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EFEITO DA SALINIZAÇÃO ARTIFICIAL NA PRODUÇÃO DE JUVENIS DE CAMARÕES *Litopenaeus vannamei* (BOONE, 1931) EM SISTEMA DE MÍNIMA TROCA DE ÁGUA.

Agatha Catharina Limeira

Dissertação apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Mestre.

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Resumo

O *Litopenaeus vannamei,* popularmente conhecido como camarão branco do pacífico lidera o ranking da espécie de crustáceos mais produzida no mundo, ao qual tem sido cultivado em sua maioria em áreas litorâneas. Entretanto, devido aos altos custos de implantação, problemas de conflitos ambientais, surtos de enfermidades, observa-se uma migração da produção de camarões marinhos para regiões interiores com águas mesohalinas e oligohalinas. As águas destas regiões são provenientes de lagos, poços, rios, açudes e aquíferos subterrâneos, apresentando grande variação em sua composição iônica (cátions e ânions). Entretanto, estes íons precisam que suas proporções sejam ajustadas para valores semelhantes aos da água do mar, para que os camarões tenham um bom desenvolvimento zootécnico durante o cultivo, promovendo um maior conforto osmótico aos animais, equilibrando os gastos energéticos. A compensação iônica realizada por meio de fertilizantes minerais na água pode ser bastante onerosa dependendo do volume aplicado, como uma alternativa para redução desses custos é a utilização de sistemas de mínima troca de água, oferecendo um reaproveitamento dos minerais utilizados, assim como uma redução no descarte de água e um menor reinvestimento na aplicação dos fertilizantes minerais. Portanto, esse estudo teve por objetivo avaliar diferentes formas de salinização artificial da água para a produção de juvenis do camarão marinho *L. vannamei* em sistema simbiótico durante 40 dias, em unidades experimentais de 60 L, com densidade de 2000 PL's m^3. Quatro tratamentos em triplicata por meio de um delineamento inteiramente casualizado foram estabelecidos: SD - água do mar diluída; LCSM - Mistura de sais de baixo custo com água doce; CS - Sal marinho comercial e SW - água do mar. Foi realizado um estudo prévio sobre a eficiência minerais quanto os íons disponibilizados na água de cultivo e posteriormente realizada a salinização artificial para obtenção da salinidade de 2,5 g/L e as proporções dos cátions e ânions. Utilizou-se um substrato artificial de conchas de *Anomalocardia brasiliana* (1% do volume total da unidade experimental) em cada unidade experimental para acelerar os processos de nitrificação. além da adição de mix comercial de bactérias à base de *Bacillus* na água e na ração. Para verificar a resistência dos juvenis de camarões foi realizado um teste de estresse de amônia ao término do cultivo e, também, foram realizadas no início e fim do experimento, análises microbiológicas para contagem total de *Vibrio spp.* e *Fungos* do intestino dos juvenis. A utilização do substrato artificial juntamente com o sistema simbiótico mostrou-se efetivo no controle dos compostos nitrogenados com média de 0,21 mg NAT/L e 0,32 mg $N\text{-}NO_2$/L. Os

valores de cálcio, magnésio e dureza total se mantiveram acima de 30, 75 e 490 mg/L, respectivamente em todos os tratamentos e a alcalinidade total manteve seus valores superiores a 120 mg $CaCO_3$/L. Com relação aos parâmetros de sobrevivência nos tratamentos com baixa salinidade, observou-se diferenças significativas entre os tratamentos, com uma maior sobrevivência no tratamento LCSM. O peso médio final observados na baixa salinidade foram superiores ao valor encontrado na água do mar, entretanto as demais variáveis de desempenho zootécnico foram similares. O uso de probiótico a base de *Bacillus* na água e ração ofertada aos camarões neste trabalho, mostrou-se eficiente na colonização da microbiota intestinal dos camarões cultivados em todos os tratamentos. Com isso, as diferentes formas de salinização artificial obtiveram semelhantes resultados de desenvolvimento zootécnico e resistência ao estresse de amônia quando comparado à água do mar.

Palavras-chave: baixa salinidade; juvenis; berçários intensivos; simbiótico, proporção iônica.

Abstract

Litopenaeus vannamei, popularly known as Pacific white shrimp, leads the ranking of the most produced crustacean species in the world, which has been cultured mostly in coastal areas. However, due to the high costs of implantation, environmental conflict problems, disease outbreaks there is a migration of production of marine shrimp to inland waters with mesohaline and oligohaline waters. The waters of these regions come from lakes, wells, rivers, dams and underground aquifers, presenting great variation in their ionic composition (cations and anions). However, these ions need their proportions to be adjusted to values similar to those of seawater, so that the shrimp have a good zootechnical development during culture, promoting greater osmotic comfort to the animals, balancing energy expenditure. The ionic compensation performed by means of mineral fertilizers in the water can be quite expensive depending on the volume applied, as an alternative to reduce these costs is the use of systems with minimal water exchange, offering a reuse of the minerals used, as well as a reduction in the disposal of water and less reinvestment in the application of mineral fertilizers. Therefore, this study aimed to evaluate different forms of artificial salinization of water for the production of juveniles of marine shrimp *L. vannamei* in a synbiotic system for 40 days, in experimental units of 60 L, with a density of 2000 PL's m^3. Four treatments in triplicate using a completely randomized design were established: SD - diluted seawater; LCSM - Low cost salt mix with freshwater; CS - Commercial sea salt and SW - sea water. A previous study was carried out on the mineral efficiency regarding the ions available in the culture water and later artificially salinized was carried out to obtain a salinity of 2.5 g/L and the proportions of cations and anions. An artificial substrate of *Anomalocardia brasiliana* shells (1% of the total volume of the experimental unit) was used in each experimental unit to accelerate the nitrification processes. in addition to the addition of a commercial mix of *Bacillus*-based bacteria in water and feed. To verify the resistance of shrimp juveniles, an ammonia stress test was carried out at the end of the culture and, also, microbiological analysis were carried out at the beginning and end of the experiment for the total count of *Vibrio spp*. and *fungi* Juvenile intestinal. The use of the artificial substrate together with the synbiotic system proved to be effective in the control of nitrogen compounds with an average of 0.21 mg NAT/L and 0.32 mg $N\text{-}NO_2$/L. The values of calcium, magnesium and total hardness remained above 30, 75 and 400 mg/L, respectively, in all treatments and the total alkalinity maintained its values above 120 mg $CaCO_3$/L. Regarding the survival parameters in the treatments with low salinity,

significant differences were observed between the treatments, with a greater survival in the LCSM treatment. The final average weight observed in the low salinity treatments were higher than the value found in the seawater treatment, however the other variables of zootechnical performance were similar. The use of *Bacillus*-based probiotic in the water and feed offered to the shrimp in this work, proved to be efficient in the colonization of the intestinal microbiota of shrimp cultured in all treatments. Thus, the different forms of artificially salinized obtained similar results in terms of zootechnical development and resistance to ammonia stress when compared to seawater.

Key words: low salinity; intensive nursery; synbiotic, ionic ratio.

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Sumário

1. Introdução

A produção mundial de crustáceos da aquicultura em 2020 foi de aproximadamente 11.237 milhões de toneladas, sendo 4.477 milhões de toneladas provenientes de cultivos em águas interiores (FAO, 2022). No grupo dos crustáceos, o camarão-cinza *Litopenaeus vannamei* (Boone, 1931) lidera o ranking, com 51,7% do total produzido pela aquicultura (5.812 mil de toneladas), sendo cultivada em aproximadamente 80% das fazendas comerciais do mundo (FAO, 2022).

No Brasil, segundo dados da Associação Brasileira de Criadores de Camarão, a produção brasileira de camarões marinhos no ano de 2019 foi de 90.000 toneladas, com projeções para atingir patamares próximos de 120.000 toneladas nos anos seguintes (ABCC, 2020). Essa produção é basicamente realizada na região Nordeste, que é responsável por 99,4% dessa produção (IBGE, 2018), pois possui condições climáticas bastante favoráveis para a prática da carcinicultura.

