Aquaculture Research

Aquaculture Research 2014, **45**, 270–277 doi: 10.1111/j.1365-2109.2012.03223.x

Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a Biofloc technology system

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Abstract

The present work evaluated the use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* in a Biofloc technology system. During a 30 days trial, three replicate tanks were randomly assigned to the following treatments: 1.) molasses (with molasses addition) and 2.) control (without molasses addition). Bacteriological analysis was used to quantify the abundance of presumptive *Vibrio* spp. between control and molasses treatment. The concentration of this microorganism was lower in molasses compared with control. For the immunological analysis, shrimp haemolymph was collected to determine the total haemocyte count and the total protein concentration. The immunological results were not different between treatments. The performance results of shrimp reared with molasses addition showed that the survival rate (88.87 ± 6.36), the mean final weight (1.22 ± 0.38) and the specific growth rate (0.0309 ± 1.06) were significantly higher compared with control (80.5 ± 2.42; 1.03 ± 0.13; 0.0256 ± 0.97 respectively). Moreover, the addition of molasses contributed to the maintenance of water quality and lower concentration of presumptive *Vibrio* spp. The control presented an unstable variation of *Vibrio* spp. reaching values of 80×10^2 CFU/ml, while the highest result of molasses was 20×10^2 CFU/ml, confirming the beneficial effects of molasses addition.

Keywords: Molasses, BFT, zero exchange, shrimp culture, *Farfantepenaeus brasiliensis*

Introduction

Forty per cent of world aquatic products are based on aquaculture, which is valued at US$ 78 billion (FAO. Fisheries & Aquaculture Department 2007). In 2006, crustacean production represented 9% of the total aquaculture production and 23% of the total value commercialized (FAO – Fisheries & Aquaculture Department 2008). In Brazil, an exotic species of shrimp, *L. vannamei*, is cultured; however, some indigenous species such as *Farfantepenaeus brasiliensis* have shown potential for culture (Lopes, Peixoto, Wasielesky & Ballester 2009). The pink shrimp *F. brasiliensis* is an indigenous species in Southeast and Southern Brazil and its distribution ranges from North Carolina (USA) to the coast of Rio Grande do Sul (Brazil) (D'Incao 1999).

Due to rapid development of aquaculture, researchers around the world are considering the need to develop environmentally friendly culture systems because the expansion of shrimp farming has been criticized for the damage to the ocean and coastal resources, destruction of surrounding ecosystems, effluent discharge, invasion of exotic species and the spread of pathogens (Naylor, Goldburg, Primavera, Kautsky, Beveridge, Clay, Folke, Lubchenco, Mooney & Troell 2000; Boyd 2003). Thus, the systems called BFT (Bio-floc technology) with zero water exchange, reduce not only the water

use, but also the issuance of effluent into the environment avoiding the environmental damage (Burford, Thompson, Bauman & Pearson 2003).

The Biofloc technology is based on the manipulation of microbial community through the addition of a carbon source that promotes the development of heterotrophic bacteria. These bacteria use the organic carbon and the inorganic nitrogen present in the water to produce their biomass removing toxic ammonia from the culture system (Avnimelech, Weber, Millstien, Hepher & Zoran 1986; Hargreaves 2006; Schryver, Crab, Defoirdt, Boon & VerstraeteW. 2008).

The basic principle of the BFT system is the retention of waste and its conversion into biofloc as a natural food source within the culture system (Azim & Little 2008). One of the benefits of this system is the bacterial uptake of nitrogen, including ammonia (Burford *et al.* 2003), and its conversion into cellular protein, which also provides a supplemental source of nutrition (McIntosh 2000; Burford, Thompson, McIntosh, Bauman & Pearson 2004b; Wasielesky, Atwood, Stokes & Browdy 2006) and possibly reducing the demand for protein on feed (Burford *et al.* 2003; Crab, Avnimelech, Defoirdt, Bossier & Verstraete 2007; Ballester, Abreu, Cavalli, Emerenciano, Abreu & Wasielesky 2010).

