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

Supono Supono, Ayu N. Yanti, Anggita P. Pertiwi, Tarsim Tarsim, Wardiyanto Wardiyanto

Department of Aquaculture, Faculty of Agriculture, Lampung University, Jl. Sumantri Brjonengor No. 1 Bandar Lampung 35141, Indonesia.

**ARTICLE INFO**

**Keywords:**
- Black tiger shrimp
- Whiteleg shrimp
- Polyhydroxy butyrate
- Natural feed
- Survival rate

**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⁻¹: 98% and 1.4 mg day⁻¹: 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 Hudaiah, 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 (*Juhasz 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...
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 contain peptidoglycan and lipopolysaccharide (LPPS) as immunostimulant (Matsuura, 2013), and the bacteria produce polyhydroxybutyrate as antibacterial 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 (15 cm × 15 cm × 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 × 10⁶ CFU mL⁻¹ 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 15 cm × 15 cm × 25 cm) 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 3 L 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⁻¹, 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:

\[ W = W_t - W_0 \]

Where, \( W \) = body weight gain (mg); \( W_t \) = weight of post-larvae at the beginning (mg); \( W_0 \) = 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:

\[ \text{DGR} = \frac{(W_t - W_0)}{T} \]

Where, \( \text{DGR} \) = Daily growth rate (g day⁻¹); \( W_t \) = Average weight of post-larvae at the end of study (g); \( W_0 \) = 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:

\[ \text{SR} (%) = \frac{(N_0 - N_t)}{N_0} \times 100 \]

Where, \( \text{SR} \) = Survival rate (%); \( N_t \) = Number of post-larvae at the end of study (pcs); \( N_0 \) = The number of post-larvae dead during the study (pcs).

The water quality parameters, including dissolved oxygen (mg L⁻¹), temperature (°C), and pH were measured every three days interval, and ammonia
content (mg L\(^{-1}\)) 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 body weight 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\(^{-1}\)) and salinity at 25 to 26 ppt, while the ammonia content was less than 0.001 mg L\(^{-1}\) (Table 2).

![Table 1. The results of the proximate analysis of *Artemia* sp. used in this study](image)

| No. | samples       | 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.](image)

83
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⁻¹) | 3.3 to 5.5 |
| Salinity (ppt)    | 25-26          |
| Ammonia (mg L⁻¹)  | <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⁻¹ 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 β-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\(^{-1}\) : 99%, and 0.86 mg day\(^{-1}\) : 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.

References

Akbary, P., S.A. Hosseini, M.R. Imanpoor. 2011. Enrichment of Artemia nauplii with essential fatty acids and vitamin C: effect on rainbow trout (Oncorhynchus mykiss) larvae performance. Iranian Journal of Fisheries Sciences, 10(4): 587-590.

Avnimelech, Y. 2015. Biofloc Technology – a practical guidebook. Third edition. The World Aquaculture Society, Baton Rouge, Louisiana, United State.

Azim, M.E., D.C. Little, I.E. Bron. 2007. Microbial protein production in activated suspension tanks manipulating C/N ratio in feed and implications for fish culture. Bioresource Technology, 99: 3590-3599.

Boon, N., T. Defoirdt, W. de Windt, T. Van De Wiele, W. Verstraete. 2010. Hydroxybutyrate and Polyhydroxybutyrate as Components of Animal Feed or Feed Additives. Patent Application Publication, April: 1-4.

Chen, K., E. Li, Z. Xu, T. Li, C. Xu, J.G. Qin, L. Chen. 2015. Comparative transcriptome analysis in the hepatopancreas tissue of pacific white shrimp Litopennum vannamei fed different lipid sources at low salinity. Plos One, 10(12):1-22.

Crab, R., A. Lambert, T. Defoirdt, P. Bossier, W. Verstraete. 2010. The application of bioflocs technology to protect brine shrimp (Artemia franciscana) from pathogenic Vibrio harveyi. Journal of Applied Microbiology, 109: 1643-1649.

De Schryver, P., R. Crab, T. Defoirdt, N. Boon, W. Verstraete. 2008. The basics of bioflocs technology: the added value for aquaculture. Aquaculture, 277: 125-137.

Furuta, H., M. Takeuchi, M. Toyota, T. Watanabe. 1996. EPA and DHA requirement in early juvenile red sea bream using HUFAs enriched Artemia nauplii. Aquaculture, 62(2): 246-251.

Hamsah, Widanarni, Alimuudin, M. Yuhana, Jr. M. Zairin. 2017. The nutritional value of Artemia sp. enriched with the probiotic Pseudomonas putida and the prebiotic mannan-oligosaccharide. AACL Bioflux, 10(1):8-17.

Hargreaves, J.A. 2013. Biofloc Production System for Aquaculture. Southern Regional Aquaculture Center Publication. No. 4503. pp. 12.

