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Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817)

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Abstract

In a 30-day experiment, *Farfantepenaeus brasiliensis* PL_{25} (25 ± 10 mg; 17.9 ± 1.6 mm) were raised in nine circular floating cages with a stocking density of 1000 shrimp m^{-3}. Three treatments were evaluated: (1) culture in BFT system plus a commercial feed supply (BFT+CF); (2) culture in BFT system without feed supply (BFT) and (3) culture in clear water with feed supply (control). Post-larvae (PL) final weight (218.9, 236.5 and 176.0 mg, for BFT+CF, BFT and control respectively), final biomass (17.9, 15.7 and 8.2 g) and weight gain (193.9, 211.5 and 151.0 mg) were similar in the BFT regardless of whether they were fed a commercial diet ($P > 0.05$), but were both significantly higher than the control ($P < 0.05$). Survival (81.5%, 67.0% and 84.8% respectively) and final length did not differ between treatments ($P > 0.05$). The biofloc analysis identified five main microorganism groups: protozoa (ciliate and flagellate), rotifers, cyanobacteria (filamentous and unicellular) and pennate diatoms. Free living bacteria and attached bacteria in bulk were 25.73 ± 8.63 and 0.86 ± 3.17 × 10^6 mL^{-1} respectively. Proximate analysis in the biofloc indicated high levels of crude protein (30.4%). Results confirmed favourable nutritional quality of biofloc, and enhanced growth and production of *F. brasiliensis* PL in biofloc systems.

Keywords: biofloc, environmental friendly, *Farfantepenaeus brasiliensis*, nursery, nutrition

Introduction

The aquaculture industry is growing fast, at a rate of ~ 9% per year since the 1970s (Food and Agriculture Organization of the United States 2008). However, due to environmental concern, the requirement for more ecologically sound management and culture practices is also growing fast. Moreover, the expansion of aquaculture production is currently restricted due to the pressure it causes to the environment by the discharge of waste products to natural water bodies and by its strong dependence on fishmeal and fish oil (Browdy, Bratvold, Stokes & Mcintosh 2001; De Schryver, Crab, Defoirdt, Boon & Verstraete 2008). Such ingredients are one of the prime constituents of feed for commercial aquaculture (Naylor, Goldburg, Primavera, Kautsk, Beveridge, Clay, Folke, Lubchencoi, Mooney & Troell 2000). Furthermore, feed costs represent at least 50% of the total aquaculture production costs, which is predominantly due to the cost of the protein component in commercial diets (Bender, Lee, Sheppard, Brinkley, Philips, Yeboah & Wah 2004).

Another important concern is water use. In the 1980s, water exchange was identified as an important factor contributing to several epizootic diseases in shrimp farms (Sandifer, Stokes & Hopkins 1991; Hopkins, Hamilton II, Sandifer, Browdy & Stokes 1993). In addition, water discharge of nutrient-rich effluent from intensive culture systems can contribute to eutrophication, potentially impacting both natur-

al biota and adjacent culture operations (Browdy *et al.* 2001). Consequently, some shrimp producers have reduced water exchange, becoming more conservative in water use (Hargreaves 2006).

Biofloc technology (BFT) is an emerging alternative towards more environment friendly aquaculture production systems. This technology was developed to create economical and environment benefits via reduced water use, effluent discharges, artificial feed supply and improved biosecurity (Wasielesky, Atwood, Stokes & Browdy 2006; Avnimelech 2007; Mishra, Samocha, Patnaik, Speed, Gandy & Ali 2008). The microbial community, mainly heterotrophic bacteria, can improve water quality (Asaduzzaman, Wahab, Verdegem, Huque, Salam & Azim 2008) and control pathogenic bacteria, thereby reducing the potential spread of diseases (Horowitz & Horowitz 2001; Cohen, Samocha, Fox, Gandy & Lawrence 2005). The biofloc as a food source are available 24 h day^{-1} (Avnimelech 2007), improving production per hectare and economic benefits through lower artificial feed inputs (Hopkins, Sandifer & Browdy 1995; Browdy *et al.* 2001). This kind of system can be defined as one for which the maintenance of water quality depends mainly on an active phytoplankton bloom, free and attached bacteria, aggregates of living and dead particulate organic matter and microbial grazers (rotifers, ciliates and flagellates and protozoa) maintained in suspension. Nitrogen compounds are controlled by a combination of phytoplankton uptake, nitrification and by heterotrophic bacteria immobilization inside the culture unit (Hargreaves 2006). Consumption of natural productivity (biofloc, phytoplankton and associated grazers) by cultured organisms can increase the efficiency of feed utilization by recovery of some fraction of excreted nutrients (Burford,Thompson, McIntosh, Bauman & Pearson 2004; Wasielesky *et al.* 2006). Consumption of biofloc can increase nitrogen retention from added feed by 7–13% (Hari, Kurup, Varghese, Schrama & Verdegem 2004; Schneider, Sereti, Eding & Verreth 2005; Hargreaves 2006).

