JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Effect of Probiotic Culture Physico-chemical Parameters on Growth, Mortality and FCR of *Penaeus vannamie* shrimp (Boone,1931)

Dr. KAULU SUJATHA

LECTURER IN ZOOLOGY, S.G.A GOVERNMENT DEGREE COLLEGE (A), YEALLAMANCHILI, ANAKAPALLI DISTRICT, ANDHRA PRADESH.

Abstract

This study was aimed to determine the effect of various types of probiotics in the culture water to the growth and mortality of Vaname shrimp. This study consist of treatment control and treatment of various dose of probiotics. This probiotic consist of *Lactobacillus plantarum, Lactobacillus fermentum, Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Nitrobacter sp*., and *Nitrosomonas sp*. Dependent variables in this study are weight of shrimp, length of shrimp, mortality and feed conversion ratio. The results had different of various dose probiotics application in the water showed significance for each treatment on growth and mortality of Vanname shrimp. The best results were shown in treatment P2 (2 mL/10 water) with mean value of Vaname shrimp weight is 7.447 ± 1.193 g/shrimp, the length is 10,390 ± 0,469 cm/shrimp, mortality is 41%, and the value of FCR is 0.91.

Key words: Probiotics, Penaeus vannamei, Feed conversion ratio, Growth,Lactobacillus.

.

INTRODUCTION.

Aquaculture practices date back to at least the 15th century in India and are often typified by the Tambak, a traditional brackish-water pond system integrating shrimp, milkfish and other finfish [2]. Demand for shrimp is increasing steadily, while the world shrimp fishery is decreasing or remaining static; only aquaculture can meet this demand [3]. In Indonesia, while the shrimp aquaculture industry was being affected by white spot syndrome virus (WSSV), a bacterial pathogen that causes early mortality syndrome, *Vibrio,* appears with devastating consequences [4]. Thus affecting the process of shrimp farming. Such as in China, shrimp production was in decline from 200.000 tons in 1992, had lower to only 55.000 tons in 1994 [5]. This drastic decline is caused by several factors, such as infection of pathogenic bacteria and waste from feed remains that affect growth, mortality, and quality of culture water. Various pathogenic microorganisms live in aquatic environment such as *Vibrio* which commonly attack shrimp larvae at zoea, mysis, and the beginning of post-larvae stadium [6]. Thus it become an obstacle in supplying high number of shrimp hatchling for shrimp production.

According to Verschuere *et al.* [7], probiotic is living microbial agent that is able to give various advantages for its host by modifying microbe community or associating with the host, enhancing nutrition value and food utilization, improving host response to disease, and elevating quality of the environment. Thus, application of probiotic can be a solution to obtain optimal growth and feeding efficiency, lower production cost such as feeding cost, substitute use of antibiotic, and reduce environment burden due to waste accumulation in the water [8]. Several species of probiotic microbes can be used for breaking down organic material from feed remains, including *Bacillus subtilis, Bacillus licheniformis,* and *Bacillus megaterium*. In addition, nitrification bacteria such as *Nitrobacter sp*. and *Nitrosomonas sp*., can also be added to reduce ammonium level. Similarly, lactic acid-producing bacteria, such as *Lactobacillus plantarum* and *Lactobacillus fermentum,* can also be utilized to improve shrimp digestive system and maintain water pH at optimal level.

Application of probiotic cannot be claimed as effective and efficient yet, due to lack of clarity in terms of dose and different application results. Generally, application in laboratory scale is much more controlled compared to field studies due to controlled

environment of aquarium used in laboratory approach that is different from aquaculture ponds and eventually causes differences in probiotic dose, water quality, and aquaculture management.

This study was aimed to determine the effect of probiotic dose variation in aquaculture water on growth, mortality, and Feed Conversion Ratio (FCR) of Vaname shrimp (*Litopenaeus vannamei* Boone).

EXPERIMENTAL METHOD

Time and Place of Study

This study was conducted in two locations: Microbiology Laboratory Dr.B.R.A.U. for probiotic preparation and fish ponds in Sri durga for fish culturing and data collection. This study was conducted for 9 months, starting from November 2023 to July 2024.