Toda a produção de camarões marinhos no Brasil é baseada na monocultura do *L. vannamei*, sendo sua grande parcela em áreas litorâneas, porém nos últimos anos observa-se um avanço da produção desta espécie em águas interiores. O camarão *L. vannamei* suporta uma ampla faixa de salinidade (0,5 – 60 ppt), o que contribui para sua adaptação às diversas condições de qualidade de água (Ramiro, 2017).

Entretanto, as variações de salinidade podem afetar o mecanismo de osmorregulação, assim como, alterar a estratégia de absorção de nutrientes e comprometer o crescimento dos camarões (Romano e Zeng, 2012). As consequências do cultivo de crustáceos marinhos em baixas salinidades com perfil iônico inadequado podem incluir baixa sobrevivência, crescimento reduzido e/ou altas taxas de conversão alimentar (Ye et al., 2009), que podem ser minimizadas com ajuste na suplementação mineral (Ca, Mg, Na e K), mesmo com o cultivo dessa espécie em águas com salinidades inferiores a 0,5 ppt (Moraes et al., 2020).

Para a salinização artificial da água é necessário ajustar as proporções iônicas para que fiquem semelhantes à água do mar, proporcionando um maior conforto osmótico aos animais, evitando gastos energéticos (Palheta, 2013). As principais relações de cátions (íons positivos) e ânions (íon negativos) no caso do *L. vannamei* são Na:K, Mg:Ca e Ca:K. A aplicação desses íons na água é com o uso de fertilizantes agrícolas ou por sais marinhos artificiais, podem auxiliar no aumento do crescimento e sobrevivência do camarão em água de baixa salinidade (Boyd, 2002; Oliveira, 2016; Nehru et al., 2018).

Atualmente a produção de camarões marinhos ocorre majoritariamente em regiões litorâneas, entretanto nos últimos anos, incrementa o uso de áreas interiores para a produção de camarões marinhos, com uso de água subterrânea salobra ou que seja feita salinização artificial através de diluição e/ou adição de sal marinho (Tlusty, 2002). Na Tailândia, já se utiliza a mistura de água doce para diluir a salmoura (água hipersalina) para o cultivo dos animais, enquanto nos EUA, utilizam águas salinas subterrâneas e correções iônicas, para que haja suplementação de sais, podendo ocorrer essa adição de sais marinhos na água e/ou na dieta dos animais (Boyd and Thunjai, 2003; Sowers et al., 2006; Roy et al., 2010).Entretanto, dependendo do volume aplicado, as correções iônicas na água são onerosas, podendo inviabilizar a produção, mas podem ser minimizadas em sistemas de mínima troca de água.

Os sistemas de mínima troca de água, através da relação carbono e nitrogênio (C:N) estimula as comunidades de bactérias heterotróficas e nitrificantes que assimilam e oxidam os compostos nitrogenados tóxicos presentes na água (Crab et al., 2012; Xu e Pan, 2012). Inicialmente, é promovido um aumento da razão C:N na água do cultivo, mantendo-a entre 10 a 20:1 (Avnimelech, 1999; Avnimelech, 2007), com a finalidade de estimular o crescimento dessas comunidades microbianas. O perfil microbiano que compõe os agregados é diverso, sendo composto por leveduras, bactérias, protozoários e microalgas (Monroy-Dosta et al., 2013).

Dentro dos sistemas de mínima troca de água, podemos destacar o sistema simbiótico, que consistem na distribuição das bactérias heterotróficas e nitrificantes de forma homogênea, afim de possibilitar a formação de flocos bacterianos e a remoção do nitrogênio residual do sistema, também estimula o crescimento do fito e zooplâncton proporcionando um aumento da disponibilidade de alimento natural, além de reduzir a carga orgânica. Esse sistema também é caracterizado por possuir baixas concentrações de sólidos suspensos e propiciar uma maior estabilidade dos nitrogenados quando comparado a outros sistemas intensivos (Brito, et al., 2019; De Andrade et al., 2021; Pimentel et al., 2022).

A combinação adequada de íons na água e sistemas de mínima troca de água para a produção de juvenis de camarões marinhos ampliar a possibilidade de cultivos de camarões marinhos longe da costa, incrementar a imunidade e resistência dos animais para a segunda etapa da produção de camarões marinhos, aumento na taxa de sobrevivência durante a engorda, aumento do número de ciclos durante o ano, além de proporcionar melhor aclimatação às condições dos viveiros de engorda (Mishra et al., 2008; Nunes, 2020).

Porém, a utilização de berçários de sistema intensivos em águas oligohalinas (salinidades entre 0,5 até 3 g/L) como mínima troca de água, ainda não é bem estabelecido,

devido à alta toxicidade de compostos nitrogenados, que afetam diretamente a produção, uma vez que, em sistemas intensivos a alta densidade de estocagem de animais resulta em uma maior quantidade do alimento por volume de água, que por conseguinte, aumenta a deposição de compostos tóxicos para o camarão (Muhlert et al., 2013) decorrentes da degradação da matéria orgânica proveniente de alimentos não consumidos e da excreção (Alves Neto et al., 2019).

Nesse sentido, o presente estudo tem por finalidade avaliar o efeito de diferentes formas de salinização artificial na produção de juvenis de *L. vannamei* cultivadas em sistema simbiótico.

2. Objetivos do Trabalho

2.1. *Geral*

Avaliar a influência de diferentes meios de salinização artificial na produção de juvenis de *Litopenaeus vannamei* em sistema simbiótico.

2.1.1 *Específicos*

- Avaliar a sobrevivência e crescimento de juvenis do *Litopenaeus vannamei* cultivados em água salinizada artificialmente;
- Determinar a contagem presuntiva total de *Vibrio* spp. e fungos no intestino dos juvenis de *Litopenaeus vannamei* em água salinizada artificialmente;
- Avaliar as variáveis físico-químicas de qualidade de água ao longo do cultivo em água salinizada artificialmente;
- Avaliar a resistência aos estresses físico-químicos dos juvenis de *Litopenaeus vannamei* cultivados em água salinizada artificialmente.

1.2. Hipótese

O *L. vannamei* cultivado na fase de berçário em águas oligohalinas salinizadas artificialmente em sistemas simbióticos, apresenta desempenho zootécnico similar aos cultivados em águas marinhas.

2. Artigo Científico I:

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Effects of different forms of artificially salinized in *Penaeus vannamei* low-salinity water in the nursery synbiotic system

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Abstract

This study aimed to evaluate the effect of different types of artificially salinized on the zootechnical performance, TCBS and Sabouraud Dextrose counts in *Penaeus vannamei* juveniles reared in a synbiotic system, in experimental tanks of 60 L, with a density of 2,000 PL m^{-3} for 40 days. Four treatments were established in triplicate using a completely randomized design: SD, diluted seawater; LCSM, low-cost salt mix with freshwater; CS, commercial salt; and SW, seawater. Previously, a study was carried out on mineral efficiency regarding the ions available in the culture water, and later salinization was performed to obtain a salinity close to 3.0 g L^{-1}, and a proportion of cations and anions similar to the values found in seawater. An artificial substrate of *Anomalocardia brasiliana* shells (1% of the total volume of the experimental unit) was used in each experimental unit. A mix of *Bacillus*-based

bacteria was used in the water and feed offered to the animals. An ammonia stress test was performed at the end of the culture, and a,microbiological analysis was performed at the beginning and end of the experiment to obtain the total count of *Vibrio* spp. and fungi. The use of the artificial substrate together with the synbiotic system proved to be effective in the control of nitrogen compounds. The values of calcium, magnesium and total hardness remained above 30, 75 and 400 mg L^{-1}, respectively. The main ions remained stable throughout the culture; the total alkalinity values were maintained above 120 mg $CaCO_3$ L^{-1}, within the recommended range for intensive systems. Better values of final average weight were obtained with the different forms of artificial salinization (LCSM, CS and SW), but survival was lower in low salinity when compared to seawater. There was no influence of different types of salinization on bacterial counts of TCBS and fungi, and on resistance to ammonia stress. Based on the results obtained, it can be concluded that it is possible to rear *P. vannamei* juveniles in artificially salinized water of low salinity (3 g L^{-1}) in a synbiotic system.