Nitrogenous by-products can be easily taken up by microorganism biota and serve as a substrate for operating biofloc systems and production of microbial protein cells (Mishra *et al.* 2008). This approach is successfully performed by control of carbon and nitrogen (C:N) ratio to promote ammonia for heterotrophic microorganisms as a major pathway of ammonia control. Effective use of microbial biomass has collateral benefits for species that have a filtering apparatus and/or digestive system that better assimilate biofloc (Burford, Thompson, McIntosh, Bauman & Pearson 2003; Hari *et al.* 2004; Hargreaves 2006). High C:N ratio will help convert ammonium and organic nitrogenous waste into bacterial protein biomass (Avnimelech, Kochva & Diab 1994; Avnimelech 1999; Schneider *et al.* 2005). There are many considerations for carbon source selection (i.e. local availability, cost, biodegradability and efficient bacteria assimilation). Table 1 presents studies with different species and carbon source use on limited water exchange systems.

Intensive nursery systems present several benefits such as optimization of farm land, increased shrimp survival and enhanced growth performance in grow-out ponds (Apud, Primavera & Torres 1983; Sandifer *et al.* 1991; Arnold, Coman, Jackson & Groves 2009). This phase is defined as an intermediate step between hatchery-reared, early postlarvae (PL) and the grow-out phase (Mishra *et al.* 2008). Previous studies, mainly with the Pacific white shrimp *Litopenaeus vannamei*, have reported several benefits by the use of nursery phase (Moss & Moss 2004; Samocha *et al.* 2007; Mishra *et al.* 2008). Information about rearing other penaeid species in a nursery environmental friendly system are scarce in recent literature.

The pink shrimp *F. brasiliensis* (Latreille, 1817) is naturally distributed from North Carolina, USA to the north coast of Rio Grande do Sul, Brazil (29°/S) (D'Incao 1995, 1999). This species is suitable for culture in alternative structures like cages (Lopes, Wasielesky, Ballester & Peixoto 2009) and could be produced as live bait juveniles for sport fishing (Preto, Pissetti, Wasielesky, Poersch & Cavalli 2009). In terms of economics, *F. brasiliensis* is an important resource for Brazilian fisheries (D'Incao 1999), having strong local demand and commanding a relatively higher price than white shrimp species (Valentini, D'Incao, Rodrigues, Rebelo & Rahn 1991). Thus, the aim of the present study was to evaluate the potential of BFT as a food source for *F. brasiliensis* PL during the nursery phase under a limited water exchange condition.

Materials and methods

Experimental design and culture conditions

This work was conducted at the Marine Aquaculture Station (Rio Grande Federal University), located at Cassino Beach (32°12/S and 51°50/W), Rio Grande City, Rio Grande do Sul, Brazil. Before starting the experiment, a round 7000 L (7 tons) outdoor fiberglass

Table 1 Summary of the carbon source and respective species used in limited or zero water exchange culture systems

