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Sustainable Growth of Shrimp Aquaculture Through Biofloc Production as Alternative to Fishmeal in Shrimp Feeds

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Abstract

As capture fisheries are now fully exploited in Egypt, aquaculture is considered the only source for meeting the demand of seafood for rapidly growing populations in Egypt. To meet these demands for seafood, aquaculture has to grow fast and to be more intensive. Fishmeal sources from capture fisheries are fully exploited and thus become costly ingredient in fish and crustacean feed formulations. The present study evaluated biofloc technology as a sustainable alternative to fishmeal in the shrimp feeds using cheap carbon source for stimulation of the microbial biofloc growth, in addition to reduce protein content of the shrimp feeds. The shrimp were fed on three different diets (Control 45% CP, $BF_{25.10}$ and $BF_{30.10}$). The shrimp were cultured for 150 days. The water quality in biofloc-based pond was maintained with the promotion and development of biofloc through rice bran addition during the experiment. At the end of the experiment, the total yields of the shrimp in the two biofloc treatments were significantly higher than those of the shrimp in the control group ($P < 0.05$). The results indicated that promoting biofloc through carbohydrate addition in culture systems could enhance growth performance and water quality of cultured shrimp under the conditions of the present study. Feed conversion ratio (FCR) was significantly ($P < 0.05$) differed among treatments compared to control diet (1.51 ± 0.11; 1.66 ± 0.13 and 1.80 ± 0.10 for $BF_{25.10}$; $BF_{30.10}$ and control, respectively). There were significant differences ($P < 0.05$) in shrimp mean final weight between treatments and control diet (37.10 ± 2.22 g; 35.31 ± 1.52 g and 33.24 ± 2.17 g for $BF_{25.10}$; $BF_{30.10}$ and control, respectively). Mean survival (70 ± 8.30%; 66 ± 9.10% and 57 ± 11.00%, for $BF_{30.10}$; $BF_{25.10}$ and control, respectively. was significantly differed among treatments compared to control diet. The data suggests that substituting high-protein (Control 45% CP) with low-protein ($BF_{25.10}$ and $BF_{30.10}$) feed in a biofloc dominated system operated with minimal discharge may provide an alternative to improve shrimp farming technology, through improved water quality, cheaper (lower protein) feed and reduced environmental impact. The biochemical composition of the biofloc treatments did not vary significantly. Biofloc grown on $BF_{25.10}$ had higher protein content (23 ± 7 % DW) than $BF_{30.10}$ treatment (18 ± 5 %DW). The composition of the PUFAs in the biofloc showed that the treatment of biofloc with $BF_{25.10}$ contained significantly more total n-3 PUFA and total n-6 PUFA than that with treatment of $BF_{30.10}$. The results of the present study proofed that biofloc is a possible additional nutritious aquaculture feed for shrimp.

Keywords: biofloc, low protein feed, shrimp, *Fenneropenaeus indicus*, growth parameters, sustainability

1. Introduction

In the last 10 years, aquaculture production showed rapidly growth to meet seafood demand due to shortage supply of fish and crustacean from overexploited fisheries in Egypt. In 2013, more than 75% of seafood supply in Egypt comes from aquaculture and less than 25% comes from capture fisheries (GAFRD, 2013). This activity of aquaculture in Egypt requires using several resources such as seed for pond stocking and low cost feeds with high quality. Intensification of aquaculture production cannot depend on fishmeal from natural fisheries due to overexploited and high cost source for developing aquaculture production in Egypt. It requires sustainable alternative sources to fishmeal in shrimp feeds, otherwise shrimp farmers will be out of business. The white Indian shrimp *Fenneropenaeus indicus* is a commercially important shrimp species being cultured in Egypt since 2010.

There are several research efforts to increase the economic and sustainaibility of aquaculture industry. These efforts are represented in implementing cheaper alternative ingredients to fishmeal, reducing the costs of the feed and impacts on natural fisheries (Tacon et al., 2006; Glencross et al., 2007; Tacon & Metian, 2008; Tacon & Metian, 2009; Naylor et al., 2009; Kuhn et al., 2010; Bendiksen et al., 2011). The use of biofloc has been evaluated by several studies as a sustainable feed option and could enhance shrimp growth (Megahed, 2010; Xu & Pan, 2012; Xu et al., 2012a, 2012b; Xu & Pan, 2013a, 2014).

