Research Article

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Growth performance of the green tiger *shrimp penaeus semisulcatus* raised in biofloc systems

Abstract

Biofloc technology (BFT) is a technique of enhancing water quality in aquaculture to produce a microbial biomass or a "biofloc" that could be consumed by the cultured animals as a food source. For this purpose, an intensive feed trial for the green tiger shrimp *Penaeus semisulcatus* was conducted throughout an indoor system to determine the benefits of biofloc technology in water quality control and growth performance of the shrimp. The impact of substrate on the shrimps' performance in this system was also evaluated. In addition, the nutritive quality of the shrimps' flesh was assessed. Water quality was enhanced in the biofloc systems in terms of dissolved oxygen, pH, unionized ammonia and nitrate. Growth rates and weight gains of shrimps in the biofloc tanks increased significantly while the feed conversion ratios decreased with increasing the C: N (16:1) ratio. The effect of substrate on the survival rate of shrimps is also discussed.

Keywords: Biofloc, *Penaeus semisulcatus*, Microbial biomass, Substrate, Growth performance

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Abbreviations: BFT, Biofloc Technology; IBW, Initial Body Weight; FBW, Final Body Weight; WG, Weight Gain; ADG, Average Daily Gain; SGR, Specific Growth Ratio; FCR, Feed Conversion Ratio; SAFICO, Shrimp and Fish International Company; PER, Protein Efficiency Ratio; SR, Survival Rate; THB, Total Heterotrophic Bacteria; HSD, Honestly Significant Differences; THB, Total Heterotrophic Bacterial Population

Introduction

Traditional penaeid shrimp culture has a long proven history using pond culture in tropical climates. The global shrimp market has expanded from less than $1 billion in 2000 to $5.8 billion (US) in 2005.[1] To meet the growing demand on shrimp, the industry is shifting from extensive rearing systems to more intensive rearing systems.[2] Increasing stocking density of fish or shrimp in ponds usually increases the deterioration of pond sediment,[3] increases the susceptibility of shrimps to disease,[4] decreases growth of shrimp,[3] increases pressure on natural food resources,[5] reduces food conversion efficiency,[6] and rises the total food costs.[7] In aquaculture, the cost of the supplementary diets is predominantly due to the cost of protein component in general and fish meal in particular.[8] Fishmeal is considered an essential ingredient in marine shrimp diets because of its balanced amount of essential amino acids, fatty acids, vitamins, minerals and palatability.[9] Tacon & Metian[10] reported that the aquaculture industry consumed 68.2% of global fishmeal production in 2006; however, fishmeal production has remained relatively constant since 1985 at about 7 million tons per year.[11] The steady growth of aquaculture and consequent increase in demand for fishmeal has caused a significant increase in fishmeal prices in the last decade.[12] It has led to an increase in feed prices reducing the use of this ingredient in diets for animals.[13] Despite the stabilization in the production of fishmeal, aquaculture production continues to grow year after year. In part, technological advances have allowed significant improvements in the feed efficiency of fish and shrimp.[10] Nevertheless, dependence on fishmeal remains high, affecting the profitability of aquaculture.[14] Thus, the increasing demand for fishmeal, combined with over-exploitation of fish stocks, has spurred a search for cheaper and sustainable protein ingredients to reduce or eliminate the use of fishmeal in aquaculture diets.[15]

With the rapid expansion and intensification, however, there is also a growing concern about the ecological sustainability of shrimp farming.[16] The cultured shrimp retain only 20-30% of feed nutrient; therefore, 70-80% of high dietary protein is excreted and accumulated in water, which finally leads to water deterioration.[17] Moreover, worsening of water quality has resulted in disease outbreaks and heavy financial losses.[18] Such environmental issues have created a large demand for productive, efficient and sustainable shrimp farming systems that have low impact on the environment and are more likely to be free of disease.[19] One of the potential management measures to improve production and nutrients retention in shrimp farming systems is the application of biofloc technology by means of manipulating the carbon/ nitrogen ratio (C/N ratio).[20] If carbon and nitrogen are well balanced according to either the use of lower protein diet and/ or supplying additional carbon sources, e.g. glucose, sucrose, and starch to the pond, the inorganic nitrogen components (ammonia, nitrite, and nitrate) in pond will be converted into bacterial biomass.[20] As such, nutrients from excretion and remnant feed are recycled into bacterial biomass and formed biofloc which can be taken up as an additional feed for aquatic animals.[21]