Carbon source	Culture species	References
Acetate	*Macrobrachium rosenbergii*	Crab, Chielens, Wille, Bossier and Verstraete (2010)
Cassava meal	*Penaeus monodon*	Avnimelech and Mokady (1988)
Cellulose	Tilapia	Avnimelech, Mokady and Schoroder (1989)
Corn flour	Hybrid bass and hybrid tilapia	Milstein, Anvimelech, Zoran and Joseph (2001); Asaduzzaman, Rahman, Azim, Islam, Wahab, Verdegem and Verreth (2010)
Dextrose	*Litopenaeus vannamei*	Suita (2009)
Glycerol and Glycerol+*Bacillus*	*M. rosenbergii*	Crab *et al.* (2010)
Glucose	*Penaeus monodon* and *M. rosenbergii* respectively	Avnimelech and Mokady (1988); Crab *et al.* (2010)
Molasses	*L. vannamei*	Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz and Brock (2007); Burford *et al.* (2004)
Sorghum meal	Tilapia	Avnimelech *et al.* (1989)
Tapioca	*M. rosenbergii* and *L. vannamei* respectively	Asaduzzaman *et al.* (2008) Hari *et al.* (2004)
Wheat flour	Tilapia (*Oreochromis niloticus*)	Azim and Little (2008)
Starch	Tilapia (Mozambique) and tilapia *O. niloticus* × *O reochromis aureus* respectively	Avnimelech (2007); Crab, Kochva, Verstraete and Avnimelech (2009)

'macrocosm tank' was used for development of biofloc (Emerenciano, Wasielesky, Soares, Ballester, Izeppi & Cavalli 2007). The tank was inoculated with diatom (*Thalassiosira weissflogii*, $5 \times 10^4\,mL^{-1}$) to help maintain water quality until a heterotrophic community was established. Water was vigorously aerated using air diffusers in the centre of the tank and eight surrounding air lifts (2 cm diameter PVC pipe, 50 cm length). With the goal of developing a conventional BFT shrimp production system and to help develop biofloc, juveniles of *F. paulensis* (3.54 ± 0.88 g) were stocked at a density of 40 shrimp m^{-2} and maintained until the end of the experiment. Shrimp were fed three times per day (09:00, 13:00 and 18:00 hours) with 40% crude protein (CP) commercial diet (Cargill's Purina Agribrands™, Agribrands Purina do Brasil, São Lourenço da Mata, PE, Brazil) at 3% of the estimated biomass. The tank was totally covered for an approximately 80% reduction in light by shade cloth. Sugarcane molasses (90% of added carbon source) and wheat bran (remaining 10%) were added daily after the addition of the feed to maintain a high C:N ratio (20:1) to ensure optimal heterotrophic bacteria growth (Avnimelech 1999; Asaduzzaman *et al.* 2008; Crab *et al.* 2009). In the macrocosm tank, limited water exchange (not exceeding 0.5% daily) was carried out by a central drain to prevent accumulation of sludge throughout the experimental period. Dechlorinated freshwater and marine water (35 ppt), previously filtered in a 125 μm sand filter were added to compensate sludge removal and evaporation losses.

The experiment was initiated after 25 days with biofloc formation in the macrocosm tank [total suspended solids (TSS) $> 100\,mg\,L^{-1}$] (Ballester, Abreu, Cavalli, Emerenciano, Abreu & Wasielesky 2010). *Farfantepenaeus brasiliensis* PL (PL_{25}, 25 ± 10 mg; 17.86 ± 1.6 mm) were obtained from routine larviculture carried out at the University station, using previous methods (Marchiori 1996). The PL were distributed in a completely randomized experimental design with three treatments and three replicates per treatment. Shrimp were stocked at a density of $1000\,m^{-3}$ and reared for 30 days. The treatments were: (1) culture in BFT system plus a commercial feed supply (BFT+CF); (2) culture in BFT system with no feed supply (BFT); and (3) culture in clear water with commercial feed supply (control). The experimental units consisted of nine circular floating cages ($0.25\,m^{-2}$ bottom area, $\sim 0.1\,m^3$ underwater volume) made from 1.5 mm mesh size plastic screen and placed directly inside the macrocosm tank for BFT treatments or in an 800 L cement tank for clear water control treatment (with 100% water exchange daily, covered with the same shade cloth). The PL were fed at 100% biomass with a 40% CP commercial feed twice a day (09:00 and 18:00 hours). The pelleted feed was screened at 2×2 mm, sized larger than cage mesh and distributed to each cage. The water flowed freely between cages and tanks. Fifty shrimps were weighed (Sartorius®, Sartorius, Santo André, SP, Brazil) to the nearest 0.1 mg at the beginning and the end of experiment. Survival (%), final weight (mg), weight gain (mg) and final total length (mm) were

measured. Final biomass (g) was estimated on each replicate accord final weight means and survival rate.