Biofloc technology is an environmental and sustainable technology used in aquaculture to maintain water quality through converting nitrogenous waste into bacterial proteinaceous biomass after the addition of carbohydrate sources (Schneider et al., 2005; Crab et al., 2010a, 2010b; Xu & Pan, 2012; Xu et al., 2012a, 2012b) and subsequently consumed by the cultivated aquatic organisms (Avnimelech, 2005). The aim of this study was to find out sustainable and cost-effective alternative source for costly and overexploited fishmeal in the feed of marine shrimp.

2. Material and Method

2.1 Experimental Set-Up

This study was carried out at a commercial shrimp farm at AL-Deba, PortSaid, Egypt. The study was carried out on Shrimp *Fenneropenaeus indicus.*Shrimp postlarvae (PL15) obtained from commercial marine hatchery, Alexandria, Egypt in 2013. Feeding trial was carried out in 12 earthen ponds with an average area of 4200 m^2 and 1.0 m average depth each. At the start of the experiment, the shrimp had an average weight of 0.03 ± 0.001 g. Shrimp stocked at an initial stocking density of 15 individuals /m^2. Feed was given at 5% of the shrimp biomass. Feed were given in daily four meals at 7 AM, 12 PM, 4PM and 8PM. Rice bran was spread to the pond surfaces in the afternoon and completely mixed with the water of each pond by strong aeration system. The experiment consisted of two biofloc treatments in addition to control. The biofloc treatments were consisted of pelleted feed contained two different protein levels ($BF_{25.10}$ and $BF_{30.10}$). The control was fed on diet contain 45% CP. The organic C/N ratios were 8.3 in the control diet and 10.4 and 12.1 in the treatments $BF_{30.10}$ and $BF_{25.10}$, respectively.

2.2 Experimental Feed

Feed preparation was carried out at the Fish Research Center, Suez Canal University, Ismailia, Egypt (Table 1). The experimental diets were prepared by individually weighing of each component and by thoroughly mixing the mineral, vitamins and Cellulose with starch. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making granules. The wet mixture was passed through CBM granule machine with 2mm diameter. The produced pellets were dried at room temperature and kept frozen until starting the experiment.

Table 1. Feed ingredients and proximate analysis of the experimental diets

Ingredients	$BF_{25.10}$	$BF_{30.10}$	Control 45 % CP
Fishmeal (60%)	41.50	50.00	75.00
Corn starch	41.50	32.50	11.00
Fish oil	5.00	5.50	2.00
Mineral & Vitamine Mix[1].	5.00	5.00	5.00
Cellulose	5.00	5.00	5.00
Carboxymethyl cellulose (CMC)	2.00	2.00	2.00
Proximate analysis			
Moisture	8.70	9.50	9.50
Crude protein	25.20	30.10	45.00
Crude lipid	9.80	11.00	11.70
Ash	11.00	13.70	18.90
Fiber	5.30	5.20	5.10
Nitrogen free extract (NFE)	40.00	30.50	9.80

[1] Each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D_3, 0.8 million IU; E, 4 g; K, 0.8 g; B_1, 0.4 g; Riboflavin, 1.6 g; B_6, 0.6 g, B_{12}, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

2.3 Proximate Analysis of Experimental Diets and Biofloc

The proximate composition of the experimental diets and the biofloc were determined following the standard methods of AOAC (1995). The moisture content was determined by drying at 105 °C to a constant weight, and the difference in weight of the sample indicated the moisture content. Crude protein was estimated by Kjeldahl method (Kelplus, Pelican equipments, India). Crude lipid was determined by the solvent extraction method by Soxtec system (Soxtec system, SCS–6, Pelican equipments, India) using diethyl ether (boiling point, 40–60 °C) as a solvent. Ash content was determined by incinerating the samples in a muffle furnace at 600 °C for 6 h. Crude fiber was determined based on the weight loss on ignition of the oven dried residue remaining after sequential digestion of a sample with H_2SO_4 and NaOH solution using Fibretec (Foss Tecator 2022, Sweden). Total nitrogen free extract (NFE) was determined as per the formula described in Hastings and Dupree (1969).

$$\text{NFE (\%)} = 100 - (\text{Crude protein} + \text{Ether extract} + \text{Ash} + \text{Fiber} + \text{Moisture})$$

Fatty acid methyl esters (FAME) were prepared by transesterification for gas chromatography according to Coutteau and Sorgeloos (1995) and identified by a gas chromatograph equipped with temperature programmable on-column injector.

