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Research Article

Addition of sodium nitrite and biofilm in a *Litopenaeus vannamei* biofloc culture system

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ABSTRACT. This study evaluates the effects of the previous addition of sodium nitrite ($NaNO_2$) and biofilm in a *Litopenaeus vannamei* biofloc culture. Five treatments were tested in 180 L - tanks: $NaNO_2$ (-20) addition of $NaNO_2$ for 20 days before stocking and without artificial substrates; $NaNO_2$+B (-20) addition of $NaNO_2$ for 20 days before stocking, with 150% additional artificial substrates; $NaNO_2$ (Stocking) addition of $NaNO_2$ at stocking and no artificial substrates; $NaNO_2$ +B (Stocking) addition of $NaNO_2$ at stocking, with 150% additional artificial substrates and; Control, without $NaNO_2$ addition and without artificial substrates. The study was carried out in two phases: shrimps (0.21 ± 0.08 g) were stocked at a density of 400 ind m^{-2}/1,100 ind m^{-3}, for 30 days (nursery). After this period, the animals were restocked at a lower density (120 ind m^{-2}/400 ind m^{-3}) for 18 more days (grow-out). The turbidity and seattleable solids were higher in treatments without artificial substrates, independently of the addition of $NaNO_2$. The ammonia and nitrite were lower in control, and the nitrate concentrations were higher in treatments with addition of $NaNO_2$ without biofilm. At the end of the nursery, the survival was lower in treatment $NaNO_2$ (-20), and at the end of grow-out the final biomasses were higher in treatments with addition of $NaNO_2$ at the stocking day. In conclusion, the use of a dose of $NaNO_2$ at the stocking day could increase the nitrite-oxidizing bacteria, and the biofilm may contribute to the reduction in suspended particles in water, enhancing shrimp production in biofloc technology system.

Keywords: *Litopenaeus vannamei*, shrimp, biofloc, nitrogen, artificial substrates.

Adición de nitrito de sodio y biofilm a un sistema de cultivo biofloc de *Litopenaeus vannamei*

RESUMEN. Se evaluó los efectos de la adición previa de nitrito de sodio ($NaNO_2$) y biofilm al cultivo de *Litopenaeus vannamei* en sistema biofloc. Se analizaron cinco tratamientos en estanques de 180 L: $NaNO_2$ (-20) adición de $NaNO_2$ durante 20 días antes de la siembra y sin sustratos artificiales; $NaNO_2$+B (-20) adición de $NaNO_2$ durante 20 días antes de la siembra y con 150% de adición de sustratos artificiales; $NaNO_2$ (Stocking) adición de $NaNO_2$ en la siembra y sin sustratos artificiales; $NaNO_2$ +B (Stocking) adición de $NaNO_2$ en la siembra y con 150% de adición de sustratos artificiales; Control sin adición de $NaNO_2$ y sin substratos artificiales. La densidad de siembra utilizada inicialmente fue de 400 ind m^{-2}/ 1.100 ind m^{-3} durante 30 días (pre-cría). Después de este periodo, los camarones fueron sembrados a 120 ind m^{-2}/400 ind m^{-3} por más de 18 días (engorde). La turbidez y los sólidos sedimentables fueron más elevados en los tratamientos sin sustratos artificiales, independientemente de la adición del $NaNO_2$. Las concentraciones de amonio y nitrito fueron inferiores en el control, y las concentraciones de nitrato fueron mayores con la adición de $NaNO_2$ y sin sustratos artificiales. Después del periodo de pre-cría, la supervivencia fue menor en el tratamiento $NaNO_2$ (-20), y al final del período de engorde las biomasas fueron más altas en los tratamientos con adición de $NaNO_2$ en la siembra. En conclusión, la utilización de una dosis de $NaNO_2$ en la siembra puede aumentar las bacterias nitrito-oxidantes, y el biofilm puede contribuir a la reducción de partículas suspendidas en el agua, mejorando la producción de camarones en sistema biofloc.

Palabras clave: *Litopenaeus vannamei*, camarón, biofloc, nitrógeno, substratos artificiales.

