Journal of Aquaculture and Fish Health Vol. 12(3) - September 2023
P-ISSN: 2301-7309 / E-ISSN: 2528-0864
doi : 10.20473/jafh.v12i3.37996

The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System

Ardana Kurniaji[1*], Diana Putri Renitasari[1], Siti Aisyah Saridu[1], Anton[1] and Yunarty[1]
[1]Aquaculture Technique Study Program, Polytechnic of Marine and Fisheries Bone, Jl. Sungai Musi Km. 9 Waetuwo, Bone, South Sulawesi 92718, Indonesia

*Correspondence :
ardana.kji@gmail.com

Received : 2022-08-02
Accepted : 2023-07-27

Keywords :
Commercial probiotics, Independent probiotics, Laboratory scale

Abstract

An important component in the application of biofloc is the presence of probiotic bacteria as a floc-forming agent. This study aimed to analyze the differences in the yield of vannamei shrimp in a biofloc system using commercial probiotics and independent probiotics on a laboratory scale. The study design was a completely randomized design with 3 treatments and 4 replications, including independent probiotics (IP), commercial probiotics (CP), and control (C). PM utilized an independent formula with starter bacteria *Lactobacillus casei* and PK contained bacteria *Bacillus* sp., *Pseudomonas* sp., *Nitrosomonas* sp., *Aerobacter* sp., *Nitrobacter* sp. Probiotics were fermented 24 hours before their application to the maintenance medium. A total of 15 shrimp/aquarium measuring 3.29 ± 0.48 g were reared for 27 days after the floc was first formed. The results showed that the growth and feed conversion ratio of vannamei shrimp in the biofloc system was higher than that of the control (P<0.05). The growth and feed conversion ratio between probiotic treatments did not show significantly different results (P>0.05). Survival in treatment and control groups was not significantly different (P>0.05). The observed floc volumes which increased during the rearing of vannamei shrimp were up to 6.50-7.50 mL/L. Several types of organisms found in the flock included nematodes, phytoplankton, copepods, and protozoa. The observed water quality was the same in each treatment except for higher dissolved oxygen and ammonia in the control. This study recommends the use of independent probiotics in vannamei shrimp culture based on biofloc technology.

INTRODUCTION

Vannamei shrimp is a commodity with high economic value and market demand increasing every year because vannamei shrimp has a soft texture and distinctive taste (FAO, 2020). The intensification of vannamei shrimp cultivation which is increasingly being carried out causes problems, such as high operational costs, shrimp culture waste that pollutes the environment, and the problem of water needs as a cultivation medium (Amir *et al.*, 2018). One

Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

technology considered to be able to overcome these problems is biofloc technology. This technology has experienced rapid development, has been applied to various commodities that can increase feed efficiency by up to 10-20%, and is supported by optimal water quality (Pantjara *et al.*, 2010). Biofloc technology is considered environmentally friendly because it can utilize aquaculture waste in rich protein feed sources that are good for shrimp (Burford *et al*., 2004).

The principle of biofloc is to recycle the nutrients from the excretion of shrimp and the rest of the feed by heterotroph bacteria into bacterial biomass that can be utilized by the shrimp so that the water quality is better and maintained. Bioflocs consist of various microbial communities such as protozoa, bacteria, and zooplankton containing vitamins, amino acids methionin, enzymes, and minerals that can be used as food supplements to help shrimp digestion so that bioflocs will save the use of shrimp feed in a pond. One of the important elements in the application of biofloc is the presence of probiotic bacteria. Probiotic bacteria play a role in the process of recycling nutrient waste to be utilized by shrimp cultured in a biofloc system (Masithah *et al.*, 2016).

Probiotics are microbial agents that can provide beneficial effects for the host because they can increase the nutritional value of feed, improve the performance of the immune response to disease, and improve the quality of the culture environment (Verschuere *et al.*, 2000). The advantages of probiotics can stimulate the growth of cultured organisms, increase feed effectiveness (Hamka *et al.*, 2021a), improve immune performance (Hamka *et al.*, 2021b), maintain optimal water quality, increase feed conversion ratio, inhibit pathogenic bacteria growth (Hamka *et al.*, 2021b; Aly *et al.*, 2008; Kesarcodi-Watson *et al.*, 2008; Nayak, 2010). Research on the use of various types of probiotics in biofoc systems has been carried out. The results show that different types of probiotics produce different outputs. Commercial probiotics can increase growth and feed conversion ratio in biofloc-based freshwater fish farming (Suprianto *et al.*, 2019; Kurniaji *et al.*, 2021). The use of commercial probiotics in vannamei shrimp bioflocs has also been widely carried out and can have a positive effect on the growth of vannamei shrimp (Setyono *et al.*, 2019).

Various types of commercial probiotics can now be easily found. The content of bacteria and nutrients facilitates shrimp cultivation for cultivators. The development of commercial probiotics on the market makes people innovate to make independent probiotics. This kind of probiotic is made by the community because the price is relatively affordable when compared to commercial ones, it is easy to manufacture, and the ingredients obtained are derived from herbal plants from the surrounding environment. The use of independent probiotics in the biofloc system is expected to facilitate cultivators in their application in the field. In addition, the use of independent probiotics can reduce production costs because farmers generally use technology with low input costs (Putri *et al.*, 2020). The effectiveness of independent probiotics use has not been tested together with commercial probiotics in the vannamei shrimp biofloc system.

This study aimed to analyze the differences in the yield of vannamei shrimp in a biofloc system using commercial probiotics and independent probiotics carried out on a laboratory scale.

METHODOLOGY

Ethical Approval

There are no animals harmed or improperly treated during the research. The test animals in this study were treated properly according to the optimal environment, starting from handling

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

technique, water quality, availability of feed, etc.

Place and Time

This research was conducted in June-July 2022 at the Teaching Factory (TEFA) Fisheries Cultivation Engineering Study Program, Polytechnic of Marine and Fisheries Bone, Bone Regency, South Sulawesi.

