# THE EFFECT OF THE BIOFLOC TECHNOLOGY APPLICATION ON THE NONSPECIFIC IMMUNITY AND SURVIVAL RATE OF WHITE SHRIMP (*Litopenaeus vannamei*) POSTLARVAE

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## *Abstract*

Biofloc technology application in aquaculture has a huge potency to improve yields of white shrimp (*L. vannamei*). Biofloc can be used as an alternative feed for shrimp due to its high nutrition. Biofloc contains bacteria that have peptidoglycan and lipopolysaccharide on their cell walls. Bacteria are able to produce polyhydroxybutyrate. Polyhydroxybutyrate will release 3-hydroxy butyric acid (short chain fatty acid) in the gastro intestinal tract as inhibitor of pathogenic bacteria. The objectives of the research were to study the effect of biofloc technology application on the nonspecific immunity (Prophenoloxidase activity) and survival rate of *L. vannamei* postlarvae. The experiment was arranged in split plot design in three replicates. The treatments consisted of two factors namely various densities and different aquaculture systems. The aquaculture systems were autotrophic (nonbiofloc) and heterotrophic aquaculture system (biofloc), while densities were 1,000, 1,500, and 2,000 $PLm^{-3}$. The result showed that there was no significant interaction between densities and aquaculture system toward prophenoloxidase activity (nonspecific immunity) and survival rate of *L. vannamei*. Aquaculture system significantly affected prophenoloxidase activity of *L. vannamei*. Meanwhile the density only affected survival rate of *L. vannamei*. The biofloc technology was able to increase the nonspecific immunity of *L. vannamei* post larvae.

**Keywords** : Biofloc technology, *L. vannamei*,survival rate, prophenoloxidase activity

## Introduction

Shrimp culture waste in autotrophic aquaculture system is mostly inorganic nitrogen (mobile nitrogen) in form of ammonia and nitrite due to high protein content in feed (30-40%). The shrimp is only capable to recover nitrogen in feed arround 25% on harvest and 75% is released into culture pond (Crab et al., 2007). Ammonia and nitrite in culture pond is toxic to shrimp. Inorganic nitrogen built up in ponds is controlled by algae and nitrification. Inorganic nitrogen is converted to organic nitrogen to build algae cell. This process is limited by the rate carbon assimilation by algae. Nitrification is a slow process and need a few weeks to fully develop the nitrifying community (Avnimelech, 2009).

The method that can be applied to overcome the problem is heterotrophic aquaculture system (biofloc technology). Biofloc technology can be an alternative method to grow shrimp due to an an environmental friendly one. A carbon source namely sugar, molasses, and starch is added into culture pond increasing ratio of C:N to immobilize inorganic nitrogen and to stimulate the growth of heterotrophic bacteria to form biofloc. Heterotrophic bacteria, the main component of biofloc contain peptidoglycan and lipopolysaccharide on their cell walls. Peptidoglycan can be found on gram- positive bacteria, while lipopolysaccharide on gram-negative one. Live bacteria, glucans, peptidoglycan, and lipopolysaccharide have been used to stimulate the nonspecific immunity of shrimp (Smith et al., 2003). The substances are able to stimulate activation of inactive serine protease to active serine protease influencing prophenoloxidase activity. Prophenoloxidase activity plays an important role in the invertebrate immune response (Fagutao et al., 2011). Shrimp require continuous immune stimulation due to the absence of immune memory (Sharma et al., 2010).

Bacteria also are able to produce polyhydroxybutyrate as a reserve energy and carbon and to accelerate the animal growth and to inhibit pathogenic vibrio in intestine tract (de Schryver et al., 2010). The objectives of this experiment were to study the effect of biofloc technology application on the nonspecific immunity and survival rate of *L. vannamei* post larvae.

