Effect of Microorganisms on Production and Cost-Benefit Analysis of Intensive Pacific White Shrimp (*Litopenaeus vannamei*) Culture

Mongkhon Primphon, Niti Chuchird and Chalor Limsuwan*

ABSTRACT

The use of probiotics and bacterial management in shrimp pond aquaculture can be a crucial element to the success of production. The effects of probiotics on the survival, yield, and economics (cost and benefit) of Pacific white shrimp (*Litopenaeus vannamei*) farming in commercial ponds were determined. Six experimental ponds in a commercial operation were used, with three assigned as control ponds (no probiotics) and the other three as treatment ponds (with probiotics) during March-June 2011. The same stocking density and source of post larvae was used and optimum stocking density at 100-125 pl/m^2 was maintained. Three major groups of probiotics including *Bacillus* spp., nitrifying bacteria and hydrogen sulfide oxidizing bacteria were used, either by mixing with the feed, applying directly to the water, or adding to the sludge area. Some water quality parameters were measured and compared between the ponds. Production and growth data were compared after harvest. Yield, survival, mean body weight (MBW) and average daily growth (ADG) of treated ponds were higher than those in the control (2,494±319.88 kg/rai, 73.18±6.47 %, 17.21±0.50 g. and 0.14 ±0.004 g/day, respectively). Whereas in the control ponds, the figures obtained were 1,970± 205.30 kg/rai , 63.15±3.69 %, 15.78±0.61 g., and 0.13 ± 0.005 g/day, respectively. Furthermore the FCR in treated ponds was 1.71 ± 0.03, which was significantly lower ($p<0.05$) therefore better, than that in the control ponds, which was 2.01 ± 0.10. Water conditions measured by Biological Oxygen Demand (BOD) in the treatment ponds increased slowly and showed less fluctuation than in the control ponds. Total organic matter (TOM) appeared to increase slowly in the treatment ponds compared to the control. All other water parameters such as ammonia, nitrite, and nitrate were all within acceptable limits. A combination of probiotics could improve water and bottom soil conditions by balancing and maintaining water parameters, increase the yield, survival and size of shrimp at harvest, reduceng feed conversion ratio and improving the income gained. However more research is needed to improve management practices and discover better probiotics application for more effectivity according to different farming models.

Keywords: microorganisms, cost-benefit analysis, Pacific white shrimp

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Aquaculture Business Research Center, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand
*Corresponding author, e-mail: ffiscll@ku.ac.th

INTRODUCTION

Development of shrimp aquaculture has been associated with increasing occurrences of infectious diseases and environmental degradation. Prior to the occurrence of Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) in 2012, shrimp farmers for both black tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*) in Thailand had been affected by white feces syndrome. The main signs of white feces syndrome include strings of white feces floating on the water surface and a significant reduction in feed consumption. Various researchers have varying information on the causes of white feces syndrome. Mixed species of *Vibrio* including *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, *V. alginolyticus*, *V. damselae*, *V. mimicus* and *V. cholera* (non01) were found associated with diseased shrimp in intensively cultured ponds (Somboon *et al*, 2012). The use of immunostimulants such as β-1,3-glucan or organic acid followed by probiotics was observed to increase survival after challenges tests with *Vibrio* spp. associated with white feces syndrome (Jueliang *et al*, 2013). Both of these findings suggest that probiotics and bacterial management in pond culture can play an important role in controlling white feces syndrome.

