UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO – UFRPE

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

CULTIVO DO CAMARÃO DE ÁGUA DOCE *Macrobrachium rosenbergii* (DE MAN 1879) EM SISTEMAS SIMBIÓTICOS E BIOFLOCOS NA FASE DE BERÇÁRIO.

Robson Batista dos Santos

RECIFE – PE

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Robson Batista dos Santos

Tese apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como requisito para a obtenção do título de Doutor.

Prof. Dr. Luis Otávio Brito da Silva
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Robson Batista dos Santos

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Resumo

A busca por melhor produtividade e sustentabilidade tem estimulado pesquisas voltadas à intensificação no cultivo do camarão de água doce *M. rosenbergii*. Em face disso, esse trabalho teve o objetivo de aprimorar as tecnologias de cultivo dessa espécie para os sistemas bioflocos e simbióticos na fase de berçário. Para isso, foram realizados dois experimentos. No Experimento 1 avaliou-se diferentes estratégias de suprimento de carbono orgânico no cultivo dessa espécie. Os camarões (10,0 ± 2,0 mg) foram estocados (0,8 PL L^{-1}) durante 35 dias em delineamento experimental com cinco tratamentos e quatro repetições: Ct = controle; M = melaço (BFT); Mf = melaço pré-tratado com *Bacillus* spp. (simbiótico); RB = farelo de arroz (BFT); e RBf = farelo de arroz pré-tratado com *Bacillus* spp. (simbiótico). Os camarões foram alimentados cinco vezes ao dia com ração contendo 40% de proteína bruta. As variáveis de qualidade da água permaneceram dentro da faixa considerada adequada para a espécie. O simbiótico reduziu os valores médios de sólidos sedimentáveis quando comparado ao bioflocos. O teor de proteína dos flocos microbianos (peso seco) diferiu entre RBf (34,07 ± 0,54%) e RB (29,77 ± 0,48%), mas foi maior no M (43,27 ± 0,76%). Os camarões submetidos ao RBf apresentaram a melhor combinação de resultados para as variáveis peso médio final (122,85 ± 12,50 mg) e ganho de peso semanal (22,26 ± 2,97 mg). Uma vez identificado melhor desempenho do RBf (Simbiótico) no Experimento 1, investigou-se no Experimento 2 os efeitos de diferentes estratégias de preparação do simbiótico com farelo de arroz (fermentação e respiração microbiana) na qualidade da água e desempenho dos animais. Assim, novas pós larvas (10,01±2,0 mg, 1,0 PL L^{-1}) foram estocadas em 20 unidades experimentais durante 35 dias, compondo cinco tratamentos e quatro repetições. Foram utilizados farelo de arroz, probióticos, alcalinizantes e água na preparação do simbiótico. Cada tratamento correspondeu a uma estratégia de preparação: T12|12 = 12h anaeróbica e 12h aeróbica; T12|24 = 12h anaeróbica e 24h aeróbica; T24|0 = 24h anaeróbica apenas; T24|12 = 24h anaeróbica e 12h aeróbica; T24|24 = 24h anaeróbica e 24h aeróbica. Os resultados das variáveis de qualidade de água foram adequados ao cultivo da espécie. Para as variáveis peso médio final (mg) e produtividade (g m^{-3}), os tratamentos T24|24 (221,3±22 e 195,4±14,6) e T12|24 (218,2±27,6 e 196,2±33,4) foram superiores ao 24|00 (176,1±24.5 e 151,3±21.6). Pode-se concluir que é possível utilizar o sistema simbiótico com farelo de arroz na produção de juvenis de camarão de água doce *M. rosenbergii*. Além disso, a combinação do tempo de fermentação e respiração microbiana no processo de preparação do simbiótico tem efeitos na sua qualidade como fertilizante e, por conseguinte, no crescimento dos animais, conforme observado nos tratamentos T24|24 e T12|24.

Palavras chave: Fermentação, respiração microbiana, farelo de arroz, melaço, probióticos.

Abstract

The freshwater prawn species *M. rosenbergii* has shown the need for research to intensify its cultivation, especially in heterotrophic systems with minimal water exchange. In view of this, this work aimed to improve the culture technologies of this species for biofloc and synbiotic systems in the nursery phase. For this, two experiments were carried out. In Experiment 1, different strategies for supplying organic carbon in the culture of this species were tested. The prawn (10.0 ± 2.0 mg) were stored (0.8 PL L^{-1}) for 35 days in an experimental design with five treatments and four replicates: Ct = control; M = molasses (BFT); Mf = molasses pre-treated with *Bacillus* spp. without and with aeration (synbiotic); RB = rice bran (BFT); and RBf = raw rice bran pre-treated with *Bacillus* spp., without and with aeration (synbiotic). The prawns were fed five times a day with a diet containing 40% crude protein. The water quality variables remained within the range considered adequate for the species. The synbiotic reduced the mean values of settleable solids. The protein content of microbial flocs (dry weight) differed between RBf (34.07 ± 0.54%) and RB (29.77 ± 0.48%), but was higher in M (43.27 ± 0.76 %). Prawns submitted to RBf showed the best combination of results for the variables final weight (122.85 ± 12.50 mg) and weekly weight gain (22.26 ± 2,97 mg). Once better performance of the RBf was identified, Experiment 2 proposed to investigate the effects of different strategies of synbiotic preparation (fermentation and microbial respiration) on water quality and animal performance. Thus, new post larvae (10.01±2.0 mg, 1.0 PL L^{-1}) were stocked in 20 experimental units for 35 days, design with five treatments and four replicates. Rice bran, probiotics, alkalizing agents and water were used in the preparation of the synbiotic. Each treatment corresponded to a preparation strategy: T12|12 = 12 hours anaerobic and 12 hours aerobics; T12|24 = 12 hours anaerobic and 24 hours aerobic; T24|0 = 24 hours anaerobic only; T24|12 = 24 hours anaerobic and 12 hours aerobic; T24|24 = 24 hours anaerobic and 24 hours aerobic. The results of the quality variables were suitable for the culture of the species. For the variables final average weight (mg) and yield (g m^{-3}), treatments T24|24 (221.3±22 and 195.4±14.6) and T12|24 (218.2±27.6 and 196.2±33.4) were superior to 24| 00 (176.1±24.5 and 151.3±21.6). Thus, we can conclude that it is possible to use the synbiotic system with rice bran in the production of freshwater prawn *M. rosenbergii* juveniles. Furthermore, the combination of fermentation time and microbial respiration in the synbiotic preparation process has an effect on its quality as a fertilizer and, therefore, on the growth of the animals.

Keywords: Fermentation, microbial respiration, rice bran, molasses, probiotics.

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1. INTRODUÇÃO

A produção mundial do camarão de água doce *Macrobrachium rosenbergii* registrou valores próximos a 234.000 mil toneladas em 2018 (FAO, 2021). Deste total, o Brasil contribuiu com cerca de 150 toneladas (FAO, 2021). A produção tímida desta espécie denota o baixo desenvolvimento da sua cadeia produtiva e do incipiente aprimoramento de sistemas de cultivo e instalações que possibilitem maiores produtividades e lucratividade atrelado ao mínimo uso de água e controle ambiental. Há que se considerar, no entanto, que houve significativos avanços no cultivo dessa espécie, com alguns autores demonstrando a possibilidade de intensificar seu cultivo em sistemas heterotróficos e tradicionais (MARQUES et al., 2000; CRAB et al., 2010; PEREZ-FUENTES et al. 2013; BALLESTER et al., 2017; MIAO et al., 2017; NEGRINI et al., 2017; HOSAIN et al., 2021; SANTOS et al., 2022). Esses avanços devem-se às suas características biológicas, como alta fecundidade, rusticidade e crescimento satisfatório, o que permite obter viabilidade econômica no cultivo desta espécie (NEW et al., 2010).

