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SUSTAINABLE GROWTH OF SHRIMP AQUACULTURE AND PROTECTION OF NATURAL FISHERIES THROUGH BIOFLOC PRODUCTION AS ALTERNATIVE TO FISHMEAL IN SHRIMP FEEDS

Mohammed E. Megahed*

National Institute of Oceanography and Fisheries, Gulfs of Suez and Aqaba's branch, Suez, 41522, Egypt

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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. This expected growth in aquaculture require utilization of inputs mainly, sources for high quality seeds and fishmeal for production of feeds for cultured fish and crustacean. Fishmeal sources from capture fisheries are fully exploited and thus become costly ingredient in fish and crustacean feeds formulation. There is a need to develop technology that will increase economic and environmental sustainability. The present study evaluated biofloc technology as a sustainable alternative to fishmeal in the shrimp feeds. It based on using cheap carbon source for stimulation of the growth of the microbial biofloc in addition to reduce protein content of the feeds and increase carbohydrate content. This lower feed price without affecting shrimp growth. Also, shrimp are not efficient in the digestion of the carbohydrates, which will be released in the water in shrimp faeces and in turn stimulate biofloc production. The shrimp were fed diets with low fishmeal content had a slightly better growth and FCR. The biochemical composition of the biofloc treatments didn't vary significantly. Bioflocs grown on 25.20 % CP + rice bran have a high protein content 20 ± 7 DW. Regarding the composition of the PUFAs in the bio-flocs, the bio-flocs with 25.20 % CP + Rice bran treatment contained significantly more LNA (18:3(n-3)) than that with 30.10 % CP+ Rice bran treatment and the opposite for LA (18:2(n-6)). In particular bio-flocs with 25.20 % CP + Rice bran treatment had a total n-6 PUFAs. The biofloc proofed to be a possible good additional nutritious aquaculture feed and accepted by the shrimp which play a crucial role in the use of bioflocs technology in aquaculture.

Keywords: Biofloc, Shrimp, Fishmeal, Fisheries, Alternative, Sustainability

* **Correspondence to:** **Muhammed E. MEGAHED**, National Institute of Oceanography and Fisheries, Gulfs of Suez and Aqaba's branch, Suez, 41522, EGYPT

Tel: +201000717510

E-mail: aquageimprove@gmail.com

Öz:

KARİDES YEMLERİNDEKİ BALIK UNUNA ALTERNATİF OLARAK BİYOFLOK ÜRETİMİ İLE DOĞAL BALIKÇILIĞIN KORUNMASI VE KARİDES YETİŞTİRİCİLİĞİNİN SÜRDÜRÜLEBİLİR BÜYÜMESİ