Materials

Materials used in this study including Vannamei shrimp hatchlings originated from Vannamei shrimp aquaculture in Srikakulam, Andhra Pradesh India, water, microbial starter culture from Laboratory of Microbiology, Dr.B.R.A.U that consisted of *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Nitrobacter sp., Nitrosomonas sp., Lactobacillus plantarum*, and *Lactobacillus fermentum*. Growth medium used for microbial culture was Nutrient Agar (NA) (Oxoid), 1% yeast extract (YE) (Oxoid), 1% glucose, sterilized distilled water, and molasses; while medium used for Total Plate Count (TPC) were produced from Nutrient Agar (NA) (Oxoid), 1 % glucose, and sterilized distilled water.

Probiotic preparation

A. Medium and isolates preparation. Sub culturing of microbial isolates was conducted on agar slant, consisted of seven bacteria species; *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium, Nitrobacter sp., Nitrosomonas sp., Lactobacillus plantarum,* and *Lactobacillus fermentum*. From each isolate, one *ose* of bacteria culture was inoculated on NA slant in sterile condition and then incubated for 24 hours.
B. Starter preparation. Microbes previously subcultured were taken two or three *oses* to be inoculated into 100 mL YE added with 1 % glucose. Inoculation was conducted in laminar flow cabinet. Culture was homogenized using shaker at 100 rpm for 5-6 h before it was incubated for 24 h at room temperature.
C. Measurement of microbe quantity. Each microbe culture was measured for its OD_{600} = 1 using spectrophotometer before TPC analysis was performed. Pour plate was prepared in petri dish for the culture at 1 mL of microbe culture in ± 15 mL NA added with 1 % glucose. After colony grew in the dish, it was counted using colony counter and data was collected from dilution serial with results of around 30-300 colony.
D. Probiotic preparation. Liquid probiotic was prepared by mixing seven microbe starters in 100 mL YE added with 1 % glucose to obtain 700 mL of mixed starter culture composed of seven bacteria species. Probiotic consisted of 10 % starter and 90 % carrier. The carrier used for probiotic was composed of 3 % molasses and 87 % distilled water. Probiotic was prepared by mixing 700 mL of mixed starter culture with 189 mL molasses and 6111 mL distilled water.

Preparation and culture of Vaname shrimp hatchling

A. Preparation of Vaname shrimp growth medium. Five shrimp ponds were prepared from tanks with volume of 210 L each. An aerator connected to a hose was set in each pond to aerate the water.
B. Spreading of Vaname shrimp hatchling. Healthy hatchlings were selected randomly before spread into the pond in the morning. Hatchling used was PL-12 (aged 12 days) with initial weight of 0.016 g/shrimp and length of 0.7 cm. A basin filled with hatchlings was lowered slowly into the water and the hatchlings were let go into the water of the pond.

Culturing of Vaname shrimp

A. Feeding. Feed used was certified by Directorate General of Fishery and Aquaculture. It was given three times a day in the morning, noon, and afternoon.
B. Measurement of pH of water . Measurement of pH was performed at the start of the study and later on, once a week.

C. Measurement of temperature. Measurement of temperature was performed at the start of the study and later on, once a week.
D. Measurement of salinity. Salinity was measured the start of the study and later on, once a week after probiotic was applied.

A. Mortality of *Vaname* shrimp

Mortality was counted using hand counter and then entered into a formula based on [14] as follows:

$$M = \frac{A}{B} \times 100\% \quad (3)$$

In which:
M = Percentage of mortality
A = Number of dead shrimp during culture period
B = Number of shrimp spread into the pond

B. Feed Conversion Ratio

Feed Conversion Ratio (FCR) of shrimp was quantified using the following formula developed by Effendi [14]:

$$FCR = \frac{\text{Weight of feed given}}{\text{Growth of shrimp weight}} \quad (4)$$

Data analysis

Data of Vannamei shrimp growth were compared and analyzed statistically using SPSS version 21 while mortality and FCR were analyzed descriptively.

