Effect of probiotic addition on survival and growth performance of white-leg shrimp (*Litopenaeus vannamei*) in earthen pond

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DOI: 10.29322/IJSRP.10.04.2020.p10054
http://dx.doi.org/10.29322/IJSRP.10.04.2020.p10054

Abstract- Study was conducted to evaluate the effect of probiotic on the survival and growth performance of white-leg shrimp (*Litopenaeus vannamei*) in earthen ponds. Two treatments were carried out including treatment 1 where probiotic was added in every 7 days and a control where probiotic was not added, triplicate for each treatment, shrimps (2.9 cm in length; 0.26 g in weight) were stocked at 50 ind/m^2. After 60-day of culture, the result showed that shrimps in the probiotic added treatemnt were significantly better performance in term of length and weight than without probiotic added treatment. However, the survival and production of shrimp was not significantly higher than the without probiotic added treatment.

Index Terms- white-leg shrimp, Litopenaeus vannamei, probiotic, growth performance, survival, production.

I. INTRODUCTION

Shrimp farming is now increasingly intensive, making the water environment polluted by the use of many feeds and chemicals. At the same time, it affects the quality of shrimp after harvest so the study of using biological agents is a positive trend to contribute to environmental stability and limit disease in ponds through farming models combined with biofloc. According to Avnimelech (2012) the waste treatment system of suspended substances in water containing heterotrophic bacteria predominates, with high potential for use in limiting water exchange and as a food source for shrimp.

Probiotics is used very popular to balance bacteria and increase water quality in ponds. Probiotics can help to reduce toxins in ponds such as NH_3. NO_2^-. H_2S ... Improving water color, balancing ecosystems, decomposing organic substances, preventing algae blooms and absorption of the nurient from decomposed algae, helping shrimp absorb good feed, reducing feed consumption, stimulating immune system and disease resistance (Farzanfar, 2006; Hari *et al*., 2006; Adel *et al*., 2017). Application of probiotics is limit use of chemicals and antibiotics in aquaculture. Probiotics have advantages over chemicals and antibiotics in that minimizing toxins that are harmful to shrimp as well as affecting shrimp quality in the future. But now, most shrimp farmers use probiotics unreasonable, only applied when the pond encounters environmental problems or shrimp health, without the need to periodically fertilize to create the initial erosion in overwhelming the pathogen. In addition, the improvement of immune, survival and production of shrimp by probiotics addition has been demonstrated under laboratory conditions in many previous studies (Garriques and Arevalo, 1995; Vaseeharan and Ramasamy, 2003; Hari *et al*., 2004). However, probiotics in the market is vary in quality. So that, the present study was tested the effect of local probiotics on shrimp performance rearing under earthen pond condition.

II. MATERIALS AND METHOD

2.1 Experimental design

The study was arranged with two treatments (T) including the treatment with probiotics addition and other without addition of probiotic (control treatment). triplicate for each. Shrimp seeds were reared in earthen ponds at 50 ind./m^2 in water salinity of 30 ‰. Probiotic powder including *Lactobacillus* sp. 10^8 CFU/g. *Bacillus subtilis* 10^8 CFU /g. *Saccharomyces cerevisiae* 10^8 CFU/g. *Nitrobacter* sp. 10^7 CFU/g. *Nitrosomonas* sp. 10^7 CFU/g và Enzyme of *Aspergillus oryzae* was added to the culture at 10 g/m^3 in the every 7 days. Shrimps were reared for 60 days and no water exchange was applied.

Management

A commercial feed (Grobest; 40% protein) was provided for shrimp at 2-3 times/day by 5-7% of shrimp's body weight.

Sample collection and analysis

Temparature, pH of culture water was measured in every day at 7:00 and 14:00. Total ammonium nitrogen (TAN), nitrite (NO_2^-), alkalinity was measured in every 7 days by test-kit (Sera. Gemany)

Individual length and weight of shrimp was measured in every 15 days, 30 shrimps from each treatment was randomly sampled and measured for body length and determined individual length by analystical balance (Sartorius. 0.00 g), then shrimps were put back to the ponds when the data was completely obtained. Afterwards, growth performance data of experimental shrimp consisting of daily weight gain (DWG). specific growth rate (SGR) and survival; was calculated using the following equations:

Weight gain (g) = final weight - initial weight

Daily weight gain (DWG; g/day) = $\frac{final\ weight - initial\ weight}{culured\ days}$

Specific growth rate (SGR; %/day)= $\frac{100(Ln\ final\ weight - Ln\ initial\ weight)}{cultured\ days}$

FCR = feed provided (dry weight)/weight gain (wet weight)

Survival of shrimp was determined at the end of experimental period.

Survival (%) = $100\ x\ \frac{final\ number\ of\ shrimps}{initial\ number\ of\ shrimps}$

2.2 Statistical analysis

Dataset of each treatment was calculated to get mean and standard deviation by Microsoft Excel software and statistically processed one-way ANOVA factor and the Tukey-HSD test by Statistica 7.0.