Research Materials

The research material used was vannamei shrimp measuring 3.29 ± 0.48 g, which was obtained from the training pond of the Bone Marine and Fisheries Polytechnic after 35 days of intensive rearing (DOC 35) in plastic ponds. The container used for maintenance was an aquarium measuring 70×50×50 cm which could accommodate 100 L of water. The water used came from sea waters around Waetuwo Urban Village, Bone Regency. Bacterial cultures were sampled from the maintenance media using Tryptone Soya Agar/TSA (HiMedia Laboratories Pvt. Ltd, India) and TCBS media (HiMedia Laboratories Pvt. Ltd, India), which were prepared with sterile aquadest (Onemed, Indonesia). The other tools used were: digital scale with an accuracy of 0.01 g, fishnet, jar, imhoff cone, aerator, DO meter (YSI Pro20, USA), pH meter (Eutech, USA), refractometer (Bright Scientific Co., Japan), thermometer (YSI Pro20, USA), ammonia, nitrate, nitrite, and phosphate test kits (Merck KgaA, Germany).

The probiotics used were commercial probiotics and independent probiotics. The commercial probiotic used was Super NB™ (PT CP Prima Tbk, Indonesia) which contained several types of bacteria, namely *Bacillus* sp., *Pseudomonas* sp., *Nitrosomonas* sp., *Aerobacter* sp., *Nitrobacter* sp. The independent probiotics used were Yakult™ (PT. Yakult Indonesia Persada, Indonesia) which contained *Lactobacillus casei* bacteria, and Fermipan™ (S.I.L, France) containing *Saccharomyces cerevisiae*. Before use, probiotics were fermented for 24 hours. The materials used for the manufacture of probiotic fermentation as an independent probiotic can be seen in the following table.

Table 1. Composition of ingredients for fermentation in the independent probiotic.

Independent Probiotic (PM)		Commercial Probiotic (PK)	
Material Type	Amount	Material Type	Amount
Brackish water	1 L	Brackish water	1 L
Molasses	40 mL	Molasses	100 mL
Yakult™	1 Bottle	Super NB™	25 mL
Fermipan™	2 g	Fermipan™	2 g
Ginger	10 g	Flour	100 g
Turmeric	10 g		
Curcuma	10 g		

The addition of ginger, turmeric, and temulawak as fermenters for probiotic production according to the research before in fish. The fermenter also plays a role in increasing feed absorption and triggering fish appetite (Mansyur and Tangko, 2008). This material is a good immunostimulant for the body's resistance to fish (Hariani and Purnomo, 2017).

Research Design

This study used a completely randomized design (CRD) with 3 treatments and 4 replications. Each treatment unit was placed randomly and conditioned homogeneously. The treatment in this study is as follows; Independent Probiotics (IP): vannamei shrimp reared in a biofloc system using probiotic fermentation made with an independent formula, Commercial

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

Probiotics (CP): vannamei shrimp reared in a biofloc system using commercial probiotic fermentation, Control (C): vannamei shrimp reared without using a biofloc system.

Work Procedure

The research container was an aquarium cleaned using a brush and detergent. Then, the aquarium was filled with water sterilized using 15 ppm of chlorine™ (Tjiwi Kimia, Indonesia) and given strong aeration for 7 days to remove residual chlorine. Each aquarium was equipped with an aeration system and filled with 100 L of water. To ensure that the water was sterile, bacterial sampling was carried out using TCBS and TSA. The bacteria sampling procedure followed the method of Kurniaji *et al.* (2020). The probiotics used were firstly cultured using several materials according to Table 1. Fermentation was carried out for 24 hours before the growth of biofloc on the maintenance media. Biofloc was grown by adding the material gradually to all aquariums according to Table 2. The water was left aerated for 10 days for floc formation (Sumitro *et al.*, 2020). Feed pellets were used as a source of N to grow flocks with a C:N ratio of 15 according to the modified method of Husain *et al.* (2014). After the floc was formed, the number of shrimps included in the aquarium was 15 shrimps/aquarium. During 27 days of rearing, the shrimps were fed with a feeding rate of 10% using commercial pellet feed of the Irawan™ brand containing 30% protein in the morning, afternoon, and evening.

Measurements of water quality parameters according to the method of Yassien *et al.* (2019) included temperature using thermometer, pH using pH meter, salinity using refractometer and DO using DO meter, those which were carried out every morning and evening. Measurements of ammonia, nitrate, nitrite, and phosphate using test kit were carried out at the beginning, middle, and end of the study. Every 7 days, growth sampling was in the form of floc volume, and flock observations were carried out under a microscope.

Table 2. Composition of ingredients for biofloc.

Material Type	Amount	Time of Application
Water	100 L	Initial Time of Research
Coarse Salt	100 g	Initial Time of Research
Probiotic (due to treatment)	3 mL	During The Research
Molasses	10 mL	During The Research
Calcium Oxide	5 g	Initial Time of Research
Tapioca Flour	10 g	During The Research
Shrimp Pellet	10 g	Initial Time of Research

Note: initial time is the application of material at the time of starting the research (day 1), during the research is the application of materials during of study.

Parameters observed included shrimp weight gain, daily growth rate, absolute growth rate, survival, feed conversion ratio, flock volume, flock condition, and water quality. Measurement of flock volume referred to Avnimelech (2012) using an imhoff cone. Flock observation refers to the method of Rajkumar *et al.* (2015) using a microscope. Daily growth rate (DGR) was calculated using a formula based on Kurniaji *et al.* (2018) as follows:

$$DGR = \frac{\text{Ln Wt} - \text{Ln Wo}}{t} \times 100$$

Where:
DGR = daily growth rate (%)
Wt = final weight (g)
Wo = initial weight (g)
t = maintenance time (days)

The absolute growth rate was obtained from the difference between the

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

final and initial weight of the shrimp. The equation used to measure the absolute growth rate (AGR) was based on Effendie (1997) as follows:

$$AGR = Wt - Wo$$

Where:
AGR = absolute growth rate (g)
Wt = final weight (g)
Wo = initial weight (g)

Survival was the number of live shrimps at the end of rearing. The equation used to calculate the survival rate (SR) referred to Effendie (1997) as follows:

$$SR = \frac{Nt}{No} \times 100$$

Where:
SR = survival rate (%)
Nt = final number of fish (tails)
No = initial number of fish (tails)

Feed conversion ratio was the ratio of feed amount needed to produce shrimp weight. The equation used to calculate the feed conversion ratio was based on Zonneveld *et al.* (1991) as follows:

$$FCR = \frac{F}{(Wt + D) - Wo}$$

Where:
FCR = feed conversion ratio
F = amount of feed (g)
Wt = final shrimps' biomass (g)
D = dead shrimps (g)
Wo = initial shrimps' biomass (g)

Data Analysis

Data on growth, survival, feed conversion ratio, bacterial population, and floc volume were statistically analyzed by the ANOVA method (Kolmogorov-Smirnov) and Duncan test using the SPSS Version 16.0 program. The data from the observation of flocks and water quality were analyzed descriptively and quantitatively.

