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Gênison Carneiro Silva

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Resumo

O cultivo do camarão branco do pacífico *Litopenaeus vannamei* tem sido realizado em áreas litorâneas, com altos custos de implantação em razão do elevado valor das terras, além de enfrentar problemas com a redução da produtividade devido ao surgimento de doenças. Diante dessa dificuldade, vários esforços têm sido desenvolvidos para o cultivo de camarão marinho em águas interiores de baixa salinidade. As águas destas regiões afastadas da costa são provenientes de lagos, poços, rios, açudes e aquíferos subterrâneos, apresentando grande diversidade em sua composição iônica (cátions e aniôns). Entretanto, estes íons importantes no cultivo precisam que suas proporções sejam ajustadas para valores semelhantes aos da água do mar, para que os camarões tenham um bom desenvolvimento zootécnico. A compensação iônica realizada por meio de fertilizantes minerais na água demanda um alto investimento, uma alternativa para redução desses custos é a utilização de sistemas de mínima troca d'água, que oferecem uma redução no descarte de água e oferecem uma maior biosseguridade ao cultivo e reaproveitamento dos minerais. Portanto, esse estudo teve por objetivo realizar um cultivo na fase de engorda para camarão marinho *L. vannamei* em sistema simbiótico durante 60 dias, em unidades experimentais de 60 L, com densidade de 416 camarões/m³. Quatro tratamentos em triplicata por meio de um delineamento inteiramente casualizado foram estabelecidos: SD - Seawater diluted; LCSM - Low cost salt mixture with freswater; CS - Commercial salt e SW - Seawater. Foi realizado um estudo prévio sobre a eficiência minerais quanto os íons disponibilizados na água de cultivo e posteriormente realizada a salinização para obtenção da salinidade de 2,5 g/L e as proporções dos cátions e ânions. Um substrato artificial de conchas de *Anomalocardia brasiliana* (um porcento do volume total da unidade experimental) e um inóculo inicial de água proveniente de um berçário em sistema de simbiótico (5%) foram adicionados nas unidades experimentais para auxiliar no processo de oxidação dos compostos nitrogenados do sistema. Um mix de bactérias a base de *Bacillus* foi utilizado na água como fertilizando e misturado a ração ofertada aos animais. A análise de composição proximal foi realizada ao término e, também, foram realizadas no início e fim do experimento, análises microbiológicas para contagem total de *Vibrio spp.* e *Bacillus spp.*. A estratégia utilizada (substrato artificial, inoculo e sistema simbiótico) mostrou-se efetivo no controle dos compostos nitrogenados. Os valores de cálcio, magnésio e dureza total se mantiveram acima de 35, 90 e 400 mg/L, respectivamente em todos os tratamentos. Os principais íons tenderam a aumentar ao longo cultivo, em especial o potássio, ocasionando uma redução da relação Na:K (6,28; 8,72; 7,48) proporcionando valores abaixo de 10:1, que somados aos valores de alcalinidade total abaixo de 100 mg $CaCO_3$/L, podem ter ocasionado redução da sobrevivência nos tratamentos de baixa salinidade. As diferentes formas de salinização não influenciaram no ganho de peso, peso final e taxa de crescimento específico dos camarões em baixa salinidade em relação a água do mar. A produtividade, fator de conversão alimentar e sobrevivência foram similares nos tratamentos em baixa salinidade, mas foram inferiores em relação a água marinha. 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. Quanto a composição proximal dos animais e floco microbianos, não se observou efeito da forma de salinização, mas sim da salinidade sobre os mesmos. Com base nos resultados obtidos, para se obter um melhor desempenho no cultivo de *L. vannamei* em águas interiores de baixa salinidade é necessário ajuste na relação Na:K e manutenção da alcalinidade acima de 100 mg/L.

Palavras-chave: baixa salinidade; cultivo intensivo; simbiótico, proporção iônica.

Abstract

The Pacific white shrimp *Litopenaeus vannamei* culture has been carried out in coastal areas, with high implantation costs due to the high value of the land, in addition to facing problems of reduced productivity due to the emergence of diseases. Faced with this difficulty, several efforts have been made for the marine shrimp culture in low salinity inland waters. The waters of these regions away from the coast come from lakes, wells, rivers, dams and underground aquifers, presenting great diversity in their ionic composition (cations and anions). However, these important ions in the culture need their proportions to be adjusted to values similar to those of seawater, so that the shrimp have a good zootechnical development. The ionic compensation carried out through mineral water fertilizers demands a high investment, an alternative to reduce these costs is the use of systems of minimum water exchange, which offer a reduction in the disposal of water and offer greater biosecurity to the culture and reuse of the minerals. Therefore, this study aimed to evaluate the effect of different forms of artificial salinization on the zootechnical performance and proximate composition of *L. vannamei* in the grow-out phase in a synbiotic system, during 60 days in experimental tanks with 60L, with of density 416 shrimp/m^3. Four treatments in triplicate using a completely randomized design were established: SD - Seawater diluted; LCSM - Low cost salt mixture with freshwater; CS - Commercial salt e SW - Seawater. A previous study was carried out on the efficiency of the ions and later the salinization was carried out taking into account the salinity of 2.5 g/L and the proportions of cations and anions. An artificial substrate of *Anomalocardia brasiliana* shells in an initial inoculum of water from a nursery in a synbiotic system (5%) was added to the experimental units to aid in the oxidation process of the nitrogenous compounds in the system. Proximate composition analysis was performed at the end and microbiological analyzes for *Vibrios spp*. and *Bacillus spp.*.The strategy used (artificial substrate, inoculum and synbiotic system) proved to be effective in controlling nitrogen compounds. The values of calcium, magnesium and total hardness remained above 35, 90, 400 mg/L, respectively. The main ions tended to increase along the culture, especially potassium, this increase caused a reduction in the Na:K ratio (6.28; 8.72; 7.48) providing values below 10:1, adding to total alkalinity values below 100 mg $CaCO_3$/L may have reduced survival in low salinity treatments. The different forms of salinization did not influence the weight gain, final weight and specific growth rate of shrimp in low salinity in relation to seawater. Yield, feed conversion rate and survival were statistically equal in treatments at low salinity, but were different in relation to marine water. There was no influence of the different forms of salinization on the intestinal microbiota of shrimp and as for the proximate composition of the animals and microbial floc, there was no effect of the form of salinization, but of the salinity on them. Based on the results obtained, it can be concluded that it is feasible to culture *L. vannamei* in low salinity inland waters with adjustments in the Na:K ratio and maintenance of alkalinity above 100 mg/L.

Keywords: low salinity; intensive culture; synbiotic.

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1- **Introdução**

A produção mundial de crustáceos provenientes da aquicultura no ano de 2018 foi de 9,3 milhões de toneladas, sendo o camarão branco do pacífico *Litopenaeus vannamei* o mais cultivado, com produção de 4,9 milhões de toneladas, onde representa 52,9% dos crustáceos cultivados no mundo (FAO, 2020). Tal produção se deve a boa aceitação no mercado consumidor e ótimas características zootécnicas como, rápido crescimento, tolerância a altas densidades de estocagem, capacidade de osmorregulação e resistência às variações ambientais, como salinidade e temperatura (BRIGGS et al., 2004; XU et al., 2014; LIN e CHEN, 2003; ESPARZA-LEAL et al., 2016).

O cultivo do camarão branco do pacífico tem sido realizado em áreas litorâneas, com altos custos de implantação em razão do elevado valor das terras, além de enfrentar problemas de redução da produtividade devido ao surgimento de doenças (FONSECA et al., 2009; EMERENCIANO et al., 2013). A legislação ambiental desse tipo de empreendimento em países como Brasil e Estados Unidos é rígida, o que dificulta a aquisição das licenças para práticas da atividade (MCGRAW, 2002; DAVIS et al., 2002). Diante dessa dificuldade, vários esforços tem sido desenvolvidos para o cultivo de camarão marinho em águas interiores de baixa salinidade (BOYD, 2006).