Assessment of water quality parameters

Throughout the experimental period, temperature (mercury thermometer, precision $\pm$ 0.5 °C), salinity (Atago™ optical refractometer, Atago Co., Tokyo, Japan; $\pm$ 1 g L^{-1}), pH and dissolved oxygen concentration (YSI Model 556, YSI Incorporated, Yellow Springs, OH, USA) were measured daily at 08:00 hours in both the biofloc and clear water macrocosm tanks. Total ammonia nitrogen (TAN), nitrite (NO_2-N) and reactive phosphorous (RP) (PO_4) (UNESCO 1983) were measured three times per week. Total suspended solids were measured two times per week (first 2 weeks) and after this period, once a week (Strickland & Parsons 1972).

Assessment of microorganisms

Well-mixed water samples from the macrocosm tank were collected at the end of the experiment in 30 mL glass bottles and preserved with borate-buffered formalin (4% v/v) (Thompson, Abreu & Wasielesky 2002). The identification and abundance of microorganisms were determined in 2.1 mL samples poured into sedimentation chambers using an Olympus inverted light microscope (Olympus, Tokyo, Japan) equipped with phase contrast (Utermöhl 1958). For bacterial and flagellate counts, volumes of 0.1 mL were concentrated in darkened polycarbonate nuclepore membrane filtres (0.2 μm), stained with the fluorochrome 4′,6-diamin-2-phenyl-indol (DAPI) (15 μg mL^{-1}) (Ballester *et al.* 2010) and directly counted using a Zeiss Axiolpan epifluorescence microscope (Zeiss, Oberkochen, Germany) equipped with a 487701 light filter set (BP 365/11; FT 395; LP 397) at $\times$ 1000 final magnification (Porter & Feig 1980). Counts were made in at least 30 fields chosen at random. Free living bacteria in the water column and attached bacteria on any substrate type (i.e. organic and inorganic particles, shrimp faeces, etc.) were considered. Fifteen random floc size measures were made in the macrocosm tank water samples at least once a week.

Proximate analysis of carbon source, commercial feed and biofloc

Proximate analysis was carried out from the carbon sources (sugarcane molasses and wheat bran), commercial feed and biofloc biomass (macrocosm tank water filtered in a 100 μm mesh every 10 days). All samples were dried in an oven at 102 °C (constant weight) and then stored in a refrigerator ($-$ 20 °C). At the end of the experiment, pooled samples were ground and processed according to AOAC (2000). The total carbohydrate was estimated by difference and gross energy content was calculated according to Tacon (1990).

Statistical analysis

Water quality parameters and growth performance were analyzed by one-way ANOVA. If main effects were significant, differences among the treatments were tested with Tukey's multi-comparison test of means at 5% significance level. Survival was arcsine transformed for analysis, but only original values are presented.

Results

Water quality values are shown in Table 2. There were significant differences ($P < 0.05$) between treatments in terms of some water quality parameters (salinity, dissolved oxygen and pH). Total ammonia nitrogen, nitrite (NO_2^--N) and reactive phosphorous (PO_4) in the BFT macrocosm tank are given in Fig. 1. Total suspended solids results in the macrocosm tank described maximum and minimum values of 123 and 414 mg L^{-1}, respectively, with an average ($\pm$ SD) of 257.88 $\pm$ 105.21 mg L^{-1}. Fluctuations of TSS and pH are shown in Fig. 2.

Shrimp growth performance is presented in Table 3. Post-larvae final weight, final biomass and weight gain in BFT+CF versus BFT treatments were not significantly different ($P > 0.05$) but were higher than the control ($P < 0.05$). Survival and final length results did not differ between all treatments ($P > 0.05$).