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INTRODUCTION

In conventional aquaculture systems, one of the most used management techniques to kept good water quality for cultured animals is the exchange of large volumes of water (Hopkins *et al.*, 1993). Due to the negative environmental impact that the emission of these effluents cause, it is necessary to find alternatives to avoid or reduce this release to water bodies. Rearing in biofloc culture systems has proved to be an effective alternative for the reduction of aquaculture effluent emission. This occurs due to the possibility of reusing water for several cycles, using the microbial community to maintain good water quality and as additional food for the reared animals (Wasielesky *et al*., 2006; Silva *et al.*, 2013; Krummenauer *et al.*, 2014).

The removal of inorganic nitrogen dissolved in water made by the microbial community can occur via two types of systems: suspended or attached. When suspended, microorganisms move freely in the water, providing direct contact between bacterial cells and the water mass. When attached, microorganisms grow on a layer adhered to a solid surface, which can be artificial or natural depending on the rearing system that is used (Fitch *et al*., 1998; Nogueira *et al*., 1998). In the biofloc culture systems, both the microbial community that is formed suspended in the water (bioflocs) and the biofilm (formed on the artificial substrates) can contribute to the metabolism of the nitrogen compounds generated within the culture (Schveitzer *et al*., 2013; Viau *et al*., 2013).

However, if there is no balance between the rates of formation and removal of ammonia and nitrite within the system, an accumulation of these compounds may occur, causing production losses. Particularly, high nitrite concentrations seem to be one of the general problems in biofloc culture facilities. The toxic effects of this compound to shrimp are related to a number of different species, causing effects in metabolism, oxygen and food consumption and, immune function (Liao *et al*., 2012; Campos *et al*., 2013, 2014; Jiang *et al*., 2014). In biofloc culture systems, high concentrations of nitrite were reported in many studies (Cohen *et al*., 2005; Vinatea *et al*., 2010), and until now, few effective techniques to deal with this problem were found.

Therefore, the use of artificial substrates to increase the surface area for attachment of bacteria was one of the alternatives to minimize ammonia and nitrite spikes in culture (Audelo-Naranjo *et al*., 2011; Zhang *et al.*, 2016). Moreover, the prior development of the bacterial community through the addition of compounds that may serve as microbial growth stimulators before initiating culture can be another alternative. Based on the view that the population of microorganisms is already mature or stable when the animals are stocked, perhaps the spikes in toxic nitrogen compounds could be avoided (Sesuk *et al*., 2009). Otoshi *et al*. (2011) showed a higher efficiency of the nitrification process with the addition of sodium nitrite in *L. vannamei* biofloc culture. However, these authors do not describe any procedure for the application of this compound in the rearing. Considering the described factors, the aim of this study was to evaluate the effects of the addition of sodium nitrite ($NaNO_2$) and artificial substrates on water quality and growth of *Litopenaeus vannamei* in a biofloc culture system.

MATERIALS AND METHODS

Experimental design

The study was performed at the Marine Station of Aquaculture, Federal University of Rio Grande (FURG), Southern Brazil. The experimental culture system was installed inside a greenhouse, in tanks with 180 L of useful volume (with a lateral surface area of 1.0 m²). Filtered marine seawater (sand filter), chlorinated at 10 ppm and dechlorinated with ascorbic acid (1 ppm) was used to fill the tanks. The study was divided in two phases: initially, juvenile *Litopenaeus vannamei* (mean weight of 0.21 ± 0.08 g) were stocked at a density of 400 ind m^{-2}, value equivalent to 1.100 ind m^{-3} (n = 198). This period lasted 30 days and was designated as nursery phase. After this period, the animals were removed from the tanks, counted and re-stocked in the same tanks at a lower density (120 ind m^{-2} equivalent to 400 ind m^{-3}; n = 72), for more 18 days (grow-out phase). The culture water (bioflocs) was fully recycled from nursery to the grow-out phase.

