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Strategies for control of nitrogenated compound concentrations in the *Penaeus paulensis* BFT culture system

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ABSTRACT: The present study is of practical significance as it aimed to analyze nitrite reduction through two management strategies, potentially leading to a proposal for controlling this compound and improving water quality in the BFT system. The study focused on achieving an optimal organic carbon-to-alkalinity ratio and an optimal feed rate in the rearing of *P. paulensis*. Two experiments were conducted. While the study did not conclusively develop a tool for nitrite reduction by oxidizing nitrite bacteria, it did find that higher alkalinities promote the action of ammonia-oxidizing bacteria, creating a more conducive environment for biofloc development and improved water quality control. In a study aimed at determining the optimal feed rate, it was observed that higher feed reductions (30%) enhanced the activity of nitrite-oxidizing bacteria, thereby accelerating the nitrification process and potentially revolutionizing shrimp farming practices.
Keywords: aquaculture; shrimp farming; water quality; nitrification; nitrite.

Estratégias para controle das concentrações de compostos nitrogenados no cultivo de *Penaeus paulensis* em sistema de bioflocos

RESUMO: O presente estudo tem significativa relevância prática, pois teve como objetivo analisar a redução de nitrito por meio de duas estratégias de manejo, potencialmente levando à proposição de um método para o controle desse composto e melhoria da qualidade da água no sistema BFT. O estudo focou na obtenção de uma relação ideal entre carbono orgânico e alcalinidade, bem como em uma melhor taxa de alimentação no cultivo de *P. paulensis*. Dois experimentos foram conduzidos. Embora o estudo não tenha alcançado conclusivamente uma ferramenta para a redução de nitrito por bactérias oxidantes de nitrito, observou-se que maiores níveis de alcalinidade favorecem a ação de bactérias oxidantes de amônia, criando um ambiente mais propício para o desenvolvimento do biofloco e o controle da qualidade da água. No experimento que visava determinar a melhor taxa de alimentação, verificou-se que maiores reduções na alimentação (30%) potencializam a ação das bactérias oxidantes de nitrito, acelerando o processo de nitrificação e possivelmente revolucionando as práticas de cultivo de camarão.
Palavras-chave: aquicultura; carcinicultura; qualidade de água; nitrificação; nitrito.

1. INTRODUCTION

The species *Penaeus paulensis*, popularly known as pink shrimp, has a restricted distribution from Ilhéus, Bahia, Brazil (14º50'S) to the region of Mar Del Plata, Buenos Aires, Argentina (38º30'S), and is an important fishing resource in these regions (IWAI, 1973). However, capture pressure on fish stocks has reduced shrimp production since the 2000s (D'INCAO, 2002). According to Machado; Mendonça (2007), *P. paulensis* is among the main species landed on the south coast of São Paulo. In addition, fishing for a particular species may result in capturing non-targeted species, such as *P. paulensis* (SEVERINO-RODRIGUES et al., 2002).

An alternative to producing marine shrimp for restocking natural environments and commercial activity is shrimp farming. Using the native species *P. paulensis* as live bait creates opportunities for small producers, offering a high-value product since the shrimp are commercialized per unit at attractive prices (HENRIQUES et al., 2014).

Some studies have demonstrated the possibility of cultivating *P. paulensis* in captivity under different conditions of temperature, salinity, concentrations of nitrogen compounds and stocking density, different levels of crude protein, cage culture, and recirculating system (BALLESTER et al., 2010; HENRIQUES et al., 2014; WASIELESKY et al., 2016). However, to optimize shrimp production, the conditions of the farming environment must meet the requirements for water quality and effluent reduction. In this regard, the use of a Biofloc Technology System (BFT) in Brazil has yielded a technological package with excellent results over time (FURTADO et al., 2014; GAONA et al., 2015; XAVIER et al., 2022; RAMIRO et al., 2025). This highlights the importance of conducting new studies on technologies that enable the exploration of the productive potential of *P. paulensis*.

