Research Article

The effect of biofloc-supplemented diets on the Pacific white shrimp (*Litopenaeus vannamei*): Analysis of water quality, growth performance, and biochemical composition

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Abstract

This study has investigated the impacts of biofloc on water quality, growth parameters, and whole-body composition of the *Litopenaeus vannamei* juveniles (initial average weight 5.23±0.20 g). Five experimental treatments were designed: Shrimp fed basal diet (Control), 5 and 10% wet biofloc-supplemented diets (W5 and W10), 5 and 10% dried biofloc-supplemented diets (D5 and D10) for 32 days. Experimental tanks (50 liters) as triplicate for each treatment, was stocked 12 shrimps. During the experiment, chemical and physical water parameters were examined and were not different statistically among experimental treatments. At the end of the experiment, in W10 treatment, the growth parameters were significantly higher than the control treatment. Also, in W10 and D5 treatments, feed conversion efficiency (FCE) and feed conversion ratio (FCR) were significantly better than the control. Compared to other treatments, significantly better protein efficiency ratio (PER) and lipid efficiency ratio (LER) were observed in W10 treatment. Results indicated that in W10 and D5 treatments, the protein, ash, and dry matter contents of the shrimp were significantly higher compared to the control treatment. Also, in W10, D5, and D10 treatments, carcass lipid and fiber contents were significantly higher compared to the control. Overall, the best performance was observed in shrimp fed on 10% wet biofloc-supplemented diet.

Keywords: White leg shrimp, Microbial flocs, Growth, Body composition, Water treatment

Introduction

Biofloc system (BFT) is considered as a technique to improve water quality in the aquaculture industry so that it can produce microbial biomass or a "biofloc" which the cultured animals consume as a food source.

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In this system, microbial protein can be made by regulating the carbon to nitrogen ratio (C/N) (Emerenciano *et al.,* 2011; Maciel *et al.,* 2018). Biofloc is the accumulation of diverse microorganisms such as microalgae, fungi, bacteria, and protozoa, also the remaining food, and the aquatic substances are suspended in the water column (Browdy *et al.,* 2012). The primary purpose of this system is to consume nitrogenous wastes and convert ammonia into microbial proteins as a dietary supplement and bacterial activity to improve water quality (Burford *et al.,* 2004). Various studies have indicated the efficiency of these systems in aquaculture.

In general, the use of biofloc technology is a tool for water quality management in Aquaculture. Its benefits include reduced water consumption and reduced wastewater discharge (Wasielesky *et al.,* 2006). Hence, this technology is an environmentally friendly aquaculture system because of the reduced production of nitrogen and phosphorus waste. Frequent water exchanges are required to control nitrogenous wastes in intensive and super intensive systems, which lead to environmental problems (Avnimelech, 1999). Therefore, the development of new technologies such as biofloc can help reduce water use in aquaculture. Bioflocs collects microorganisms that can help the absorption of the nutrients by the stimulation of the digestive enzyme (Shyne Anand *et al*., 2014). It can also be used as a microbial protein source in the digestive system of fish and shrimp (Ekasari *et al*., 2014). There are bioactive compounds such as free amino acids, essential fatty acids, chlorophylls, minerals, and vitamins in the biofloc (Ju *et al*., 2008; Tacon *et al*., 2002; Crab *et al*., 2012) that can improve the immune system, antioxidant status, growth, and reproduction in aquatic animals (Ekasari *et al*., 2014; Abbaszadeh *et al*., 2019; Adineh *et al*., 2019).

It should be noted that all aquatic animals are not suitable candidates for the biofloc system, some features are essential in choosing culture species, such as high-density compatibility, tolerance of oxygen fluctuations, sedimentary solids, and nitrogen compounds in water (Taw, 2010). The white leg shrimp (*Litopenaeus vannamei*) in the Western Hemisphere is considered the extensively reared penaeid (Saoud *et al.,* 2003). This species rapid growth, disease tolerance, and high survival in high-density culture cause it a good candidate for intensive and, or bio-secure closed grow-out strategies (Cuzon *et al.,* 2004). Currently, *L.vannamei* culture has the first rank on the southern and northern (Gomishan-Golestan province) coasts of Iran. Shrimp in biofloc system depends on intensive and super-intensive which in such a situation where there is at least water exchange and, or with zero-water exchange (Tacon *et al.,* 2002). Although many researchers have highlighted the favorable impacts of promoted biofloc on shrimp production, the pathways which influence growth improvement and feed utilization are mainly unknown. The aim of this research was to investigate the effects of biofloc (wet and dried) on physical and chemical factors of water, feeding and growth performance, and also body composition of the *L. vannamei*.