Günümüzde Mısır' da doğadan balık avcılığından tamamen faydalanıldığı gibi su ürünleri yetiştiriciliği de Mısır' da hızla büyüyen nüfuslar için su ürünleri talebini karşılamada tek kaynak olarak düşünülmektedir. Su ürünleri için bu talepleri karşılamada, su ürünleri yetiştiriciliği hızla büyümeli ve çok entansif olmalıdır. Su ürünleri yetiştiriciliğindeki beklenen bu büyümede esas olarak kültürü yapılan balıklar ve kabuklular için yemlerin üretiminde balık unu ve yüksek kaliteli yavrular için girdilerin kullanımını gerektirmektedir. Avcılık yoluyla elde edilen balıklardan sağlanan balıkunu kaynakları tamamen faydalanılmaktadır. Böylece balıklar ve kabukluların yemlerinin formülasyonlarındaki içerik bileşim maddesini pahalı hale getirmektedir. Teknolojiyi geliştirmek için bir ihtiyaç vardır. Bu ekonomik ve çevresel sürdürülebilirliğini artıracaktır. Mevcut çalışma karides yemlerindeki balık ununa sürdürülebilir bir alternatif olarak biyoflok teknolojisi değerlendirildi. Bu yemlerin protein içeriğini azaltmak ve karbonhidrat içeriğini arttırmaya ilaveten mikrobiyal biyoflokun büyümesini uyarmak için ucuz karbon kaynağını kullanmaya dayalıdır. Bu karideslerin büyümesini etkilemeksizin daha düşük yem fiyatına neden olur. Ayrıca, karbonhidratlar karides dışkılarından suya bırakılacaklar ve sırasıyla biyoflok üretimini uyarıp karidesler karbonhidratlarının sindiriminden etkilenmezler. Karidesler daha düşük balıkunu içerikli diyetlerle beslendiler sırayla daha iyi bir büyüme ve FCR' ye sahiptiler. Biyoflok tedavilerinin biyokimyasal kompozisyonlarında önemli ölçüde değişiklik yoktu. % 25.20 ham protein + pirinç kepeğinde biyofloklarla büyütme 20 ± 7 kuru ağırlık içeren yüksek bir proteine sahiptir. Biyofloklardaki PUFA' ların kompozisyonuna göre, % 25.20 ham protein + pirinç kepeği uygulamasıyla biyofloklar % 30.10 ham protein + pirinç kepeği uygulamasındakinden belirgin olarak çok fazla LNA (18:3(n-3)) ve tersi olarakta LA (18:2(n-6)) içermekte idiler. Özellikle % 25.20 ham protein + pirinç kepeği uygulamasıyla biyofloklar toplam n-6 PUFA' lara sahiptiler. Biyoflok besleyici su ürünleri yemlerine mümkün olarak iyi şekilde ilave edilmesiyle yemleri dayanıklı hale getirdi. Karidesler tarafından alındığında su ürünlerindeki biyofloklarn teknolojisini kullanmada önemli bir rol oynamaktadır.

Anahtar Kelimeler: Biyoflok, Karides, Balıkunu, Balıkçılık, Alternatif, Sürdürülebilirlik

Introduction

In the 10 years, aquaculture production in Egypt showed rapid 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. This rapid expansion of aquaculture to meet seafood supply in Egypt requires the use of several resources namely; seed for pond stocking and least cost- high quality-feeds. Intensification of aquaculture production cannot depend on fishmeal from natural fisheries which is both overexploited and costly source. For the country to continue in increasing aquaculture production, it requires sustainable alternative source to fishmeal in shrimp feeds, otherwise shrimp farmers will be out of business.

In order for aquaculture to be completely successful, the industry will need to develop technology that will increase economic and environmental sustainability (Kuhn *et al*., 2010). This technology implements cheaper alternative ingredient to fishmeal and this will effectively reduce the costs of feed as feed costs can account for more than 50% of operational expense, while reducing their impact on overexploited natural fisheries (Naylor *et al*., 2009). Thus, it is important to determine if alternative source, biofloc, could be a suitable replacement ingredient in marine shrimp diets. If implemented successfully, this option would offer a sustainable option to fishmeal. Over the period of January 2008 to May 2009, the global fishmeal market varied from a low mean of about $900 to a high mean of $1250 per metric ton. During the same time frame, soybean meal varied approximately from a low mean of $375 to a high mean of $550 (FAO, 2009). Thus, 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 pressure on wild fisheries by reducing at least some of the demand for fishmeal (Avnimelech, 2005).

The bioflocs technology (BFT) is a sustainable technique used in aquaculture to maintain good *in situ* water quality through the development and control of dense heterotrophic microbial bioflocs by adding carbohydrate to the water (Crab *et al*., 2010). If organic carbon and nitrogen are well balanced in a culture system, ammonium and nitrogenous waste will be converted into bacterial proteinaceous biomass (Schneider *et al*., 2005). Hence, developing and controlling dense heterotrophic microbial bioflocs in the water column by adding carbohydrates improves the water quality in the pond and in addition to this, the bioflocs can subsequently be consumed and used as a source of feed 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 over-exploited fishmeal in the feed of marine shrimp.

