

## EFFECT OF PROBIOTICS ON WHITE SHRIMP (*Litopenaeus vannamei*) GROWTH

RATU SAFITRI<sup>1\*</sup>, SUNENDI<sup>2</sup>, YULI ANDRIANI<sup>2</sup>, ISKANDAR<sup>2</sup> AND IBNU DWI BUWONO<sup>3</sup>

<sup>1</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang 45363, Indonesia

<sup>2</sup>Fisbery Studies Program, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Sumedang 45363, Indonesia

Received 4 September 2018 / Accepted 28 May 2019

### ABSTRACT

Indonesia is the second largest supplier of white shrimp (*Litopenaeus vannamei*, Boone 1931) in the USA market. Hence, the need for its sustainable production and improved growth. Probiotics, among others, are known for their growth enhancing attributes. Therefore, this study was conducted to determine the effects of powder and liquid probiotics on the growth of white shrimps at the Minaloka Jaya shrimp ponds, Grabag District, Purworejo Regency, Central Java. The shrimps were cultivated for 60 days and applied with three probiotic treatments, namely commercial liquid probiotics with dosage of 10 mL/kg feed, powder probiotics with dosage of 10 g/kg feed and liquid probiotic with dosage of 10 mL/kg feed. Each probiotic preparation was administered four times a day to over 150,000 vannamei shrimps which were cultured in a semi-intensive system. Probiotics in powder and liquid forms contain *Lactobacillus fermentum*, *L. acidophilus*, *L. plantarum*, *L. curvatus*, *Bacillus licheniformis*, *B. subtilis*, and *B. polymyxa*. *B. megaterium*, *B. coagulans*, *Pseudomonas putida*, *Nitrosomonas* sp. and *Nitrobacter* sp. Using the Randomized Block Design (RBD), the three treatments were replicated five times. The application of probiotics in both powder and liquid forms had increased the growth yield of the vannamei shrimp. However, the powder probiotic had shown better growth performance than the commercial liquid probiotics and liquid preparation of probiotics. Probiotic powder form provides a specific growth rate (SGR) of 8.18%, absolute body length of 9.68 cm, absolute biomass of 6.78 g, and feed conversion ratio (FCR) of 1.93.

**Keywords:** liquid probiotic, powder probiotic, white shrimp performance

### INTRODUCTION

The aquaculture industry is one of those industries that contribute the most significant economic value for Indonesian. According to the Food and Agriculture Organization (FAO) in 2016, Indonesia is the second only to China in worldwide aquaculture production (BPS, 2015).

One of the leading export commodities from brackish water fisheries is the white shrimp (*Litopenaeus vannamei*) of which Indonesia is the second largest supplier in the United States shrimp market at 504,000 Metric Ton (MT) (Asia Pacific Aquaculture 2015). White shrimps are preferred more than tiger shrimps, as they are easy to cultivate, fast growing and have higher stocking density hence, greater production.

Very high stocking density, however, results in the accumulation of residual organic material from feed and metabolism at the bottom of the waters. High waste accumulation increases the concentration of various toxic compounds and results in changes in water quality and further increases the growth of opportunistic pathogens that can disrupt the life cycle of white shrimps (Gao *et al.* 2016). High stocking densities would result in unfavorable growth, low survival rate, low production, poor water quality and the emergence of pathogen outbreaks (Gao *et al.* 2016). Therefore, probiotics are needed, particularly those which can work directly against hosts and microbes that degrade leftover feed, in order to improve the pond water quality. In shrimp aquaculture, probiotics are mainly considered as a disease control agent and also stimulate the dominance of microbiota that benefits the host.

\*Corresponding author, email: ratusafitrie@yahoo.com

Probiotics are living microorganisms, which, when administered in sufficient quantities, will provide health benefits to their host. Probiotics can improve the microbes balance in the digestive tract of shrimps and can increase the nutritional value of feed and nutrient absorption rate thereby allowing the shrimps to achieve maximum growth (Fijan 2014). These probiotic bacteria produce antimicrobial compounds, such as bacteriocin, which can be used as natural preservatives (Karami *et al.* 2017). A combination of probiotics composed of *Bacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp. and *Lactobacillus* when applied to *Penaeus vannamei* ponds has mitigated nitrogen and phosphate pollution in the ponds as shown in the reduction of total phosphorus, total inorganic phosphorus, total nitrogen and total organic carbon (Lazado *et al.* 2015).

Powdered probiotics are another common format for friendly bacteria. The great thing about powder is its ability to incorporate them into all kinds of substances, including juice, yoghurt and sauce. The powdered probiotics or encapsulated probiotics, is made by spray-drying technique using the Spray Dryer. In this study, maltodextrin was used as the encapsulation material, because this is a source of oligosaccharides for the growth of probiotic bacteria that can facilitate the process of feed nutrient degradation that takes place in the digestive tract of shrimp (Andriani *et al.* 2017).