Another successful strategy utilized in modern shrimp culture systems is the nursery phase (a transitional system between the hatchery and growout) that allows the production of larger shrimp during growout while maintaining or increasing harvest yields (Samocha, Hamper, Emberson, Davis, McIntosh, Lawrence & Wyk 2002; Arnold, Coman, Jackson & Groves 2009). Culturing postlarvae in nurseries to a more robust size before stocking growout ponds has been suggested to increase the initial survival rate (Samocha, Lawrence & Bray 1993), which eliminates the need to overstock in anticipation of high mortality, a practice that can lead to the production of small prawns. In addition, nurseries can potentially increase the number of annual crops through reduced growout duration and head-start production during the cooler months in controlled temperature systems (Samocha *et al.* 1993; Peterson & Griffith 1999; Samocha, Blacher, Cordova & Wind 2000; Arnold *et al.* 2009).

Therefore, the goal of the present work was to compare two treatments, with and without molasses addition as a carbon source to evaluate the effects of carbon addition on the water quality, microbial floc formation, immunological parameters, and the performance of *F. brasiliensis* reared in a zero-water-exchange, microbial-floc-based nursery system.

Material and methods

A 30 days trial was conducted at the Marine Aquaculture Station (EMA/FURG). The experimental system consisted of 6 rectangular plastic tanks (40 L) with a bottom area of 0.20 m^2. *F. brasiliensis* early juveniles (0.46 g ± 0.13) were stocked in the tanks at a density equivalent to 150 shrimp.m^{-2} (30 shrimp/tank). Three replicate tanks were randomly assigned to the following treatments: 1.) molasses (with molasses) and 2.) control (without molasses).

To promote the development of the microbial flocs, all experimental tanks of molasses treatment received an inoculum (500 mL) from a heterotrophic shrimp culture system. For these tanks, the amount of organic supplementation was calculated based on the methods of Ebeling, Timmons and Bisogni (2006) and Avnimelech (1999), assuming that 6 g of carbon is needed to convert 1 g of TAN (total ammonia nitrogen), generated from feed, into bacterial biomass. Therefore, when the ammonia concentration in the experimental tanks of the molasses treatment reached values of 1 mg/L or higher, these tanks received a molasses dose calculated according to the equations proposed by (Ebeling *et al.* 2006) and (Avnimelech 1999).

Shrimp were fed twice daily a commercial diet containing 38% crude protein (Guabi®) via a specially designed feeding tray (Wasielesky *et al.* 2006). The initial feeding rate was 15% of the total tank biomass and was adjusted daily according to shrimp consumption. At the end of the trial, shrimp remaining in each tank were counted to determine the survival rate and were weighted to the nearest 0.01 g to determine the mean final weight and the specific growth rate (SGR). The SGR was calculated as described by Bagenal (1978):

$$SGR = (\ln(W_f) - \ln(W_i)) \times 100/t$$

where: W_f, final weight; W_i, initial weight; t, timeNo water exchange was carried out during the experimental period; only dechlorinated freshwater was added to compensate for evaporation losses.

Throughout the experimental period, the water temperature (mercury thermometer, precision ± 0.5°C), salinity (optical refractometer model

RTS – 101, Atago® US, Bellevue, WA, US, ± 1 g L^{-1}), pH (digital pH meter model Handylab 2 BNC, ± 0.01 precision, Schott®, Hattenbergstr, Germany) and dissolved oxygen (dissolved oxygen meter model Handylab/OXI/set ± 0.01 mg L^{-1} precision, Schott® Cambridge, UK) were measured every day.

Water samples were collected every 2 days to determine the concentrations of TAN (NH_3 + NH_4^+-N; Unesco 1983) and nitrite (NO_2; Bendschneider & Robinson 1952). The chlorophyll α concentration was measured twice weekly according to the method of Strickland and Parsons (1972). The reactive phosphorus concentration was determined three times during the experimental period (PO_4; Aminot & Chaussepied 1983), and alkalinity and total suspended solids (TSS) were measured once per week (Eaton, Cleserci & Greenberg 1995).