RESULTS AND DISCUSSION

Weight Gain

The weight gain of vannamei shrimp was measured at each observation time, namely on days 1, 7, 14, 21, and 27. The measurement results showed an increase in individual weight at each observation time (Figure 1).

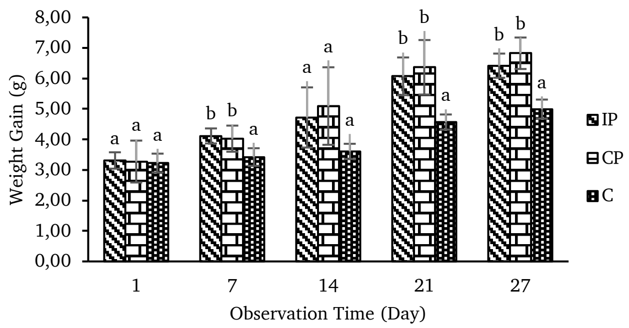

Figure 1. Weight gain of vannamei shrimp during 27 days of rearing (differences in letter notation indicate significant differences between treatments). Description: IP (independent probiotic), CP (commercial probiotic), C (control).

The results of the ANOVA test showed that the weight gain of vannamei shrimp per individual on the 1st day of observation was not significantly different between treatments (P>0.05) and the weight of each treatment was 3.31±0.27 g, 3.28± 0.69 g and 3.23 ± 0.31 g, where the shrimp used were uniform at the beginning of the treatment. This is by the statement of Karimah *et al.* (2018) that measuring growth performance requires a uniform size of the test organism. The weight gain observed was significantly different (P<0.05) on days 7, 21 and 27.

The weight of shrimp on IP (independent probiotic) and CP (commercial probiotic) was significantly different from C (control). The results of this study showed that the treatment with probiotics in the biofloc system was able to

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

significantly increase the growth of vannamei shrimp. This is by the results of research by Pantjara *et al.* (2010) that the application of biofloc in shrimp farming intensively can increase growth. The activity of probiotic bacteria in the waste cycle process becomes a food source that can trigger better shrimp growth (Avnimelech, 2012).

The treatment of commercial and independent types of probiotics had no significant effect on the weight gain of vannamei shrimp at each observation time. This shows that the two types of probiotics used could increase the growth of shrimp. The commercial probiotics used contained *Bacillus* sp. which played a role in growth. According to Lestari *et al.* (2021), the addition of *Bacillus* sp. in biofloc can accelerate catfish growth and feed efficiency. *Bacillus* sp can work on the digestive system and produce exogenous enzymes that can improve digestive performance (Ekasari *et al.*, 2010). Probiotics *Nitrosomonas* sp, *Aerobacter* sp., and *Nitrobacter* sp. were nitrifying bacteria that improved water quality in the biofloc system and supported growth (Suprapto, 2012). The independent probiotic used was derived from *L. casei* bacteria which was added with fermipan containing *S. cerevisiae*. According to Putri *et al.* (2015), probiotic EM-4 containing *L. casei* and *S. cerevisiae* can play a role in the biofloc system and increase the growth of cultured organisms, so that the commercial and independent probiotics play a role in the growth of shrimp.

Daily Growth Rate and Absolute Growth Rate

The daily growth rate and absolute growth rate were observed to be different between the treatment and control. The results of observations of the daily growth rate and absolute growth rate of vannamei shrimp can be seen in Figure 2.

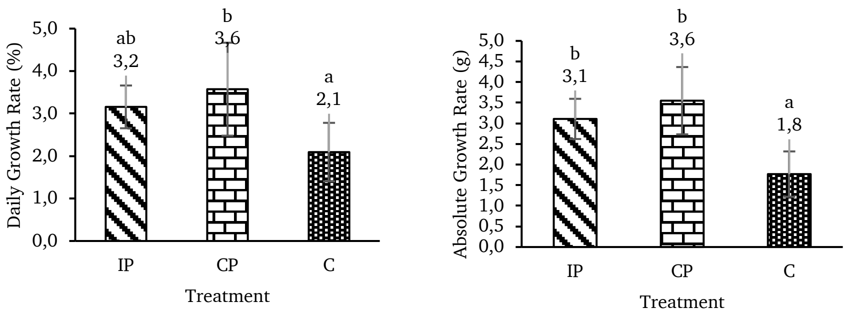

Figure 2. Daily growth rate and absolute growth rate (differences in letter notation indicate significant differences between treatments). Description: IP (independent probiotic), CP (commercial probiotic), C (control).

The results of the ANOVA test showed that the daily growth rate and absolute growth rate were significantly different between treatments. Duncan's further test showed that the daily growth rate of IP (independent probiotic) was not significantly different from CP (commercial probiotic) and C (control). In addition, CP (commercial probiotic) was significantly different from C. The absolute growth rate of IP (independent probiotic) was not significantly different from CP (commercial probiotic) but significantly different from C ($P<0.05$). This study showed that the use of probiotics, both commercial and independent, was able to increase the growth of vannamei shrimp. Probiotics *Bacillus* sp., *Pseudomonas* sp., *Nitrosomonas* sp., *Aerobacter* sp., *Nitrobacter* sp., and *Lactobacillus* sp were bacteria that played a role in the biofloc system.

Bacillus sp. could work on the digestive system, whereas bacteria *Nitrosomonas* sp., *Aerobacter* sp., and *Nitrobacter* sp. were nitrifying bacteria that improved water quality. *Lactobacillus* sp is an aerobic heterotrophic bacterium that plays a role in floc formation (Suprapto, 2012). Biofloc is a cultivation system focusing on more effective nutrient inputs requiring a nutrient recycling process carried out by heterotrophic bacteria. Several types of heterotrophic bacteria are bacteria *Bacillus* sp and *Lactobacillus* sp. (Widanarni *et al.*, 2012).