Coating is one way to protect the probiotics and deliver them safely. Encapsulated probiotics containing polyalcohols of glycerine, sorbitol, mannitol, and prebiotic oligosaccharides inulin, starch and dextrin were effective in the protection of probiotic bacteria during freeze-drying with little change in bacterial cell counts when the bacteria were stored at 4 °C for five months. Probiotics also, have ideal moisture between 2.8 - 5.6%. Microencapsulation using polysaccharide or protein-based systems is far more effective in the protection of bacteria during freeze-drying and storage as compared to traditional cryo-protection. Polysaccharides, are prebiotics that protects the probiotic delivered bacteria and that produce a synbiotic formulation (Govender *et al.* 2013). Many different probiotic supplements are in the form of powder, capsule, juice, yoghurt, sachet and tablets.

Currently, among the commercial products of probiotic is liquid probiotics, which is used in ponds. The popular commercial liquid probiotic is EM-4. Probiotics in liquid form are less efficient compared to powder form in terms of expiry issues, storage or packaging, besides the possibility of greater overgrowth of other bacteria (Govender *et al.* 2013). In addition, it is less practical, especially in remote areas or inter-island transport. The liquid probiotics are unstable in warm temperatures. Therefore, the powder probiotic is more preferred. Some people, however, claim that liquid probiotics are the freshest and most potent form.

Probiotics application is expected to increase the growth of white shrimps. Therefore, it is necessary to study about the effect of commercial probiotic, liquid preparation and powder preparation that can improve the growth performance of white shrimp in intensive ponds.

## MATERIALS AND METHODS

The research was conducted for three months from October 5 to December 16, 2017, at the Minaloka Jaya shrimp pond, Shrimp Club Indonesia (SCI) Grabag Subdistrict, Purworejo District, Central Java.

### Materials

The materials used in this research included: a) White prunes (PL-9) weighing  $70 \pm 0.02$  mg and of  $7.57 \pm 0.8$  mm long obtained from PT. Suri Tani Pemuka Hatchery Unit Indramayu; b) Shrimp feed in the form of starter and grower pellets of the 'Pakan Udang' brand; c) Commercial probiotics (EM-4), (Powder and liquid probiotic preparations) from the Laboratory of Microbiology, Universitas Padjadjaran. The liquid probiotics was composed of *L. fermentum*, *L. acidophilus*, *L. plantarum*, *L. curvatus*, *B. licheniformis*, *B. subtilis*, *B. polymyxa*, *B. coagulans*, *B. megaterium*, *P. putida*, *Nitrosomonas* sp., and *Nitrobacter* sp. These probiotics were prepared in the microbiology laboratory at a cell density of  $10 \times 10^{10}$  cfu/g.

The instruments used included the following: refractometer to measure the salinity of pond water, pH meter, Lutron brand with type of PH-

201 and accuracy of 0.1, to measure water pH in the farm pond, DO meter of Hanna brand, type of HI 9146 (0.1 mg/L) to measure dissolved oxygen, mercury thermometers to measure the ponds temperature, the analytical scale (0.01 g) to measure the white shrimp weight, calipers (0.1 mm) to measure the length of the white shrimp, fish scoop net to pick up white shrimp, and digital cameras to document every research activity.

Using the Randomized Block Design (RBD) the three treatments were replicated five times. Commercial probiotic applications, liquid probiotics and powder probiotics were used as the treatments.

### Procedures

The research was conducted for 60 days using three ponds, each of which used a different treatment, namely treatment plot A (area of 0.15 ha), treatment plot B (area of 0.18 ha), and treatment plot C (area of 0.18 ha) with a stocking density of 150,000 white shrimps per pond plot. Vannamei shrimp cultivation was carried out using a semi-intensive culture system, with a maximum area of 1 ha per plot, water depth of 80 - 100 cm and a water mill for aeration.

Feeding was adjusted to the feeding rate (FR) of 15% of the weight of the vannamei shrimp biomass. The feed dose was calculated once every week. Probiotic applications from each treatment were:

1. Liquid commercial probiotics (EM-4) was mixed with feed at the beginning of stocking until harvest time with a dose of 10 mL/kg of feed. Probiotics applied to the water media with a dose of 900 mL/0.15 ha at the beginning of stocking, then when the shrimps were 30 days old until the harvest time, the dose was increased to 2.4 L/0.15 ha, with EM-4 containing *L. casei* and *Saccharomyces cerevisiae*.
2. Liquid probiotics which were produced in laboratory and applied from the beginning of the stocking until the harvest time was used as much as 10 mL/kg of feed. Probiotics applied to the waters used a dose of 5.5 L/0.18 ha at the beginning of stocking, then when the shrimps were 30 days old until the harvest, the dose used was 2.5 L/0.18 ha.

The liquid probiotics was composed of *L. fermentum*, *L. acidophilus*, *L. plantarum*, *L. curvatus*, *B. licheniformis*, *B. subtilis*, *B. polymyxa*, *B. coagulans*, *B. megaterium*, *P. putida*, *Nitrosomonas* sp., and *Nitrobacter* sp. These probiotics were prepared in the microbiology laboratory at a cell density of  $10 \times 10^{10}$  cfu/g.