The concentration of presumptive *Vibrio* spp. in water was monitored at day 0, 10, 15, 22 and 30 according to the spread plate technique using thiosulphate citrate bile salt sucrose (TCBS) Difco® (Difco Laboratories, Detroit, MI, USA) (Lennette, Spaulding & Truant 1974). At the beginning of the experiment, the water was chlorinated and the absence of *Vibrio* spp. was verified.

For the immunological analysis, haemolymph was collected on days 0, 15 and 30 by inserting a Hamilton syringe (50 µl) into the shrimp's ventral sinus, transferring the haemolymph to a polyethylene tube and leaving it to coagulate for 24 h at 4°C. The clot was then centrifuged at 2000 × *g* for 10 min to obtain the serum, which was either immediately used or aliquoted and stored at –20°C (Maggioni, Andreatta, Hermes & Barracco 2004).

The granular and hyaline haemocyte counts were determined using a Neubauer chamber after collecting the haemolymph (six animals per treatment) directly into an anticoagulant solution (1 : 4) (modified Alsever's solution or MAS: 27 mM sodium citrate, 336 mM sodium chloride, 115 mM glucose, 9 mM EDTA, pH 7.0) (Maggioni *et al.* 2004).

The total protein concentration (TPC) in shrimp serum (six animals per treatment) was determined according to the (Bradford 1976) method using bovine serum albumin as a standard (Maggioni *et al.* 2004).

A T-test was used to identify significant differences ($P < 0.05$) in shrimp performance. A two-way analysis of variance (ANOVA, $\alpha = 0.05$) (time × treatment) was used to detect differences in the bacteriological, immunological and water quality parameters between treatments. The Tukey test was applied when significant differences were detected. All tests were conducted after the confirmation of the homogeneity of variance (Cochran test) and the normality of the distribution of the data (Kolmogorov–Smirnov's test).

Results

The survival rate (88.87 ± 6.36), the final weight (1.22 ± 0.38) and SGR (0.0309 ± 1.06) of shrimp reared in molasses treatment were significantly higher than those of shrimp reared in control (80.5 ± 2.42; 1.03 ± 0.13; 0.0256 ± 0.97 respectively) (Table 1).

According to the bacteriological analysis, the molasses treatment resulted in a significantly lower and stable concentration of *Vibrio* spp. during the experimental period (Figure 1). The control presented an unstable variation of *Vibrio* spp. reaching concentrations of 80×10^2 CFU/ml, while the highest molasses result was 20×10^2 CFU/ml.

The water quality parameters presented statistical differences during the experimental period. The mean concentration of ammonia in the control tanks (1.02 ± 0.83 mg/L) was nearly twofold the concentration recorded in the molasses tanks (0.71 ± 0.65 mg/L); the same trend was recorded for the mean concentration of nitrite for control (8.28 ± 6.37 mg/L) and Molasses (3.65 ± 3.11 mg/L) (Table 2). The highest ammonia concentration was recorded for both treatments on day 12.

Table 1 Mean (± SD) of the survival rate, final weight and specific growth rate of *F. brasiliensis* reared during the nursery phase with and without molasses addition in a Bio-floc technology culture system

Treatment	Survival (%)	Final Weight (g)	Specific growth rate (%day)
Molasses	88.87 ± 6.36[a]	1.22 ± 0.38[a]	0.0309 ± 1.06[a]
Control	80.5 ± 2.42[b]	1.03 ± 0.13[b]	0.0256 ± 0.97[b]

Different superscript letters indicate significant differences.

In the control treatment, the concentration reached 9.12 mg/L and in the molasses treatment, 5.06 mg/L. Additionally, the ammonia concentration decreased to zero after 15 days of culture in the molasses tanks, whereas the same was not observed for the control treatment until the 19th day (Fig. 2).

The fluctuations in nitrite concentrations are shown in Fig. 3. After 9 days, the nitrite concentration reached to 10 mg/L in both treatments, dropped to zero at 16th day and then increased again. Significantly higher nitrite concentrations were recorded for the control treatment. The chlorophyll *a* was significantly higher in molasses treatment (94 μg L^{-1}) only on the last experimental day compared with control (65 μg L^{-1}). The development of microbial flocs was followed by determining the amount of total suspended solids analysis (Figure 4). There were no significant differences between treatments for this parameter.