Proximate analysis of commercial feed, sugarcane molasses, wheat bran and biofloc are given in Table 4. Levels of CP in artificial feed and biofloc were 39.7% and 30.4% respectively. Ash content in biofloc was 39.2% and crude lipid level was 0.47%.

During the experimental period, biofloc varied in size ranging from 50 to 1000 μm. The biofloc samples indicated mainly presence of microorganisms such as protozoa (ciliate and flagellate), rotifers, cyanobacteria (filamentous and single cell) and pennate diatoms (Fig. 3). Free living bacteria and attached bacteria in bulk were 25.73 $\pm$ 8.63 and 0.86 $\pm$ 3.17 $\times$ 10^6 mL^{-1} respectively (Fig. 4).

Table 2 Water-quality parameters of 30 days experimental period for *Farfantepenaeus brasiliensis* reared from PL25 and stocked at a density of 1000 shrimp m^{-3}

Parameter	BFT+CF and BFT (macrocosm tank)	Control (clear-water tank)	*P*-value
Temperature (°C)	23.7 (± 1.7)	24.3 (± 1.5)	>0.05
Salinity ($g\,L^{-1}$)	35.9^{A} (± 1.0)	34.5^{B} (± 0.9)	<0.001
Dissolved oxygen ($mg\,L^{-1}$)	5.9^{A} (± 1.0)	7.1^{B} (± 0.9)	<0.001
pH	6.5^{A} (± 0.2)	8.3^{B} (± 0.3)	<0.001
TAN ($mg\,L^{-1}$)	0.4 (± 0.5)	0.1 (± 0.1)	>0.05
NO_2–N ($mg\,L^{-1}$)	0.9 (± 0.6)	0.5 (± 0.7)	>0.05
PO_4^+ ($mg\,L^{-1}$)	1.3 (± 0.7)	1.1 (± 1.0)	>0.05

Data are means (± standard error) and significance level, along experiment. Different letters indicate means are significantly different ($P < 0.05$).

Treatments: BFT+CF, BFT culture system plus a commercial feed supply; BFT, BFT with no feed supply; control, clear water condition with commercial feed supply.

TAN, total ammonia nitrogen.

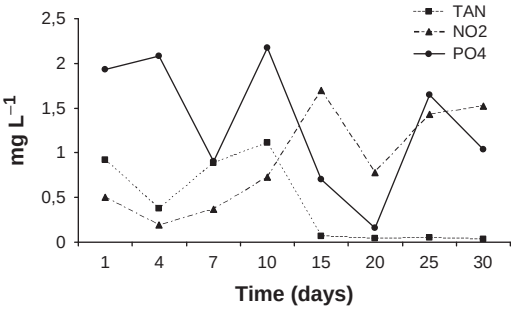

Figure 1 Total ammonia nitrogen (TAN), nitrite and reactive phosphorous concentrations in a biofloc technology (BFT) macrocosm tank during a 30 days experimental period.

Discussion

Water quality parameters

The pink shrimp *F. brasiliensis* is a new species in aquaculture literature. No data were found about optimal water quality for *F. brasiliensis*. Some parameters showed significant differences between the macrocosm tank and the clear water tank. Salinity was slightly higher in the biofloc treatments compared with the control. This possibly occurred due to evaporation in BFT limited water exchange systems. Dissolved oxygen and pH values were low in BFT treatments. This was likely a result of higher respiration rates due to the presence of a heterotrophic community that increased the carbon dioxide concentration in limited water exchange systems (Tacon, Cody, Conquest, Divakaran, Forster & Decamp 2002; Wasielesky *et al.* 2006).