III. RESULTS AND DISCUSSION

The average temperature in the morning and afternoon was much different in whole experimental period. The average temperature in the morning ranged from 27.2 to 27.6 and the afternoon in the range of 30.6 - 31 ℃. pH level was not widely fluctuated, the morning pH of the treatments was 7.9, for the afternoon pH ranged from 8.3 to 8.4. The temperature for suitable growth of shrimps is reccomendated from 25 - 32 ℃, pH is from 7.5 to 8.5 (Boyd, 2002). Thus, the temperature and pH values of the current experiments are within the appropriate limits for whiteleg shrimp growth.

Table 1. Physical parameters

Treatment	Temperature (℃)		pH		TAN (mg/L)	NO_2^- (mg/L)
	7:00	14:00	7:00	14:00		
T1	27.2±0.6	31.0±1.2	7.9±0.3	8.3±0.2	0.14±0.29	0.14±0.61
T2	27.6±1.1	30.6±1.4	7.9±0.4	8.4±0.2	0.10±0.24	0.06±0.21

TAN concentration was in range 0.10 – 0.14 mg/L and NO_2^- was 0.06 - 0.14 mg/L. In probiotic added treatments, the NO_2^- concentration was 0.04 mg/L, it was lower than in the control treatment (0.08 mg/L). The appropriate TAN concentration for shrimp's growth is less than 2 mg /L (Chanratchakool, 2003) and NO_2^- is less than 4.5 mg/L (Chen and Chin. 1988). Thus, the TAN and NO_2^- in current study was within the allowed range for shrimp. The results of alkalinity was recorded from 116 to 126 mg/L, this level is suitable range for shrimp beacause the alkality level for shrimp is reccommendated from 80 to 200 mg$CaCO_3$/L (Ching, 2007).

An average initial length of shrimp was 2.9 cm/ind. The size of shrimp increased through experimental period. The length of shrimp with 7.6 cm/individual on 30th day was recorded in probiotics added treatment, but there was significantly higher than the control (Table 2). However, the individual length of shrimp on 45th day to the day end of experimental peeriod, the size of shrimp in probiotics a dded treatment was significantly better than the control ($p < 0.05$).

Table 2. Individual length (cm), weight (g) and production (ton)

Biological parameters	T1	T2
Length of shrimp on 30th day	7.1 ± 0.8^a	7.6 ± 0.7^a
Length of shrimp on 45th day	8.3 ± 0.8^a	9.2 ± 0.9^b
Length of shrimp on 60th day	9.3 ± 0.97^a	10.3 ± 0.91^b
Weight of shrimp on 30th day	3.2 ± 1.1^a	3.8 ± 1.0^a
Weight of shrimp on 45th day	5.1 ± 1.5^a	6.0 ± 1.9^b
Weight of shrimp on 60th day	6.8 ± 2.0^a	8.2 ± 2.0^b
DWG (%/ngày)	0.11 ± 0.02^a	0.13 ± 0.02^b
SGR (g/ngày)	5.43 ± 0.18^a	5.57 ± 0.15^b
Survival (%)	96.4 ±3.3^a	98.4 ± 2.1^a
FCR	1.2±0.1^a	1.1±0.1^a
Production (ton/hecta)	3.758±213^a	4.350±304^a

Figure in the same line having the same letters are not significantly different ($p>0.05$); SGR: specific growth rate; DWG: daily weigth gain; Food conversion ratio: FCR

The initial weight was 0.26 g/individual. The results showed that the shrimp in the probiotic supplementation treatments were better growth than the control treatments for whole experimental period (Table 2). Ziaei-Nejad (2006) reported that the presence of useful microorganisms can stimulate endogenous enzymes produced by shrimp that to increase the activity of digestive enzymes in shrimp digestve tract, and lead to increase food absorption. It therefore contributes to improved survival growth and FCR reduction.

The daily weight gain (0.13 g/individual) and the specific growth rate (5.57 %/day) was the highest in T2 (supplemented with probiotics) which was statistically significant ($p<0.05$) compared to the control. Because the presnce of probiotics in ponds helps to reduce harmful factors like NH_3, H_2S… In order to

improve the living environment for shrimp to maintain the density of organic decomposing bacteria.

Survival of shrimp after 60 days of culture between treatments ranged from 98% - 99% of which in the probiotic supplementation treatment reached 99% higher than the control treatment, but there was no statistically significant ($p > 0.05$) between the two treatments.

Feed conversion ratio (FCR) of shrimp in the probiotic supplemented treatment was lower than the control treatments because the shrimp in the probiotic supplementation had better growth than the shrimp in the control treatment. should have led to a low feed coefficient. according to Toi et al. (2013), probiotics are a source of digestive enzyme, this enzyme also with the host enzyme will help digest food better, thus leading to better shrimp growth of target animal.

Due to the higher growth and survival rate of the probiotic supplement than the control treatment, this resulted in a higher shrimp yield, as well. Shrimps fed with probiotic supplements experienced growth in length. The weight and survival rate was higher than the control treatment. Probiotic-treated shrimp have lower feed conversion ratios and higher yields than non -probiotic reared shrimp. The results of this study indicated that probiotic for shrimp culture is recommended to achieve the best yield results.

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