## Materials and Methods

### Experimental design

The experiment was conducted at laboratory scale using split plot design with two factors in three replicates. The treatments were aquaculture system (subplot) and density (mainplot). The aquaculture systems were heterotrophic system (HS/biofloc) and autotrophic system (AS/nonbiofloc) while stocking densities *of L. vannamei* were 10, 15, and 20 PL in 10 litres container equivalent to 1,000, 1,500, and 2,000 PL $m^{-3}$.

### Shrimp culture experiment

The experiment used eighteen plastic containers that were filled with sterile saline water. Water salinity of culture media was adjusted to osmolarity of *L. vannamei* haemolymph at intermolt phase. Haemolymph osmolarity of PL 11-*L. vannamei* on premolt phase was 933,89 mOsm/l $H_2O$, equivalent to 32 ‰, meanwhile haemolymph osmolarity of *L. vannamei* on intermolt phase was 861,00 mOsm/l $H_2O$, equivalent to 29,5 ‰ (Supono et al., 2014). Applied salinity of media was 30 ‰. Osmorality of haemolymph on premolt phase and intermolt phase is the best range for growth (Anggoro and Muryati, 2006).

Nine containers were used to culture *L. vannamei* in autotrophic system and nine containers in heterotrophic one. In heterotrophic system, biofloc was grown by adding shrimp feed and glucose to get C:N ratio of 20 (Supono et al., 2014). Heterotrophic bacterium of *Bacillus cereus* ($10^6$ CFU/ml) was inoculated into media to stimulate heterotrophic system. Aeration was installed on each container bottom to maintain dissolved oxygen min. at 4 mg/l and to make water movement. White shrimp *L. vannamei* post larvae (PL 17) with an average body weight of 11±1.0 mg and average length of 1.36±0,15 cm was stocked into culture media After 10 days of growing biofloc. Shrimp were cultured in 30 days and were fed by formulated feed of 38% protein on feeding rate of 5% accompanied by adding glucose (32.3% of carbon) to maintain C:N ratio of 20. There was no water exchange in heterotrophic system.

### Measurement of prophenoloxidase activity

Nonspecific immunity (prophenoloxidase activity) was analyzed with a method described by Liu and Chen (2004). Prophenoloxidase activity (PO activity) was measured spectrophotometrically by recording the formation of dopachrome from L-dihydroxyphenylalanine (L-DOPA). Haemolymph (0.1 ml) was withdrawn using 1-ml sterile syringe and placed in a microtube containing 0.9 ml of an anticoagulant solution (Trisodium citrat 0.01 M, Sodium chloride 0.34 M, EDTA 10mM, Glucose 0.12 M). Haemolymph-anticoagulant mixture was centrifuged at 1000 x g at 4$^o$C for 20 min. After discarding the supernatant, the pellet was rinsed with cocodylate-sitrate buffer (Sodium cacodylate 0.01 M, Sodium chloride 0.45 M, Trisodium sitrat 0.10 M, pH 7,0), and then centrifuged again. The supernatant was discarded. The pellet was resuspended in 200 µl cacodylate buffer (0.01 M Sodium cacodylate, 0.45 M Sodium chloride, 0.01 Calcium chloride, 0.26 M $MgCl_2$). The cell suspension was equally divided into two tubes. One tube was used to measure PO activity and the other tube was used to measure the background PO activity. The cell suspension (100 µl) was incubated for 10 min at 25-26$^o$C with 50 µl of trypsin which served as elicitor. Fifty microlitres of L-DOPA was added, followed by 800 µl of cacodylate buffer 5 min later. The control solution which consisted of 100 µl of cell suspension, 50 µl cacodylate buffer (to replace the trypsin), 50 µl L-DOPA was used for the background PO activity in all test conditions. The shrimp's PO activity was measured at optical density of 490 nm

using a spectrofotometer. The results are expressed as dopachrome formation per 50 µl of haemolymph.

**Survival rate analysis**

The survival rate of *L. vannamei* was calculated with the method conducted by Tseng et al. (1988) :

$$\frac{Nt}{N_0} \times 100\%$$

Where $N_0$ and Nt are the number of shrimps cultured in each container at initial time and t time, respectively.