O cenário promissor para a carcinicultura tem sido estimulado pela adoção de sistemas superintensivos de produção com pouca ou sem renovação de água, os quais têm apresentado resultados positivos em termos de produtividade e sustentabilidade nos cultivos (CRAB et al., 2012). Dentre essas novas tecnologias com o uso mínimo de água destacam-se os RAS (Recirculating Aquaculture System – Sistema de Recirculação de Água), BFT (Biofloc Technology ou Sistema de Bioflocos) e simbióticos (Timmons e Ebeling, 2010; AVNIMELECH et al., 2012; ROMANO et al., 2018).

Apesar da eficiência, o RAS apresenta alto custo de operação, manutenção e baixa adoção nos países em desenvolvimento (BADIOLA et al., 2012; AHMAD et a., 2017). Já o cultivo em meio heterotrófico ou mixotrófico, também chamado de sistema BFT, é um sistema superintensivo de produção com pouca ou sem renovação de água, caracterizado pela alta produtividade e reduzido impacto ambiental (DE SCHRYVER et al., 2008; CRAB et al., 2012; AVNIMELECH et al., 2012; SAMOCHA et al., 2017; EMERENCIANO et al., 2017). Nesse ambiente, a água contém uma grande variedade de microorganismos, alimentos não consumidos, fezes, detritos e partículas mantidas em suspensão por aeração constante (DE SCHRYVER et al., 2008).

Por outro lado, sistemas intensivos são também caracterizados pelo grande incremento de nitrogênio inorgânico, em especial, o nitrogênio amoniacal (NH_4^+ e NH_3) oriundo de alimentos não consumidos e excretas dos animais em cultivo (AVNIMELECH et al., 2012). Para reverter esse

quadro no BFT, são adicionadas fontes externas de carbono na água por meio da modificação do teor de carboidratos na ração ou pela adição de uma fonte externa de carbono orgânico para aumentar a relação C:N e, assim, estimular o crescimento de bactérias heterotróficas, que são responsáveis pela metabolização dos compostos nitrogenados e servem como proteína microbiana para os animais no ambiente de cultivo (EBELING, et al., 2006; DE SCHRYVER et al., 2008; AVNIMELECH et al., 2012; CRAB et al., 2012).

Não obstante, para que o crescimento bacteriano ocorra de forma satisfatória é necessário o ajuste dos fatores ambientais, como temperatura, oxigênio e pH, mas também de fontes de carbono facilmente degradáveis, como melaço, dextrose, ou até mesmo aquelas de menor solubilidade, como é o caso dos farelos vegetais (AVNIMELECH, 2012; CRAB et al., 2010; ROMANO et al., 2018, SANTOS et al., 2022). No caso das fontes de carboidratos de menor solubilidade, trabalhos recentes têm demostrado a eficácia do uso de microorganismos como um pré-tratamento para diminuir o teor de fibras, aumentar o teor de proteína e melhorar a solubilidade em água. (SUPRIYATI et al., 2015; ROMANO, 2017; ROMANO et al., 2018, SANTOS et al., 2022).

Tradicionalmente, o termo simbiótico é empregado como uma combinação de probióticos e prébióticos. Os probióticos são microrganismos vivos benéficos, como bactérias láticas, *Bacillus*, leveduras, etc. que conferem vantagens à saúde e ao crescimento dos hospedeiros quando administrados adequadamente. Os prebióticos, por sua vez, são açúcares (oligo ou polissacarídios) não digeríveis que estimulam o crescimento desses microrganismos vivos benéficos no trato gastrointestinal dos animais (HUYNHTG et al., 2017; AMENYOGBE et al., 2020; CIENFUEGOS-MARTÍNEZ et al., 2020; YILMAZ et al. 2022). Nesse sentido, diversos trabalhos foram realizados utilizando probióticos, prebióticos e simbióticos em rações, os quais foram responsáveis por importantes avanços em termos de crescimento e saúde dos animais cultivados (HUYNHTG et al., 2017; AMENYOGBE et al., 2020). Apenas recentemente, alguns autores também começaram a atribuir o termo simbiótico ao sistema de cultivo que utiliza como fertilizante da água um carboidrato (normalmente farelos vegetais) fermentado com microrganismos probióticos (ROMANO, 2017; ROMANO et al., 2018; SANTOS et al., 2022).

Os simbióticos, nesse sentido, consistem na formulação de um biofertilizante utilizando resíduos agroindustriais (principalmente farelos vegetais) e microrganismos probióticos submetidos à fermentação semi-sólida ou líquida. Como resultado, obtêm-se um produto melhorado nutricionalmente e rico em microrganismos benéficos ao ambiente de cultivo (HUYNHTG et al., 2017; ROMANO et al., 2018; DEEPAK et al., 2020; ANDRADE et al., 2021; LIÑAN-VIDRIALES et al., 2021; SILVA et al., 2021; SANTOS et al., 2022).

A aplicação do farelo de arroz fermentando na fertilização de viveiros de aquicultura é relativamente recente, datada de meados da década de 1990 na Tailândia, em que posteriormente viria a ganhar o nome de Aquamimicry (ROMANO, 2017). Desde então, a popularização desses sistemas de cultivo ao redor do mundo veio acompanhado de variações protocolares de fertilização aquícola utilizando farelo de arroz, soja e trigo fermentado, as quais geraram diferentes efeitos na sua composição centesimal, na composição dos flocos e no desempenho dos animais cultivados (ROMANO, 2017; ROMANO et al., 2018; ANDRADE et al., 2020; HUSSAIN et al., 2021; LIÑAN-VIDRIALES et al 2021; SILVA et al., 2021; SANTOS et al., 2022).

Sabe-se, contudo, que para os processos de preparação dos simbióticos (fermentação e/ou respiração microbiana) ocorrerem de forma eficiente, é necessário conhecer as condições ambientais que favorecem o metabolismo dos microrganismos, já que variáveis como temperatura, aeração, pH e tempo de fermentação possuem grande influência no crescimento microbiano, produção e atividade enzimática (POTUMARTHI et al., 2007; AGUILAR et al., 2019; DAWOOD e KOSHIO, 2019; SHIM et al., 2010; SUGIHARTO e RANJITKAR, 2019).

Independente do sistema adotado (BFT ou Simbiótico), a fonte de carbono orgânico como um promotor do crescimento microbiano tornou-se um dos parâmetros mais pesquisados e sua aplicação tenta associar eficiência, baixo custo e disponibilidade no mercado local (DAUDA, 2019; ROMANO et al., 2018). Assim, dependendo da região, as fontes de carbono orgânico mais comumente utilizadas são os carboidratos - $(CH_2O)n$ - desde os monossacarídeos e dissacarídeos (glicose, melaço, sacarose, glicerol) quanto os polissacarídeos (farelo de arroz, trigo, amido, etc.) (AVNIMELECH et al., 2012; CRAB et al., 2010; EMERENCIANO et al., 2017; DAUDA et al., 2017; KUMAR et al. 2017; ABAKARI et al. 2020b; HOSAIN et al. 2021; SANTOS et al. 2022).

Tendo isso em vista, surge a necessidade de contribuir com informações que auxiliem no entedimento de sistemas de cultivo (bioflocos ou simbiótico) que possam otimizar o desempenho do *Macrobrachium rosenbergii* na fase de berçários utilizando diferentes fontes de carbono orgânico.

1.1.Objetivos

Geral:

Identificar os sistemas de cultivo e as estratégias de implementação que promovem melhores resultados para o cultivo do camarão de água doce *M. rosenbergii* na fase de berçário.