De acordo com Keren (2000) mais de 100 países possuem solos salinos e suas águas superficiais possuem mais de 1 g/L de salinidade, no qual permite que o cultivo do *L. vannamei* ocorra em águas com baixa salinidade. Roy et al. (2010) relatam que estados como Flórida, Texas e Arizona nos EUA, possuem fazendas produzindo camarões há mais de 10 anos, já no Brasil**,** Nunes & Lopes (2001) relatam cultivos de camarão em água de poço oligohalina desde os anos 2000. Atualmente, a utilização de sais para salinização das águas de cultivo vem se tornando cada vez mais frequente, incentivado pelo sucesso dessa prática em países como a Tailândia, que salinizou seus viveiros com a adição de uma solução de salmoura (ROY et el., 2010).

De acordo com a Organização das Nações Unidas para a Alimentação e a Agricultura (FAO) (2020), a produção mundial de crustáceos provenientes da aquicultura interior teve um incremento de 5,7% entre os anos de 2000 e 2018, representado principalmente pelos camarões, lagostins e caranguejo. No Brasil em 2014, a produção interiorana na Região Nordeste de *L. vannamei* representou entre 10 a 15 % da produção nacional, realizada por mais de 500 micros, pequenos e médios produtores, ocupando uma área de aproximadamente 1.000 hectares de viveiros (ROCHA, 2014).

A água utilizada no cultivo do *L. vannamei* em regiões afastadas da costa são provenientes de lagos, poços, rios, açudes e aquíferos subterrâneo (BOYD e THUNJAI, 2003). Normalmente, doces ou salgadas, essas águas apresentam uma grande diversidade em sua composição iônica (cátions e ânions) fazendo com que alguns tipos de água sejam adequadas para o cultivo do camarão branco do Pacífico (BOYD e THUNJAI, 2003; DAVIS et al., 2004; ROY et al., 2009).

Mesmo contendo íons importantes no cultivo, as águas interiores ainda precisam que suas proporções sejam ajustadas para valores semelhantes aos da água do mar, para que os camarões tenham um bom desenvolvimento zootécnico (BOYD e THUNJAI, 2003), pois a deficiência de íons importantes para o metabolismo do camarão como por exemplo potássio (K^+), influencia diretamente na sobrevivência dos animas, uma vez que desempenha um papel na ativação da enzima Na^+, K^+-ATPase. Essa enzima é responsável pelo transporte ativo de íons através da membrana celular, permitindo a manutenção da homeostase do metabolismo celular, regulando o volume celular e melhorando a captação de glicose e aminoácidos (NAIK, 2012). A compensação desses íons pode ser realizada pelo ajuste das proporções entre Mg:Na, Ca:Mg e Ca:K. A reposição desses cátions e ânions na água é feita por meio da aplicação de fertilizantes minerais de origem agrícola, que auxiliam no aumento do crescimento e sobrevivência do camarão cultivado em água de baixa salinidade (ROY e DAVIS, 2010; MAICÁ, 2012).

A compensação iônica realizada por meio de fertilizantes minerais demanda um alto investimento, uma vez que os protocolos atuais de correção do perfil iônico requerem mais de uma aplicação ao longo do cultivo, e ainda há a dificuldade de descarte de efluentes salinizados no meio ambiente. Outro ponto importante é a toxicidade dos compostos nitrogenados em água de baixa salinidade. Estudos relatam que existe uma relação entre a redução da salinidade com o aumento da toxicidade desses compostos, quanto menor a salinidade maior toxicidade pela amônia e nitrito (VALENCIA-CASTAÑEDA et al. 2019).

Segundo Samocha et al. (2017), uma maneira de otimizar a reciclagem de nutrientes na água é por meio da utilização de sistemas de mínima troca d'água, que oferecem uma redução no descarte de água e oferece uma maior biosseguridade ao cultivo. Esses sistemas associados a salinização artificial são uma importante ferramenta para o desenvolvimento do cultivo de camarões longe da costa, uma vez que possibilita a retenção dos sais no sistema e permite sua reutilização por vários ciclos. Outro ponto importante é que o uso dessas técnicas associadas reduz o custo com o transporte de água das regiões costeira para o

interior, para a realização dos cultivos. Além disso, essa reutilização dos efluentes permite uma redução de íons como o magnésio, na formulação da salga, tornando ainda menos oneroso essa forma de cultivo (DAVIS et el., 2002).

Dentre os sistemas fechados de troca mínima de água, o de biofloco é o mais conhecido, devido a sua assimilação dos compostos nitrogenados tóxicos pela comunidade de bactérias heterotróficas e nitrificantes através da manutenção da adequada proporção carbono e nitrogênio (C:N) na água, e a formação de flocos que servem de alimento para o camarão, complementando sua dieta (AVNIMELECH, 1999; EMERENCIANO et al., 2013). Os flocos são importantes fontes de proteína, contêm quantidades importantes de macronutrientes e micronutrientes assim como aminoácidos e ácidos graxos. A importância desse aglomerado microbiano não se limita apenas a alimentação, pois além da reciclagem dos nutrientes e manutenção da qualidade de água, o floco pode atual com um "probiótico natural", pois as bactérias presentes no biofloco podem exercer um biocontrole efetivo contra ectoparasitos e *Vibrio sp*. (EMERENCIANO et al., 2013).

Porém, esse sistema requer um sistema eficiente para suprir a alta demanda de oxigênio para suprir a consumo dos diversos organismo ali presentes e manter os flocos em suspensão, (AVNIMELECH, 2009), além da necessidade de manter controlados os sólidos suspensos, os quais em altas concentrações podem acarretar o entupimento de brânquias dos animais e deterioração na qualidade da água (SCHVEITZER et al., 2013).

Desta forma, outros sistemas derivados dos bioflocos, vem sendo utilizados com o intuito de reduzir o problema do excesso de sólidos suspensos, proporcionar um maior equilíbrio entre as comunidades bacterianas heterotróficas e nitrificantes, gerando uma melhor eficiência da assimilação do nitrito e redução da demanda de oxigênio dissolvido do sistema (DE ANDRADE et al., 2022; PIMENTETEL et al., 2022). O sistema simbiótico adotada uma nova estratégia de fertilização orgânica através da fermentação e respiração microbiana do carbono orgânico com microrganismos probióticos (ROMANO et al., 2018). Esse sistema permitir um uso mais eficiente de carboidratos polissacarídeos, como o farelo de trigo ou arroz, a partir dos processos anaeróbicos e/ou aeróbicos permitindo o crescimento diversificado de agregados microbianos, compostos por fitoplâncton, zooplâncton, bactérias autotróficas e heterotróficas (ROMANO et al., 2018; PIMENTETEL et al., 2022; SANTOS et al. 2022).

Portanto, estudos relacionados ao sistema simbiótico com mínima troca de água e a adequação iônica da mesma (processo de salinização), para o desenvolvimento de modelo

de cultivo que tenha dependência mínima ou não da água oceânica e estuarina, é uma importante etapa para expansão do cultivo de camarões marinhos em águas interiores.

1.1- Objetivos

Objetivo Geral

Avaliar o efeito de diferentes formas de salinização artificial, no desempenho zootécnico e composição centesimal do *L. vannamei* na fase de engorda em sistema simbiótico.

Objetivos específicos

- Avaliar a influência das diferentes formas de salinização da água sobre o desempenho zootécnico dos camarões cultivados;
- Analisar as diferentes formas de salinização da água na composição centesimal dos flocos microbianos e do *L. vannamei*;
- Determinar a contagem presuntiva total de *Vibrio* spp. e *Bacillus* no intestino do camarão cultivado nas diferentes formas de salinização da água;
- Avaliar as variáveis físico-químicas de qualidade de água ao longo do cultivo com diferentes formas de salinização da água.

1.2- Hipótese

O *L. vannamei* cultivado na fase de engorda em águas salinizadas artificialmente em sistemas simbióticos de baixa salinidade, apresentará desempenho zootécnico similar aos cultivados em águas marinhas.