The experiment contained four treatments (three replicates each): $NaNO_2$ (-20 days) addition of $NaNO_2$ for 20 days before stocking and without artificial substrates; $NaNO_2$+B (-20 days) addition of $NaNO_2$ for 20 days before stocking, with 150% additional artificial substrates; $NaNO_2$ (Stocking) addition of $NaNO_2$ at stocking and no artificial substrates; $NaNO_2$ +B (Stocking) addition of $NaNO_2$ at stocking, with 150% additional artificial substrates and; Control, without $NaNO_2$ addition and without artificial substrates. The additions of sodium nitrite ($NaNO_2$) were done to stimulate the growth of nitrifying bacteria to a dosage of 2 mg L^{-1}. The proposed value was based on the study of Otoshi *et al.* (2011), who reported a reduction in the nitrite peak by adding this compound during certain periods of the rearing. In this way, in treatments with previous addition of $NaNO_2$, it was established that the application would occur every two days during the 20 days before stocking, after analysis of nitrite

concentrations. Thus, when necessary, the compound was added on a regular basis in order to maintain a nitrite concentration in water of about 2 mg L^{-1}. In other treatments (except in control) the $NaNO_2$ was added only at stocking day, at a concentration of 2 mg L^{-1}.

The formation of bioflocs followed the methodologies proposed by Avnimelech (1999) and Ebeling *et al*. (2006). Liquid molasses and rice bran were used as carbon sources in order to maintain a C:N ratio of approximately 15-20:1. The shrimp were fed with a commercial diet (Guabi® 38% crude protein and 8% lipids) provided twice a day. The feeding rates were adjusted according to the methodology proposed by Jory *et al*. (2001). Initially, the shrimps were fed with a feeding rate of 30% of total biomass in each tank and, this rate decreased to 4.5% of biomass until the end of the study.

For the attachment of biofilm, "Needlona™" artificial substrates composed by non-floating nets (mesh size of 1.0 mm) were used. The percentage of substrates added to each tank was calculated according to the lateral area of the tanks, given that the substrates were fixed in the upper part of the tanks and remained submerged during the entire experimental period. The tanks were aerated with air stones placed on the bottom of each experimental tank.

In order to maintain the alkalinity levels in acceptable range to shrimp growth and both to the nitrifying community development, adjustments were made according to Furtado *et al*. (2011), which recommended alkalinities above 100 mg $CaCO_3$ L^{-1}, using hydrated lime.

Physical and chemical variables of the water

During the 48 days of rearing, the concentrations of dissolved oxygen and the water temperature were monitored, twice a day, using a digital oximeter (YSI™, Model 55). The pH was analyzed once a day, during the morning, using a counter meter (Mettler Toledo™, model FE20). The salinity was determinated weekly, using an optical refractometer (Atago™). Water sampling to monitor changes in total ammonia (UNESCO, 1983) and water nitrite (Bendschneider & Robinson, 1952) were done daily. The nitrate and phosphate concentrations were analyzed weekly and also followed the methodology proposed by Strickland & Parsons (1972). Alkalinity analyzes were also performed weekly, according to the American Public Health Association (1989) and adjustments with calcium hydroxide were done following the methodology described by Furtado *et al*. (2011).

The settleable solids (mL L^{-1}) were measured twice a week with Imhoff cones, following the methodology adapted by Avnimelech (2007). For the analysis of total suspended solids (mg L^{-1}), samples of 20 mL of each tank were collected weekly for filtering. The analysis method was adapted from Strickland & Parsons (1972), which consisted of filtering the samples with 0.45 µm mesh size membrane filters and subsequent oven drying. Turbidity (NTU) was also analyzed weekly with a digital turbidity meter (Hach™, Model 2100P).

Shrimp growth

An initial biometry (n = 100) was performed to estimate the mean weight of the shrimp to be stocked. Throughout the experiment, weekly biometrics (n = 20) were performed to evaluate weight gain (g) and also to adjust the amount of feed provided to the shrimps. Survival was evaluated at the end of the two rearing phases by counting the animals of each experimental unit. The apparent feed conversion rate was calculated by the ratio between the total feed supplied to the shrimp and the difference between the final and initial biomass in each treatment. The final biomass (g) was calculated multiplying the number of survivors shrimp in each tank by its respective final weight in grow-out.

