



## The effect of nauplii *Artemia* sp. enriched with biofloc on the performance of *Penaeus monodon* and *Penaeus vannamei* post-larvae

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### ABSTRACT

*Artemia* sp. is a common natural feed for shrimps at the post-larvae stage and is characterized by poor lipid content. Therefore, *Artemia* is commonly enriched with specific nutrition, including biofloc. This contains some useful nutrients in the form of protein and polyhydroxy butyrate (PHB). The aim of this study was to analyze the effect of *Artemia* enriched with biofloc on the growth performance of black tiger shrimp, *P. Monodon*, and whiteleg shrimp *P. vannamei*. This research used a completely randomized design (CRD), encompassing three treatments of feed and four replications. The tested treatments include: (a) *Artemia* sp. without enriched (b) *Artemia* enriched with biofloc, and (c) biofloc without *Artemia*, and the treatments were fed on black tiger as well as whiteleg shrimp post-larvae for 15 days. The results showed the significant effect of using *Artemia* sp. enriched with biofloc on the growth of *P. monodon* and *P. vannamei*, but not on the survival rate. However, both parameters were significantly influenced for post-larvae shrimp at a dose of 30 mL biofloc. This served as a nutritional source by providing the best growth of 14.57 mg and 15 mg at a daily growth and survival rate of 0.86 mg day<sup>-1</sup>: 98% and 1.4 mg day<sup>-1</sup>: 99% for *P. vannamei* and *P. monodon*, respectively.

### Introduction

A natural feed is one of the important factors in post-larvae shrimp culture. The natural feed should have nutrient content that is needed by shrimp (Supono and Hudaidah, 2018). *Artemia* sp. has been identified as the best natural feed for shrimp (Yao *et al.*, 2018), hence the nauplius is commonly adopted as the main food at for larvae hatchery. This is due to the high protein contained in suitable sizes (Jhon *et al.*, 2004), with insufficient fat or lipid. However, the Penaeid shrimp, including *Penaeus monodon* (black tiger) and *Penaeus vannamei* (whiteleg shrimp) require lipid as a source of essential fatty acids and various classes of the other fat like phospholipid and sterol. These species have the ability to utilize the fatty acid obtained from *Artemia* as an irreplaceable natural feed. Furthermore, Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are included in the category of highly unsaturated fatty acids (HUFA) featuring the most important role of serving as

support for metabolic processes, and also to facilitate the growth and survival of *P. vannamei* larvae (Chen *et al.*, 2015). Therefore, the nutritional contents of *Artemia* need to be adequately enriched before use. This is possibly achieved by introducing some materials, including probiotics (Hamsah *et al.*, 2017), essential fatty acids (Akbar *et al.*, 2011), selenium (Juhász *et al.*, 2017), and soybean meal (Putra *et al.*, 2016). However, no study has exploited enrichment using biofloc, although the application on black tiger shrimp *P. monodon* has been reported by Nurhatijah *et al.* (2016) and Supriatna *et al.* (2019).

Biofloc is an aquaculture technology known to utilize the result of fish or shrimp metabolism. Furthermore, the process involves a conversion of the nitrogen component into protein, followed by the possible utility by fish or shrimp, using carbon source and heterotroph bacterium, and the consequent provision of constant aeration. This subsequently allows for aerobic decomposition and floc bacteria

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maintenance in the suspension (Azim et al., 2007; Putra et al., 2020). The process results in the build-up of a micro-community (bacteria, protozoa, detritus (dead body cells), fungus, and zooplankton) and organic fiber particles rich in cellulose, alongside inorganic materials in the form of anhydrous calcium carbonate salt crystals, biopolymers, and polyhydroxyalkanoate (de Schryver et al., 2008; Avnimelech, 2015). Moreover, biofloc has the advantage of high protein content, the bacteria contains peptidoglycan and lipopolysaccharide (LPPS) as immunostimulant (Matsuura, 2013), and the bacteria produce polyhydroxybutyrate as anti-bacterial agent (Boon et al., 2010; Crab et al., 2010). Khanjani et al. (2015) reported on the capacity for biofloc in culture media to increase *P. vannamei* growth at the nursery stage. According to the preliminary testing, *Artemia* utilizes the biofloc as feed (Luo et al., 2017), and there is a need for further research on enrichment with biofloc as feed for *P. monodon* and *P. vannamei* post-larvae. Therefore, the aim of this study was to analyze the effect of *Artemia* enriched with biofloc on the performance of *P. monodon* and *P. vannamei*.