Biofloc technology has been applied and developed in high intensive farming systems of several shrimp species, such as *Penaeus monodon*, *Litopenaeus vannamei*, and *Macrobrachium rosenbergii*.[22-24] However, due to the specific hiding behavior of *P. semisulcatus* by day light, little is known about the practicability of biofloc technology for its intensive farming. Nevertheless, Megahed[25] was able to develop an intensive farming system for *P. semisulcatus* at the Shrimp and Fish International Company (SAFICO), South Sinai, Egypt. In his study, he showed that BFT could provide a solution to reduce feed cost and enhance water quality. The use of artificial substrates is a management strategy employed in the growth of aquatic organisms to improve efficiency in culture tanks. Polyethylene and polypropylene screens, bamboo, plastic bottles and commercial products (Aquamats TM) have been used as substrates in growth tanks.[26] In experiments with post-larvae and juvenile marine shrimp, the use of substrates is frequently associated with improved performance by the shrimp.[27] In intensive cultures, substrates have been used in an effort to mitigate the negative effects of increasing the stocking density.[28] Abdussamad

& Thampy[29] suggested that substrates provide an additional surface for the shrimps, which reduces their competition for space and negative behavioral interactions such as cannibalism. In this study, we conducted an intensive farming trial for *P.semisulcatus* using indoor biofloc systems to determine the benefits of biofloc technology in water quality control and growth performance of the shrimp. The impact of substrate on the shrimps' growth performance in this system was also evaluated. In addition, the nutritive quality of the shrimps' flesh was assessed.

Materials and methods

The objective of this experiment was to study the effect of BFT application on the water quality and growth performance of the green tiger shrimp *P. semisulcatu*s reared in the laboratory.

Preparation of experimental tanks

Eight square fiber glass tanks with dimensions of 60 × 60 × 35 cm (length× width× height) were used in this experiment. The tanks were cleaned with formalin, washed with water then left to dry. Each tank was filled with 80 liters of sea water with a salinity of 30gL-1 and aerated using a silicone tube connected to an air blower. Salinity was measured using a Refractometer. Water temperature was maintained at 30°C ± 1 by means of eight water heaters. The tanks were covered completely with plastic films (0.4 mm) to reduce evaporation. The tank volume was maintained constant by adding one liter of tap water weekly to replace the loss due to evaporation.

Experimental diet

The prepared diet was used to feed shrimps in the control tanks as well as those in the biofloc treatment tanks.

Composition: The diet was formulated to be equivalent to the levels of crude protein, crude fat, total ash and crude fiber required for the culture of this shrimp. Composition and cost of the dietary ingredients is shown in Table 1 (all ingredients were locally purchased). The proximate analysis of the diet (Table 2) was carried out at the Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Table 1 Composition and cost of the dietary ingredients used in the feeding trial

Ingredients	Experimental diet (G/ 100g Dry matter)	Cost (US$/ Kg) In 2014
Fish meal (Local)	20	0.7
Shrimp meal	20	0.7
Soybean meal	20	0.7
Wheat flour	20	0.3
Corn meal	19	0.3
Gelatin (Binder)	0.5	1.1
Vitamin premix	0.025	1.4
Mineral premix	0.025	1.4
Manufacture cost		23.3 $/ Kg

Preparation: Exact amounts of dried and finely ground ingredients were mixed thoroughly. Sufficient amount of hot water was added to dissolve the gelatin powder, and then the solution was added to the ingredients and mixed until stiff dough was produced. The dough was pressed into pellets (2mm) using a commercial food mincer/ grinder then dried for 24 hrs in a laboratory oven at 60°C. They were then packed in a plastic container at room temperature for subsequent use.