2- Artigo científico

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Effects of different forms of artificially salinized in low-salinity water of *L. vannamei* in the grow-out phase in a synbiotic system

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Abstract

This study aimed to evaluate the effect of different forms of artificially salinized on the zootechnical performance, bacterial counts in shrimp intestinal and proximate composition of *L. vannamei* in the grow-out phase in a synbiotic system, during 60 days in experimental tanks with 60 L, with a density of 416 shrimp m^{-3}. Four treatments in triplicate using a completely randomized design were established: SD - seawater diluted; LCSM - low-cost salt mixture; CS - commercial salt mixture; and SW - seawater. The values of calcium, magnesium and total hardness remained above 35, 90, 400 mg L^{-1}, respectively. The main ions tended to increase along the culture, especially potassium, which caused a reduction in the Na:K ratio, providing values below 10:1, adding to total alkalinity values below 100 mg $CaCO_3$ L^{-1} that may have reduced survival in low salinity treatments. The different forms of artificially salinized did not influence the final weight and specific growth rate, however yield, FCR and survival were lower in low-salinity as compared to seawater. There was no influence of the different forms of artificially salinized on TCBS and MYP bacterial count values, shrimp proximate composition and microbial floc. Based on the results obtained, it can be concluded that it is feasible to culture *L. vannamei* in intensive synbiotic low-salinity water with adjustments in the Na:K ratio and maintenance of total alkalinity above 100 mg L^{-1}.

Keywords: Ionic composition, Growth, *Bacillus*, *Vibrios*, Proximate composition

1. Introduction

The culture of Pacific white shrimp *Litopenaeus vannamei* occurs mainly in coastal regions using water of estuarine and oceanic origins. However, the emergence of diseases such as White Spot Disease (WSD) has brought great damage to the sector and a variety of social, economic and environmental issues, leading producers to seek areas further and further away from the coast to develop their activities (Davis et al., 2002). Added to this, increasingly onerous costs and difficulties in environmental licensing in coastal regions have also led to culture migrating to inland waters (MCGraw, 2002; Davis et al., 2002).

According to the Food and Agriculture Organization of the United Nations (FAO) (2020), world production of crustaceans from inland aquaculture increased by 5.7% between 2000 and 2018, mainly represented by shrimp, crayfish and crab. Brazil in 2014 had the inland production of *L. vannamei* in the Northeast region representing between 10% and 15% of the national production, carried out by more than 500 micro, small and medium farmings and occupying an area of approximately 1,000 hectares of ponds (Rocha, 2014).

These cultures of *L. vannamei* in regions far from the coast use water from lakes, wells, rivers, dams and underground aquifers (Boyd and Thunjai, 2003). Usually oligohaline or mesohaline, these waters present important ions (Ca^{+}, Mg^{+}, K^{+}, SO_4^{-}, HCO_3^{-}, Cl^{-}) for *L. vannamei*, allowing their culture (Boyd and Thunjai, 2003; Davis et al., 2004; Roy et al., 2009).

Even though inland waters contain important ions in culture, some sources still need their proportions adjusted to values similar to those of seawater, so that the shrimp may have a good zootechnical performance (Boyd and Thunjai, 2003), because ion

deficiency directly influences the survival of shrimp. For instance, potassium plays a role in the activation of the enzyme Na^+,K^+-ATPase, which is responsible for the active transport of ions across the cell membrane, allowing the maintenance of cellular metabolism homeostasis, regulating cell volume and improving glucose and amino acid uptake (Naik, 2012). The compensation of these ions can be performed by adjusting the proportions between Mg:Na, Ca:Mg and Ca:K. The replacement of these cations and anions in the water is done through the application of mineral fertilizers of agricultural origin, which help to increase the growth and survival of shrimp culture in low-salinity water (Roy and Davis, 2010; Maicá, 2012).

Despite the possibility of using inland waters and/or artificially salinized of the water, there is an environmental concern with this type of culture in relation to soil salinization and the source of water supply. Therefore, systems of minimum water exchange have been shown to be a viable alternative. This type of system can avoid soil salinization problems, since geomembranes are used in the soil covering or suspended tanks, in addition to reducing the costs of transporting water from coastal regions to the inland (Samocha et al., 2017).

The synbiotic is a system that can be used in semi-intensive and intensive shrimp systems with minimal water exchange, and consists of an organic fertilization strategy through fermentation and microbial respiration of organic carbon with probiotic microorganisms (Romano et al., 2018). It has been used in studies for the culture of *L. vannamei* in low salinity water and seawater, and also for *Macrobrachium rosenbergii*, proving to be effective in controlling nitrogen compounds, retaining minerals and as a source of supplementary food for shrimp and prawn farming (Andrade et al., 2022; Pimentel et al., 2022; Santos et al., 2022).

This system allows a more efficient use of polysaccharide carbohydrates, such as wheat or rice bran, from anaerobic and/or aerobic processes, allowing the diversified growth of microbial aggregates, composed of phytoplankton, zooplankton, and autotrophic and heterotrophic bacteria (Romano et al., 2018; Pimentel et al., 2022; Santos et al. 2022), in addition to improving the nutritional quality of microbial aggregates after anaerobic and/or aerobic processes (Romano et al., 2018; Santos et al., 2022).

Therefore, studies related to the process of artificially salinized in synbiotic systems with minimal water exchange are important for the development of a farming model that has minimal or no dependence on oceanic and estuarine water, being an important step for the expansion of intensive marine shrimp farming in inland waters. Thus, the objective is to evaluate the effect of different forms of artificially salinized on zootechnical performance, water quality, microbial and proximate composition in low-salinity water of *L. vannamei* in the grow-out phase in an intensive synbiotic system.

2. Material and methods

2.1 Experimental design and system

The study was carried out for 60 days at the Shrimp Farming Laboratory, at the Fisheries and Aquaculture Department of the Federal Rural University of Pernambuco, Brazil. Four treatments in triplicate using a completely randomized design were established: SD - seawater diluted; LCSM - low-cost salt mixture; CS - commercial salt mixture and SW - seawater.

The post-larvae (PL10 ~ 3.0 mg) were acquired in a commercial hatchery (Aquatec,

RN, Brazil) at a salinity of 20 g L^{-1}. The batch was divided into two tanks with a useful volume of 800 liters (7,500 PLs m^{-3}), where a part of the PLs was acclimated to a salinity of 2.5 g L^{-1} and the other to a salinity of 35 g L^{-1} for 10 days. Afterwards, the shrimp were kept under aeration (dissolved oxygen > 5 mg L^{-1}) and constant temperature (30°C; Hopar Sh-608 heater 100 w), until they reached ~2 g for the start of the experiment.

During the shrimp maintenance period, until reaching ~2 g weight, they were fed a commercial feed of 40% crude protein, 11% crude lipids, 4% crude fiber, and 14% mineral matter (Guabitech Inicial, Guabi, Brazil), four times a day (8 am, 11 am, 2 pm and 5 pm). Initially, a feeding rate of 33% was adopted, which was gradually reduced to 5.3% of the biomass after 60 days.

After this process, juvenile shrimp (~2.0 g) were stocked at a density of 416 shrimp m^{-3} (25 animals per experimental unit) in experimental units with a useful volume of 60 L (0.06 m^{-3}) and an artificial substrate (14 x 14 x 3.2 cm) corresponding to 1% of the volume of the experimental units, made with *Anomalocardia brasiliana* shells, and kept under constant aeration (dissolved oxygen > 5 mg L^{-1}), constant temperature (30°C; Hopar Sh-608 heater 100 w) and natural photoperiod. No exchange of water was carried out during the entire experiment and de-chlorinated freshwater was added to replace evaporation loss four times a week.