Key words: Low salinity, Ionic composition, Fungi, *Vibrio*, Ammonium Resistance.

Introduction

The world fish market has been fueled by aquaculture since the 1970s, working towards a sustainable economic alternative for human consumption of proteins that aim for quality, high palatability, and affordable value for the final consumer (Valenti, 2021). World aquaculture production in 2020 exceeded 122 million tonnes, of which 54.4 million tonnes came from inland waters and 68.1 million tonnes from coastal regions (FAO, 2022). The third most produced group is crustaceans, with 11.2 million tons in 2020, with 39% of this

production being inland (FAO, 2022). Among these, the most reared species is the shrimp *Penaeus vannamei*, representing 51.7% of crustacean production (FAO, 2022).

The production of *P. vannamei* is carried out mainly in coastal areas, however, in recent years it has expanded to locations far from the coast and in low salinity conditions (Roy et al., 2010a; Fierro et al., 2018). This can be related to the various economic and environmental advantages presented in these regions when compared to coastal zones, such as lower cost of land acquisition, less conflicts arising from the use of common resources, and reduced impact on not very resilient ecosystems such as mangroves (Nunes and Lopez, 2001; Jory, 2017; Lacerda et al., 2021). This is possible because the shrimp *P. vannamei* supports a wide range of salinity (0.5–60 g L^{-1}), although large variations and an inadequate ionic profile can affect the osmoregulation mechanism and change the nutrient absorption strategy of the species.

In addition, the culture of marine crustaceans in low salinities with an inappropriate ionic profile can lead to lower animal performance, such as poor survival, reduced growth and/or high feed conversion ratio (Diaz et al., 2001; Li et al., 2007; Ye et al., 2009), which can be minimized by adjusting the mineral supplementation (Ca, Mg, Na and K) so that the water in low salinity becomes similar to the proportions of ions (cations and anions) found in seawater, even with the culture of this species in waters with salinities below 0.5 g L^{-1} (Moraes et al., 2020).

When culture in low salinity uses the technique of artificially salinized and/or ionic adjustment of the water, with similarity in the proportions of ions in relation to seawater, it favors greater osmotic comfort for the animals, avoiding energy expenditure and helping to increase shrimp growth and survival (Boyd, 2002; Oliveira, 2016; Nehru et al., 2018). For *P. vannamei*, the main ratios of cations are Na:K, Mg:Ca and Ca:K, making it necessary to apply these ions in water, through the use of agricultural fertilizers or artificial sea salts.

In order to increase the growth of aquaculture activity in inland regions and minimize the high cost of transporting and/or producing saline water from saline/brackish effluents, techniques such as adding artificial sea salt or diluting the hypersaline water with freshwater, has been widely used, respectively in the USA and Thailand (Boyd and Thunjai, 2003; Sowers et al., 2006, Roy et al., 2010a). However, the use of inappropriate technologies and/or techniques can cause a series of environmental impacts on natural ecosystems (Figueiredo, 2005; Araneda et al., 2008). To minimize these impacts and increase the efficiency of water reuse, generating less application of new fertilizers and/or sea salt in the water, systems with minimum water exchange can be used.

Among the systems of minimum water exchange, the synbiotic stands out, since it stimulates the growth of phyto and zooplankton and provides an increase in the availability of natural food, reducing the organic load and enhancing the stability of nitrogen when compared to other intensive systems, in addition to having low concentrations of suspended solids (Brito et al., 2019; De Andrade et al., 2021; Pimentel et al., 2022; Oliveira et al., 2022 a,b; Santos et al., 2022a,b).

The synbiotic used in semi-intensive and intensive shrimp systems with minimal water exchange consists of the use of bran after the fermentation process and/or microbial respiration with probiotic microorganisms (Romano et al., 2018). This process allows a more efficient use of polysaccharides (such as wheat or rice bran), from aerobic and anaerobic processes, providing the diversified growth of microbial aggregates composed of phytoplankton, zooplankton, and autotrophic and heterotrophic bacteria (Romano et al., 2018; De Andrade et al., 2021; Pimentel et al., 2022).

The combination of the use of minimal water exchange systems with ion supplementation (artificially salinized) for the production of juvenile shrimp in low salinity increases the possibility of shrimp culture far from the coast, since the population of the grow-

out phase with juveniles instead of post-larvae increases the survival rate during grow-out phase and the number of production cycles during the year, in addition to providing better acclimatization and resistance to shrimp to the water quality conditions of the ponds (Mishra et al., 2008; Nunes, 2020).

In this sense, the objective of this work is to evaluate the effect of different forms of artificially salinized on zootechnical performance and water quality, and the resistance to ammonia stress in the production of *P. vannamei* juveniles in a synbiotic system.

Material and methods

Experimental design and system

The study was carried out for 40 days at the Laboratório de Carcinicultura (LACAR [Shrimp Culture Lab]), of the Departamento de Pesca e Aquicultura (DEPAq [Fisheries and Aquaculture Department]) of Universidade Federal Rural de Pernambuco (UFRPE [Rural Federal University of Pernambuco)], in Brazil. Four treatments in triplicate using a completely randomized design were established: SD, diluted seawater; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW, seawater.

A matrix tank (0.8 m^3 by treatment) with seawater and freshwater was chlorinated with 20 mg L^{-1} of active chlorine and dechlorinated through constant aeration. Twenty days before stocking the shrimp, the synbiotic started being added at two-day intervals, for a total of ten fertilizations. This synbiotic was obtained through an anaerobic (24 h) and aerobic (24 h) process, from a mixture of 20 g m^{-3} of rice bran (< 200 μm), 2 g m^{-3} of sugar, 0.5 g m^{-3} of commercial bacterial mix (*Bacillus subtilis* [2.2 × 10^9 CFU g^{-1}], *B. licheniformis* [1.8 × 10^9 CFU g^{-1}], *Bacillus* sp. (1.6 × 10^9 CFU g^{-1}), sodium chloride (NaCl), and magnesium hydroxide [$Mg(OH)_2$], from Kayros Agrícola and Ambiental, SP, Brazil), 4 g m^{-3} of sodium bicarbonate, and previously chlorinated water (20 mg L^{-1} of chlorine per 24 hours, followed

by dechlorination by aeration) in the proportion of ten times the amount of rice bran. An initial inoculum of 5% of the tank volume with water from a shrimp nursery in a synbiotic system was used.

Throughout the culture, the procedure for the preparation of the synbiotic was the same, for four times a week up until reaching 50% of the concentrations of the inputs, apart from the probiotic, which continued at 0.5 g m^{-3}. The use of the synbiotic was until settleable solids reached 5 ml L^{-1}, and applications were suspended past this limit.

The experimental units (60 L) were constantly aerated (dissolved oxygen > 5.0 mg L^{-1}), with temperature maintained at ~30°C (Hopar Sh-608 heater 100 W), a 12:12 h photoperiod, and mean luminance of 8.65 μmol photons m^{-2} (Equitherm Lux-204). No water exchange was performed during the experimental time. Dechlorinated freshwater was added four times a week to compensate for evaporation loss. To assist the growth of the nitrifying bacterial community, artificial substrate (14 × 14 × 3.2 cm) corresponding to 1% of the volume of the experimental units (60 L), made with *Anomalocardia brasiliana* shells, was added. Alkalinity correction with sodium bicarbonate ($NaHCO_3$) was also performed every 10 days after water analysis to reach values > 150 mg L^{-1}.

Salinization adjustment

Prior to the salinization of the treatments, previous studies were carried out on the efficiency of increasing ions in the water by applying chemical products. The tests were performed in experimental units of 14 L, with salinity of ~2.5 g L^{-1}, obtained by diluting seawater in freshwater under constant aeration. The increase in pH and ion concentration was analyzed after 72 hours of application of 100 g m^{-3} of potassium chloride (KCl), calcium carbonate ($CaCO_3$), magnesium sulfate heptahydrate ($MgSO_47H_2O$), magnesium chloride hexahydrate ($MgCl_26H_2O$), and *Lithothamnium* (Primasea, Bahia, Brazil) (Table 1).