In the long term, the application of beneficial bacteria (probiotics) to displace the pathogenic bacteria yields better result than applying antibiotics or other banned chemicals because over use of antibiotics causes bacteria to develop antibiotic resistances. This is the major role for bacteria in management practices in sustainable aquaculture under bio-security, food safety and environmental friendly concerns. A number of probiotics had been used in the aquaculture industry, and most of these commercial probiotics contain nitrifying bacteria and/or *Bacillus* spp. (Gatesoupe, 1999), primarily for their abilities to improve water quality (Gomes *et al*., 2009), along with providing other vitamins and nutrients (Gatesoupe, 1999; Verschuere *et al*., 2000). The majority of probiotic products in use today are bacteria spore formers, mostly of the genus *Bacillus* (Hong *et al*., 2005). The introduction of *Bacillus* spp. in close proximity to pond aerators has been shown to reduce biological oxygen demand and increase shrimp production (Porubcan, 1991). Shrimp probiotics have been isolated from many sources including shrimp culture water (Nogami and Maeda,1992; Direkbusarakom *et al*., 1997; Tanasomwang *et al*.,1998; Otta *et al*., 1999) , the intestine of different penaeid species (Rengpipat *et al*.,2000) or hepatopancreas of healthy wild shrimp (Alavandi *et al*., 2004; Gullian *et al*., 2004). Probiotics commonly associated with shrimp and easily isolated from aquatic environment include Vibrionaceae, Pseudomonads, lactic acid bacteria, *Bacillus* spp. and yeast (Moriarty,1997; Gastesoupe,1999).

The objectives of this study were to examine the effect of probiotic application on survival, yield, and cost-benefit ratio of Pacific white shrimp (*L. vannamei*) in practical commercial culture settings.

MATERIALS AND METHODS

Study site

A total of 6 earthen ponds were selected from a commercial farm located in Chanthaburi Province, a major shrimp culture area in the eastern part of Thailand. The ponds were between 5-6 rai (8,000-9,600 m^2) with fully bio-security management model. The experiment was conducted between March and June 2011. Six ponds were assigned as either control (3 ponds- receive no probiotic) and treatment (3 ponds- with probiotic). The ponds were stocked with the same number of PLs, at 100-125 pl/m^2 coming from the same source.

Selection of Microorganisms

Three groups of probiotics were selected and applied in ponds throughout the production cycle. These probiotics were: (1) 5 species of *Bacillus* spp. (*Bacillus subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. megaterium* and *B. pumilus*) to control the organic matter in water and bottom soil (PondPlus), (2) nitrifying bacteria including AOB (*Nirosomonas eutropha*) and NOB (*Nitrobacter winogradskyi*) (PondProtect) to control ammonia and nitrite and (3) hydrogen sulfide oxidizing bacteria (*Paracoccus pantotrophus*) (PondDtox) to control hydrogen sulfide. All probiotics were packed in soluble packages.

Microorganisms application protocol

Protocol for probiotics application was set up as guidelines for practical management, and the recommended dosage was adjusted according to pond area, stocking density, days of culture as well as the water and bottom conditions (Table 1). All probiotics were applied directly into the water and mixed in the pond using paddlewheel aerators for circulation.

Table 1. Probiotic application protocol (for pond area 5000-6000 m^2).

Type of probiotics	Activities	Dosage and application practices	Remark
Bacillus spp.	Organic matter digestion *Vibrio* spp. control and phytoplankton maintenance	- 200g before stocking - 200g weekly up to 60 days - 400g weekly after 60 days until harvest	Added in feed as gut probiotic during 23-30 days and 53-60 days
Nirosomonas eutropha *Nitrobacter winogradskyi*	Ammonia and nitrite control	- 400 g during the first 30 days - 400 g weekly after 50 days	Applied 1 day after *Paracoccus pantotrophus*
Paracoccus pantotrophus	Hydrogen sulfide control	- 200 g before stocking - 200 g every 10 days up to 30 days - 200 g weekly between 30 days to 60 days - 400 g weekly after 60 days until harvest	Applied at the sludge area

Post larvae stocking and farm management

Normal culture practices and pond management for each farm were followed. Post larvae (age PL12) from the same hatchery were used for both control and treated ponds and stocked at the same time (direct stocking with no nursery system on farm). Feeding management followed the normal practices being done with other ponds in operation in that farm. Manual feeding was practiced during the entire cropping period until harvest, with the proper feeding program adjusted by the farm manager. Culture period was at normal culture period of around 120 days.