Específicos:

- Identificar a fonte de carbono orgânico que melhor contribui para o controle das variáveis de qualidade da água no sistema de bioflocos e simbióticos.
- Comparar os efeitos dos sistemas de cultivo bioflocos e simbótico no desempenho produtivo do *M. rosenbergii.*
- Identificar a estratégia de preparação do simbiótico com farelo de arroz que promove melhores condições de cultivo e crescimento para o *M. rosenbergii.*
- Observar o efeito de diferentes alcalinizantes no controle do pH durante o processo de preparação do simbiótico.
- Observar a dinâmica dos compostos nitrogenados durante a preparação dos simbióticos em suas diferentes estratégias de preparação.

2. ARTIGO 1

Effects of organic carbon sources on water quality, microbial flocs protein and performance of *Macrobrachium rosenbergii* post-larvae reared in biofloc and synbiotic systems

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Effects of organic carbon sources on water quality, microbial flocs protein and performance of *Macrobrachium rosenbergii* post-larvae reared in biofloc and synbiotic systems

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Abstract

Different strategies for supplying organic carbon in biofloc and synbiotic were tested for Macrobrachium rosenbergii port-larvae (PL). The prawn (0.01 ± 0.002 g) were stocked (0.8 PL L^{-1}) during 35 days in an experimental design with five treatments and four replicates: Ct = control; M = molasses (BFT); Mf = molasses pre-treated with *Bacillus* spp. without and with aeration (synbiotic); RB = rice bran (BFT); and RBf = raw rice bran pre-treated with *Bacillus* spp., without and with aeration (synbiotic). The prawns were fed five times a day with a feed containing 40% crude protein. Water quality variables remained within the range considered suitable for the species, and the synbiotic reduced the mean values of settleable solids. The protein content of microbial flocs (dry weight) differed among RBf (34.07 ± 0.54%) and RB (29.77 ± 0.48%), but was higher in M (43.27 ± 0.76%). The prawns submitted to RBf presented the best combination of results for variables final weight (122.85 ± 12.50 mg) and weekly weight gain (22.26 ± 2.97 mg). Thus, RBf treatments (synbiotic) in addition to maintaining water quality variables at acceptable levels can improve growth of *M. rosenbergii* post-larvae.

KEYWORDS: *Bacillus*, bioflocs, molasses, pre-treated, rice bran, synbiotic

1 | INTRODUCTION

The promising scenario for shrimp farming has been stimulated by the adoption of super-intensive production systems with little or no water exchange, which have shown positive results in terms of yield and sustainability (Crab et al., 2012). Among these new technologies with the minimum use of water, recirculating aquaculture systems, biofloc technology and system synbiotic stand out (Andrade et al., 2021; Avnimelech et al., 2012; Badiola et al., 2012; Dawood & Koshio, 2019; Romano et al., 2018).

Culture in heterotrophic or mixotrophic system, namely BFT (biofloc technology), and synbiotic systems are characterized by being a super-intensive production system with minimal or no water exchange that generate high yield and have a reduced environmental impact (Andrade et al., 2021; Avnimelech et al., 2012; Crab et al., 2012; Dawood & Koshio, 2019; De Schryver et al., 2008; Emerenciano et al., 2017; Romano et al., 2018; Samocha et al., 2017). In this system, a large number of microorganisms play an important role in the maintenance of water quality and in the nutrition of animals, which positively affects the costs of feeding, survival rates, resistance to diseases and the feed conversion for shrimp and fish (Ahmad et al., 2017; Andrade et al., 2021; Avnimelech et al., 2012; Crab et al., 2012; Dauda, 2019; Dawood & Koshio, 2019; De Schryver et al., 2008; Emerenciano et al., 2017; Romano et al., 2018; Samocha et al., 2017).

The BFT system is also characterized by large increments of inorganic nitrogen, especially ammonia (NH_4^+ and NH_3) derived from unconsumed feed and the excreta of reared animals (Avnimelech et al., 2012). To revert this picture, external sources of organic carbon are added in the water, aiming to modify the organic carbon content of the feed, in the attempt of increasing the C/N ratio and stimulate the growth of heterotrophic bacteria, which are responsible for the metabolism of nitrogen compounds and serve as an suitable microbial protein biomass for animals (Avnimelech et al., 2012; Crab et al., 2012; De Schryver et al., 2008; Ebeling et al., 2006).

Organic carbon sources became one of the most studied parameters in intensive system, and its application is made in the attempt to associate efficiency with low cost and local availability (Dauda, 2019; Romano et al., 2018). Thus, depending on the region, the most commonly used sources of organic carbon vary from mono/oligosaccharides (e.g. glucose, molasses, sucrose and glycerol) to polysaccharides (e.g. rice bran, wheat, starch, among others) (Avnimelech et al., 2012; Crab et al., 2012; Dauda, 2019; Emerenciano et al., 2017). Some authors reported that polysaccharides tend to produce better results in terms of growth, but their low solubility in water can slow the removal of total ammonia nitrogen (TAN) of the water (Dauda, 2019; Dauda et al., 2017; Kumar et al., 2017; Pamanna et al., 2017; Serra et al., 2015; Vilani et al., 2016).

As an alternative to the low solubility of polysaccharide carbohydrates, pre-treated with microorganisms have been used in synbiotic systems, including the fermentation (without aeration) and/or cellular respiration (aeration) using *Bacillus* spp., which improves the solubility and can increase the content of total soluble sugars and crude protein, in addition to reducing the crude fibre of the polysaccharides (Dawood & Koshio, 2019; Romano et al., 2018). Furthermore, this type of fermentation and/or cellular respiration ingredient has known beneficial effects for the ecosystem, water quality and improvement of the gastrointestinal tract morphology. It also increases the

abundance of *Bacillus* while reducing the number of harmful bacteria and improves the performance and growth of animals that are reared in the system (Andrade et al., 2021; Dawood & Koshio, 2019; Romano et al., 2018). This kind of strategy contributes to reduced production costs and provides higher flexibility for farmers in terms of choosing organic carbon sources used in intensive systems (Dauda, 2019).

In addition to the effects of fermentation and/or cellular respiration of probiotic supplementation, studies have been demonstrating that distinct carbon sources used to stimulate floc production have an influence not only in the control of nitrogen compounds, but also in the microbial composition and nutritional properties of flocs, which in this case can lead to an increased use as microbial protein by reared animals (Abreu et al., 2019; Ahmad et al., 2017; Crab et al., 2010; Ekasari et al., 2010, 2014; Kumar et al., 2017; Miao et al., 2017; Nevejan et al., 2016; Romano et al., 2018).

Studies demonstrated the possibility of growing *Macrobrachium rosenbergii* in intensive systems throughout different stages (Ballester et al., 2017; David et al., 2016; Miao et al., 2017; Negrini et al., 2017; Perez-Fuentes et al., 2013). However, is it currently known that feed, inadequate stocking densities, water quality parameters, organic carbon sources and C/N ratio might cause problems related to mortality, food wastage, growth performance and yield, in addition to hampering animals health (Ballester et al., 2017; David et al., 2016; Marques et al., 2000; Miao et al., 2017; Negrini et al., 2017).

Based on the aforementioned, there is an increasing interest in the development of new technologies, in the attempt of understanding and improving productive aspects of the species *M. rosenbergii* in intensive nurseries with minimal water exchange. Considering the technological and environmental advances associated with aquaculture systems, it is important to expand the knowledge related to the organic carbon sources that promote higher stability of water quality parameters, optimize growth performance and improve the welfare of reared animals. Thus, this study aimed at evaluating the effects of different sources of organic carbon (molasses and rice bran) and culture system (synbiotic and BFT) on water quality and productive performance of post-larvae of *M. rosenbergii*.