3. Probiotic powder mixed with feed at the beginning of stocking until harvest time with a dose of 10 g/kg of feed and applied to the waters as much as 5 kg/0.18 ha at the beginning of stocking. The dose was reduced to 2 kg/0.18 ha when the shrimps were 30 days old until harvest time.

Shrimp growth parameters were measured once a week for each treatment, 3 - 5 vannamei shrimps were collected from five sampling points using fish scoop net, then the length and weight were measured using calipers and analytical scale.

The feed conversion ratio (FCR) was calculated at the end of the study.

The observed parameters were computed as follows:

1. Specific Growth Rate (Effendie 1997)

$$G = \frac{(\ln W_t - \ln W_o)}{t} \times 100\% \quad (1)$$

where:

G = Growth rate (%)

W<sub>t</sub> = Average of fish daily weight at the end of the research (g)

W<sub>o</sub> = Average of fish daily weight at the beginning of the research (g)

t = Duration of observation (day)

2. Absolute Length Growth (Effendie 1997)

$$L = L_t - L_o \quad (2)$$

where:

L = Absolute length growth (cm)

L<sub>t</sub> = Average of individuals length at the time refers to t (cm)

L<sub>o</sub> = Average of individuals length in the beginning of the research (cm)

3. Absolute Biomass (Effendie 1997)

$$W = W_t - W_o \quad (3)$$

where:

$W$  = Absolute growth (g)

$W_t$  = Biomass weight at the end of the research (g)

$W_o$  = Biomass weight at the beginning of the research (g)

#### 4. Feed Conversion Ratio (WWF-Indonesia 2014)

$$FCR = \frac{\text{Amount of feed}}{\text{Biomass}} \quad (4)$$

The water quality parameters observed included the following: Dissolved Oxygen, pH, temperature and salinity performed once a week, while ammonia measurement was carried out every two weeks.

#### Data Analyses

Data were analyzed using analysis of variance with 95% confidence level and Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

### Specific Growth Rate (SGR)

The specific growth rate of the shrimps critically indicates the success and effectiveness of maintenance time in shrimp aquaculture. Several factors influence the growth rate of shrimp, namely the effective maintenance time, the effective feeding dosage, disease control and control of the cultivation environment i.e., dissolved oxygen (DO), carbon dioxide (CO<sub>2</sub>), temperature, and water salinity (Susilowati *et al.* 2018).

ANOVA test results on the daily growth rate of white shrimp showed normal and homogeneous distributed data. The variance analysis result showed that the treatments applied significantly affected ( $p < 0.05$ ) the daily specific growth rate of the test shrimp. Further test results using Duncan Test showed that commercial probiotic treatment provided significantly different specific growth rate compared to those using probiotic liquid and powder preparations. The specific growth rates

of the shrimps differed significantly among treatments ( $p < 0.05$ ).

After 60 days of cultivation, the probiotic powder application produced the highest specific growth rate of 8.18%, followed by liquid probiotics at 8.07%, while the commercial probiotics produced 8.03%, (Fig. 1). These results were higher than those of Swapna *et al.* (2015), which had white shrimps SGR of 4.32% with the application of powder probiotic *B. licheniformis* ( $10 \times 10^9$  cfu/kg) and *L. rhamnosus* ( $8 \times 10^9$  cfu/kg) in the feed. In another study, the application of probiotics *Lactobacillus* sp. to as much as 10% in feed yielded a daily growth rate of 7.16% (Sandeepa *et al.* 2015).

The high SGR in powdered products was probably due to the coating or the maltodextrins encapsulated probiotics that were able to maintain viability and protected the probiotic bacteria from damage due to unfavorable environmental conditions. This was indicated by the number of colonies after the encapsulation process ( $10^{10} - 10^{12}$ ). This amount met the requirements applicable to shrimp digestive tracts, which is  $10^6 - 10^8$  cfu/mL (Andriani *et al.* 2017). The increased presence of bacterial colonies resulted in the increased activity of enzymes produced by probiotic bacteria in the digestive tract of shrimps that had thereby improved the digestibility of feed. Efficient digestion will optimize the utilization of feed consumed and thereby increase the growth of vannamei shrimps.

The probiotic bacteria *Bacillus coagulans* found in powder preparations is capable of producing amylase and lipase enzymes, the *Bacillus licheniformis* bacteria produce protease enzymes that can increase protein digestibility in feeds, hence, increased the growth rate of white shrimps. The probiotic bacteria regulate the microbial environment of the intestine and block the pathogenic microorganisms inside the intestine by releasing enzymes (proteases, lipases and amylases) that aid in the digestion of food. With the help of these enzymes, the nutrients in feed will be more readily absorbed by the shrimp digestive organs, so that shrimps can grow maximally (Salz *et al.* 2019).



