Growth performance of *Litopenaeus vannamei* grown in biofloc system produced from different carbohydrate sources

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Abstract. This study aimed to analyze the growth performance of *Litopenaeus vannamei* grown in biofloc system using different local carbohydrate sources. Shrimps with an average weight of 0.01 g were stocked in 12 aquaria (capacity 60 L each) with a density of 150 post larvae per aquarium. Larvae were fed at 5% of body weight/day, three times a day. Biofloc was prepared by fermenting molasses, palm sugar and sago flour (450 g each) in the media previously mixed with 450 g fine bran, 15 g baker's yeast, 15 g probiotics (*Bacillus* sp.). No carbohydrate was added into control media. All the ingredients were dissolved in 6 liters of sterile water and aerated for 7 days until the aroma was odorless. The floc was then distributed to each aquarium as much as 1 liter and allowed to stand for 20 days with continuous aeration. The shrimps were grown for two months in the biofloc media. The results showed that the best specific growth rate, survival rate, feed conversion ratio were obtained in shrimps grown in biofloc media supplemented with palm sugar as carbohydrate source. As conclusion, palm sugar as carbohydrate source in biofloc system had the potential to increase the biofloc nutritional value, thus improving the specific growth rate, survival rate, feed conversion ratio.
Key Words: biofloc, *L. vannamei*, palm sugar, feed convertion ratio.

Introduction. Aquaculture continues to innovate the development of intensive cultivation technology to increase production and to fulfill the protein needs of people. Intensification has caused deterioration of culture environment, increased the outbreaks of diseases and reduced productivity. Therefore, technologies that minimize the impact on environment and disease occurrence are very much needed. The use of various natural products as immunostimulants in aquaculture has been reported to be effective in reducing disease evidence in fish and shrimp aquaculture and increasing production (Manoppo et al 2015). In addition, more researches have been focused on the application of medicinal plants and spices to prevent disease and promote growth such as ginger, garlic, etc. (Nya & Austin 2009; Manoppo et al 2016; Payung et al 2017). Application of biofloc is another promising alternative and is still continued to be developed. In Indonesia, biofloc systems are applied to catfish, tilapia and shrimp aquaculture especially *Litopenaeus vannamei* with high density (Azim et al 2008; Ekasari 2009; Hermawan et al 2014).

Some definitions were put forward by some experts on the notion of biofloc technology. Crab et al (2012) stated that biofloc technology is a technique to improve water quality for aquaculture activities by adding externally carbohydrate sources through feed. According to Aiyushirota (2009), biofloc is a biological treatment of waste water which is activated sludge while Ekasari (2009) stated that biofloc technology is the conversion of inorganic nitrogen especially ammonia by heterotrophic bacteria into microbial biomass which can then be consumed by cultured organisms. Biofloc deals with waste that has the potential to damage the environment with high nutrient content continuously produced by aquaculture activities (Riani et al 2012).

Several reasons that make biofloc systems more valuable compared to conventional systems include minimizing the use of feed, preventing the spread of disease by minimizing water changes, environmentally friendly. Biofloc is important in improving water quality and preventing the disease occurrence (Choo & Caipang 2015; Nurhatijah et al 2016). Cadiz et al (2016) found that the use of biofloc could control the presence of *Vibrio* sp. and *Vibrio parahaemolyticus* in intensive culture of *L. vannamei*. In addition, biofloc can be applied at high densities aquaculture (Hargreaves 2013). In general, protein in fish feed is only absorbed around 20-25% and the rest will be secreted through waste and uneaten food that form ammonia and organic nitrogen. Manipulation of C/N ratio in aquaculture environment is required to stimulate growth of heterotrophic bacteria and to convert inorganic nitrogen into microbial protein (Avnimelech 1999).

