Vannamei Shrimp (*Litopenaeus vannamei*, Boone, 1931) Performance with *Bacillus* and *Lactobacillus* Probiotic Formulation Consortium in Dry Preparation

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Abstract

The use of probiotics in shrimp farming is one of the efforts to control diseases, to improve immune responses, to improve water quality and to contribute to host nutritional and enzymatic digestion. Probiotic preparations in the feed will be more effective when they are encapsulated in a dry preparation due to its longer durability and efficient storage. This study aims to determine the effect of dried probiotic preparation provision of Bacillus and Lactobacillus consortium in skim milk carrier material on vannamei shrimp performance. This is a descriptive experimental study through the application of four different formulations of dried probiotic preparations in vanamei shrimp fish feed. The bacterium used is the Bacillus group consisted of Bacillus licheniformis, Bacillus polymyxa and Bacillus subtilis and Lactobacillus group consisting of Lactobacillus bulgaricus and Lactobacillus curvatus. Tests were carried out on shrimp larvae vannamei PL12 size for two weeks with a stocking density of 20 individuals/L.

The probiotic treatment was performed by mixing the commercial diets with encapsulated probiotic with a ratio of 2g/kg of commercial feed. Feed was added with ad-libitum, three times a day. Parameters tested were Daily Growth Rate (DGR), survival and Feed Conversion Ratio (FCR). The results showed that administration of Bacillus licheniformis, Bacillus polymyxa, Bacillus subtilis, Lactobacillus bulgaricus and Lactobacillus curvatus probiotic consortium presented the highest daily growth rate of 0.16%. The survival of shrimp larvae with Lactobacillus probiotic consortium reached 92.5% and the feed conversion value (FCR) of vannamei shrimp larvae was 1.46.

Keywords: *Bacillus, Lactobacillus*, probiotics, skim milk, vannamei shrimp performance

Introduction

Vannamei shrimp (*Litopenaeus vannamei*) is one of the shrimp varieties that are currently widely cultivated due to several advantages including among others, fast growth, ability to be cultivated in high density and relatively high market price[1]. Factors that influence the success of vannamei cultivation are fish health, good water environment and quality feed. Probiotic use is currently a popular innovation in aquaculture because the microbes contained in the probiotics have the ability to give advantages to the host by modifying microbe community or through association with the host, by improving nutritional values and feed use, by improving the host response to diseases and by improving the environment quality[2].

The application of probiotics is to provide a biocontrol agent for reducing disease attacks, to improve environment quality through bio-remediation as well as to increase the nutrition value of feed and nutrient absorption rate to enable optimum growth. A higher feed nutrient absorption rate is expected to reduce the cannibalism trait of shrimps that may lead to death during cultivation and affect the survival rate[3].

Dried probiotic preparation using spray drying method is one of the preparations that aims to improve the microbe contact opportunity with the shrimp digestive tract. Probiotic bacterial application in feed nutrition enhancement can be done through natural feed enhancement and artificial feed enhancement[3]. Encapsulation is a process of packing probiotic bacteria as the main ingredient using certain encapsulation materials that will maintain the viability and protect the probiotics from damages due to unfavorable environmental conditions[4].

Sensitive components such as microorganism and encapsulation will increase the viability and durability[5]. A study suggested that skim milk is the best material for dried probiotic preparation[6]. The best result shows that skim milk is able to maintain the viability of *Saccharomyces cereviceae* for 4 weeks at 60^{o}C with a bacterial count of 6.67×10^{13}, *Bacillus mycoides* in pH 2 with a bacterial count of 4.72×10^{10}, *L. bulgaricus* in a bile salt concentration of 5% with a bacterial count of 3.558×10^{10} and produce a clear zone against *Vibrio harveyi* of 20.7 for *B. polymixa*.

This study aims to understand the effect of dried probiotic preparation of *Bacillus* and *Lactobacillus* consortium in skim milk carrier on the performance of vannamei shrimp. This study is useful for providing information regarding the application of *Bacillus* and *Lactobacillus* probiotics in vannamei shrimp feed and its effect on the productivity of shrimp cultivation.

Material and Methods

Materials and Tools: The tools used in this study were: aerator, laboratory glassware, stationeries, aluminum foil, autoclave, fiber container, Bunsen burner, BUCHI mini spray dryier, heater, incubator, laminar, refrigerator, micropipette, analytical scale, inoculation needle, oven, pH meter, PVC pipe, plastic wrap, tube rack, Scanning Electron Microscope (SEM), centrifuge, stirrer, jar, tube stopper, water bath and vortex.