Materials and Methods

Experimental set-up:

This study was carried out at the Shrimp and Fish International Company (SAFICO), South Sinai, Egypt in the year 2010.The study was carried out on (F1) generation produced at SAFICO from May 2010 till October 2010. The description of the different treatments (Table 1, Figure 1) and control used is as follow:

A) Control: artificial feed with 45 % CP.

B) Treatment A: artificial feed with 30.10 % CP+ Rice bran.

C) Treatment B: artificial feed with 25.20 % CP + Rice bran.

The organic C/N ratios were 8.3 in the treatments 45 % CP without Rice bran and 10.4 and 12.1 in the treatments 30.10 % CP and 25.20 % CP with Rice bran, respectively. Feeding started at 7 AM, 12 PM, 4PM and 8PM. Rice bran addition was applied on pond surface daily at 10 AM.

Feed preparation:

Feeds were prepared on-farm sites (Table 2, Figure 1) with ingredients purchased from local animal feed supplier in Alexandria, Egypt. Proximate analysis of the feeds was analyzed in Animal Feed Research Institute, Regional Centre for Food and Feed, Agricultural Research center, Giza, Egypt.

Sampling:

The impact of varying treatments was monitored by assessing Survival (SR %), Food Conversion Ratio (FCR), and Specific Growth Rate (SGR). Sampling was carried out every 7 days, to measure the average weight of the shrimp. Survival and growth were calculated based on the following formulas:

Survival (SR %) = (Nf/Ni) × 100%

Where: Ni = initial number of shrimp stocked;

Nf= final number of shrimp harvested

FCR: Total feed used/weight gain of the sampled shrimp;

Growth rate (g/week) were calculated 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).

Physico-Chemical Analysis:

The biofloc samples were analyzed for for Kjeldahl nitrogen (Kj-N), total ammonia nitrogen (TAN) total suspended solids (TSS) and volatile suspended solids (VSS). These properties were determined following APHA (1998). The difference between Kjeldahl-N and TAN was used to calculate the protein content of the bioflocs by multiplying the organic nitrogen content by 6.25. The ash content was determined using TSS and VSS values. Lipids were extracted according to Folch *et al.* (1957), using the modification of Ways and Hanahan (1964). Protein, lipid and ash content were expressed as percentage of the dry weight (% DW) of the bioflocs. The total carbohydrate was calculated according to the following formula: carbohydrate (% DW) = 100 – (crude protein (% DW) + lipid (% DW) +ash (% DW)) (Manush *et al.*, 2005) (Table 14). 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.

Figure 1. Layout of SAFICO farm and ponds used in the biofloc experiment and treatment assignment.

Table 1. Different treatments varying in protein content of the pelleted feed added and the amount of rice bran applied to the pond surface.

Treatment	Rice bran addition (Kg/day)	C/N ratio	Pond no.
45 % CP	0	8.3	1A, 3A, 5B, 2B
30.10 % CP + Rice bran	5	10.4	2A, 3B, 4A,1B
25.20 % CP + Rice bran	8	12.1	5A,6A,4B,6B

Table 2. Feed ingredients and calculated proximate composition of the experimental diets.

Ingredients	25.20 % CP	30.10 % CP	Control feed 45 % CP
Fishmeal (58%)	43.50	52.00	77.60
Starch	41.50	32.50	10.40
Fish oil	4.00	4.50	1.00
Mineral Mix.	3.00	3.00	3.00
Vitamin mix.	2.00	2.00	2.00
Cellulose	5.00	5.00	5.00
CMC[1]	1.00	1.00	1.00
Proximate analysis			
Crude protein	25.20	30.10	45.00
Crude fat	9.70	11.20	11.30
Ash	11.10	13.50	19.30
Moisture	8.70	9.50	9.50
Carbohydrate	45.30	35.70	14.90

[1]*Carboxymethyl cellulose*

Water quality:

Water quality in the culture systems was monitored daily for dissolved oxygen (DO), salinity, and temperature using a YSI 85m (YSI Inc., Ohio, US). Nitrate, nitrite, pH, and total ammonia nitrogen (TAN) were measured weekly using methods designed for seawater samples (Spotte, 1979). More detail for nitrate, nitrite, and TAN procedures can be found in Strickland and Parsons (1972), Mullin and Riley (1955), and Solorzano (1969), respectively.