Theoretically the addition of carbon sources with an increase in C/N ratio can increase the conversion of toxic inorganic nitrogen species to microbial biomass available as food for cultivated animals. The optimum C/N ratio in aquaculture systems can be maintained by adding a variety of cheap carbon sources or reducing protein content in feed (Avnimelech 1999; Hargreaves 2006). The use of biofloc is urgently needed for the development of environment friendly aquaculture. This study aimed to analyze the growth and survival of shrimp *L. vannamei* cultivated in biofloc systems using different local carbohydrate sources.

Material and Method

***Experimental fish**.* The shrimp used was *L. vannamei* post larvae with an average weight of 0.01 g. Larvae were stocked in 12 aquaria (capacity 60 L each) with a density of 150 larvae per aquarium. Water parameters and the corresponding values were temperature (28.19-31.24°C), pH (5.21-8), dissolved oxygen (3.89-6.76 mg L^{-1}) and salinity (32.98-34.85 ppt). Larvae were fed three times a day at 07:00 a.m., 11:00 a.m. and 16:00 p.m. Feeding dose was 5% of body weight/day. This research was conducted from January to April 2017.

***Biofloc fermentation**.* Biofloc was prepared by fermenting three different sources of carbohydrates added into the water. The ingredients used in preparing the fermentation were 450 g fine bran, 15 g baker's yeast, 15 g probiotics (*Bacillus* sp.) and 450 g of carbohydrate sources (molasses, sagu flour, palm sugar) (Gunarto et al 2010). Control treatment was not added with any carbohydrate source. All the ingredients were dissolved with 6 liters of sterile water, and aerated for 7 days until the aroma was odorless. After 7 days the floc was distributed to each aquarium as much as 1 liter and allowed to stand for 20 days with continuous aeration. The post larvae were stocked after the floc was formed.

***Experimental design**.* This study used a completely randomized design with four treatments, each with three replications. Carbohydrate sources as treatments consisted of molasses, sago flour, palm sugar. Control medium was not added with any carbohydrate.

Growth performance

Specific growth rate. Calculation of shrimp daily growth was done by the formula as follows (Effendie 1997):

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W_t = final weight of shrimp (g);
W_0 = initial weight of shrimp (g);
t = rearing time (days).

Survival rate. Shrimp survival rate was calculated by the formula as follows:

$$SR = \frac{N_t}{N_0} \times 100$$

where: SR = survival rate (%);
N_t = number of live shrimp at the end of the study;
N_0 = number of live shrimp at the beginning of the study.

Feed conversion ratio. Feed conversion ratio was calculated using the formula (Marlida et al 2014):

$$FCR = \frac{\text{Feed given (dry weight)}}{\text{Weight gain (wet gain)}} \times 100$$

Floc volume (FV). It was calculated by the following formula:

$$FV = \frac{\text{Water sample volume}}{\text{Sediment volume}} \times 100$$

Data analysis. All data were analyzed by one-way variance analysis (ANOVA). The different effect between treatments was analyzed using least significant difference (LSD) test.

Results and Discussion

Specific growth rate. The use of different carbohydrate sources in shrimp aquaculture (*L. vannamei*) using biofloc systems had a significant effect on the SGR ($p < 0.05$). The best SGR was obtained in shrimp treated with palm sugar as carbohydrate source (4.72%) as compared to malasses (3.26%), sago flour (2.87%) and control shrimp (2%) (Figure 1). Statiscally, the SGR of shrimp treated with palm sugar was different as compared to control shrimp as well as to molasses and sago flour treatments. SGR of shrimp treated with molasses was also different as compared to control shrimp but not with sago flour treatment. There was no significant differences in SGR between shrimp treated with sago flour and control (Figure 1).