At this stage, the shrimp were fed a commercial pelleted feed of 35% crude protein, 11% crude lipids, 4% crude fiber, and 14% mineral matter (Guabitech Inicial J, Guabi, Brazil), plus a mix of commercial probiotic (Colony Forming Units (CFU) g^{-1} containing: *Bacillus subtilis* (8.5 x 10^8 CFU g^{-1}), *Bacillus licheniformis* (8.5 x 10^8 CFU g^{-1}), *Bacillus pumilus* (5.0 x 10^8 CFU g^{-1}), *Bacillus toyoi* (8.0 x 10^8 CFU g^{-1}), *Bacillus amyloliquefaciens* (8.5 x 10^8 CFU g^{-1}), *Lactobacillus acidophilus* (3.7 x 10^8 CFU g^{-1}), *Lactobacillus*

plantatarum (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, three times a day (8 am, 1 pm and 5 pm).

The initial feed rate was 5.3%, which was gradually reduced to 2.6% of the biomass over a 60 day period. Both feed rates were calculated according to Van Wyk (1999), taking into account the results of biometrics, feed leftovers and shrimp mortality. Alkalinity correction with sodium bicarbonate ($NaHCO_3$) was also performed every 10 days after water analysis to reach values > 100 mg L^{-1}.

2.2 *Salinization adjustment*

Prior to the salinization of the treatments, previous studies were carried out on the efficiency of increasing ions in the water from the application of chemical products. The tests were carried out 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).

Location of the table 1

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 2.5 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., 2010).

Location of the table 2

Location of the table 3

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.

2.3 Water fertilization

Twenty-one days before stocking the shrimp, four matrix tanks (one tank for each treatment) were fertilized with three-day intervals between each application. This synbiotic was obtained through an anaerobic (24 h) and aerobic (24 h) process, for a mixture of 10 g m^{-3} of rice bran (< 200 μm), 2 g m^{-3} of sugar, 0.05 g m^{-3} of commercial bacterial mix (*Bacillus subtilis* (2.2 x 10^9 CFU g^{-1}), *Bacillus licheniformis* (1.8 x 10^9 CFU g^{-1}), *Bacillus* sp. (1.6 x 10^9 CFU g^{-1}), sodium chloride (NaCl) and magnesium hydroxide ($Mg(OH)_2$) (Kayros Agrícola and Ambiental, SP, Brazil), 1 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 and three artificial substrate (14 x 14 x 3.2 cm) in each matrix tanks corresponding to 1% of the volume of the experimental units (60 L), made with *A. brasiliana* shells was used.

After this period, the fertilized water and artificial substrate was added to the experimental units according to their respective treatments. During the experimental time, the synbiotic was added at 5 g m^{-3} of rice bran (< 200μm), 0.5 g m^{-3} of sugar, 0.5 g m^{-3} of sodium bicarbonate and 0.05 g m^{-3} of commercial bacterial mix), application three times a week until settleable solids reached 5 ml L^{-1} and when the settleable solids exceeded applications were suspended, and with the aid of settling chamber, reduced to levels below 5 ml L^{-1} to avoid clogging of shrimp gills.

2.4 Water Quality

Dissolved oxygen and temperature (Asko multiparameter meter, model AZ86031) were monitored twice a day (8:00 am and 4:00 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.

2.5 Presumptive total count on Thiosulphate Citrate Bile Sucrose - TCBS (Vibrio spp.) and Mannitol Agar Egg Yolk Polymyxin - MYP (Bacillus spp.)

Presumptive total count were performed at the Aquatic Animal Health Laboratory/Fishery and Aquaculture Department of the Rural Federal University of Pernambuco for the quantification of the colony-forming units (CFUs g^{-1}) of *Vibrio* sp. and *Bacillus* sp..

Shrimp samples for analysis in TCBS (*Vibrio* spp.) and MYP (*Bacillus* spp.) were collected at the start (pools of five shrimp from low-salinity water and seawater - gut samples), and at the end of the experiment (pools of five shrimp - gut samples each

treatment), with three replications of each treatment. The shrimp were euthanized by cutting the nerve cord, and their external body surface was disinfected by immersion in 70% ethanol (15 seconds), followed by a solution of sodium hypochlorite (1.5%) with 0.1% tween-80 (15 minutes), and then washed in sterile distilled water three times.

The intestines were deposited in sterile crucibles and 600 µL of sterile alkaline peptone water solution was added, followed by maceration and homogenization of the samples. Afterwards, they were serially diluted from 10^{-1} to 10^{-5} and 100 µL were seeded in the plates containing the culture media Thiosulfate Citrate Bile Sucrose (TCBS) and Mannitol Egg Yolk Polymyxin Agar (MYP), with the aid of an "L" loop, and then incubated at 30°C for 24 hours in triplicate. After the incubation period, colonies of sucrose-fermenting bacteria, non-sucrose-fermenting and *Bacillus* spp. (CFU g^{-1}) were counted using a colony counter.

2.6 Zootechnical performance

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

Biomass gain (g) = final biomass (g) − initial biomass (g);

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/biomass gain;

Survival (%) = (number of individuals at the end of evaluation period/initial number of individuals) × 100;

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

2.7 Proximate composition

Moisture, crude protein, crude lipid and ash analyzes for shrimp samples and microbial floc were performed in triplicate according to the AOAC (2012) at the end of experimental time. The microbial floc samples were collected with a cylindrical mesh net (50 μm) for the retention of solids and whole body of the shrimp samples were washed with distilled water to remove encrusted material for the proximate composition analysis.

The samples were oven-dried at 60°C. For moisture content, samples were oven-dried at 105°C for 24 h until reaching a constant weight (model TE - 393, Tecnal, São Paulo, Brazil). The difference in weight before and after drying was recorded and expressed as percentage. Protein content was determined by measuring nitrogen (N x 6.25) using the Kjeldahl method (TE 0363/180L model; Tecnal, São Paulo, Brazil). Total lipid content was determined using the Soxhlet extraction method with pure hexane (98%) as the solvent (model TE - 1881/6, Tecnal, São Paulo, Brazil). Ash was determined by oven incineration at 550°C (Q318 D24 model; Quimis, São Paulo, Brazil).

2.8 Data analysis

Data analyzes were performed using the IBM SPSS Statistic version 25.0 software. Data were tested for normality using the Shapiro-Wilk test ($p < 0.05$), and for homoscedasticity using the Levene test ($p < 0.05$). Subsequently, a one-way ANOVA was applied for the zootechnical performance and proximate composition and a repeated measures ANOVA ($p \leq 0.05$) was applied for dissolved oxygen, temperature, pH, K^+ followed by Tukey post-hoc test ($p \leq 0.05$) for the comparison of means. Non-parametric data were analyzed using the Friedman's test with Conover's multiple comparison test with Holm–Bonferroni correction for salinity, TAN, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, settleable solids, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{-2}, Na^+, magnesium hardness, calcium hardness, TH and TA for the comparison of median. For *Vibrio* and *Bacillus* counts at the beginning were analyzed using the non-parametric Mann-Whitney test (seawater x low salinity) and at the end of experimental time were analyzed using the non-parametric the Kruskal-Wallis test ($p \leq 0.05$) followed by Dunn's post-hoc test ($p \leq 0.05$).

3. Results

3.1 Water Quality

Data on water quality variables are summarized in Table 4. No significant differences were observed for TAN, dissolved oxygen (DO) and temperature. However, significant differences ($p \leq 0.05$) were observed for salinity, pH and settleable solids (SS). The highest value of SS was observed in the CS treatment (4.74 ± 3.54 ml L^{-1}), and the lowest, in the SW treatment (1.22 ± 1.52 ml L^{-1}). The highest concentration of $N\text{-}NO_2^-$ was found in the SW treatment (2.41 ± 2.96 mg L^{-1}), and the lowest, in the CS treatment (0.24 ± 0.25 mg L^{-1}).

Location of the table 4

Calcium and calcium hardness concentrations did not differ between treatments at artificially salinized in low-salinity water ($p > 0.05$), being different only in relation to treatment with seawater. The other variables showed significant differences between the artificially salinized in low-salinity water treatments and also regarding the SW treatment (Table 5). Potassium showed an increase in its concentrations throughout the culture time, and among the artificially salinized in low-salinity water treatments, the highest value observed was in the LCSM treatment (125.49 mg L^{-1} ± 67.04), and the lowest, in the CS (89.02 mg L^{-1} ± 46.66), whereas the total alkalinity values remained close to 100 mg $CaCO_3$ L^{-1} with the exception of the CS treatment, which presented 83.14 mg $CaCO_3$ L^{-1}. There was an increase in the concentrations of ions in relation to the concentrations measured at the beginning of the experiment (Table 5).