### Effect of probiotics preparation on white shrimp growth

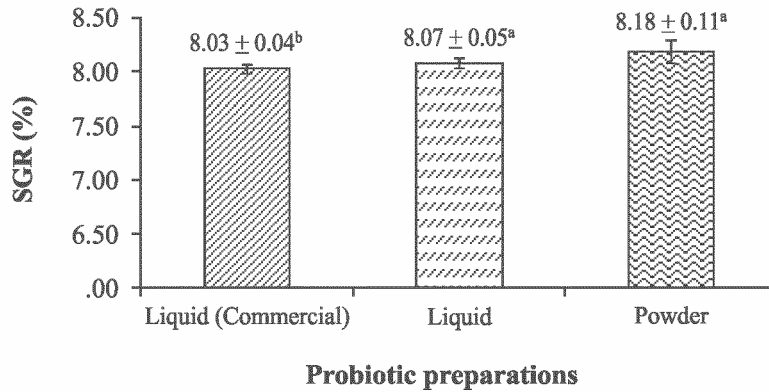


Figure 1 Specific growth rate of vannamei shrimp fed with commercial liquid, liquid and powder probiotics  
Note: Average values with different superscripts indicate significant differences at  $p < 0.05$ .

### Absolute Growth Length (AGL)

The absolute growth length of vannamei shrimps differed significantly among treatments. After 60 days of cultivation, AGL was the highest at 9.68 cm with the application of powder probiotic, followed by 9.58 cm with liquid probiotics and 9.13 cm with commercial probiotics (Fig. 2).

### Absolute Biomass Growth

The absolute biomass growth of the white shrimps also differed significantly among the treatments (Fig. 3). The powder probiotic produced the highest biomass growth (6.78 g)

compared to liquid probiotic (6.37 g) and commercial (6.20 g) probiotic treatments (Fig. 3).

The addition of probiotics to the shrimp feed had also increased the growth length and biomass of the shrimp after 60 days of cultivation (Figs. 4 & 5). Shrimp growth is influenced by heredity, gender, age, density, parasites and diseases and, the ability to digest the feed (Fendjalang *et al.* 2016). Weight gain is influenced by feed consumption as the latter determines the input of nutrients into the body for growth and metabolism. Length and biomass increase indicated that the application of probiotics had improved nutrient utilization by the shrimps.

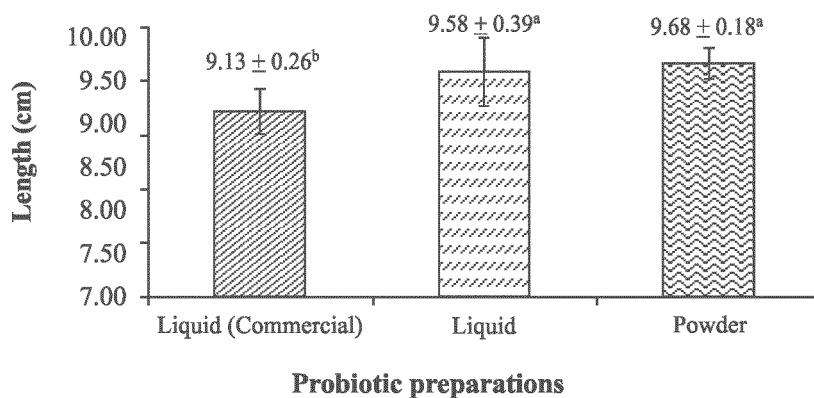


Figure 2 Absolute growth length of vannamei shrimp fed with commercial liquid, liquid and powder probiotic  
Note: Mean values with different superscripts indicate significant differences at  $p < 0.05$ .

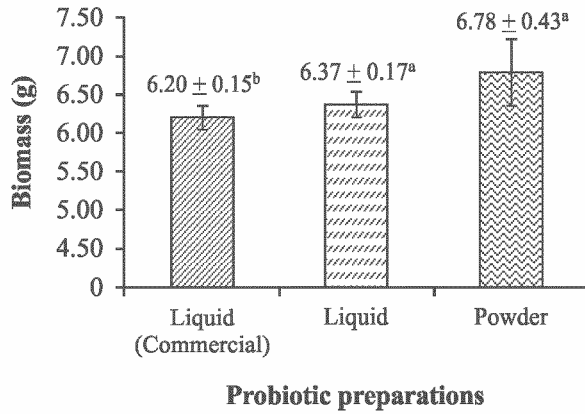


Figure 3 Absolute biomass growth of vannamei shrimp fed with commercial, liquid, and powder probiotic  
 Note: Mean values with different superscripts indicate significant differences at  $p < 0.05$ .