Table 1. Increase in percentage of ionic concentration (after 72 hours of application of 100 g m^{-3}) of chemical fertilizers used in ionic adjustment.

	Fertilizers	KCl	$MgSO_4 7H_2O$	$MgCl_2 6H_2O$	*Lithothamnium*	$NaCl^-$
% target ion increment	$pH^{\#}$	0.41 ± 0.04	0.40 ± 0.07	0.17 ±0.01	0.00 ± 0.00	-
	Ca^{2+}	-	-	-	26.70 ± 1.80	-
	K^+	51.10 ± 11.38	-	-	-	-
	Mg^{2+}	-	9.40 ± 0.51	10.52 ± 1.37	5.20 ± 2.00	-
	Na^+	-	-	-	-	39.42 ± 0.08
	Cl^-	-	-	-	-	60.33 ± 0.02

Data correspond to the mean of two replicates ± standard deviation. KCl: potassium chloride; $CaCO_3$: calcium carbonate; $MgSO_4 7H_2O$: magnesium sulfate heptahydrate; $MgCl_2 6H_2O$: magnesium chloride hexahydrate. $^{\#}$pH in absolute value.

In view of the results obtained from the previous study on the increase of ions in the water from the application of mineral fertilizers, the salinization adjustments of the treatments were carried out with commercial products and a commercial salt mixture (Veromix, São Paulo, Brazil) (Table 2), taking into account the salinity of 3.0 g L^{-1} and the proportions of cations (sodium, potassium, calcium and magnesium) and anions (bicarbonate, chloride, sulfate) (Table 3). The adjustment was based on the proportion factors referring to the salinity of the seawater (Boyd and Thunjai, 2003; Roy et al., 2010a).

Table 2. Fertilizers used in the artificially salinized low-salinity water used in the of *P. vannamei* nursery synbiotic system.

Treatment	Inputs	Ions made available

Commercial salt mixture (CS)	Commercial salt mixture (830 g m^{-3}, Veromix) and coarse salt (1,660 g m^{-3})	Sodium, magnesium, potassium, strontium, chlorides, carbonates, bicarbonates, borates, sulfates, bromides, fluorides, and trace elements
Low-cost salt mixture (LCSM)	Coarse salt (1,951 g m^{-3}), potassium chloride (52.45 g m^{-3}), *Lithothamnium* (96.67 g m^{-3}), magnesium chloride (466.67 g m^{-3}), magnesium sulfate (515.13 g m^{-3})	Sodium, chloride, potassium, magnesium, sulfate, and calcium

Table 3. Initial ionic profile in the artificially salinized low-salinity water and seawater used in the *P. vannamei* nursery in synbiotic system.

Variables	**Treatments**			
	LCSM	**CS**	**SD**	**SW**
Ca^{2+} (mg L^{-1})	37.87 ± 9.38[a]	26.67 ± 0.92[a]	38.40 ± 4.23[a]	600.00 ± 105.83[b]
Mg^{2+} (mg L^{-1})	90.07 ± 7.55[a]	115.67 ± 3.50[a]	77.76 ± 3.89[a]	1,514.70± 248.21[b]
K^{+} (mg L^{-1})	34.94 ± 0.98[a]	22.22 ± 0.90[a]	26.85 ± 1.06[a]	322.67 ± 66.89[b]
Na^{+} (mg L^{-1})	646.46 ± 19.58[a]	679.11 ± 39.59[a]	705.23 ± 19.59[a]	13,323.53±607.77[b]
SO_4^{2-} (mg L^{-1})	230.53±14.37[a]	257.97 ± 29.90[a]	212.17 ± 44.86[a]	3,135.67 ± 212.01[b]
Cl^{-} (mg L^{-1})	1,169.85±35.45[a]	1,228.93±71.63[a]	1,276.20±35.45[a]	20,561.00±937.92[b]
TA (mg $CaCO_3$ L^{-1})	61.67 ± 2.89[a]	80.00 ± 0.01[a]	63.33 ± 5.77[a]	135.00 ± 5.00[b]

TH (mg $CaCO_3$ L^{-1})	417.33±20.13[a]	542.67 ± 16.65[a]	400.00 ± 16.00[a]	7,733.33 ± 184.62[b]
Salinity (g L^{-1})	3.01 ± 0.09[a]	2.99 ± 0.12[a]	3.12 ± 0.36[a]	35.07 ± 0.54[b]
Mg:Ca	2.38	4.34	2.03	2.52
Mg:K	2.58	5.20	2.90	4.69
Ca:K	1.08	1.20	1.43	1.86
Na:K	18.50	30.56	26.26	41.29
TH:TA	6.80	6.78	6.32	57.28
Error (%)	0.88	0.90	2.24	13.88

Data correspond to mean (n = 3) ± standard deviation. SD, seawater diluted; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW, seawater. TA: total alkalinity; TH: total hardness.

After analyzing the water in all treatments, ion concentrations in milliequivalent L^{-1} (mEq L^{-1}) were calculated to check the cation and anion equilibrium. The calculation was performed by determining the difference between the sum of the cation mEq L^{-1} (Na^+ = 23 mg mEq^{-1}; K^+ = 39.1 mg mEq^{-1}; Ca^{2+} = 20 mg mEq^{-1}; and Mg^{2+} = 12.15 mg mEq^{-1}) and sum of the anion mg mEq^{-1} (HCO_3^- = 61 mg mEq^{-1}; Cl^- = 35.45 mg mEq^{-1}; and SO_4^{2-} = 48.03 mg mEq^{-1}) (Boyd, 2020). A balance error lower than 15% between cations and anions was adopted as a standard for certifying the accuracy of the analysis of these major ions (Boyd, 2002). This error was calculated using the following equation:

$$Error\ (\%) = \frac{|\Sigma\ cations\ -\ \Sigma\ anions|}{\Sigma\ cations\ +\ \Sigma\ anions} \times 200$$

In which:

- Σ cations: sum of cations
- Σ anions: sum of anions.

Water Quality

Dissolved oxygen and temperature (Asko multiparameter meter, model AZ86031) were monitored twice a day (8 am and 4 pm). Salinity and pH (Asko multiparameter meter, model AZ86031) were monitored twice a week. The settleable solids (Avnimelech, 2009) were monitored three times a week. Total ammonia nitrogen (TAN; APHA, 2012), nitrite-nitrogen (NO_2^--N; Fries, 1971), nitrate nitrogen (NO_3^--N; APHA, 2012), orthophosphate (PO_4^{3-}; APHA, 2012), total alkalinity (TA; APHA, 2012), total hardness (TH; APHA, 2012), Ca^{2+} (APHA, 2012), Mg^{2+} (APHA, 2012), Na^+ (APHA, 2012), Cl^- (APHA, 2012), SO_4^{2-} (APHA, 2012), and K^+ (Fries and Getrost, 1977) were monitored every 10 days. All water samples were previously filtered through a 45µm paper filter before performing the analyses.

Shrimp stocking, feeding, and monitoring

The post-larvae (PL_{10} ~ 2.0 mg) were acquired in a commercial hatchery (Aquatec, RN, Brazil), produced in water with salinity at 20 g L^{-1}. The batch was divided into two tanks with a useful volume of 800 liters (7,500 PL m^{-3}), where a part of the PL was acclimated to a salinity of 3.0 g L^{-1} and the other to a salinity of 35 g L^{-1} for 10 days. At the end of the acclimatization period, the PL_{24} (12 mg ± 1.0 mg) were randomly selected and stocked at a density of 2,000 PL m^{-3} in experimental units.