Statistical analysis

Temperature, dissolved oxygen, pH, salinity, alkalinity and performance of shrimp data were analyzed by one-way ANOVA ($P < 0.05$). The results of ammonia, nitrite, nitrate, turbidity, settleable solids and total suspended solids were analyzed by two-way ANOVA ($P < 0.05$) in order to determine if there were significant differences among treatments in different experimental days. All tests were performed after confirmation of homogeneity of variances (Levene's test) and data normality (Kolmogorov-Smirnov test). The Tukey test was applied to detect significant differences among means of treatments (Sokal & Rohlf, 1969).

RESULTS

The main results of the physical and chemical water parameters during the 48 days of study are presented in Table 1. The results (global mean ± standard deviation) observed for dissolved oxygen (6.46 ± 0.43), temperature (26.26 ± 1.85), pH (8.04 ± 0.11), salinity (29.76 ± 2.85) and alkalinity (147.87 ± 25.89) did not differ significantly among treatments ($P > 0.05$). The results of turbidity were higher in treatments where artificial substrates were not used; similarly the settleable solids in these treatments were higher ($P < 0.05$). The total suspended solids were lower in treatments with substrates, when compared to the treatments with addition of $NaNO_2$ ($P < 0.05$).

Table 1. Means ± standard deviation of turbidity, settleable solids (SS), total suspended solids (TSS), total ammonia nitrogen (TAN), nitrite-nitrogen ($N\text{-}NO_2$), and nitrate-nitrogen ($N\text{-}NO_3$) over the 48 experimental days. Different letters in the same line correspond to significant differences among treatments by Tukey test ($P < 0.05$).

Treatment	$NaNO_2$ (-20 days)	$NaNO_2$ + BF (-20 days)	$NaNO_2$ (Stocking)	$NaNO_2$ + BF (Stocking)	Control
Turbidity (NTU)	270.84 (±113.62)^b	112.11 (± 63.72)^a	228 (±121.02)^b	83.22 (±57.70)^a	211.61 (±117.73)^b
SS (mL L^{-1})	46.12 (±16.34)^b	12.78 (±6.62)^a	35.78 (±15.16)^b	8.61 (±5.07)^a	33.83 (±12.43)^b
TSS (mg L^{-1})	1166.06 (±420.16)^c	805.11 (±482.49)^b	818.83 (±541.05)^b	646.44 (±507.30)^a	976.22 (±487.77)^{bc}
TAN (mg L^{-1})	1.16 (±2.13)^b	0.99 (±1.75)^b	0.69 (±1.19)^a	1.10 (±1.60)^b	0.67 (±1.29)^a
$N\text{-}NO_2$ (mg L^{-1})	5.68 (±8.62)^b	7.04 (±8.64)^c	5.72 (±8.04)^b	8.07 (±9.08)^c	3.65 (±6.40)^a
$N\text{-}NO_3$ (mg L^{-1})	2.95 (±7.08)^b	1.42 (±3.84)^a	2.71 (±6.54)^b	1.69 (±3.37)^a	1.64 (±3.62)^a

The mean concentrations of ammonia (N-AT) were significantly lower in treatment with addition of $NaNO_2$ at stocking day and without artificial substrate and in control treatments ($P < 0.05$). Nitrite concentrations ($N\text{-}NO_2$) were significantly higher ($P < 0.05$) in treatments with use of artificial substrates, and lower in the control. Nitrate ($N\text{-}NO_3$) concentrations were lower in these same treatments ($P < 0.05$). Additionally, some differences can be observed between the treatments in which substrates were used. These treatments presented compartmentalization with respect to the nitrogen cycle. In the treatments with biofilm, the nitrite peak occurred from the 42nd experimental day and from this moment the nitrite started decreasing followed by an increase in nitrate. Meanwhile, in the treatments in which only sodium nitrite was added, the concentrations of nitrite and nitrate increase almost simultaneously. Figures 1, 2 and 3 represent the changes throughout time for nitrogen compounds.

