UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

EFEITO DO AJUSTE IÔNICO EM ÁGUA DE BAIXA SALINIDADE NO DESEMPENHO ZOOTÉCNICO E COMPOSIÇÃO CENTESIMAL DO *Litopenaeus vannamei* CULTIVADO EM SISTEMA SIMBIÓTICO

CAIO RUBENS DO RÊGO OLIVEIRA

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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RECIFE,

Fevereiro/2022

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Dedico este trabalho a todos que confiam em mim, principalmente a minha mãe, Regina Célia e minha tia, Roseneide Augusta.

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Resumo

O cultivo de camarão marinho em águas interiores ocorre em diferentes salinidades e perfis iônicos em todo o mundo. Apesar da contribuição desta modalidade na aquicultura, diversos obstáculos são observados para a realização de um ciclo de cultivo completo. Este estudo teve como objetivo a avaliação de diferentes composições iônicas em água oligohalina sobre o desempenho zootécnico e composição centesimal do *Litopenaeus vannamei*, cultivado em sistema simbiótico. O experimento foi realizado durante 56 dias (densidade: 300 camarões/m³) em unidades experimentais de 60 L, em um delineamento experimental inteiramente casualizado, sob três tratamentos: PI – água na salinidade ~ 2,7 g/L com sua relação Ca:Mg:K ajustada para 1:3:1, C1 (controle) – água na salinidade ~ 2,7 g/L e C2: água na salinidade ~ 2,7 g/L com correção de magnésio (Mg^{2+}) equivalente a proporção a água do mar, todos em triplicata. Os indivíduos atingiram um peso final (g) de 3,79 ± 0,07 – 4,72 ± 0,09 e uma produtividade (Kg/m³) de 0,61 ± 0,14 - 1,22 ± 0,06, apresentando diferenças significativas entre os tratamentos. O melhor resultado em peso médio final e produtividade foram observados nos tratamentos C1 e C2, por outro lado, o tratamento PI apresentou os menores resultados, isso pode ser influenciado pela adição de cloreto de potássio (KCl) para aumentar a concentração de potássio (K^{+}), resultando em uma redução da razão Na:K. Em relação à qualidade da água, foi observado um aumento da alcalinidade total (mg $CaCO_3$/L) em todos os tratamentos durante o experimento, também foram observadas baixas concentrações de compostos nitrogenados, o que pode ter sido acarretado pelo sistema simbiótico e o uso de substratos artificiais compostos por conchas de *Anomalocardia brasiliana*, que funcionaram como substrato para bactérias e uma fonte de carbonato de cálcio. A composição centesimal dos camarões não apresentou diferença significativa entre os tratamentos, porém, a composição dos flocos microbianos apresentou diferenças significativas nas cinzas (%). A relação Na:K mostrou uma relação positiva com o desempenho zootécnico dos camarões neste estudo. Nossos resultados indicam que juvenis de camarão apresentam desempenho satisfatório em água oligohalina em sistema simbiótico.

Palavras-chave: Baixa salinidade; Perfil iônico; Composição bioquímica; Flocos microbianos; Sistema intensivo.

Abstract

Shrimp farming in inland waters is carried out under different salinity and ionic water profiles worldwide. Despite the contribution of this culture modality, several obstacles are observed to accomplish a complete farming cycle. This study aimed the evaluation of different ionic compositions in oligohaline water on the zootechnical performance and proximal composition of *Litopenaeus vannamei* reared in synbiotic system. A 56-day trial (300 shrimp/m³) was performed in 60 L experimental units in a completely randomized experimental design, under three treatments: IP – water at a salinity ~2,7 g/L with its Ca:Mg:K ratio adjusted to 1:3:1, C1 (control) - water at a salinity ~2,7 g/L, and C2 - water at a salinity ~2,7 g/L with a magnesium (Mg^{2+}) adjusted seawater equivalent concentration, all in triplicates. The individuals reached a final weight (g) of 3.79 ± 0.07 – 4.72 ± 0.09 and an average yield (kg/m³) of 0.61 ± 0.14 - 1.22 ± 0.06, showing significant differences among treatments, the best overall growth and average yield were observed at C1 and C2, in the other hand PI treatment presented the worst results, this may be influenced due to potassium chloride (KCl) addition to increase potassium (K^+) concentration, resulting in a Na:K ratio reduction. Regarding the water quality, an increase of total alkalinity (mg $CaCO_3$/L) was observed in all low salinity treatments during the experiment, also, low concentrations of nitrogenous compounds were observed, this may be accomplished due to the presence of artificial substrates composed by *Anomalocardia brasiliana* shells, that worked as a substrate for bacteria and a calcium carbonate source. Shrimps' proximal compositions did not show significant differences among treatments, however, microbial floc composition presented significant differences in ashes (%). Na/K ratio showed a positive relationship to shrimps zootechnical performance in this study. Our results indicate that shrimp juveniles present satisfactory performance in oligohaline water in synbiotic system.

Key Words: Low salinity; Ionic profile; Proximal composition; Microbial flocs; Intensive system

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Sumário

1. Introdução

O camarão branco do pacífico *Litopenaeus vannamei* (Boone, 1931) é a espécie de camarão marinho mais produzida no mundo. No ano de 2018 a produção da espécie representou 52,9% da produção total de crustáceos, atingindo 4,9 milhões de toneladas, com um incremento de 22,8% em relação à produção do ano de 2016 (FAO, 2020). Esse incremento na produção de crustáceos também se deve pela produção do camarão marinho em zonas interioranas, em água oligohalina e mesohalina, visto que a produção em zonas litorâneas tem bastante conflitos relacionados ao uso de terras e de água com agências ambientais. Dessa forma o cultivo em zonas interiores vem sendo considerado como alternativa para uma contínua expansão da carcinicultura (FAO, 2020). Segundo Jaime-Ceballos et al. (2012), outro fator que auxilia na expansão desta atividade para zonas interiores é o aspecto sanitário, visto que nelas não ocorrem a presença de outros crustáceos marinhos, que são vetores de doenças virais como a mancha branca (WSSV), síndrome de Taura (TS) e a doença da cabeça amarela (YHV). Entretanto, nos últimos anos observa-se o aumento nos casos de surtos de doenças em águas interiores.

O *Litopenaeus vannamei* é nativo da costa norte do Peru ao noroeste do México e por ser uma espécie eurialina, pode tolerar altas variações de salinidade, desde 0,5 até 60 g/L (DAVIS et al., 2004; ESPARZA-LEAL et al, 2010; ROY et al., 2010; CHONG-ROBLES et al., 2013, JAFFER et al., 2019). Aliado à adaptação do cultivo em diferentes salinidades, o sucesso do *L. vannamei* está atrelado a sua alta taxa de crescimento, grande aceitação no mercado consumidor, tolerância a altas densidades de estocagem e a variações ambientais (LIN e CHEN, 2003; RACOTTA et al., 2003; BRIGGS et al., 2004; XU e PAN., 2014). Essas qualidades, conferem ao *L. vannamei* como espécie preferida nos cultivos de camarões marinhos em águas de baixa salinidade, seja esta advinda de rios, lagos, poços e afloramentos subterrâneos. A implementação deste cultivo auxiliou no desenvolvimento da carcinicultura em diversos países como Brasil, China, Equador, Estados Unidos, México e Venezuela (SAMOCHA et al., 2001; BOYD e THUNJAI, 2003; MIRANDA et al., 2010; NUNES e LÓPEZ, 2001; ROY et al., 2010; GODÍNEZ-SIORDIA et al., 2011; ARUNA e FELIX, 2017).

No Brasil, especialmente no Nordeste, o cultivo do *L. vannamei* é uma importante fonte de renda, principalmente para os produtores rurais (MARQUES et al., 2016). Principalmente quando a atividade ocorre em zonas interiores, auxiliando a retenção de mão de obra local, além de fortalecer e modificar a economia regional. Esta contribuição das zonas interioranas é desenvolvida pela abundância de aquíferos subterrâneos, com mais de 330 mil poços registrados no Serviço Geológico do Brasil e 83 mil destes poços apresentam salinidade próxima a 0,5 g/L (CPRM, 2020), apresentando dessa forma condições para o cultivo do *L. vannamei*.