The Biofloc Technology System (BFT) allows the intensification of the culture by increasing stocking density. Still, it also results in the higher excretion of nitrogenous compounds, such as ammonia in the early stages of cultivation and nitrite from ammonia oxidation, which directly influences water quality due to the excess organic load generated by animal excretion in cultivation (AVNIMELECH, 2014). This technology maintains the water quality within the cultivation unit, thereby minimizing water exchange in the system and providing protein-rich bioflocs that can serve as supplemental food for cultivated animals (ROBLES-PORCHAS et al., 2020).

Feeding marine shrimp in a BFT system increases the organic carbon present in the feed (AVNIMELECH, 2014). In the interaction between organic carbon and the nitrification process, one of the main problems in BFT systems is the increase in inorganic nitrogen in the initial stages of cultivation (Avnimelech, 2014), which can result in toxicity of ammonia and nitrite (VALENCIA-CASTAÑEDA et al., 2018). For nitrifying bacteria to be effective in reducing nitrogen compounds, there is a dependence on an inorganic carbon source that comes from alkalinity, with the recommendation being between 100-150 mg $CaCO_3$ L^{-1} (EBELING et al., 2006).

Total ammoniacal nitrogen (TAN) comprises the sum of ionized ammonia ($NH4^+$) and non-ionized ammonia (NH_3), the latter being the most toxic form due to its ability to diffuse through cell membranes (AVNIMELECH, 2014). Ammonia, depending on concentrations, can reduce both the growth and survival of cultivated shrimp (WASIELESKY et al., 2016). Based on the safety level assessment of ammonia's chronic effect on *P. paulensis*, Wasielesky et al. (2016) recommended a value less than 0.91 mg L^{-1} in a salinity of 15‰.

Nitrite (NO_2^-) in high concentrations can reduce growth and be lethal to shrimp, produced due to the oxidation of the copper atom in the hemocyanin molecule, which turns it into meta-hemocyanin, unable to transport oxygen to the tissues (GROSS et al., 2004). Under conditions of chronic exposure in *P. paulensis*, Wasielesky et al. (2016) estimated a value of 2.55 mg L^{-1} of NO_2^- in a salinity of 15‰ as a safety level.

In the initial rearing phase of a BFT system, ammonia concentrations can be controlled by adding an organic carbon source in a 6:1 carbon-to-nitrogen ratio (EBELING et al., 2006). However, there is a need to develop techniques to control nitrite concentrations in the culture medium.

Serra et al. (2015) used water exchange to reduce nitrite levels in *Penaeus vannamei* farming in a BFT system. In this context, in contrast to the use of water exchange, where water and bacteria are discharged in the process of establishment, beginning the cultivation in reduced water volume may be feasible, since, with the emergence of nitrite, the dilution of this compound is carried out, completing the volume available in the cultivation unit.

The appropriate relationship between organic and inorganic carbon can facilitate the establishment of nitrifying bacteria, allowing the nitrification process to occur without peaks of ammonia and nitrite concentrations that are lethal to shrimp (LARA et al., 2021; RAMIRO et al., 2024). In addition, an adequate feed rate can reduce ammonia from animal excretion and feed deposition, leading to less oxidation of this compound to nitrite (BRANDÃO et al., 2024; CHEN et al., 2024).

In this context, this paper aimed to analyze the reduction of nitrite through two management strategies that may result in a proposal for controlling this compound and improving water quality in the BFT system: an adequate organic carbon-to-alkalinity ratio and a more suitable feed rate for the rearing of *P. paulensis*.

2. MATERIAL AND METHODS

The experiments were conducted at the Laboratory of Biology and Culture of Crustaceans (LABCRUST) at the School of Agricultural Sciences (FCAVR) of São Paulo State University (UNESP), located in Registro, SP, Brazil. Rectangular tanks of 20 L (useful volume of 18 L) were used. The stocking density of the animals was 24 shrimp m^{-2} (166 shrimp m^{-3}). The aeration of the water was supplied by a 3/4hp blower coupled to a system with porous stones positioned in the center of the tank for oxygen diffusion, but also maintaining the movement of the water in the system, allowing the particles present in the tanks to be kept in suspension and distributed throughout the water column.

The shrimp were fed a commercial diet containing 38% crude protein twice daily. Thermostatic heaters maintained the water temperature at approximately 28 °C.