The immunological analysis presented no statistical differences between treatments, although shrimp reared in the molasses contained higher levels of total protein compared with those reared in control (Table 3).

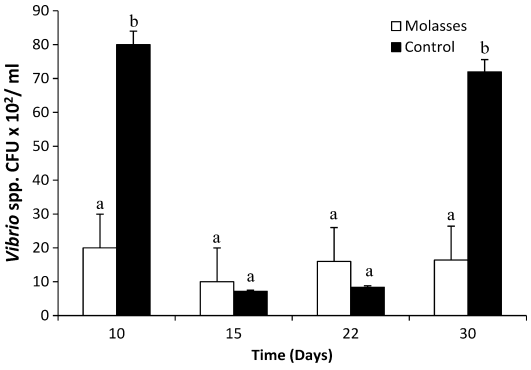

Figure 1 Comparative abundance of presumptive *Vibrio* spp. in the water during the nursery rearing of *Farfantepenaeus brasiliensis* for different treatments * Different superscript letters indicate significant differences (p 0.05).

Table 2 Mean (± SD) of water quality parameters of the tanks of *F. brasiliensis* reared during the nursery phase with and without molasses addition in a BFT culture system

Treatment		
Parameter	**Molasses**	**Control**
Temperature (°C)	26.7 ± 0.25	26.63 ± 0.11
pH	8.10 ± 0.006	7.99 ± 0.02
Salinity (g L^{-1})	31.47 ± 0.23	31.73 ± 0.56
DO (mg L^{-1})	6.11 ± 0.01	6.13 ± 0.03
TSS (mg L^{-1})	538.09 ± 444.7	522.61 ± 375.81
Alkalinity (mg L^{-1})	184.16 ± 12.4	172.08 ± 21.58
Ammonia (mg L^{-1})	0.71 ± 0.65[a]	1.02 ± 0.83[b]
Nitrite (mg L^{-1})	3.65 ± 3.11[a]	8.28 ± 6.37[b]
Phosphate (mg L^{-1})	3.27 ± 1.32	3.94 ± 1.11
Chlorophyll α (μg L^{-1})	6.96 ± 3.15[a]	2.07 ± 1.63[b]

Different superscript letters indicate significant differences ($p < 0.05$).

Discussion

In aquaculture systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo & Rimon 1982; Diab & Shilo 1988). According to Boyd and Clay (2002), the water quality of a heterotrophic microbial-based production system containing bacterial flocs is more stable than that of a phytoplankton-based production system.

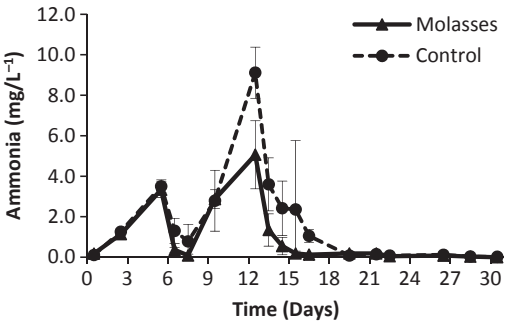

Figure 2 Fluctuations in the ammonia concentration during the nursery rearing of *Farfantepenaeus brasiliensis* under different conditions.

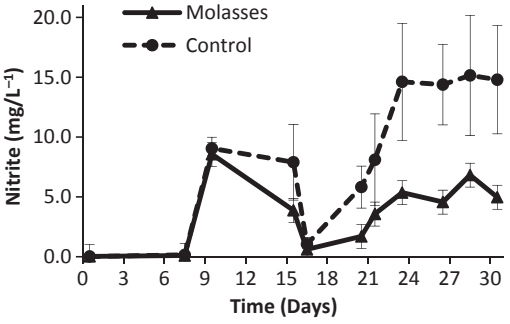

Figure 3 Fluctuations in the nitrite concentration during the nursery rearing of *Farfantepenaeus brasiliensis* under different conditions.