2.4 Determination of Water Quality Parameters

Water quality in the culture systems was monitored for dissolved oxygen (DO), salinity, and temperature using an YSI 85m (YSI Inc., Ohio, US). Total ammonia-N (TAN), nitrite-N (NO_2) and nitrate-N (NO_3) were analyzed immediately after sample collection following the procedures described in APHA (1998).

2.5 Growth Parameters

The growth performance was assessed in terms of survival rate %, feed conversion ratio (FCR), average final body weight (ABW) and weight gain (g week-1) were recorded to assess dietary effects on shrimp performance. At the end of the experiment, the above mentioned indicators were estimated in addition to final yield (kg/ha). The parameters were calculated based on the following formulas:

$$\text{FCR} = \text{Feed applied} / \text{Body weight gain}$$

$$\text{Survival} = (\text{Total number of shrimps survived} / \text{Total number of shrimp}$$

Growth rate (g/week) were estimated for each sampling interval by the formula G = (Wf _ Wi)/T, where G = growth rate in g/week, Wf = mean final weight (g), Wi = mean initial weight (g) and T = time (weeks).

2.6 Statistical Analysis

Statistical analysis was performed using SPlus v 8.0 for Windows (Insightful Corp.). One way ANOVA followed by Tukey's test was employed to test the significant differences between treatments. The Student 't-test' was used to determine differences between treatments in survival (arcsine transformed), feed convertion ratio and mean final weight, according to Zar (1996). All differences were analyzed at significance level of $\alpha = 0.05$.

3. Results and Discussion

3.1 Growth Performance

To reduce the cost of shrimp feeds and utilization of marine proteins (fishmeal, squid meal, and shrimp meal), biofloc were used to recycle waste protein and nitrogen in the shrimp ponds to complement the pelleted feeds with live organisms. The biofloc experiment was carried out in the growout ponds using cheap carbon source to develop least cost feed for sustainable shrimp farming. Growth performance of *F. indicus* postlarvae over the time period are presented in Table 2. Final body weight among treatments differed significantly ($p < 0.05$) between control and biofloc treatments. Recently, it has been demonstrated that dietary inclusion of biofloc enhanced the growth performance of *L. vannamei* (Ju et al., 2008b; Kuhn et al., 2010; Bauer et al., 2012). Moreover, it has been reported that use of biofloc as a dietary ingredient in shrimp diet enhances the growth rate of *L. vannamei* (Kuhn et al., 2009, 2010). The present study illustrates the role of biofloc as a dietary supplement on growth performance in *F. indicus.* As a supplemental food source available for cultured shrimp, the biofloc can be consumed and provide a significant fraction of protein demand (Burford et al., 2004; Wasielesky et al., 2006; Ballester et al., 2010; Crab et al., 2010a; Xu et al., 2012a, 2012b). Besides providing supplemental nutrition, like protein, lipid, mineral and vitamin (Izquierdo et al., 2006; Ju et al., 2008b; Moss et al., 2006; Xu et al., 2012b), the biofloc is a source of abundant natural microbes and bioactive compounds that could exert a positive effect on the physiological health and improved the digistiability of cultured shrimp (Xu et al., 2012a, 2012b).

3.2 Feed Utilization

Results of feed utilization in terms of FCR are presented in Table 2. The average of feed conversion ratio (FCR) in biofloc treatments were significantly ($P < 0.05$) improved in comparison with the control. The FCR was found to be 1.51 ± 0.11, 1.66 ± 0.13 and 1.80 ± 0.10 in $BF_{25.10}$, $BF_{30.10}$ and control, respectively. These results indicated that the best ($P < 0.05$) FCR values were obtained by groups of shrimp fed on $BF_{25.10}$ followed by $BF_{30.10}$. The best FCR values observed with biofloc treatments suggested that the biofloc improved feed utilization (Megahed, 2010; Xu & Pan, 2013a, 2014).