In limited water exchange biofloc-based systems the accumulation of toxic inorganic nitrogen species can be prevented by maintaining a high C:N ratio (Avnimelech 1999) and inducing the uptake of ammonium by the microbial community (Avnimelech *et al.* 1994). In this study, the concentration of inorganic nitrogen by-products (TAN and nitrite) and RP presented no significant differences between the macrocosm tank and the clear water tank ($P > 0.05$). The C:N ratio of 20:1 by addition of a carbon source (molasses and wheat bran) seems to be a helpful tool to reduce and maintain optimal levels of inorganic N concentrations (Asaduzzaman *et al.* 2008). Reactive phosphorous levels decreased sharply from 10th to 20th days and then increased. This trend could indicate a period of phytoplankton uptake and consequently growth. Hargreaves (2006) showed that algal assimilation could temporarily reduce RP levels, but these would increase again following algal crashes. Despite differences in water quality parameters among treatments, all of them were within acceptable ranges for most penaeid species (Wickins 1976; Van Wyk & Scarpa 1999).

The control of TSS in the BFT tanks is an important issue closely related to optimum levels of dissolved oxygen and inorganic N compounds (Ray, Lewis, Browdy & Leffler 2010), as well as the prevention of gill clogging. Total suspended solids in the macrocosm tank fluctuated greatly (123–414 $mg\,L^{-1}$), with a mean of 257.88 ± 105.21 $mg\,L^{-1}$. As observed with RP, TSS levels decreased quickly from 15th to 20th days. This trend could indicate that shrimp juveniles consumed a large fraction of the bioflocs present in the macrocosm tank or shifts in the composition and abundance of the microbiota due to ecological succession in the food web (Moriarty 1997). Samocha *et al.* (2007) recommended TSS values below 500 $mg\,L^{-1}$ for shrimp culture. Mishra *et al.* (2008)

recorded TSS values below 300 mg L^{-1} in an intensive shrimp nursery system when evaluating foam fractionators as a solids removal device. In a recent study, Ray *et al.* (2010) demonstrated that controlling the abundance of solids by a simple settling low-tech solids removal device can benefit shrimp yields. In tilapia culture, Avnimelech (2007) and Azim and Little (2008) recorded values ranging from 460 to 643 mg L^{-1} and 597 and 560 mg L^{-1} respectively.

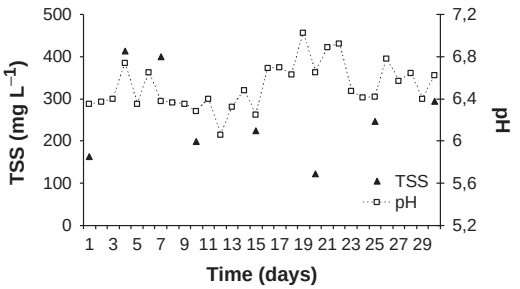

Figure 2 Total suspended solids and pH measurement in a biofloc technology (BFT) macrocosm tank during a 30 days experimental period.

Growth performance

The BFT limited water exchange system is considered to be a suitable approach for sustainable and efficient aquaculture production of high fish or shrimp biomass. In our study, final weight, final biomass and weight gain results were better in BFT treatments as compared with clear water ($P < 0.05$). Higher growth rates of PLs were often associated with higher grow-out performance or at least during PL pond stocking (Aquacop, Le Moullac & Damez 1991; Fegan 1992; Castille, Samocha, Lawrence, He, Frelier & Jaenike 1993). Also an interesting finding was that pelletized feed supply did not change shrimp performance. The PL in the BFT tank had similar growth performance regardless of whether they were fed a commercial feed. Some nutrients (as vitamins and minerals) derived from the commercial feed used to feed shrimp juveniles in the macrocosm tank could have 'enriched' the biofloc, enlarging the nutrient supply to the PLs.

In this study, survival rates (67–84%) were similar to *F. paulensis* results. Survival rates were 54–82% for *F. paulensis* PL reared in a clear-water nursery (Hennig & Andreatta 1998), 90–95% in estuarine cages

Table 3 Performance parameters of 30 days experimental period for *Farfantepenaeus brasiliensis* reared from PL25 and stocked at a density of 1000 shrimp m^{-3}