Materials used in this study were: sea water, 70% alcohol, distilled water, peptone buffer, *Bacillus licheniformis* isolates, *Bacillus polymyxa* isolates, *Bacillus subtilis* isolates, *Lactobacillus bulgaricus* isolates, *Lactobacillus curvatus* isolates, *Saccharomyces cerevisiae* isolates, physiological salt solution, 1 N sodium hydroxide solution, vannamei shrimp postlarva 12 (PL-12), Nutrient Agar (NA) medium, Nutrient Broth (NB) medium, vannamei shrimp commercial shrimp, Phosphate Buffer Saline (PBS) and egg whites.

Methods; The method used in this study was a descriptive experimental method in laboratory scale. Four treatments were given for vannamei shrimp larva feed testing:

P_1 : Commercial feed
P_2 : Commercial feed + *Bacillus* consortium in the best carrier material formula
P_3 : Commercial feed + *Lactobacillus* consortium in the best carrier material formula
P_4 : Commercial feed + *Bacillus* and *Lactobacillus* consortium in the best carrier material formula

Data collection for the growth rate data was performed on the first day of the vannamei shrimp larva cultivation and at the end of the cultivation. Data collected were analyzed descriptively.

Procedures: This study consisted of two stages: 1) bacterial biomass production for *Bacillus* and *Lactobacillus* in dry preparation with skim milk as the coating material and 2) probiotic testing on vannamei shrimp larvae.

Probiotic Bacterial Biomass Dry Preparation Production: The probiotic biomass production was conducted by re-cultivating probiotics that had been cultivated in MRS agar slants on liquid MRS media for 24 hours at 37ºC which were then used as a culture intermediary. Ten milliliters of culture intermediary were cultivated on 1,000 liquid MRS (1:100) which was used for biomass production. After that, the biomass was harvested using centrifugation (5,000xg) for 10 minutes at 4ºC and washed twice using phosphate buffer[7]. The probiotic suspension was gained by re-cultivating the probiotics in sterile 10% liquid carrier material media for 24 hours at 37ºC, which was then used as culture intermediary. As much as 2.5 mL of culture intermediary was put into 250 mL of sterile 10% carrier material solution (b/v). This was incubated for 24 hours at 37ºC.

The probiotic culture that was used before encapsulation was in the form of biomass and suspension. The biomass gained was re-suspended into sterile distilled water and was encapsulated using skim milk. The ratio of biomass and encapsulation material used was 3:7 (b/b)[8]. The mixture was homogenized and then dried using BUCHI mini spray dryer with an inlet temperature of 100ºC and outlet temperature of 50ºC. The encapsulated probiotics (microcapsules) were kept in a sterile bottle at 4ºC.

Probiotic testing of vannamei shrimp larvae: The shrimp larvae of postlarva-12 (PL-12) were cultivated in a 3L jar containing 2L of sea water. The jars were arranged in the fiber container and each jar was aerated. The number of shrimp kept in the jar was 40 (20 shrimps/L). The fiber container used to hold the jars was filled with fresh water up to the cultivation media surface height. A heater was used to maintain stable media temperature of 28 – 31.5ºC during cultivation[9].

Probiotic bacteria were given by mixing commercial feed with encapsulated probiotic bacteria with a ratio of 2 g/Kg of commercial feed used. Feed was added with *ad-libitum* three times a day at 08.00, 12.00 and 18.00. The shrimps were maintained for two weeks. Each week, 30% of water was replaced.

Observation Parameters

1. Daily Growth Rate: The daily growth rate was calculated based on the formula[5] below:

$$SGR = \frac{Wt - Wo}{t} \text{ x } 100\%$$

where SGR = Specific Growth Rate (%/day); Wt = Weight of test animal at the end of the study (gr); Wo = Weight of test animal at the beginning of the study (gr) and t = Length of study (day).

2. Survival Rate: The survival rate of the shrimps was calculated at the end of the cultivation period using the following formula[5]:

$$SR = \frac{Nt}{No} \text{ x } 100\%$$

where SR = Survival Rate (%); = Number of shrimps survived at the end of cultivation period (individul) and N_0 = Number of shrimps at the beginning of the cultivation period (individual).

3. Feed Conversion Ratio (FCR): The Feed Conversion Ratio (FCR) during the cultivation period was calculated using the following formula[10]:

$$FGR = \frac{F}{(Bt - D) - Bo}$$

where FCR = Feed Conversion Ratio; F = Amount of feed (g); B_t = Shrimp biomass at the end of the cultivation period (g); D = Shrimp biomass that died during the cultivation period (g) and B_0 = Shrimp biomass at the beginning of the cultivation period (g).