There were significant differences ($P > 0.05$) among the two treatments with two dietary protein levels and control diet in the mean survival rate (Table 2). The high survival and improved growth of the shrimp in the two biofloc treatments support the view that the shrimp grew in a healthy condition in biofloc-based ponds with rice bran addition as a crbon source. These results agree with results obtained by (Xu & Pan, 2012). It should be noted that biofloc contains an appropriate amount of antioxidants such as carotenoids and fat-soluble vitamins (Ju et al., 2008a), which could contribute to sustaining sufficient antioxidant status of the shrimp (Xu & Pan, 2013b). For example, carotenoids have been reported to improve animal immune systems, increase stress tolerance and perform an antioxidant function (Linan-Cabello et al., 2002; Babin et al., 2010). Since the biofloc not only can provide supplemental nutrition, like protein, lipid, mineral and vitamin for the growth of cultured shrimp (Izquierdo et al., 2006; Ju et al., 2008b; Moss et al., 2006; Xu et al., 2012a), but also is a source of abundant natural microbes and bioactive compounds that could exert a positive effect on the physiological health of the shrimp in biofloc-based culture systems (Ju et al., 2008a; Xu & Pan, 2013), there is a great potential in reducing crude protein level of feeds while maximizing the contribution of biofloc to the growth and health of the shrimp.

In addition, the result suggests that it's possible to manipulate an appropriately high C/N ratio of feed input through carbohydrate addition to achieve a well-performing biofloc system, and has a positive application prospect in large-scale shrimp aquaculture. The biofloc supplementing the inadequate portion of protein intake and the proper use of low protein feed can reduce dietary fishmeal inclusion, thus reducing the cost of feed and improving the efficiency of production.Some studies suggested that using low protein feeds in biofloc-based culture systems could also achieve good survival and growth performance of cultured shrimp (Ballester et al., 2010; Megahed, 2010; Moss, 2002; Wasielesky et al., 2006).

Table 2. Growth performance parameters of shrimp *F. indicus* fed on exprememtal diets and biofloc

	$BF_{25.10}$	$BF_{30.10}$	Control 45 % CP
Initial Weight (g)	0.031 ± 0.001^a	0.033 ± 0.001^a	0.03 ± 0.001^a
Final Weight (g)	37.10 ± 2.22^a	35.31 ± 1.52^a	33.24 ± 2.17^a
Average growth (g/week)	1.85 ± 0.22^a	1.76 ± 0.20^b	1.66 ± 0.40^c
FCR	1.51 ± 0.11^a	1.66 ± 0.13^b	1.80 ± 0.10^c
Survival (SR %)	70 ± 8.30^a	66 ± 9.10^a	57 ± 11.00^b
Yield (kg) /ha	3672.23 ± 13.29^a	3300.34 ± 17.83^b	2695.71 ± 15.33^c

3.3 Nutritional Value of the Biofloc

The biochemical composition of the biofloc is shown in Table 3. Protein content was high in the $BF_{25.10}$, being 23 ± 7 % DW, while 17 ± 5 in the $BF_{30.10}$. Crude lipid was also high in the $BF_{25.10}$ 22 ± 4 % DW.High ash content was noted in the $BF_{25.10}$, up to 4 ± 3 % DW. The carbohydrate content of the biofloc was high in the $BF_{30.10}$ 62 ± 5% DW.The composition of the biofloc of the two treatments (Table 3) did not vary significantly between sampling dates. This microbial protein can serve as an additional high value feed for fish or shrimp, recycling the non-utilized fraction of the added conventional feed (Avnimelech et al., 1989; Megahed, 2010; Xu & Pan, 2012, 2013a, 2014). Protein levels in conventional feeds generally average 20-40% on the DW (Craig and Helfrich, 2002). Proteins are required in the diet to provide essential amino acids. Lipids are high-energy nutrients that can be utilized to partially substitute for protein in aquaculture feeds (Craig & Helfrich, 2002). Lipids supply about twice the energy as proteins and carbohydrates. Lipids typically comprise about 15% on the DW of fish diets, supplying essential fatty acids. In this biofloc experiment, about 22 ± 4% lipid on the DW was measured in the $BF_{25.10}$ and it was 17 ± 1% DW in the $BF_{30.10}$. The developed biofloc could provide an important source of food protein for cultured shrimp (Decamp et al., 2002; Ballester et al., 2010; Hari et al., 2004), and thereby supplemented the relatively inadequate portion of dietary protein intake in the present experiment. Therefore, the dietary protein level could be reduced from 45% to 25% without affecting the growth performance of the shrimp in this study.