The performance of the shrimp is presented in Table 2. At the end of the nursery phase, the mean weight of shrimp did not show significant differences among treatments ($P > 0.05$). The survivals were significantly lower in the treatment where $NaNO_2$ was added 20 days before stocking and without using biofilm, compared to the other treatments ($P < 0.05$). At the end of the grow-out period, the mean weight was significantly higher ($P < 0.05$) in treatments with addition of sodium nitrite in the stocking, when compared to treatments with previous addition of sodium nitrite. The control treatment was equal to the other treatments, representing an intermediate value ($P > 0.05$). The survival was lower in the control and did not show significant differences among the other treatments ($P > 0.05$). The FCR values (means between nursery and grow-out phases) were significant different ($P < 0.05$) only between treatments with previous addition of $NaNO_2$ without biofilm and addition of $NaNO_2$ at stocking with biofilm, presenting the higher and the lower values, respectively. The other treatments presented intermediate values and did not differ statistically among them ($P > 0.05$). The production obtained (g) at the end of the study was significantly higher in treatments with addition of sodium nitrite at stocking day ($P < 0.05$) and did not present significant differences among the other treatments ($P > 0.05$).

DISCUSSION

The mean temperature, dissolved oxygen concentrations and pH were maintained within the optimum range for growth and survival of *L. vannamei* (Ponce-Palafox *et al*., 1997; Van Wyk & Scarpa, 1999). The mean values obtained for ammonia, nitrite and nitrate remained within the range considered as safe for the performance and survival of the shrimp (Lin & Chen, 2001, 2003; Furtado *et al*., 2015).

Some studies report the improving in water quality when artificial substrates are used, mainly in the removal of ammonia in intensive shrimp cultures (Huang *et al*., 2013; Zhang *et al*., 2016). In contrast, in the present study, it can be observed that the artificial substrates could increase the concentrations of this compound in the rearing environment. The same was related in the study of Ferreira *et al.* (2016), which observed higher ammonia concentrations in treatments with use of artificial substrates. In addition, it can be observed that the previous addition of sodium nitrite

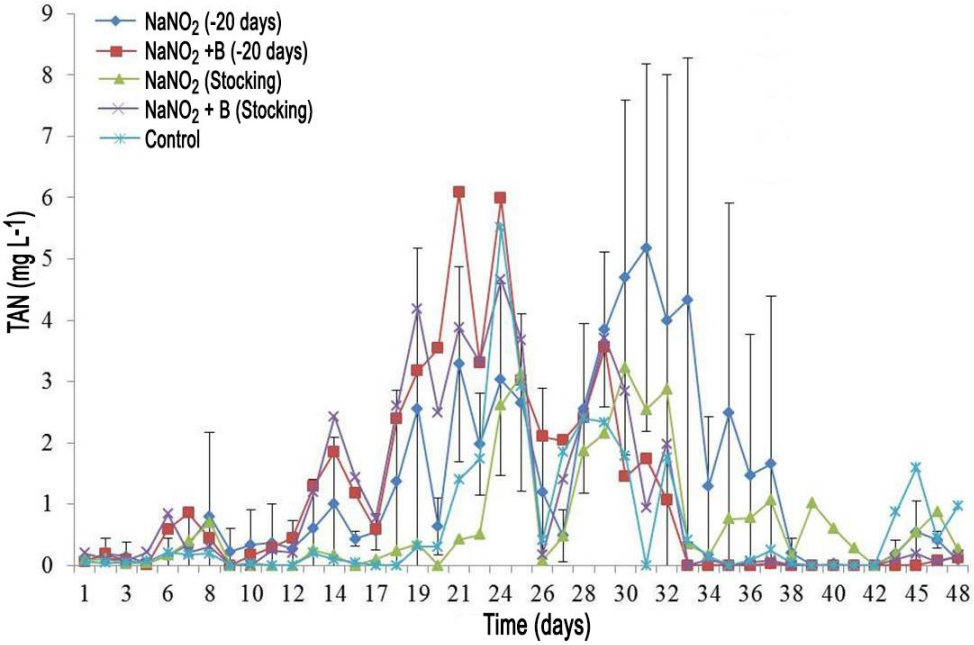

Figure 1. Mean variations of total ammonia nitrogen (TAN) during the study in different treatments.