2.1. Biological material

For the experiments, *Penaeus paulensis* was acquired through artisanal fishing in the estuarine-lagoon region of Cananéia-Iguape-Ilha Comprida, a coastal region in the state of São Paulo, and was transported to the FCAVR.

2.2. Experimental design

2.2.1. Experiment 1. Adjusting C:N ratio and alkalinity

The effect of two C:N ratios on ammonia reduction, combined with two alkalinity concentrations, on the nitrification process, was evaluated, particularly the establishment of nitrifying bacteria and the subsequent behavior of ammonia and nitrite concentrations over a six-week period.

Four treatments were designed with three replications (18 L of useful volume) each, being: (1) Treatment with 6:1 C:N ratio and 100 mg $CaCO_3$ L^{-1} of alkalinity (6/100); (2) Treatment with a 3:1 C:N ratio and 100 mg $CaCO_3$ L^{-1} of alkalinity (3/100); (3) Treatment with a 6:1 C:N ratio and 200 mg $CaCO_3$ L^{-1} of alkalinity (6/200); (4) Treatment with a 3:1 C:N ratio and 200 mg $CaCO_3$ L^{-1} of alkalinity (3/200). Commercial sodium bicarbonate (used as a swimming pool alkalizer) was employed as the alkalizer.

The alkalinity adjustment was made as suggested by Furtado et al. (2011). In this work, new biometry was performed if any mortality was noticed during the biometric process, and this mortality was replaced immediately afterward. In this way, biomass was kept similar throughout the experiment to maintain identical ammonia concentrations in all treatments (OSTRENSKY; WASIELESKY, 1995).

2.2.2. Experiment 2. Adjustment in feed rate:

The effect of two feeding rates (percentage of estimated biomass in each biometry, calculated using the methodology reviewed by Jory et al. (2001)) was used to evaluate the establishment of nitrifying bacteria and the consequent behavior of ammonia and nitrite concentrations over eight weeks. One control and two treatments were designed with three repetitions each: (1) Control with 100% of the feeding rate adjusted throughout the experiment (C); (2) Treatment with a 10% reduction in the feed rate when nitrite concentrations reached 5 mg L^{-1} (10%); (3) Treatment with a 30% reduction in the feed rate when nitrite concentrations reached 5 mg L^{-1} (30%). The daily amount of feed provided was divided into two equal portions in all treatments.

2.3. Physical and chemical parameters

Parameters such as temperature, pH, dissolved Oxygen, and salinity were monitored twice daily using a multiparameter device (ASKO®, AK88 model). Salinity was maintained at 20‰ and adjusted as necessary due to water loss by evaporation. The concentrations of total ammonia (NH_4^+ + NH_3) were analyzed using the methodology described by UNESCO (1983), and nitrite (NO_2^-) was analyzed as described by Bendschneider; Robinson (1952). Initially, total ammonia was measured daily during the increasing period and weekly when levels decreased to less than 1 mg L^{-1}. Nitrite was measured initially once a week until it reached 1 mg L^{-1}. After that, daily measurements were performed until the compound was reduced. Alkalinity was measured weekly following the methodology described in APHA (2005).

2.4. Organic carbon source and Alkalinity

The organic carbon source used was liquid sugar cane molasses. When the concentration of total ammoniacal nitrogen (TAN) reached 1 mg L^{-1} in each experimental unit, fertilization was performed using a C:N ratio of 6:1 (Ebeling et al., 2006) and 3:1 (for specified treatments in experiment 2). The amount of carbon source needed to reduce total ammoniacal nitrogen was calculated as follows:

Correction (g) = [TAN] × C:N × EF × Volume of the tank (L) ÷ 1000

where: [TAN] = concentration of total ammoniacal nitrogen (mg L^{-1}); C:N = 6 or 3 grams of organic carbon for each 1 g of nitrogen; EF = equivalence factor (100% of desired carbon content ÷ percentage of carbon present in the source to be used). The organic carbon content in molasses was the same as that reported by Serra et al. (2015).

According to Furtado et al. (2014), the alkalinity of each treatment was adjusted using Sodium bicarbonate ($NaHCO_3$).