Embora o interesse no cultivo do *L. vannamei* em água de baixa salinidade esteja em pleno crescimento nos últimos anos, pesquisas relatando o desempenho zootécnico do *L. vannamei* em cultivos utilizando água oligohalina ocorrem desde os anos 90, tendo em vista que nessa década já havia uma preocupação com o custo de terras em zonas costeiras e questões ambientais (BRAY et al., 1994; LARAMORE et al., 2001; SAMOCHA et al., 2004). Os primeiros resultados referem-se ao efeito da idade das pós-larvas, tempo de aclimatação e taxa de redução da salinidade na sobrevivência e desempenho dos camarões (MCGRAW et al., 2002; MCGRAW e SCARPA, 2004; ROY et al., 2009; ESPARZA-LEAL et al., 2010). A partir dos resultados obtidos, diversos estudos apresentaram dados satisfatórios sobre o desempenho do camarão marinho cultivado em ambientes oligohalinos (SANTOS et al., 2009; MAICÁ et al., 2014; ESPARZA et al., 2016; VALENZUELA-MADRIGAL et al., 2017). Em contrapartida, Ray e Lotz (2017), afirmam que ainda há uma falta de informação a respeito do desempenho do *L. vannamei* em sistemas intensivos com diferentes perfis iônicos, demonstrando dessa forma um desafio para novas pesquisas a respeito do cultivo intensivo e superintensivo do camarão marinho em baixa salinidade.

Um dos principais desafios no cultivo do camarão marinho nessas condições diferenciadas, é a determinação dos requisitos iônicos ideais da água, visto que a composição iônica e a salinidade podem variar amplamente entre os locais de cultivo. Além disso, para potencializar o desenvolvimento de espécies marinhas em águas de baixa salinidade, as proporções iônicas devem-se manter semelhantes à água marinha (BOYD e THUNJAI, 2003). A preocupação com a composição iônica na água advém da sua importância no desenvolvimento do camarão, visto que as concentrações dos cátions e ânions, atuam na osmorregulação, sobrevivência, metabolismo e crescimento do *L.*

vannamei (BOYD e THUNJAI, 2003; SAOUD et al., 2003; DAVIS et al., 2005). Por outro lado, o desequilíbrio ou deficiência na relação entre esses íons, proporcionam limitações no desenvolvimento ou até na mortalidade do animal (VALENZUELA-MADRIGAL et al., 2017; NEHRU et al., 2018). Por isso, a partir destes fundamentos científicos, independente da salinidade em que o cultivo é realizado, é imprescindível a observação das concentrações individuais e relativas destes íons, principalmente os íons majoritários que são responsáveis por grande parte dos sólidos totais dissolvidos nas águas naturais (BOYD e THUNJAI, 2003), sendo os cátions: cálcio (Ca^{2+}), magnésio (Mg^{2+}), sódio (Na^{+}) e potássio (K^{+}) e os ânions: cloreto (Cl^{-}), sulfato (SO_4^{2-}), bicarbonato (HCO_3^{-}) e carbonatos (CO_3^{2-}).

Assim, visando à correção da concentração dos íons na água, fertilizantes minerais estão sendo utilizados com o intuito de manter proporções iônicas ideais para o cultivo do *L. vannamei* em sistemas semi intensivos utilizando água de baixa salinidade (ROY e DAVIS, 2010; SUGUNA, 2020). Neste sentido, Aruna e Felix (2017) adicionaram fertilizantes a base de cálcio (Ca^{2+}) e magnésio (Mg^{2+}) em tanques de cultivo a fim de compensar a deficiência desses íons e avaliar o efeito desta adição no crescimento e sobrevivência do *L. vannamei*. Enquanto Liu et al. (2016) utilizaram fertilizantes minerais em águas de poço com baixa salinidade para testar diversas razões de sódio (Na^{+}) e potássio (K^{+}) no crescimento e composição centesimal do *L. vannamei.*

A adição de fertilizantes minerais na água, pode modificar a composição centesimal de camarões peneídeos, pois esta varia de acordo com a salinidade do cultivo (HUANG et al., 2014). Estudos apontam que quanto mais baixa a salinidade maior será a umidade do *L. vannamei* (LIANG et al., 2008; PEREZ-VELAZQUEZ et al., 2007). No entanto, resultados controversos em relação ao conteúdo de proteínas, lipídios e cinzas foram obtidos por Liu et al. (2016) que obtiveram maiores valores destas três características em salinidades mais altas, enquanto Li et al. (2007), Perez-Velazquez et al. (2007) e Liang et al. (2008) observaram um comportamento inverso. Além disso, há também uma falta de conhecimento em como a adição de fertilizantes minerais pode influenciar na composição centesimal do *L. vannamei* em sistemas intensivos.

A utilização dos fertilizantes minerais exige alto investimento financeiro. Segundo Boyd (2018), são necessárias 2 ou 3 aplicações de fertilizantes minerais para manutenção das concentrações iônicas em um cultivo de camarões com duração entre 100 a 160 dias, sendo esta frequência influenciada tanto pelo perfil iônico da água quanto o tamanho do tanque e/ou viveiro de cultivo e as renovações de água. Dessa forma, para otimizar o uso dos fertilizantes minerais e a compensação iônica, é prudente utilizar sistemas com mínima troca de água, visto que estes sistemas reduzem o descarte de água, sendo também um método que oferece maior biosseguridade ao cultivo (WASIELESKY et al., 2006; SAMOCHA et al., 2017).

O sistema de mínima troca de água consiste na reciclagem de parte dos resíduos e/ou nutrientes dos ambientes de cultivo para produção de biomassa microbiana, que ocorre devido à adição de uma fonte de carbono orgânico e inorgânico (EMERENCIANO et al., 2013). A redução na troca de água e a adição do carbono (orgânico e inorgânico) proporciona o surgimento de agregados microbianos que são responsáveis pelo controle da qualidade da água, influenciando diretamente no controle dos compostos nitrogenados (EKASARI et al., 2014; KRUMMENAUER et al., 2014; XU et al., 2016; CARDONA et al., 2016; ESPARZA-LEAL et al., 2016). Desta forma, o sistema simbiótico vem sendo utilizado na aquicultura, provendo a redução na concentração de amônia e o aumento abrupto nos sólidos suspensos na água. Este sistema se baseia na fermentação ou respiração microbiana de carboidratos como, farelos de arroz, soja e trigo, proporcionando a formação de agregados microbianos em sistemas com mínima troca de água, que além de melhorar a qualidade da água, também servem como alimento aos organismos cultivados, auxiliando no crescimento. (ROMANO et al., 2018; LIMA et al., 2021; SILVA et al., 2021, PIMENTEL et al., 2022; SANTOS et al., 2022). Apesar de vários estudos em sistemas semi intensivos e mais informações adquiridas a respeito do cultivo do *L. vannamei* em água oligohalina nos últimos anos, ainda existe uma necessidade de novos estudos em sistemas intensivos, dessa forma, essa pesquisa demonstra-se importante na compreensão de como diferentes composições iônicas podem influenciar no desempenho zootécnico e composição centesimal do camarão marinho em água oligohalina em sistema simbiótico.

1.1. Objetivos

1.1.1. Objetivo Geral

Avaliar o ajuste iônico no desempenho zootécnico e a composição centesimal do *L. vannamei* em água oligohalina no sistema simbiótico.

1.1.2. Objetivos Específicos

- Avaliar o efeito do ajuste iônico nas variáveis físico-químicas da água durante o cultivo em águas oligohalinas;
- Avaliar o índice zootécnico dos camarões cultivados com ajustes iônicos em águas oligohalinas;
- Analisar a composição centesimal do floco microbiano e do *L. vannamei* em cultivo utilizando ajustes iônicos em águas oligohalinas;

2. Hipóteses

- Os ajustes iônicos na água melhoram o desempenho zootécnico do *L. vannamei* em águas oligohalinas.
- Os ajustes iônicos na água alteram a composição centesimal do *L. vannamei*.