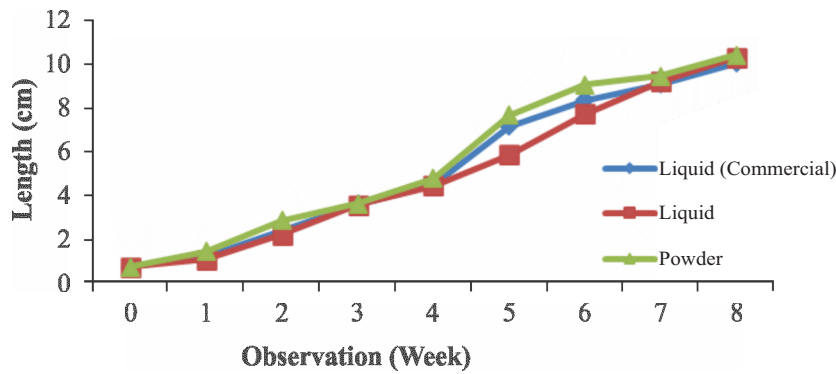


Figure 4 Average growth length of vannamei fed with commercial, liquid and powder probiotic applications in feed after 60 days of cultivation

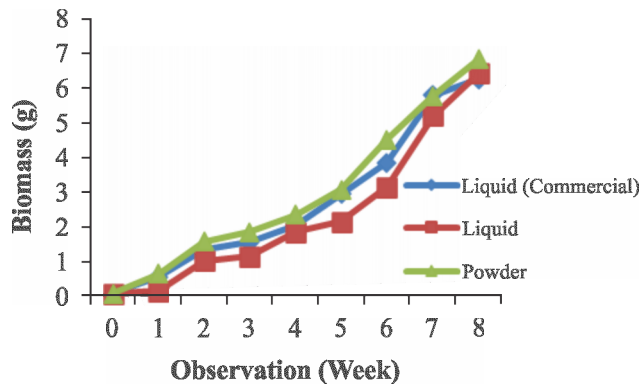


Figure 5 Average biomass growth of vannamei fed with commercial, liquid and powder probiotic applications in feed after 60 days of cultivation

The proteases and amylases enzymes contributed to better feed digestion of the shrimps resulting in more nutrients being absorbed by the shrimp digestive tract, thereby improving growth. *Bacillus megaterium* bacteria play an effective role in the digestive process by producing extracellular enzymes such as

proteases, carbohydrases and lipases that can maintain beneficial bacterial growth in the shrimp tract, thereby increasing growth (Widanarni *et al.* 2012).

The *Pseudomonas putida* consortium contained in the probiotic preparations was able to produce several exogenous enzymes to digest

feed, such as amylase, protease, lipase, and cellulose. The *Bacillus licheniformis* produced the protease enzymes, which can increase the digestibility of feed proteins. The exogenous enzyme will help endogenous enzymes in the host to hydrolyze feed nutrients. The available enzymes produced by probiotics will increase the availability of nutrients that are readily absorbed from the digestive tract into the blood vessels and are circulated to all parts of the body and tissues for further metabolic processes. The *Bacillus pumilus* probiotic bacteria with proper concentration and dosage can increase the number of hemoglobin (Rajikkannu *et al.* 2015), which is believed to be the one indicator of increased shrimp ability to supply nutrients to the body in order to repair digestive system of white shrimp and increase the growth of white shrimp. The higher the nutrient content of the digestible feed, the greater is the possibility of nutrients being used by the shrimp for its growth. According to Rajikkannu *et al.* (2015), fish treated with *B. pumilus* showed maximum percentage of total erythrocyte count, haemoglobin concentration and haematocrit concentration than in other groups. The result suggests that *B. pumilus* could be used effectively as a probiotics in aquaculture.

### Feed Conversion Ratio (FCR)

Feed conversion ratio is a description of the effectiveness level of the given feed to shrimp growth response. FCR is an indicator of how far the given feed can be utilized by the shrimp to raise 1 kg of shrimp weight. The highest conversion rate of white shrimp feed was that of

commercial probiotic liquid (2.46), followed by powder probiotic (1.93), and lastly, liquid probiotic (1.83) (Fig. 6).

The lowest FCR value (1.83) for the liquid probiotic was not much different from that of Susilowati *et al.* (2017) i.e., FCR (1.42) of white shrimp. Generally, an FCR value of less than 2 is still declared good in semi-intensive shrimp farming (Tahe *et al.* 2015). Judging from the FCR values obtained, the pond plots with liquid probiotic treatment and powder preparation were able to produce good feed conversion ratio to support the successful cultivation of white shrimps.

Low feed conversion is believed to be a result of a consortium of different probiotic bacteria added to pond waters and into feed as well. The added probiotic bacteria will increase the activity of digestive enzymes significantly in the shrimp body, thereby increasing the nutrient digestion and resulting in low FCR value. Probiotic treatments increased the activity of amylase and trypsin enzymes in shrimp (Valsamma *et al.* 2014). The addition of probiotic bacteria in the diet can improve the growth performance and feed conversion ratio; which is related to the cellulose and amylase enzymes produced by *Bacillus* sp. (Tahe *et al.* 2015). The use of probiotics in shrimp farming can improve nutrient digestibility, feed efficiency and tolerance to stress (Cruz *et al.* 2012).