**Data analysis**

All data were further analyzed statistically using Two-Way-Anova after testing of normality and homogenity using SPSS statistical software. Statistical significance of differences required that $p < 0.05$.

## Results And Discussion

**Results**

Dissolved oxygen, total ammonia nitrogen (TAN), and pH of culture media of heterotrophic system and autotrophic system are performed at Table 1. The values were in range for growing *L. vannamei* (Far et al., 2009).

Table 1. Water quality

| Water quality | AS | HS |
|---|---|---|
| DO (mg/l) | 5.0-8.0 | 5.0-8.0 |
| TAN (mg/l) | 0.02-0.05 | 0.01-0.05 |
| pH | 6.9-7.3 | 6.9-7.3 |

In general, nonspecific immunity (PO avtivity) and survival rate of *L. vannamei* postlarvae in heterotrophic aquaculture system were better than those in autotrophic one. Prophenoloxidase activities and survival rate of *L. vannamei* at harvest are summarized in Table 2.

Table 2. Prophenoloxidase activities and survival rate of *L. vannamei*

| Density ($PL/m^3$) | System | Prophenoloxidase activities (/50 µl haemolymph) | SR (%) |
|---|---|---|---|
| 1000 | AS | 0.055±0.003 | 60.0±10.0 |
| | HS | 0.135±0.029 | 73.3± 5.8 |
| 1500 | AS | 0.045±0.004 | 60.0± 6.7 |
| | HS | 0.107±0.056 | 66.7± 6.7 |
| 2000 | AS | 0.041±0.034 | 41.7±10.4. |
| | HS | 0.111±0.020 | 50.0±10.0. |

According to statistical analytic (Anova), there was no significant interaction between densities dan aquaculture systems toward PO activity and survival rate of *L. vannamei*. Aquaculture system significantly affected PO activities and survival rate of *L. vannamei.* However density only affected survival rate of *L. vannamei*. Observed data showed that PO activity and survival rate of *L. vannamei* cultured in heterotrophic aquaculturee system tended to increase on all of densities.

**Discussion**

Shrimp culture industry has experienced loss of production by infectious diseases mainly viral diseases. Disease outbreak is frequently caused by pond water quality deterioration. Ammonia in pond is produced as a major end product of the metabolism due to high content

protein of feed and is excreted across the gill of shrimp (Ebeling et al., 2006). In biofloc system, the bacteria are going to immobilize inorganic nitrogen present in the pond by adding organic carbon source to the water. Inorganic nitrogen was recycled in the culture pond resulting in microbial protein biomass needed for cell growth and multiplication. At high ratio of C:N, heterotrophic bacteria will assimilate ammonium nitrogen directly from water metabolized to cell biomass.

Prophenoloxidase activities of *L. vannamei* cultured in autotrophic aquaculture system were about 0,041/50 µl haemolymph to 0,055/50 µl haemolymph. Sharma et al. (4) investigated that PO activity of *L. vannamei* with out treatment was 0,060/50 µl haemolymph, meanwhile Yeh et al. (2010) reported that prophenoloxidase activity of *L. vannamei* was around 0,040-0,100/50 µl haemolymph.

Prophenoloxidase activities of *L. vannamei* cultured in heterotrophic aquaculture system were about 0,107/50 µl haemolymph to 0,135/50 µl haemolymph. The values increased on all of densities compared to autotrophic one (Fig 1). Increasment of prophenoloxidase activity describes increasment of nonspecific immunity (Fagutao et al., 2011).