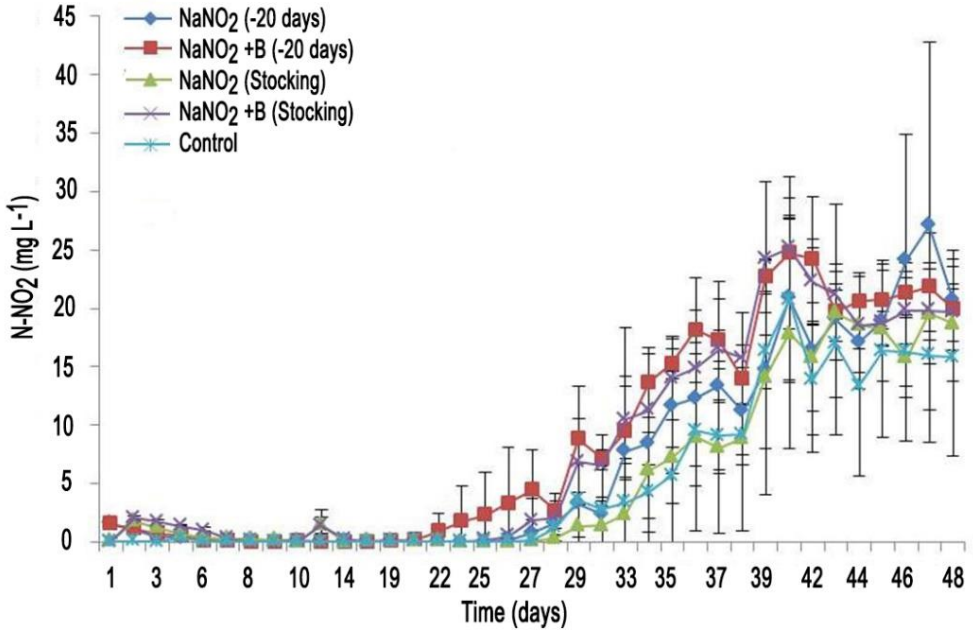

Figure 2. Mean nitrite concentrations during the study in different treatments.

did not contribute to the reduction of this compound in biofloc culture.

Studies dealing with artificial substrates demonstrate higher nitrite peaks when artificial substrates are used in shrimp culture (Bratvold & Browdy, 2001). This authors relates this phenomenon with higher nitrification rates coupled with an unbalance in development of nitrifying community, when the rates of ammonia oxidation exceeds the nitrite oxidation, leading to generation of toxic nitrite concentrations in cultures. Although toxic levels of nitrite were not reached in this study, a similar phenomenon seems to be occurred, where significantly higher concentrations of nitrite were observed in treatments using artificial substrates. The mean concentrations of this compound were lower in the control treatment, indicating that the addition of $NaNO_2$ also did not influence in reducing the nitrite peaks in the system. These results are contrary