Location of the table 5

3.2 Presumptive total count for TCBS - Vibrio spp. and MYP - Bacillus spp.

At the beginning, the highest values the presumptive *Vibrio* counts were observed in shrimp from seawater (128.7 ± 95.4 x 10^4 CFU g^{-1}), and the lowest, in shrimp from low-salinity water (59.7 ± 64.2 x 10^4 CFU g^{-1}), with the highest percentage of sucrose-fermenting bacteria. At the end of the culture time, an increase in non-sucrose-fermenting was observed in all treatments. The presumptive *Vibrio* counts at the end of the culture time ranged from 233.9 ± 268.4 x 10^4 CFU g^{-1} (LCSM) to 42.1 ± 34.7 x 10^4 CFU g^{-1} (SD), however there were no significant differences between treatments (Figure 1).

At the beginning, the highest values presumptive *Bacillus* spp. counts were observed in shrimp from low-salinity water (555.5 ± 530.0 x 10^5 CFU g^{-1}), and the lowest, in shrimp from seawater (15.2 ± 14.9 x 10^5 CFU g^{-1}), differing significantly from each other. The presumptive *Bacillus* spp. counts at the end of the culture time ranged from 186.6 ± 135.0 x 10^5 CFU g^{-1} (CS) to 70.2 ± 43.2 x 10^5 (SD), however there were no significant differences between treatments (Figure 1).

Location of the figure 1

3.3 Shrimp Zootechnical performance

The zootechnical variables of final weight and specific growth rate (SGR) did not show significant differences ($p > 0.05$) (Table 6). However, significant differences ($p \leq 0.05$) were observed between treatments for shrimp survival, where the SW treatment had the highest survival (96.00 ± 6.92%) and LCSM had the lowest (64.00 ± 4.00%). As for the FCR, the SW treatment resulted in the lowest value (1.46 ± 0.11), with CS being the treatment with the highest value (2.27 ± 0.21) ($p < 0.05$). Yield (kg m^{-3}) in the LCSM, CS and SD treatments (2.14 ± 0.21; 2.11 ± 0.24; 2.12 ± 0.21 kg m^{-3}, respectively) did not differ from each other, but were significantly lower in relation to SW (3.19 ± 0.20 kg m^{-3}) ($p \leq 0.05$).

Location of the table 6

3.4 Proximate composition

The proximate composition of shrimp and microbial floc at the end of the

culture time are shown in Table 7. Shrimp moisture and crude protein showed no significant differences between treatments. However, the other results indicate an influence of the form of artificially salinized in low-salinity water on the shrimp and microbial floc proximate composition.

Location of the table 7

4. Discussion

The variables temperature, dissolved oxygen and pH remained within the ideal values the shrimp species (Van Wyk and Scarpa, 1999; Samocha, 2019). In systems with minimal water exchange, greater attention should be given to dissolved oxygen, due to the large consumption by the microbial community (Ebeling et al., 2006). The levels of dissolved oxygen during the experiment were always above 5.0 mg L^{-1}, and the concentrations obtained were not limiting for the good development of shrimp and bacteria in the culture system.

According to Ebeling et al. (2006), heterotrophic and nitrifying bacteria require carbonate and bicarbonate ions for the oxidation of nitrogen compounds, which can lead to a reduction in total alkalinity and, consequently, a drop in pH levels. The pH values, despite showing a significant difference between treatments, remained within the recommended for the shrimp, not showing large fluctuations throughout the experimental period (Boyd, 2001).

The total alkalinity values were higher than 80 mg $CaCO_3$ mg L^{-1}, which are recommended for autotrophic systems (Van Wyk and Scarpa, 1999), however, in more intensive systems bacteria require higher total alkalinity values (>100 mg

$CaCO_3\ L^{-1}$) (Avnimelech et al., 2015), as it improves conditions for microbial activity and increases the availability of carbon dioxide, phosphorus and other nutrients (Ferreira et al., 2011).

Furthermore, Pimentel et al. (2021) found significant positive correlations of total alkalinity and calcium on final weight and specific growth rate, explained by the consumption of large amounts of calcium by the animal during the exoskeleton mineralization process (Boyd & Tucker, 1998). This shows the importance of adjusting the total alkalinity and calcium to minimum levels in water with low-salinity water, in order to obtain a good development of the shrimps during the productive cycles (Samocha, 2019).

Corrections with sodium bicarbonate ($NaHCO_3$) were performed every ten days to try maintain values above 100 mg $CaCO_3\ L^{-1}$, however, in artificially salinized in low-salinity water the average values of total alkalinity were below 100 mg $CaCO_3\ L^{-1}$, indicating the need for a smaller alkalinity adjustment interval in a system with higher stocking density, mainly due to the greater conversion of nitrogen compounds into low-salinity, when compared to seawater treatment, due to the processes of nitrification and ammonia absorption by nitrifying and heterotrophic bacteria.

Due to high stocking densities, uneaten food remains, high biomass, and accumulation of organic matter during culture, concentrations of nitrogen compounds in intensive systems tend to increase (Furtado et al., 2011). In all treatments in this study, TAN and $N\text{-}NO_2$ levels were maintained within the ideal levels for seawater (TAN < 3 mg L^{-1} and $N\text{-}NO_2$ < 10 mg L^{-1}) and for artificially salinized in low-salinity water (TAN < 0.81 mg L^{-1} and $N\text{-}NO_2$ < 0.45 mg L^{-1}), as recommended by Samocha

(2019) and Valencia-Castañeda et al. (2018).

The increase in $N\text{-}NO_3$ concentrations in relation to TAN and $N\text{-}NO_2$ in the LCSM, CS and SD treatments indicates the presence of nitrifying bacteria (Luo et al., 2013). The SW treatment did not show an increase in nitrate in relation to nitrite like the other treatments, which is related to a smaller community of nitrifying bacteria. Many nitrifying bacteria have a greater capacity for growth and transformation in nitrogen compounds at low-salinity water, as they do not require high concentrations of salts (Costa and Campos, 2015). Increased nitrate concentrations in synbiotic systems with low-salinity water were also observed by Pimentel et al. (2022).

These results indicate the efficiency of the combination of organic carbon input (anaerobic and aerobic from rice bran with probiotic bacteria), which, combined with the use of 5% inoculum with artificial substrate, proved to be efficient for the control of nitrogen compounds in the culture of *L. vannamei* in the grow-out for the different forms of artificially salinized in low-salinity water used in this study.

High concentrations of settleable solids can cause accumulation of organic matter in the gills of shrimp, which can affect the diffusion of oxygen and suppress the growth (Gaona et al, 2011). The values of settleable solids in this study, due to the use of settler chamber, remained below the critical limit of 14 ml L^{-1} (Samocha et al., 2019), with averages ranging from 1.22 to 4.74 ml L^{-1}.

As for ions, it was possible to observe an increase between the start and the end of the experimental period. The main ion for growth and survival as well as osmoregulation is potassium, which associates with sodium to form the most important relationship for ionic balance (Roy et al., 2007). According to Sowers et al.

(2006), the ratio between sodium and potassium in natural marine water is approximately 28:1, and changes in up to 10 points do not interfere with the osmoregulatory capacity of *L. vannamei.* The increase in potassium values throughout the culture caused a reduction in the Na:K ratio, providing values below 10:1, which may have had a negative effect on the enzyme Na^+, K^+-ATPase, which is responsible for the active transport of ions across the cell membrane. This enzyme allows the maintenance of cellular metabolism homeostasis and improves the uptake of glucose and amino acids. These changes, in addition to the total alkalinity values below 100 mg $CaCO_3\,L^{-1}$, may have caused a reduction in survival in low-salinity water treatments. Studies show that it is possible to have good survival with lower Na:K rations, however, higher values of total alkalinity are necessary. Pimentel et al. (2022) in low-salinity water in the *L. vannamei* nursery observed Na:K ratios below 20:1 and alkalinity above 100 mg L^{-1}, and Esparza-Leal et al. (2009) in the *L. vannamei* grow-out found Na:K ratios between 8.61 and 185:1, with total alkalinity values between 115.70 and 314.6 mg $CaCO_3\,L^{-1}$.