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Materials and methods

Biofloc production and experimental diets

Filtered water and shrimp were transported from the northeastern part of the Caspian Sea, Gomishan, Iran, to the Aquatic Engineering Laboratory of the Gonbad Kavos University. During the feeding trial, biofloc produced in indoor tanks was used as dietary supplement in shrimp feed. Biofloc production was performed in four fiberglass tanks with a water volume of 50 liters in the laboratory environment. In each tank, three air stones were used to make a circular water flow for complete mixing. Biofloc materials for each 50 liters tank contains shrimp food of 10g (protein content 36 %), the mixture of flour and wheat bran of 2.5g, sugar beet molasses of 12.5g, the soil of the shrimp farm bed of 0.25g, urea of 0.25g (46% nitrogen) were transferred to the tank, and total ammonia nitrogen (TAN) concentration was measured. The ratio of Carbon/Nitrogen (C:N) in the biofloc tanks was monitored regularly and adjusted by adding the above-mentioned carbon sources at the rate of 15 times the total nitrogen according to Crab *et al.* (2012). On the 10, the air is cut off to biofloc the deposition and subsequently harvested by passing water in micro screen filter plastic with 10 µm pore size. This floc was used as a wet biofloc for 5% and 10% wet biofloc-supplemented diets. The collected flocs was centrifuged at 2000 rpm and throw away the supernatant water. Bioflocs were then washed twice with filtered brackish water of the same salinity to remove the traces of ammonia nitrogen level (Shyne *et al.,* 2014). Flocs were dried in an oven at 40 °C. This floc was used as a dried biofloc for 5% and 10% dried biofloc-supplemented diets. The dried flocs were ground to a fine powder (less than 200 microns) and then preserved in the fridge to make an experimental diet.

Shrimp and experimental protocol

L. vannamei, (average body weight and length 5.23±0.20 g and 9.70± 0.27 cm, respectively) were selected and randomly distributed into fifteen fiberglass tanks (Water volume 50 liters). Caspian Seawater was used for this experiment at a temperature 25.77 °C, pH = 7.23 and Salinity = 17.13 ppt. The shrimp were fed for 32 days using the control or corresponding experimental diets. Commercial shrimp diet with 36% protein, 8% lipid, 4% fiber, 14% ash, and 10% moisture (aquatic food factory of Hatami, Iran) was ground to a powder and used as basal diet.

Five experimental diets were considered containing the basal diet, including: Control (basal diet), W5 and W10 (5% and 10% wet biofloc-supplemented diets, respectively), D5 and D10 (5% and 10% dried biofloc-supplemented diets, respectively). Daily water change was 5% of the total volume of the tank. The photoperiod was performed of 12 h light and 12 h darkness. During the experiment, shrimp were fed four times per day. Daily feeding ration is determined in terms of the 5 to 4.5% body weight for all treatments.

Assessment of water quality parameters

During the experiment, water quality parameters such as temperature and dissolved

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oxygen measured with multiparameter meter, Hack, model 2000. Salinity and electrical conductivity measured with Martini instruments, model EC60, Italy. The pH was recorded using a Palin test device, model 7500, England. Water quality parameters including total alkalinity, total ammonia nitrogen (TAN), nitrite-nitrogen (NO2-N), nitrate-nitrogen (NO3-N), total phosphorous (TP), and total suspended solids (TSS) were analyzed following the standard methods for water and wastewater analysis on days 8, 16, 24, and 32 experiment (APHA, 1998).

Calculations and statistics

The growth performance and feed utilization, at the end of the experiment, were evaluated based on percentage weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), lipid efficiency ratio (LER) and condition factor (CF) using the following formulae:

Weight gain = (final weight (g) - initial weight (g)) ×100 / initial weight.

Specific growth rate = (Ln final weight (g) - Ln initial weight (g)) / experimental days.

Feed conversion ratio = (feed consumed / weight gain).