Table 3. Nutritional value of biofloc produced in shrimp ponds with rice bran as a carbon source

Parameters	$BF_{25.10}$	$BF_{30.10}$
Crude protein (% DW)	23 ± 7^a	18 ± 5^b
Crude lipid (% DW)	22 ± 4^a	17 ± 1^b
Ash (% DW)	4 ± 3^a	3 ± 2^b
Carbohydrate (% DW)	51 ± 4^b	62 ± 5^a
Total n-3 PUFA	0.9	0.8
Total n-6 PUFA	19.6	15.4

The measured lipid content of the two types of biofloc was high. The advantage of using biofloc as feed will not need to be supplemented with lipid-containing material, since diets deficient in lipid lead to lower growth and poor food conversion efficiency in fish (Tacon, 1990). High energy diets are widely used in salmon and trout farming; given the significant benefits of high levels of non-protein energy on improved protein retention and lower nitrogen extraction (Cho et al., 1994). High dietary lipid can lead to an increase of the whole body lipid content. This increase in body lipid is mainly due to an increase in lipid content of liver and digestive tract, and not of the muscle tissue (Boujard et al., 2004). The ash content of the biofloc in the $BF_{30.10}$ treatment was low 3 ± 2 % on the DW.

The PUFAs content is presented in Table 3. The total n-3 PUFAs of the biofloc produced from $BF_{25.10}$ and $BF_{30.10}$ treatments was not significantly different from each other and fall in the range of 0.6 – 0.9 mg/g DW. The total n-6 PUFAs in the biofloc grown with $BF_{25.10}$ treatment had a higher level than those grown with $BF_{30.10}$ treatment (Table 3).

The biofloc were also rich in 16:0, 16:1n-7 and 18:1n-7 fatty acids which were similar to that reported for bacterial-based microbial communities form biological phosphate removal systems (Liu et al., 2000; Izquierdoet al., 2006). A study carried out by Izquierdo et al. (2006) showed that the biofloc collected from a shrimp tank, where the shrimp were fed with a diet containing fish oil, contained a higher total n-3, n-6 and n-9 PUFAs than those fed with a feed that did not contain fish oil. Certik and Shimizu (1999) pointed out that besides de novo synthesis of fatty acids from glucose, the microbial fatty acid biosynthesis can be also carried out by the incorporation of exogenous fatty acids directly into lipid structures followed by desaturation and elongation of lipid sources. This suggested that the fatty acid profile of the biofloc is affected by the dietary lipid composition.

Complete diets are advised to contain less than 8.5% on the DW ash (Craig & Helfrich, 2002). High ash content lowers the digestibility of other ingredients in the diet resulting in poor growth of the fish. Carbohydrates are the most economical and inexpensive sources of energy for fish diets (Craig & Helfrich, 2002). Yet, most of the fish species have a poor ability to utilize carbohydrates and they only represent a minor source of energy for fish. The biofloc grown on $BF_{25.10}$ showed low carbohydrate content 51 ± 4% on the DW. Besides that biofloc are overall a possible good additional nutritious aquaculture feed, the acceptance by the cultured species will play a crucial role in the use of biofloc technology in aquaculture. The beneficial influence of biofloc on the water quality in aquaculture systems has been investigated, but not all fish will be able to utilize biofloc. Experimental evidence however, showed that certain aquaculture species can effectively utilize biofloc (Avnimelechet al., 1989, Crab et al., 2009, 2010a, 2010b; Megahed, 2010, Xu & Pan, 2012, Xu et al., 2012a). Besides herbivores, more general detritus and benthos feeders can thrive on biofloc.

Biofloc developed in the shrimp ponds composed of macroaggregates –diatoms, macroalgae (Bacterial filament, *Chroococcus* – cyanobacterium, – green alga), fecal pellets, exoskeleton, remains of dead organisms, bacteria, protest and invertebrates (Figure 1). Previous studies has proven that the presence of biofloc can increase growth and decrease FCR which means that shrimp can benefit from the nutritional quality of biofloc (Xu & Pan, 2013a, 2014). A good balanced feed can be produced without the utilization of marine proteins, as long as digestible protein sources are used and amino acids are balanced. The diet without marine proteins is about 4500 Egyptian pound (EGP) per ton using cheaper raw materials; compared to 8000- 11000 EGP per ton for commercial shrimp feeds available locally. The utilization of a biofloc system enables sustainable production of shrimp. In addition to economic characteristics, the biofloc is environmental friendly because no water exchange during farming, recycling nutrient feces via biofloc and limited utilization of natural resources.