The post-larvae were fed with a commercial feed of 0.3–0.6 mm granulometry (45% crude protein and 7% lipids, Inve Aquaculture Inc) between day 1 and day 17 of the experimental time. Between day 17 and day 40 of the experimental time, the animals were fed with a commercial feed of 0.8–1.3 mm granulometry (45% crude protein, and 9.5% lipids,

ADM Animal Nutrition Company). The feed was offered four times a day (8 am, 11 am, 2 pm and 5 pm). Initially, a 27.2% daily feeding rate was adopted, which was gradually reduced to 4.8% of body weight for 40 days. The feeding rate was adjusted daily according to the estimated shrimp feed consumption and mortality rate in each experimental unit (Van Wyk et al., 1999), plus a mix of commercial probiotic (2 g/kg feed) with colony forming units (CFU) g^{-1} containing *Bacillus subtilis* (8.5×10^8 CFU g^{-1}), *B. licheniformis* (8.5×10^8 CFU g^{-1}), *B. pumilus* (5.0×10^8 CFU g^{-1}), *B. cereus* var. toyoi (8.0×10^8 CFU g^{-1}), *B. amyloliquefaciens* (8.5×10^8 CFU g^{-1}), *Lactobacillus acidophilus* (3.7×10^8 CFU g^{-1}), *L. plantarum* (3.7×10^8 CFU g^{-1}), yeast extract, magnesium, and mannan oligosaccharide, in addition to a dispersing agent (Kayros Agrícola and Ambiental, São Paulo, Brazil), manually added to the feed using a commercial binder based on digestive cellulose.

Shrimp weight was monitored every 10 days to determine growth and adjust the amount of feed offered. At the end of the experimental period, final weight, specific growth rate (SGR), feed conversion ratio (FCR), survival, and yield were determined using the following equations:

Final weight (g) = final biomass (g)/number of individuals at the end of evaluation period

SGR (% day^{-1}) = 100 × [ln final weight (g) – ln initial weight (g)]/time (days)

FCR = feed supplied/ (final biomass – initial biomass)

Survival (%) = (final number of individuals/initial number of individuals) × 100

Yield (Kg m^{-3}) = final biomass (Kg)/volume of experimental unit (m^3).

*Presumptive total count on Thiosulphate Citrate Bile Sucrose – TCBS (*Vibrio *spp.) and Sabouraud dextrose agar (fungi)*

Presumptive total count were performed at the Laboratório de Sanidade de Organismos Aquáticos (LASAq [Aquatic Animal Health Lab]), of the Fisheries and

Aquaculture Department] of Rural Federal University of Pernambuco, Brazil for the quantification of the colony-forming units (CFUs g^{-1}) of Thiosulphate Citrate Bile Sucrose – TCBS (*Vibrio* spp.) and Sabouraud dextrose agar (Fungi). Shrimp samples for analysis in TCBS (*Vibrio* spp.) and Sabouraud dextrose agar (Fungi) were collected at the 20th and 40th days (pools of 50 mg gut samples each experimental units) (Vandenberghe et al., 1999).

The shrimp were disinfected by immersion in 70% ethanol for 15 seconds, followed by immersion for 15 minutes in sodium hypochlorite solution (1.5%) with 0.1% tween-80 and rinse with sterile distilled water. Subsequently, the biological samples were weighed, macerated and homogenized with a solution of peptone water (2%) in the 1:10 ratio, for the 10^{-1} dilution, serially diluted from 10^{-1} to 10^{-5} and 100 µL were seeded in the plates containing the culture media Thiosulfate Citrate Bile Sucrose and Sabouraud dextrose agar (fungal count made of yeast and filamentous fungi), with the aid of an "L" loop, and then incubated at 30°C for 24 hours (TCBS) and 36°C for 72 hours (Sabouraud dextrose agar), all in triplicate (APHA, 2017). After incubation, total colonies were counted using a colony counter, and macromorphological colonial aspects for the characterization of yeast and filamentous fungi were analyzed based on Trabulsi and Alterthum (2015). The conversion to CFU g^{-1} for the shrimp samples and gut samples was performed using the following formula: CFU g^{-1} = number of colonies × dilution factor / weight (g) gut of the sample.

Stress tests

At the end of the culture time, shrimp were submitted to resistance test for water ammonia nitrogen concentration (NH_3-N). For the NH_3-N resistance test, at the end of the experiment, 10 shrimps were randomly collected in each experimental unit and were stocked to experimental units containing 10 L of water salinity 3.0 g L^{-1} (LCSM, CS and SD), and 10 L of water salinity 35 g L^{-1} (SW) with NH_3-N concentrations between 0.39 and 0.42 mg L^{-1}

(Table 4), water temperature close to 29°C, and pH close to 8.1–8.5. The NH_3-N concentration was achieved by applying a stock solution of 10 g L^{-1} of NH_4Cl. The test was carried out for 96 h, and survival was measured every 24 h (Zhang et al., 2012).

Table 4. Total ammonia nitrogen (TAN) concentration and non-ionized ammonia (NH_3-N) produced in the experimental units from NH_4Cl solution application.

	TAN (mg L^{-1})		**NH_3-N (mg L^{-1})**	
	SW	**LCSM, CS and SD**	**SW**	**LCSM, CS and SD**
Initial	2.57	4.91	0.41	0.42
24hrs	2.44	4.79	0.39	0.41
48hrs	2.56	4.88	0.41	0.42
72hrs	2.48	4.77	0.40	0.41
96hrs	2.56	4.80	0.41	0.41

Data correspond to the mean concentration of TAN and NH_3-N ± standard deviation.

Total hemocyte count (THC)

Hemolymph (10 shrimp per treatment) was collected for the blood analysis before and after the stress tests of ammonia nitrogen concentration (NH_3-N following the protocol described by Guertler et al., 2013). For such, the hemolymph (200 µL) was collected from the hemocoel located in the ventral region of the surviving animals with the aid of a syringe (1 ml) containing anticoagulant (modified Alsever's solution [MAS: 336 mmol L^{-1} of NaCl, 115 mmol L^{-1} of glucose, 27 mmol L^{-1} of sodium citrate, and 9 mmol L^{-1} of EDTA, pH 7.2]) at a proportion of 1:2 (v:v). One aliquot of hemolymph was separated and stored in MAS and 4% formaldehyde (1:3). The total hemocyte count was performed in a Neubauer chamber in a similar manner used for white blood cells in triplicate (Hameed et al., 2006).

Data analysis

Statistical analyses were performed using the IBM SPSS Statistic 25.0 software. Data were checked for homogeneity of variances using the Levene test ($p \leq 0,05$) and for normality using the Shapiro-Wilk test ($p \leq 0,05$). For normal and homogeneous data, parametric one-way ANOVA (shrimp zootechnical performance and total hemocyte count) and repeated measures ANOVA (water quality – dissolved oxygen, temperature, pH, salinity, Ca^{2+}, Mg^{2+}, Cl^{-}, K^{+}, Na^{+}, magnesium hardness, calcium hardness, TH, and TA) were used, followed by Tukey's mean comparison test ($P \leq 0.05$). Non-parametric data were analyzed using the Friedman's test with Conover's multiple comparison test with Holm-Bonferroni correction for TAN, $N\text{-}NO_2^{-}$, $N\text{-}NO_3^{-}$, settleable solids, and SO_4^{-2}.

TCBS and Sabouraud dextrose agar counts were analyzed using the non-parametric Kruskal-Wallis test ($P \leq 0.05$) followed by Dunn's post-hoc test with Holm-Bonferroni correction ($P \leq 0.05$).

Results

Ions

Ion concentration had no significant differences ($P > 0.05$) among low salinity treatments, but it did have ($P < 0.05$) when regarding seawater (SW) (Table 5). The water ions values were similar between the beginning and the end, however the alkalinity increased significantly (Table 5).

Table 5. Concentrations (mg L^{-1}) of ions from artificially salinized low-salinity water and seawater used in the of *P. vannamei* nursery in synbiotic system.