## Materials and Methods

### Materials

The equipments used include Aquarium (15cm ×15cm ×25 cm), aerator, scope net, digital scales, Imhoff cone, DO meter, pH meter, Thermometer, lamp, plastic containers (vol. 15 L), and hatching media. The materials encompass the whiteleg shrimp PL-4, black tiger shrimp PL-10, *Artemia* sp., saline water, molasses, commercial feed, and *Bacillus* sp.

### Experimental design

The experimental design was completely randomized, comprising of 3 treatments and 4 replications. In addition, the treatments, including feeding with (1) *Artemia* sp. alone, (2) enriched with biofloc, and (3) biofloc only were tested on the black tiger and whiteleg shrimp post-larva.

### Biofloc culture

Biofloc production was conducted in plastic containers with a volume of 15 L, filled with 10 L saline water, and equipped with an aerator for oxygen supply. A total of 5 g feed (protein content of 40%), 5 g molasses (carbon content of 50%), and bacterium *Bacillus* sp. with a density of  $2,5 \times 10^5$  CFU mL<sup>-1</sup> were placed inside, further allowing for floc formation in 10 days.

### Artemia enrichment

*Artemia* sp. hatching is performed in a cone container filled with 1 l of seawater and reserved for 24 hours. Therefore, the *Artemia* sp. was harvested and transferred to the enrichment container and further placed into 4 containers (size of 15cmx15cmx25cm) for the respective treatments. These include P1: *Artemia* sp. without enrichment of biofloc (control), P2: *Artemia* sp. enriched with 10 mL biofloc, P3: *Artemia* sp. with 20 mL biofloc, and P4: *Artemia* sp. and 30 mL biofloc. These augmentations were conducted in 5 hours, followed by proximate analysis (fat, protein) on all 4 samples. Therefore, the best nutrition is adopted as feed for black tiger and whiteleg shrimp post-larvae.

### Shrimp culture

The culture of post-larvae black tiger and whiteleg shrimp used were presented in 24 containers filled with 3L seawater (saline water) equipped with aeration. Furthermore, 30 PL's (PL-10) black tiger shrimp was used with a stocking density of 10 PL's L<sup>-1</sup>, which was similar to whiteleg shrimp at 30 PL's (PL-4). The feeding was adjusted according to the treatment, as follows (1) *Artemia* sp., (2) *Artemia* sp. enriched by biofloc, and (3) biofloc. This was administered 4 times a day (06.00, 12:00, 18.00, and 24.00), with a dose of 20-80 individuals/larvae/day. Therefore, both samples were cultured for 15 days, and observed for growth at the beginning and end of the study, using a digital scale with 0.001 grams precision. The weight gain was calculated as follow:

$$W = W_t - W_o$$

Where, W = body weight gain (mg); W<sub>o</sub> = weight of post-larvae at the beginning (mg); W<sub>t</sub> = weight of post-larvae at the end of the study (mg).

The daily growth observation of Post-larvae black tiger and whiteleg shrimp was calculated based on Muchlisin et al. (2016) as follow:

$$DGR = (W_t - W_o) / T$$

Where, DGR = Daily growth rate (g day<sup>-1</sup>); W<sub>t</sub> = Average weight of post-larvae at the end of study (g); W<sub>o</sub> = Average weight of post-larvae at the beginning (g); T = days of culture. The survival rate was calculated based on Muchlisin et al. (2016) as follow:

$$SR (\%) = (N_o - N_t / N_o) \times 100$$

Where, SR: Survival rate (%); N<sub>t</sub>: Number of post-larvae at the end of study (pcs); N<sub>o</sub>: The number of post-larvae dead during the study (pcs).

The water quality parameters, including dissolved oxygen (mg L<sup>-1</sup>), temperature (°C), and pH were measured every three days interval, and ammonia

content (mg L<sup>-1</sup>) was assessed at the beginning, middle and end of the experiment.

**Statistical analysis**

The data on survival and growth rate for Post-larvae black tiger and whiteleg shrimp were analyzed using ANOVA, with a confidence level of 95%. Subsequently, the Least Significant Difference (LSD) test is performed when there are significant differences amongst treatments.