2 | MATERIAL AND METHODS

2.1 | Experimental conditions

The experiments were conducted in the Laboratory of Carcinology of the Fishing Engineering course of the Federal University of Alagoas (LabCarci). Floc maturation tanks were installed in this area, as well as the experimental units. A radial air compressor (0.28 hp) and porous stones (2 units,

2.5 cm diameter/tank) were used to oxygenate the water. The water containing flocs that was used in the experimental units came from four circular maturation tanks (unitary volume of 250 L), each corresponding to an organic carbon source: tank 1 had molasses as organic carbon source (BFT), tank 2 had molasses pre-treated with *Bacillus* spp. without and with aeration (synbiotic), in tank 3 the carbon source was raw rice bran (BFT) and tank 4 the carbon source was raw rice bran pre-treated with *Bacillus* spp., without and with aeration (synbiotic).

The maturation tanks were prepared by stimulating the formation of microbial flocs, during 30 days. In the first 20 days of maturation, a C:N ratio of 15:1 was used to stimulate and stabilize the heterotrophic community (Emerenciano et al., 2017), and in the last 10 days, this relation was reduced to 5:1, according to the recommendations of Ebeling et al. (2006). In these maturation tanks, the temperature was maintained between 28 and 30°C, pH between 8 and 9 and alkalinity between 120 and 130 mg L^{-1} $CaCO_3$ (sodium bicarbonate addition).

The experimental units composed of rectangular tanks (55 × 35 × 30 cm) totaling a bottom area of 0.19 m^2, useful internal surface area of 0.64 m^2 and useful volume of 50 L.

The water inside the experimental units was prepared one day before stocking the animals. This procedure was carried out by filling 30% of the tanks with floc water from the maturation tanks and 70% of filtered water (10 µm), treated with sodium hypochlorite in a concentration of 10 ppm of activated chloride. Alkalinity was initially adjusted (120-130 mg $CaCO_3$ L^{-1}) with the addition of sodium bicarbonate. No water exchange in biofloc and synbiotic treatments only the dechlorinated freshwater was added to compensate for evaporation losses.

2.2 | Synbiotic preparation

The synbiotic (molasses and rice bran) were pre-treated with a commercial microbial blend (*B. subtilis* 2.2 × 10^9 UFC g^{-1}, *B. licheniformes* 1.8 × 10^9 UFC g^{-1} and *Bacillus* sp. 1.6 × 10^9 UFC g^{-1}) (Kayros Ambiental and Agrícola, São Paulo, Brazil). The proportion used was defined as follows: for every 100 g of carbohydrate (molasses or rice bran), 20 g of sodium bicarbonate, 0.2 g of the microbial blend and 1 L of freshwater were added. Subsequently, the mixture was homogenized, placed inside a plastic Becker (500 ml), covered with a black cloth and maintained during 24 h without aeration, so that the anaerobic fermentation process occurred. Then, the aerobic process was initiated by uncovering the Becker and inserting an aeration porous stone that oxygenated the mixture during 24 h. Throughout the procedure, the mixture had its pH maintained ≥6.0 and temperature between 27 and 29°C. Therefore, this pre-treated process lasted for 48 h and then the synbiotics (molasses and

rice bran) were added in the maturation tanks. Subsequently, this process was repeated and the product was added to the experimental units three times a week.

2.3 | Control and maintenance of flocs in the experimental units

The concentration of organic carbon present in each sample of carbohydrates (molasses 37.3% C and rice bran 43% C) was performed in the Laboratory of Applied Electrochemistry (LEAp) of the Institute of Chemistry and Biophysics (IQB) of the Federal University of Alagoas (UFAL), by means of the method of chemical oxidation with dichromate (APHA, 1995).

The carbohydrates were weighted and added into the experimental units by keeping a C:N ratio of 5:1, as suggested by Ebeling et al. (2006). The determination of the amount of nitrogen added to the experimental tanks through the feed was made using the following equation: $\Delta N = Q_{feed} \times \%CP_{feed} \times DM \times \%N_{feed} \times \%N_{excretion}$, in which Q_{feed} is the amount of feed provided based on the percentage of biomass of stocked animals, $\%CP_{feed}$ is the percentage of crude protein of the feed, DM is the dry matter of the feed, N_{feed} is the percentage of nitrogen in the feed (approximately 16% of crude protein), and $N_{excretion}$ is the ammonification of the non-metabolized feed plus the excretion, which is equivalent to approximately 75% (Crab et al., 2012; De Schryver et al., 2008; Emerenciano et al., 2017).

The amount of carbohydrates (wet weight) added in the experimental units to obtain a C/N ratio of 5:1 was determined according to the equation: $\Delta carbohydrate = [(\Delta N \times C/N) \times \%C^{-1})]$, in which ΔN is the amount of nitrogen introduced in the system and $\%C^{-1}$ represents the percentage of organic carbon present in the respective carbohydrate, i.e. 0.37 for molasses and 0.43 for rice bran. The amount of carbon present in the feed was not taken into account.

2.4 | Experimental design

Newly metamorphosed *M. rosenbergii* post-larvae (PL) were obtained in a commercial prawn hatchery (Aquamarão, PE, Brazil) and acclimatized to experimental conditions during five days. Then, initial biometrics was performed (mean weight 10.01 ± 2.1 mg), and the animals were stocked (0.8 PL L^{-1}) in the experimental units, arranged in a completely randomized design with five treatments and four replicates. The experiment lasted for 35 days, which corresponds to the average culture time of a primary nursery (New et al., 2010). Treatments were identified as follows: M = molasses as organic carbon source (BFT); Mf = molasses pre-treated with *Bacillus* spp., without and with aeration (synbiotic); RB = rice bran as organic carbono source (BFT); RBf = rice bran pre-treated with *Bacillus* spp., without and with aeration (synbiotic); Ct = clear water with a 20% daily water

exchange. Floating substrates made of rectangular PVC (Polyvinyl Chloride), and plastic meshes (10 mm) were added to the control treatment (Ct). The sum of these structures had an area of 0.037 m^2, corresponding to 19% of the bottom area or 5.8% of the useful internal surface area of the experimental unit.

A factorial scheme was also considered, comparing two factors: source of organic carbon (molasses or rice bran) and system (BFT and Synbiotic). Both the M and RB treatments received the same amount of carbohydrates and microbiological blend (*B. subtilis* 2.2 × 10^9 UFC g^{-1}, *B. licheniformes* 1.8 × 10^9 UFC g^{-1} and *Bacillus* sp. 1.6 × 10^9 UFC g^{-1}) (Kayros Ambiental and Agrícola, São Paulo, Brazil), but without pre-treated.

The photoperiod was maintained as a light/dark regime of 12 h (12:12), using artificial lighting, and the temperature was maintained within the range 27–30 °C, with the aid of heaters (100 W) with thermostats in each experimental unit.

2.5 | Monitoring and water quality analysis

The water quality variables pH, temperature (°C), dissolved oxygen - DO (mg L^{-1}), total dissolved solids - TDS (mg L^{-1}) and electrical conductivity (µS cm^{-1}) were determined on a daily basis, with the aid of a multiparameter probe (YSI ProOD). The variables total ammonia–nitrogen (TAN) (mg L^{-1}), nitrite-nitrogen (N-NO_2) (mg L^{-1}) and nitrate-nitrogen (N-NO_3) (mg L^{-1}) were weekly measured, using a spectrophotometer (HANNA® Instruments, model HI 83399, Belgium) and the reagents of the analysis kit HI93700-01. Settleable solids were also measured according to the adapted methodology of Samocha et al. (2017), through which 1000 ml samples were collected from each experimental unit, transferred to Imhoff cones and decanted for 50 min. Total alkalinity (mg $CaCO_3$ L^{-1}) and hardness (mg $CaCO_3$ L^{-1}) were analysed in the same frequency, being determined through titration, using sulphuric acid (H_2SO_4) and EDTA (Na_2H_2Y), respectively (BRASIL, 2013).