Variables	Treatments			
	LCSM	**CS**	**SD**	**SW**

Ca^{2+} (mg L^{-1})	39.36 ± 5.03[b]	34.45 ± 6.42[b]	39.78 ± 5.78[b]	626.66 ± 46.18[a]
Mg^{2+}(mg L^{-1})	122.92±20.32[b]	86.44±19.05[b]	102.38±19.91[b]	1,747.80±329.39[a]
K^{+} (mg L^{-1})	28.33 ± 5.19[b]	24.00 ± 5.86[b]	25.37 ± 2.87[b]	231.69 ± 71.14[a]
Na^{+} (mg L^{-1})	714.37±347.97[b]	692.82±294.57[b]	767.26±359.91[b]	16,482.12±4,052.30[a]
SO_4^{2-} (mg L^{-1})	333.18 ± 91.95[b]	317.34 ± 54.52[b]	316.82±108.13[b]	3,881.13 ± 691.12[a]
Cl^{-} (mg L^{-1})	1,127.30±552.83[b]	1,132.03±455.60[b]	1,207.66±549.45[b]	25,169.50±6,371.14[a]
TA (mg $CaCO_3$ L^{-1})	165.00 ± 11.92[b]	151.00 ± 72.35[b]	157.66 ± 104.80[b]	200.80 ± 86.65[a]
TH (mg $CaCO_3$ L^{-1})	613.33 ± 10.27[b]	498.67±90.25[b]	497.33±99.06[b]	9,500.00±568.37[a]
Calcium hardness (mg $CaCO_3$ L^{-1})	96.00 ± 26.54[b]	81.33 ± 16.13[b]	97.33 ± 16.46[b]	1,500.00±110.23[a]
Magnesium hardness (mg $CaCO_3$ L^{-1})	512.00 ± 80.30[b]	390.67±89.86[b]	400.00±85.92[b]	8,000.00±464.48[a]
Mg:Ca	3.12	2.50	2.57	2.79
Mg:K	4.33	3.60	4.03	7.54
Ca:K	1.39	1.43	1.57	2.70
Na:K	25.22	28.87	30.24	71.13
TH:TA	3.72	3.30	3.15	47.31
Error (%)	0.94	0.46	0.79	13.32

Data correspond to mean ± SD. The results were analyzed by means of a repeated measures ANOVA ($P \leq 0.05$) followed by the Tukey test, Friedman's test and Conover's multiple comparison test ($P \leq 0.05$) for non-parametric data. SD, seawater diluted; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW, seawater. TH, total hardness; TA, total alkalinity.

Water quality

Data on water quality variables are summarized in Table 6. No significant differences among the treatments were found regarding nitrogen compounds (TAN, $N\text{-}NO_2$, $N\text{-}NO_3$).

However, significant differences were found for salinity, pH, and SS. The higher values for SS were found in CS treatment (1.64 ± 0.80 ml L^{-1}) and the lowest in SW treatment (0.20 ± 0.08 ml L^{-1}).

Table 6. Water quality variables in the artificially salinized low-salinity water and seawater used in the of *P. vannamei* nursery in synbiotic system.

Variables	Treatments			
	LCSM	**CS**	**SD**	**SW**
Temperature (ºC)	30.50 ± 0.46[a]	30.15 ± 0.33[a]	30.30 ± 0.44[a]	30.01 ± 0.15[a]
Oxygen (mg L^{-1})	5.28 ± 0.30[a]	5.35 ± 0.22[a]	5.21 ± 0.29[a]	4.67 ± 0.15[a]
Salinity (g L^{-1})	3.00 ± 0.07[b]	2.96 ± 0.15[b]	2.99 ± 0.34[b]	36.50 ± 0.03[a]
pH	8.89 ± 0.01[a]	8.86 ± 0.08[a]	8.90 ± 0.08[a]	8.11 ± 0.22[b]
TAN (mg L^{-1})	0.18 ± 0.12[a]	0.20 ± 0.31[a]	0.24 ±0.12[a]	0.23 ± 0.27[a]
N-Nitrite (mg L^{-1})	0.42 ± 0.90[a]	0.24 ± 0.50[a]	0.23 ± 0.10[a]	0.42 ± 0.90[a]
N-Nitrate (mg L^{-1})	2.84 ± 1.44[a]	2.48 ± 1.60[a]	3.00 ± 1.66[a]	0.82 ± 1.24[a]
Settleable solids (ml L^{-1})	1.43 ± 0.56[a]	1.64 ± 0.80[a]	0.49 ± 0.62[ab]	0.20 ± 0.08[b]

Data correspond to mean ± standard deviation. The results were analyzed by means of a repeated measures ANOVA ($P \leq 0.05$) followed by the Tukey test for the parametric data, and Friedman's test and Conover's multiple comparison test($P \leq 0.05$) for non-parametric data. SD, seawater diluted; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW, seawater.

Shrimp zootechnical performance

The shrimp zootechnical performance data at the end of the 40 days of experimental culture are summarized in Table 7. Significant differences among the treatments were found in final weight, with highest value in LCSM (0.87 ± 0.24 g) and lowest in SW (0.65 ± 0.10 g),

however, regarding survival, LCSM (70.00 ± 5.00%) showed lower values when compared to SW (90.55 ± 10.58%). No significant differences among the treatments were found regarding yield, FCR and SGR (Table 7).

Table 7. Zootechnical performance of *P. vannamei* juveniles from artificially salinized low-salinity water and seawater in synbiotic system.

Variables	Treatments			
	LCSM	**CS**	**SD**	**SW**
Final weight (g)	0.84 ± 0.18^{a}	0.84 ± 0.04^{a}	0.72 ± 0.03^{a}	0.65 ± 0.10^{b}
Survival (%)	70.00 ± 5.00^{b}	78.61 ± 3.15^{ab}	82.77 ± 3.93^{ab}	90.55 ± 10.58^{a}
FCR	1.04 ± 0.31^{a}	1.18 ± 0.01^{a}	1.25 ± 0.01^{a}	1.16 ± 0.05^{a}
Yield (Kg m^{-3})	1.01 ± 0.42^{a}	1.14 ± 0.02^{a}	1.05 ± 0.06^{a}	1.09 ± 0.05^{a}
SGR (% dia^{-1})	4.25 ± 0.36^{a}	4.34 ± 0.81^{a}	4.56 ± 0.56^{a}	4.52 ± 0.27^{a}

Data correspond to mean ± standard deviation. The results were analyzed by means of ANOVA ($P \leq 0.05$) followed by the Tukey test. Mean values on the same line with different superscripts differ significantly. SD, seawater diluted; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW, seawater. FCR, feed conversion ratio; and SGR, specific growth rate.

Thiosulphate Citrate Bile Sucrose (TCBS, Vibrio *spp.) and Sabouraud dextrose agar (fungi)*

The *Vibrio* spp. samples from the shrimp gut at 20 days of culture, were highest in the SW ($7.44 \pm 9.73 \times 10^{4}$ CFU g^{-1}), and the lowest in CS ($0.91 \pm 1.89 \times 10^{4}$ CFUg^{-1}). The counts at the end of the culture cycle ranged from $15.6 \pm 15.5 \times 10^{4}$ CFU g^{-1} (LCSM) to $0.72 \pm 1.27 \times 10^{4}$ CFU g^{-1} (SD), however there were no significant differences between treatments (Figure 1).

Regarding fungi, the SW treatment presented the highest concentration levels (260.0 ± 244.0 × 10^4 CFU g^{-1}) at 20 days of culture. At the end, an increase in filamentous fungi was observed in all low salinity treatments, but they were still less representative when compared to yeasts. The final counts varied between 323.0 ± 287.0 × 10^4 CFU g^{-1} (LCSM) and 40.2 ± 41.1 × 10^4 CFU g^{-1} (SW), however, there were no significant differences between treatments (Figure 1).