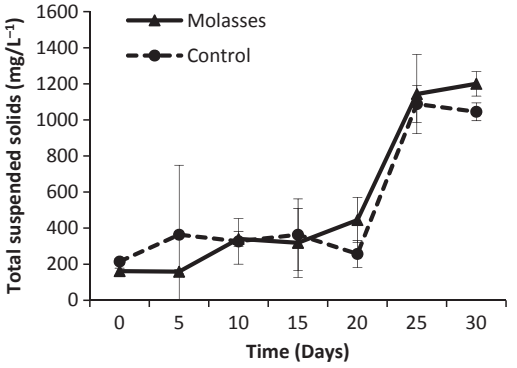

Figure 4 Fluctuations in the amount of total suspended solids during the nursery rearing of *Farfantepenaeus brasiliensis* under different conditions.

The most promising features of BFT systems (zero water exchange) are that they increase biosecurity (Bullis & Pruder 1999), reduce feed costs and water use (Chamberlain & Hopkins 1994; Boyd 2000). In these systems, the manipulation of the C/N ratio by the addition of carbohydrate significantly reduced inorganic N concentrations in the water column and total nitrogen in the sediment (Azim & Little 2008). At high carbon and nitrogen (C/N) ratio, heterotrophic microorganisms dominate autotrophic microorganisms and assimilate total ammonia nitrogen, nitrite and nitrate to produce cellular proteins that can serve as a supplemental feed source for shrimp (Avnimelech 1999; Moss, Pruder & Samocha 1999; Browdy, Bratvold, Stokes & McIntosh 2001; Burford & Lorenzen 2004).

Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz and Brock (2007) tested the molasses as a carbon source for *L. vannamei* and demonstrated that the use of molasses resulted in stimulation of heterotrophic bacterial floc formation that successfully competed with the algal population in this environment with an augmented carbon concentration. In addition, the low levels of TAN and nitrite suggest that molasses addition was an effective tool in controlling these nitrogen compounds.

Gao, Shan, Zhang, Bao and Ma (2012) tested sucrose as a carbohydrate to increase C/N ratio to evaluate the water quality improvement and shrimp performance. These authors observed that the concentrations of TAN and NO_2 were kept at significantly lower levels with the certain adding quantity of sucrose (75% and 100% based on the formula proposed by Avnimelech (1999). Their results indicated that 75% and 100% could effectively increase the C/N ratios of the water. Furthermore, they suggested that 75% of the carbohydrate quantity addition may be appropriate for the *L. vannamei* intensive culture in a zero-water exchange system presenting higher survival and the lowest FCR.

Boyd and Clay (2002) observed that bacterial flocs provide more stable water quality, and Avnimelech (1999) reported that the addition of carbohydrate to the production systems reduces the TAN concentration through immobilization of bacterial biomass. Our results of water quality follow the same pattern as those recorded by Samocha *et al.* (2007) and are in agreement with Boyd and Clay (2002), Avnimelech (1999) and (Gao *et al.* 2012). Moreover, in the present work, low levels of TAN and nitrite in molasses treatment compared with control were also observed, suggesting that molasses addition as a carbon source in *F. brasiliensis* culture was effective in controlling these nitrogen compounds.

Although in both treatments the mean values were within the suitable range for penaeid shrimp culture, TAN and nitrite reached higher concentrations during the culture period in the control treatment. These higher concentrations may have resulted in lower survival and growth rates because

Table 3 Granular and hyaline haemocyte counts and total protein concentration in the haemolymph of *F. brasiliensis* reared during the nursery phase with and without molasses addition

Treatment	Days	Granular haemocytes (%HG)	Hyaline haemocytes (%HH)	Total protein (mg/ml)
Molasses	0	72.16 ± 2.92	27.83 ± 2.92	121.16 ± 1.72
Control	0	72.16 ± 2.92	27.83 ± 2.92	121.16 ± 1.72
Molasses	15	69 ± 2.36	31 ± 2.36	121.66 ± 1.96
Control	15	71 ± 3.28	29 ± 3.28	119.16 ± 1.94
Molasses	30	70.83 ± 2.31	29.16 ± 2.31	120.66 ± 3.07
Control	30	70.83 ± 3.86	29.5 ± 3.39	118.83 ± 2.13

these compounds have long-term effects on shrimp (Cavalli, Peixoto & Wasielesky 1998; Lin & Chen 2001). At the end of the experiment, the water quality parameters were not significantly different, probably because flocs had formed in both treatments.