2.6 | Protein microbial floc

The protein content of microbial flocs was also analyzed at the end of the experiment, by means of standard methods (AOAC, 2016) that measure the Kjeldahl nitrogen (N × 6.25), at the Laboratory of Physico-Chemical Analysis (LAFQA) of the Department of Economic Sciences, at the Federal Rural University of Pernambuco (UFRPE), Brazil. Water samples from each experimental unit were filtered using different meshes of 20 and 100 µm, and dried (60°C) for 24 h.

2.7 | Feeding and growth performance

The prawns were fed five times a day (8h00, 11h00, 14h00, 16h00 and 19h00), with a commercial feed formulated for marine shrimp (Camaronina CR2, 40% crude protein and 8% lipids, Presence Animal Nutrition, Brazil). Throughout the rearing period, daily observations and weekly biometrics (n = 25% of the population) were performed in order to adjust the daily amount of feed provided. The experimente was initiated with a total supply of 100% of the animals' biomass, which was gradually reduced until reaching 12% of the biomass during the last week.

At the end of the experimental period (35 days), all animals from each experimental unit were counted and individually weighted. The following parameters were measured: survival (S% = (number of live animals/number of stocked animals) × 100); yield (g m^{-3}), mean final weight, weekly weight gain (WWG = weight gain/weeks of culture); specific growth rate (SGR = (ln final weight - ln initial weight/days) × 100); and feed conversion ratio (FCR = provided feed/biomass gain).

2.8 | Statistical analysis

Possible interactions between treatments were verified by means of a variance analysis (one-way ANOVA) considering all treatments. Additionally, a two-way ANOVA was carried out, comparing two factors: source of organic carbon (molasses or rice bran) and system (BFT and Synbiotic). For this purpose, the homogeneity of variances and normality of data were verified with the tests of Cochran and Shapiro–Wilk, respectively. In case significant differences were observed among treatments, the Duncan's test was applied at a 5% probability level. When the data did not follow the appropriate premises, the non-parametric test of Kruskal–Wallis or Friedman (for the factorial scheme) was performed, and when significant differences were found among treatments, the Dunn's test was applied considering a 5% probability level. Statistical analysis and graphic preparations were performed with the aid of the software R Project 3.1.0 and SigmaPlot 12.0, respectively.

3 | RESULTS

All variables related to water quality were significantly affected ($p < 0.05$) by the factors organic carbon sources and/or system, except for dissolved oxygen, temperature and pH. Both the temperature and dissolved oxygen remained above 25°C and 4 mg L^{-1}, respectively. In addition, all treatments presented minimum pH of 8.0 and alkalinity above 100 mg L^{-1} throughout the experimental period (Table 1).

Tabela 1 Means and SD (±) of the parameters monitored during the period of the nursery experiment with different organic carbon sources and system (BFT and Synbiotic).

Variables	**Treatments**					**(*p* value)^{Int}**		
	Ct	**M**	**Mf**	**RB**	**RBf**	**C**	**S**	**C-S**
DO (mg L^{-1})	5.50±0.06	5.67±0.11	5.48±0.16	5.55±0.05	5.52±0.17	ns	ns	ns
T (°C)	28.49±0.13	28.19±0.53	28.82±0.75	28.38±0.37	28.66±0.84	ns	ns	ns
pH	8.88±0.06	8.75±0.07	8.80±0.06	8.72±0.08	8.85±0.11	ns	ns	ns
Alkalinity (mg $CaCO_3$ L^{-1})	117.47±18.42^{c}	126.67±7.46^{b}	149.53±11.79^{a}	120.43±6.80^{bc}	153.06±15.06^{a}	ns	*	*
Hardness (mg $CaCO_3$ L^{-1})	49.55±7.19^{c}	102.29±9.36^{b}	126.11±9.13^{a}	125.41±11.53^{a}	107.12±13.94^{b}	ns	ns	*
TAN (mg L^{-1})	0.14±0.14^{b}	0.15±0.12^{b}	0.15±0.10^{b}	0.30±0.35^{a}	0.27±0.16^{ab}	*	ns	ns
N-NO_2 (mg L^{-1})	0.019±0.01^{c}	0.092±0.11^{ab}	0.163±0.20^{a}	0.062±0.06^{ab}	0.034±0.04^{bc}	*	ns	ns
N-NO_3 (mg L^{-1})	0.016±0.06^{a}	3.15±1.30^{b}	2.52±1.52^{b}	3.35±1.81^{b}	3.12±2.05^{b}	ns	ns	ns
Conductivity (µS cm^{-1})	294.06±34.07^{c}	731.56±40.84^{b}	811.06±33.72^{a}	782.75±46.45^{a}	800.31±44.92^{a}	ns	*	*
TDS (mg L^{-1})	184.93±0.61^{a}	452.61±11.18^{b}	510.29±6.28^{c}	484.06±12.10^{d}	497.41±8.53^{c}	ns	*	*
*SS (mL L^{-1})	-	*28.88±16.18^{a}	20.75±16.03^{ab}	19.25±8.22^{ab}	14.16±8.51^{b}	*	*	*

Note: The data correspond to the mean ± SD. Results from Duncan's test and Dunn's test. Means in the same row with different superscripts differ significantly ($P < 0.05$). Ct = clear water with a 20% daily water exchange; M = molasses as organic carbon source (BFT); Mf = molasses pre-treated with *Bacillus* spp., with and without aeration (Synbiotic); RB = rice bran as organic carbon source (BFT); RBf = rice bran pre-treated with *Bacillus* spp., with and without aeration (Synbiotic); TAN: total ammonium nitrogen; N-NO_2: nitrite-nitrogen; N-NO_3: nitrate-nitrogen; TDS: total dissolved solids; *SS: settleable solids (There was removal of solids in treatment M in the second and fourth week of culture); (p value)^{Int} = interaction between C (carbon source) and S (system). Non-parametric analysis: DO, Temperature, pH, Alkalinity, Hardness, TAN, N-NO_2, N-NO_3.

The nitrogen compounds monitored in this study revealed significant differences among treatments, with the lowest means and variations being observed in the Ct. The concentrations of TAN and $N\text{-}NO_2$ significantly varied, with peaks being recorded during the third week of culture in treatments RB (0.79 ± 0.57 mg L^{-1}) and Mf (0.53 ± 0.2 mg L^{-1}) (Figure 1). In relation to $N\text{-}NO_3$, all treatments showed a tendency to accumulate this compound over time, except for Ct.

Significant differences were found for settleable solids levels, with the highest mean registered for the treatment M, which exceeded 15 ml L^{-1} at the end of the first week of experiment. The treatment RBf presented values greater than 15 ml L^{-1} in the fourth week, while RB registered higher values in the third week and Mf in the second week (Figure 2). In the second and fourth week of experiment, settleable solids were reduced with the aid of settling chambers in the treatment M. Nevertheless, this treatment revealed values that were significantly higher in comparison with others, in relation to this variable.