Water and bottom soil parameters analysis

Water samples were collected and analyzed every 2 weeks, while bottom soil samples were collected monthly. Both water and soil samples were collected from two areas of the pond, i.e. 2 feeding areas and 2 edges of sludge areas (from both sides of the culture ponds). Water samples consisted of 500ml from the surface and 500 ml from the bottom. The samples from both areas were mixed before the analyses of ammonia by phenol-hypochlorite method (APHA *et al*., 1995), nitrite, nitrate by colorimetric method (APHA *et al*., 1995), biological oxygen demand (BOD) (APHA *et al*., 1995) and chlorophyll *a* by spectrophotometric method (Strickland and Parsons, 1972). Soil samples were analyzed for total organic matter (TOM) using the Walkly-Black method (Nelson and Sommers, 1996)

Statistical analysis

Crop data and results (yield, survival rate, size and FCR) as well as the water and bottom soil parameters from both control and treatment ponds were compared using one-way analysis of variance (ANOVA) (Steel and Torrie, 1980).

RESULTS

Yield and survival

Yield, survival, MBW and average daily growth (ADG) of treatment ponds (2,494±319.88 kg/rai, 73.18±6.47 %, 17.21 ±0.50 g. and 0.14 ±0.004 g/day, respectively) were higher than the control (1,970±205.30 kg/rai, 63.15±3.69 %, 15.78±0.61 g. and 0.13 ±0.005 g/day, respectively). Furthermore, the FCR of treatment ponds (1.71 ± 0.03) was significantly lower ($p<0.05$) than that of the control ponds (2.01 ± 0.10, Table 2).

Shrimp farmers can gain higher profits not only from the higher yield of shrimp but also from the increase in price per kg for the larger shrimp and the saving from feed due to a lower FCR. The results showed an outcome of 38,365 Baht/rai from the increase in total profit gain and the cost of feed saved from lower FCR. The difference in total profit between treated ponds and the control was around 7,211 Baht/rai. In treated ponds, the market price at harvest period was 145 Baht/kg (based on 17.21 g shrimp) with production cost of 129 Baht/kg, whereas in control ponds, the market price was 140 Baht/kg (based on 15.78 g size shrimp) with a production cost of 136 Baht/kg. The cost of microorganisms spent for the entire culture period was around 4,000 Baht/rai. Overall, the cost of microorganisms for producing 1 kg of shrimp was about 1.60 Baht.

Table 2. Production data, growth, survival, size, FCR and ADG results from the experimental farm in Chanthaburi Province.

Ponds	DOC (days)	Stocking density	Yield/rai (kg)	Survival rate (%)	MBW (g)	FCR*	ADG (g)
Control	120	125	1,970±205.30	63.15±3.69	15.78±0.61	2.01±0.10	0.13±0.005
Treatment	120	125	2,494±319.88	73.18±6.47	17.21±0.50	1.71±0.03	0.14±0.004

* means are significantly different at $p<0.05$.

Biological Oxygen Demand (BOD) analysis

The results for BOD levels during culture are shown in Table 3 and Fig. 1. The BOD of the control and treated ponds at feeding and sludge areas slightly increased overtime during the early stages of culture. After 40 days, the BOD in both areas of the control ponds increased rapidly whereas the BOD in the treated ponds slightly changed and fluctuated less than the control ponds (Figure 1). From stocking till harvest, the BOD in the treated ponds increased from 8.07 ± 0.83 ppm. to 19.63 ± 4.96 ppm. and 9.83 ± 1.04 ppm. to 25.77 ± 2.62 ppm. at feeding and sludge areas, respectively. Meanwhile the BOD in control ponds increased from 9.97 ± 1.09 ppm. to 42.27 ± 6.76 ppm. and 10.63 ± 0.58 ppm. to 45.47 ± 2.91 ppm. at feeding and sludge areas, respectively. The percentage of BOD increment in treated ponds at the end of the culture period (118 DOC) was significantly lower ($p< 0.05$) than control ponds at the feeding area and was significantly lower ($p< 0.05$) than control ponds at the sludge area (Table 3).