In addition, there was no difference in growth between vannamei shrimp reared in a biofloc system with commercial probiotics and independent probiotics. The types of probiotic bacteria used in both treatments were bacteria that played a role in floc formation so these probiotic bacteria had the potential to increase the growth of vannamei shrimp. The use of *Bacillus* sp. on shrimp culture with biofloc technology has been evaluated by Adipu *et al.* (2019). The results showed that these bacteria were able to improve the growth performance of shrimp. Likewise, the use of *L. casei* bacteria was evaluated by Putri *et al.* (2015) which showed positive results on the growth of cultured organisms. Other bacteria were heterotrophic bacteria those which played a role in recycling wastewater so that they could be utilized by shrimp. Manan *et al.* (2020) stated that biofloc is an aggregation of algae, bacteria, protozoa, and organic particles such as uneaten feed. Heterotrophic bacteria such as *Pseudomonas* sp. are commonly found in biofloc.

Survival Rate

The results of the ANOVA test showed that the observed survival rates were not significantly different between the treatment and control (P>0.05). The results of observations on the survival of vannamei shrimp can be seen in Figure 3.

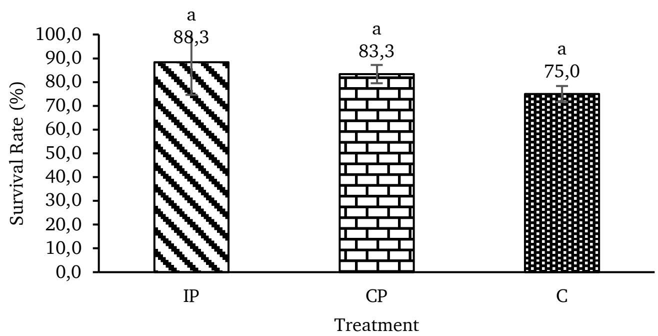

Figure 3. Vannamei shrimp survival after 27 days of rearing (same letter notation shows results that are not significantly different between treatments). Description: IP (independent probiotic), CP (commercial probiotic), C (control).

Based on the results of the study, probiotic treatment on biofloc media did not affect the viability of vannamei shrimp. The survival rate of vannamei shrimp was not significantly different, presumably due to the absence of pathogenic bacterial infection in culture media. Manan *et al.* (2020) stated that biofloc media contaminated with pathogenic bacteria such as *Vibrio* sp. can reduce the survival rate of vannamei shrimp. Although numerically the results of statistical tests were not significantly different, there was a tendency for higher survival in shrimp reared in biofloc systems. According to Chethurajupalli and Tambireddy (2022), the application of biofloc can increase the viability of vannamei shrimp which is higher than the control or without biofloc.

Shrimp survival without biofloc was 62% and in biofloc >70%. The ability of

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

doi : 10.20473/jafh.v12i3.37996

biofloc to increase survival and growth has long been reported by several studies (Xu dan Pan, 2012; Shilta *et al.,* 2020; Panigrahi *et al.,* 2020). The presence of probiotic bacteria can help improve digestive performance, improve air quality, and suppress bacterial pathogens so that shrimp can grow properly (Long *et al.,* 2015). This is due to the ability of the biofloc system to form an optimal environment for the growth and survival of white shrimp.

Feed Conversion Ratio

The observed feed conversion ratio was significantly different between the treatment and control (P<0.05). The shrimp feed conversion ratio in PM (independent probiotic) treatment was not significantly different from PK (commercial probiotic) (P>0.05) but significantly different from K (control) (P<0.05).

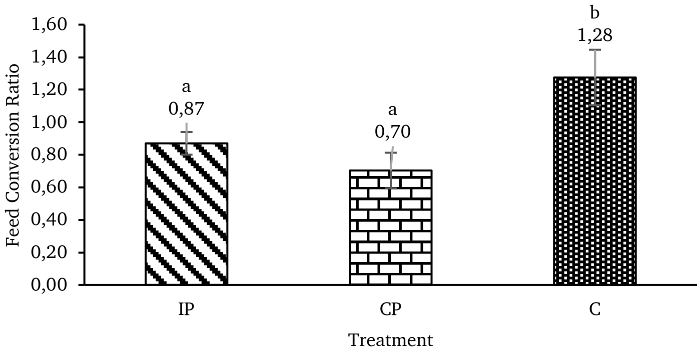

Figure 4. Vannamei shrimp feed conversion ratio (differences in letter notation indicate significant differences between treatments). Description: IP (independent probiotic), CP (commercial probiotic), C (control).

The results of observations of feed conversion ratios showed that the use of probiotic bacteria in biofloc was able to increase the effectiveness of feed utilization. This had an impact on increasingly optimal growth. Similar results were found by Adipu *et al.* (2019), stating that the application of biofloc technology in shrimp farming can reduce the value of the feed conversion ratio and increase growth. Several previous studies also found that the vannamei shrimp feed conversion ratio was better in the biofloc system (Xu and Pan, 2012; Emerenciano *et al.*, 2013; Krummenauer *et al.*, 2014). This effective use of feed is thought to be due to the presence of probiotic bacteria that can work on the shrimp digestive system so that feed digestibility is better. Ridho and Subagiyo (2013) stated that the low feed conversion ratio is caused by the performance of probiotic bacteria to produce extracellular enzymes that increase food digestibility in the shrimp intestines. *Bacillus* sp. are probiotic bacteria that can work on the digestive system thereby increasing nutrient digestibility (De *et al.*, 2018).

The feed conversion ratio between commercial and independent probiotic treatments showed results that were not significantly different. Although the feed conversion ratio data showed a higher trend in commercial probiotics. Agustama *et al.* (2021) stated that the probiotic *Bacillus* sp. mixed in the feed showed a better performance than *L. casei* in the growth performance of vannamei shrimp. In addition to the presence of bacteria acting on the digestive system, the low feed conversion ratio is also thought to be due to the presence of floc which is an additional food for shrimp. According to Ju *et al.* (2008), biofloc provides additional protein, fat, minerals, vitamins, and other bioactive compounds such as

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

carotenoids, bromophenols, chlorophyll, phytosterols, and amino sugars.

Floc Volume

The observed floc volume increased from the 1st day to the 27th day of observation. Floc volume was not significantly different between treatments during the 27-day vannamei shrimp rearing period (P>0.05). The results of the observation of floc volume can be seen in the following figure.