Results and discussion

The effect of probiotic application at various doses towards Vannamei shrimp growth was determined based on several parameters such as body weight, length, mortality, and FCR. Based on statistical test,

application of probiotic to Vaname shrimp significantly affected its growth, indicated by significant increase in shrimp weight and length compared to control that was given no probiotic, as presented in table 1.

Table 1. The effect of various probiotic doses applied into aquaculture water towards growth (body weight and length) of Vaname shrimp (*Penaeus vannamei* Boone).

No	Group	Mean weight (g)	Mean length (cm)
1	K (0 mL/10 L water)	5.301 ± 0.321[a]	7.431 ± 0.543[a]
2	P1 (1 mL/10 L water)	6.321 ± 0.201[a]	8.232 ± 0.321[b]
3	P2 (2 mL/10 L water)	7.504 ± 1.181[b]	11.323 ± 0.543[c]
4	P3 (3 mL/10 L water)	7.430 ± 0.483[b]	11.296 ± 0.341[c]
5	P4 (4 mL/10 L water)	5.108 ± 0.542[a]	9.865 ± 0.247[ab]

*Difference of letters following number indicated statistical difference result of Duncan test at α = 0.05

Data of Vaname shrimp mortality was collected by comparing the number of shrimp after 60 days of caring period with initial number of shrimp spread into the ponds. Mortality percentage was compared among groups treated with probiotic at various doses and untreated control. Mortality percentage was quantified using the formula based on [14]. The highest number of living shrimp after 60 days was found from P2 (2 mL/10 L), amounting of 59 shrimps with lowest mortality rate of 41 %; while the lowest number of living shrimp after 60 days was from P4 (4 mL/10 L), amounting of 50 shrimps with the highest mortality rate of 50 %. Effect of probiotic application at various doses on mortality is presented in table 2.

Feed Conversion Ratio (FCR) of shrimp was also quantified using formula based on [17]. FCR was the amount of feed given to the shrimp converted into its unit. it is measured from weight of feed given during caring period and the increase of shrimp weight from the start up to the end of caring period. Lowest FCR was found from P2 (2 mL/10 L) at 1 : 0.91, while highest FCR was K (0 mL/10 L) at 1 : 1.66 (table 2).

Table 2. Mortality percentage of Vaname shrimp (*Litopenaeus vannamei* Boone) and Food Conversion Ratio(FCR) after probiotic application at various doses for 60 days.

No	Group	Starting shrimp number	Shrimp number after 60 days	Mortality (%)	Food Conversion Ratio(FCR)
1	K (0 mL/10 L water)	100	52	50 %	1.65
2	P1 (1 mL/10 L water)	100	55	50 %	1.317
3	P2 (2 mL/10 L water)	100	60	45 %	0.9
4	P3 (3 mL/10 L water)	100	59	47 %	0.976
5	P4 (4 mL/10 L water)	100	51	51 %	1.615

The varying percentage of mortality as presented in table 2 could be caused by environment impact, feed remains, and shrimp aggressiveness. Accumulated feed remains in the water medium could produce ammonium toxic for the shrimp. Stated that the growth of Vaname shrimp was affected by two factors; molting frequency (interval period) and growth. On every molting time, hard layer of carapace was released by the shrimp before it secreted new carapace. When the carapace was still soft, shrimp had higher chance to be preyed upon by other shrimp.

Feed conversion ratio was used in this study to determine the effectiveness of feeding, indicating level of feed remains and also profit of aquaculture. FCR was found to be varying from acrosstreatment groups given different probiotic dose. FCR of P2 (2 mL/10 L) was found to be 1 : 0.91, meaning that for every 1 g of shrimp weight, 0.91 g feed is required (table 2).