Table 2 Proximate composition of the experimental diet

Proximate analysis	%
Dry matter (DM)	92.8
Moisture	7.2
Crude protein	23.4
Crude fat	3.2
Crude Fiber (CF)	6.6
Ash	15.4
Nitrogen Free Extract (NFE)	44.3
Gross energy*	3707.7

*Gross energy was calculated on the basis of 5.64, 4.11 and 9.44 Kcal GE/g protein, NFE and fat, respectively.

Transportation of shrimps

Wild specimens were obtained from Shatta, Damietta, Egypt and transferred in a closed plastic tank supplied with aeration using a portable battery air pump.

Acclimatization

Upon reaching the laboratory, shrimps were transferred to the prepared tanks using small containers. The containers were allowed to float on the water surface of the tank for 30 minutes. They were lowered gradually into the tanks and the tank water was sprinkled slowly into the containers for 10 minutes to equalize with tank water quality. The shrimps were considered acclimatized when they started to get out of the container and swim into the tanks. Shrimps were left without feeding during the first day then adapted gradually to the prepared diet and aquarium conditions for one week.

Experimental design

The experiment was performed in the Mariculture laboratory, at the Department of Marine Science, Suez Canal University, Ismailia, Egypt, for eight weeks (from April 16th -June 16th 2014). The previously prepared fiber glass tanks were used. Thirty PVC pipes of 1 inch diameter and 20cm length were placed at the bottom of each tank to act as artificial burrows (substrate) for shrimps. Three replicates for two biofloc treatments (with and without substrate) were made. A control tank for each treatment was used and those were equipped with an automatic mechanical filtration filter (Model Jinzlian, China). Thirty juvenile shrimps (with a mean weight (± standard deviation) of 0.5g ±0.1) were stocked per tank, resulting in a density of 100 shrimps/ m2. The daily feeding rate was 10% of their body weight at the beginning of experiment, and reduced gradually to 5% body weight at the end of the culture period. The amount of feed given to the shrimps increased gradually every two weeks of the experimental period (1.5g, 3.15g, 4g and 5.5g, respectively). In order to stimulate bacterial growth, an amount of rice meal was added daily after the addition of the feed in the biofloc tanks to maintain a C: N ratio of (16:1) and increased gradually every two weeks of the experimental period (0.7g, 1.5g, 2g and 2.7g).

Water quality parameters

Water quality in each tank was monitored twice a week between 10:00 am and 12:00 pm for dissolved oxygen (DO), pH, temperature, nitrate (NO_3) and unionized ammonia (NH_3). pH and temperature were measured by means of a Multimeter (Crizon, mm 40). Dissolved oxygen was measured by a digital oxygen meter. Nitrate and unionized ammonia were measured using colorimetric estimations of a commercial kit (API® master test kit). Total heterotrophic bacteria

(THB) count in the water was estimated following the standard procedures[30] and expressed as colony-forming units per ml (CFU ml-1).

Growth performance analysis

Upon termination of the 8-week growth trial, final weights of the remaining shrimp were obtained as shrimp weight per tank (FBW). Weight gain (WG = final weight [FBW] - initial weight [IBW]); average daily gain (g/shrimp/day): (ADG= [FBW - IBW]/ days); specific growth rate (100×[ln FBW - ln IBW]/ days); feed conversion ratio (FCR = Dry feed consumed/ WG); protein efficiency ratio (PER = total WG / total protein consumed) and survival (SR = 100 × final shrimp number /initial shrimp number) for each dietary treatment were determined according to Ricker;[31] Felix & Sudharsan.[32] and Venkat et al.[33]

Nutritive quality of the shrimps' flesh

The shrimps were washed with deionized water to remove any adhering contamination, and then drained over filter paper. For each group of individuals the exoskeletons were peeled off and the flesh was oven dried at 70°C then ground into fine powder. Protein, carbohydrate and lipid contents were estimated by adopting the standard methods of Lowry et al.[34] Dubois et al.[35] and Folch et al.,[36] respectively. Shrimp flesh was analyzed for moisture and ash content.[37] The carbohydrate content was estimated according to Merrill & Watt:[38] carbohydrate=100− (ash+ crude protein+ moisture+ total fat). Samples were analyzed at the Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Suez Canal University, Egypt. The obtained data was pooled for all replicate samples and the average was calculated.

