The effects of various doses of probiotics on growth and survival rates of white shrimp larva (*Litopenaeus vannamei*)

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Abstract. The purposes of this research is to know the effective dose of probiotics that can optimize the growth of postlarva (PL15) white shrimp (*Litopenaeus vannamei*). This research was conducted in January 2020 in Kasetsart University, Bangkok, Thailand for seven days using completely randomized design (CRD) research method with 4 treatments and 6 replications. Commercial probiotic powder application with *Bacillus* content (10⁸ CFU / g) is given consisting of treatments are C (0 mg/L), P1 (1 mg/L), P2 (3 mg/L) and P3 (5 mg/L). A one way analysis of variance (ANOVA) was applied to compare the control and treated groups at a significance level of P<0.05. Duncan’s New Multiple Range Test (DMRT) was used to identify significant differences of growth, survival, and feed efficiency parameters. The results show that the highest SGR, DGR, absolute weight growth, absolute length growth, SR, FCR, and efficiency of feed utilization average respectively 22.76%, 4.32 mg/day, 11.20 mg, 7,68 mm, 77%, 1.78, 0.56. However, all parameters of shrimp growth, survival, and feed efficiency are not significantly different.

1. Introduction
White shrimp (*Litopenaeus vannamei*) is a valuable export commodity high in the fisheries sector. Consumer demand for shrimp rises on average 11.5 percent every year. Although there are still many obstacles, but until now this shrimp producing country is a new competitor with Indonesia shrimp exports continue to emerge. At present white shrimp is still a main commodity in aquaculture ponds. Apart from variety the problem in the cultivation business is the failure to grow in ponds, but until now white shrimp commodity is still the main choice to be cultivated by farmers. This is due white shrimp has a good and relatively stable market price [1].

The rapid development of aquaculture activities with the application of intensive systems has raised problems in the form of decline pond carrying capacity for the life of fish / shrimp that are cultivated. Impact further inflicted was a series of disease attacks cause huge losses. Anticipatory steps through the application of technology cultivation based on the principle of ecosystem balance is a solution to prevent more serious damage. In between these steps is through probiotic applications that have proficiency in maintain water quality and inhibit the growth of microorganisms pathogens in order to create a sustainable aquaculture system [2].

We know that the main purpose of cultivation is to produce optimal commodity, this can be
obtained through application probiotics with the right dosage. Not all organisms are able to grow and develop optimally with the same probiotic dosage, and therefore it is necessary to do research on the right dosage in the enlargement process of white shrimp (Litopenaeus vannamei).

2. Materials and methods

2.1 Experimental Design

The research was conducted for seven days using completely randomized design (CRD) research method with four treatments and six replications. Each bottle contained postlarva (PL15) white shrimp with a stocking density of five shrimp / 750 ml. Commercial probiotic powder application with Bacillus content \((10^8\text{ CFU / g})\) is given every four days in each bottle containing 750 ml of brackish water (20 ppt) containing white shrimp with the appropriate concentration of treatment is

- \(P_1 = 1\text{ mg / L}\)
- \(P_2 = 3\text{ mg / L}\)
- \(P_3 = 5\text{ mg / L}\)
- \(C = \text{control (0 mg / L)}\)

2.2 Media Preparation

Each bottle has been sterilized with 70% methanol and filled with 750 ml of brackish water (20 ppt) with an aerator connected by a hose to aeration each bottle. Each bottle was labeled according to its concentrations of probiotic.

2.3 Probiotic Administration and Cultivation

White shrimp (PL15) with an average weight of 8 mg were acclimated in an aquarium containing 20 ppt brackish water equipped with continual aeration for 24 hours, it can increased shrimp survival by 27\% [3]. Shrimp will be fed a commercial diet twice a day as much as 3\% of body weight (6 am and 6 pm) [4]. 50\% of the water has been changed in every treatment on the fourth day of the experiment. Commercial probiotic powder \((10^8\text{ CFU/g})\) was applied at the beginning of the research, which is after the shrimp were stocked. Probiotic also given on the fourth day, which is after the water substitution was carried out. The probiotic dose was applied according to the treatment in this research.

2.4 Measurement of Shrimp Growth and Survival

Experimental shrimp in the control and treatments groups were measured for length, weight, growth rate and survival rate. Length and weight were measured for all shrimps from each bottle on a vernier caliper and digital balance, respectively. Shrimp growth and survival rate were calculated according to Nimrat et al. (2011) [5]. The shrimp growth calculated based on the SGR and DGR formulas.