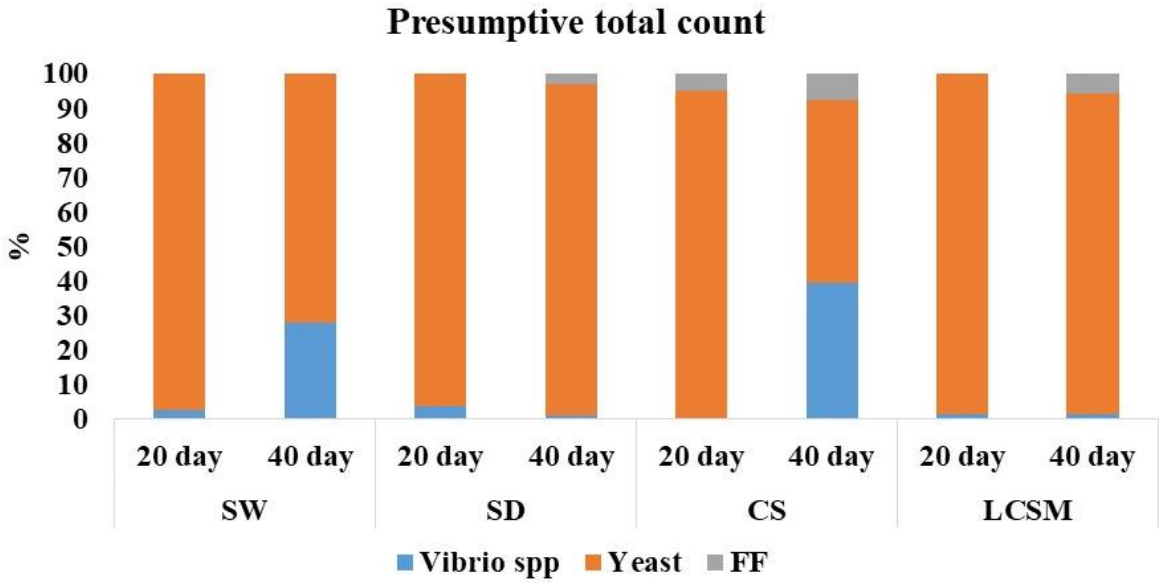

Figure 1. Presumptive total count for Thiosulphate Citrate Bile Sucrose (TCBS, *Vibrio* spp.) and Sabouraud dextrose agar (fungi) in *P. vannamei* gut. SD, seawater diluted; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW, seawater. FF = filamentous fungi.

Stress tests

Regarding the N-NH_3 resistance test, no significant differences were observed between treatments. After being submitted to the stress test, 100% survival was observed in all treatments, and total hemocyte counts did not differ significantly between treatments. Juveniles had hemocyte counts ranging between 5.00 ± 1.77 × 10^6 cells mL^{-1} (LCSM) and 2.00 ± 1.17 × 10^6 cells mL^{-1} in the SD treatment (Figure 2).

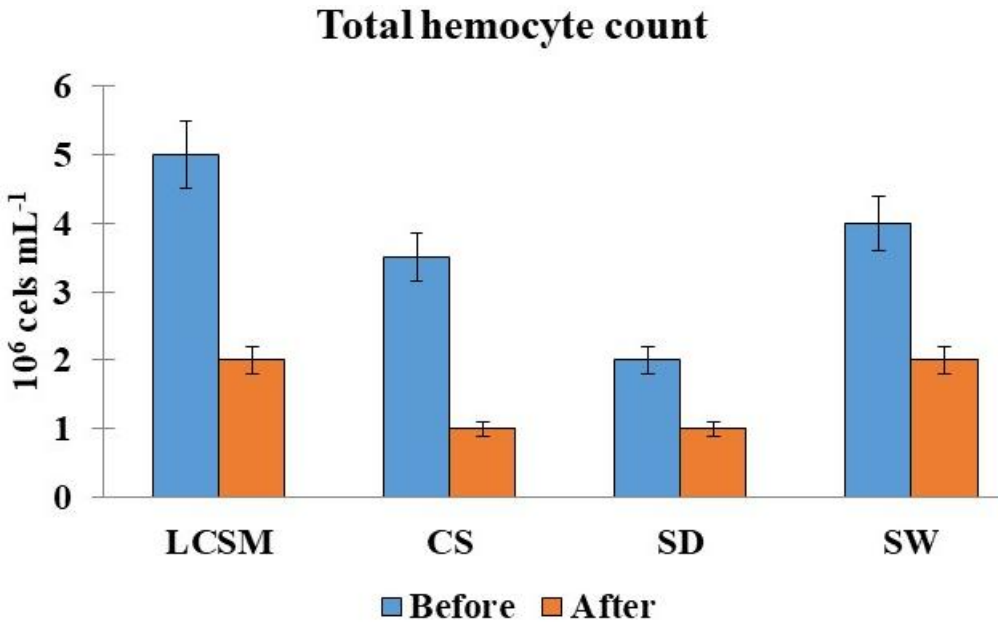

Figure 2. Total hematocyte count (THC) of *Penaeus vannamei* juveniles under stress for water ammonia nitrogen concentration (NH_3-N) after nursery in the artificially salinized low-salinity water and seawater used synbiotic system. SD, seawater diluted; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW, seawater.

Discussion

Regarding ions, the different forms of artificially salinized used in this experiment managed to maintain appropriate levels during the nursery phase, even with the observed ecdysis processes, unlike Huong et al. (2010), who described a reduction of ions (calcium and magnesium) in the culture water during the growth of shrimp by the ecdysis process, as they have a high demand for shell formation. This observed maintenance of ions is probably related to the use of the natural substrate of *A. brasiliana* and rice bran as a source of organic carbon. This maintenance of calcium and magnesium ions provides greater total water hardness and, in addition to higher concentrations of total alkalinity, favor the growth of shrimp. According to Pimentel et al. (2022), there are positive correlations for the

concentrations of total alkalinity and calcium regarding final average weight and specific growth rate of *P. vannamei* juveniles in oligohaline water.

The ratio of potassium to sodium in seawater is approximately 28:1, although the shrimp *P. vannamei* support variations of up to 10 points without interfering with its osmoregulatory capacity (Sowers et al., 2006), especially when alkalinity is greater than or equal to 100 mg $CaCO_3$ L^{-1} (Pimentel et al., 2022). Potassium is one of the main ions related to the growth and survival of shrimp in low salinity, in addition to influencing osmoregulation when associated with sodium, contributing to the activation of the Na^+, K^+-ATPase enzyme, responsible for the active transport of ions through the animal's cell membrane (Roy et al., 2007; Galkanda-Arachchige et al., 2021). The Na:K ratio in the low salinity and total alkalinity treatments during the shrimp nursery remained close to the recommended and may have contributed to the activation of the Na^+, K^+-ATPase enzyme, allowing the maintenance of cellular metabolism homeostasis and improving the uptake of glucose and amino acids by shrimp (Galkanda-Arachchige et al., 2021).

Dissolved oxygen, temperature and pH remained within the ideal values for the production of *P. vannamei* (Van Wyk and Scarpa, 1999; Samocha, 2019). The levels of dissolved oxygen in the SW treatment were lower than 5 mg L^{-1}, due to the higher concentrations of salts, generating lower solubility of dissolved oxygen (Fiorucci and Filho, 2005). However, these concentrations were not limiting for a good development of the shrimp and the transformation of nitrogen compounds by the bacteria in the culture system.

The pH values showed a significant difference between the low salinity treatments (LCSM, CS and SD) in relation to that of seawater (SW). The application of mineral fertilizers for artificially salinized, in addition to the use of sodium bicarbonate in low salinity, contributed to increase the pH of the water. According to Brito et al. (2021), low salinity sodium bicarbonate behaves differently when compared to its use in a marine water system,

as it has greater power to increase the pH. Alkalinity adjustments with sodium bicarbonate ($NaHCO_3$) every ten days in the nursery phase maintained mean concentrations greater than 150 mg $CaCO_3$ L^{-1}, within the recommended range for intensive systems (Van Wyk and Scarpa, 1999; Avnimelech et al., 2015), helping to keep the oxidation of nitrogen compounds via nitrifying bacteria and/or the transformation of ammonia into microbial biomass (Ebeling et al., 2006; Ferreira et al., 2011), however, it is necessary to pay attention to the pH values.

In addition to contributing to the control of nitrogen compounds, total alkalinity and total hardness (calcium and magnesium) are of paramount importance for a good development of shrimp, as they directly influence the animal's ecdysis process, since the amount of calcium consumed by shrimp will supply the exoskeleton mineralization process (Boyd and Tucker, 1998; Samocha, 2019).