Results and Discussion

Daily Growth Rate: The effect of probiotic provision through feed on the average weight of the vannamei shrimp is depicted in figure 1. The results of the observation on the vannamei shrimp weight gain for more or less than 2 weeks of cultivation presented increasing weight in time for all treatments. The weight of the vannamei increased from average 0.003-0.004 g at the beginning of the cultivation period to 0.024-0.027g at the end of the cultivation period. However, the average weight gain in shrimps with probiotic-added feed treatment was higher than the control. The highest average weight gain was seen in the group that received commercial feed added with *Lactobacillus* consortium and the group that received commercial feed added with *Bacillus* and *Lactobacillus* consortium.

During the cultivation period, the weight growth rate of the vannamei shrimp was between 0.15% and 0.16% while the length growth rate of the vannamei shrimps ranged between 3.5% and 4.2%. The shrimp growth rates in probiotic-added feed treatment groups were higher than that of the control and the length growth rate was higher than the weight growth rate. This is due to the fact that shrimp growth is allometric, meaning than the length grows faster than the weight[11]. Many studies report the positive effects of probiotics on fish larvae by bioencapsulation of live food[6] and supplementation by diets[12].

Based on the observation results, weight gain happened every day due to probiotic provision. The probiotic bacteria have specific enzymes that help in breaking down complex molecules into simple molecules, making it easier for digestion and nutrition absorption in the shrimp digestive tract[13]. The high absorption of nutrition is expected to increase growth and production; hence, supports optimum cultivation efforts. *Lactobacillus* spp is the most frequently used bacterium for probiotics in food quality enhancement and improvement and, in general, has the potential to be a beneficial probiotic agent for human and animal health.

The faster weight gain in the groups with probiotics is in line with the result of a study showing that the bacterial supplementation of bacteria consortium (*B. licheniformis*, *B. subtilis*, *B. circulans*, *B. laterosporus* and *B. polymyxa*) of 2×10^5 CFU/g in rainbow trout larvae diet resulted in better growth performance and feed utilization[14].

Survival Rate: The number of vannamei shrimp larvae used in this study was 40 per one jar with a 3-liter water volume. Each treatment was repeated three times. The average number of vannamei shrimp larvae after the cultivation period and the survival rates are depicted in figure 2.

