Influence of commercial probiotics on bacterial (Vibrio) load and total yield in semi intensive Penaeus monodon culture ponds

CH. VENKATARAYULU, V. KALA RANI AND D.C. REDDY

ABSTRACT

The major limiting factor - the mass mortality in Penaeus monodon culture has been attributed to the presence of pathogenic bacteria Vibrio in the rearing environment. This study presents results on the effect of commercial water and feed probiotics (Wunapuo-15; Aqualact) on the population density of Vibrio (yellow and green colonies) bacteria in rearing pond waters and total yield in semi intensive shrimp culture ponds. The results showed that the use of probiotics has reduced Vibrio bacterial count and improved total yield of shrimp. The average Vibrio bacterial (yellow and green colonies) counts were found to be significantly (P<0.01) lower in probiotic treated (PB) ponds compared to control ponds (CP). The total yield (TY), survival rate (SR%) and average body weight (ABW) were significantly (P<0.01) higher but food conversion ratio (FCR) was significantly (P<0.01) lower in probiotic treated (PB) culture ponds than in the control ponds (CP). Cumulative feed (CF) did not differ (P>0.05) significantly between control (CP) and probiotic treated (PB) culture ponds. Possible impact of using probiotics on sustainable shrimp culture is discussed.

Key words : Probiotics, P. monodon, Vibrio, Total yield, Survival rate, FCR

Semi-intensive farming of the black tiger shrimp, Penaeus monodon, is widely practiced in India and other South East Asian countries. However increasing intensity of shrimp farming was inevitably paralleled by an increase in the incidence of diseases in the farming system (Balakrishnan et al., 2003). Of late shrimp culture all over the world is being increasingly affected with pathogenic microbial diseases inflicting considerable economic losses in several countries (Vaseeharan et al., 2003). Opportunistic shrimp pathogenic bacteria present in sea water cause of ecological changes when the water is used in aquaculture (Moriarty, 1998). Vibrios which are members of the normal bacterial flora of shrimps, induce mass mortalities in affected shrimp populations (Lightner, 1993). Bacterial diseases caused by members of genus Vibrio such as V. parahemolyticus, V. alginolyticus, V. anginillarum have often been reported among cultured penaeid shrimps (Sung et al., 1999). V. harveyi is the major cause of luminous vibriosis in crustaceans world wide (Austin and Austin, 1993). It has emerged as a serious pathogen of penaeid shrimp in hatcheries and farms in South America, Australia and SE Asia (Lavilla–Pitogo et al., 1990; Liu et al., 1996a,b). It produces green colonies on TCBS agar medium. Vibrio have been studied for many years and have been reported to cause serious infections and lower shrimp production (Lightner 1996). It has been implicated as being the major cause of disease problem in shrimp culture. Although the use of antibiotics to control vibrios in shrimp farming system leads to the development of antibiotic resistant pathogenic bacterial strains, an alternate method for control of vibrios in shrimp farming system is the use of probiotics (Moriarty, 1997). Probiotics are mainly composed of groups of Bacillus spp. and are being increasingly used aquaculture systems to promote the health of shrimp. The beneficial bacteria have the capacity to breakdown complex organic matter, oxidize toxic substances from water (Arunkumar Nayak and Pawar, 2003) and inhibit wide range of pathogens (Irianto and Austin, 2002). While in most cases only water or feed probiotic effects have been studied separately over short periods, synergistic effects of both water and feed probiotics have not been studied in parallel over long periods exclusively under natural field conditions.

Unfortunately most published work on the influence of probiotics was confined only to a single beneficial bacterial strain rather than to multiple strains. Consequently this study aims at studying the long term synergistic effects of multiple beneficial bacterial strains having commercially available water and feed probiotics on Vibrio bacterial load and total yield in P. monodon culture ponds exclusively in natural field conditions.
MATERIALS AND METHODS

The present work was carried out in a private coastal shrimp farm, Sharat Sea Foods Industries Ltd., near Venkannapalem village (14°.27'E; 80°.5? N) of Nellore District, Andhra Pradesh, India between March and July, 2006 (Summer crop). Six modified extensive shrimp culture ponds (~ 1 ha) were adopted for this purpose. They were uniformly prepared, following usual practices like ploughing, liming etc., and were filled with filtered, chlorinated (20ppm) and dechlorinated water upto 1.2m depth. This was followed by manuring and fertilization and the water quality variables were maintained at optimum levels. After proper preparation and maintenance, the control and experimental ponds were simultaneously stocked with P. monodon post larvae (PL20)@ 12/m2 obtained from Sharat Shrimp Hatchery (SSF Industries Ltd.) after PCR screening for white spot syndrome virus (WSSV) free seed.