Statistical analysis

Statistical analysis was performed using SYSTAT (V.10.2.05, 2002). Differences in water quality were considered significant at $P< 0.05$. Analysis of Variance (ANOVA) was used to determine the effect of different culture systems on shrimp performance. When appropriate, a Turkey's HSD (Honestly Significant Differences) post-hoc test was employed to check for differences between means. The 5% significance level was applied for all tests.

Results

Water quality parameters

Dissolved oxygen (DO): DO concentrations in the control tanks were higher than the biofloc treatment tanks throughout the culture period (Figure 1). The values decreased gradually from 6.07 mg/L until 5.2 and 4.07 mg/L in the control and biofloc tanks, respectively. Variations in DO concentrations between the two treatments were significantly different ($P <0.001$). However, no significant difference was recorded in the treatments with the presence or absence of substrate ($P <0.070$).

Hydrogen ion concentration (pH): The pH values in the control tanks were higher compared to the biofloc treatment tanks (Figure 2). The values in the biofloc tanks showed minor differences during the culture period ranging between 7.6-7.8. On the other hand, values in the control tanks increased relatively from 7.6 to 8.2. They remained more or less constant in both treatments (ranging between 8.13-8.17; 7.67-7.63, respectively) from the 7th week until the end of the experiment. Differences in pH values between the two treatments were significantly different ($P <0.001$) but not significantly different with the presence or absence of the substrate in both treatments ($P <0.367$).
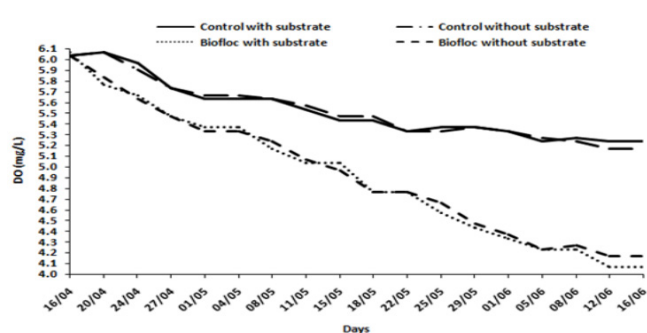

Figure 1 Variations in DO concentrations in the different treatments throughout the experimental period.
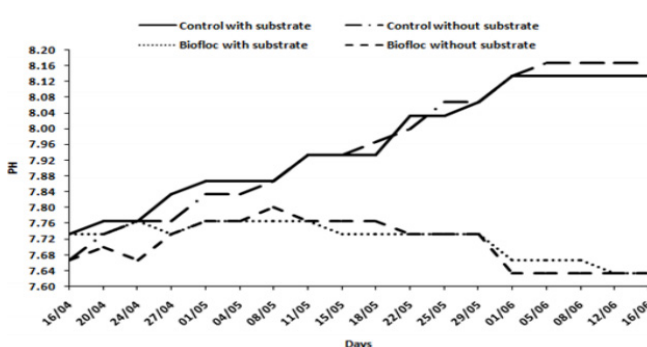

Figure 2 Variations in pH values in the different treatments throughout the experimental period.