Specific Growth Rate (SGR) is calculated using the formula [5]:

\[
SGR = \frac{\ln Wt - \ln Wo}{t} \times 100\%
\]

\(SGR\) = Specific Growth Rate (\%),
\(\ln Wt\) = \(\ln\) final body weight (g),
\(\ln Wo\) = \(\ln\) initial body weight (g),
\(t\) = feeding time (day)

Daily Growth Rate (DGR) is calculated using the formula [5]:

\[
DGR = \frac{Wt - Wo}{t}
\]

\(DGR\) = Daily Gain Rate (g / day),
\(Wo\) = initial body weight (g),
\(Wt\) = body weight (g),
\(t\) = time of calculation (days)
2.5 **Measurement of Feed Efficiency**

Effectiveness of feed utilization can be calculated using formula \( \frac{1}{FCR} \). Calculation for feed utilization parameters was as follows [6]:

\[
\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}
\] (3)

2.6 **Water Quality Analysis**

Water quality monitoring is carried out on the first, fourth, and end of the research at 17.00. pH and dissolved oxygen were monitored using a regularly calibrated Multi-Probe Model (Horiba, W-22XD, Kyoto, Japan). Temperature and salinity were measured by thermometer and salinometer (Atago, S/Mill, Kyoto, Japan) respectively. Ammonia-nitrogen was measured also along with nitrate-nitrogen and nitrite-nitrogen [7,8,9].

2.7 **Statistical Analysis**

Data were expressed as mean standard deviation. A one way analysis of variance (ANOVA) was applied to compare the control and treated groups at a significance level of \( P<0.05 \). Duncan’s New Multiple Range Test (DMRT) was used to identify significant differences of growth, survival, and feed efficiency parameters in the ANOVA [10]. All statistics were performed using SPSS for Windows version 11.5 (SPSS, Chicago, USA).

3. **Result and discussion**

3.1 **Shrimp Growth and Survival**

There were no difference (\( P>0.05 \)) in SGR, DGR, absolute weight growth and absolute length growth. The highest average of SGR was in the P1 (1 mg / L) which reached 22.76%, while the lowest average of SGR was in the control (0 mg / L) which reached 14.66%. The highest average of DGR was in the P1 (1 mg / L) which reached 4.32 mg/day, while the lowest average of DGR was in the control (0 mg / L) which reached 2.07 mg/day. The highest average of absolute weight in the P1 (1 mg / L) which reached 11.20 mg, while the lowest average of absolute weight was in the control (0 mg / L) which was 5.40 mg. The highest average of absolute length was in the P1 (1 mg / L), which reached 7.68 mm, while the lowest average of absolute length was P2 (3 mg / L) of 6.94 mm (Table 1).

| Treatments | SGR (%) | DGR (mg/day) | Absolute Weight Growth (mg) | Absolute Length Growth (mm) |
|------------|---------|--------------|-----------------------------|-----------------------------|
| CONTROL    | 14.66   | 2.07         | 5.40                        | 7.63                        |
| 1 mg/L     | 22.76   | 4.32         | 11.20                       | 7.68                        |
| 3 mg/L     | 18.48   | 2.74         | 7.10                        | 6.94                        |
| 5 mg/L     | 20.89   | 3.34         | 8.70                        | 7.64                        |

There were no difference (\( P>0.05 \)) in survival rate among treatment groups, however the average of survival rate in the P1 (1 mg / L) reached 77%, while the lowest average of survival rate was in the P2 (3 mg / L) and P3 (5 mg / L) which were both 43% (Table 2).

| Treatments | SR (%) |
|------------|--------|
| CONTROL    | 53%    |
| 1 mg/L     | 77%    |
| 3 mg/L     | 43%    |
| 5 mg/L     | 43%    |
3.2 Feed Efficiency
There were no difference (P>0.05) in FCR and efficiency of feed utilization, however the average of FCR in the control (0 mg / L) reached 3.71, while the lowest average of FCR was in the P1 (1 mg / L) which reached 1.78. The average of efficient use of feed in the P1 (1 mg / L) reached 0.561, while the lowest average of efficient use of feed was in the control (0 mg / L) reached 0.269 (table 3).

| Treatments | FCR  | Efficiency of Feed Utilization |
|------------|------|------------------------------|
| CONTROL    | 3.71 | 0.27                         |
| 1 mg/L     | 1.78 | 0.56                         |
| 3 mg/L     | 2.80 | 0.36                         |
| 5 mg/L     | 2.30 | 0.43                         |

3.3 Water Quality
The range of pH obtained during maintenance is in accordance with values 7.12 - 8.40. The temperature range is 28.97°C - 30.20°C. The dissolved oxygen content (DO) is in the range of 4.05 - 5.10 mg / L. The salinity range is at 20-23.70 ppt. The ammonia content is in the range of 0.25 – 5.00 mg/L. Nitrite content during the research were in the range of <0.30 - 0.8 mg/L (table 4).

| Treatments | pH     | DO (mg/L) | Salinity (ppt) | Temperature (°C) | Ammonia (mg/L) | Nitrite (mg/L) |
|------------|--------|-----------|----------------|------------------|----------------|----------------|
| CONTROL    | Day 1  | 7.82      | 4.80           | 20.00            | 29.60          | 0.25           | <0.30          |
|            | Day 4  | 7.50      | 5.10           | 23.30            | 29.00          | 3.00           | 0.30           |
|            | Day 7  | 8.35      | 4.15           | 23.30            | 29.90          | 5.00           | 0.30           |
| 1 mg/L     | Day 1  | 7.82      | 4.80           | 20.00            | 29.60          | 0.25           | <0.30          |
|            | Day 4  | 7.57      | 5.00           | 22.80            | 29.47          | 3.00           | 0.30           |
|            | Day 7  | 8.34      | 4.20           | 22.80            | 30.20          | 5.00           | 0.30           |
| 3 mg/L     | Day 1  | 7.82      | 4.80           | 20.00            | 29.60          | 0.25           | <0.30          |
|            | Day 4  | 7.12      | 4.90           | 23.67            | 28.97          | 3.00           | 0.30           |
|            | Day 7  | 8.38      | 4.30           | 23.70            | 29.15          | 3.00           | 0.30           |
| 5 mg/L     | Day 1  | 7.82      | 4.80           | 20.00            | 29.60          | 0.25           | <0.30          |
|            | Day 4  | 7.59      | 5.05           | 20.80            | 29.08          | 3.00           | 0.30           |
|            | Day 7  | 8.40      | 4.05           | 20.80            | 29.50          | 3.00           | 0.80           |