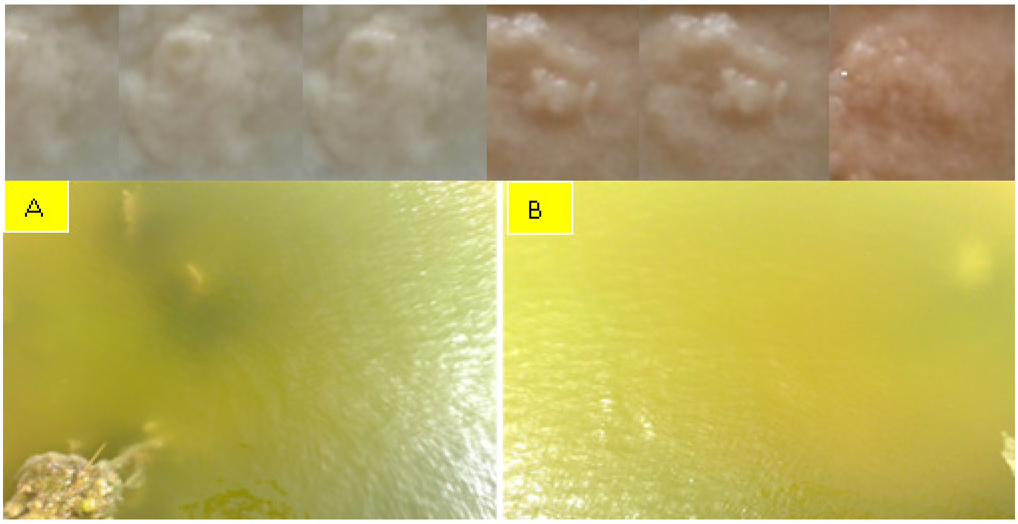

Figure 1. Biofloc developed in the shrimp ponds. A) Biofloc developed in the $BF_{25.10}$ treatment, B) Biofloc developed in the $BF_{30.10}$ treatment

The shrimp fed with control diet showed greenish digestive tracts similar to the color of the feed whereas those fed with biofloc revealed whitish and brownish digestive tracts, similar to the colors of the biofloc.

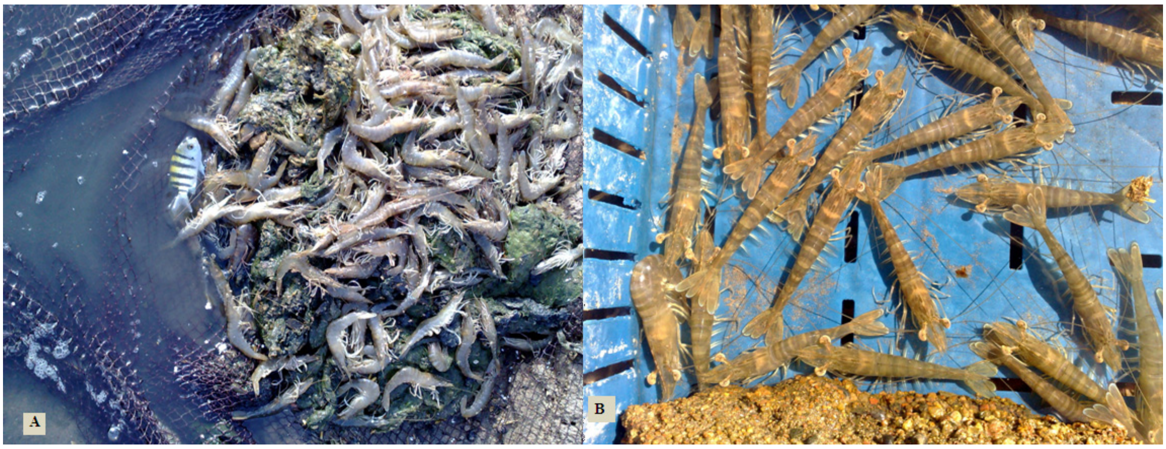

Figure 2. A) Shrimps fed with biofloc shows whitish with no clear bands on the body; B) Shrimps fed with artificial feed (control) showing dark clear banded body

3.4 Water Quality

The water quality results for pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_2^--N) were measured and are represented in Table 4. The temperature of the biofloc treatments was around 25°C. In the present study, mature biofloc developed with the addition of rice bran in the two biofloc treatments; meanwhile, main water quality parameters (especially TAN and NO_2^-–N concentrations) were maintained within suitable ranges for shrimp culture (Fast & Lester, 1992; Cuzon et al., 2004; Fox et al., 2006) throughout the 150-day experimental period (Table 4).