Feed conversion efficiency = (weight gain / Feed consumed) × 100.

Protein efficiency ratio = wet weight gain (g) / protein intake.

Lipid efficiency ratio = wet weight gain (g) / lipid intake.

Condition factor = (fish weight (g) / fish length $(cm)^3$) × 100.

Shrimp body composition

Crude lipid, crude protein, fiber, ash, and dry matter contents were calculated based on AOAC (1995). Through Kjeldahl method, crude protein content was analyzed in triplicate (Kjeltec 1030 Auto Analyzer, Tector, Sweden); using a Soxtec extraction unit, crude lipid determination was performed (model 1043 Extraction Unit; Tecator, Sweden); after burning the shrimp sample in a muffle furnace at 550 °C for 6 h, crude ash determination was performed by weighing the crude ash residue (Heraeus, Germany). Finally, fiber was analyzed using a Fibertec System Tector 1010 (Sweden).

Research statistical analysis

This study used the statistical software SPSS version 16 to analyze the data statistically. In this line, through Shapiro–Wilk test, the data on water quality, growth parameters, feed performance, and body composition were examined to check their normality. Then, the comparison of mean values were evaluated among experimental and control by One-way analysis of variance (ANOVA) and Duncan tests. It is noted that the significance level of data analysis in this study was $p<0.05$.

Results

As table 1 indicates, there was no significant difference ($p>0.05$) among the treatments in water quality parameters. During the experiment, in different treatments were registered the mean temperature 27.51 °C,

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salinity 15.97 g/L, dissolved oxygen 7.58 mg /L, and pH 8.45. Electrical conductivity (EC) and total dissolved solids (TDS) were not significantly different between treatments (*p>0.05*). The phosphate was not significantly different between experimental treatments (*p>0.05*). Its range was between 0.28± 0.07 to 0.41± 0.22 mg/L. Based on the measurement of ammonia, no statistical difference between treatments was observed (*p>0.05*), but the highest amount was 0.45 ± 0.11 mg/L in the D10 treatment. The highest and lowest nitrite levels were obtained 1.05±0.17 and 0.26±0.16 mg/L in the D10 and W5 treatments, respectively. There were no significant differences in Nitrate concentration among treatments (*p>0.05*). The nitrate concentration was ranged from 42.03± 12.39 to 81.71± 12.20 mg/L in the experimental diets. There was no significant difference in alkalinity and total suspended solids (TSS) between the two feed types and control (*p>0.05*).

Table1. Overall water quality parameters in the control and two bioflocs experiments (wet and dried) during 32 days experimental period

	Control	Wet Biofloc		Dried Biofloc	
		W5	W10	D5	D10
Temperature (°C)	27.50±0.90	27.42± 0.83	27.52± 0.95	27.50± 0.94	27.62± 0.82
Salinity (ppt)	15.90± 0.43	16.11± 0.26	15.83± 0.57	16.17± 0.41	15.85± 0.24
Dissolved oxygen (ppm)	7.77± 0.50	7.54± 0.47	7.55± 0.43	7.47± 0.63	7.56± 0.65
pH	8.36±0.22	8.45±0.17	8.18±0.25	8.46±0.35	8.84±0.24
EC (×10^{-3} mho)	25.94±0.58	26.15± 0.46	25.80± 0.41	26.12± 0.34	25.77± 0.37
TDS(×10)	15.38± 0.44	15.53± 0.25	15.33± 0.53	15.47± 0.22	15.28± 0.25
Phosphate (mg/L)	0.32± 0.18	0.28± 0.07	0.26± 0.14	0.41± 0.22	0.31± 0.08
Total ammonia-N (mg/L)	0.37± 0.21	0.23±0.11	0.20± 0.10	0.28± 0.18	0.45± 0.11
Nitrite-N (mg/L)	0.87± 0.39^{ab}	0.26± 0.16^{b}	0.41± 0.22^{ab}	0.67± 0.28^{ab}	1.05± 0.17^{ab}
Nitrate-N (mg/L)	59.70± 21.75	62.42± 20.62	42.03± 12.39	81.71± 12.20	71.83± 29.45
TSS (mg/L)	432.25±113.78	430.00±67.82	320.50±110.27	452.25±104.05	419.49±93.73
Alkalinity (mg CaCO3/L)	204.33±58.80	187.50±26.87	178.43±42.39	216.37±58.97	222.02±77.38

Data are presented as mean ± SD. Data with different superscript letters (a, b and c) in the same row mean significant differences among experimental treatments (*p<0.05*).