Unionized ammonia (mg/L): Unionized ammonia concentrationsin the control tanks were higher than the biofloc treatment tanks, increasing gradually from 0.07 to 1.1 mg/L (Figure 3). In contrast, ammonia in the biofloc tanks remained relatively constant (ranging between 0.07-0.09 mg/L). Concentrations in both treatments were significantly different ($P <0.001$), but no significant difference was recorded in the treatments with the presence or absence of the substrate ($P <0.945$).
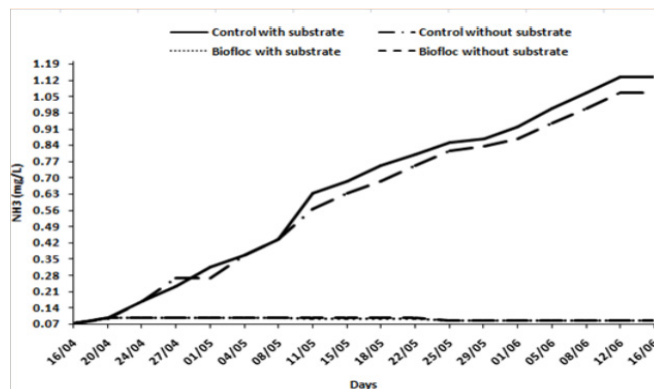

Figure 3 Variations in concentrations of unionized ammonia in the different treatments throughout the experimental period.

Nitrate (mg/L): Nitrate concentrationsin the control tanks increased from less than 5, 10, 20, 40 reaching 60mg/L, respectively. In contrast, the levels of nitrate in the biofloc treatment tanks showed approximately the same values (less than 5 mg/L) throughout the culture period.

Total heterotrophic bacterial population (THB): The total numbers of heterotrophic bacterial cells in the biofloc treatment tanks increased

greatly from 25 to 125 CFU/ ml (Figure 4). In contrast, their numbers in the control tanks remained more or less constant (ranging between 21 and 23 CFU/ ml). The total numbers of heterotrophic bacteria in both treatments were significantly different ($P < 0.001$) but no significant difference was recorded in the treatments with the presence or absence of the substrate ($P < 0.640$).
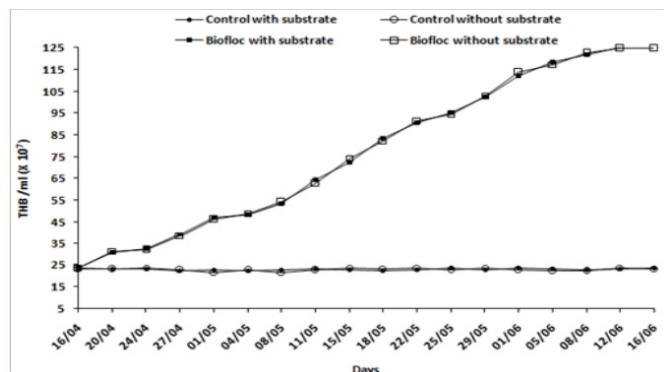

Figure 4 Variations in the total numbers of heterotrophic bacterial cells in the different treatments throughout the experimental period.

Growth performance analysis

Final body weight (FBW), weight gain (WG), average daily gain (ADG) and specific growth ratio (SGR) values showed minor differences between the two treatments (Table 3). However, the feed conversion ratio (FCR), protein efficiency ratio (PER) and survival ratios (SR) in both treatments (in the presence of substrate) showed better values than those without substrate. Differences in the final weights of *P. semisulcatus* in both treatments were significantly different ($P < 0.001$).

Values in the same row with different letters are significantly different ($P < 0.05$). SD: Standard Deviation; FBW: Final Body Weight; WG: Weight Gain; ADG: Average Daily Gain; SGR: Specific Growth Ratio; FCR: Feed Conversion Ratio; PER: Protein Efficiency Ratio; SR: Survival Rate

Nutritive quality of the shrimps' flesh

Moisture was the highest component in the shrimps' flesh in both treatments (Table 4). The flesh of shrimps raised in the biofloc tanks exhibited relatively higher values of crude protein percentages (20.19 %) than those raised in the control tanks (18.99 %). Carbohydrates, fat and ash were the lowest components obtained with minor differences between both treatments.

Discussion

The consumption of biofloc by shrimp has demonstrated several benefits such as the improvement of the growth rate,[39] decrease of food conversion ratio (FCR) and associated costs in feed.[40] Growth enhancement has been attributed to the bacterial nutritional components of the biofloc; up to 30% of the conventional feeding ration can be lowered due to biofloc consumption in shrimp.[40] The present study aimed to determine the benefits of biofloc technology in water quality control and growth performance of the green tiger shrimp. The impact of substrate on the shrimps' performance in the biofloc system was also evaluated in the presence of high stocking density. In addition, the nutritive quality of the shrimps' flesh was assessed.