At low salinity, nitrogen compounds are more toxic, causing large mortalities of shrimp. This problem with nitrogen concentrations can be more accentuated in intensive systems with minimal water exchange in the first days of culture, due to the high concentrations of ammonia and nitrite. According to Furtado et al. (2011), high densities contribute to the accumulation of organic matter from feed waste (leached or not consumed) and high biomass, among others, in addition to the reduced concentrations of nitrifying and heterotrophic bacteria at the beginning of the production cycles. In all treatments in this study, TAN and N-NO_2 levels were maintained within the ideal levels for low salinity treatments (< 0.81 mg TAN L^{-1} and < 0.45 mg N-NO_2 L^{-1}) and for the treatment of seawater (< 3 mg TAN L^{-1} and < 10 mg N-NO_2 L^{-1}) (Valencia-Castañeda et al., 2018; Samocha, 2019).

This control of nitrogen in different forms of artificial salinization of water is related to the combination of the use of a 5% water reuse inoculum, a synbiotic system and an artificial substrate, as also observed by Pimentel et al. (2022) and Oliveira et al. (2022b), with production of *P. vannamei* juveniles in oligohaline water. These results contribute to the

installation of intensive nurseries with minimal water exchange in oligohaline condition, with salinity close to 3 g L^{-1}, reducing production costs with artificial salinization, since many researches and productions in artificial waters use higher or close salinity to 10 g L^{-1} (Pinto et al., 2020; Fleckenstein et al., 2022), which makes the cost per m^3 of water economically onerous for the production of shrimp in artificially salinized water in some countries.

The synbiotic system, when compared to other systems with application of molasses in natura in the nursery phase, produces less settleable solids, due to the application of the source of organic carbon after the processes of fermentation and/or microbial respiration, which make it more soluble in water (Santos et al., 2022a). Higher concentrations of solids in a culture system can cause accumulation of organic matter in the gills of cultivated shrimp, which can affect the diffusion of oxygen and suppress the growth of organisms (Gaona et al., 2011; Schveitzer et al., 2013), requiring the control for different concentrations according to the shrimp growth phase.

The concentrations of settleable solids were not different between the forms of artificially salinized, however they were higher than the concentrations with seawater, mainly in the LCMS and CS treatments, due to the application of minerals, similar to the data observed by Oliveira et al. (2022b), in synbiotic with oligohaline water. These results are probably related to the lower solubility of these ions in water, as well as the increase in pH, which hinders the solubility of some ions, especially calcium carbonate (Boyd, 2020).

Regarding the zootechnical performance of *P. vannamei* juveniles, we observed significant differences in survival between treatments, with the lowest survival in LCSM (70%). Expressive result in relation to those observed by Pinto et al. (2020), who compared only mineral fertilizers and obtained a low survival (15%) compared to artificial sea salt (81%). The application of *Lithothamnium* (marine algae fossils), which has some important macro and micro minerals for shrimp, probably contributed to the survival of close to 70% in

the LCSM treatment, and its application may be increased in future evaluations, since artificial sea salts have a series of micro-constituents and trace elements (Atkinson and Bingman, 1997), which contribute to better survival (Pinto et al., 2020).

The final weight was significantly higher in the low salinity treatments (LSCM, CD to SD) when compared to seawater, which may be related to the higher survival rates found in the same treatment (SW). However, the other variables of zootechnical performance were similar without the effect of the form of **artificially salinized**, demonstrating the possibility of producing juveniles in low salinity synbiotic system. The final weight and productivity results were similar to those observed by Pimentel et al. (2022), with marine water diluted to oligohaline water (3 g L^{-1}), with and without the correction of ionic ratios (Ca:Mg:K), by Oliveira et al. (2022b) with diluted marine water (3 g L^{-1}), with different frequencies of correction of the ionic ratios (Ca:Mg:K) and by Brito et al. (2016) with marine water with plankton application as a food supplement.

The FCR is an important variable from an economic point of view in the production of juveniles, and the values observed were satisfactory for intensive nurseries, in the different forms of artificially salinized, even in the LCSM treatment with lower survival, since it was compensated by the final weight. FCR values at low salinity can usually be higher, due to the energy expenditure that shrimp use for osmoregulation, needing to use diets with higher carbohydrate contents or application of mineral supplements (Roy and Davis, 2010b). However, in the system of minimum water exchange as the synbiotic in conditions of low salinity, the complementation with natural food has contributed to maintain the values in adequate ranges (Pimentel et al., 2022; Oliveira et al., 2022b).

The genus *Vibrio* is present in the shrimp gut in different farming systems, contributing negatively to food digestion and mortality (Fan et al., 2019a; Fan et al., 2019b; Fan and Li, 2019c; Gao et al., 2019). These bacteria are pathogenic and opportunistic and can

cause disease and mortality in shrimp when they are physiologically debilitated (Valente and Wan, 2021).

In a system with minimal water exchange, *Vibrio* spp. have their development favored due to the large amount of organic matter present in the crop cycle (Ferreira et al., 2011; Yanong and Erlaher-Reid, 2012). This increase in organic matter is very common due to the high C:N ratios used for the development of heterotrophic bacteria (Avnimelech, 2007), in addition to the carbon sources such as the hexose, glucose and fructose sugars used (Emerenciano et al., 2017).

Thus, in a synbiotic system, we can reduce the proportion of *Vibrio* in shrimp, as rice bran (prebiotic) used as a source of organic carbon contributes to the supply of beneficial microorganisms (probiotics), and can therefore alter the host's microbiota, increasing competition with pathogens and production of enzymes and organic acids, in addition to the reduction of intestinal pH (Huynh et al., 2017; Dawood and Koshio, 2020), which can be used as a preventive tool to control *Vibrio* (Valente and Wan, 2021). However, artificially salinized of water does not seem to have a direct influence on *Vibrio* spp. concentration in the shrimp gut when compared to seawater, probably due to the use of the feed with the application of commercial probiotic and the synbiotic system.

As well as bacteria, the presence of fungi (filamentous and yeasts) is observed in synbiotic systems (water and hepatopancreas) of shrimp, however with yeast dominance (de Andrade et al., 2021). These data are similar to the observed in this study, with a synbiotic system and application of probiotics via nutrition, with no significant effect of salinity and the type of artificially salinized. This dominance of yeasts in different forms of artificially salinized and seawater is important, because yeasts have a high protein value and are rich in B vitamins, with an important effect on the digestive system, helping the functioning of the gut,

and playing a role in the defense of the organism against pathogens (Agboola et al., 2021), as the health of shrimp is closely linked to the gut microbiota (Holt et al., 2021).

Shrimp can be in constant challenge in intensive systems in relation to nitrogen compounds, until the bacterial community is stabilized, or even when the amounts of feed used are rapidly increased, generating toxic compounds more quickly. According to Perazollo et al. (2002), we can use total hemocyte count (THC) as a health indicator for shrimp, as its concentration responds to factors such as ecdysis, environmental stress or infections. Furthermore, higher concentrations of circulating hemocytes in the hemolymph indicate lower effects of environmental stress or infection (Le Moullac et al., 1998).

After 96 hours of the ammonia stress test, the juveniles showed 100% survival, with reduced circulating THC in the hemolymph, indicating an effect of N-NH_3 levels on the immune response. However, with the survivals obtained, we can infer that the shrimp tolerated the evaluated concentrations and the exposure time well, even though the N-NH_3 values were approximately 3 to 4 times higher than the safety limit (concentrations equivalent to 10% of the PL_{50}) for *P. vannamei* (post-larvae and juveniles) in low salinity (0.12 mg N-NH_3 L^{-1}; Valencia-Castaneda et al., 2018) and higher salinity (0.16 mg N-NH_3 L^{-1}; Lin and Chen, 2001).

Conclusion

The different forms of artificially salinized used in the present study were efficient for the maintenance of the main water ions. Regarding the production of *P. vannamei* juveniles in nurseries with a synbiotic system in artificially salinized water, they obtained similar zootechnical results and resistance to ammonia stress when compared to seawater. Regarding the presumptive count of bacteria in TCBS (*Vibrio* spp.) the use of the synbiotic and the

commercial probiotic in the feed were efficient to keep the concentrations low, however the artificially salinized increased the participation of *Vibrio* spp.

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Credit author statement

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Declaration of competing interest

548 The authors declare that they have no known competing financial interests or personal
549 relationships that could have appeared to influence the work reported in this paper.
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