Total ammonia, nitrite, nitrate and some other parameters were regularly checked during the culture period. All parameters were in the normal range for shrimp culture, with treated ponds having lower concentrations of ammonia, nitrite and nitrate than the control ponds.

Table 3. The level of BOD during culture period between control and treatmemt ponds at both feeding area (A) and sludge area (B).

Feeding area (A)	3 days	22 days	38 days	52 days	76 days	87 days	103 days	118 days
Control	9.97 ± 1.09	12.43 ± 1.09	14.00 ± 1.89	28.03 ± 8.00	30.67 ± 8.71	29.13 ± 6.78	44.77 ± 9.59	42.27 ± 6.76
Treatment	8.07 ± 0.83	11.43 ± 1.39	12.60 ± 2.39	12.47 ± 1.98	19.17 ± 3.44	21.17 ± 8.03	28.20 ± 10.55	19.63 ± 4.69*
Sludge area (B)								
Control	10.63 ± 0.58	18.10 ± 4.52	15.53 ± 3.24	28.20 ± 8.40	36.17 ± 11.53	36.87 ± 12.61	42.03 ± 8.52	45.47 ± 2.91
Treatment	9.83 ± 1.04	11.07 ± 2.31	12.60 ± 1.31	19.60 ± 5.80	31.33 ± 9.35	28.43 ± 7.63	27.77 ± 5.63	25.77 ± 2.62*

* means significantly different at $p < 0.05$.

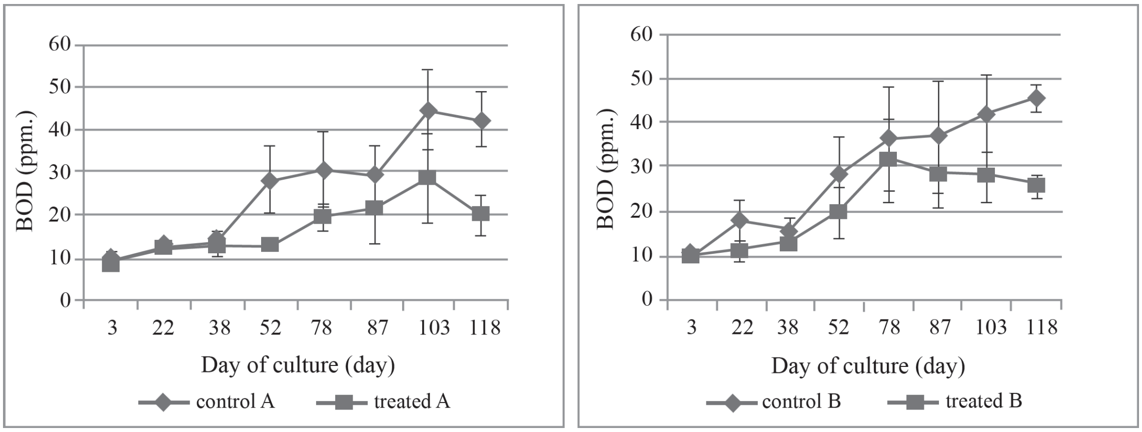

Figure 1. Changes in BOD levels during culture period in control and treatment ponds at both feeding area (A) and sludge area (B).

Bottom soil analysis during culture period

The TOM level at the feeding area decreased in both control and treated ponds from stocking till 60 days and then gradually increased at 90 days. At 120 days, the TOM in treated ponds slightly decreased and was lower than the initial level. Whereas in the control pond, TOM increased and was slightly higher than the initial level (Figure 1). On the other hand, the TOM at the sludge area of both control and treated ponds slightly increased during the first 60 days, and then rapidly increased until shrimp were harvested. The TOM in treated ponds was slightly higher than that of the control ponds from stocking till 60 days. However, at 120 days TOM in treated ponds was lower than the control ponds (Figure 2). At the sludge area, the TOM level at the end of the crop in treated ponds (2.43 ± 0.358%) was lower than that of the control ponds (2.99 ± 0.381%). Similarly, the TOM at the feeding area in treated ponds (0.17 ± 0.003%) was significantly lower ($p<0.05$) than that of the control ponds (0.25 ± 0.003%) (Table 4).