3.4 Discussion
Provision of probiotics with the right concentration has benefits for host animals by balancing the microbiological conditions of the host, modifying the form of association with the host or environmental microbial community, increasing the utilization of feed nutrition, increasing the host's immune response to pathogens, and improving the quality of the environment [11,12,13].

Weight is greatly influenced by feed consumption, because feed consumption determines the input of nutrients into the body and used for growth and other purposes. Lactobacillus group is able to increase the appetite of shrimp so as to optimize the utilization of feed nutrition with the help of Bacillus which is proteolytic. Probiotic microbes being able for balancing the microbiological conditions of the host and modifying the form of association with the host or environmental microbial community [14]. The provision of probiotics in feed with the right concentration can reduce white shrimp mortality. The addition of probiotic bacteria to shrimp rearing containers can function to suppress the population of pathogenic bacteria that cause shrimp death [11]. The use of probiotic bacteria was able to reduce the death of shrimp postlarva through controlling the population of Vibrio sp [15]. However, the results from this research shown that all parameters of shrimp growth, survival,
and feed efficiency are not significant (P>0.05). Treatment of *Litopenaeus vannamei* with a commercial *Bacillus* probiotic did not significantly improve neither survival nor growth, this is supported by Shariff et al. (2001) and McIntosh et al. (2000) [16, 17]. based on research conducted by Srinivasa et al. (2000) explained that bacteria are regularly abundant in aquaculture environments and play an important role in many geochemical cycles and in the health of aquatic animals. Similar culturable bacterial counts in hepatopancreas, intestine and water samples between treated and control groups indicated that the addition of mixed *Bacillus* probiotic bacteria did not affect culturable bacterial number [18].

Ammonia control in waters can increase survival rate, administration of probiotics containing *Nitrobacter* and *Nitrosomonas* helps control the levels of ammonia and organic matter in the culture media. *Nitrosomonas* plays a role in the oxidation process of ammonia to nitrite. Furthermore nitrite is converted into nitrate which is not harmful to shrimp with the help of *Nitrobacter* [14].

Fluctuation of pH with this value is still feasible for white shrimp cultivation as, the optimum pH for white shrimp cultivation is 7.3 - 8.5 with a tolerance of 6.5 - 9.0 [19]. Such temperature range is still suitable for white shrimp life because according to the proper temperature for white shrimp cultivation is 28 - 31 °C with a tolerance of 16 - 36 °C [20].

The results showed that there was a decrease in dissolved oxygen content on the last day with the lowest concentration of 4.05 mg / L. Even so, the range of dissolved oxygen content is still suitable for white shrimp cultivation with a value of > 4 mg / L tolerance of 0.8 mg / L and > 3 with a tolerance of 2 mg / L. The decrease in oxygen content at the end of the research was caused by the available oxygen besides being used by aquaculture shrimp, it was also used by microbial decomposers that needed oxygen to decompose organic materials, the amount of which increased with the length of time of maintenance or cultivation. The increase in the amount of organic matter is not only due to the addition of the amount of feces as a result of metabolism of cultured shrimp as well as the result of accumulation of feed that is not utilized optimally [19].

Salinity ranges are still very suitable for shrimp farming. The range of ammonia obtained causes damage to the range shrimp and juveniles. This is proven by the existence of dead shrimp with damaged body condition. Ammonia content, one of which is derived from the high level of feed waste in the waters caused by inaccurate method in the application of feed. Because of miscalculation in feeding as much as 3% of the total biomass, causing a high content of ammonia in water.

The range of nitrite obtained was relatively high for white shrimp, the optimum range of nitrite in white shrimp ponds was 0.01 - 0.05 ppm [21]. The nitrite content in this study was high, this is usually caused by high ammonia content [20]. The addition of probiotics with a dose of 5 mg / L has a higher nitrite content because at that dose the content of bacteria decomposes more, so that the nitrification process that produces nitrates occurs more quickly [22].

4. Conclusion

The use of probiotic bacteria was able to reduce the death of shrimp postlarva through controlling the population of *Vibrio* sp. However, the treatment of *Litopenaeus vannamei* with a commercial *Bacillus* probiotic did not significantly improve all parameters of shrimp growth, survival, and feed efficiency. One of the factors is because of miscalculation in feeding as much as 3% of the total biomass causing a high content of ammonia in water.

5. References

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