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4. Artigo Científico

Os resultados obtidos durante o trabalho experimental desta dissertação estão apresentados no artigo intitulado **“Growth performance and proximal composition of *Litopenaeus vannamei* reared in low salinity water with different ionic composition in a synbiotic nursery system”** (manuscrito), que se encontra anexado.

Artigo científico a ser submetido à Revista: Aquaculture International

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Growth performance and proximal composition of *Litopenaeus vannamei* reared in low salinity water with different ionic composition in a synbiotic nursery system

ABSTRACT

This study aimed the evaluation of different ionic compositions in oligohaline water on the growth and proximal composition of *Litopenaeus vannamei* reared in the synbiotic system. A 56-day trial (300 shrimp/m³) was performed in 60 L units in a completely randomized experimental design, with three treatments (each with three repetitions): C1 – seawater diluted to a salinity ~2.7 g/L (control); IP – seawater diluted to a salinity ~2.7 g/L with Ca:Mg:K ratio adjusted to 1:3:1 and C2 - seawater diluted to a salinity ~2.7 g/L with Mg^{2+} adjusted to approximate seawater equivalent concentration. The shrimps reached a final weight of 3.70 ± 0.07 – 4.72 ± 0.01 g and an average yield of 0.61 ± 0.14 - 1.22 ± 0.06 Kg/m³, showing significant differences among treatments. The best overall growth was observed at C2 treatment. Ionic profile IP treatment presented the worst results, this may be influenced due to Na:K ratio reduction after K^+ corrections. Low concentrations of nitrogenous compounds were observed, accomplished due to the synbiotic system and substrate composed by *Anomalocardia brasiliana* shells, functioning as a substrate for bacteria and a calcium carbonate source. Shrimp's proximal compositions did not show significant differences among treatments. Microbial flocs showed differences in ash, where the PI treatment had a higher percentage of ash than treatments C1 and C2. Na/K ratio showed a positive relationship to shrimp zootechnical performance in this study. Our results indicate that shrimp juveniles present satisfactory performance in oligohaline water in a synbiotic system.

Key words: Oligohaline water; Ionic profile; Biochemical composition; Microbial flocs; Intensive system.

1. Introduction

Shrimp farming production is expanding worldwide, showing an increase of 22.8% in its production between 2016 and 2018. Among the most cultivated crustacean species, the Pacific white shrimp *Litopenaeus vannamei* stands out with a production of approximately 4.9 million tons, equivalent to 52.9% of world production in 2018 (FAO, 2020). Part of this crustacean production (38.9%, equivalent to 3.7 million tons) comes from inland water, data that demonstrate a trend towards the growth of shrimp farming in areas further away from the coast (FAO, 2020). This increase is closely linked to the *L. vannamei* farming, since this species is able to tolerate a wide saline gradient (0.5 to 60 g/L), allowing it to be cultivated in oligohaline and hypersaline waters (Davis et al. 2004; Esparza-Leal et al. 2010; Roy et al. 2010; Chong-Robles et al. 2013; Jaffer et al. 2019).

However, there are obstacles such as the wide variation of water ionic profiles among the culture sites (Boyd and Thunjai 2003; Valenzuela-Madrigal et al. 2017), lack of information about *L. vannamei* performance in intensive and super intensive systems using oligohaline waters with different ionic profiles (Ray and Lotz 2017) and how the water ionic condition can limit the shrimp productive potential in different regions (Valenzuela-Madrigal et al. 2017). Thus, culture using oligohaline water in intensive systems becomes challenging.

The importance of the water ionic composition in the culture of *L. vannamei* comes from the role that cations concentration [Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Potassium (K^{+}) and Sodium (Na^{+})] play in the *L. vannamei* ecdysis, osmoregulation and metabolism. On the other hand, are the anions [bicarbonate (HCO_3^{-}), carbonate (CO_3^{2-}), sulfate (SO_4^{2-}) and chloride (Cl^{-})], where HCO_3^{-} and CO_3^{2-} are closely connected to alkalinity which helps phytoplankton and bacterial communities' maintenance in the culture environment, while SO_4^{2-} and Cl^{-} contribute to osmotic pressure and salinity. However, they play a limited role in physiological

processes when compared to the main cations (Boyd and Thunjai 2003; Saoud et al. 2003; Davis et al. 2005; Antony et al. 2015). In addition, Cl^- presents itself as a fundamental anion in water quality, being responsible for reducing nitrite toxicity, due to it greater affinity for this ion absorption by the gill cells in relation to nitrite (Tomasso 2012).

In this context, mineral fertilizers have been used to maintain the ideal ionic proportions for *L. vannamei* culture in oligohaline waters in semi-intensive systems (Roy and Davis 2010; Suguna 2020). Liu et al. (2016) used mineral fertilizers in well water in order to test different ratios of Na^+ and K^+ on the growth and proximate composition of *L. vannamei* grown in low salinity. While Aruna and Felix (2017), added mineral fertilizers containing Ca^{2+} and Mg^{2+} to the water in order to compensate the deficiency of these cations and evaluate its effect on the growth and survival of *L. vannamei*. In addition, it is necessary to understand the possible changes in the proximal composition of these animals while culture in low salinity, due to variations according to mineral concentrations in the water (Maicá et al. 2014) and possible modifications through the addition of minerals using chemical fertilizers.

However, the addition of mineral fertilizers in the water to increase these ions concentrations requires a high financial investment, requiring two or three applications to maintain ionic concentrations in a *L. vannamei* culture, being application frequency and concentration influenced by cycle duration, pond size, and water ionic profile (Boyd 2018). Therefore, in order to optimize fertilizer application in the water, it is advisable to use systems with minimal water exchange, which provide a reduction in water discharge, in addition to offering greater biosecurity to the crop (Samocha et al. 2017; Abdel-Tawwab et al. 2020; Xu et al. 2021).

Minimal water exchange systems are characterized by their contribution to sediment and/or nutrients cycling, from the production of microbial biomass, which occurs according to

the addition of an organic carbon source (Emerenciano et al. 2013). The reduction in water exchange and carbon addition provides the growth of microbial aggregates that are responsible for maintaining water quality and directly influencing the control of nitrogen compounds (Ekasari et al. 2014; Krummenauer et al. 2014; Xu et al. 2016; Cardona et al. 2016; Esparza-Leal et al. 2016; El-Sayed 2021).

On the other hand, in oligohaline waters, the control of these nitrogen compounds must be performed regularly, due to the greater toxicity of nitrogen compounds [Total ammoniacal nitrogen (TAN) and Nitrite (NO_2^-)] in low salinity (Tomasso, 2012). From this, aiming at greater control of these nitrogenous compounds, the synbiotic system can be used, being characterized by the use of polysaccharide carbohydrates such as rice, soybean and wheat bran, submitted to fermentation processes and/or microbial respiration with *Bacillus*, *Lactobacillus* and yeasts in water fertilization (Lima et al. 2021; Silva et al. 2021; Pimentel et al. 2022; Santos et al. 2022). Thus, the present study aimed to evaluate the zootechnical performance and proximal composition of marine shrimp *L. vannamei* cultured in a synbiotic system, using oligohaline water with different ionic compositions.

2. Material and Methods

2.1. Experimental structures and facilities

This study was conducted for 56 days at the Shrimp Culture Laboratory (LACAR) of the Fisheries and Aquaculture Department of the Rural Federal University of Pernambuco (UFRPE), Brazil. The experimental design was completely randomized with three treatments (all in triplicate): C1 (control) – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1}; IP – (ionic profile adjustment) *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with ionic adjustment to obtain a Ca:Mg:K ratio of 1:3:1; C2 – *L. vannamei* reared in seawater

diluted to salinity of 2.7 gL^{-1} with Mg^{2+} adjusted to a concentration approximately equivalent to that of seawater.