The addition of carbon source reduced water TAN to 1.0 mg N/L, while nitrite-N and nitrate-N were both 0 mg N/L. One can calculate the concentration of ammonia (NH_3^-) and ammonium (NH_4^+) derived from the TAN concentration and the pH. Ammonia is toxic to most commercial fish at concentrations above 1.5 mg N/L (Neori et al., 2004). The practical usage of biofloc technology is essential strategy for environmental protection due to strict legislation regarding the discharge of farm effluents into the neighboring water bodies. Biofloc has been sought as a means of enhancing water quality through microbial manipulation, thereby facilitating the growth and health of cultured shrimp. In biofloc zero-water exchange systems, carbohydrate addition can promote the development of diverse and balanced microbial communities originating from the rearing water (Haslun et al., 2012).

Table 4. Water quality parameters measured in the control and the biofloc treatments

Parameters	$BF_{25.10}$	$BF_{30.10}$	Control 45 % CP
pH	7.4 ± 0.4^a	7.6 ± 0.5^a	7.3 ± 0.3^a
DO (mg O_2/L)	7 ± 10^a	7 ± 30^a	7 ± 0.0^a
TAN (mg N/L)	1.0 ± 0.7^a	1.0 ± 1^a	1.5 ± 0.6^b
NH_3-N (mg N/L)	0.03 ± 0.01^c	0.051 ± 0.01^a	0.012 ± 0.02^b
NH_4^+-N (mg N/L)	0.8 ± 0.4^a	1.0 ± 10^b	1.1 ± 0.3^b
NO_2^--N (mg N/L)	0.00 ± 0	0.10 ± 0.00	0.00 ± 0
NO_3^--N (mg N/L)	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.00

3.5 Benefits of Biofloc in Shrimp Farming

There is a need to develop diets for shrimp cultured in intensive farming systems that will provide sufficient protein for shrimp production while minimizing the amount of nitrogen being introduced into the culture medium (McIntosh et al., 2001). Shrimp typically have a higher dietary protein requirement during the different growth phases (Chen et al., 1985; Velasco et al., 2000). However, there is a wide range in reported dietary protein requirements for *L. vannamei*, typically from 300 to 480 g kg^{-1} (30–48%), with an optimum for PL of 340 g kg^{-1} (34%) (Hu et al., 2008). In intensive nursery systems *L. vannamei* have been fed diets with protein levels as high as 40–55% (Samocha et al., 1993; Velasco et al., 2000).

Biofloc produced in this study could offer the shrimp industry a novel alternative feed and reduction in the dependency on fish oil and fishmeal in feeding marine shrimp. In this study, biofloc were produced in shrimp ponds using rice bran as a carbon source. Feed was applied at 5% of the total shrimp biomass in daily four rations. The nutritional quality of biofloc was appropriate for shrimp. There was significant difference ($P < 0.05$) in shrimp growth/production between control and biofloc treatments of varying protein levels. Survival rate between biofloc treatments and control diet was significantly different ($P < 0.05$), indicating no increased shrimp stress due to the presence of biofloc. The shrimp growth and yield were acceptable in terms of commercial feasibility. During the feeding experiment, it can be seen that the shrimp were able to consume the biofloc (Figure 2). This was visually observed by the color of the digestive tract of the shrimp.

Besides the nutrient recycling aspect, the dense bacterial community that develops in such systems plays a significant role in the production of single cell microbial protein (biofloc) that can provide supplemental natural feed for the shrimp (Avnimelech et al., 1994, 1999; Browdy et al., 2001). Wasielesky et al. (2006) suggested that this enhanced natural production in zero exchange production systems allows the use of low protein feeds with no adverse effect on shrimp performance compared to high protein feeds. However, penaeid shrimps are highly valued seafood commodity in domestic and international markets. Economics of shrimp farming is largely dependent on the feed which constitutes 40–60% operational expenses (Tan et al., 2005). Dietary supplements are widely used in shrimp culture to enhance the growth, immune response and digestive enzyme activities. Commonly used dietary supplements in penaeid shrimp are microalgal products (Boonyaratpalin et al., 2001; Ju et al., 2009; Supamattaya et al., 2005), macroalgae (Yeh et al., 2006), probiotics (Ziaei-Nejad et al., 2006; Wang, 2007), prebiotics (Zhang et al., 2012) and periphyton (Anand et al., 2013b). Recently, manipulation of carbon nitrogen ratio (C: N ratio) for development of biofloc has shown promise in aquaculture (Anand et al., 2013a). The C: N ratio is manipulated by supplementation of external carbon source or elevated carbon level in the feed (Ballester et al., 2010; Crab et al., 2012; McIntosh, 2000). At high C: N ratio, heterotrophic bacteria immobilize the ammonium ion for production of microbial protein and maintain inorganic nitrogen level within the limit (Avnimelech, 1999). Biofloc enhances the growth performance of *Penaeus monodon* (Anand et al., 2013a; Arnold et al., 2009; Hari et al., 2006), *Litopenaeus vannamei* (Wasielesky et al., 2006; Xu & Pan, 2012), *Farfantepenaeus paulensis* (Ballester et al., 2010) and *Marsupenaeus japonicus* (Zhao et al., 2012). Apart from being a source of quality proteins, biofloc are rich source of growth promoters and bioactive compounds (Ju et al., 2008a) which enhance digestive enzymes (Xu & Pan, 2012) and health status of the cultured shrimps (Singh et al., 2005).