Growth parameters and feed utilization of shrimps fed with biofloc and control diets were evaluated for 32 days (Table 2). In both biofloc treatments, the shrimp growth was significantly better (*p<0.05*) than that obtained in control (except D10). The highest final weight was observed in W10 (8.91± 0.63 g) and the lowest in D10 (7.34± 0.33 g). The final length was highest in shrimp fed on W10 diet (11.23± 0.30 cm), while the lowest (10.60±0.34 cm) was found in shrimp fed on D10 diet. The highest specific growth rate (SGR %) of 1.65± 0.14 was obtained in fish fed 10% wet biofloc (W10), while the D10 treatment showed the lowest value of 1.05±0.14. In biofloc treatments (W10 and D5), the feed conversion ratio (FCR) of the shrimp was significantly lower (*p<0.05*) than that obtained in other treatments (Table 2). Among the experimental treatments, significantly higher (*p<0.05*) protein efficiency ratio (PER) and lipid efficiency ratio (LER) were recorded in W10 treatment compared to other treatments.

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Table 2. Growth parameters and feed utilization of the *L. vannamei* reared in the control and two bioflocs experiments (wet and dried) during 32 days

	Control	Wet Biofloc		Dried Biofloc	
		W5	W10	D5	D10
final weight (g)	7.83± 0.64^{bc}	8.12± 0.59^{b}	8.91± 0.63^{a}	8.35± 0.32^{b}	7.34± 0.33^{c}
Final length (cm)	10.77±0.22^{cd}	11.05± 0.28^{bc}	11.23±0.30^{a}	11.12±0.18^{b}	10.60± 0.34^{d}
Weight Gain (%)	49.82± 8.38^{bc}	55.36±6.55^{b}	74.59± 7.61^{a}	59.65± 6.24^{b}	40.39± 6.49^{c}
SGR (%/day)	1.25± 0.15^{bc}	1.37± 0.14^{b}	1.65± 0.14^{a}	1.45± 0.11^{b}	1.05± 0.14^{c}
CF	0.62±0.03^{a}	0.60± 0.01^{a}	0.62± 0.02^{a}	0.60± 0.01^{a}	0.61± 0.03^{a}
FCR	1.84± 0.32^{a}	1.78± 0.20^{a}	1.21± 0.11^{b}	1.42± 0.13^{b}	2.02± 0.30^{a}
FCE	56.81± 10.12^{c}	57.43± 8.90^{c}	85.53± 10.02^{a}	71.05± 7.36^{b}	50.47± 8.11^{c}
PER	0.21±0.016^{bc}	0.22± 0.011^{b}	0.24± 0.010^{a}	0.23±0.008^{b}	0.20± 0.009^{c}
LER	0.97± 0.060^{bc}	1.01± 0.052^{b}	1.11± 0.041^{a}	1.04± 0.040^{b}	0.91± 0.042^{c}

Data are presented as mean ± SD. Data with different superscript letters (a, b and c) in the same row mean significant differences among experimental treatments (*p<0.05*).

Proximate composition whole body of the shrimp is presented in Table 3. The crude protein content ranged from 70.46±1.50% to 66.03±1.15% in the experimental treatments. The dried biofloc (D5) treatment contained 70.46±1.50% crude protein and 4.56±0.42% crude lipid. In biofloc W10 and D5 treatments, the protein, ash, and dry matter contents of the shrimp were significantly higher compared to the control treatment. Also, in biofloc W10, D5, and D10 treatments, carcass lipid and fiber contents were significantly higher compared to the control.