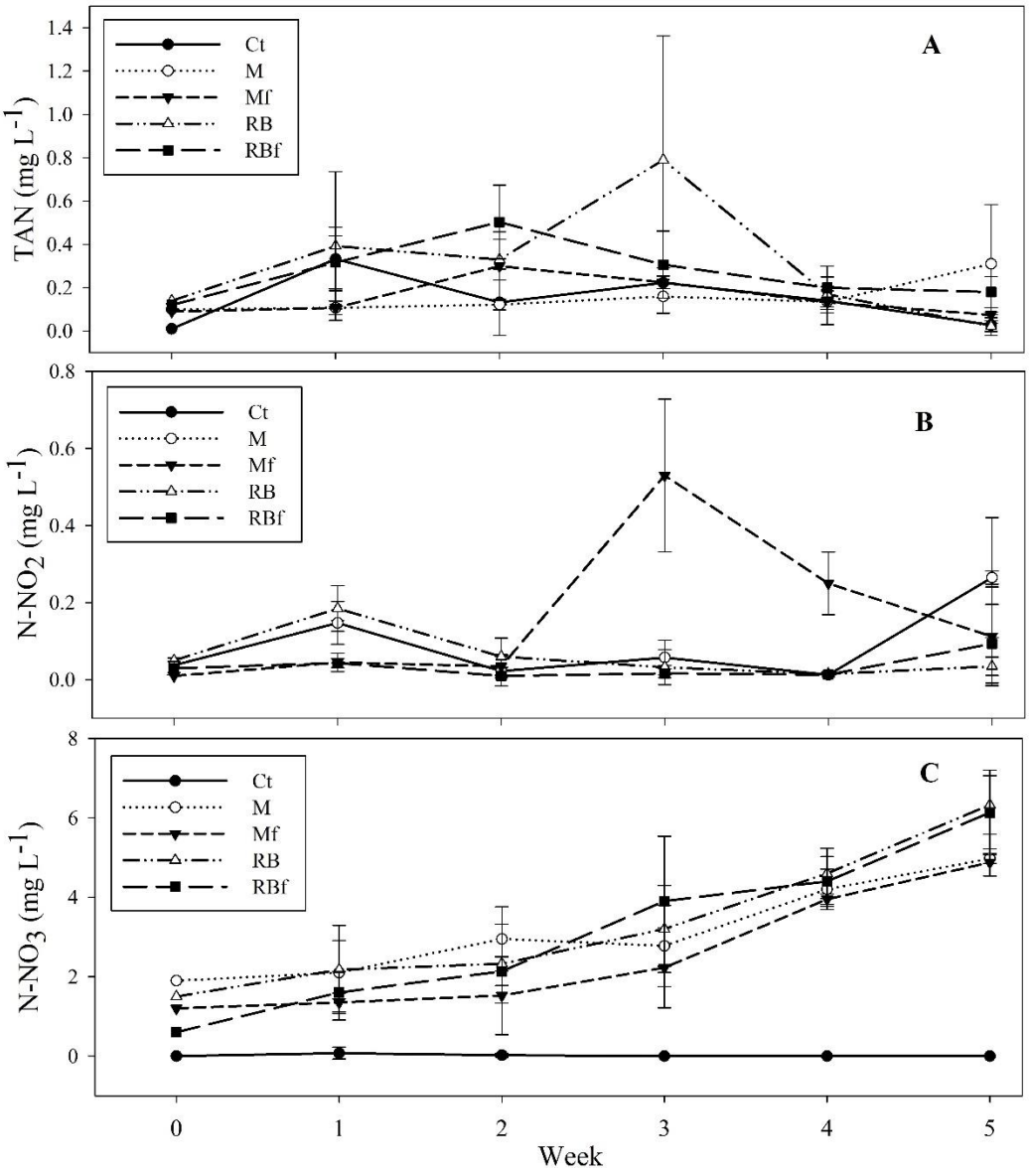

Figure 1 Variations in weekly concentrations of TAN, N-NO2 and N-NO3 for the different organic carbon sources and system in nurseries used to *M. rosenbergii*.

The values of electrical conductivity and TDS were lower in Ct (294.06 ± 34.07 µS cm^{-1} and 184.93 ± 0.61 mg L^{-1}, respectively) in relation to other treatments, which ranged from 651 to 862 µS cm^{-1} for electrical conductivity and from 415 to 591 mg L^{-1} for TDS.

The protein content of microbial flocs differed among treatments and factors, with the following values being observed: M (43.27 ± 0.76% dry weight), RBf (34.07 ± 0.54% dry weight), Mf (30.08 ± 0.40% dry weight) and RB (29.77 ± 0.48% dry weight). No significant differences were observed only between treatments Mf and RB (Figure 3).

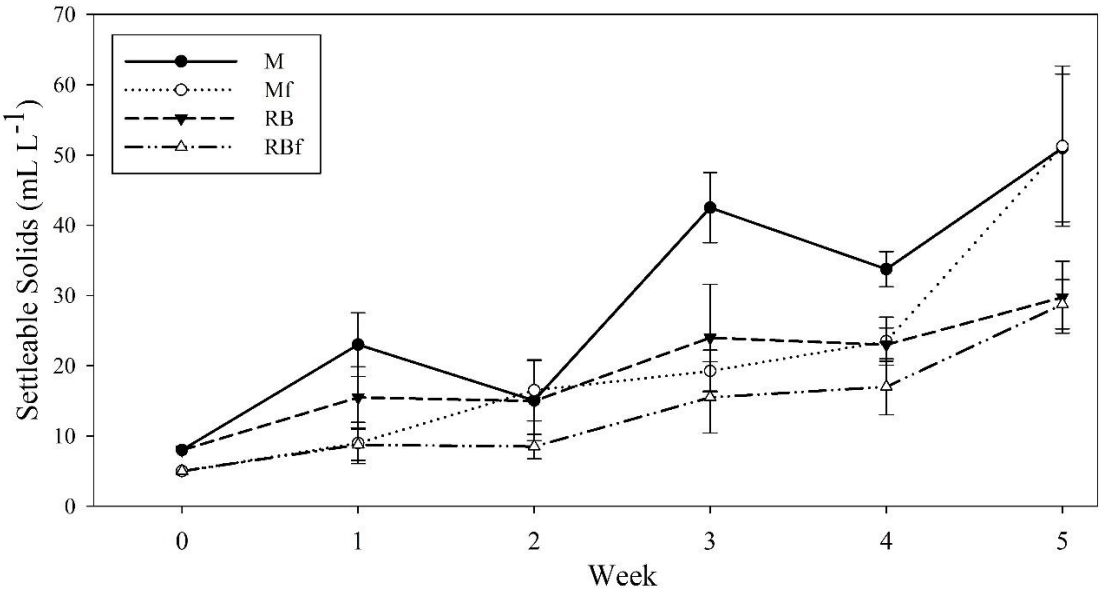

Figure 2 Variations in weekly concentrations of settleable solids (SS) for different sources of organic carbon and system in nurseries used to M. rosenbergii.

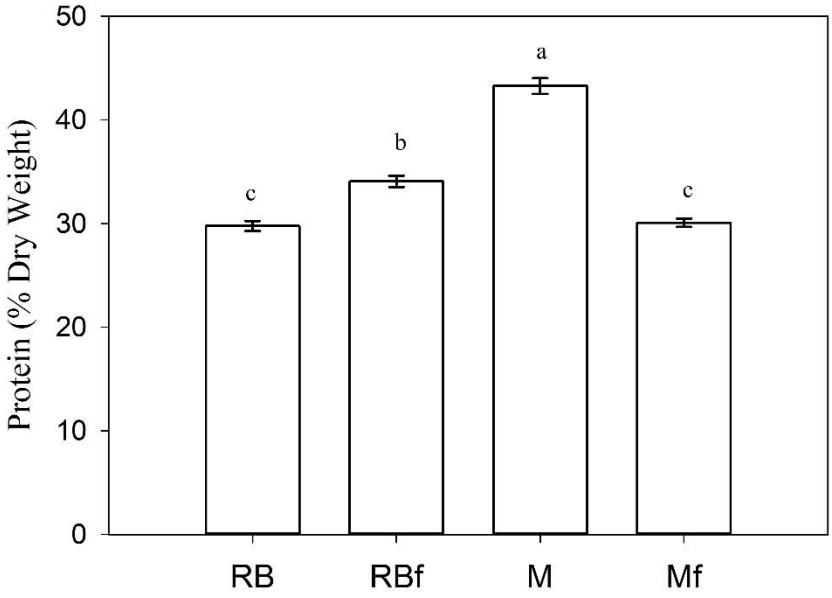

Figure 3 Protein concentration (% dry weight) of the flocs analyzed at the end of the experimental period. Different letters indicate statistical differences between treatments (p <0.05).