The average body weight (ABW) of biofloc treatments was significantly ($P < 0.05$) higher than control diet. Based on visual observation made during the experiment, the shrimps in control diet reduced feed intake which showed by sampling, checking feeding trays and by observing the empty digestive tract. This can be due to several reasons such as the palatability of feed, stress due to disease infection or water quality deterioration. Also, Tacon (1987) found that the absence of feed attractant and low palatability may also have been the cause of less feed consumption in control diet. During the culture period, the biofloc treatments showed good floc formation. Crab et al. (2007) pointed out that at moderate mixing rate as practiced in aquaculture system (1 – 10 W/m^3), microbial cells in permeable aggregates grow better than single dispersed cells due to higher accessibility to the nutrients. There was no abnormality or disease symptoms observed in control and biofloc treatments. Growth of shrimp as well as other aquatic organisms is mostly affected by water quality, culture systems (Tacon et al., 2002), nutrition (Chen et al., 2006) and health condition (Argue et al., 2002).

As dietary protein is the most important factor affecting growth performance of shrimp (Kureshy & Davis, 2002), most shrimp farmers prefer to use high protein feeds, especially in intensive culture systems. In China, for example, shrimp feeds are typically formulated to contain 38% ~ 42% of crude protein. In most of the cases, however, those formula feeds do not consider the contribution of natural production in practical culture systems, such as biofloc-based system, and the use of high protein feeds could be unnecessary and wasteful. Reduction of dietary protein level without affecting growth performance of cultured shrimp in the presence of biofloc has been reported by several authors (Decamp et al., 2002; Hari et al., 2004; Ballester et al., 2010). For example, Ballester et al. (2010) demonstrated that the dietary protein level of *Farfantepenaeus paulensis* can be reduced up to 10% (from 45% to 35%) without impairing shrimp growth performance when the shrimp were nursed in a suspended biofloc system. In the present study, the development of biofloc not only maintained the water quality suitable for shrimp culture but also supplemented a portion of protein nutrition, thereby resulting high survival and better growth performance (growth and FCR) of the shrimp fed with $BF_{25.20}$ and $BF_{30.10}$ compared to the control diet contained 45% dietary protein levels.

Production results obtained in this study are within the range of commercial shrimp production in intensive production systems (Megahed, 2010). This study supports the theory that natural biota can provide a nitrogen source for shrimp, and that flocculated particles are likely to be a significant proportion of this nitrogen source. If this biofloc technology proved to be successful; it could offer the shrimp industry a new culture option. A very significant further justification is the need to have alternative to lower feed cost, replacing marine fish and shrimp meals. For these reasons, this study investigated if it would be possible to produce biofloc as a potential ingredient for reducing fishmeal in shrimp feeds**.** Several advantages to using the low protein feed can be shown. Firstly better water quality, as nitrite, nitrate were lower in the biofloc treatments than in the control; second, feed with lower protein content is cheaper; and third the use of lower protein feed in this system can reduce the environmental impacts from shrimp culture, through lower protein use and water exchange requirement.

4. Conclusion

The results of the present study showed that the use of biofloc represents a viable and more sustainable feed option due to cost, the manner in which it is generated, and the potential that it can decrease the capture wild fisheries by reducing at least some of the demand for fishmeal. Also, the biofloc technology is a sustainable technique used in aquaculture to maintain water quality through the development and control of dense heterotrophic biofloc by adding carbohydrate to the water. Other nutritional parameters of the biofloc such as amino acids profile, lipid class, vitamins and minerals content should be measured. The use of carbon sources with low price such as molasses, tapioca flour, etc. should be investigated. The effect of biofloc on the survival and pathogenicity of *Vibrio* should be monitored.

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