Table 4. TOM readings during culture period, compared between control and treatment ponds at both feeding and sludge areas.

Feeding area	**0 day**	**30 days**	**60 days**	**90 days**	**120 days**
Control	0.19 ± 0.028	0.14 ± 0.009	0.12 ± 0.003	0.19 ± 0.003	0.25 ± 0.003
Treatment	0.20 ± 0.043	0.15 ± 0.038	0.14 ± 0.006*	0.18 ± 0.015	0.17 ± 0.003*
Sludge area					
Control	0.19 ± 0.000	0.25 ± 0.012	0.29 ± 0.035	1.84 ± 0.179	2.99 ± 0.381
Treatment	0.26 ±0.006*	0.53 ± 0.027*	0.70 ± 0.196	1.69 ± 0.326	2.43 ± 0.358

* means significantly different at $p < 0.05$.

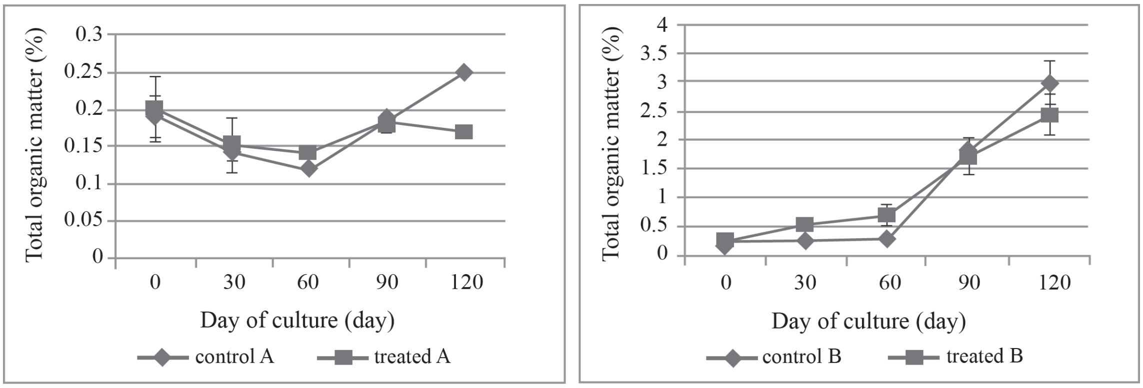

Figure 2. Total organic matter (TOM) during culture period between control and treated ponds in both feeding area (A) and sludge area (B).

DISCUSSION

Probiotic effects on yield, survival, FCR and economics

From the results of the trial, treated ponds gave better yield, survival and size as well as lower FCR, with significant differences at $p<0.05$ (Table 2). This was likely due to the effectiveness of *Bacillus* spp., nitrifying bacteria as well as *Paracoccus pantotrophus* in improving water and bottom soil quality by maintaining and balancing the level of organic matter and water quality parameters during the culture period. This results in healthy shrimp leading to lower infection and mortality during the later stage of culture, especially during molting period. With well-managed pond environment by effective micro-organisms, other reasons for using *Bacillus* spp. as the putative probiotics (Verchuere *et al*., 2000; Ziaei-Nejad *et al*., 2006) include degrading accumulated organic sludge in shrimp ponds (Verchuere *et al*., 2000; Nimrat *et al*., 2008), improving water quality, activating immune response, reducing the outbreak of pathogenic microbes and providing the essential nutrients as well as increasing the survival and growth rate of shrimp (Gatesoupe,1999; Verschuere *et al*., 2000; Irianto and Austin, 2002; Balcazar *et al*.,2006). It has been reported that using *B. coagulans* to treat the water increased survival and digestive activities of *L. vannamei* larvae (Zhou *et al*., 2009). Generally, bacteria in aquaculture can be categorized as either beneficial or pathogenic. Beneficial bacteria are helpful in nutrient recycling and organic matter degradation, resulting in a cleaner environment (Moriarty, 1997). On the other hand, pathogenic bacteria are the causative agents for diseases, poor water quality, and stress as they act as primary and secondary pathogens (Karunasagar, *et al*., 1996). The reasons mentioned above could also explain the reduction in feed conversion ratio (FCR) and the benefit gained from higher yield and bigger size of shrimp (Table 2). The lower FCR has a direct impact on feed cost saving, higher yield and bigger size of shrimp, resulting in more income, since the net profit depends on yield, survival, size at harvest, and feed cost. Probiotics can address all of the above parameters as well as an additional biological control option that is safer and reduces the risk of disease outbreak during culture.