Optimal feed digestion and absorption will result in low feed conversion value to change the feed into meat in optimal time. Probiotic bacteria that can help in feed absorption and shrimp growth include the genus *Bacillus*

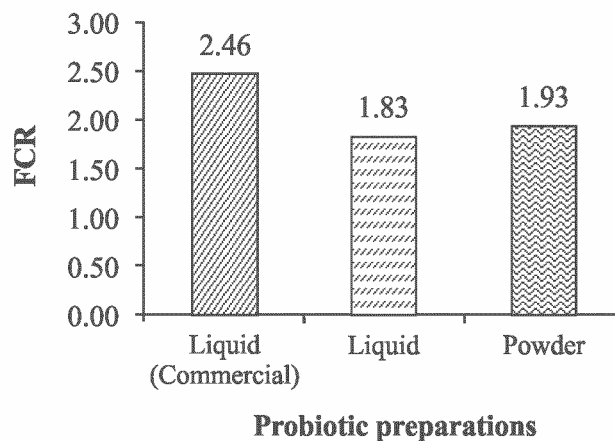


Figure 6 Feed conversion ratio of vannamei fed with commercial, liquid and powder probiotic applications in feed after 60 days of cultivation

(Rajikkannu *et al.* 2015). In the powder probiotics, there are several species of the genus *Bacillus*, among which are *Bacillus circulans*, *B. subtilis*, *B. coagulans*, *B. amyloliquefaciens*, *B. pumilus*, *B. licheniformis* and *B. megaterium*. *Bacillus* bacteria will increase feed absorption and subsequently increase the weight of white shrimp and lower the feed conversion value. Increased intake of feed is caused by the balance of microbes in the digestive tract of shrimps.

In addition, *Lactobacillus* sp. are lactic acid-producing bacteria that also serve to increase the enzyme's effectiveness and help digest the food (Buruina *et al.* 2014). Increased fat metabolism due to increased enzyme activity, one of which is lipase, will increase the utilization of fat-rich diet as an energy source so that the conversion of protein to meat will be more optimal (Dhanalakshmi *et al.* 2015). The bacterial activity during digestion will change rapidly when there are beneficial microbes entering through the feed or water causing changes in the balance of existing bacteria with bacteria entering in the digestive tract of shrimps, so the process of feed protein absorption will be more optimal. Based on the current study, the application of liquid and powder probiotic preparations which contain several beneficial bacterial consortiums were able to improve feed efficiency which is indicated by the low feed conversion value of the white shrimps.

### Water Quality Parameters

Water quality has an important role as life support and growth of vannamei shrimp. The observation results on several water quality parameters, including temperature, dissolved oxygen, salinity, pH, alkalinity and ammonia in all treatments during the study are presented in Table 1.

Water quality has a very influential impact on shrimp health. Low water quality of the culture media can result in lower growth rate, survival rate, molting frequency and an increase in harmful bacteria. Water quality of the culture media during the study was still in the proper range for the growth and survival of vannamei shrimp. Nevertheless, in the solid probiotic treatments, the pH and alkalinity values were less than optimal.

The temperature range for commercial (29.6 - 31.6 °C), liquid (29.9 - 32.4 °C), and probiotic powder (29 - 31.8 °C) treatments were still within the optimal range for the white shrimps (Table 1) as compared to the optimal temperature range of 28 - 32 °C with tolerance range of 20 - 35 °C (WWF-Indonesia 2014). The optimal temperature for white shrimps ranged from 27 - 32 °C (Tahe *et al.* 2015). If the temperature is higher than the range of tolerance, then the metabolism in the body of the shrimp will take place quickly, so that the demand for dissolved oxygen increases.

For all treatments, the dissolved oxygen content (DO) in the white shrimp culture medium were 6.57 - 8.47 mg/L, 5.87 - 7.47 mg/L and 6 - 7.2 mg/L, which were still in the optimal category as compared to the minimum tolerance value of 3 mg/L (WWF-Indonesia 2014). The minimum DO for shrimp health is 3.0 mg/L, whereas DO < 2.0 mg/L would cause death to shrimp (Tahe *et al.* 2015).

The salinity values for commercial (9.6 - 19.63 ppt), liquid (13.4 - 19.8 ppt) and powder (9.87 - 19.7 ppt) probiotic were still in the optimal conditions for the white shrimps as salinity tolerance values are 0 - 35 or < 35 ppt (WWF-Indonesia 2014).

Table 1 Water quality parameters in the white shrimp aquaculture farm

Parameters	Probiotic Preparations			Acceptable <sup>a</sup>
	Liquid (Commercial)	Liquid	Powder	
Temperature (°C)	29.6 - 31.6	29.9 - 32.4	29 - 31.8	20 - 35
DO (mg/L)	6.57 - 8.47	5.87 - 7.47	6 - 7.2	> 3
Salinity (ppt)	9.6 - 19.63	13.4 - 19.8	9.87 - 19.7	0-35 or < 35
Alkalinity (mg/L)	110 - 142	108 - 132	62 - 140	100
pH	7.7 - 8.4	7.73 - 8.33	6.7 - 8.37	7 - 8.5
Ammonia (mg/L)	0.1023 - 0.259	0.001 - 0.191	0.019 - 0.180	0.1 - 0.5

Note: <sup>a</sup>WWF-Indonesia (2014).