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. Significance different was considered when *P*-value <0.05. A paired Student's test was used for analyzing significant changes between two treatments with carbon source (when $P<0.05$).

Results and Discussion

Biofloc as a sustainable alternative to fishmeal in shrimp feed:

The biofloc experiment carried out in the growout ponds using cheap carbon source with the aim of developing least cost feed technology for sustainable shrimp farming in Egypt. Shrimp showed a similar growth with all diets. The shrimp were fed diets with low Fishmeal content had a slightly better growth and FCR, and the results were statistically different (Table 3; $P<0.05$).

The aim was to reduce the cost of feeds used to produce marine shrimp and reduction the utilization of marine proteins (fishmeal, squid meal, and shrimp meal) in the feeds and use the bioflocs to recycle waste protein and nitrogen in the shrimp ponds to complement the pelleted feeds with live organisms. Two feeds were formulated and pressed on a 2 mm die and a reference diet with 45 % CP (Table 3) available locally and commercially for feeding marine fishes. The shrimp trial lasted for 150 days. Biofloc shrimp system yields high density production, low FCR's, and superior survival rates using strong aeration and high quality probiotics and vitamins & premixes mixed with the carbon source. The biochemical composition of the bioflocs is shown in Table (4). Protein content was high in the 25.20 % CP + rice bran bioflocs, being 20 ± 7 % DW, while 16 ±5 in the 30.10 % CP+ rice bran. Crude lipid was also high in the 25.20 % CP + rice bran bioflocs 22 ±4 % DW. High ash content was noted in the 25.20 % CP + rice bran bioflocs, up to 4 ±3 % DW. The carbohydrate content of the bioflocs was high in the 30.10 % CP+ rice bran treatment 61 ± 5% DW.

The composition of the bioflocs of the two treatments (Table 4) didn't vary significantly between sampling dates; therefore mean values are discussed here. Bioflocs grown on 25.20 % CP + rice bran have a high protein content 20 ±7 DW. 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). 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. Fats are high-energy nutrients that can be utilized to partially substitute for protein in aquaculture feeds (Craig and Helfrich, 2002). Fats 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 bioflocs experiment about 22 ±4 % fat on the DW was measured in the 25.20 % CP + rice bran treatment, 17 ±1 % DW in the 30.10 % CP+ rice bran.

The measured lipid content of all two types of bioflocs is high. The advantage is that the bioflocs, when used as feed, will not need to be supplemented with a fat-containing material, since diets deficient in fats 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 fat can lead to an increase of the whole body fat 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 flocs in the 30% CP+ rice bran treatment was low 3 ± 2 % on the DW.

The PUFAs content is presented in Table (5). The total n-3 PUFAs of the bio-flocs produced from 25.20 % CP + Rice bran and 30.10 % CP+ Rice bran treatments was not significantly different from each other and fall in the range of 0.6 –

0.8 mg/g DW. With respect to the total n-6 PUFAs, the bio-flocs grown with 25.20 % CP + Rice bran treatment had a higher level than those grown with 30.10 % CP+ Rice bran treatment (Table 5). Regarding the composition of the PUFAs in the bio-flocs, the bio-flocs with 25.20 % CP + Rice bran treatment contained significantly more LNA (18:3(n-3)) than that with 30.10 % CP+ Rice bran treatment and the opposite for LA (18:2 (n-6)). In particular bio-flocs with 25.20 % CP + Rice bran treatment had a total n-6 PUFAs.

The bio-flocs 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; Izquierdo *et al*., 2006). A study carried out by Izquierdo *et al.* (2006) showed that the bio-flocs 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 bio-flocs is affected by the dietary lipid composition. In this experiment the total n-3 PUFAs ranged from 0.6 to 0.8 mg/g DW whereas in the other study, where fish oil was included in the shrimp diet (Izquierdo *et al*., 2006). On the other hand, the total n-6 PUFAs content of the bio-flocs in this experiment, particularly with 25.20 % CP + Rice bran.