2.5. Water exchange

To preserve the animals, 50% of the total volume (9 liters) was exchanged in all treatments whenever nitrite concentrations in the replicates reached levels between 10 and 15 mg L^{-1}.

2.6. Zootechnical performance

Every fifteen days, biometrics were performed to analyze the growth of the shrimp by measuring individual wet weight using a digital scale, allowing for the estimation of the average weight and biomass of the shrimp and the adjustment of the feeding rate (based on the estimated biomass percentage), as suggested by Jory et al. (2001). Other zootechnical parameters were checked at the end of the first and third experiments according to the following calculations:

Feed conversion rate: FCR = feed given / biomass gained;

Survival: S% = (total count of shrimps harvested / number of stocked shrimps) x 100;

Weekly weight gain: WWG = [(Final weight - initial weight) / number of weeks)];

2.7. Biomass and stocking density of experiment (2)

Every fifteen days, biometrics were performed to analyze the growth of the shrimp by measuring individual wet weight using a digital scale, allowing for the estimation of the average weight and biomass of the shrimp and the adjustment of the feeding rate (based on the estimated biomass percentage), as suggested by Jory et al. (2001). Animals were stocked in a density of 24 shrimps m^{-2} (166 shrimp m^{-3}). When mortality was noticed in the replicas, new biometry was performed, and this mortality was replaced shortly after. In this way, biomass was kept similar throughout the experiment among all treatments to maintain similar ammonia concentrations (OSTRENSKY; WASIELESKY, 1995).

2.8. Statistical analysis

For the statistical analysis, conditions of homoscedasticity of the variances and normality of the data distribution were verified. For this, Levene and Kolmogorov-Smirnov tests were applied, respectively. The survival data were transformed (arcsine x0.5) before analysis (Zar, 2010). The data were analyzed using a one-way analysis of variance (ANOVA). Then, the Tukey test was applied to detect any possible differences ($p < 0.05$) between treatments.

3. RESULTS

3.1. Adjusting C:N ratio and alkalinity

Table 1 shows the means and standard derivations of the physical and chemical parameters. No significant differences ($p > 0.05$) were found between the means of the dissolved oxygen, temperature, and pH parameters.

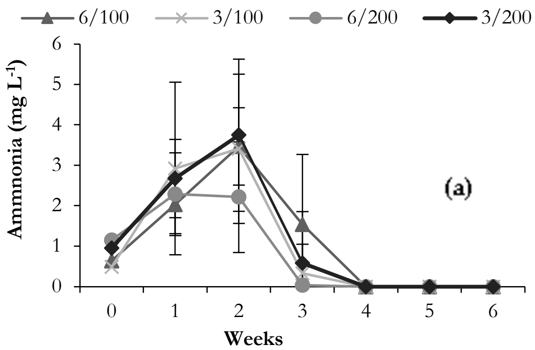
Alkalinity was monitored and adjusted throughout the experiment according to the specifications for each treatment. There were no significant differences ($p>0.05$) between treatments with 100 mg $CaCO_3$ L^{-1} of alkalinity (6/100 and 3/100), and the same happened between treatments with 200 mg $CaCO_3$ L^{-1} of alkalinity (6/200 and 3/200).

There were no significant differences ($p>0.05$) between the means of ammonia concentrations. However, it is possible to observe an early reduction of the values in the treatment with a C:N ratio of 6:1 and 200 mg $CaCO_3$ L^{-1} of alkalinity (6/200), with a peak occurring in the first week. On the other hand, in the treatment with a C:N ratio of 6:1 and 100 mg $CaCO_3$ L^{-1} of alkalinity (6/100), a delay in the reduction of total ammonia is observed, with a peak occurring in the second week, unlike in the other treatments. The variation in ammonia concentrations throughout the experiment is shown in Figure 1a.