The water used in this study was obtained by diluting seawater (ionic profile: n= 3*:* Ca^{2+} = 437.33 ± 36.95 mgL^{-1}; Mg^{2+} = 1,380.24 ± 25.72 mgL^{-1}; K^{+} = 364.80 ± 4.33 gL^{-1}; Na^{+} = 13,843.37 ± 299.24 gL^{-1}; Cl^{-} = 25,051.33 ± 250.67 gL^{-1}; SO_4^{2-} = 977.87 ± 10.25 gL^{-1}; total alkalinity = 173.33 ± 2.89 mg $CaCO_3L^{-1}$; and total hardness = 6,773.33 ± 180.37 mg $CaCO_3L^{-1}$) in freshwater to a salinity of 2.7 g L^{-1} (Table 1).

Table 1. Initial water ionic compositions of *L. vannamei* reared in a synbiotic system in low-salinity water.

Variables	Treatments		
	IP	C1	C2
Ca^{2+} (mg L^{-1})	43.20 ± 5.76	46.40 ± 11.53	45.33 ± 6.66
Mg^{2+} (mg L^{-1})	132.52 ± 16.39	98.82 ± 11.34	109.70 ± 13.23
K^{+} (mg L^{-1})	37.07 ± 4.28	35.37 ± 4.87	35.16 ± 3.28
Na^{+} (mg L^{-1})	881.50 ± 129.57	799.91 ± 130.43	852.10 ± 110.38
SO_4^{2-} (mg L^{-1})	46.20 ± 3.38	34.19 ± 10.42	113.00 ± 6.71
Cl^{-} (mg L^{-1})	1,595.20 ± 234.47	1,447.00 ± 236.03	1,542.00 ± 199.75
HCO_3^{-} (mg L^{-1})	111.83 ± 12.20	115.90 ± 12.20	95.56 ± 18.63
Total alkalinity (mg L^{-1})	63.30 ± 2.88	56.60 ± 2.88	60.00 ± 5.00
Total hardness (mg L^{-1})	653.00 ± 67.21	522.60 ± 38.85	532.00 ± 69.74
Salinity (g/L)	2.66 ± 0.16	2.61 ± 0.13	2.75 ± 0.23
Mg:Ca	3.11	2.26	2.43
Mg:K	3.58	2.81	3.12
Ca:K	1.17	1.32	1.29
Na:K	23.73	22.56	24.28
TH: TA	10.34	9.22	8.86
Error (%)	10.28	7.96	4.25

Data correspond to the mean (n=3) ± standard deviation. C1 (control) – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1}; IP – (ionic profile adjustment) *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with ionic adjustment to obtain a Ca:Mg:K ratio of 1:3:1;

C2 – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with Mg^{2+} adjusted to a concentration approximately equivalent to that of seawater.. Ca^{2+} : calcium; Mg^{2+}: magnesium; K^{+} : potassium; Na^{+}: sodium; $SO4^{2-}$: sulfate ; Cl^{-}: chloride; HCO_3^{-}:bicarbonate; TH:TA: total hardness:total alkalinity.

The water in the experimental units was prepared 15 days prior to animal stocking. This procedure was carried out by filling the tanks with 20% (12 L) microbial synbiotic (Total ammonia nitrogen -TAN = 0.39 mgL^{-1}, nitrite nitrogen -N-NO_2^{-}= 0.41 mgL^{-1}, nitrite nitrogen -N-NO_3^{-}= 12.1 mgL^{-1}) and 80% (48 L) water treated with sodium hypochlorite at a concentration of 15 gm^{-3} of activated chloride. The synbiotic was then added at two-day intervals for a total of seven fertilizations. The fertilizer was submitted to an anaerobic phase (24 h) and an aerobic phase (24 h). The synbiotic was composed of 10 $gm^{-3}$of rice bran (< 200 μm), 1 $gm^{-3}$of sugar, $gm^{-3}$of sodium bicarbonate and 0.05 $gm^{-3}$of a commercial bacterial mix [6.5×107 colony-forming units (CFUs)g^{-1}] containing *Bacillus subtilis, B. licheniformis, Bacillus* sp., sodium chloride (NaCl) and magnesium hydroxide ($Mg(OH)_2$) (Kayros Agrícola and Ambiental, São Paulo, Brazil) and chlorinated water [20 mg L^{-1} of active chlorine (60%), then unchlorinated through aeration] at a proportion of 10 × the amount of rice bran. During experimental time, the synbiotic was added to the experimental treatments every three days until settleable solids reached 5 mL L^{-1}.

The experimental units (60 L) were constantly aerated (dissolved oxygen > 5.0 mg L^{-1} with the temperature maintained at ~31°C (Hopar Sh-608 heater 100 W), a 12:12-h photoperiod and mean luminance of 8.65 μmol photons m^{-2} s (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. The artificial substrate added to the experimental units was composed of mollusk shells (*Anomalocardia brasiliana*), which

covered ≈ 28.12% of the bottom area (25 × 24 × 5 cm, width × height × depth) and corresponded to ≈ 3.36% of the useful volume of the experimental unit.

2.2. Shrimp stocking, feeding and monitoring

Post-larvae (PL10: 3.0 ± 0.12 mg) were acquired from a commercial hatchery (Aquasul, Nísia Floresta, Rio Grande do Norte, Brazil). Shrimps had been produced in water with salinity of 35 gL^{-1}. Prior to the experiment, the PL10 were acclimated to salinity of 2.7 gL^{-1} for 14 days. At the end of the acclimatization period, PL24 (9.24 ± 1.38 mg) were randomly selected and stocked at a density of 2000 PLm^{-3} in nursery tanks (800 L) for 35 days. No water exchange was performed during the entire experimental period and de-chlorinated freshwater was added to compensate for evaporation.

For the nursery phase, post-larvae were fed a commercial feed with 40% crude protein, 11% lipids, 4% crude fiber and 14% mineral matter (Guabitech Inicial, Guabi, Brazil) four times a day (8:00 am, 11:00 am, 2:00 pm and 5:00 pm). A daily feeding rate of 33.75% of body weight was initially adopted, which was gradually reduced to 10.5% over 35 days (Van Wyk et al. 1999). The feeding rate was adjusted daily in accordance with the estimated feed consumption and mortality rate.

The experimental units (60 L) were stocked with *L. vannamei* juveniles (initial weight: 0.42 ± 0.01 g) at a density of 300 shrimps m^{-3}. The animals were fed three times a day (8:00 am, 12:00 pm and 4:00 pm) using a commercial shrimp feed with 35% crude protein, 10% lipids, 4% crude fiber and 14% mineral matter (Guabitech Active, Guabi, Brazil). The amount of feed offered to the animals was calculated based on Garza de Yta et al. (2004), considering a weekly growth rate of 0.5 g, weekly mortality of 2% and FCR 1.1.

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

2.3. Ionic adjustment

Commercial products were used for the ionic adjustment of the water: potassium chloride (KCl), calcium carbonate ($CaCO_3$) and magnesium sulfate ($MgSO_4$). The addition of ions was performed based on previous tests of the efficiency of the chemical compounds at increasing the concentration of ions in the water. The test was performed in experimental units containing 14 L of water with salinity of 2.5 gL^{-1} (seawater diluted with freshwater) under constant aeration. The pH and ion concentrations were analyzed before and 72 h after application of the chemicals at a concentration of 100 gm^{-3} (Table 2).

Table 2. Percentage % increment of the target ion (after 72 h of the application of 100 gm-^{3}) by chemical compounds used for ionic adjustment in intensive nursery synbiotic system.