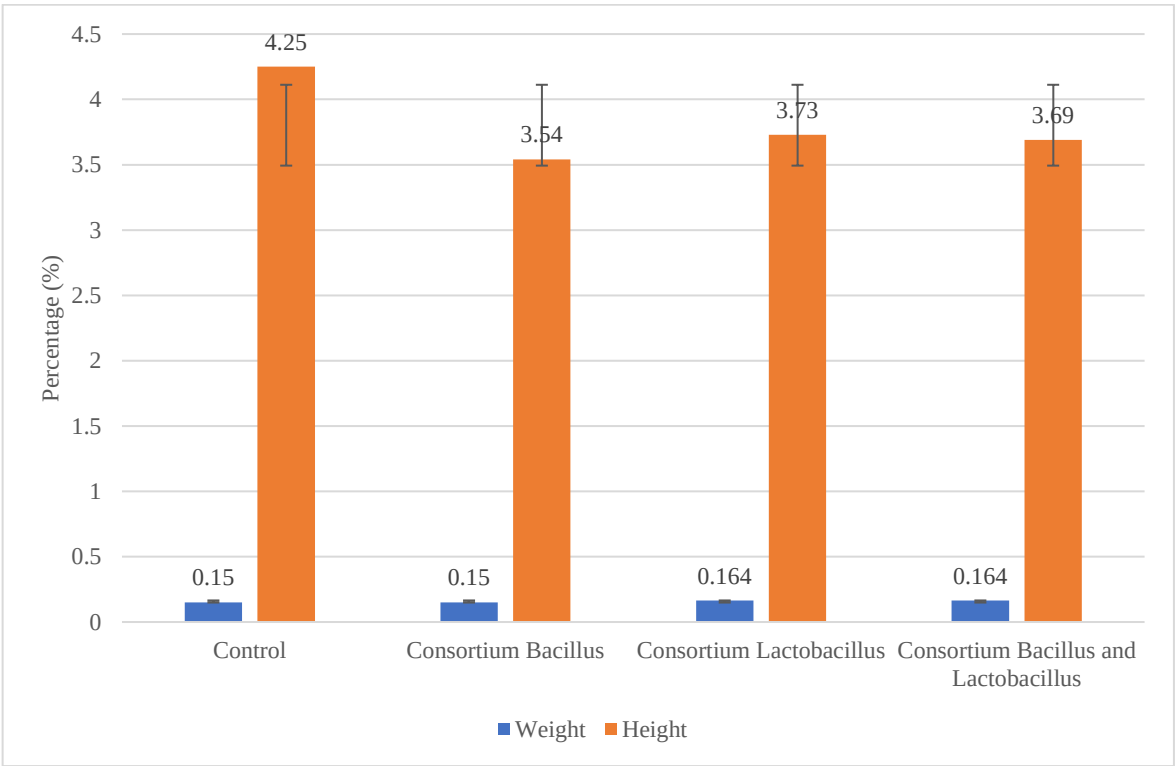

Figure 1: Daily growth rate of vannamei shrimp larvae was affected by probiotic provision through feed. The best results of the observation on the vannamei shrimp weight gain was obtained by consortium of Lactobacillus (3.73%)

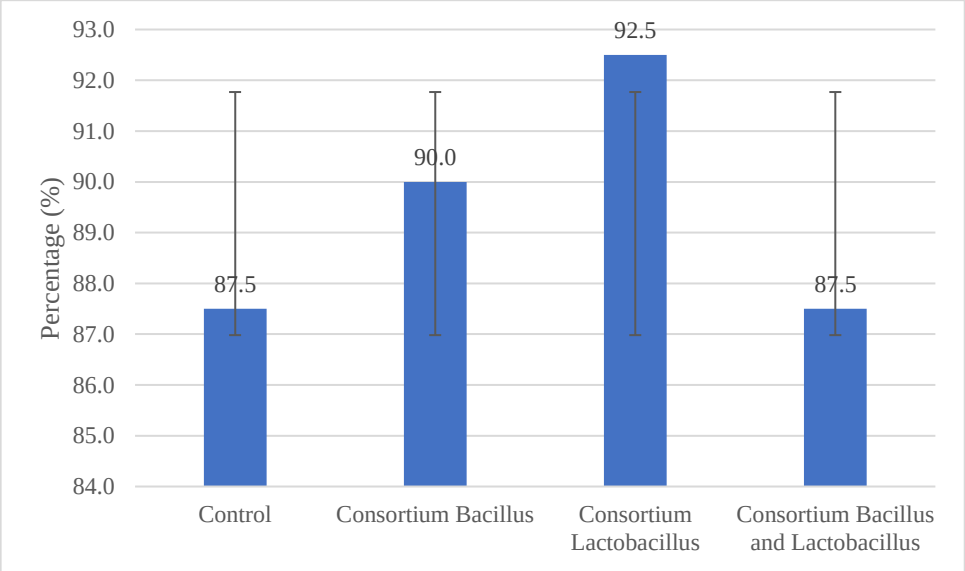

Figure 2: Survival rate of vannamei shrimp larvae was affected by probiotic provision through feed. The best result during the study period was obtained by consortium of Lactobacillus (92.5%). One of the probiotic roles in aquaculture is to improve water quality and immune response.

The Survival Rate (SR) of the shrimps during the study period is considered high for all treatment groups with an average range of 87.5% – 92.5%. The vannamei shrimp larvae survival can be influenced by density due to competition for moving space and dissolved oxygen in the transport container. This is in line with the statement mentioning that density very much influences the survival during transport because the denser is the fish/shrimp transported, the tighter is the competition for space and dissolved oxygen is[15]. The survival rate is also influenced by external factors such as competition for moving space, feed quality and quantity, improper and careless handling especially during sampling[10].

Probiotics have been used widely in aquaculture to control diseases, increase immune response, contribute to nutrition and digestive enzymes of the host and improve water quality[16] . Probiotics can stimulate plankton growth, degrade organic materials and shrimp residual dirt and suppress adverse bacteria population in the pond.

The selection of bacteria included in the probiotic formulation will affect the quality of water and, in turn, will support the survival of the shrimps. *Bacillus sp* is a gram positive bacterium that has the ability to improve the quality of water because it is able to convert inorganic materials into carbondioxide. *Bacillus sp* also suppresses the formation of dissolved organic carbon in the water. It is further explained by a study that *Bacillus sp* has been proven to improve water quality, survival, growth rate and health status of juvenile *Penaeus monodon*[17].

Feed Conversion Ratio (FCR): Feed was provided for the shrimp larvae three times a day. The amount of feed given was 20% of the shrimp larva's weight. The effect of probiotic provision through feed on the Feed Conversion Ratio (FCR) is depicted in figure 3.