Table 3 Mean values of the final weights and growth performance parameters of *Penaeus semisulcatus* in the four treatments at the end of the eight weeks experimental period

Treatment	Control		Biofloc	
	With substrate	**Without substrate**	**With substrate**	**Without substrate**
FBW ± SD	4.73 ± 0.3a	5.36 ± 0.4 b	5.52 ± 0.4 c	5.71 ± 0.5 d
WG (g)	4.23	4.86	5.02	5.21
ADG (g)	0.071	0.081	0.084	0.087
SGR (%/ day)	3.75	3.95	4	4.06
FCR	3.31	2.88	2.79	2.69
PER	2.41	1.19	3.23	1.44
SR (%)	76.67	33.33	87.78	37.78

Table 4 Nutritive quality of the flesh of *P. semisulcatus* in the two treatments (expressed as % fresh weight basis)

Parameter	Treatments	
	control	**Biofloc**
Moisture	75.07	73.64
Crude protein	18.99	20.19
Crude carbohydrate	2.13	2.22
Crude fat	1.48	1.54
Ash	2.33	2.41

Water quality parameters

Formulated diets and water quality values were considered optimal for shrimp culture.[41,42] This allowed direct comparisons between the water quality parameters in the control and biofloc treatments to be made.

Dissolved oxygen (DO): The recorded values of DO were within the good range for all the aquatic organisms.[43] DO concentrations decreased in both treatments over time. This could be attributed to the high stocking density (30 pcs per tank, resulting in a density of 100 shrimps/ m2) which agrees with the study of Allan & Maguire[5] who reported a decline in the growth of *P. monodon* juveniles as densities increased in model farming ponds. The concentrations of dissolved oxygen in the biofloc tanks tended to decrease more rapidly than the control tanks. This could be due to the increased C: N ratio which stimulates the growth of the heterotrophic bacteria which in turn require oxygen for their growth. This finding is in accordance with that of Landman et al.[44] who determined the hypoxia sensitivity of ten

fish and invertebrate species in plastic aquaria in New Zealand and found that there was a decrease in dissolved oxygen concentrations with the increase of C: N ratio. Sun et al.[45] studied the supplementation and consumption of dissolved oxygen and their seasonal variations in a shrimp pond. They proved that the concentrations of dissolved oxygen had a significant negative correlation with the numbers of heterotrophic bacteria. Bacteria have been reported to contribute as much as 77 % of the total oxygen consumption in fish ponds.[46]

Hydrogen ion concentration (pH): Values of pH in the present study were within the preferable range for penaeid shrimps.[41] The values in the control tanks increased relatively from 7.6 to 8.2. Ebeling et al.[47] reported that the photosynthesis and nitrification processes that are likely to occur in control system might have resulted in pH increase. These processes could alter CO2 concentration and the buffering capacity of the water.

On the other hand, levels of the C: N ratios in the biofloc tanks have significantly influenced the values of pH during the culture period by keeping them more or less constant. This could be related to the presence of heterotrophic bacteria which consume organic matter and cause the increase in the level of water inorganic carbon (CO2) and decrease the values of pH. pH usually declines as the redox potential declines as a result of microbial activity.[48] Ebeling et al.[47] stated that nitrogen uptake by heterotrophic process that likely to dominate BFT system consumes alkalinity half than nitrification (3.57 g alkalinity/g NH_4^+-N). They also concluded that as alkalinity concentration relates to the buffering capacity of water, the effect of the high concentration of CO2 resulted from fish and microbial respiration on water pH could sufficiently buffered in BFT systems.