The alkalinity values of the white shrimps environment were still within the tolerance value of 100 mg/L as prescribed by WWF-Indonesia (2014). The commercial probiotics recorded 110 - 142 mg/L, liquid probiotics at 108 - 132 mg/L and powder probiotics at 62 -140 mg/L. The powder probiotics lowest alkalinity value of 62 mg/L was temporary as it was a result of fairly high rainfall intensity. Rain water has a pH value of about 5; whose presence had decreased the pH value and total alkalinity in the pond, so that with low alkalinity value, the buffer (water resistance) to water pH fluctuations is very low, resulting in pH shocks with low pH value.

The pH values from each treatment, commercial (7.7 - 8.4), liquid (7.73 - 8.33) and powder (6.7-8.37) probiotics, were still within the optimum range compared to the standard optimum pH value of 7.5 - 8 with a tolerance range of 7 - 8.5 (WWF-Indonesia 2014). The low alkalinity value had decreased the pH in the culture media. pH value affects the osmotic pressure between the maintenance environment and the shrimp body. If the pH value range is not optimum then the shrimps will require more energy to do the osmoregulation process and the energy that should be earmarked for growth is then diverted for the process of adapting to fluctuating pH changes.

The ammonia levels from each treatment were still at tolerable range for the white shrimps; 0.1023 - 0.25, 0.001 - 0.191 and 0.019 - 0.180 mg/L, for respective treatments, as compared to the standard tolerance level of 0.1 mg/L to 0.5 mg/L (WWF-Indonesia 2014).

## CONCLUSION

The application of liquid and powder probiotics has improved the growth performance of white shrimps under intensive scale culture. Provision of probiotics in powder and liquid forms resulted in a relatively equal growth increase in support of successful shrimp farming activities. However, the powder probiotic preparation produced the best results in increasing white shrimps growth, at a daily growth rate of 8.18%, absolute growth of 9.68 cm, and absolute biomass of 6.78 g, and feed conversion ratio of 1.93.

## ACKNOWLEDGEMENTS

The authors would like to express their appreciation to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia who has funded the research through the Scheme of Prospective Technology-Based Beginner Entrepreneurship (*Calon Perusahaan Pemula Berbasis Teknologi, CPPBT*) in 2017 with theme 'Probiotic Production as Anti-Pathogen Agent and Growth Promotion to Improve Production of Vannamei Shrimp'.

## REFERENCES

- Andriani Y, Safitri R, Kanza AA, Rustama MM. 2017. Karakterisasi *Bacillus* dan *Lactobacillus* yang Dienkapsulasi dalam Berbagai Bahan Pembawa untuk Probiotik Vannamei (*Litopenaeus vannamei* Boone, 1931). [Characterization of Encapsulated *Bacillus* and *Lactobacillus* in Various Forms of Probiotics for Vannamei (*Litopenaeus vannamei* Boone, 1931) Shrimp]. J. Perikanan dan Kelautan 7(2):150.
- Andriani Y, Safitri R, Shaiyanne F. 2018. Vannamei Shrimp (*Litopenaeus vannamei*, Boone, 1931) Performance with *Bacillus* and *Lactobacillus* Probiotic Formulation Consortium in Dry Preparation. Res. J. Chem. Environ. Special Issue August (2018):307-12.
- Asia Pasific Aquaculture [Internet]. 2015. Akuakultur Indonesia di Level Asia Pasifik. [cited 2017 June 18]. Available from: <http://www.djpb.kkp.go.id>
- Badan Pusat Statistik [Internet]. 2015. Produksi Perikanan Menurut Subsektor. [cited 2017 May 16] Available from: <http://www.bps.go.id/linkTabelStatis/view/id/1711>
- Buruina CT, Profir AG, Vizireanu C. 2014. Effects of Probiotic *Bacillus* species in Aquaculture - An Overview. The Annals of the University Dunarea De Jos Of Galati Fascicle. J. Vi-Food Technology 38(2):9-17.
- Cruz PM, Ibanez HOA, Monroy, Saad HCR. 2012. Use of Probiotics in Aquaculture. Review Article. International Scholarly Research Network ISRN Microbiology. 2012:13 p.
- Dhanalakshmi G, Reniprabha A, Chandarakala A. 2015. Studies on the Effect of Commercial Probiotic Application in the Growth of the Fish. Int J Adv Res 3(8):708-12.
- Effendie I. 1997. Biologi Perikanan. Jakarta (ID): Yayasan Pustaka Nustama.