Complete diets are advised to contain less than 8.5% on the DW ash (Craig and Helfrich, 2002). High ash content lowers the digestibility of other ingredients in the diet resulting in poor growth of the fish. In the acetate bioflocs we noted higher ash content, i.e. 20% on the DW. This can decrease the digestibility of the acetate bioflocs. The ash content of the glucose and starch bioflocs was lower than 8.5% on the DW. Carbohydrates are the most economical and inexpensive sources of energy for fish diets (Craig and 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 flocs grown on 25.20 % CP + rice bran showed low carbohydrate content 35 ± 4% on the DW. Besides that bioflocs are overall a possible good additional nutritious aquaculture feed, the acceptance by the cultured species will play a crucial role in the use of bioflocs technology in aquaculture. The beneficial influence of bioflocs on the water quality in aquaculture systems has been investigated, but not all fish will be able to utilize bioflocs. Experimental evidence however, showed that certain aquaculture species can effectively utilize bioflocs (Crab *et al*., 2009; Crab *et al*., 2010). Besides herbivores, more general detritus and benthos feeders can thrive on bioflocs.

Table 3. Production performances of the different treatments of the biofloc study on selected F1 shrimp postlarvae (Mean ± SD).

	Control 45 % CP	30.10 % CP+ Rice bran	25.20 % CP + Rice bran
Initial ind. Weight (g)	0.03 ± 0.001^{a}	0.03 ± 0.001^{a}	0.03 ± 0.001^{a}
Final ind. Weight (g)	36.24 ± 2.17^{a}	37.31 ± 1.52^{a}	36.80 ± 2.22^{a}
Average growth (g/week)	1.56 ±0.40^{a}	1.61 ± 0.20^{a}	1.55 ±0.22^{a}
FCR	1.80 ±0.10^{b}	1.66 ±0.13^{b}	1.61 ±0.11^{a}
Survival (%)	76 ±11.00^{b}	84 ±15.02^{a}	85 ±8.30^{a}
Yield (kg) /ha	1735.65 ±15.33^{b}	1976.56 ±17.83^{a}	1971.71 ±13.29^{a}

Table 4. Overall average of the nutritional qualities (Mean ± SD) of the bioflocs. Samples were taken over a 150 days period on regular time intervals (30 days).

Parameters	**30.10 % CP+ Rice bran**	**25.20 % CP + Rice bran**
Crude protein (% DW)	16 ± 5^{b}	20 ± 7^{a}
Crude lipid (% DW)	17 ± 1^{b}	22 ± 4^{a}
Ash (% DW)	3 ± 2^{b}	4 ± 3^{a}
Carbohydrate (% DW)	61 ± 5^{a}	35 ± 4^{b}

Microscopic Observations of Observations of Bio-Floc:

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 2). It is unknown but certainly doubtful if the same results would have been obtained in clear water without bioflocs. Research in the past has proven that the presence of bioflocs can increase growth and decrease FCR which means that shrimp can benefit from the nutritional quality of bioflocs. 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 3500 L.E per tonne using cheaper raw materials; compared to 6000- 10000 L.E per tonne for commercial shrimp feeds available locally. The combination of such a diet and utilization of a biofloc system enables sustainable production of shrimp anywhere in Egypt. In addition to economic characteristics, the biofloc is environmental friendly because no water exchange during farming, recycling nutrient feces via bioflocs and limited utilization of natural resources.

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 (6). The temperature of the biofloc reactor water was around 25°C.

Table 5. PUFAs content (mg/g DW) of bio-flocs grown in 25.20 % CP + Rice bran and 30.10 % CP+ Rice bran treatments.