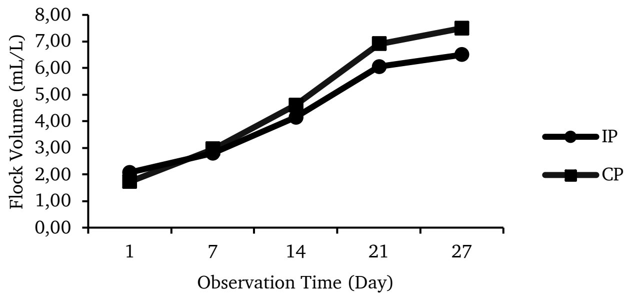

Figure 5. Floc volume during 27 days of maintenance.

The flock volume increased gradually from the beginning to the end of the observation. Maximum floc volume was observed up to 6.50 mL/L in independent probiotic aquariums and 7.50 mL/L in commercial probiotics. The floc volumes observed in all treatments were still in the optimal range for the growth of vannamei shrimp. According to Avnimelech (2012), the maximum floc volume for shrimp growth is 50 mL/L. Flock volume greater than 50 mL will cause depletion of dissolved oxygen and interfere with shrimp growth. Efforts to maintain the volume of floc to remain in the optimal range are to add water to dilute the floc content.

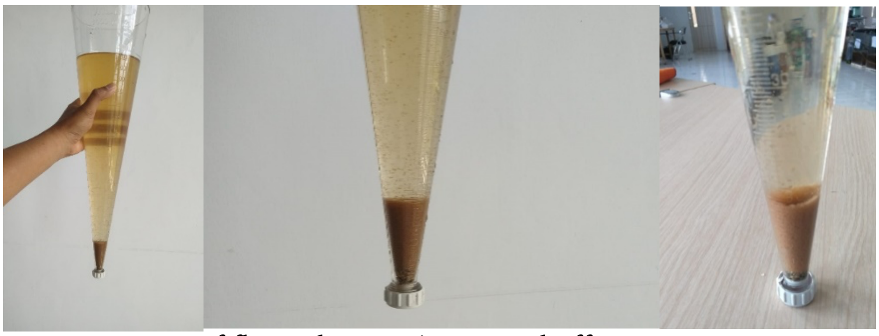

Figure 6. Measurement of floc volume using an Imhoff cone.

Floc Observation

The observation of the flocks showed that there were differences in the density of the flocks at each time of observation. This was in line with the increasing floc volume during the vannamei shrimp rearing period. The floc observed under the microscope was formed from various elements of organisms and organic matter. Observations showed the presence of copepods, nematodes, protozoa and several types of phytoplankton (unidentified). This is in accordance with Rajkumar *et al.* (2015) who found that there were several types of organisms in the vannamei shrimp floc which were dominated by zooplankton and phytoplankton, including tintinids, copepods, spirulina, ciliates and nematodes.

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

doi : 10.20473/jafh.v12i3.37996

This community is generally present in the maintenance media of the biofloc system and will decrease with shrimp rearing. Shrimp take advantage of the presence of plankton as a food source. Plankton contains nutrients that are important for shrimp (Moss *et al.*, 2001). Chethurajupalli and Tambireddy (2022) also reported the presence of rotifers, copepods, nematodes, and ciliated protozoa in the biofloc system. Biofloc consists of 98% flagellates, 1.5% rotifers and 0.5% amoeba (Ju *et al.*, 2008). This is similar to the report of Manan *et al.* (2017) who stated the presence of nematodes, gastrotrichs, euplotes, protozoa, rotifers, and copepods in the vannamei shrimp biofloc system (Figure 7).

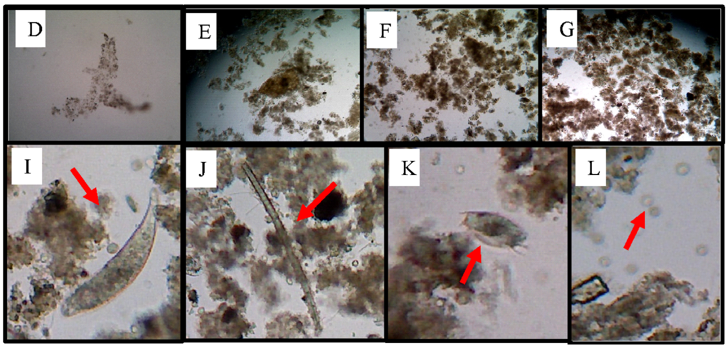

Figure 7. Observations of flocks under a microscope with a magnification of 10×/0.25. The condition of the flock on the 7th day (D), the condition of the flock on the 14th day (E), the condition of the flock on the 21st day (F), and the condition of the flock on the 27th day (G). Microorganisms in the floc: nematodes (I), phytoplankton (J), copepods (K), protozoa (L).

Water Quality

Water quality measurement data includes temperature, salinity, dissolved oxygen, pH, ammonia, nitrite, nitrate, and phosphate. The water quality during the study was still within the standard range except nitrate and nitrite according to Boyd and Gautier (2000). Temperature and salinity are below MMF standards (2016) because maintenance is carried out indoors and water comes from brackish waters which are commonly used in the surrounding area. The other parameters are in accordance with the standard.

Measurements of temperature, salinity, and pH showed the same results in the treatment and control. The measurement of dissolved oxygen was observed to be different in the treatment and control. This indicates that there was a higher utilization of dissolved oxygen in the treatment than in the control. According to Chethurajupalli and Tambireddy (2022), dissolved oxygen in biofloc media is lower than without biofloc. This is due to the high activity of heterotrophic bacteria that utilize oxygen to remodel organic matter. The rate of nitrification and respiration of microorganisms results in an increase in carbon dioxide levels in the floc and a decrease in dissolved oxygen levels. Ammonia, nitrite, nitrate, and phosphate levels were also observed to be no different in the biofloc and control media. The difference lay in the range of ammonia levels of independent and commercial probiotics which were lower at the beginning of the observation.

According to Tong *et al.* (2020), the input ratio of C:N 12-15 helps heterotrophic bacteria to decompose more ammonia in the biofloc system. The activity of nitrifying bacteria is able to keep ammonia at low levels (Anand *et al.*, 2014). Previous studies also found that low ammonia was found in biofloc media (Rajkumar *et al.*, 2015; Bossier and Ekasari, 2017). The results of measuring water quality in all treatment units during

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

the rearing of vannamei shrimp are as follows.