Water quality during caring period had important role to support shrimp life and growth. Parameters of water quality were temperature, salinity, and pH or acidity level. Water quality in caring medium could affect growth level, mortality, molting frequency, and number of disadvantageous bacteria. As observed during the caring period of current study, the quality of aquaculture water used was still in the acceptable range for growth and mortality of Vaname shrimp with recorded salinity of 5 ppt while water temperature was at range of 28-32ºC. Based on Haliman and Adijaya [13], fluctuation of temperature during caring period could be affected by the environment. during rainy season, temperature relatively normal, but rain water could affect water pH and salinity. Optimal temperature for Vaname shrimp caring was at a range of 26-31ºC, while optimal pH was 7.5-8.1. Suitable water pH for intensive aquaculture of Vaname shrimp was at pH 7.4-8.9, with the most optimum pH of 8.0 [15]. Fluctuation of water pH during Vaname shrimp caring period was mainly influenced by the environment in the form of rain , which could be predicted to lower pH of culture water. Normal limit of acidity of rain was at pH 5.6, while pure water was produced at pH balances with global CO_2 concentration (330 ppm) in the atmosphere [16]. Activity of BAL microbe could lower pH
periodically and also inhibit the growth of pathogenic bacteria [17].

1. Conclusions

- Treatment of P2 (2 mL/10 L) resulted in the highest value compared with other treatments in Vaname shrimp growth, which has an average weight of Vaname shrimp of 7.4 grams and a shrimp length of 10.40 cm.
- Treatment with P2 (2 mL/10 L) resulted in 59 live Vaname shrimp and a low percentage mortality rate of 41%.
- Treatment P2 (2 mL/10 L) had the lowest feed conversion ratio value with FCR value of 1: 0.91.

2. References

[1] Patrik J G H, Nhuong T, Chadag V M, Chin Y C, U-Primo R, Sharon S, Lara D M, Nur B P U, Stephen H, Michael J P 2017 *J. Cleaner Prod.***17** 3

[2] Troell M 2009 *FAO Fish. Aquac. Tech. Paper* **529** 1-86

[3] Bondad-Reantaso M G, Subasinghe R P, Josupeit H, Cai J and Zhou X 2012 *J. Inver Pathol.* **110** 158-165

[4] Flagel T, Lightner D V, Lo C F and Owens L 2008 *Diseases in Asian Aquac.* **6** 355-378

[5] Sugita H Ahibuya K 1996 *Fish. Aquac.* **145** 195-203

[6] Lavilla-Pitogo CR, Baticados MCL, Cruz-Lacierda ER, De Ia Pena LD. 1990 *J. Aquac.* **91** 1-13

[7] Boyd C E 1990 *Water Quality in Ponds for Aqua Culture* (Auburn University : Alabama Agricultural Experiment Station) 482 p

[8] Raccota I, S E Palacios and A M Ibbara 2003 *J. Aquac.* **227** 107 – 130

[9] Raj P R and Raj P J S 1982 *Proc. Symp. Coastal Aquac.***1** 236–243

[10] Venkataramaiah A, Laksmi G J and Gunter G 1972 *The Effect of Salinity, Temperature and Feeding Levels on the Food Conversion, Growth and Survival Rates of the Shrimp Penaeus aztecus* (Marine Technology Society : Food-Drugs from the Sea Proceedings) p 29–42

[11] Verschuere L, G Robaut, P Sorgeloos and W Verstraete 2000 *Microbiol. Mol. Bio.* **64** 655-671

[12] Iribarren D, P Dagá, M T Moreira, G Feijoo 2012 *Aquac. Int.* **20** 779-789

[13] Haliman R W dan D Adijaya S 2005 *Vaname Shrimp* (Jakarta: Penebar Swadaya)
[14] Effendi I 1997 *Fisheries Biology* (Yogyakarta: Yayasan Pustaka Nusantara)
[15] Wyban J A and Sweeney J N 1991 *Intensive Shrimp Production Technology* (USA: The Oceanic Institute Press)
[16] Seinfeld J H and Pandis S N 1998 *Atmospheric Chemistry and Physics from Air Pollution to Climate Change* (New York: John Wiley and Sons INC)
[17] Markas and De Vyust 2006 *Int.Dairy J.* **16** 1049-1057