Table 3. Comparative proximate body composition of the *L. vannamei* in the control and two bioflocs experiments (wet and dried) during 32 days

	Control	Wet Biofloc		Dried Biofloc	
		W5	W10	D5	D10
Protein	67.11±1.55^{c}	66.03±1.15^{d}	69.20±1.56^{b}	70.46±1.50^{a}	67.98±1.43^{c}
Lipid	3.50±0.41b^{c}	3.00±0.30^{c}	4.11±0.22^{b}	4.56±0.42^{a}	3.67±0.20^{ab}
Fiber	1.18±0.10^{b}	0.87±0.05^{c}	1.77±0.15^{a}	1.58±0.12^{a}	1.08±0.19^{ab}
Ash	6.21±0.25^{c}	6.46±0.28^{c}	7.31±0.15^{b}	7.74±0.13^{a}	6.48±0.26^{c}
Dry matter	24.92±0.63^{b}	23.64±0.49^{c}	26.96±0.57^{a}	27.24±0.47^{a}	24.70±0.36^{b}

Data are presented as mean ± SD. Data with different superscript letters (a, b and c) in the same row mean significant differences among experimental treatments (*p<0.05*).

Discussion

This study highlighted that biofloc technology is based on the growth of microorganisms, which can be a stress-free environment in aquaculture systems (Adineh *et al*., 2019) by maintaining water quality and feed supply (Crab *et al.,* 2012). As recommended for the white shrimp species, the water quality parameters were maintained in the present study (Ponce-Palafox *et al.,* 2013). Water quality factors such as dissolved oxygen, temperature, and pH showed no considerable differences between treatments. The nitrogen compounds (Nitrite) showed variations between different treatments during the experimental period (from 0.26± 0.16 to 1.05± 0.17 mg/L). According to Lin and Chen

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(2003), the NO2–N levels in this experiment were within a safe range, and they recommend a safe level of nitrite–N concentration for *L. vannamei* at 15% salinity of 6.1 mg NO_2–N/L. This study employed frequent use of biofloc to maintain ammonia and nitrite concentrations levels below the recommended for shrimp (Lin and Chen, 2003), to facilitate the establishment and proliferation of nitrifying bacteria (Krummenauer *et al*., 2014) by steering the C/N ratio in water by modifying the carbohydrate content of the feed or by adding an external carbon source to the water. Soluble solids (TDS) is the total concentration of all ions (organic and inorganic) in water. Therefore, measuring it is very important for controlling the quality of water. To reduce the amount of organic and inorganic matter in the environment, it is essential to eliminate accumulating nutrients such as nitrate and phosphate. There was no significant difference in the amount of phosphate between experimental treatments. Its range was between 0.28± 0.07 to 0.41± 0.22 mg/L. There was no significant difference in TSS levels between experimental treatments during the experiment. The TSS concentrations suggested changes between 200 and 600 mg/L for *L. vannamei* in a biofloc system (Samocha *et al.,* 2007; Avnimelech and Kochba, 2009; Ray *et al.,* 2011; Gaona *et al.,* 2011; Schveitzer *et al.,* 2013). In this study, concentrations of TSS were ranged between 222 and 690 mg L^{-1}. Various studies indicated differences in TSS values, which may be because of consumption and stocking density, cultured growth, microbial community tank, aeration design, and biological conditions of the microorganisms (Emerenciano *et al.,* 2011; Adineh *et al*., 2019). Ray *et al* (2010) reported that water quality and shrimp production can be improved by controlling the particle concentration in shrimp culture systems.

The biofloc as a feed for aquatic animals are available 24 hours a day (Xu and Pan, 2012). Different carbon sources play the role of stimulus in microbiota development; therefore, they have a distinctive impact on water quality and feed production by the cultured organisms. Organisms can simultaneously maintain good water quality in aquaculture systems and produce additional food for aquaculture (De Schryver *et al*., 2008). In this study, to change the efficiency of inorganic nitrogen to microbial protein, the C: N ratio was kept at 15: 1 (Avnimelech, 1999). The consumption of biofloc by shrimp has verified various merits, including the decline of food conversion ratio (Burford *et al.,* 2004), the growth rate improvement (Wasielesky *et al.,* 2006; Abbaszadeh *et al.,* 2019). At the end of our experiment, in biofloc W10 treatment, the growth performance parameters were significantly higher than the control treatment. Also, in biofloc treatments (W10 and D5), FCE and FCR were significantly better than the control. Correspondingly, compared to other treatments, significantly better PER and LER were observed in W10 treatment. Consequences of dietary supplementation with graded levels of biofloc from 0% to 20% on growth performance and physiological stress showed that levels of 10%–15% biofloc had