Commercial product		MgSO4	$CaCO_3$	KCl
% increment of the target ion	pH[a]	0.4 ± 0.07	0.32 ± 0.06	0.41 ± 0.05
	Ca^{2+}	-	30.4 ± 0.73	-
	K^+	-	-	51.1 ± 11.38
	Mg^{2+}	9.4 ± 0.51	-	-

Note: The data correspond to the mean of 2 replicates ± standard deviation. $MgSO_4$: magnesium sulfate: KCl: potassium chloride; $CaCO_3$: calcium carbonate; [a]pH in absolute value; Ca^{2+}: calcium; K^+: potassium; Mg^{2+}: magnesium

The application of chemical compounds to the experimental units to adjust the ion concentration was performed according to the following equation (Samocha 2019):

$$Amount\ of\ product = \frac{Fc - Ic}{TI} \times V$$

in which:

- Fc: Final concentration;
- Ic: Initial concentration;
- TI: % increment of the target ion (decimal value);
- V: tank volume (m^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 10% between cations and anions was adopted as a standard for certifying the accuracy of the analysis of these major ions (Custodio and Llamas 1983). 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.

In the C1 treatment, seawater was diluted with freshwater to reach salinity of 2.7 g L^{-1}. This treatment was used as a control treatment and no ionic adjustment was performed during the experimental period. In the C2 treatment, Mg^{2+} supplementation was performed at the

beginning of the experiment to achieve a concentration equivalent to that of seawater. Optimal ion concentrations in farming ponds are determined by multiplying the salinity of the water in the tank by the ratio of the ion concentration in seawater and mean seawater salinity (Boyd and Thunjai, 2003). The Mg^{2+} concentration was adjusted from 98 mg L^{-1} to 109 mg L^{-1} by adding magnesium sulfate ($MgSO_4$).

In the IP treatment, adjustments were made to the Ca:Mg:K ratio on Days 0, 14, 28 and 42, maintaining the ratio at 1:3:1 to simulate Ca:Mg:K ratios found in seawater (Boyd, 2020). At the beginning of the experiment (Day 0), the Mg^{2+} concentration was increased from 98.8 to 132.5 mg L^{-1}. On Day 14, Mg^{2+} was increased from 130.2 mg L^{-1} to 148.8 mg L^{-1} and K^+ was increased from 31.9 mg L^{-1} to 49.6 mg L^{-1}. On Day 28, Mg^{2+} was increased from 142.5 mg L^{-1} to 203.2 mg L^{-1} and K^+ was increased from 53.5 mg L^{-1} to 67.7 mg L^{-1}. On Day 42, Ca^{2+} was increased from 62.4 mg L^{-1} to 72.9 mg L^{-1} and K^+ was increased from 58.8 mg L^{-1} to 72.9 mg L^{-1}. Magnesium sulfate ($MgSO_4$), potassium chloride (KCl) and calcium carbonate ($CaCO_3$) were used to achieve the desired Ca:Mg:K ratio of 1:3:1.

2.4. Water Quality

Dissolved oxygen (DO; mg L^{-1}) and temperature (°C; YSI model 100, Yellow Springs, Ohio, USA) were monitored twice a day (8:00 am and 4:00 pm). Salinity (salinity meter AZ, model 8371), pH (pH meter Akso, model A90) and settleable solids (mL L^{-1}; Imhoff cone) (Avnimelech 2012) were monitored three times a week. Total ammonia nitrogen (TAN; APHA 2012), nitrite nitrogen (NO_2^--N; Fries 1971) and total alkalinity (TA; APHA 2012) were monitored every seven days. Nitrate nitrogen (NO_3^--N; APHA 2012), orthophosphate (PO_4^{3-}; 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 14 days.

2.5. *Proximate composition*

At the end of experimental time, floc samples were collected with a cylindrical mesh net (50 μm) for the retention of solids and whole body of the shrimps for the proximate composition analysis (crude protein, lipids, moisture, ash and fiber contents) using standard methods (AOAC 2012) (all in triplicate). The animals were washed in the laboratory with filtered freshwater and carefully inspected to eliminate encrusted organisms. 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 (315 SE model, Fanem). 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 model; Tecnal, São Paulo, Brazil). Total lipid content was determined using the Soxhlet extraction method with pure hexane (98%) as the solvent (Ma 044/8/50 model, Marconi, São Paulo, Brazil). Crude fiber content was determined using the enzymatic-gravimetric method by measuring the residue after acid and alkaline digestions (Vasconcelos et al. 2010). Ash was determined by oven incineration at 550°C (Q318 D24 model; Quimis, São Paulo, Brazil).

2.6. Statistical analyses

The data were performed using the Statistica software version 13.0 (StatSoft). The data were checked for homogeneity of variance using the Cochran test ($p < 0.05$) and normality was determined using the Shapiro-Wilk test ($p < 0.05$). One-way analysis of variance (ANOVA) was performed to evaluate the growth performance variables and proximate composition. Repeated-measures ANOVA was used to compare water quality data, followed by Duncan's test ($p < 0.05$) for the comparison of means. Nonparametric data were analyzed using the Kruskall–Wallis test ($p < 0.05$) followed by Dunn's test (for SGR data) and Friedman's test with

Conover's multiple comparison test with Holm–Bonferroni correction (for DO, TAN, NO_2^--N, NO_3^--N, PO_4^{3-}, salinity, SS, Cl^-, TA and TH).

3. Results

3.1. Water Quality

No significant differences were found among the treatments with regards to water quality variables (Table 3). Dissolved oxygen and temperature were approximately 6.0 mgL^{-1} and 30 ºC, respectively, throughout the experimental time in all treatments. TAN ranged from 0.19 to 0.25 mgL^{-1} and $N\text{-}NO_2^-$ ranged from 0.20 to 0.27 mgL^{-1} throughout the experiment ($p > 0.05$; Table 3). pH was similar among the treatments, with no substantial changes between morning and afternoon (Table 3).

Table 3. Water quality variables measured during *Litopenaeus vannamei* grow out in a synbiotic system with different ionic composition in low-salinity water.

Variáveis	Tratamentos		
	PI	C1	C2
OD manhã (mg/L)	6.26 (7.31 – 4.91)	6.22 (7.16 – 4.90)	6.25 (7.37 – 5.37)
OD tarde (mg/L)	6.19 (7.35 – 4.52)	6.11 (7.38 – 4.05)	6.17 (7.55 – 4.20)
Temperatura (ºC)	30.60 (34.00 – 28.20	30.66 (33.10 – 28.30)	30.85 (32.90 – 28.80)
Salinidade (g/L) *	2.67 (3.30 – 2.51)	2.85 (3.80 – 2.23)	2.76 (3.50 – 2.35)
pH manhã *	7.31 (8.34 – 7.01)	7.32 (8.45 – 6.98)	7.32 (8.38 – 6.98)
pH tarde *	7.29 (8.10 – 6.97)	7.29 (8.13 – 6.87)	7.33 (8.12 – 6.92)
NAT (mg/L)	0.23 (0.71 – 0.04)	0.25 (0.93 – 0.04)	0.19 (0.57 – 0.05)
$N\text{-}NO_2^-$ (mg/L)	0.20 (0.85 – 0.04)	0.27 (1.39 – 0.04)	0.23 (0.93 – 0.02)
$N\text{-}NO_3^-$ (mg/L)	0.41 (1.12 – 0.30)	0.67 (2.27 – 0.10)	0.56 (2.08 – 0.11)
PO_4^{3-} (mg/L)	15.37 (21.21 – 8.71)	15.52 (23.52 – 9.02)	13.66 (23.53 – 8.99)
SS (ml/L)	5.75 (14.00 – 0.50)	6.88 (23.00 – 0.90)	6.28 (20.00 – 0.70)

Data correspond to the mean over the experimental time (maximum - minimum). Repeated measures ANOVA for parametric statistical data and Friedman's test for no-parametric statistical data. C1 (control) – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1}; IP

– (ionic profile adjustment) *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with ionic adjustment to obtain a Ca:Mg:K ratio of 1:3:1; C2 – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with Mg^{2+} adjusted to a concentration approximately equivalent to that of seawater.. DO: dissolved oxygen; pH: hydrogen potential; TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; PO_4^{3-}: phosphate; SS: settleable solids.