PUFA (mg/g DW)	Treatment	
	25.20 % CP + Rice bran	30.10 % CP+ Rice bran
LNA (18:2(n-6))	15.8	13.4
LA (18:3(n-3))	0.7	0.3
AA (20:4(n-6))	1.0	0.3
EPA (20:5(n-3))	0.2	0.2
DHA (22:6(n-3))	0.0	0.1
Total n-3 PUFA	0.8	0.7
Total n-6 PUFA	17.6	14.4

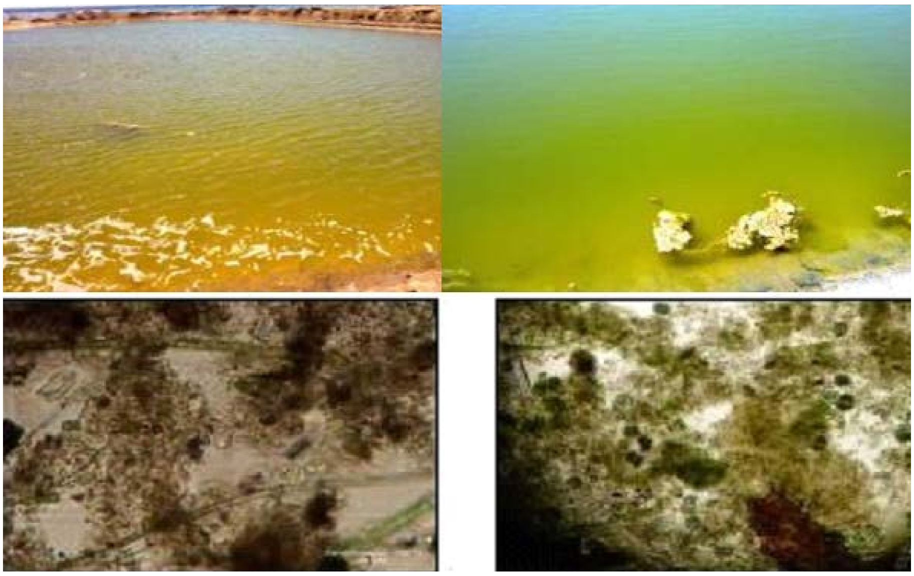

Figure 2. Biofloc developed in the shrimp ponds. macroaggregates –diatoms, macroalgae (Bacterial filament, *Chroococcus* – cyanobacterium, – green alga), fecal pellets, exoskeleton, remains of dead organisms, bacteria, protest and invertebrates.

Table 6. Water quality measured in the biofloc treatments after addition of carbon source.

Parameters	Control 45 % CP	30.10 % CP+ Rice bran	25.20 % CP + Rice bran
pH	7.5 ± 0.3^a	7.5 ± 0.5^a	7.3 ± 0.4^a
DO (mg O_2/L)	8 ± 0.0^a	8 ± 30^a	8 ± 10^a
TAN (mg N/L)	1.5 ± 0.6^b	1.0 ± 1^a	1.0 ± 0.7^a
NH_3-N (mg N/L)	0.011 ± 0.002^b	0.055 ± 0.001^a	0.03 ± 0.01^c
NH_4^+-N (mg N/L)	1.2 ± 0.3^b	1.0 ± 10^b	0.7 ± 0.4^a
NO_2^--N (mg N/L)	0.00 ± 0^a	0.10 ± 0.01^b	0 ± 0^a
NO_3^--N (mg N/L)	0.03 ± 0.01^b	0.00 ± 0.0^a	0.00 ± 0^a

Samples were taken over a 150 days period on regular time intervals (30 days). Based on TAN concentrations, pH and temperature, concentrations of ammonia and ammonium were calculated. The water quality parameters (Table 6) were for two treatments and control within acceptable range over the whole testing period of 150 days. Sampling dates didn't significantly affect water quality parameters; hence mean values are discussed here. The addition of carbon source reduced water column 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. The calculated values indicate that for the carbon source treatments toxic levels are not exceeded. Ammonia is toxic to most commercial fish at concentrations above 1.5 mg N/L (Neori *et al.*, 2004). Microbial flocs 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, microbial flocs were produced in intensive shrimp greenhouses using wheat flour 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 low protein levels.