Unionized ammonia and nitrate (mg/L): Nitrogenous constituents were at safe levels for ammonia[49] and nitrate.[50] Ammonia and nitrate concentrationsin the control tanks increased gradually from 0.07 to 1.1 mg/L and 5 to 60 mg/L, respectively throughout the culture period which can be referred to the increase in the stocking density of shrimps. Similar results have been reported in studies performed in different parts of the world.[51,52]

Concentrations of ammonia and nitrate in the biofloc tanks decreased as the levels of C: N ratio (16:1) increased. This result implies that rice meal addition (as a carbon source) had an obvious effect on the inorganic nitrogen reduction through stimulation of the bacterial growth. Furthermore, concentrations of ammonia and nitrate were negatively associated with the numbers of bacteria (Figures 3, 4). These findings are in agreement to those reported by Tezuka[53] and Hoch et al.[54] who stated that addition of carbon diminished inorganic nitrogen due to increasing uptake of NH_4^+ by bacteria. Zero water exchange ponds using carbon source enabled the control of the inorganic nitrogen accumulation through a balanced ratio of carbon to nitrogen in the feed.[20,55,56] In addition, Stuart et al.[57] raised the tiger shrimp *Penaeus monodon* in zero water exchange model using a daily carbon source (tapioca powder) and concluded that the addition of carbon promoted the microbial community and improved the water quality.

Total heterotrophic bacterial population (THB): There was a significant increase in the total number of heterotrophic bacteria with increased levels of C: N ratio in the biofloc tanks (Figure 4). This indicates that bacteria required carbon from the carbon source (rice meal) in order to multiply their cells. Middelboe et al.[58] studied bacterial utilization of dissolved free amino acids, dissolved combined amino acids and ammonium in the Delaware Bay estuary in the US and stated that the addition of glucose increased the number of heterotrophic

bacteria in water. Similarly, some previous investigators[22,55,56] found that the numbers of heterotrophic bacteria increased in response to increasing levels of C: N ratio in penaeid shrimp culture.

Growth performance analysis

Impact of the different treatments on growth parameters: The mean final weight, weight gain (WG), average daily gain (ADG) and the specific growth rate (SGR) in the control tank (without substrate) showed relatively higher values than that with substrate. On the other hand, the same parameters were slightly higher in biofloc tanks that had no substrate than those with substrate. This could be attributed to the high mortality of shrimps in absence of the substrate which resulted in decreasing the stocking density. Wyban et al.[59] studied the impact of stocking density on *Penaeus vannamei* growth rates in manure-fertilized ponds and reported that growth declined while both yield and density increased. The low shrimp performance in intensive cultures has been attributed to the negative social interactions between individuals which may result in an inhibitory effect such as aggressiveness[60] or stimulate cannibalism.[29] Otoshi et al.[61] and Zhang[26] suggested that substrates diminish the negative effects of intensification as they provide more space for the shrimp and reduce the stress that can lower their performance.

Growth rates and weight gains of shrimps in the biofloc tanks increased significantly while the feed conversion ratios decreased with increased C: N (16:1) ratio compared to those obtained from the control tanks. These results are in accordance with Avnimelech[20] who reported that the growth rates of fishes in tilapia commercial-scale ponds in Israel treated with carbon: nitrogen ratio of 16.6:1 was significantly higher than those grown in ponds with carbon: nitrogen ratio of 11.1: 1, while mortality of fishes, feed conversion rate and feed cost coefficient in treatment with carbon: nitrogen of 16.6:1 were significantly lower than in treatment with carbon: nitrogen of 11.1: 1.

Protein efficiency ratios of the shrimps in the biofloc tanks were higher than the control tanks. Hari et al.[62] conducted one indoor and one on-farm trial in India to evaluate the effect of control of carbon/nitrogen ratio (C/N ratio) by addition of carbohydrate to the water column in extensive types of *Penaeus monodon* culture systems and reported that protein conversion efficiency of the shrimps in carbohydrate added ponds were higher, revealing that the input feed protein along with the microbial protein was effectively converted by shrimps into biomass.