Calcium and magnesium are other important ions for osmoregulation and ecdysis processes, directly influencing survival. Their values remained stable during the culture time, even without the addition of mineral fertilizers and with the consumption of these ions during ecdysis, since at this stage there is a high demand for carapace formation (Huong et al., 2010). This stabilization of calcium and magnesium concentrations throughout the experiment is probably related to the addition of rice bran used as a source of organic carbon and the use of *A. brasiliana* substrate in the system, as reported by Pimentel et al. (2022).

The different forms of artificially salinized in low-salinity water used were

efficient in maintaining the main ions. However, the increase of these ions, especially potassium, caused an imbalance between the main ionic ratios for the culture of *L. vannamei* in low-salinity water. The increase in potassium throughout the experimental period may be related to the rice bran-based synbiotic offered throughout the experiment, since rice bran is rich in potassium.

Minimal water exchange systems can favor the development of pathogenic and opportunistic bacteria such as the genus *Vibrio*, due to the large amount of organic matter accumulated during culture (Ferreira et al., 2011; Yanong and Erlacher- Reid, 2012). However, there was a predominance of *Bacillus* in the intestine of shrimp, with no significant effect of salinity and the form artificially salinized in low-salinity water. The predominance in *Bacillus* concentrations can be explained by the application of the *Bacillus*-based probiotic added to the feed and water (Synbiotic) throughout the culture. Bacteria of the genus *Bacillus* have the ability to colonize the gastrointestinal tract of shrimp, controlling the proliferation of *Vibrio* spp., through the production of inhibitory compounds (natural antibiotics), competition for nutrients, adhesion sites, and the secretion of digestive enzymes, making the animals more resistant to infections caused by *Vibrio* (Liu et al., 2009; Ninawe & Selvin, 2009; Nakayama & Nomura, 2009; Sapcharoen and Rengpipat, 2013).

The zootechnical performance of final weight and SGR did not differ significantly between the SW treatment and the artificially salinized in low-salinity water (CS, LCSM and SD), however, there was a lower survival in the artificially salinized in low-salinity water treatments, causing a reduction in the yield. Despite the reduction in survival observed in the LCSM, CS and SD treatments, these results are expressive when compared

with the survival found by Maicá et al. (2012), who obtained a survival of 22% in an intensive system of minimum water exchange at a salinity of 2 g L^{-1}, and by Pinto et al. (2020) who obtained 12% and 56% survival for salinities of 1 and 5 g L^{-1} in the culture of *L. vannamei*, with a stocking density of 250 shrimp m^{-3} in the growt-out.

Survival directly influences FCR and yield, which can be observed by the results of these zootechnical parameters in the treatments artificially salinized at low salinity water, compared to the seawater treatment. However, the FCR values of this study were similar to those found by Maicá et al. (2014), who evaluated the performance of *L. vannamei* juveniles at low salinity in a intensive minimum water exchange system with an FCR of 2.14 and 2.26 for salinities of 4 and 16 g L^{-1}, respectively. In order to improve the zootechnical results in artificially salinized at low salinity water, it is necessary to monitor and adjust the Na:K ratios more frequently, as well as increase the levels of total alkalinity during the entire culture time, since higher stocking biomass seems to require more bicarbonate and carbonate ions for the processes of osmoregulation. According to Maicá et al. (2014), there is a positive correlation of increasing concentration of total alkalinity and total suspended solids on the growth and survival of shrimp at low-salinity water. However, it can be observed that survival was not affected by the different forms of artificially salinized, since there were no significant differences between treatments.

As for the proximate composition of this study, some literatures report (Huang et al., 2004 and Liang et al., 2008) the influence of salinity on the protein, lipids and moisture of *L. vannamei*, with an increase in this composition as salinity increases. Protein levels for shrimp, despite not showing significant differences, were numerically higher in the seawater treatment compared to those with artificially

salinized low-salinity water, and these values are close to those found by Khanjani et al. (2019). The lipid values found for shrimp were higher in treatments LCSM and SD, which corroborates the studies by Li et al. (2007) and Liang et al. (2008), who reported a decrease in the lipid index of *L. vannamei* juveniles with increasing salinity. The ash content for the shrimp may have suffered the effect of salinity, as reported by Huang et al. (2004), where SW was the treatment with the highest value, however, the amounts of ash were similar to those found by Maicá et al. (2014) and Khanjani et al. (2019). According to Khanjani et al. (2019), the increase in ash levels found in shrimp in systems with the presence of flocs is related to the continuous availability of minerals and trace elements, in particular the phosphorus present in the floc.

The proximate composition of the flocs showed that the protein values tend to be higher with decreasing salinity. Similar results were obtained by Ju et al. (2008), who collected floc from the culture of juveniles of *L. vannamei* from systems with minimal water exchange and open tanks at 5, 18 and 32 g L^{-1} of salinity and found that the microbial aggregates formed at the lowest salinity had a greater protein content than those formed at higher salinities, also corroborating the results found by Maicá et al. (2014) and Khanjani et al. (2019), who observed higher levels of floc proteins formed at lower salinities.

The literature reports a variety of findings on the lipid content of microbial aggregates. The amounts of lipids found in this study between 3–5% of dry matter are similar to those found by Silva et al. (2013) in *L. vannamei* cultures in marine water BFT systems, but higher than those found by Maicá et al. (2012) with a stocking density of 250 shrimp m^{-3} in the grow-out phase at salinities of 0, 2, 4 and 25 g L^{-1}.

The ash values found in the microbial flocs of this study were similar to those observed in the works by Wasielesky et al. (2006) and Silva et al. (2013), following the trend of higher percentages of ash in higher salinities. Thus, it can be observed that there was an influence of salinity on the shrimp and microbial floc proximate composition, but it cannot be said that this influence is related to the form of artificially salinized low-salinity water used in this study.

5. Conclusion

The artificially salinized low-salinity water used in this study are similar regarding the shrimp zootechnical performance at high stocking densities in synbiotic systems, but lower to shrimp cultured in seawater. Thus, adjustments in the total alkalinity and in the Na:K ratio to improve the zootechnical performance indices in intensive systems (> 400 shrimp m^{-3}) are necessary. The use of an initial inoculum of 5% with a synbiotic system based on rice bran (anaerobic and aerobic), with probiotic bacteria and artificial substrate *Anomalocardia brasiliana* (1% of volume), is efficient in controlling nitrogen compounds. Water salinity and forms of artificially salinized low-salinity water did not influence the predominance of *Bacillus* in relation to *Vibrio* in the intestine of shrimp, however, there was an influence of salinity on the proximate composition of shrimp and microbial floc, but it cannot be said that this influence is related with the form of artificially salinized low-salinity water used in this study.

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Declaration of competing interest

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

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

	Fertilizers	**KCl**	**MgSO47H2O**	**MgCl26H2O**	***Lithothamnium***	**$NaCl^{-}$**
% of target ion increment	$pH^{\#}$	0.41 ± 0.04	0.40 ± 0.07	0.17 ±0.01	0.0 ± 0.0	-
	Ca^{2+}	-	-	-	26.7 ± 1.8	-
	K^{+}	51.10 ± 11.38	-	-	-	-
	Mg^{2+}	-	9.40 ± 0.51	10.52 ± 1.37	5.2 ± 2.0	-
	Na^{+}	-	-	-	-	39.42 ± 0.08
	Cl^{-}	-	-	-	-	60.33 ± 0.02

Data correspond to the mean of two replicates ± standard deviation. KCl: potassiumchloride; $CaCO_3$: calcium carbonate; $MgSO_47H_2O$: magnesium sulfate heptahydrate; $MgCl_26H_2O$: magnesium chloride hexahydrate. $^{\#}$pH in absolute value.