Feed management:
Feeding for the initial 30 days of culture duration is dependent on survival in hapas installed and maintained in the culture ponds. 1.15kg feed was applied on day one to a pond with a stocking density of one lakh and increased @400-500 g/day for the same density till 30th day. After 30 days of culture duration, feed consumption is regularly monitored through check trays and depending on this feeding rate could be adjusted. The body weight of shrimp is measured every 7-10 days by random sampling. Feed quantity from then on would be calculated depending upon the survival rate and average body weight of shrimps. Throughout the culture duration, shrimp were fed with CP shrimp feed (CP Aquaculture (India) Pvt., Ltd.). Feeding schedules and monitoring water quality parameters were uniformly done for all culture ponds.

Probiotics:
Most widely used water and feed probiotics viz. “Wunapuo-15” (TEAM AQUA Corporation, Taiwan) and “Aqualact” (WOCHHARDT, Mumbai, India Pvt., Ltd.), respectively were used in the present study. Six ponds enmarked for the present study were divided into two groups of three each. The first group of ponds was not treated with probiotics and hence, designated control ponds (CP). Each of the second group of culture ponds was treated with water probiotic (Wunapuo-15) @ 30kg / ha every 15 days from 15th day to 115th day of culture duration. Simultaneously shrimp in culture ponds were also fed with feed probiotic (Aqualact) supplementation containing 5 g/kg feed once in two days from 30th day till the end of culture as per routine feeding schedules at evening meal.

Vibrio count was determined following the procedure of Dalmin et al. (2001). TCBS agar medium was used for isolating Vibrios (yellow and green colonies) from the pond water samples. 1 ml sample was spread over solidified surface of TCBS agar medium using a sterilized bent glass rod. After inoculation the petriplates were incubated in an inverted position at 36°C for 20-24 hr. Bacterial colonies could be seen after 18 hr. The Vibrio colonies count (yellow and green) was expressed as cfu/ml.

Harvesting of shrimp:
Shrimp were harvested using a selective bag not after 130-137 days of culture. The harvested shrimp were weighed using standard balance and shrimp count was also estimated for taking random samples from harvested shrimps. ANOVA was performed using the statistical tool package of Microsoft Excel 97 software. Student’s t-test was used to evaluate the significance of difference between the means (P<0.05).

RESULTS AND DISCUSSION

The total vibrio (yellow and green) count obtained during initial days of culture (30 days after stocking the post larvae) and at the end of culture (before harvest) in control (CP) and probiotic treated culture ponds are presented in Table 1. It is clear from the results that the vibrio (yellow and green colonies) count was significantly higher (P<0.05) in control ponds than in probiotic treated ponds both at initial and final stages of culture (at harvest). The probiotic treated ponds showed almost nill count at the end of the culture. Results obtained on the per cent survival (%SR), cumulated feed (CF), average body weight, food conversion ratio (FCR) and total yield (TY) of P. monodon from control (CP) and probiotic treated (PB) ponds at harvest are presented in Fig. 1, 2, 3, 4 and 5. The results clearly show that there were significant differences (P<0.01) in survival percentages of shrimp between control and probiotic treated culture ponds. Shrimp from control (CP) ponds exhibited a significantly (P<0.01) lower per cent survival than those from probiotic treated (PB) ponds. Data on cumulated feed (CF) show that there were no significant (P>0.05) differences between to control and probiotic treated ponds.

The average body weight (ABW) also differed significantly (P<0.05) between the control and probiotic treated shrimp (Fig. 3). FCR was significantly higher (P<0.05) in control (CP) shrimp than in probiotic treated shrimp (Fig. 4). Total yield was significantly lower
Vibrios are by far the most numerous of the reported bacterial agents of penaeid shrimp and reported to constitute majority bacteria present in the normal microflora of cultured and wild penaeid shrimp (Dempsey et al., 1989; Hameed, 1993; Leano et al., 1998; Singh et al., 1998). Infections due to them are characterized by massive colonization of the appendages and foregut followed by infection of the midgut, hepatopancreas and a terminal septicaemia (Ajitha et al., 2004). The total Vibrio count (yellow as well as green colonies) was significantly lower (P<0.05) in probiotic treated ponds than in control ponds suggesting that the application of

\[\text{Table 1: Mean (±SD; n=3) values of vibrio count (yellow and green colonies) in control (CP) and probiotic treated (PB) culture ponds}\]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yellow Colonies Count (cfu/ml)</th>
<th>Green Colonies Count (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial At Harvest</td>
<td>Initial At Harvest</td>
</tr>
<tr>
<td>Control pond (CP)</td>
<td>$1.66 \times 10^1 \pm 0.15 \times 10^1$</td>
<td>$3.63 \times 10^2 \pm 5.0 \times 10^1$</td>
</tr>
<tr>
<td>Probiotic treated pond (PB)</td>
<td>$0.6 \times 10^1 \pm 0.26 \times 10^1$</td>
<td>$0.06 \times 10^2 \pm 0.11 \times 10^1$</td>
</tr>
</tbody>
</table>

* indicates significance of value at P=0.01 @ : Not significant
cfu / ml : Colony forming units / millilitre
probiotics reduced Vibrio population through competitive exclusion process (Matias et al., 2002). These results are in consonance with those of Moriarty (1999) who found that routine use of commercial probiotics in a shrimp farm in West Java, resulted in reduced incidence of Vibriosis and other viral outbreaks. In addition, Moriarty (1998) found that the use of probiotics could prevent luminescent Vibrio infestation either by lowering or completely eliminating luminous Vibrios in pond waters and sediments as well.