Significant effects of the system ($p < 0.05$) were observed for all variables related to growth performance, with exceptions for survival and FCR (Table 2). Regarding the mean final weight, the treatment RBf displayed the best result in comparison with others (122.85 ± 12.50 mg). In relation to SGR, RBf also presented higher mean values ($p < 0.05$) as than to Ct and RB, but no diferences ($p >$

0.05) to the treatments that had molasses as the source of organic carbon (M and Mf). Finally, the interaction between factors, the best results were observed for the main growth variables when synbiotic was adopted, mainly for rice bran as organic carbon source. For the system factor, the synbiotic presented better result in relation to the BFT for the variables mean final weight (mg) (106.27 ± 21.48 vs 82.41 ± 21.75), weekly weight gain (mg) (18.64 ± 4.25 vs 14.48 ± 4.35), yield (g m^{-3}) (75.80 ± 10.98 vs 55.08 ± 20.13) and SGR (% day^{-1}) (6.61 ± 0.61 vs 5.93 ± 0.79). Regarding the organic carbon source factor (molasses or rice bran), there was no significant difference ($p > 0.05$) between treatments for these variables (Table 2).

Tabela 2 Growth performance of *M. rosenbergii* during nursery stage, in tanks fertilized with different organic carbon sources and system.

Variables	Treatments					(*p* value)^{Int}		
	Ct	M	Mf	RB	RBf.	C	S	C-S
Survival (%)	76.75±18.13	88.50±9.29	96.50±3.3	78.00±18.60	89.66±7.50	ns	ns	ns
Mean final weight (mg)	72.47±18.43^{c}	97.73±15.50^{b}	89.70±13.69^{bc}	67.47±16.40^{c}	122.85±12.50^{a}	ns	*	*
Weekly weight gain (mg)	17.47±3.09^{b}	17.47±3.10^{b}	15.94±2.73^{bc}	11.49±3.28^{c}	22.26±2.97^{a}	ns	*	*
SGR (% day^{-1})	5.58±0.75^{bc}	6.47±0.45^{ab}	6.23±0.47^{abc}	5.39±0.68^{c}	7.11±0.35^{a}	ns	*	*
FCR	4.83±3.05	3.31±0.71	3.33±0.58	4.55±1.63	2.83±0.13	ns	ns	ns
Yield (g m^{-3})	44.38±17.68^{b}	67.45±11.23^{a}	68.86±9.43^{a}	42.71±20.27^{b}	85.02±2.15^{a}	ns	*	*

Note: The data correspond to the mean ± SD. Results from Duncan's test and Dunn's test. Means in the same row with different superscripts differ significantly ($P < 0.05$). Ct = clear water with a 20% daily water exchange; M = molasses as organic carbon source (BFT); Mf = molasses pre-treated with *Bacillus* spp., with and without aeration (Synbiotic); RB = rice bran as organic carbon source (BFT); RBf = rice bran pre-treated with *Bacillus* spp., with and without aeration (Synbiotic); FCR: feed conversion ratio; SGR: specific growth rate; (p value)^{Int} = interaction between C (carbon source) and S (system).

4 | DISCUSSION

All water quality parameters monitored in this study remained within the adequate ranges for the *M. rosenbergii* during nursery stage (New et al., 2010). In intensive systems, the heterotrophic, chemosynthetic and autotrophic bacteria consume significant amounts of inorganic carbon; thus, it is recommended that alkalinity remains between 100 and 150 mg $CaCO_3$ L^{-1}, in order to stimulate the microbial activity and avoid low values of pH that might impair the growth of reared animals (Avnimelech et al., 2012; Ebeling et al., 2006; Emerenciano et al., 2017; Samocha et al., 2017). Therefore, because of the microbial activity, it is natural that alkalinity levels tend to be reduced in those systems, which was observed in treatments M and RB during the last weeks of experiment, even though it did not reach concentrations below 100 mg $CaCO_3$ L^{-1}. The highest mean values observed for the RBf and Mf treatments are related to the constant addition of sodium bicarbonate in pre-treated organic carbon. In this study, no differences were found between the factors organic carbon source and system for hardness in the water. The Ct treatment presented the lowest concentration (49.55 mg $CaCO_3$ L^{-1}), while the highest ones were registered in the experimental units containing bioflocs, with emphasis for Mf (126.11 mg $CaCO_3$ L^{-1}) and RB (125.41 mg $CaCO_3$ L^{-1}). However, this variation is within the limits recommended by New et al. (2010) (50–150 mg $CaCO_3$ L^{-1}) and Vasquez et al. (1989) (20–200 mg $CaCO_3$ L^{-1}).

Despite the fluctuations observed in the levels of TAN and N-NO_2 throughout the experiment, which is considered normal in intensive systems (Avnimelech et al., 2012; Ballester et al., 2017), the values remained within the recommended range for the species (TAN < 0.54 mg L^{-1} and N-NO_2 < 1.0 mg L^{-1}) (Dong et al., 2020; New et al., 2010; Perez-Fuentes et al., 2013). Probiotic supplementation with *Bacillus* spp. together with additional organic carbon sources are notably effective in relation to the metabolization of nitrogen compounds (Crab et al., 2010; Miao et al., 2017; Romano et al., 2018), as observed in this study. Carbon sources such as molasses, glycerol and acetate for instance are more soluble in water and tend to more readily available to the microbial community, thus resulting in a higher efficiency of the metabolization of nitrogen compounds (Crab et al., 2010; Dauda et al., 2017; Kumar et al., 2017; Vilani et al., 2016). This trend was also observed in this study, seen that the treatment RB presented TAN concentrations that were higher as than to other treatments that used molasses as organic carbon source (M and Mf). The behaviour of N-NO_3 levels in treatments with intensive system followed the same trend observed by Luo et al. (2019), accumulating throughout the experimental period, without reaching values considered harmful (8.6 mg L^{-1}) for the animals (Dutra et al., 2019).

The electrical conductivity provides information on the metabolism of the entire aquatic ecosystem (Sipaúba-Tavares, 1995), so that high conductivity values such as the ones found in biofloc and synbiotic treatments indicate an elevated degree of decomposition, which in this case makes it possible to infer on the nutrients' availability in the rearing system. In intensive systems, feed consumption and animal excreta associated with minimal water exchange contribute to an increased concentration of solids (Samocha et al., 2017). Hence, settleable solids reached concentrations above the recommended by some authors (5–15 ml L^{-1}) (Emerenciano et al., 2017; Samocha et al., 2017), especially during the last weeks of experiment. Nevertheless, the growth and survival of animals did not seem to be negatively affected by the concentration of this variable in this study, seen that the treatments M and RBf were higher than the Ct in terms of mean weight, weight gain and yield. Apart from that, it should be noted that the effects of organic carbon pre-treated with *Bacillus* spp., without and with aeration in increasing the solubility of organic carbon sources resulted in the differences found for the concentrations of settleable solids between treatments synbiotic in comparison with BFT.

The higher protein content in flocs of treatment M (43.37 ± 0.76% dry weight) as than to others treatments might not have been sufficient to raise their growth rates of animals. The greatest protein content found in flocs of RBf (34.07 ± 0.54% dry weight) in relation to RB (29.77 ± 0.58% dry weight) might be an indicative of the synbiotic system, corroborating the results reported by Romano et al. (2018). The quality of this protein is, however, related to the composition of flocs, which might be poor in essential amino acids and fatty acids when bacteria and other heterotrophic microorganisms dominate the microbial community, or even abundant in these elements, when there is a predominance of certain groups of photoautotrophic organisms (Abreu et al., 2019; Ekasari et al., 2014). The higher settleable solids in the treatment M, associated with low luminosity, may have contribute to the establishment of this scenario of predominance of heterotrophic organisms, generating a high protein content that is, however, deficient in methionine and fatty acids. The size of microbial flocs is another factor that strongly influences its nutritional value, i.e. larger flocs (>100 µm) can contain a higher content of protein, lipids and a better profile of essential amino acids (Ekasari et al., 2014). In addition to containing a high settleable solids, visual observations also noted larger flocs in treatment M.