Reasonable profits can be obtained by appropriately using probiotics in shrimp culture. However, profits vary depending on the location, internal culture system and management, production season as well as the market price at harvest period. In addition, production season can also impact on the culture pond system and water quality. This experiment shows that there is a potential benefit in using effective micro-organisms or probiotic treatment in shrimp culture, to assist in the management and minimizing the risk factors in water, pond bottom as well as shrimp health condition.

Using probiotics for water and bottom soil quality improvement in shrimp culture

The results from this experiment indicate that water and soil quality could be controlled and managed by probiotics. Ponds applied with *Bacillus* spp, nitrifying bacteria and *Paracoccus pantotrophus* had less organic matter deposit in water and bottom soil as indicated by BOD and TOM analysis, respectively. BOD levels in treated ponds (Table 3, Fig. 1) showed that during the later stage of culture and the organic matter deposit in water in treated ponds could be controlled by the *Bacillus* group as shown by the lower BOD in treated ponds compared to control ponds. The probiotic group of bacteria applied in this experiment could digest and minimize total organic waste deposited (TOM) at both the feeding and sludge areas of pond bottom in treated ponds more effectively (Table 4, Fig. 2). Improving the pond environment can result in better shrimp health. The mode of action for these probiotics is not only enhancing the natural process such as organic matter degradation, nitrification, ammonia removal and de-nitrification, sulfide oxidation and degradation of toxic pollutants, but also increasing the abundance of useful bacteria, competitive exclusion of undesirable species to help reducing the incidence of fish and shrimp disease in culture ponds (Boyd and Gross, 1998). The application of commercial probiotics hase been shown to reduce organic matter accumulation, improve water quality, and enhance environmental conditions for shrimp culture (Suhendra *et al*., 1997).

In the probiotics treated ponds, the dissolved oxygen improved in water at the bottom as well especially at the edge of sludge area (the critical area for shrimp molting). During molting period, the toxic gas (ammonia, nitrite and hydrogen sulfide gas) can affect the shrimp in the area, especially when oxygen is low. This induces stress to the shrimp resulting in bacterial infection at the later stage of culture. Paracoccus pantotrophus takes the major role to effectively remove and prevent further building up of hydrogen sulfide. *Paracoccus* spp. can also increase the redox potential (Panichakornkul, 2007) to prevent and reduce mortality as well as improve size, yield and FCR. Furthermore, even with poor bottom condition during the early stage of culture and with benthic algae or sudden crashes of phytoplankton, this probiotic could manage and improve the bottom soil and water condition and reduce shrimp mortality during emergency conditions.

CONCLUSION

According to this study, a combination of probiotics composed of *Bacillus* spp. group, nitrifying bacteria group and *Pacracoccus pantotrophus* could improve shrimp aquaculture production by, (1) managing water and bottom soil conditions, balancing and maintaining water parameters, (2) increase the yield, survival and size of shrimp at harvest, (3) reducing feed conversion ratio and (4) improving the income gained. It can be postulated that the result may come from the improvement in the pond environment with more stable water and bottom soil conditions, which could make the shrimp feed better, increase the digestibility of feed and improve shrimp health.

In the future, more research could be designed according to different management practices and find new probiotics application for more effectivity according to different farming models. The new probiotic strains and proper application dosage should also be selected and implemented in various shrimp culture seasons and in wider scale of culture areas.

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