**Results**

The proximate test results indicate an increase in the value of protein, fat, and crude fiber for each treatment. This indicates the ability for *Artemia* sp to properly digest biofloc, thus resulting in nutrient amplification. Table 1 shows the test results. Based on the research, data on the survival rate of black tiger shrimp post-larvae are as follows: *Artemia* feed without enrichment (96%), *Artemia* enriched with biofloc (99%), and biofloc only (86%) (Figure 1a). Meanwhile, the respective values for whiteleg shrimp were 97%, 98%, and 83% (Figure 1b).

The statistical analysis with ANOVA shows a significant difference in terms of survival rate of samples between treatments. In addition, data from LSD (least significant difference) showed no substantial variation between the *Artemia* treatment without enrichment and those enriched with biofloc on black tiger shrimp post-larvae, but both were significantly different from biofloc only treatment. Furthermore, the best survival rate was observed in

the enriched media, indicating *Artemia* as the best feed for both species at the post-larvae stage. The sample body weight was evaluated at the inception and end of the study, and Figure 2a, Figure 2b demonstrate the trend of absolute growth.

According to Fig. 2a-b, LSD (least significant difference) data showed a significant difference between the absolute growth of post-larvae black tiger shrimp for each treatment. However, the highest value was achieved using *Artemia* feed enriched with biofloc, at an average bodyweight of 15 mg, while biofloc alone produced the lowest outcome at approximately 5 mg. Furthermore, similar output was observed with the shrimp whiteleg, at 14.57 mg and 4.09 mg, respectively. Therefore, enriched *Artemia* sp. significantly influences the average body weight of both specimens. The daily growth rate is obtained by dividing the result of average body weight by the length of culture time, as shown in Figure 3a and Figure 3b. In addition, the measure of important water quality parameters for shrimp farming included temperature at a range of 27 to 29.5°C, acidity, at pH 7, dissolved oxygen (3.3 to 5.5 mg L<sup>-1</sup>) and salinity at 25 to 26 ppt, while the ammonia content was less than 0.001 mg L<sup>-1</sup> (Table 2).

Table 1. The results of the proximate analysis of *Artemia* sp. used in this study

No.	samples	Content (%)					
		Moisture	Ash	protein	Fat	Fiber	Carbohydrate
1	P1	5.50	2.25	24.04	1.76	2.30	64.15
2	P2	7.04	3.83	31.59	3,31	4.13	50.10
3	P3	10.42	7.43	53.60	6.03	6.53	15.99
4	P4	9.40	7,82	54.41	9.21	7.58	11.57

Description: P1 (*Artemia* without enriched with biofloc), P2 (*Artemia* enriched with 10 ml/l biofloc), P3 (*Artemia* enriched with 20 ml/l biofloc), P4 (*Artemia* enriched with 30 ml/l biofloc).

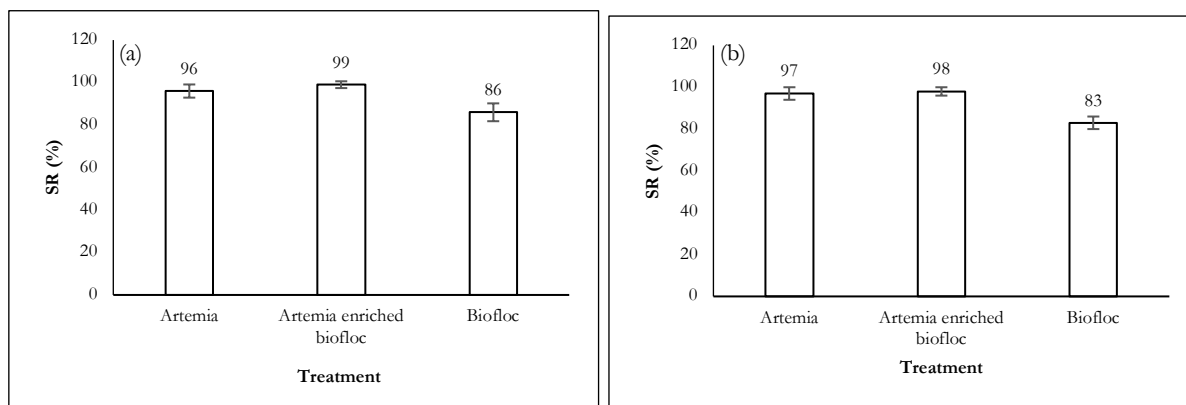


Figure 1. (a) The survival rate of black tiger shrimp post-larvae with different feed; (b) The survival rate of whiteleg shrimp post-larvae with different feed.

