Effect of probiotic culture water on growth, mortality, and feed conversion ratio of Vaname shrimp (*Litopenaeus vannamei* Boone)

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Abstract. This study was aimed to determine the effect of various dose 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. Control (0 mL/10 L water), P1 (1 mL/10 L water), P2 (2 mL/10 L water), P3 (3 mL/10 L water) and P4 (4 mL/10 L water) treatment, given to the Vaname shrimps with intervals once per week. 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 Vaname 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.

1. Introduction
The Republic of Indonesia is nation set on an archipelago of islands and populated by some of the most frequent fish consumers in the world [1]. Aquaculture practices date back to at least the 15th century in Indonesia 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.
In addition, the decline of shrimp production was also caused by contamination of organic materials in the aquaculture water. These materials originated from uneaten feed, dead plankton, fertilizer, and accumulated shrimp feces at the base of the pond [7]. Accumulation of organic matters at high level will raise the anaerobic decomposition rate, resulting in various toxic compounds, such as ammonium. Anaerobic condition at the base of the pond will also lower shrimp appetite and make it susceptible to disease. Level of ammonium can affect pH of the water; if ammonium level rises, pH will also elevate [8]. According to Raj and Raj [9], salinity is one of the environment factors that plays an important role on growth and survival of shrimp in facing rapid change of salinity and extreme condition of the environment. Feed consumption and efficient feed conversion are main components of growth and survival of Vaname shrimp that are affected by salinity and temperature [10].

According to Verschuere et al. [11], 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 [12]. 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).

2. Methodology
2.1. Time and Place of Study
This study was conducted in two locations: Microbiology Laboratory Airlangga University for probiotic preparation and fish ponds in Sidoarjo for fish culturing and data collection. This study was conducted for 9 months, starting from September 2015 to May 2016.

2.2. Materials
Materials used in this study including Vaname shrimp hatchlings originated from Vaname shrimp aquaculture in Lamongan, Indonesia, water, microbial starter culture from Laboratory of Microbiology, Airlangga University 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.

2.3. Design of Study
This study was designed as experimental study using Completely Randomized Design. Probiotic was applied into aquaculture water every week with 5 varying doses; 1 mL/10 L (P1), 2 mL/10 L (P2), 3 mL/10 L (P3), 4 mL/10 L (P4), and a control treatment of 0 mL/10 L water (K). Each treatment consisted of 6 replications and for each replication, 5 shrimp samples were collected.
2.3.1. Variables of study

Three types of variables in this study could be elaborated as follows:

A. Independent variable : Dose of probiotic (mL/10 L water)
B. Dependent variable : Growth of shrimp including body length (cm), weight (g), and mortality of Vaname (percentage of dead shrimp).
C. Controlled variable : Variety of shrimp hatchling, shrimp age (days), OD$$_{600}$$=1 of microbes in the probiotic.

2.4. Methods

2.4.1. 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 oases 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.

2.4.2. 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.

2.4.3. 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 after probiotic was applied.

D. Measurement of salinity. Salinity was measured the start of the study and later on, once a week after probiotic was applied.
2.4.4. Vaname shrimp harvest
Vaname shrimp was ready for harvest after 60 days. Growth of shrimp was measured, including body length and weight, and so was shrimp mortality. Shrimps were collected in net and then put into collection tank for data collection.

2.4.5. Data collection
A. Measurement of Vaname shrimp growth
   Following [13], formula used for quantifying growth rate (GR) was as follows:

   $$\text{GR} = \text{Wt} - \text{W}_0$$  \hspace{1cm} (1)

   In which:
   \(\text{GR}\) = growth rate of weight (g)
   \(\text{Wt}\) = Mean weight of shrimp at the end of caring period (g)
   \(\text{W}_0\) = Mean weight of shrimp at the start of caring period (g)

   Growth of length was quantified using formula based on Effendi [14], as following:

   $$\text{P} = \text{Pt} - \text{P}_0$$  \hspace{1cm} (2)

   In which:
   \(\text{P}\) = Absolute growth of shrimp (cm)
   \(\text{Pt}\) = Length of shrimp at the end of culture period (cm)
   \(\text{P}_0\) = Length of shrimp at the beginning of culture period (cm)

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

   $$\text{M} = \frac{\text{A}}{\text{B}} \times 100\%$$  \hspace{1cm} (3)

   In which:
   \(\text{M}\) = Percentage of mortality
   \(\text{A}\) = Number of dead shrimp during culture period
   \(\text{B}\) = Number of shrimp spread into the pond

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

   $$\text{FCR} = \frac{\text{Weight of feed given}}{\text{Growth of shrimp weight}}$$  \hspace{1cm} (4)

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

3. Results and discussion
The effect of probiotic application at various doses towards Vaname 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 (*Litopenaeus vannamei* Boone).

| No | Group | Mean weight (g) | Mean length (cm) |
|----|-------|----------------|-----------------|
| 1  | K (0 mL/10 L water) | $4.701 \pm 0.341^a$ | $8.450 \pm 0.688^c$ |
| 2  | P1 (1 mL/10 L water) | $5.467 \pm 0.305^a$ | $9.357 \pm 0.395^b$ |
| 3  | P2 (2 mL/10 L water) | $7.447 \pm 1.193^b$ | $10.390 \pm 0.469^c$ |
| 4  | P3 (3 mL/10 L water) | $7.204 \pm 0.498^b$ | $10.300 \pm 0.302^c$ |
| 5  | P4 (4 mL/10 L water) | $5.277 \pm 0.607^a$ | $8.914 \pm 0.322^b$ |

*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                   | 51                         | 49 %          | 1.66                      |
| 2  | P1 (1 mL/10 L water) | 100                   | 56                         | 44 %          | 1.307                     |
| 3  | P2 (2 mL/10 L water) | 100                   | 59                         | 41 %          | 0.9                       |
| 4  | P3 (3 mL/10 L water) | 100                   | 57                         | 43 %          | 0.974                     |
| 5  | P4 (4 mL/10 L water) | 100                   | 50                         | 50 %          | 1.516                     |

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 across
treatment 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 28-32°C. 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 26-31°C, 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 CO2 concentration (330 ppm) in the atmosphere [16]. Activity of BAL microbe could lower pH periodically and also inhibit the growth of pathogenic bacteria [17].

4. 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.

5. References

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