Hernández, C., M.A. Olvera-Novoa, D.M. Smith, R.W. Hardy, B. Gonzalez-Rodriguez. 2011. Enhancement of shrimp Penaeus vannamei diets based on terrestrial protein sources via the inclusion of tuna by-product protein hydrolysates. Aquaculture, 317:117-123.
Supono et al.

Irianto, K. 2006. Mikrobiologi, Menguak Dunia Mikroorganisme. CV. Yrama Widya, Bandung.

John C. J. A., T.J. Abatzopoulos, P.M. Marian. 2004 Characterization of a new parthenogenetic Artemia population from Thamarakulam, India. Journal of Biological Research, 2:63-74.

Juhász, P., S. Lengyel, Z. Udvari, A.N. Sándor, L. Stándl. 2017. Optimized selenium enrichment of Artemia sp. feed to improve red drum (Seriema ocellata) larvae rearing. Acta Biologica Hungarica, 68(3):255-266.

Kangpanich, C., J. Pratoomyot, W. Senanan. 2016. Effects of alternative oil sources in feed on growth and fatty acid composition of juvenile giant river prawn (Macrobrachium rosenbergii). Agriculture and Natural Resources, 51 (2):103-108.

Khanjani, M.H., M.M. Sajjadi, M. Alizadeh, I. Sourinejadi. 2015. Study on nursery growth performance of Pacific whiteleg shrimp (Penaeus vannamei Boone, 1931) under different feeding levels in zero water exchange system. Iranian Journal of Fisheries Sciences, 15(4):1465-1484.

Lakshmi, B., B. Viswanath, D.V.R.S. Gopal. 2013. Probiotics as Antiviral Agents in Shrimp Aquaculture. Journal of Pathogen, 1-13.

Luo, G.Z., M.L. Yao, H.X. Tan, W.H. Wu. 2017. The performance of microbial flocs produced with aquaculture waste as food for Artemia. Aquaculture Nutrition, 23(6): 1440-1448.

Matsuura, M. 2013. Structural modifications of bacterial lipopolysaccharide that facilitate Gram-negative bacteria evasion of host innate immunity. Frontiers in Immunology, 4(109): 1-9.

Muchlisin, Z.A., A.A. Arisa, A.A. Muhammadr, F. Fadli, I.I. Arisa, M.N. Siti Azizah. 2016a. Growth performance and feed utilization of keureling (Tor tambra) fingerlings fed a formulated diet with different doses of vitamin E (alpha-tocopherol). Archives of Polish Fisheries, 24: 47-52.

Nurhatijah, N., Z.A. Muchlisin, M.A. Sarong, A. Supriatna. 2016. Application of biofloc to maintain the water quality in culture system of the tiger prawn (Penaeus monodon). AACL Bioflux, 9(4): 923-928.

Putra, D. F., M. Fanni, Z.A. Muchlisin, A.A. Muhammadr. 2016. Growth performance and survival rate of climbing perch (Anabas testudineus) fed Daphnia sp. enriched with manure, coconut dregs flour and soybean meal. AACL Bioflux, 9(5):944-948.

Putra, I., I. Effendi, E. Lukintowo, U.M. Tang, M. Fauzi, I. Suharman, Z.A. Muchlisin. 2020. Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia Oreochromis niloticus system. F1000Research, 9: 293.

Supono, J. Hutabarat, S.B. Prayitno, Y.S. Darmanto. 2013. The Effect of different C:N and CP ratio of media on the content of polyhydroxybutyrate in biofloc inoculated with bacterium Bacillus cereus. Journal of Coastal Development, 16(2): 114-120.

Supono. 2017. Teknologi Produksi Udang. Plantaxia, Yogyakarta, pp. 168.

Supono, S. Hudaidah. 2018. Short Communication: The diversity of epipelic diatoms as an indicator of shrimp pond environmental quality in Lampung Province, Indonesia. Biodiversitas, 19(4): 2085-4722.

Supriatna, A., N. Nurhatijah, M. A. Sarong, Z. A. Muchlisin. 2019. Effect of biofloc density and crude protein level in the diet on the growth performance, survival rate, and feed conversion ratio of black tiger prawn (Penaeus monodon). IOP Conf. Series: Earth and Environmental Science, 348: 012131.

Venero, J.A., D.A. Davis, D.B. Rouse. 2007. Variable feed allowance with constant protein input for the pacific whiteleg shrimp Penaeus vannamei reared under semi-intensive conditions in tanks and ponds. Aquaculture, 269(1): 490-503.

Yao, M., G. Luo, H. Tana, L. Fana, H. Meng. 2018. Performance of feeding Artemia with bioflocs derived from two types of fish solid waste. Aquaculture and Fisheries, 5:246–253.

Zhang, S.P., J.F. Li, X.C. Wu, W.J. Zhong, J.A. Xian, S.A. Liao, Y.T. Miao, A.L. Wang. 2013. Effects of different dietary lipid levels on the growth, survival and immune-relating genes expression in Pacific white shrimp, Litopenaeus vannamei. Fish & Shellfish Immunology, 34(5): 1131-1138.