Table 2. Fertilizers used in the artificially salinized low-salinity water used in the of *L. vannamei* grow-out in synbiotic system.

Treatment	Inputs	Ions made available
CS - commercial salt mixture	commercial salt mixture (0.83 g m^{-3}) (Veromix) and coarse salt (1.66 g m^{-3}).	Sodium, magnesium, potassium, strontium, chlorides, carbonates, bicarbonates, borates,sulfates, bromides, fluorides and trace elements
LCSM - Low-cost salt mixture	Coarse salt (1.95 g m^{-3}), potassium chloride (0.052 g m^{-3}), *Lithothamnium* (0.96g m^{-3}), magnesium chloride (0.466 g m^{-3}), magnesium sulfate (0.515 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 of *L. vannamei* grow-out in synbiotic system.

Variables	Treatments			
	LCSM	**CS**	**SD**	**SW**
Ca^{2+} (mg L^{-1})	24.00 ± 9.00	28.8 ± 6.40	38.40 ± 5.27	580 ± 17.09
Mg^{2+} (mg L^{-1})	92.34 ± 8.02	83.59 ± 9.26	102.06 ±13.02	1,395 ± 69.55
K^{+} (mg L^{-1})	17.94 ± 4.04	18.83 ± 5.16	17.87 ± 3.07	278.30 ± 12.50
Na^{+} (mg L^{-1})	626.86 ± 34.66	705.22 ± 10.02	685.63 ± 41.88	13,061.09 ± 68.77
$SO4^{2-}$ (mg L^{-1})	245.13 ± 12.12	225.20 ± 40.70	261.80 ± 23.94	3,730.00 ± 45.30
Cl^{-} (mg L^{-1})	1,134.10 ± 42.01	1,276.20 ± 26.59	1,240.75 ± 38.97	20,156.00 ± 33.18

Total alkalinity (mg L^{-1})	120.00 ± 5.00	90.00 ± 4.36	95.00 ± 2.65	130.00 ± 5.29
Total hardness (mg L^{-1})	440.00 ± 29.82	416.00 ± 7.81	516.00 ± 7.81	7,000.00 ± 87.40
Salinity (g L^{-1})	2.40 ± 0.20	2.50 ± 0.26	2.70 ± 0.10	34.46 ± 0.35
Mg:Ca	3.84	2.90	2.65	2.40
Mg:K	5.14	4.43	5.71	5.01
Ca:K	1.33	1.52	2.14	2.08
Na:K	34.94	37.45	38.36	46.93
DT:Alc	3.66	4.62	5.43	53.84
Error (%)	7.84	7.39	4.26	10.23

Data correspond to mean (n = 3) ± standard deviation. SD - seawater diluted; LCSM - low-cost salt mixture; CS - commercial salt mixture and SW - seawater.

Table 4. Water quality variables in the artificially salinized low-salinity water and seawater used in the of *L. vannamei* grow-out in synbiotic system.

Variables	Treatments			
	LCSM	**CS**	**SD**	**SW**
Temperature (ºC)	30.79 ± 0.71^{aA}	30.78 ± 0.83^{aA}	30.87 ± 0.84^{aA}	30.80 ± 0.92^{A}
Oxygen (mg L^{-1})	6.47 ± 0.89^{aA}	6.48 ± 0.88^{aA}	6.47 ± 0.81^{aA}	6.19 ± 0.77^{B}
Salinity (g L^{-1})	2.74 ± 0.22^{aB}	2.63 ± 0.15^{bB}	2.62 ± 0.17^{bB}	34.72 ± 1.00^{A}
pH	8.50 ± 0.20^{aA}	8.29 ± 0.22^{bB}	8.34 ± 0.25^{bB}	8.22 ± 0.40^{B}
TAN (mg L^{-1})	0.75 ± 0.75^{aA}	0.66 ± 0.61^{aA}	0.70 ± 0.64^{aA}	0.61 ± 0.65^{A}
N-Nitrite (mg L^{-1})	0.28 ± 0.28^{aA}	0.24 ± 0.25^{aA}	0.32 ± 0.32^{aA}	2.41 ± 2.96^{B}

N-Nitrate (mg L^{-1})	2.42 ± 2.17^{aA}	3.17 ± 4.05^{aA}	2.76 ± 3.46^{aA}	1.27 ± 1.72^{B}
Suspended solids (mL L^{-1})	3.75 ± 2.04^{aA}	4.74 ± 3.54^{aA}	3.70 ± 2.79^{aA}	1.22 ± 1.52^{B}

Data correspond to mean ± standard deviation. The results were analyzed by means of a repeated measuresANOVA ($p \leq 0.05$) 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. The average values on the same line with different lowercase superscripts did not take into account the SW treatment, while the uppercase superscripts consider all treatments. SD - seawater diluted; LCSM - low-cost salt mixture; CS - commercial salt mixture and SW - seawater.

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

Variables	Treatment			
	LCSM	**CS**	**SD**	**SW**
TH (mg $CaCO_3$ L^{-1})	494.32 ± 44.96^{aB}	434.74 ± 39.64^{bC}	476.84 ± 35.11^{aAB}	7,652.63 ±796.77^{A}
TA (mg $CaCO_3$ L^{-1})	99.43 ± 24.50^{aA}	83.14 ± 25.04^{bA}	92.29 ± 23.22^{abA}	106.00 ± 35.26^{A}
Calcium hardness (mg $CaCO_3$ L^{-1})	97.47 ± 18.72^{aB}	103.37 ± 16.07^{aB}	100.21 ± 16.78^{aB}	1,372.11 ± 380.65^{A}
Magnesium hardness (mg $CaCO_3$ L^{-1})	396.84 ± 47.75^{aB}	331.37 ± 41.95^{bC}	376,63 ± 35,68^{aBC}	6,209.47 ± 775.87^{A}
Ca^{2+} (mg L^{-1})	38.98 ± 7.48^{aB}	41.34 ± 6.42^{aB}	40.08 ± 6.71^{aB}	565.26 ± 152.58^{A}
Mg^{2+} (mg L^{-1})	96.43 ± 11.60^{aB}	80.52 ± 10.19^{bC}	91,52 ± 8,67^{aBC}	1,508.78 ± 188.61^{A}
Cl^{-} (mg L^{-1})	1,426.37 ± 157.93^{aB}	1,271.53 ± 44.15^{bB}	1,272.46 ± 79.83^{bB}	21,705.80 ± 1,904.30^{A}
Na^{+} (mg L^{-1})	788.21 ± 87.27^{aB}	702.64 ± 24.39^{bB}	703,16 ± 44,11^{bB}	14,065.40 ± 1,293.38^{A}
K^{+}(mg L^{-1})	125.49 ± 67.04^{aB}	89.02 ± 46.66^{bC}	93,92 ± 48,74^{bC}	594.76 ± 313.86^{A}
SO_4^{2-} (mg L^{-1})	446.94 ± 102.94^{bB}	409.53± 106.25^{bB}	481,59 ± 162,62^{aAB}	2,276.75 ± 851.30^{A}
Mg:Ca	2.47	1.94	2.28	2.66

Mg:K	0.76	0.90	0.97	2.53
Ca:K	0.31	0.51	0.42	0.95
Na:K	6.28	8.72	7.48	23.64
TH:TA	4.97	5.22	5.16	72.19
Erro (%)	8.42	10.36	11.64	13.56

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 and Friedman's test and Conover's multiple comparison test ($p \leq 0.05$) for non-parametric data. The average values on the same line with different lowercase superscripts did not take into account the SW treatment, while the uppercase superscripts consider all treatments. SD - seawater diluted; LCSM - low-cost salt mixture; CS - commercial salt mixture and SW - seawater. TH - total hardness; TA - total alkalinity

Table 6. Zootechnical performance from artificially salinized low-salinity water and seawater used in the of *L. vannamei* grow-out in synbiotic system.