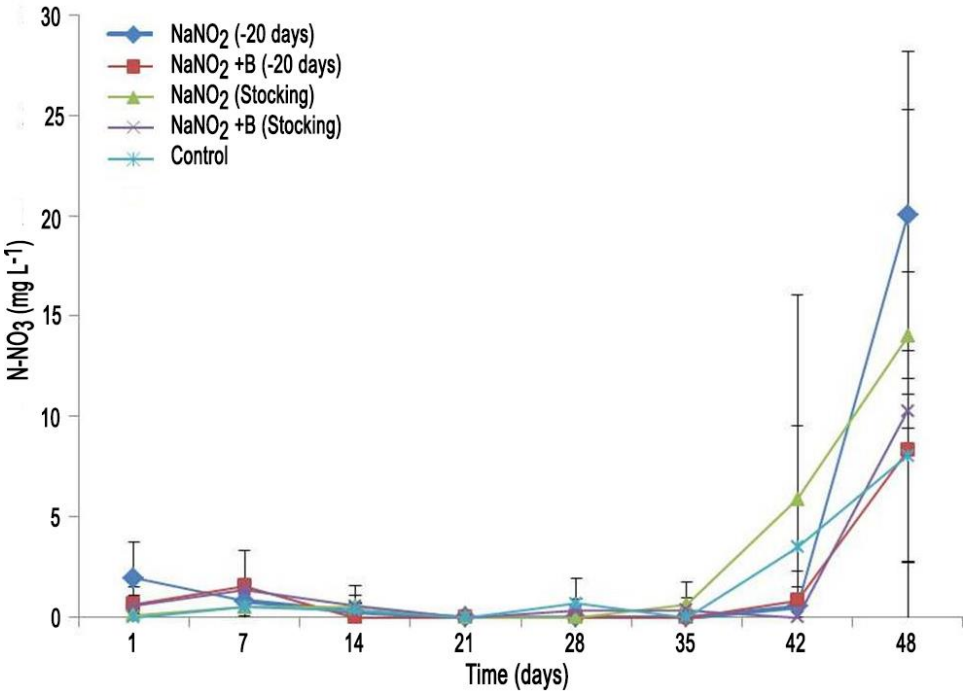

Figure 3. Mean nitrate concentrations during the study in different treatments.

Table 2. Growth parameters (mean ± SD) of shrimp observed in the two phases of the study (nursery and grow-out). Different letters in the same line correspond to significant differences among treatments by Tukey test ($P < 0.05$). W_i: initial weight (g); W_f: final weight (g); $S_{(\%)}$: survival; FCR: mean feed conversion rate of nursery + grow-out phases.

Treatment	$NaNO_2$ (-20 days)	$NaNO_2$ + BF (-20 days)	$NaNO_2$ (Stocking)	$NaNO_2$ + BF (Stocking)	Control
W_i(g) nursery	0.21 (±0.08)	0.21 (±0.08)	0.21 (±0.08)	0.21 (±0.08)	0.21 (±0.08)
W_f(g) nursery	0.82 (±0.40)	0.95 (±0.40)	1.13 (±0.45)	1.11 (±0.49)	1.06 (±0.35)
$S_{(\%)}$ nursery	79.67 (±19.34)^a	94.83 (±5.84)^b	87.83 (±1.62)^b	92.83 (±2.47)^b	91.33 (±3.52)^b
W_f(g) grow-out	1.95 (±0.96)^a	1.67 (±0.73)^a	2.55 (±0.85)^b	2.77 (±0.90)^b	2.37 (±0.92)^{ab}
$S_{(\%)}$ grow-out	97.22 (±3.43)^b	93.33 (±10.13)^b	100^b	97.78 (±3.85)^b	77.78 (±16.70)^a
FCR	1.24 (±0.49)^b	1.07 (±0.21)^{ab}	0.91 (±0.13)^{ab}	0.84 (±0.12)^a	0.99 (±0.15)^{ab}
Final biomass (g)	135.42 (±43.99)^a	112.49 (±24.77)^a	183.60 (±35.51)^b	195.18 (±37.71)^b	132.64 (±34.15)^a

to the obtained by Otoshi *et al*. (2011), who observed lower fluctuations in the nitrite levels throughout the rearing of *L. vannamei* in bioflocs systems with addition of $NaNO_2$ at the same concentrations that were added in this study.