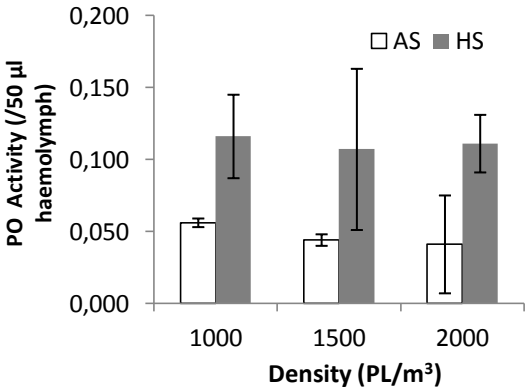


Fig. 1. PO activities of *L. vannamei*

White shrimp has an ability to consume bacteria in biofloc. Bacterium's size is very small, less than 5µ which is too small to be benefited by fish or shrimp. In biofloc form, the size one can reach 500µ to 2 mm, consequently it could be fed by shrimp or fish. Bacterium *Bacillus cereus* inoculated into the culture media is gram-positive one containing mostly peptidoglycan. According to Volk and Wheeler (1993), gram-positive bacteria contain 60 % of peptidoglycan and 20% of lipopolysaccharride.

Peptidoglycan and lipopolysaccharride are immunostimulants that are able to stimulate the immune system of shrimp (Zhou, 2003). The substances are able to activate inactive serine protease to active serine protease as prophenoloxidase activating enzyme. Prophenoloxidase activating enzyme is used to encourage activation of prophenoloxidase to phenoloxidase (prophenoloxidase activity). The process results in protein opsonin factor that stimulate phagocyte of hyaline cell (Yeh et al., 2010). Sharma et al. (2010) reported that biofilm of *V. alginolyticus* was capable to improve the immune response and resistance to diseases in *P. monodon* by influencing various immune response such as changes in haemocyte count, PO activity and antibacterial activity.

Enhancement of immune system corresponded to survival rate of *L. vannamei* in the experiment. Survival rate of *L. vannamei* in heterotrophic system experienced enhancement for

all of densities (Fig. 2). survival rate of *L. vannamei* in autotrophic aquaculture system was 41.7% µl haemolymph to 60% and in heterotrophic aquaculture system was 50 to 73,3%, respectively.

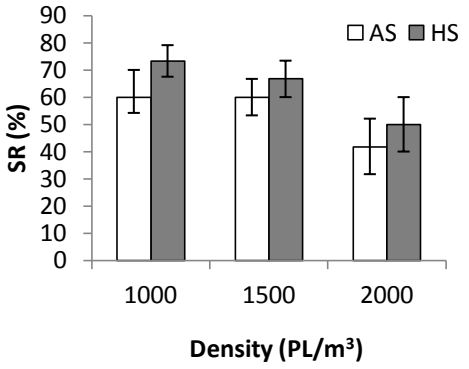


Fig. 2. Survival rate (SR) of *L. vannamei*

Besides containing immunostimilant, bacteria in biofloc are able to produce polyhydroxybutyrate. Polyhydroxybutyrate will release 3-hydroxy butyric acid (short chain fatty acid) in the gastro intestinal tract as inhibitor of pathogenic bacteria. According to several researches, PHB is capable to inhibit pathogen in the intestinal tract and to be antimicrobial against *Vibrio, E. coli, and Salmonella,* to control pathogen of *Vibrio harveyi,* and to enhance survival rate of *Artemia franciscana* larvae (Crab ae al., 2010). Far et al. (2009) investigated that *Bacillus* is able to increase survival rate of *L. vannamei* and to decrease luminous *Vibrio* densities in the pond water.

## Conclusions

Biofloc technology (heterotrophic aquaculture system) can be an alternative method to culture *L. vannamei* in pond. The system is able to improve nonspecific immunity and survival rate of *L. Vannamei* postlarvae. Prophenoloxidase activitiy of *L. vannamei* in autotrophic aquaculture system was 0,041/50 µl haemolymph to 0,055/50 µl haemolymph and in heterotrophic aquaculture system was 0,107/50 µl haemolymph to 0,135/50 µl haemolymph, respectively. Meanwhile survival rate of *L. vannamei* in autotrophic aquaculture system was 41.7% to 60% and in heterotrophic aquaculture system was 50 to 73,3%, respectively.

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