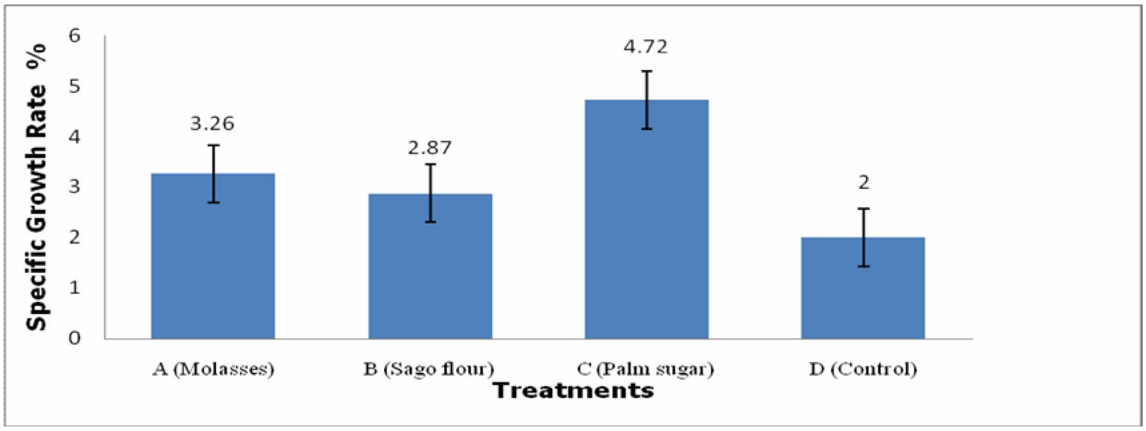

Figure 1. Specific growth rate of *L. vannamei* treated with biofloc produced from different carbohydrate sources.

Carbohydrate content of palm sugar is high. Heryani (2016) reported that the nutritional value of 100 g palm sugar was 95 g carbohydrates, 75 mg of calcium and 35 mg of phosphorus, iron 3 mg, with total calory of 368 cal. According to Avnimelech (1999) and Ebeling et al (2006), heterotrophic bacteria will grow optimally by increasing the C/N ratio through continuous supplementation of organic carbon such as molasses, wheat flour and tapioca flour. The low value produced by sago flour was probably caused by the texture of sago which is easy to stick when mixed with water. The occurrence of floc attaching on the part of the aquarium wall caused the decomposition process would not maximal compared to molasses and palm sugar. In this study the control gave the lowest response on the growth of shrimp.

Research result showed that supplementation of different carbohydrate sources had significant effect on the SGR of the shrimp. The growth of shrimp from the 1 cm post larvae to an average of 6 cm post larvae in treatment C (palm sugar) was resulted from the nutritional content of the biofloc. Biofloc also provides additional proteins, lipids, minerals, vitamins and many bioactive compounds such as carotenoids, chlorophyll, phytosterols, bromophenol and amino sugars (Avnimelech 1999; Tacon et al 2002; Ju et al 2008). Moreno-Arias et al (2018) suggest that biofloc could provide high quality protein and essential fatty acids *in situ* to compensate the partial fishmeal substitution. In addition, biofloc has a positive effect on the activity of shrimp digestive enzymes thus enhance the growth of shrimp (Xu & Pan 2012). In fish, Bakhshi et al (2018) reported that microbial flocs formed in biofloc system using corn starch as carbon source can improve growth performance of common carp and tanks water quality under zero water exchange and hence ensures sustainability. In nutritional and economical points of view, biofloc system using agriculture by product with high cellulose content as a carbon source is more reasonable, guarantee positive environmental impact and aquaculture sustainability (El-Husseiny et al 2018).

Survival rate. Survival rate of shrimp treated with palm sugar, molasses and sago flour was presented in Figure 2.