Parameter	BFT+CF	BFT	Control	*P*-value
Mean final weight (mg)	218.9^{A} (± 13.9)	236.5^{A} (± 13.5)	176.0^{B} (± 4.7)	<0.001
Final length (mm)	27.6 (± 1.3)	27.7 (± 1.1)	27.2 (± 0.4)	>0.05
Mean weight gain (mg)	193.9^{A} (± 13.9)	211.5^{A} (± 13.5)	151.0^{B} (± 4.7)	<0.001
Final biomass (g)	17.9^{A} (± 0.8)	15.7^{A} (± 1.3)	8.2^{B} (± 0.1)	<0.001
Survival (%)	81.5 (± 3.5)	67.0 (± 7.0)	84.8 (± 4.2)	>0.05

Data are means (± standard error) and significance level, along experiment. Different letters indicate means are significantly different ($P < 0.05$).
Treatments: BFT+CF, BFT culture system plus a commercial feed supply; BFT, BFT with no feed supply; control, clear water condition with commercial feed supply.

Table 4 Proximate analysis of commercial diet (Cargill's Purina Agribrands^{TM}), sugarcane molasses and wheat bran (carbon source) and biofloc

Composition	Commercial feed	Molasses	Wheat bran	Biofloc
Dry matter (%)	90.4	61.1	88.1	87.1
Crude protein (% DW)	39.7	5.6	18.9	30.4
Crude lipid (% DW)	13.1	0.2	2.7	0.47
Crude fibre (% DW)	2.6	0.2	11.3	0.8
Carbohydrate (% DW)	35.6	81.6	61.1	29.4
Ash (% DW)	9.0	12.4	6.0	39.2
Gross energy (kJ g^{-1} DW)	20.2	15.4	15.9	12.2

Carbohydrates estimates by difference. Gross energy content were calculating according to Tacon (1990).
DW, dry weight.

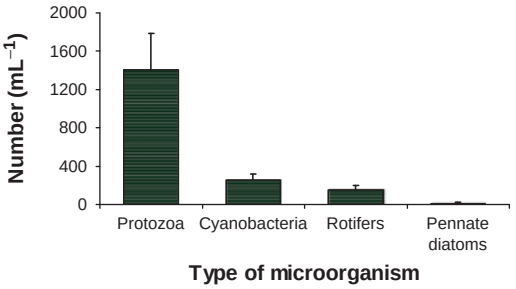

Figure 3 The number of associated microorganisms (mean ± SD) in water from a biofloc technology (BFT) macrocosm tank at the end of a 30 days experimental period.

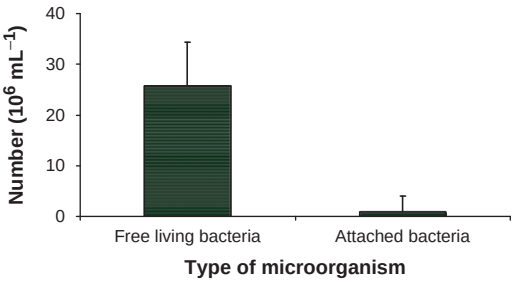

Figure 4 The number of free living and attached bacteria (mean ± SD) in a biofloc technology (BFT) macrocosm tank at the end of a 30 days experimental period.

(Ballester, Wasielesky, Cavalli & Abreu 2007), and 89–95% in a similar biofloc device (Ballester *et al.* 2010). For *L. vannamei* reared in an intensive raceway nursery system, Mishra *et al.* (2008) and Cohen *et al.* (2005) reported survival rates ranging from 55.9% to 100% and 97% and 100% respectively. Even though survival rates and final biomass were not significantly different between BFT and BFT+CF (67% and 15.7 g and 81.5% and 17.9 g respectively), the lower values of BFT could indicate cannibalism, increasing the nutrient supply to the survivors. Further studies are needed to better understand the biofloc as a nutritional resource in *F. brasiliensis* nurseries, aiming to improve the percentage survival. Mineral content and lipid profile in biofloc seems to need further investigations.

In relation to improved growth performance in BFT treatments, the cage mesh possibly provided an added substrate for colonization of biota (biofilm), thereby enhancing the amount of microorganisms available for shrimp intake as compared with the clear-water tank. Moreover, no biofilm formation was observed in control cages. Many authors reports that biofilm enhances shrimp performance (Moss & Moss 2004; Ballester *et al.* 2007), decreases feed conversion ratio (FCR) (Bratvold & Browdy 2001; Tidwell, Coyle, Arnum & Weibel 2007) and improves water quality (Asaduzzaman *et al.* 2008; Arnold *et al.* 2009). Further investigations are needed to better understand the role of substrate for *F. brasiliensis* PL as substrate type, size and display.