Parameters	Treatments			
	LCSM	**CS**	**SD**	**SW**
Final weight (g)	8.46 ±1.23^{a}	8.42 ±1.45^{a}	7.97 ± 1.04^{a}	8.01 ± 0.65^{a}
Survival (%)	64.00 ± 4.00^{b}	65.33 ± 4.61 ^{b}	69.33 ± 12.85^{b}	96.00 ± 6.92^{a}
FCR	2.14 ± 0.32^{ab}	2.27 ± 0.21^{a}	2.23 ± 0.40^{a}	1.46 ± 0.11^{b}
Yield (kg m^{-3})	2.14 ± 0.21^{b}	2.11 ± 0.24^{a}	2.12 ± 0.21^{b}	3.19 ± 0.20^{a}
SGR (%/day)	3.18 ± 0.35^{a}	2.70 ± 0.91^{a}	3.01 ± 0.40^{a}	3.01 ± 0.22^{a}

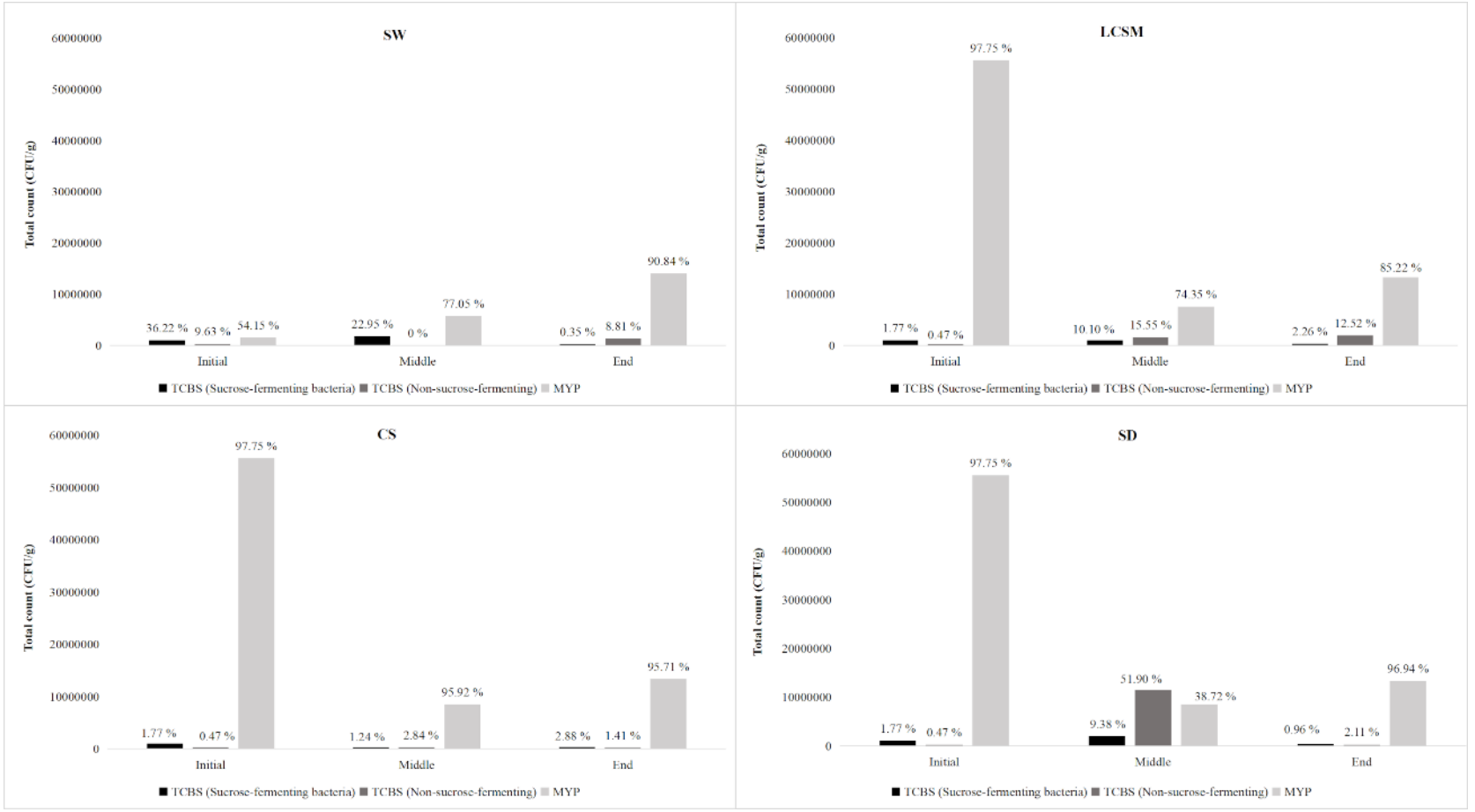
Data correspond to mean ± standard deviation. The results were analyzed by means of ANOVA ($p \leq 0.05$) followed by the Tukey test. Mean values on the same line with different superscripts differ significantly. SD - seawater diluted; LCSM - low-cost salt mixture; CS - commercial salt mixture and SW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

Table 7. Proximate composition (dry matter weight) of shrimp and microbial floc from artificially salinized low-salinity water and seawater used in the of *L. vannamei* grow-out in synbiotic system.

Proximate Composition		Treatments			
		LCSM	**CS**	**SD**	**SW**
Shrimp	Moisture (%)	74.34 ± 0.80[a]	74.12 ± 0.16[a]	73.68 ± 0.29[a]	74.13 ± 0.25[a]
	Lipids (%)	9.42 ± 0.70[a]	8.32 ± 0.25[b]	9.75 ± 0.23[a]	7.47 ± 0.06[b]
	Protein (%)	76.80 ± 0.71[a]	77.53 ± 0.72[a]	74.36 ± 4.46[a]	82.12 ± 1.93[a]
	Ash (%)	11.99 ± 0.06[b]	11.41 ± 0.04[c]	12.00 ± 0.14[b]	12.58 ± 0.13[a]
Microbial floc	Moisture (%)	85.46 ± 2.06[a]	84.33 ± 0.66[a]	86.76 ± 0.60[a]	83.03 ± 2.06[a]
	Lipids (%)	5.14 ± 0.62[b]	7.27 ± 0.49[a]	4.91 ± 0.12[b]	3.97 ± 0.43[b]
	Protein (%)	28.72 ± 0.13[a]	30.34 ± 0.08[a]	30.84 ± 1.40[a]	20.62 ± 0.10[b]
	Ash (%)	49.00 ± 0.49[b]	50.25 ± 0.08[b]	50.28 ± 0.09[b]	60.30 ± 0.15[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.

Figure 1. Total bacterial count in TCBS - *Vibrio* spp. and MYP - *Bacillus* spp. from artificially salinized low-salinity water and seawater used in the of *L. vannamei* grow-out in synbiotic system. SD - seawater diluted; LCSM - low-cost salt mixture; CS - commercial salt mixture and SW - seawater.



3- Considerações finais

As estratégias de salinização artificial usadas neste estudo são similares em relação ao desempenho zootécnico de camarões marinhos cultivados em altas densidades em sistemas simbióticos de baixa salinidade, porém inferiores aos camarões cultivados na água do mar. Desta forma, ajustes na alcalinidade total e na relação Na:K para melhorar os índices de desempenho zootécnico em sistemas superintensivos (> 400 camarões/m^3) são necessários.

Uso de inoculo inicial de 5% com o uso de sistema simbiótico com farelo de arroz (anaeróbio e aeróbio) com bactérias probióticas e substrato artificial *Anomalocardia brasiliana* (1 % do volume) são eficientes no controle dos compostos nitrogenados.

A salinidade e formas de salinização artificial utilizadas neste estudo não influenciaram na predominância de *Bacillus* em relação a *Vibrios* no intestinos dos camarões, Entretanto, pode-se perceber que houve influência da salinidade sobre a composição proximal do camarão e floco microbiano, porém não se pode afirmar que essa influência tem relação com a forma de salinização utilizada neste estudo.

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