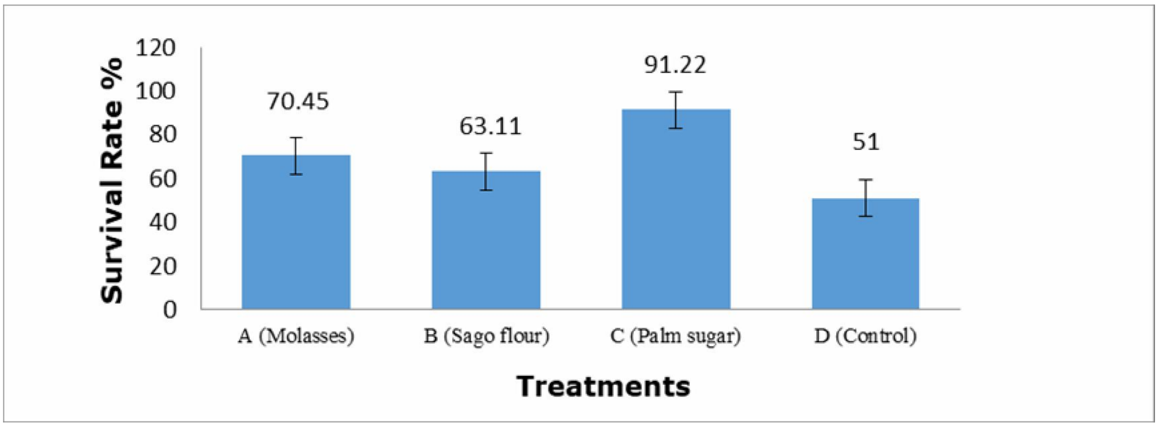

Figure 2. Survival rate of *L.vannamei* treated with biofloc from different carbohydrate sources.

The highest SR of *L. vannamei* was achieved in shrimp treated with palm sugar as carbohydrate source. Schveitzer et al (2013) stated that in the early stages of post larval development, high density could cause failure in cultivation. Agustina et al (2015) reported the level of stocking density affected the survival of *L. vannamei* due to the competition occurred between shrimp. The higher the stocking density, the smaller space available for each individual. It was found in this research that shrimp treated with palm sugar had high SR even though they were cultured in high density. Agustina et al (2015) stated that high stocking density did not affect shrimp growth but the availability of quality feed in sufficient quantities will reduce the percentage of shrimp larvae mortality. Crab et al (2012) reported that immunostimulants from biofloc lead to increased immunity and antioxidant status of shrimp and fish to provide broad-based resistance to many infections. Thus, shrimp that are cultivated with biofloc systems have a high level of tolerance to the environment, especially those that use carbohydrate sources of palm sugar.

Feed conversion ratio. Feed conversion ratio (FCR) of shrimp treated with palm sugar, molasses and sago flour was presented in Figure 3. The best FCR was observed in shrimp treated with palm sugar. According to Widanarni et al (2016), supplementation of different carbon sources to biofloc media could increase the growth rate and reduce the value of FCR for tiger shrimp (*Penaeus monodon*) ranging from 2.28 to 3.67%.

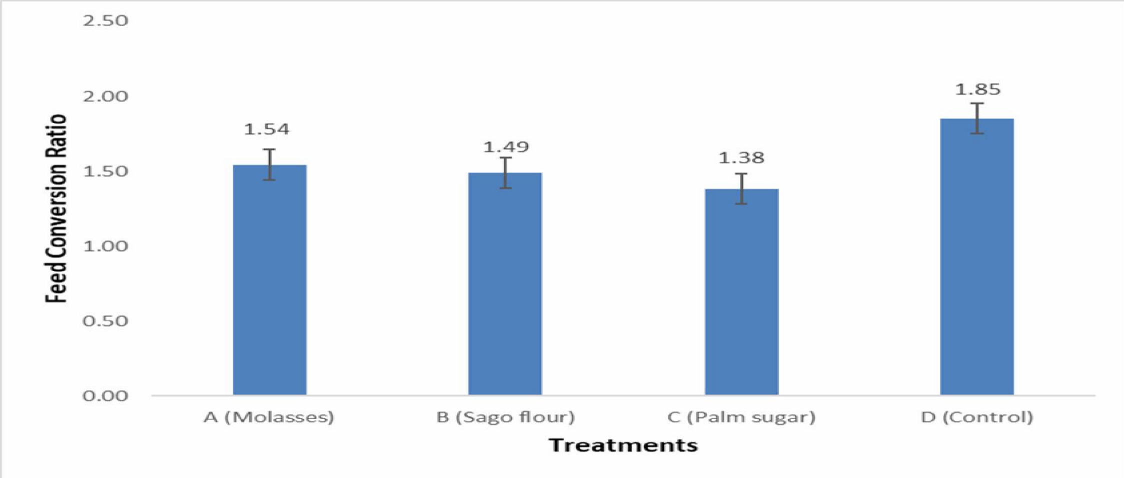

Figure 3. Feed conversion ratio of *L. vannamei* treated with biofloc from different carbohydrate sources.