Impact of the different treatments on survival rates

In our study, although the water quality parameters were appropriate for shrimps' growth, the survival rate of the shrimps in the control and biofloc tanks that lacked the substrate was lower to a great extent than those with substrate. Suresh & Lin[63] studied the effect of stocking density on the water quality and production indices of red tilapia in a recirculated water system and reported a negative correlation between stocking density of fish with growth as well as other production parameters. In intensive culture systems, however, the use of shrimp that are genetically selected for fast growth in high densities and the use of formulated feed for such conditions are considered key factors for successful cultivation.[61]

Here, the use of the green tiger shrimp that was not specifically selected for intensive systems may have contributed considerably to the lower survival rate recorded in the tanks that had high stocking density and lacked the substrate. Araneda et al.[64] and Otoshi et al.[61] suggested that crowding at high densities causes stress on the shrimp, and can compromise performance. Stressed shrimp can be more

susceptible to facultative pathogenic microorganisms that are part of their natural microbial flora and aquatic environment,[65] which has been noticed in the control tanks of the present experiment. Crab et al.[66] studied the role of biofloc to protect the brine shrimp (*Artemia franciscana*) from the pathogen *Vibrio harveyi* and concluded that biofloc technology constituted a possible alternative measure to fight pathogenic bacteria.

Our study showed that the survival rates in the control and biofloc tanks (with substrate) were higher than those without substrate. Schveitzer et al.[67] studied the effects of using artificial substrates in the culture of *Litopenaeus vannamei* (Biofloc system) at different stocking densities on the microbial activity, water quality and production rates. They concluded that the presence of substrates increased the surface area of the tanks and reduced the relative stocking densities; and consequently reduced the stress level of the shrimps, indicated by the higher survival rates. A positive impact of substrates on the production indices for the culture of post-larvae and juvenile shrimp has been reported by several authors.[26-28,61] Those authors concluded that the increase in the growth of the shrimp resulted in the reduction in the stocking density because of the presence of substrates. Nevertheless, the survival rates in the biofloc tanks (with substrate) were higher (87.78%) than the control one (76.67%) (Table 3). Numerous studies have reported enhanced survival, health, and growth rates of shrimp raised in ponds with high activity of algae, microbial floc, and other natural biota.[20,23,24,39,40,42,68,69]

Nutritive quality of the shrimps' flesh

Although the culture of shrimp in zero exchange systems is an increasing and extensive research that has been carried out in different areas of the world, the impact of biofloc on the proximate composition and flesh quality has not extensively been studied. In our study, shrimps in both treatments had suitable moisture contents (73.64-75.07%). Moisture of fresh shrimp is generally reported as 75 to 80%.[70] Protein was found as the major constituent indicating that shrimp flesh can be a good source of amino acids.[71] Crude protein levels showed a tendency to increase in the shrimps raised in the biofloc treatments (Table 4). It has been reported that protein content of shrimp ranged between 17-21% depending on shrimp species.[72] Only slight differences were found in the carbohydrate, fat and ash compositions among shrimps grown in the control and biofloc tanks, representing that the quality of flesh was not affected by any of the culture conditions used in this study. Proximate compositions in shrimps' flesh are affected by several factors such as species, growth stage, feed and season.[73] It has been shown that organoleptic characteristics, quality of flesh, odor, texture and color of fish/shrimp depend on the conditions where they were captured or cultivated.[74] In our study, the proximate compositions or nutritive quality parameters found for *P. semisulcatus* were within the range of other shrimp species.[75-76]

Conclusion

Our results corroborate with those of other studies that reported the positive impact of artificial substrates on the production indices of cultured shrimps; however, the substrates had no impact on the water quality parameters. Substrates served in increasing the surface area of the tank, thus reducing the relative stocking density, which accordingly lowered the stress levels on the shrimps indicated by the higher survival rate. In the biofloc system, carbon source addition in combination with the low protein diet (23%) improved the sustainability of *P. semisulcatus* farming through increased growth rates, weight gain and reduced FCR. Consumption of microorganisms in BFT reduces FCR and consequently the costs of feed to nearly one-third. This work has demonstrated that the green tiger shrimp *P. semisulcatus* can be cultured in a biofloc system without losing its flesh quality.

Acknowledgments

Conflicts of interest

None.

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