Nitrite was significantly lower ($p <0.05$) in treatment with C:N of 6:1 ratio and 100 mg $CaCO_3$ L^{-1} of alkalinity (6/100) compared to treatment with C:N of 6:1 ratio and 200 mg $CaCO_3$ L^{-1} of alkalinity (6/200) in the third week. There was also a significant difference ($p <0.05$) in treatment 6/100 compared to the other treatments in the sixth week. It is possible to observe an early appearance of nitrite in treatment 6/200, with a peak occurring in the third week, whereas a delay in the appearance of this compound is noted in treatment 6/100, with a peak in the fourth week. The variation in nitrite concentrations throughout the experiment is shown in Figure 1b.

Table 1. Means and standard deviations of the physical and chemical parameters were monitored during the experiment of adjustments in the C:N ratio and alkalinity for the treatments rearing *Penaeus paulensis* in a BFT system.
Tabela 1. Médias e desvio padrão dos parâmetros físico-químicos da água, monitorados durante o experimento com ajuste da relação C:N no cultivo de *Penaeus paulensis* em sistema BFT.

Parameters	6/100	3/100	6/200	3/200
Temperature (°C)	28.51 ± 0.31	28.15 ± 0.48	28.8 ± 0.48	28.16 ± 0.43
Dissolved Oxygen (mg L^{-1})	7.05 ± 0.86	7.01 ± 1.00	7.27 ± 0.86	7.17 ± 0.99
pH	7.98 ± 0.22	7.92 ± 0.22	8.00 ± 0.25	7.90 ± 0.27
Ammonia (mg L^{-1})	1.10 ± 1.34	1.02 ± 1.60	0.81 ± 1.12	1.14 ± 1.56
Nitrite (mg L^{-1})	6.86 ± 7.42	7.23 ± 6.57	7.98 ± 7.02	7.20 ± 6.69
Alkalinity (mg $CaCO_3$ L^{-1})	97.96 ± 12.33	97.41 ± 12.64	176.67 ± 21.18	181.48 ± 23.83



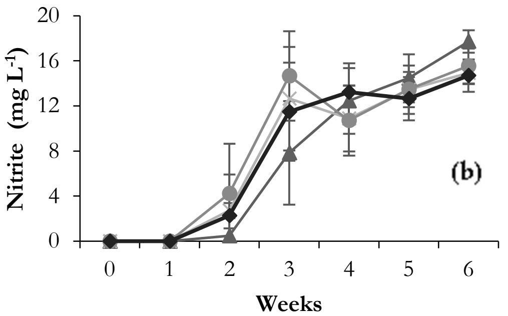


Figure 1. Variation in ammonia concentrations (a) and nitrite concentrations (b) throughout the experiment of adjustments in the C:N ratio and alkalinity for different treatments. The vertical bars indicate the standard deviation.
Figura 1. Variações nas concentrações de amônia (a) e nitrito (b) ao longo do experimento com ajuste da relação C:N para os diferentes tratamentos. As barras verticais indicam o DP.

There were no significant differences in the volumes of water used by each treatment for water exchange. 12 ± 5.20 L of water was used in treatment 6/100, 15 ± 5.20 L in treatment 3/100, 15 ± 5.20 L in treatment 6/200 and 15 ± 10.39 L in treatment 3/200.

During the experiment, there were no significant differences ($p>0.05$) between the means of the biomasses in the treatments, which were 4.44 ± 1.68 g (6/100), 3.55 ± 1.73 g (3/100), 3.14 ± 1.68 g (6/200) and 4.50 1.30 g (3/200).

3.2. Adjustments in feed rate:

The mean values of the physical and chemical parameters did not show significant differences ($p > 0.05$) between the control and treatment groups (Table 2).

There were no significant differences ($p>0.05$) between the means of total ammonia concentrations, with peaks in control and other treatments occurring similarly in the second week (Figure 2a). Values close to zero were recorded in the fourth week for control and treatment 30% and in the fifth week for treatment 10% but without significant differences ($p>0.05$) between all.

The mean nitrate concentrations in the experiment were similar to those of the control and other treatments. However, throughout the study, peaks in nitrite concentrations (Figure 2b) were observed in the third week for the C, 10%, and 30% treatments. Nitrite levels were significantly lower ($p < 0.05$) in the 30% treatment compared to C in the eighth week.