3.2. Major ion and cation ratios

The major ion and cations ratios are summarized in Table 4. Cation concentrations (Ca^{2+}, Mg^{2+} and K^+) varied throughout the experimental period (Figure 1). Significant differences in Mg^{+2} ($p < 0.05$) were found among the treatments on Days 0 and 56, with higher concentrations in the IP treatment compared to the C1 treatment ($p < 0.05$; Figure 1). On Day 42, the concentration of Mg^{+2} was significantly higher in the IP treatment ($p < 0.05$) compared to the C1 and C2 treatments. A significant difference in K^+ was also found among treatments ($p < 0.05$), with higher concentrations in the IP treatment on Days 28, 42 and 56 compared to the C1 and C2 treatments (Figure 1). Significant differences among the treatments were found in SO_4^{2-} ($p < 0.05$) on Day 56 as well as TH on Days 42 and 56, with higher concentrations in the IP treatment compared to the C1 and C2 treatments (Figure 1). Ca:K and Na:K^+ differed significantly among treatments ($p < 0.05$), with lower ratios in the IP treatment (Table 4).

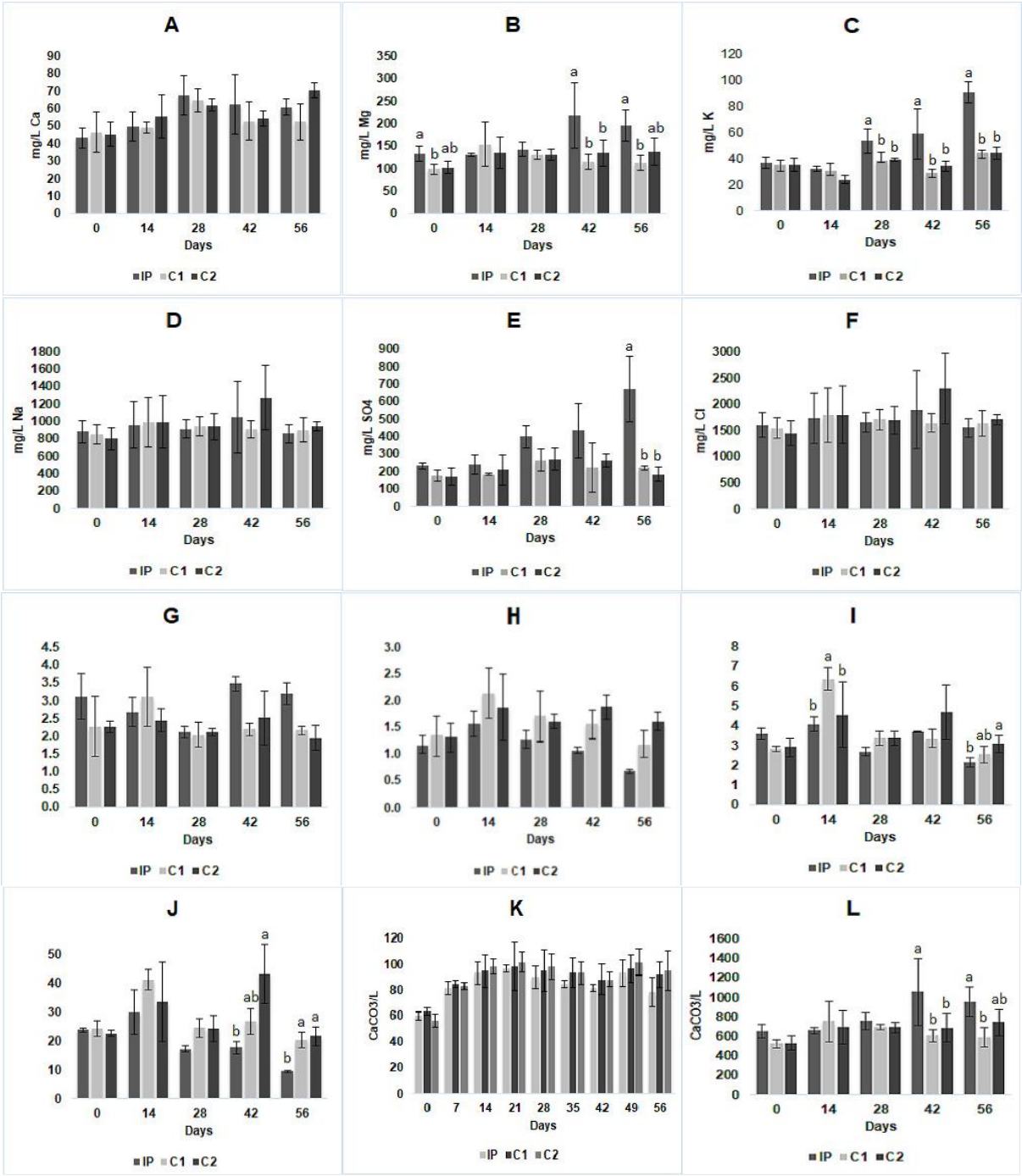
Table 4. Ion concentration (mg L^{-1}) and ratios among cations on *Litopenaeus vannamei* in a synbiotic system with different ionic composition in low-salinity water.

Variables	Treatments		
	PI	C1	C2
Ca^{2+} (mg L^{-1})	56.74 ± 9.44	53.01 ± 8.60	57.49 ± 6.13
Mg^{2+} (mg L^{-1})	163.81 ± 28.63	121.82 ± 20.72	127.85 ± 23.78
K^+ (mg L^{-1})	54.42 ± 8.52 [a]	35.27 ± 3.96 [b]	35.57 ± 3.51 [b]

Na^+ (mg L^{-1})	929.86 ± 200.70	917.45 ± 148.01	988.63 ± 201.98
SO_4^{2-} (mg L^{-1})	394.35 ± 95.30 [a]	212.96 ± 49.96 [b]	219.13 ± 55.20 [b]
Cl^- (mg L^{-1})	1,682.69 ± 363.30	1,660.24 ± 267.84	1,789.04 ± 365.52
TA (mg $CaCO_3$ L^{-1})	89.62 ± 6.20	90.74 ± 10.76	84.44 ± 7.93
TH (mg $CaCO_3$ L^{-1})	816.00 ± 136.61	634.66 ± 85.32	669.06 ± 114.72
Mg:Ca	2.91 ± 0.34 [a]	2.35 ± 0.46 [b]	2.25 ± 0.33 [b]
Mg:K	3.24 ± 0.22	3.68 ± 0.34	3.72 ± 0.85
Ca:K	1.14 ± 0.13 [b]	1.58 ± 0.37 [a]	1.65 ± 0.28 [a]
Na:K	19.55 ± 2.34 [b]	27.39 ± 3.39 [a]	29.03 ± 6.48 [a]
Error (%)	5.06 ± 0.01	5.07 ± 0.01	3.31 ± 0.01

Data correspond to the mean (n=15) ± standard deviation. Different capital letters represent significant differences ($p < 0.05$) among the treatments. Repeated measures ANOVA results followed by Duncan's test ($p < 0.05$) for the comparison of means. C1 (control) – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1}; IP – (ionic profile adjustment) *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with ionic adjustment to obtain a Ca:Mg:K ratio of 1:3:1; C2 – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with Mg^{2+} adjusted to a concentration approximately equivalent to that of seawater. TA = total alkalinity; TH = total hardness.

Figure 1. Variation of cations, anions, ionic ratios, total hardness and total alkalinity during a *Litopenaeus vannamei* grow out using a synbiotic system with different ionic composition in low-salinity water.



3.3. Proximate composition

The proximate composition of the shrimps and microbial floc at the end of the experimental time are summarized in Table 5. No significant differences among treatments were found for crude protein, lipids, moisture or ash in the shrimps ($p > 0.05$). No significant

differences in the proximate composition of the microbial floc were among treatments ($p > 0.05$) for crude protein, lipids or moisture. However, ash content was higher ($p < 0.05$) in the IP treatment (Table 5).