- Fendjalang SNM, Budiardi T, Supriyono E, Effendi I. 2016. Produksi Udang Vaname (*Litopenaeus vannamei*) pada Karamba Jaring Apung dengan Padat Tebar Berbeda di Selat Kepulauan Seribu. [Production of White Shrimp (*Litopenaeus vannamei*) in Floating Cage System with Different Stocking Density at Thousand Island Strait]. J. Ilmu dan Teknologi Kelautan Tropis 8(1):201-14.
- Fijan S. 2014. Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature. International Journal Environment Res. Public Health 11:4745.
- Food and Agriculture Organization [Internet]. 2016. Fishery for Statistical Time Series. [cited 2017 May 16]. Available from: <http://www.fao.org/fishery/statistics/software/fishstatj/en>
- Gao Y, He1 Z, Vector H, Zhao1 B, Li Z, He J, Lee JY, Chu Z. 2017. Effect of Stocking Density on Growth, Oxidative Stress and HSP 70 of Pacific White Shrimp *Litopenaeus vannamei*. Turkish J Fish Aquat Sci 17:877-84.
- Govender, M. Yahya EC, Pradeep K, Lisa C. du Toit, Sandy van Vuuren, and Viness Pillay. 2014. A Review of the Advancements in Probiotic Delivery: Conventional vs. Non-conventional Formulations for Intestinal Flora Supplementation. AAPS PharmSciTech 15(1):29-43.
- Karami S, Roayaei M, Hamzavi H, Bahmani M, Azar HH, Leila M, Kopaei M. 2017. Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens. Int J Pharm Investig 7(3):137-41.
- Lazado CC, Lacsamana JI, Caipang CMA. 2015. Mechanisms of probiotic actions in shrimp: Implications to tropical aquaculture. Biotechnological Advances in Shrimp Health Management in the Philippines. p. 89-114.
- Luthfi MZ, Sri R, Tita E. 2017. Analisa Kelayakan Usaha Budidaya Polikultur Udang Windu (*Penaeus monodon*) dan Ikan Koi (*Cyprinus carpio*) di Desa Bangsri, Kabupaten Brebes. Jurnal Sains Akuakultur Tropis 1:62-71.
- Nandhini RS, Sahana E, Dhanakkodi B. 2015. Beneficial and destructive effects of probiotics in aquaculture systems-A review. Intl. J. Fish Aqua Stud 2(3): 153-9.
- Paul P, Rahman A, Hossain M, Islam S, Mondal S, Haq M. 2016. Effect of Stocking Density on the Growth and Production of Freshwater Prawn (*Macrobrachium rosenbergii*). Intl. J. of Fish Aq Sci 6(1):77-86.
- Sandeepa MG, Ammani K. 2015. Effect of Probiotic Bacterium on Growth and Biochemical Parameters of Shrimp *Litopenaeus vannamei*. Int J Recent Sci Res 6(2):2872.
- Sivakumar N, Sundararaman M, Selvakumar G. 2012. Probiotic effect of *Lactobacillus acidophilus* against vibriosis in juvenile shrimp (*Penaeus monodon*). Afr. J. of Biotechnol 11(91): 15811-18.
- Susilowati T, Herawati VE, Basuki F, Yuniarti T, Rachmawati D, Suminto. 2017. Performa Produksi Udang Vaname (*Litopenaeus vannamei*) yang dibudidayakan pada Tambak Sistem Semi Intensif dengan Aplikasi Probiotik. [Performance of Vannamei Shrimp (*Litopenaeus vannamei*) Cultured in Semi Intensive System with Probiotics Applications]. J. PENA Akuatika 16(1):22-37.
- Susilowati T, Desrina, Hutabarat J, Anggoro S, Zainuri M, Sarjito, Basuki F, Yuniarti T. 2018. The effects of season, aeration and light intensity on the performance of Pacific whiteleg shrimp (*Litopenaeus vannamei*) polycultured with seaweed (*Gracilaria verrucosa*). IOP Conf. Series: Earth and Environmental Science 137(2018):1-7.
- Swapna B, Venkatrayulu CH, Swathi AV. 2015. Effect of probiotic bacteria *Bacillus licheniformis* and *Lactobacillus rhamnosus* on growth of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). Eur J Exp Biol 5(11):31-6.
- Tahe S, Suwoyo HS, Fahrur M. 2015. Aplikasi Probiotik Rica dan Komersial pada Budidaya Udang Vaname (*Litopenaeus vannamei*) Pola Intensif. Proceedings: Forum Inovasi Teknologi Akuakultur. p.435-45.
- Valsamma J, Haseeb M, Ranjit S, Anas A, Brigh IS. 2014. Shrimp Production under Zero Water Exchange Mode Coupled with Bioremediation and Application of Probiotics. J. Fisheries International 9(1):5-14.
- Widanarni D, Yuniasari, Sukenda, Ekasari J. 2012. Nursery Culture Performance of *Litopenaeus vannamei* with Probiotics Addition and Different C/N Ratio under Laboratory Condition. J. Biosciences 17(3):115-9.
- WWF-Indonesia. 2014. Budidaya Udang Vaname Tambak Semi Intensif dengan Instalasi Pengelolaan Air Limbah (IPAL). Jakarta (ID).