The higher nitrate levels were detected in treatments with only addition of $NaNO_2$, although it was not observed a significant reduction of this compound compared to the control, could be evidence that this management practice may accelerate the growth of nitrite oxidizing bacteria. This result is in accordance with the observed by Otoshi *et al.* (2011), which evidenced that growth of nitrite-oxidizing bacteria was increased by the addition of sodium nitrite in shrimp biofloc culture. However, longer and more detailed studies are needed to identify these bacteria and the factors which influence its growth rates. Studies with use of artificial substrates are contradictory in regard to the metabolism of nitrogen compounds. In recent studies, Huang *et al.* (2013), Ferreira *et al.* (2015) and Zhang *et al.* (2016) observed an apparently more efficient process in NO_2 oxidation using biofilm. Nevertheless, Audelo-Naranjo *et al.* (2011) did not observed significant differences in nitrogen compounds concentrations with and without biofilm in rearing system, demonstrating that the effects of biofilm in water quality could be variable according to each culture system.

The reduced values in turbidity and settleable solids in treatments with use of artificial substrates are in

accordance with the studies of Ferreira *et al.* (2016) and Zhang *et al.* (2016). These authors reported a few reasons why this can occur in tanks as lower turbulence in water, resulting in increased floc size, and, absorption or adhesion of the suspended solids by the artificial substrates cloths. The formation of bioflocs occurred more rapidly in the treatments with previous addition of $NaNO_2$, showing that possibly this compound is a stimulator of the microbial community and, therefore, of the development of the bioflocs. Studies conducted by Ray *et al*. (2010, 2011) and Gaona *et al*. (2012) demonstrate that the maintenance of low concentrations of total suspended solids can contribute to the development of nitrifying bacteria, likely as a result of competition for substrate with heterotrophic bacteria. Furthermore, these same authors highlight that the management of the solids is also fundamental for the growth of shrimp. The results of total suspended solids obtained in this work are above those recommended by these authors in order to obtain the best results of zootechnical performance in the biofloc system.

In nursery phase, the lower survival observed in treatment with previous addition of sodium nitrite and without artificial substrates could be a result of the elevated concentrations of total suspended solids in water that could cause the clogging of gills, which may retard shrimp growth and affect survival (Ray *et al.*, 2010). Many studies highlight the importance of the natural productivity provided by the biofilm as a supplementary food item to shrimp, enhancing the body weight, final biomass and FCR obtained (Audelo-Naranjo *et al*., 2011; Huang *et al.*, 2013). In the present study, the use of artificial substrates did not enhance the shrimp growth in neither of the two phases, however could be contributed to high survival in treatment with previous addition of $NaNO_2$ and biofilm, reducing the negative effect of the excess of total suspended solids. A similar positive effect occurred in the study performed by Viau *et al.* (2013), which observed no significant differences in mean body weight of pink shrimp (*Farfantepenaeus brasiliensis)*, but an increase in survival when biofilm was provided to the culture tanks.

With respect to the addition of the nitrogen compounds to the culture, there are not many studies reporting those effects on growth performance of the reared animals. A study conducted by Sesuk *et al*. (2009), with previous acclimation (78 days) of a type of surface for bacterial adhesion and addition of ammonium chloride (NH_4Cl) proved to be efficient in improving growth results for tilapia in the biofloc technology system. The authors relate that acclimated biofilters with this compound are effective in maintaining acceptable ammonium and nitrite concentrations in water and thus, higher tilapia growth was obtained. Otoshi *et al.* (2011) reported better results in water quality using sodium nitrite, but did not presented results of shrimp growth in order to compare with the results obtained in this study. The treatments with addition of $NaNO_2$ at the stocking day presented globally better growth results, probably due to the slower growth of the bacterial biomass. The higher final biomasses obtained for these two treatments comparing with others could corroborate this observation.

Analyzing the results obtained for water quality and growth performance of shrimp, the addition of $NaNO_2$ at the stocking day could enhance the growth of nitrite oxidizing bacteria besides improving the growth of shrimp. The use of artificial substrates coupled with the addition of $NaNO_2$, may contribute in reduce the turbidity and settleable solids in rearing system, reducing the risk of losses in growth and survival caused by the excess of suspended particles. More studies are necessary in order to determine the secure dosages of this compound and the time for maturation of microbial community, in order to develop a protocol for the application of $NaNO_2$ in shrimp biofloc culture without losses in production.

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