Table 5. *Litopenaues vannamei* (wet base) and microbial floc (dry base) proximal composition in a synbiotic system with different ionic composition in low-salinity water.

	Proximal composition	Treatments		
		IP	C1	C2
Shrimp	Moisture (%)	77.68 ± 0.92	77.92 ± 2.55	77.65 ± 1.26
	Crude protein (%)	17.80 ± 0.34	17.25 ± 0.30	18.04 ± 1.16
	Lipids (%)	5.60 ± 0.04	5.41 ± 1.25	5.69 ± 0.09
	Ash (%)	4.06 ± 0.88	3.96 ± 0.31	3.94 ± 0.23
Microbial floc	Moisture (%)	92.57 ± 0.69	93.43 ± 1.63	93.77 ± 1.24
	Crude protein (%)	25.61 ± 9.20	26.02 ± 5.33	30.9 ± 7.43
	Lipids (%)	4.55 ± 0.09	5.40 ± 0.44	5.52 ± 0.12
	Ash (%)	28.23 ± 2.14^{a}	20.31 ± 3.10^{b}	22.13 ± 2.40^{b}

Data correspond to the mean ± standard deviation. Different capital letters represent significant differences ($p < 0.05$) among the treatments by One-way variance analysis (ANOVA) followed by Duncan's test. C1 (control) – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1}; IP – (ionic profile adjustment) *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with ionic adjustment to obtain a Ca:Mg:K ratio of 1:3:1; C2 – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with Mg^{2+} adjusted to a concentration approximately equivalent to that of seawater.

3.4. Shrimp growth performance

Growth performance data at the end of the 56 days of experimental time are summarized in Table 6. No significant differences ($p > 0.05$) were found among treatments with regards to survival or FCR. Performance was greater in C2 treatment (final weight: 4.72 g; weekly growth: 0.59 g; SGR: 4.10 $\%day^{-1}$; yield; 1.22 Kg/m^{-3}) compared to the IP treatment.

Table 6. Shrimp zootechnical performance in a synbiotic system with different ionic composition in low-salinity water during 56 days.

Zootechnical Performance	Treatments		
	IP	C1	C2
Survival (%)	53.70 ± 11.56	70.37 ± 19.51	81.48 ± 8.49
Final weight (g)	3.79 ± 0.07 ^{b}	4.55 ± 0.37 ^{a}	4.72 ± 0.09 ^{a}
Yield (Kg m^{-3})	0.61 ± 0.14 ^{b}	0.96 ± 0.28 ^{ab}	1.22 ± 0.06 ^{a}
Wg (g)	0.47 ± 0.01 ^{b}	0.57 ± 0.06 ^{a}	0.59 ± 0.10 ^{a}
SGR (% day^{-1})	4.08 ± 0.03 ^{b}	4.27 ± 0.18 ^{a}	4.33 ± 0.10 ^{a}
FCR	1.32 ± 0.07	1.26 ± 0.16	1.20 ± 0.05

* Shrimp Initial weight (0.42 ± 0.01g). Data correspond to the mean ± standard deviation. Different capital letters represent significant differences ($p < 0.05$) among the treatments by One-way variance analysis (ANOVA) followed by Duncan's test. C1 (control) – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1}; IP – (ionic profile adjustment) *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with ionic adjustment to obtain a Ca:Mg:K ratio of 1:3:1; C2 – *L. vannamei* reared in seawater diluted to salinity of 2.7 gL^{-1} with Mg^{2+} adjusted to a concentration approximately equivalent to that of seawater.. Wg: weekly growth; SGR: specific growth rate; FCR: feed conversion ratio.

4. Discussion

Water quality variables (temperature, DO and pH) remained within the recommended ranges for the reared of *L. vannamei* (Van Wyk and Scarpa 1999). In low-salinity systems with minimal water exchange, high concentrations of nitrogen compounds, especially TAN and NO_2^--N, are considered the main cause of mortality due to their high toxicity in low-salinity environments (Laramore et al. 2001; Maicá et al. 2012; Esparza-Leal et al. 2016). In this study, the concentration of nitrogen compounds remained lower than 0.3 mgL^{-1}, which is below the upper limit of the safe range for this species in water with salinity of 2.7 gL^{-1} (Sowers et al. 2004; Gross et al. 2004; Ramirez-Rochin et al. 2016; Valencia-Castaneda et al. 2018). The present results differ from those reported in other studies involving the farming of *L. vannamei*

in low-salinity water with minimal water exchange (Esparza-Leal et al. 2010; Maicá et al. 2012 and 2014; Khanjani et al. 2020).

The control of these nitrogen compounds may be related to the strategy implemented to lower the stress of the shrimps reared in a low-salinity environment with minimal water exchange, which was the use of the fermentation and microbial respiration of polysaccharide carbohydrates by a mix of probiotic bacteria, stimulating the growth of heterotrophic and chemoautotrophic bacteria, enhancing the oxidation of nitrogen compounds (Lima et al. 2021; Dos Santos et al. 2022; Silva et al. 2021; Pimentel et al. 2022) and increasing the availability of nutrients in the rice bran through the breakdown of organic molecules, such as polysaccharides, into simpler molecules, such as sucrose, resulting in the formation of organic acids, such as lactic acid (Wainwright 1995; Feddern et al. 2007). In contrast, the addition of polysaccharide carbohydrates with no fermentation or microbial respiration leads to a greater biomass production of heterotrophic bacteria rather than chemoautotrophic bacteria, causing an initial reduction in the nitrification process (Serra et al. 2015; Khanjani et al. 2017).

The use of inoculum from water of a previous culture is a strategy used in several studies to accelerate the stabilization of heterotrophic and autotrophic bacterial communities in tanks and/or ponds. Hostins et al. (2017) and Santos et al. (2019) also obtained TAN and NO_2^--N concentrations below 0.3 mgL^{-1} with water inoculum from previous crops. Another efficient strategy for the control of nitrogen compounds is the use of artificial substrates composed of the shells of the mollusk *Anomalocardia brasiliana*. According to Navada et al. (2019), chemoautotrophic bacteria need substrates for their fixation and are more efficient in the oxidation of nitrogen compounds compared to heterotrophic bacteria. Studies on the grow out of *L. vannamei* with salinity between 25 and 32 gL^{-1} have reported the effectiveness of artificial substrates at controlling nitrogen compounds in intensive systems (Schveitzer et al. 2013; Santos et al. 2019; Satanwat et al. 2019).

In addition to being used as a substrate for nitrifying bacteria, the substrate in the present study was composed of calcium carbonate ($CaCO_3$), which contributes to the increase of total alkalinity in the water, enabling a lower fluctuation of pH, the variation of which was less than 0.1 between morning and afternoon throughout the experimental period (Boyd 2020; Furtado et al. 2015). The addition of this substrate also assisted in the increase of the total alkalinity of the water in the IP, C1 and C2 treatments, which was 63.3, 56.6 and 60 mg $CaCO_3$ L^{-1} for 90, 95 and 85 mg $CaCO_3$ L^{-1}, respectively, at the end of the experimental period, even though the compound was consumed through nitrification processes (Chen et al. 2006; Ebeling et al. 2006) and ecdysis of the shrimps (Boyd and Tucker 1998), which require bioavailable calcium carbonate in the water. According to Van Wyk and Scarpa (1999), the recommended total alkalinity concentration for *L. vannamei* farming is $\geq$ 100 mg $CaCO_3$ L^{-1}, which was not reached in the treatments, although total alkalinity increased gradually.