Table 3. Results of water quality measurements during vannamei shrimp rearing.

Parameter	Independent Probiotic (IP)	Commercial Probiotic (CP)	Control (Without Biofloc)	Standard (Boyd and Gautier, 2000)	Standard (MMF, 2016)
Temperature (°C)	25,70-31,20	26,20-31,40	25,90-31,10	24 – 28	28 -30
Salinity (ppt)	18,00-25,00	18,00-25,00	18,00-25,00	15 – 25	26 – 32
Dissolved Oxygen (ppm)	5,70-6,90	5,58-6,20	6,12-6,88	>5	> 4
pH	6,70-8,40	6,58-8,17	6,83-8,54	6,0 – 9,0	7,5 – 8,5
Ammonia (NH_3) (ppm)	0,00-0,80	0,00-0,80	0,40-0,80	< 0,1	< 0,1
Nitrite (NO_3) (ppm)	0,15-0,50	0,15-0,50	0,15-0,50	0,05	< 1,0
Nitrate (NO_2) (ppm)	2,30-5,60	2,30-5,60	2,30-5,60	0,3	< 0,5
Phosphat (PO_4) (ppm)	0,25-1,50	0,25-0,50	0,25-0,50	< 0,3	< 0,1-5

The results of this study provide information that independent probiotics have the potential to be used because of various advantages, namely the growth and feed conversion ratio produced were no different from biofloc using commercial probiotics. In addition, independent probiotics use ingredients derived from herbal plants in the surrounding environment, making it easier for cultivators to produce. The use of independent probiotics in the biofloc system is expected to make it easier for cultivators to apply them and not incur large costs to procure the ingredients.

CONCLUSION

The results showed that there was no difference in growth performance, feed conversion ratio, viability, floc volume, and water quality on biofloc media from commercial and independent probiotic treatments. This study recommends for use of independent probiotics because it can produce the same result performance as commercial probiotics. Vannamei shrimp reared in the biofloc system showed better growth and feed conversion ratios compared to those without biofloc.

CONFLICT OF INTEREST

There is no conflict of interest among all authors upon writing and publishing the manuscript.

AUTHOR CONTRIBUTION

The contribution of each author is the following: Ardana Kurniaji, Diana Putri Renitasari, and Siti Aisyah Saridu were collecting, analyzing data, and drafting the manuscript. Yunarty and Anton participated in the conception of experimental design.

ACKNOWLEDGMENT

Thank you to Polytechnic of Marine and Fisheries of Bone students who have helped carry out this research.

REFERENCES

Adipu, Y., Lumenta, C., Mangindaan, R.E.P. and Manoppo, H., 2019. Growth performance of *Litopenaeus vannamei* grown in biofloc system produced from different carbohydrate sources. *AACL Bioflux*, *12*(2), pp.472-479. http://www.bioflux.com.ro/docs/2019.472-479.pdf

Agustama, Y., Lestari, T.A., Verdian, A.H., Witoko, P. and Marlina, E., 2021. Maintenance of *Litopenaeus vannamei* post larva stadia with

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

addition probiotics EM4 and *Bacillus* sp. to artificial feed on the growth and survival. *Jurnal Perikanan Terapan (PERANAN), 2*, pp.39-44. https://jurnal.polinela.ac.id/PERANAN/issue/view/123

Aly, S.M., Mohamed, M.F. and John, G., 2008. Effect of probiotics on the survival, growth and challenge infection in tilapia nilotica (*Oreochromis niloticus*). *Aquaculture Research*, *39*(6), pp.647-656. https://doi.org/10.1111/j.1365-2109.2008.01932.x

Amir, S., Setyono, B.D.H., Alim, S. and Amin, M., 2018. Aplikasi Teknologi Bioflok pada Budidaya Udang Vaname (*Litopenaeus vannamei*). *Prosiding PKM-CSR*, *1*, pp.660–666. http://www.prosiding-pkmcsr.org/index.php/pkmcsr/article/view/245

Anand, P.S.S., Kohli, M.P.S., Kumar, S., Sundaray, J.K., Roy, S.D., Venkateshwarlu, G., Sinha, A. and Pailan, G.H., 2014. Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture, 418-419*, pp.108-115. http://dx.doi.org/10.1016/j.aquaculture.2013.09.051

Avnimelech, Y., 2012. *Biofloc technology - A practical guide book*, 2nd Edition. The World Aquaculture Society, Baton Rouge, Louisiana, United States. p.258.

Bossier, P. and Ekasari, J., 2017. Biofloc technology application in aquaculture to support sustainable development goals. *Microbial Biotechnology*, *10*(5), pp.1012–1016. https://doi.org/10.1111/1751-7915.12836

Boyd, C.E. and Gautier, D., 2000. Effluent composition & water quality standards. *Global Aquaculture Alliance*, pp.61-66. https://www.researchgate.net/profile/Claude-Boyd/publication/285843894_Effluent_composition_and_water_quality_standards/links/57bdf35a08aeb95224d05fb1/Effluent-composition-and-water-quality-standards.pdf

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. and Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture, 232*(1-4), pp.525–537. https://doi.org/10.1016/S0044-8486(03)0054 1-6

Chethurajupalli, L. and Tambireddy, N., 2022. Rearing of white leg shrimp *Litopenaeus vannamei* (Boone, 1931) in biofloc and substrate systems: microbial community of water, growth and immune response of shrimp. *Turkish Journal of Fisheries and Aquatic Sciences*, *22*(3), TRJFAS20130. http://doi.org/10.4194/TRJFAS20130

De, D., Raja, R.A., Ghoshal, T.K., Mukhrjee, S. and Vijayan, K.K., 2018. Evaluation of growth, feed utilization efficiency and immune parameters in tiger shrimp (*Penaeus monodon*) fed diets supplemented with or diet fermented with gut bacterium *Bacillus* sp. DDKRC1 isolated from gut of Asian seabass (*Lates calcalifer*). *Aquaculture Research, 49*(6), pp.2147-2155. https://doi.org/10.1111/are.13669

Effendie, M.I., 1997. *Biologi Perikanan*. Yayasan Pustaka Nusatama. Yogyakarta. p.163.