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significantly higher SGR than the control treatment (diet B0). Therefore, digestive enzyme activity increased with dietary biofloc levels (Chen *et al.,* 2018). Shyne Anand *et al* (2014) examined the impact of dietary supplementation of biofloc (0, 4, 8, and 12%) on growth and feed performance in *Penaeus monodon* juveniles. Their results showed that the best weight, FCR, and PER were obtained in B4 (8% dried biofloc) compared to control. In Taw (2010), lower FCR (1.3 to 1.6) with *L. vannamei* was observed in intensive ponds with liners and zero or minimal exchange water. Also, Maicá *et al.,* 2014 evaluated the impact of salinity on the culture water quality and the body composition of *L. vannamei* reared in an intensive system (200 animals in 140/m^2) without water exchange. The results showed that WG and SGR of shrimp in this system increased and FCR reduced (Maicá *et al.,* 2014). These systems minimize the possibility of pathogens transmission and spread in the environment (Wasielesky *et al.,* 2006) and the discharge of effluent. Furthermore, biofloc is a supplemental dietary source for farmed aquatic animals produced in this system by the microbial community (Burford *et al.,* 2004).

The results of determining the effect of biofloc microbial community on the water quality and growth of *Penaeus monodon* showed the treatment contains diatom and bacteria had statistically significant ($p<0.05$) highest weight 7.5 gr and lowest FCR 1:1.5 (Sakkaravarthi, 2015). In another study, the effect of biofloc dietary supplementation showed that use at the 4% level in feed improves metabolic activities in black tiger shrimp (Anand *et al*., 2017). Huang *et al* (2017) reported that add biofloc supplements for Penaeus monodon reduced the feed coefficient and improved water quality. Various published studies show that a supplemental food source for the farmed shrimp can be accompanied by biofloc (Kuhn *et al.,* 2009 and 2010; Bauer *et al.,* 2012). The bioflocs can provide extra protein, minerals, vitamins, and lipids as a natural and essential nutrient in the culture systems of shrimp (Valle *et al.,* 2015; Jatobá *et al.,* 2017). Also, the microbial protein had a significant effect on the total body composition of cultured shrimp. In this study, there was a significant difference in protein and lipid levels between biofloc treatments and control. The highest amounts of carcass compositions were obtained in D5 and W10 treatments compared to other treatments. The examination of the impacts of bioflocs on the growth performance of *L. vannamei* in zero-water indicated that biofloc could increase growth rate, feed utilization, and digestion; however, a considerable difference in the body composition was not observed (Xu and Pan, 2012). Although shrimp growth is less in D5 treatment than W10 treatment in this study, body composition (such as protein, lipid, and ash) tended to enhance in this treatment (D5) compared to those obtained in the other biofloc treatments and the control. Our results indicated that in W10 and D5 treatments, the protein and ash contents of the shrimp were significantly higher compared to the control treatment. Also, in W10, D5, and D10

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treatments, carcass lipid and fiber contents were significantly higher compared to the control. Similarly, Izquierdo *et al.,* (2006) reported that *L. vannamei* reared in green water containing floc had a higher increase in the whole body lipid content than clear water systems. It is acceptable to judge that due to the nutritional elements more significant amount supplied by the bioflocs, the shrimp fed on the artificial feed and the bioflocs might have better nutrient assimilation than with those fed only the commercial feed (Izquierdo *et al.,* 2006). According to Khanjani, *et al.,* (2017), the use of biofloc led to increased protein, fat, and ash composition in the *L vannamei* postlarvae; one of the alternatives that have been used in aquaculture is the use of microbial communities (biofloc). Indeed, the efficiency of this systems in aquaculture have indicated by many researchers (Ray and Lotz, 2017; Xu *et al.,* 2018) and the positive consequence are assigned to the presence of microorganisms, especially the metabolites and bacteria they produce (Crab *et al.,* 2010).

Our results indicate that the addition of 10% wet biofloc (W10) in water environment minimal exchange, intensive culture systems contributed to increased shrimp growth, feed utilization, and body composition; because the heterotrophic bacteria used carbon and nitrogen to produce microbial protein and improve the water quality. The highest amount of protein and lipid was obtained in biofloc treatments (D5 and W10). In general, the biofloc amount and its type are very important in aquaculture systems that should be taken into consideration.

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Conflicts of interest

Authors have no conflict of interest on this work.

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