Regarding the cation ratios, despite having a Ca:Mg:K ratio close to 1:3:1, the IP treatment led to a poorer growth performance compared to the other treatments. This result may be related to the combination of the lower Na:K ratio and total alkalinity concentrations below 100 mg $CaCO_3$ L^{-1}. According to Roy et al. (2007), it is extremely important to maintain the Na:K ratio close to 28:1 to promote better shrimp development and growth, as the proper ratio of these elements is involved in processes such as osmoregulation and ionic transport (Valenzuela-Madrigal et al. 2017). Zhu et al. (2004) found that Na:K ratios lower than 25.6:1 and higher than 119.1:1 negatively affected the performance of *L. vannamei* in salinity of 30 gL^{-1} at 25 ºC. Likewise, Liu et al. (2014) reported that Na:K ratios between 23:1 and 33:1 resulted in the better growth and survival of *L. vannamei* in salinity of 4 gL^{-1}. However, Pimentel et al. (2022) found no loss of production efficiency in *L. vannam*ei nurseries with a Na:K ratio below 20:1 and mean alkalinity above 100 mg $CaCO_3$ L^{-1}. Esparza-Leal et al. (2009) also found no loss of production with the Na:K ratio between 8.6:1 to 185:1 and total alkalinity

values above 200 mg $CaCO_3$ L^{-1} during the grow-out phase of *L. vannamei*. Thus, the combination of the Na:K ratio and alkalinity concentration below 100 mg $CaCO_3$ gL^{-1} may have negatively influenced shrimp performance.

During the experimental period, the ionic adjustments of the Ca:Mg:K ratio did not influence the performance of *L. vannamei* in low-salinity using a synbiotic system and artificial substrate. *L. vannamei* growth was similar when the initial concentrations of the main ions were Ca^{2+} = 45 mgL^{-1}, Mg^{2+} = 98 mgL^{-1}, K^{+} = 35 mgL^{-1} and total hardness was close to 522 mg $CaCO_3L^{-1}$. During grow out, however, the combination of a lower Na:K (< 22:1) and lower total alkalinity (< 100 mg $CaCO_3$ L^{-1}) limited shrimp growth and survival. However, the final weight (4.55 to 4.72 g) in the C1 and C2 treatments was close to that reported by Roy et al. (2007) in systems with 100 shrimp m^{-3} and 4 gL^{-1} of salinity (49 days) and Furtado et al. (2014) in a BFT system with 425 shrimp m^{-3} and 25 gL^{-1} of salinity (56 days). The survival rate (> 70%) and yield (0.96 and 1.22 kg m^{-3}) in the C1 and C2 treatments can be considered high when compared to other studies with low-salinity water (0.25 to 4 gL^{-1}), which obtained a survival rate of less than 25% for *L. vannamei* reared in intensive system (300 shrimp m^{-3}) with minimal water exchange (Maicá et al. 2012) and average yield up to 0.5 kg m^{-3} (Roy et al. 2007; Maicá et al. 2012).

The FCR was similar to that found by Furtado et al. (2014), who tested the effect of different levels of $Ca(OH)_2$ on the growth of *L. vannamei* juveniles (425 shrimp m^{-3}) in water with salinity of 25 gL^{-1} for 56 days, reporting an FCR ranging from 1.2 to 1.7. Likewise, Esparza-Leal et al. (2009) evaluated the effect of different ionic compositions in low-salinity water (~1 gL^{-1}) on the growth of *L. vannamei* juveniles (200 shrimp m^{-3}) for 84 days and found an FCR between 1.3 and 1.5.

Satisfactory results observed with the salinity used in the present study are similar to Moura et al. (2021) that found that the addition of an inoculum of 6% natural marine water

reaching salinity of 2.9 gL^{-1} positively affected the growth performance of *L. vannamei* post-larvae reared in a biofloc system for 27 days.

According to Huang et al. (2014), the shrimp proximate composition can vary according to salinity. In this study, no differences were found in the proximate composition of *L. vannamei* reared in low-salinity water (~2.7 gL^{-1}) with different ionic concentrations. Moisture, proteins and ash in this study were similar to those found in several studies involving using low-salinity water (Li et al. 2007; Maicá et al. 2014; Wang et al. 2014). However, the concentration of lipids was higher than that reported in studies involving shrimp reared in seawater (Huang et al. 2004; Ashild et al. 2004; Li et al. 2007). According to Chen and Nan (1994), the high concentration of lipids in *L. vannamei* at low-salinity water may be related to the use of this nutrient by the shrimp for processes such as osmoregulation and enzyme activation. In *L. vannamei* reared in salinity of 3 gL^{-1}, Huang et al. (2019) found a reduction in the concentration of the lipid metabolite phosphatidylcholine, which is related to the elevation of the hormone choline under hyposmotic stress, the regulation of gill permeability and the functioning of gill cells. However, the reasons behind the shrimp's need to store lipids when living in low salinity conditions remain unclear.

According to Ekasari et al. (2009), microbial flocs larger than 100 μm have higher concentrations of crude protein (27.8%) and lipids (7.5%). The present findings are in agreement with this statement, as flocs collected with a 50-μm mesh had lower concentrations than those reported by Maicá et al. (2014) and Khanjani et al. (2014). The literature reports different results regarding the lipid content of microbial flocs. In the present study, the concentration of lipids ranged from 4.55 to 5.52%, which is higher than the concentration found by Khanjani et al. (2020) and similar to values reported by Tacon (2000) and Wasielesky et al. (2006), who found lipid concentrations in microbial flocs ranging from 5 to 6%. This may be linked to the source of organic carbon and the microbial composition found in the floc as well

as the size of the mesh in which the flocs were collected. Regarding the ash content, the IP treatment had a higher concentration than the other treatments, which may be related to the addition of mineral fertilizers to the water to achieve the Ca:Mg:K ratio of 1:3:1.

5. Conclusion

The ionic adjustment performed in this study demonstrated that the addition of mineral fertilizers ($CaCO_3$, $MgSO_4$ and KCl) to maintain the Ca:Mg:K ratio close to 1:3:1 caused a reduction in the Na:K ratio, culminating in a poorer growth performance and survival. The study also demonstrated that the combination of an artificial substrate composed of *Anomalocardia brasiliana*, a synbiotic system (anaerobic and aerobic processing of rice bran) and an inoculum of 20% of the nursery water were sufficient to promote the control of nitrogen compounds during the shrimp *L. vannamei* reared in low-salinity water. In the C1 and C2 treatments, the initial ionic concentration, total alkalinity close to 90 mg $CaCO_3$ L^{-1} and the Na:K ratio close to 28:1 produced conditions that did not limit the growth of *L. vannamei* in low-salinity water with a synbiotic system. Moreover, the ionic adjustments did not alter the proximate composition of the shrimp. On the other hand, the addition of mineral fertilizers ($CaCO_3$, $MgSO_4$ and KCl) altered the ash concentration in the microbial floc. Further studies addressing the ionic concentration and cation ratios in low-salinity water using a synbiotic system could help clarify the results of the present investigation.

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5. Considerações Finais

No presente estudo foi observado que os diferentes ajustes iônicos não resultaram em um efeito positivo no desempenho zootécnico do camarão marinho, a adição de fertilizantes minerais para o controle da proporção Ca:Mg:K (1:3:1), causou um desequilíbrio na relação Na:K, culminando em resultados inferiores de desempenho zootécnico em relação aos demais tratamentos. O estudo também demonstrou que a concentração iônica dos tratamentos C1 e C2, conjuntamente a utilização de substratos artificiais de *Anomalocardia brasiliana*, utilização do sistema simbiótico com fertilização anaeróbica e aeróbica do farelo de arroz e um inóculo de 20% da água do berçário, foram suficientes para prover um crescimento adequado ao camarão marinho *L. vannamei* em baixa salinidade, sem necessidade de correções iônicas durante todo o período experimental. Foi observado também que para os tratamentos C1 e C2, as concentrações iônicas iniciais, alcalinidade total próxima a 90 mg/L $CaCO_3$ e a relação Na:K próxima a 28:1 proferiram condições que não limitaram o crescimento do *L.* vannamei em água oligohalina em sistema simbiótico. No entanto, mais estudos relacionando a concentração iônica e relações catiônicas em água oligohalina, utilizando sistema simbiótico pode auxiliar e agregar aos resultados deste presente estudo.