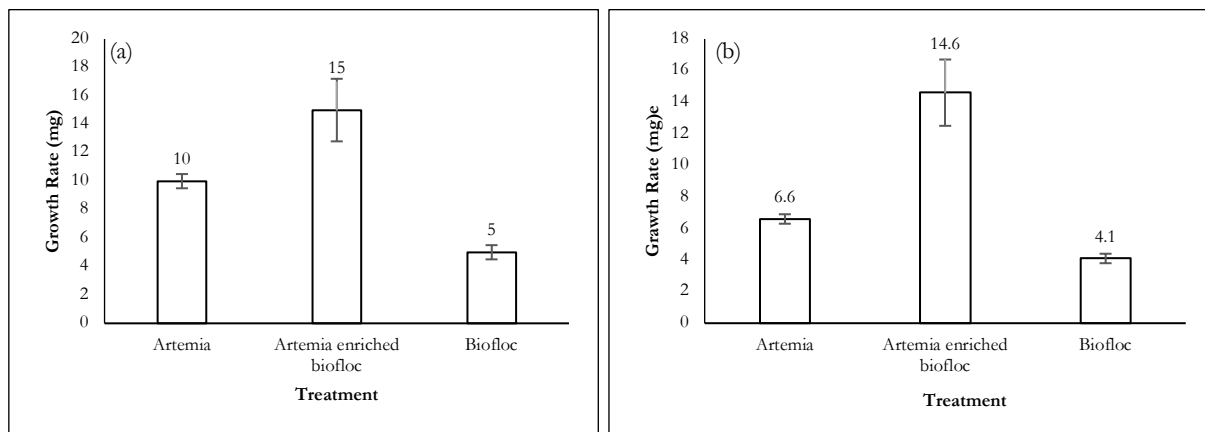


Figure 2. (a) The growth rate of Black tiger shrimp post-larvae with different feed; (b) The growth rate of Whiteleg shrimp Post-larvae with different feed.

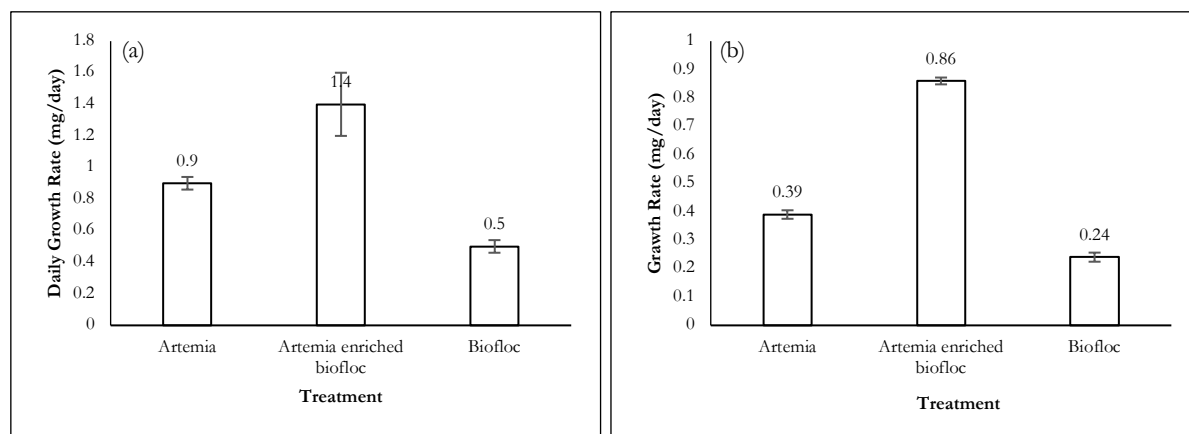


Figure 3. The daily growth rate of (a) Black tiger shrimp, and (b) Whiteleg shrimp post-larvae with different feed.