The role of particulate organic matter and microorganisms in the microbial food web as a potential food source for cultured aquatic animals has been discussed since the early 1960s (Baylor & Sutcliffe 1963) and more recently by Moriarty (1997). Natural productivity is a likely avenue to decrease feed input in aquaculture systems (Browdy *et al.* 2001). High-intensity production has been most successful with *L. vannamei* (McIntosh, Samocha, Jones, Lawrence, McKee, Horowitz & Horowitz 2000; Moss 2002; Burford *et al.* 2003) and more recently with *Penaeus monodon* (Arnold *et al.* 2009). Burford *et al.* (2004) reported that more than 29% of daily food consumed for *L. vannamei* could be biofloc. Wasielesky *et al.* (2006) described the effect of natural productivity in a heterotrophic super-intensive system for *L. vannamei* juveniles. The authors showed a decrease in FCR (1.54–1.03) and increased growth rate (0.39–1.25 g week^{-1}) derivate from biofloc consumption when compared with clear-water conditions.

Proximate analysis and assessment of biofloc

The nutritional benefits of biofloc as a natural food source for *F. brasiliensis* is still under investigation. The biofloc can contain high levels of CP and other nutrients such as essential fatty acids and amino acids (Azim & Little 2008; Ju, Forster, Conquest, Dominy, Kuo & Horgen 2008). Proximal analysis of biofloc from the current study indicates the presence of a high CP level (30.4%). However, Froés, Abe, Wasielesky, Prentice and Cavalli (2006) demonstrated that when reared in clear water, the optimal dietary protein level for *F. paulensis* pink shrimp juveniles is 45% CP. In contrast, Ballester *et al.* (2010) showed that microorganisms present in BFT system played an important role for provision of essential nutrients for *F. paulensis*, thereby enabling the CP content in practical diets to be reduced from 45% to 35% without any reduction in growth. The CP level of 31% found in our study is consistent with levels reported by Tacon *et al.* (2002) and Wasielesky *et al.* (2006), but less than the 43% and 49% CP described by Jory (2001)

and Kuhn, Boardman, Lawrence, Marsh and Flick (2009) respectively. In addition, Crab *et al.* (2010) found that using glucose or a combination of glycerol and *Bacillus* as a carbon source in 'bioreactors' led to higher biofloc protein content, higher n-6 fatty acids, which resulted in improved survival rates of freshwater prawns PLs. Further investigations are needed to compare different carbon sources and external bioreactor devices versus *in situ* biofloc tank production in terms of biofloc nutritional quality and economical benefits.

The biofloc samples showed a large quantity of grazers. Protozoa appeared to be the most abundant biofloc microorganism group (Fig. 3). The same trend was observed by Azim and Little (2008) and Ballester *et al.* (2010). The great number of protozoa and rotifers may contribute to better shrimp performance in BFT treatments compared to the control (Thompson *et al.* 2002). In terms of bacteria counts, the number of free living bacterial cells was almost 20 times higher than attached bacteria. This may be a satisfactory free living/attached bacteria ratio for good biofloc aggregation as corroborated with TSS levels. These results make a few assumptions and further studies are needed to better understand the differences in microorganism profiles and their benefits for shrimp nutrition. Microbial ecology of bioflocs (i.e. using different carbon source) certainly merits further investigation.

Conclusion

In summary, the BFT limited water exchange system benefited *F. brasiliensis* by improving growth performance and also maintenance of water quality. Postlarvae that did not receive a commercial feed had the same growth as those that received feed supply. The microorganism community, mainly represented by protozoa grazers, rotifers, cyanobacteria and diatoms, provided a continuous natural food source. The biofloc could also provide 'extra components', such as growth factors, that enhance the growth rates. Further research efforts are highly recommended to detect these extra components in biofloc and also to compare the economical benefits with other alternative culture systems.

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