:

1. The outcome of this study could be applied for the practice in the real situation especially in shrimp farm.
2. This study is important to the additional information on shrimp culture

## 1.6 Objectives of Study

The purpose of this study was to investigate the effectiveness of the use of probiotics in promoting the growth and survival of *L. vannamei* in a closed recirculation water system. There are two main objectives:

1. To observe the improvement on the survival and growth performances of post larvae with probiotics.
2. To determine the effects of probiotics on post larvae after challenging with *Vibrio* sp.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Classification of White Leg Pacific Shrimp, *Litopenaeus vannamei* (FAO, 2004).

| | |
|---|---|
| Kingdom | :Animalia |
| Phylum | :Arthropoda |
| Subphylum | :Crustacea |
| Class | :Malacostraca |
| Order | :Decapoda |
| Suborder | :Dendrobranchiata |
| Family | :Penaeidae |
| Genus | :*Litopenaeus* |
| Species | : *Litopenaeus vannamei* |

| | |
|---|---|
| FAO name | : White Leg Pacific Shrimp |
| Local name | : Udang Putih |

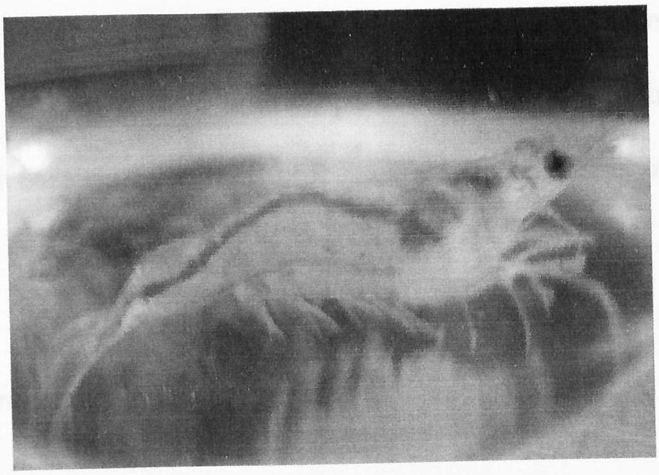

**Photo 2.1** White Leg Shrimp (*Litopenaeus vannamei*) post-larvae

## 2.2 Distribution and General Characteristic

White leg pacific shrimp, which is naturally distributed throughout the Pacific coast from Gulf of California to northern Peru, has become the primary species currently being cultured even in several places in the East hemisphere including Malaysia. *L. vannamei* can be found at the depth at 0 to 72 m. Adult stage can be found in the marine area while the juveniles are found in the estuarine. The maximum total length is 230 mm; maximum carapace length 90 mm (FAO, 2004).

## 2.3 Definition of Probiotics

The term "probiotics" first used as antonym of the term "antibiotic" (Verschuere *et al.*, 2000), and it is derived from Greek words προ and βιοτοσ and translated as "for life" (Hamilton *et al.*, 2003). But, the original definition of probiotics is defined as live microorganisms, which when consumed in adequate amounts, confer a health benefit for the host (FAO, 2001). In aquaculture probiotics is a very well known aquatic commercial term which describing an application amount of beneficial bacteria in rearing ponds or tank of aquatic animal, particularly shrimp culture in purpose to eliminate harmful bacteria by certain processes and mechanisms of action either *in vitro* and *in vivo*.

**2.3.1 Types of Probiotics in Host Animals**

Basically, probiotics strains have been isolated from indigenous and exogenous micro biota of aquatic animals, this including, the gastro-intestinal tract, from surrounding water and mud (Balcazar *et al*, 2006). The abundance of different strains also different in species, for marine fish, the predominant indigenous microbiota which constitute is the gram-negative facultative anaerobic bacteria such as *Vibrio* and *Pseudomonas* (Hansen *et al.*, 1999).

Different from seawater fish, the genera *Aeromonas*, *Plesiomonas*, representatives of the family Enterobacteriaceae, and obligate anaerobic bacteria of the genera *Bacteroides*, *Fusubacterium*, and *Eubacterium* are dominant in the indigenous microbiota of freshwater fish. Lactic acid producing bacteria, which are prevalent in the mammal or bird gut, are generally sub-dominant in fishes and represented essentially by the genus *Carnobacterium* (Irianto *et al.*, 2003).

**2.3.2 Mechanisms of action and effects of probiotics**

Probiotics strains have been shown to inhibit pathogenic bacteria both *in vitro* and *in vivo* through several different mechanisms, suggested as followed.