During the cultivation period, the FCR value of the vannamei shrimp ranged between 1.46 and 1.66. FCR is a ratio between the amount of feed given and the amount of shrimp biomass produced. The appropriate use of probiotics in the aquaculture industry has been shown to improve intestinal microbial balance and also to improve feed absorption, thus leading to increased growth rate[3] and reduced feed conversion ratio (FCR) during the cultural period[18]. Factors that influence shrimp's appetite are among others, water quality, weather, dirty pond bottom, temperature, feed condition, mass moulting period, diseases and feed mixing technique during feed number replacement[19].

The conversion ratio is inversely proportional with weight gain; hence, the lower is the conversion value, the more efficient the shrimp uses the feed it consumes[20]. The lower is the FCR value, the more efficient the feed use is. In general, an FCR value that is less than 2 is considered good. A high FCR might be due to several factors such as over feeding, certain nutrient deficiency and poor water quality. Those factors need to be monitored continuously so that the feed provision program can be more efficient.

The feed conversion ratio value gained in this study is good because it is less than 2 and compared to the feed conversion ratio of the control feed group; most groups with probiotic addition in the feed had lower ratio. This lower feed conversion ratio is due to the role of bacteria that can produce extracellular enzyme in improving feed digestibility in the shrimp intestine that it is easier to be absorbed by the shrimp. *Bacillus* spp. are commonly used commercially as probiotics and it can act positively on cultured organisms by enhancing survival and growth[21]. Many studies indicate that growth performance and feeding efficiency of fish larvae are promoted by the use of probiotic *Bacillus* spp.

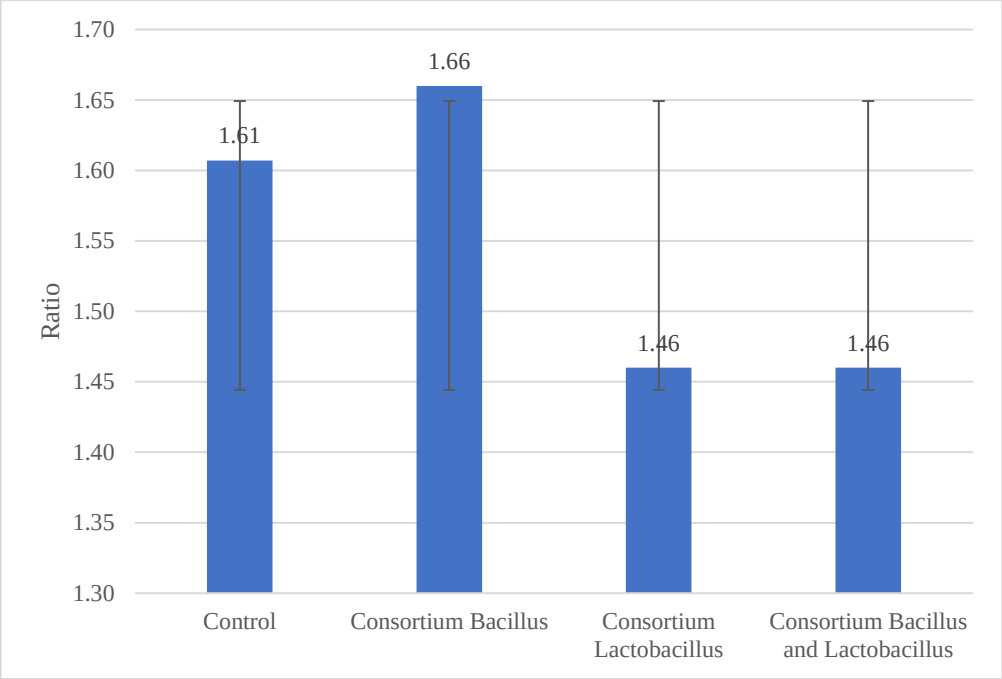

Figure 3: The best FCR value of the vannamei shrimp was obtained by consortium Lactobacillus and consortium Bacillus-Lactobacillus (1.46). The use of probiotics improves intestinal microbial balance and feed absorption, thus leads to an increase of growth rate and feed conversion ratio reduction(FCR)

Conclusion

The results of the observation show that the provision of *B. licheniformis, B. polymyxa, B. subtilis, L. bulgaricus and L. curvatus* probiotic consortium led to the highest daily growth rate of 0.16% while the shrimp larva survival rate with the provision of Lactobacillus probiotic consortium reached 92.5% with a feed conversion rate (FCR) of 1.46.

It is necessary to do testing on shrimps in a higher stadium and refinement of the probiotic formulation by using Nitrosomonas bacteria to improve the quality of water.

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