Table 2. Mean values and standard deviations of the physical and chemical parameters were monitored while adjusting feed rates for control (C) and treatments (10% and 30%) in the rearing of *Penaeus paulensis* in a BFT system.
Tabela 2. Médias e desvio padrão dos parâmetros físico-químicos da água monitorados durante o experimento com ajuste da taxa alimentar para o Controle (C) e tratamentos (10% e 30%) no cultivo de *Penaeus paulensis* em sistema BFT.

Parameters	C	10%	30%
Temperature (ºC)	28.05 ± 0.15	27.80 ± 0.09	28.09 ± 0.24
Dissolved Oxygen (mg L^{-1})	7.85 ± 0.20	7.78 ± 0.22	7.77 ± 0.18
pH	8.0 ± 0.06	8.23 ± 0.05	8.23 ± 0.04
Ammonia (mg L^{-1})	0.36 ± 0.43	0.46 ± 0.53	0.36 ± 0.45
Nitrite (mg L^{-1})	3.96 ± 2.34	3.51 ± 2.20	2.25 ± 1.82
Alkalinity (mg $CaCO_3$ L^{-1})	131.83 ± 31.04	134.17 ± 34.40	135.70 ± 38.24
Salinity	20.45 ± 0.29	20.51 ± 0.28	20.37 ± 0.33

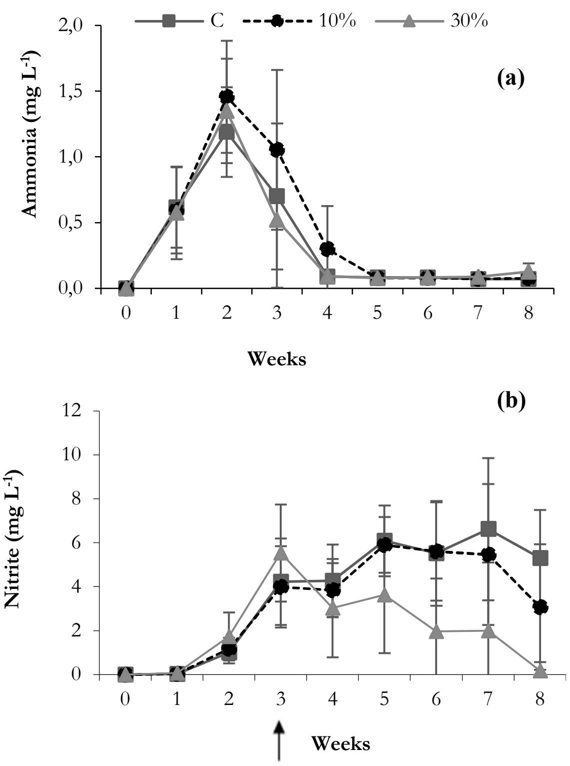


Figure 2. Variation in ammonia concentrations (a) and nitrite concentrations (b) throughout the experiment, showing adjustments in feed rate for control and treatment groups. The vertical bars indicate the standard deviation. The arrow indicates feed restrictions.
Figura 2. Variações nas concentrações de amônia (a) e nitrito (b) ao longo do experimento com ajuste da frequência alimentar para o controle e tratamentos. As barras verticais indicam o DP. As restrições alimentares são indicadas pela seta.

There were no significant differences ($p > 0.05$) between the volumes of water used by each treatment for water exchange. The experiments were conducted for both the control and the treatments in the third and fifth weeks of the experiment. Mean volumes of 21.6 ± 6.49 L of water were used in the control, 22.8 ± 4.52 L in the 10% treatment, and 18.6 ± 4.52 L in the 30% treatment.

For the shrimp's zootechnical performance parameters, there was no significant difference ($p > 0.05$) in feed conversion ratio, final weight, weekly weight gain, and survival between the control and treatment groups (Table 3).

Table 3. Means and standard deviations of the initial and final weights feed conversion ratio (FCR), weekly weight gain, and survival per treatment were used in the experiment to adjust the feed rate.
Tabela 3. Médias e desvios-padrão do peso inicial e final, taxa de conversão alimentar (FCR), ganho de peso semanal e sobrevivência por tratamento utilizados no experimento de ajustes na taxa de alimentação.