In the treatment with molasses addition, the microbial flocs were formed earlier than control, due to the higher levels of carbon that stimulate the heterotrophic bacteria development. The concentrations of ammonia and nitrite in molasses decreased faster than control because this microbial community was able to utilize the nitrogen contributing to the maintenance of water quality. Moreover, the improvement in shrimp performance observed in molasses can be a result of supplemental food source of flocs available in the system. Several works have reported the benefits of microorganisms as food source. (Ballester, Wasielesky, Cavalli & Abreu 2007) indicate that the microorganisms on the biofilm served as complementary food source providing nutritional benefits for shrimp, improving survival and biomass.

However, apart from serving as a direct source of nutrients to shrimp, there is evidence that the microorganisms present in the flocs also exert a positive effect on shrimp digestive enzyme activity and gut microflora Moss, Divakaran and Kim (2001). Emerenciano, Ballester, Cavalli and Wasielesky (2012) presented higher levels of final weight, final biomass and weight gain of *F. brasiliensis* reared in BFT treatments compared with clear water. Moreover, these authors confirmed a favourable nutritional quality of biofloc-enhancing shrimp performance. Therefore, it is reasonable to assume that the addition of carbon led to a microbial community with proprieties that contributed to shrimp performance.

Shrimp reared in the environment with molasses addition exhibited a survival rate, final weight and SGR significantly higher than those of the control. These results are in agreement with Krummenauer (2008), who demonstrated the efficacy of the BFT culture system in high-intensity shrimp culture with a production above 2.5 kg/m^2. Other authors (Otoshi, Tang, Dagdaban, Holl, Tallamy, Moss, Arce & Moss 2006; Otoshi, Scott, Naguwa & Moss 2007 a) have reported production values ranging from 4.5 to 10 kg/m^2, confirming the success of this system for shrimp production.

According to the immunological analysis, no statistical differences were found between treatments, although the molasses was associated with a higher level of hyaline haemocytes on day 15 and a higher concentration of total protein. (Pascual, Sánchez, Sánchez, Vargas-Albores, LeMoullac & Rosas 2003) asserted that the haemolymph protein levels of shrimp are affected by nutritional stress.

Crustaceans have only an innate immune system, and the immunological components of this system (total proteins and haemocytes) play a fundamental role in the immune defence of animals. Some authors believe that hyaline haemocytes are the main phagocytic cells (Johansson, Keyser, Sritunyalucksana & Söderhäll 2000; Barracco, Perazzolo & Rosa 2008) and that these cells are an intermediate form in the granular haemocyte lineage (Braak, Botterblom, Liu, Knaap & Rombout 2002 a). Based on final growth indicators, the addition of molasses had no effect on shrimp under conditions of limited water discharge (Samocha *et al.* 2007). These findings demonstrated that this system has no negative effect on shrimp.

The bacteriological analysis revealed a stable concentration of presumptive *Vibrio* bacteria during the experimental period in molasses treatment. This result could be explained by the maintenance of the water quality and the favourable conditions of the shrimp culture.

This study demonstrated that molasses can be used as a tool to prevent increases in the TAN and nitrite concentrations during the nursery phase of *F. brasiliensis* culture under conditions of limited water discharge. Additionally, the molasses treatment presented lower concentrations of presumptive *Vibrio* spp., and the shrimp performance results suggest that the microbial community served as a complementary food and improved the rearing conditions and the shrimp growth and survival.

Acknowledgements

The authors acknowledge the financial support provided by CNPq (Conselho Nacional de Pesquisa e Desenvolvimento Científico) and Capes (Coordenação de aperfeiçoamento de Pessoal de Nível Superior), Brazil. Wilson Wasielesky Jr and Eduardo Ballester are research fellows from CNPq.

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