Survival and abnormality were compared and no significant differences ($P > 0.05$) between BFT and control diet indicating no increased shrimp stress due to the presence of biofloc. Overall shrimp growth and production was good in terms of commercial feasibility. During the feeding experiment, it can be seen that the shrimp in all the biofloc treatments were able to consume the flocs (Figure 3). This was visually observed by the color of the digestive tract of the shrimp. The shrimp fed with control feed showed greenish digestive tracts similar to the color of the feed whereas those fed with bioflocs revealed whitish and brownish digestive tracts, similar to the colors of the bioflocs.

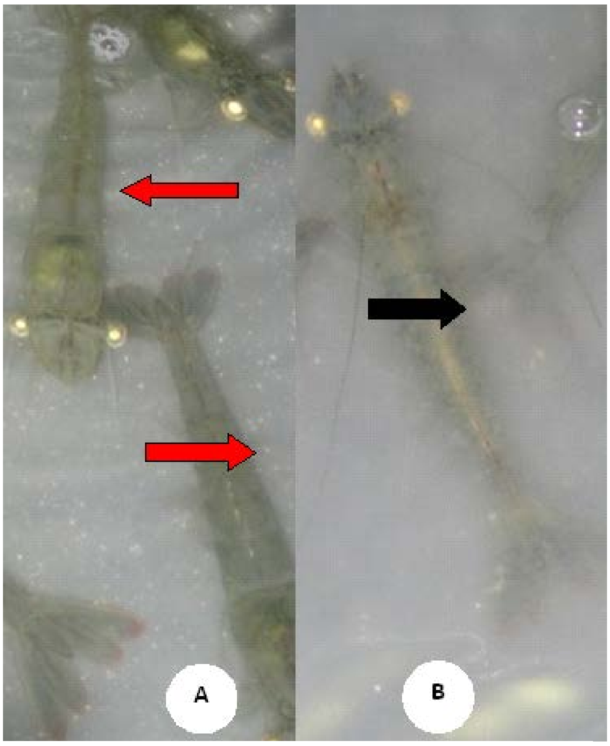

Figure 3. Biofloc consumption. (A) Shrimps fed with artificial feed (control) show greenish digestive tracts (black arrow); (B) Shrimps fed with bio-flocs shows whitish digestive tracts (red arrow).

There was no significant difference ($P > 0.05$) in survival between the control and all other treatments. The ABW of biofloc treatments was significantly ($P < 0.05$) higher than control and there was no significant difference ($P > 0.05$) among biofloc treatments. 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. Intense aeration on the other hand minimizes the advantage of growing in flocs and free cells show a higher nutrient uptake. 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).

In terms of shrimp growth and feed utilization, it can be seen that shrimp growth was better in the low CP treatment, most likely due to the lower concentrations of toxic inorganic nitrogen species. In addition to a lower feed conversion ratio (FCR). According to Avnimelech (1999), the protein conversion ratio (PCR) was markedly reduced in the 20% protein treatment. The PCR in the conventional 30% protein feed treatment was 4.35–4.38, meaning that only 23% of the feed protein was recovered by the fish. The PCR in the low CP% was twice as high. The increased protein utilization is due to its recycling by the microorganisms. It may be said that the proteins are eaten by the fish twice, first in the feed and then harvested again as microbial proteins. It is possible that protein recycling and utilization can be further increased. Production results obtained in this study are within the range of commercial shrimp production in intensive production systems. 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 lower cost feeds replacing marine fish and shrimp meals. For these reasons, this study investigated if it would be possible to produce microbial floc as a potential ingredient for reducing fishmeal in shrimp feeds.

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 pressure on wild fisheries by reducing at least some of the demand for fishmeal. Also, the bioflocs technology is a sustainable technique used in aquaculture to maintain good water quality through the development and control of dense heterotrophic microbial bioflocs by adding carbohydrate to the water. The results offered biofloc as a sustainable and cost-effective alternative source for costly and overexploited fishmeal in the feed of marine shrimp.

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