Zootechnical	C	10%	30%
Initial weight (g)	1.9 ± 0.4	2.4 ± 0.5	2.3 ± 0.5
Final weight (g)	2.7 ± 0.2	3.4 ± 0.7	3.1 ± 0.7
Weekly Weight Gain (g)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Survival (%)	55.6 ± 38.5	44.4 ± 19.3	55.6 ± 19.3
FCR	2.8 ± 1.1	2.01 ± 0.7	2.32 ± 1.09

4. DISCUSSION

Temperature, salinity, dissolved oxygen, pH, and alkalinity were maintained within the ideal conditions for the growth of *P. paulensis* and the bacterial communities involved in the nitrification process in all experiments (EBELING et al., 2006; BALLESTER et al., 2010; AVNIMELECH, 2014).

During the adjustment of the C:N ratio and alkalinity, reductions in the pH of the water were observed as alkalinity was consumed, which was corrected according to the experimental design. Ebeling et al. (2006) reported this relationship of alkalinity and pH. This decrease in pH levels is related to the nitrification process, caused by the consumption of alkalinity ($CaCO_3$) and the release of CO_2 into the environment. In aquatic environments, CO_2 reacts with water to form carbonic acid, which dissociates, releasing hydrogen protons (H^+). As verified by Hargreaves (1998), two hydrogen protons (H^+) are released for each mole of oxidized ammonia, explaining the drop in pH observed in the treatments. The accumulation of CO_2 in biofloc systems is maximized by the increase in living biomass within the cultivation tank, primarily through the non-exchange of water. However, pH and alkalinity were maintained at ranges recommended for penaeid shrimps (EBELING et al., 2006; AVNIMELECH, 2014; XU et al., 2024).

The ammonia present in the system is a result of the decomposition of uneaten feed and metabolites (ROLES-PORCHAS et al., 2020). The toxicity of this compound can occur at different concentrations for different species of marine shrimp (WASIELESKY et al., 2016). In all experiments, the concentrations of this compound were below the values considered toxic for the species (WASIELESKY et al., 2016). Ammonia-oxidizing bacteria were established after the second week of cultivation in the experiment, which adjusted the C:N ratio and alkalinity, thereby reducing the concentration of this compound and generating nitrite (AVNIMELECH, 2014; CHEN et al., 2024). In the experiment involving feed rate adjustment, the ammonia-oxidizing bacteria established themselves in the second week of cultivation, coinciding with higher alkalinity values, which reduced the concentration of this compound and consequently generated nitrite. This was due to the greater availability of inorganic carbon (alkalinity) to ammonia-oxidizing bacteria (EBELING et al., 2006; AVNIMELECH, 2014; PIMENTEL et al., 2024).

Nitrite is the product generated through the oxidation of ammonia, a result of the nitrification process by nitrifying autotrophic bacteria (FERREIRA et al., 2020; MELO FILHO et al., 2020; CHEN et al., 2024). In the experiment of feed rate adjustments, the concentrations remained below the values considered toxic for the species, as described by Wasielesky et al. (2016).

In the third week of the experiment in which the C:N ratio and alkalinity were adjusted, the higher alkalinity maintained in treatment the 6/200 may have favored the action of ammonia-oxidizing bacteria when compared to treatment 6/100, owning the appearance of nitrite initially in this treatment and need of consumption of alkalinity by these bacteria. The lower alkalinity in the 6/100 treatment compared to treatments 6/200 and 3/200 may have hindered the action of nitrite-oxidizing bacteria, resulting in a higher nitrite concentration in this treatment and a negative effect on the final stages of the nitrification process. The 6/100 and

3/100 treatments were maintained with similar alkalinities. However, the higher proportion of carbon in treatment 6/100 may have negatively impacted the action of nitrite-oxidizing bacteria, potentially favoring heterotrophic bacteria. The relationship between organic and inorganic carbon can interfere with the action of nitrifying bacteria, considering that at the beginning of the cycle in a BFT system, the control of ammonia concentrations through the addition of an organic carbon source can result in the inhibition of nitrifying bacteria (Chen et al., 2025), due to the rapid growth of heterotrophic bacteria stimulated by organic substrate, which compete for dissolved oxygen, space, ammonia, and micronutrients (BRANDÃO et al., 2021; XU et al., 2024). In the present study, even with the higher amount of organic carbon in the 6:1 (C:N) ratio, the higher alkalinity favored the activity of ammonia-oxidizing bacteria.