Ekasari, J., Crab, R. and Verstraete, W., 2010. Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. *HAYATI Journal of Biosciences, 17*(3), pp.125–130. https://doi.org/10.4308/hjb.17.3.125

Emerenciano, M., Gaxiola, G. and Cuzon, G., 2013. Biofloc Technology (BFT): A review for aquaculture application and animal food industry. In: Matovic M. D. (ed) Biomass now–

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

cultivation and utilization. *InTech,* pp.301-328. http://dx.doi.org/10.5772/53902

[FAO] Food and Agriculture Organization. 2020. *The state of world fisheries and aquaculture (2020)*. Sustainability in action, FAO, Rome.

Hamka, M.S., Meryandini, A., Widanarni, Wahidin, L. and Kurniaji, A., 2021a. Peran probiotik *Bacillus megaterium* PTB I.4 dan *Pediococcus pentosaceus* E2211 dalam meningkatkan pertumbuhan dan konsumsi pakan ikan lele (*Clarias* sp.). *Jurnal Perikanan Darat dan Pesisir, 1*(2), pp.46-54. https://www.journal.univbinainsan.ac.id/index.php/jpdp/article/view/52

Hamka, M.S., Meryandini, A., Widanarni and Kurniaji, A., 2021b. Efek probiotik *Bacillus megaterium* PTB I.4 dan *Pediococcus pentosaceus* E2211 terhadap respons imun dan kelangsungan hidup ikan lele (*Clarias* sp.) selama uji tantang *Aeromonas hydrophila. Journal of Fisheries and Marine Research, 5*(3), pp.567-577. https://doi.org/10.21776/ub.jfmr.2021.005.03.9

Hariani, D. and Purnomo, T., 2017. Pemberian probiotik dalam pakan untuk budidaya ikan lele. *Stigma Journal of Science, 10*(1), pp.31-35. https://doi.org/10.36456/stigma.vol10.no1.a582

Husain, N., Putri, B. and Supono, 2014. Comparasion of carbon and nitrogen in the biofloc system on the growth of red tilapia (*Oreochromis niloticus*). *e-Jurnal Rekayasa dan Teknologi Budidaya Perairan, 3*(1), pp.343-350. https://jurnal.fp.unila.ac.id/index.php/bdpi/article/view/472

Ju, Z.Y., Forster, I., Conquest, L., Dominy, W., Kuo, W.C. and Horgen, F.D., 2008. Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. *Aquaculture Research*, *39*(2), pp.118-133. https://doi.org/10.1111/j.1365-2109.2007.01856.x

Karimah, U., Samidjan, I. and Pinandoyo, 2018. Growth perfomance and survival of gift tilapia (*Oreocromis niloticus*) given different amounts of feed. *Journal of Aquaculture Management and Technology, 7*(1), pp.128-135. https://ejournal3.undip.ac.id/index.php/jamt/article/view/20378

Kesarcodi-Watson, A., Kaspar, H., Lategan, M.J. and Gibson, L., 2008. Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, *274*(1), pp.1–14. https://doi.org/10.1016/j.aquaculture.2007.11.019.

Krummenauer, D., Poersch, L., Romano, L.A., Lara, G.R., Encarnaçạo, P. and Wasielesky, Jr, W., 2014. The effect of probiotics in a *Litopenaeus vannamei* biofloc culture system infected with *Vibrio parahaemolyticus*. *Journal of Applied Aquaculture*, *26*(4), pp.370-379. https://doi.org/10.1080/10454438.2014.965575

Kurniaji, A., Idris, M. and Muliani, 2020. Inhibition test of mangrove leaf extract (*Sonneratia alba*) on bacteria *Vibrio harveyi* secara *in vitro. Jurnal Sains Teknologi Akuakultur, 3*(2), pp.84-92. https://jmai.aquasiana.org/index.php/jmai/article/view/60

Kurniaji, A., Nuryati, S., Murtini, S. and Alimuddin, 2018. Maternal immunity response and larval growth of anti cyhv-3 dna vaccinated common carp (*Cyprinus carpio*) at different pre-spawining time. *Pakistan Journal of Biotechnology, 15*(3), pp.689-698. https://pjbt.org/index.php/pjbt/article/view/362

Kurniaji, A., Yunarty, Anton, Usman, Z., Wahid, E. and Rama, K., 2021. Pertumbuhan dan konsumsi pakan ikan nila (*Oreochromis niloticus*) yang dipelihara pada kepadatan berbeda dengan sistem bioflok. *Jurnal Sains Akuakultur Tropis, 5*(2), pp.197-203. https://doi.org/10.14710/sat.v5i2.11824

Lestari, D.P., Azhar, F. and Marzuki, M., 2021. The effect of biofloc with the addition of different commercial probiotic in catfish (*Clarias* sp.). *Jurnal Biologi Tropis, 21*(2), pp.361-367. https://doi.org/10.29303/jbt.v21i2.2552

Long, L., Yang, J., Li, Y., Guan, C. and Wu, F., 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). *Aquaculture*, *448*. https://doi.org/10.1016/j.aquaculture.2015.05.017

Manan, H., Safwan, A.A., Kasan, N.A. and Ikhwanuddin, M., 2020. Effects of biofloc application on survival rate, growth performance and specific growth rate of pacific whiteleg shrimp, *Penaeus vannamei* culture in closed hatchery system. *Pakistan Journal of Biological Sciences, 23*(12), pp.1563-1571. https://doi.org/10.3923/pjbs.2020.1563.1571

Manan, H., Moh, J.H.Z., Kasan, N.A., Suratman, S. and Ikhwanuddin, M., 2017. Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system. *Applied Water Science*, *7*, pp.2437–2446. https://doi.org/10.1007/s13201-016-0421-4

Mansyur, A. and Tangko, A.M., 2008. Probiotik: Pemanfaatannya untuk pakan ikan berkualitas rendah. *Media Akuakultur, 3*(2), pp.145-149. http://dx.doi.org/10.15578/ma.3.2.2008.145-149

Masithah, E.D., Octaviana, Y.D. and Manan, A., 2016. Pengaruh perbedaan probiotik komersial terhadap rasio C:N dan N:P media kultur bioflok pada bak percobaan. *Journal of Aquaculture and Fish Health, 5*(3), pp.118-125. http://dx.doi.org/10.20473/jafh.v5i3.11333

[MMF] Ministry of Marine Affairs and Fisheries Republic of Indonesia. *General guidelines for raining tiger shrimp (Penaeus monodon) and vaname shrimp (Litopenaeus vannamei)*. Ministerial regulation number 75/Permen-KP/2016.