These results showed that FCR of shrimp treated with sago flour and palm sugar gave a good response and the FCR values were not much different as compared to control treatment. According to Avnimelech (1999) and Crab et al (2007), single cell proteins synthesized by heterotrophic bacteria could be directly used as a source of feed for cultured fish, thereby reducing the demand for fish feed protein. In addition, Ridho & Subagiyo (2013) reported that the low value of the FCR was obtained because the bacteria in the form of probiotics could produce extracellular enzymes that increase the digestibility of food in shrimp intestine, thus increase nutritional absorption.

Floc volume. The FV during the study was listed in Table 1. The best value of FV was achieved in palm sugar treatment with a value of 20.87 mg L^{-1}, followed by the use of molasses 17.49 mg L^{-1}, and sago flour 10.17 mg L^{-1}.

Table 1

The volume of floc produced in different carbohydrate sources

Treatments	*Floc volume (mg L^{-1})*	*Average (mg L^{-1})*
A (molasses)	15-20	17.49±2.50
B (sago flour)	8-13	10.17±2.56
C (palm sugar)	18-23	20.87±2.57

According to Avnimelech (2009), floc density could be distinguished by per liter of volume. Biofloc is classified as low if the volume ranges from 1 t o10 mg L^{-1}, classified as medium if the range is 10-20 mg L^{-1}, and high if the biofloc volume is > 20 mg L^{-1}, and is very low if < 1 mg L^{-1}. In this research, the value of the floc in each treatment was included in the medium and high categories. The volume of floc formed showed that bacteria in the culture medium could utilize carbohydrate sources for growth and as an energy source. The growth of floc was marked by the presence of foam on the surface of the water and the grain of floc floating in the water column. The composition of organisms in the floc would affect the structure of biofloc and nutrient content of biofloc (Ju et al 2008). According to Crab et al (2010), biofloc produced in glycerol gave the best results. In addition, Azim & Little (2008) stated that the quality of biofloc is strongly influenced by the quality of feed used for biofloc production. Thus changes in carbon sources would change the nutrient composition and floc quality index. This was related to the quality of fermentation used at the begining of cultivation. According to Hapsari (2016), floc which accumulates in fermented biofloc is very dense and large, compared to non-fermented biofloc. This was similar to the result of this research in which the biofloc produced in water treated with molasses and palm sugar was more dense than biofloc produced in the treatment of sago flour. The color of the water generated at the beginning of the study was light brown and finally became dark brown until the end of the study except in control treatment. Aiyushirota (2009) stated that the floc was formed if visually the color of water turned to light brown, and formed lumps that moved with the flow of water. In the first 30 days, dissolved oxygen was crucial for the formation of biofloc and in this stage, application of minimal exchange water was very important. In this study, there was no water exchange during the first 20 days after the water treated with different types of carbohydrates and stocked the larvae.

Conclusions. Supplementation of molasses, sago flour and palm sugar as carbohydrate sources in biofloc system influenced the biofloc nutrition value and thus improved the specific growth rate, survival rate and feed conversion ratio of shrimp. The best SGR was ontained in shrimp treated with palm sugar as carbohydrate source (4.72%) as compared to molasse (3.26%), sago flour (2.87%) and control shrimp (2%). The highest survival rate (91.22%) and the lowest FCR (1.38) were also achieved in shrimp treated with palm sugar.

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Received: 24 October 2018. Accepted: 29 December 2018. Published online: 13 April 2019.
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How to cite this article:
Adipu Y., Lumenta C., Mangindaan R. E. P., Manoppo H., 2019 Growth performance of *Litopenaeus vannamei* grown in biofloc system produced from different carbohydrate sources. AACL Bioflux 12(2):472-479.