Table 2. Water quality parameters during the study

Parameter	Value
Temperature ° C)	26 to 29.5
pH	7
Dissolved oxygen (mg L <sup>-1</sup> )	3.3 to 5.5
Salinity (ppt)	25-26
Ammonia (mg L <sup>-1</sup> )	<0.01

Discussion

The proximate analysis showed elevated *Artemia* sp absorption following an increase in biofloc provided as feed. Therefore, treatments with 30 mL L<sup>-1</sup> demonstrated the highest outcome, because of the high protein supported by the biofloc actively used as food, as well as fat, ash, and fiber composition. Hargreaves (2013) reported on the protein and fat content in biofloc, estimated to reach 30-45%, and 1-5%, respectively. Furthermore, bacteria as the main component served as a highly

significant contributor due to the 60% protein content (Irianto, 2006), which is needed for growth, maintenance, and also as a source of energy for crustaceans (Venero et al., 2007). Zhang et al. (2013) reported on the need for fat (10 to 12%) in the diet of *P. vannamei*, because the fatty acids present are critical for survival. Kangpanich et al. (2016) reported a similar result. The fatty acids are possibly elevated due to the presence of polyhydroxybutyrate produced by biofloc bacteria (de Schryver et al., 2008), with a dry weight of about 29% (Supono et al., 2013).

Furuita et al. (1996) reported on the tendency for essential fatty acid deficiency to disrupt in the growth and survival of post-larvae shrimp. In addition, the proximate test show a higher percentage of fat content in *Artemia* enriched with biofloc compared to other treatment. Therefore, an increase in the enrichment concentrations produces positive correlation, observed with the improved survival rate of both specimens at the post-larvae stage.

The highest survival rate in black tiger shrimp was achieved in *Artemia* feeds enriched with biofloc (99.13%), while the least was observed in treatments with biofloc only (85.83%). Moreover, a similar outcome was recorded with whiteleg shrimp, at 96.67% and 82.5%, respectively. This finding was due to the fact that using wet biofloc as feed is not appropriate because the shrimp larvae are not able to utilize it directly. However, the *Artemia* feed administered with or without biofloc-enrichment was better and is thus considered the best live feed for both fish and shrimp larvae (Yao et al. 2018).

The high survival rate possibly results from the optimum condition of water quality during maintenance, hence no effect is observed. These parameters are critical success factors in black tiger and whiteleg shrimp post-larvae farming. The data on water quality was within the normal limit during the culture period (Supono, 2017). However, poor conditions have been affiliated with a decline in the shrimp immune system, leading to disease susceptibility, as well as an increased infection process speed, and even death. Therefore, maintaining the environmental conditions is vital, in order to ensure the proper control of shrimp (Lakshmi et al., 2013).

*Artemia* sp enriched with biofloc possessed elevated nutrient compositions for use by the post-larvae shrimps. In addition, the protein and fat content serve as an energy source in the metabolic process (Hernandez et al., 2011), needed for growth and the replacement of damaged tissues. Therefore, faster growth was produced compared to treatments without enrichment. In addition, Yao et al. (2018) reported on the increased crude protein content from 40-60% to 65-70% in *Artemia* sp combined with biofloc.

Bacteria cell wall, including those in biofloc contains peptidoglycan, which serves as the building block. This comprises of lipopolysaccharide (Irianto, 2006), a combination of lipids and polysaccharides (carbohydrates). Besides, biofloc also contains biopolymers of proteins, lipopolysaccharides, and nucleic acids as well as polyhydroxyalkanoate (PHA), and the lipid, polyhydroxybutyrate (PHB), which is a member of the PHA, comprising  $\beta$ -hydroxybutyrate acid. These compounds have been affiliated with the increased protein and fat contents in the enriched media, subsequently augmenting the weight and growth rate of post-larvae black tiger and whiteleg shrimp. Akbary et al. (2011) proved the ability of *Artemia* enriched with fatty acids and vitamin C to

increase growth in rainbow trout (*Oncorhynchus mykiss*).

## Conclusions

Enrichment with biofloc was estimated to cause an increase in the protein and fatty acid content of *Artemia* sp. Hence, the feed treatments significantly influence the growth and survival rate of post-larva of the black tiger (*P. monodon*) and whiteleg shrimp (*P. vannamei*). The best effect was produced with the 30 mL biofloc dose, used as a nutritional source, with corresponding absolute growth of 15 and 14.57 mg. Meanwhile, the daily growth rate and survival percentages were 1.4 mg day<sup>-1</sup> : 99%, and 0.86 mg day<sup>-1</sup> : 99%, respectively

## Declarations of interest

The author(s) declare that there is no conflict of interest with regards to the research, authorship and/or publication of this article.

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