Further, Rengpipat et al. (1998), Dalmin et al. (2001), Sambasivam et al. (2003) and Vaseeharan et al. (2004) obtained similar results reflecting higher survival of shrimp treated with probiotics. It has been shown that the alimentary tract of penaeids provides a congenial environment for Vibrios to multiply and activation of any stress factors in the culture system may make the animal susceptible to the invasion of pathogenic strains of the genus (Singh et al., 1998).

Better survival is a crucial factor in achieving good production in shrimp culture. Probiotics have been reported to increase the survival and net production of P. monodon larvae and post larvae. Application of probiotic bacteria improved microbial balance of pond waters apart from stabilizing the useful microbial flora in the guts of cultured animals (Yousuke Taoka et al., 2006). The results of the present study clearly show that there were significant differences in the survival percentages between the control and probiotic treated shrimp (P<0.01) (Fig. 5). The higher survival rate in probiotic treated ponds could be attributed to the fact that the water and feed probiotics used have beneficial bacteria like Lactobacillus, Streptococcus and Bifidobacterium which are reported to inhibit the growth of harmful bacteria through competition for nutrients, space and adhesion sites in the shrimp gut and surrounding water there by enhancing the survival rates (Gatesoupe, 1999; Moriarty, 1998, 1999; Ajitha et al., 2004). Probiotics when inoculated into the rearing water of an aquaculture pond, act as bioremediation agents in improving water and sediment quality, as well as biocontrol agents in eliminating pathogens (Queiroz and Boyd, 1998; Prabhu et al., 1999).

Commercial probiotics are also reported to improve growth and survival rates in cultured organisms and enhance the immune response and digestive enzyme activities (Rengpipat et al., 1998; 2000).

Cumulated feed (CF) is an important factor in estimating food conversion ratio (FCR) of cultured shrimp. The results showed no significant difference (P>0.05) in CF between probiotic treated and control shrimp (Fig. 2). It is probable that probiotics may stimulate appetite and improve nutrition by the production of vitamins and by the breakdown of indigestible compounds in the diet (Irianto and Austin, 2002) thus enhancing food consumption and yield. Similar results have also been reported by Uma et al. (1999) in P. indicus and Suralikar and Sahu (2001) and Himabindu et al. (2004) in M. rosenbergii treated with commercial probiotics.

There were significant differences (P<0.01) in average body weight (ABW) with probiotic treated shrimp showing higher ABW than the control shrimp (Fig. 3). This could again be ascribed to the ability of probiotics to improve water quality, enhance digestive enzyme activities and food consumption and minimize feed wastes (Moriarty, 1999). FCR was found to be significantly higher (P<0.01) in control shrimp than in probiotic treated shrimp. This is only expected because water and feed probiotics are reported to improve water quality, enhance the production of vitamins and digestive enzymes and improve the overall nutritional status of the shrimp thus contributing to a decrease in FCR and an increase in FCE (Irianto and Austin, 2002; Ajitha et al., 2004).

Similar results have also been reported by Rengpipat et al. (2000) in P. monodon, Uma et al. (1999) in P. indicus and Suralikar and Sahu (2001) in M. rosenbergii. Higher FCE and better FCR reduce feed wastes and promote growth and average body weight. It has been reported that probiotic supplemented feed improve food conversion efficiency by preventing intestinal disorders and predigestion of antinutritional factors present in the feed ingredients (Himabindu et al., 2004).

Finally the total yield (kg) was found to be significantly higher in probiotic treated ponds than in control
ponds (P<0.01) reflecting better growth performance of shrimp due to probiotics. Similar results were obtained by Rengpipat et al. (1998), Ravi et al. (1998), Dalmin et al. (2001) and Sambasivam et al. (2003) in P. monodon; Uma et al. (1999) in P. indicus and Suralikar and Sahu (2001) in M. rosenbergii. It is possible that probiotics provide a healthy microbial environment, improve water quality, provide better digestion and nutritional security leading to better growth performance and total yield as has been reported by by Yousuke Taoka et al. (2006).

The results of the present investigation demonstrate that probiotic treatment has improved internal and external environment of the shrimp leading to better growth. Water and feed probiotics used in this study are mixed strains having bacteria belonging to Bacillus, LAB and Bifidobacterium which are reported to influence the growth and survival of shrimp in a significant manner and enhance the total yield at harvest in semi intensive culture ponds.

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Abbreviations:

CP : Control pond, PB : Probiotic treated pond, g : grams, Kg : Kilograms, PCR : Polymerase chain reaction, ppm : Parts per million, FCE : Food conversion efficiency, LAB : Lactobacillus, m : Meter, ha : hectre, cfu/ml : colony forming units / milliliter

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