According to Avnimelech (2014), nitrite concentrations can be reduced within 40 days of cultivation due to nitrite oxidation to nitrate by nitrite-oxidizing bacteria. Although the best result was achieved in June 200, the experiment was terminated due to the prolonged exposure of the shrimps to nitrite concentrations, which may have caused the mortality of some animals. Therefore, zootechnical performance indexes were not considered in this experiment. Additionally, water exchange was necessary to reduce nitrite concentrations in the system and mitigate the toxicity of this compound (SERRA et al., 2015).

In the experiment on adjusting the feed rate, peaks in nitrite concentrations were observed in the third week of cultivation in all treatments, a characteristic process of the BFT system (AVNIMELECH, 2014; LARA et al., 2021; RAMIRO et al., 2024; PIMENTEL et al., 2025). As a proposal to reduce nitrite levels in BFT systems, reductions in feed rates were implemented in the third week for the treatments at 10% and 30%. By the fourth week, the nitrite-oxidizing bacteria were established, resulting in a 30% reduction in the concentration of this compound over time until the end of the study. This reduction was observed to be faster than that of C, particularly in the last week of the experiment. At the end of the study survey, nitrite concentrations reached values close to zero in the treatment (30%), probably due to the lower feed rate. This reduction in feed supply is likely reflected in lower consumption and a consequent decrease in metabolism rate and ammonia excretion by shrimps, as well as less leftover feed and subsequent decomposition, which would also enter the nitrification route (AVNIMELECH, 2014). Additionally, the nitrification process is closely linked to the organic load in the water, which can lead to the inhibition of nitrifying bacteria and the rapid growth of heterotrophic bacteria (BRANDÃO et al., 2024). Reducing the organic load from the feed has probably favored nitrifying bacteria over heterotrophic bacteria.

In addition to this reduction in feed rate, the higher alkalinity levels in the seventh week contributed to a reduction in nitrite, favoring the establishment of nitrite-oxidizing bacteria (EBELING et al., 2006). Nevertheless, water exchange was necessary to decrease nitrite concentrations in the system, thus reducing the toxicity of this compound, as described in Serra et al. (2015).

According to Wasielesky et al. (2016), ammonia is a more toxic compound than nitrite; however, the ammonia levels recorded in the present study were below the safety level (3.65 mg L^{-1}). The high nitrite concentrations in an experiment with adjusted feed rates were possibly responsible for the low zootechnical indexes due to the chronic effect of the animals' exposure to these compounds, which was reflected in lower feed consumption. Consequently, there was a low weekly weight gain and a high feed conversion ratio (FCR), as shown by Vinatea et al. (2010), who observed that concentrations between 0.72 and 9.49 mg L^{-1} were sufficient to reduce the growth rate of shrimp. Additionally, Serra et al. (2015) observed a reduction in shrimp growth and survival due to high nitrite concentrations for eight weeks during the growth phase of *P. vannamei*. However, since there were no differences in zootechnical performance between the control and treatment groups, it was demonstrated that reducing feed by 30% was feasible for controlling nitrite concentrations. In an experiment where the C:N ratio and alkalinity were adjusted, it was not possible to observe the optimal nitrite reduction strategy accurately.

5. CONCLUSIONS

The proposed method of reducing the feed rate by 30% demonstrated a faster response, indicating it to be more effective in reducing nitrite in the production of *Penaeus paulensis* in a BFT system. Although differences in nitrite concentrations were observed during certain periods of the study, adjustments in the C:N ratio and alkalinity, it was not possible to conclude that a tool for nitrite reduction through the action of nitrite-oxidizing bacteria was achieved. However, it was found that higher alkalinities favor the action of ammonium-oxidizing bacteria, resulting in a more favorable environment for developing bioflocs and controlling water quality.

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Data availability: Study data can be e-mail from the corresponding author or the second author upon request. It is not available on the website as the research project is still under development.

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