Moss, S.M., Divakaran, S. and Kim, B.G., 2001 Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp *Litopenaeus vannamei* (Boone). *Aquaculture Research, 32*(2), pp.125–131. https://doi.org/10.1046/j.1365-2109.2001.00540.x

Nayak, S.K., 2010. Probiotics and immunity: A fish perspective. *Fish & Shellfish Immunology*, *29*(1), pp.2–14. https://doi.org/10.1016/j.fsi.2010.02.017

Panigrahi, A., Das, R.R., Sivakumar, M.R., Saravanan, A., Saranya, C., Sudheer, N.S., Vasagam, K.P.K., Mahalakshmi, P., Kannappan, S. and Gopikrishna, G., 2020. Bio-augmentation of heterotrophic bacteria in biofloc system improves growth, survival, and immunity of Indian white shrimp *Penaeus indicus*. *Fish & Shellfish Immunology*, *98*, pp.477-487. https://doi.org/10.1016/j.fsi.2020.01.021

Pantjara, B., Nawang, A., Usman and Rachmansyah, 2010. Budidaya udang vaname sistem bioflok. *Media Akuakultur*, *5*(2), pp.93–97. https://doi.org/10.15578/ma.5.2.2010.93-97

Putri, B., Wardiyanto and Supono, 2015. Efektivitas penggunaan beberapa sumber bakteri dalam sistem bioflok terhadap keragaan ikan nila

(*Oreochromis niloticus*). *e-Jurnal Rekayasa dan Teknologi Budidaya Perikanan, 4*(1), pp.433-438. https://jurnal.fp.unila.ac.id/index.php/bdpi/article/view/1348

Putri, D.S., Affandi, M.I. and Sayekti, W.D., 2020. Analysis of business performance and risk of vanname shrimp farmers in traditional system and intensive systems in Labuan Manringgai District, East Lampung Regency. *Jurnal Ilmu-Ilmu Agribisnis, 8*(4), pp.625-631. https://doi.org/10.23960/jiia.v8i4.4707

Rajkumar, M., Pandey, P.K., Aravind, R., Vennila, A., Bharti, V. and Purushothaman, C.S., 2015. Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research*, *47*(11), pp.3432-3444. https://doi.org/10.1111/are.12792

Ridho, A. and Subagiyo, 2013. Pertumbuhan, Rasio Konversi Pakan dan Kelulushidupan Udang Litopenaeus vannamei yang Diberi Pakan dengan Suplementasi Prebiotik FOS (Fruktooligosakarida). *Buletin Oseanografi Marina*, *2*(4), pp.1-8. https://ejournal.undip.ac.id/index.php/buloma/article/view/11166

Setyono, B.D.H., Azhar, F. and Paryono, 2019. The impact of bioflocs application combined with probiotics on the growth perfomance of white leg shrimp (*Litopenaeus vannamei*). *Buletin Veteriner Udayana*, *11*(1), pp.7-13. https://doi.org/10.24843/bulvet.2019.v11.i01.p02

Shilta, M.T., Christina L., Lalrinsanga, P.L. and Bindu, P.R., 2020. Biofilm developed on plant substrates enhances growth and survival of post larvae of *Macrobrachium rosenbergii*. *Fisheries Technology*, *57*, pp.88–97. http://epubs.icar.org.in/ejournal/index.php/FT

Sumitro, Afandi, A., Hidayat, K.W. and Pratiwi, R., 2020. Evaluation of several microporous pipe designs as an aeration system in intensive catfish farming (*Clarias gariepenus*) based biofloc technology. *Journal of Aquaculture and Fish Health, 9*(2), pp.114-121. https://doi.org/10.20473/jafh.v9i2.16692

Suprapto, 2012. *Application of biofloc technology in vannamei shrimp farming in Indonesia*. Shrimp Club Indonesia, p.88.

Suprianto, Redjeki, E.S. and Dadiono, M.S., 2019. Optimization of probiotic doses on the growth rate and survival of tilapia (*Oreochromis niloticus*) in biofloc system. *Journal of Aquaculture and Fish Health, 8*(2), pp.80-85. https://doi.org/10.20473/jafh.v8i2.13156

Tong, R., Chen, W., Pan, L. and Zhang, K., 2020. Effects of feeding level and C/N ratio on water quality, growth performance, immune and antioxidant status of *Litopenaeus vannamei* in zero –water exchange bioflocsbased outdoor soil culture ponds. *Fish & Shellfish immunology*, *101*, pp.126-134. https://doi.org/10.1016/j.fsi.2020.03.051

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W., 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology And Molecular Biology Reviews, 64*(4), pp.655–671. https://doi.org/10.1128/mmbr.64.4.655-671.2000

Widanarni, Ekasari, J. and Maryam, S., 2012. Evaluation of biofloc technology application on water quality and production performance of red tilapia *Oreocrhromis* sp. cultured at different stocking densities. *HAYATI Journal of Biosciences, 19*(2), pp.73-80. https://doi.org/10.4308/hjb.19.2.73

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.

Xu, W.J. and Pan, L.Q., 2012. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture*, *356-357*, pp.147-152. https://doi.org/10.1016/j.aquaculture.2012.05.022

Yassien, M.H., Khoreba, H.M., Mohamed, M.A. and Ashry, O.A., 2019. Effect of biofloc system on the water quality of the white leg shrimp *Litopenaeus vannamei* reared in zero water exchange culture tanks. *Egyptian Journal of Aquatic Biology and Fisheries, 23*(2), pp.133-144. https://doi.org/10.21608/ejabf.2019.33952

Zonneveld, N., Huisman, E.A. and Boon, J.H., 1991. *Prinsip-prinsip budidaya ikan.* PT Gramedia Pustaka Utama, Jakarta, p.318.

Cite this document as Cite this document as Kurniaji, A., Renitasari, D.P., Saridu, S.A., Anton and Yunarty, 2023. The Effect of Different Probiotic Sources on Vannamei Shrimp (*Litopenaeus vannamei*) Cultivation with Biofloc System. *Journal of Aquaculture and Fish Health*, *12*(3), pp.405-420.