The animals' survival was not affected by the rearing system, nor by the strategies of organic carbon supply in the systems. Means ranged between 76.75% and 96.5%, which were similar to the studies of Marques et al. (2000), Crab et al. (2010) and Ballester et al. (2017), using the same species in the same developmental stage. Therefore, the satisfactory results for this variable might be related

to an efficient feeding strategy and maintenance of water quality parameters in adequate levels (Dauda, 2019; Dong et al., 2020; Dutra et al., 2019; New et al., 2010). Concomitant to this, and given the aggressive and territorial aspects of *M. rosenbergii* (New et al., 2010), the stocking densities and the use of artificial substrates (Ct) are variables that may also have influenced the animals' survival, seen that rearing *M. rosenbergii* at high densities tend to result in lower survival (Coyle et al., 2010; Mamun et al., 2010; Marques et al., 2000; Negrini et al., 2017).

Differently from survival, several other growth performance parameters presented significant differences ($p < 0.05$) among treatments, with emphasis on the RBf, which registered the highest mean final weight. These results indicate that this strategy of rice bran (organic carbon source) pre-treated with *Bacillus* spp., without and with aeration (synbiotic system) may have generated a better synergistic effect between animal welfare and supplying an additional feed by microbial community.

The treatments RB, Ct and Mf had the lowest performance in relation to mean weight and did not differ statistically among each other, even though the treatment M was significantly different from RB and Ct. In line with the results obtained in this study, other studies also reported the effects of different organic carbon sources in the growth of animal's culture in intensive systems. Khanjani et al. (2017) culture *Litopenaeus vannamei* in biofloc system with diferente carbon sources and found a higher performance of animals in the tanks containing molasses instead of more complex carbon sources without pre-treated, such as starch and wheat flour.

Other studies evaluated the growth of the same species with different sources of carbon (tapioca starch, wheat flour, rice bran and molasses) and reported higher performance when polysaccharides such as wheat flour and rice bran were used (Pamanna et al., 2017; Serra et al., 2015; Vilani et al., 2016). However, in this experiment, synbiotic proved to be a major factor to increase the growth indexes in the treatment using rice bran as organic carbon source, seen that this strategy brings beneficial effects on solubility, total soluble sugar contents, crude protein and reduction of the crude fibres present in this polysaccharide (Dawood & Koshio, 2019; Romano et al., 2018).

Improvements in the yield performance of prawn culture with minimal exchange water in relation to conventional systems with regular water exchanges were extensively documented over the last few years (Ahmad et al., 2017; Crab et al., 2012). On the other hand, and similarly to this experiment, in which animals culture in clear water (Ct) presented similar results for some growth variables to the animals reared in RB and Mf, other studies reported comparable results (Ballester et al., 2017; Rajkumar et al., 2016). It is noteworthy that this dissonance in the results may be linked to the distinct effects that each organic carbon source has on the amount of flocs and in the composition

of microorganisms in the system, which also interfere its nutritional value (Crab et al., 2010; Dauda et al., 2017; Ekasari et al., 2014; Rajkumar et al., 2016).

The pre-treated with *Bacillus* spp., without and with aeration (synbiotic) efficiently improved the solubility of the polysaccharides (organic carbon–rice bran) used in this study and facilitated the conversion of dissolved nitrogen compounds by heterotrophic bactéria into microbial biomass, thus contributing for the growth of reared animals (Dauda, 2019; Dauda et al., 2017; Romano et al., 2018). In addition, Dawood and Koshio (2019) and Andrade et al. (2021) stated that fermented feeds and rice bran (synbiotic) used as fertilizers in intensive systems have beneficial effects on ecosystems, on the morphology of the gastrointestinal tract, intestinal microbiota and growth of animals. In this sense, these nutritional improvements made possible by the pre-treated with *Bacillus* spp., may also have directly contributed to the animals' performance in the treatment RBf. On the other hand, the effects of synbiotic in the treatment Mf were more noticeable in improving the solubility of the carbohydrate in relation to the lower accumulation of settleable solids in relation to the treatment M.

The values of FCR observed in this study were higher in comparison with other studies on the same species, in both clear water and intensive system (Ballester et al., 2017, 2018; Mamun et al., 2010; Negrini et al., 2017). Despite the best results of FCR presented by those authors, it is worth noting that different rearing stages (primary or secondary nursery) generate distinct growth rates, which is natural for this species, seen that older individuals remain for longer periods in the same developmental stage (New et al., 2010). In addition, the amount of feed provided is another factor that can influence the FCR. While those authors considered percentages of the biomass between 30% and 7%, in this experiment the amount supplied varied from 100% to 12%; therefore, this amount of feed might have contributed for the higher animals' survival; however, it provided an increase in FCR.

It is possible to conclude that the sources of organic carbon used in this study were effective in controlling nitrogen compounds in the rearing system. The synbiotic increased the solubility of carbohydrates, and this effect reduced the mean values of settleable solids in the system. In addition to maintaining the RBf treatment (synbiotic) with *Bacillus* spp. also improved the prawn mean final weight and weekly weight gain. Conversely, without synbiotic, the use of rice bran did not prove to be advantageous in relation to the conventional system with water exchanges. The animals cultivated in the synbiotic system showed better results of final weight in relation to BFT. However, future research should be carried out in the attempt of understanding the effects of pre-treated with *Bacillus* spp on the biochemical alterations of carbohydrates, its influence in the composition of flocs, morphology of the gastrointestinal tract and intestinal microbiota.

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AUTHOR CONTRIBUTION

Robson Batista dos Santos: Investigation, conceptualization, methodology, experiment development, nutrients analysis, water quality analysis, data analysis, and writing (original draft); Alex Pereira Gonçalves, Rafaela Alves dos Santos, Mariana Lins Rodrigues and Valdemir Queiroz de Oliveira: Experiment development, nutrients analysis, water quality analysis, and writing (review and editing); Petrônio Alves Coelho Filho and Eudes de Souza Correia: Methodology, and writing (review and editing); Luis Otavio Brito: Supervision, methodology, conceptualization, and writing (review and editing).

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this study.

ETHICAL APPROVAL

The research undertaken complies with the current animal welfare laws in Brazil. The experimental animals with *Macrobrachium rosenbergii* does not need approval previously approved by the Ethics Committee on Animal Use of Brazil.

DATA AVAILABILITY STATEMENT

The authors confirm that all data involved in this study are available for publication.

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3. ARTIGO 2

The effect of different synbiotic preparation strategies on water fertilization and zootechnical performance of *Macrobrachium rosenbergii* reared in the nursery stage.

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The effect of different synbiotic preparation strategies on water fertilization and zootechnical performance of *Macrobrachium rosenbergii* reared in the nursery stage.

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Abstract

The reared of *Macrobrachium rosenbergii* was evaluated using a synbiotic system with different fermentation (anaerobic) and microbial respiration (aerobic) strategies. Post larvae (10.01 ± 2.0 mg) were stored (1 pl L^{-1}) in 20 experimental units for 35 days, comprising five treatments and four replications. Rice bran, a mix of probiotic microorganisms, alkalizing agents and water were used in the preparation of the synbiotic. Each treatment corresponded to a synbiotic preparation strategy: T12|12 = 12 h anaerobic and 12 h aerobic; T12|24 = 12 h anaerobic and 24 h aerobic; T24|0 = 24 h anaerobic only; T24|12 = 24 h anaerobic and 12 h aerobic; T24|24 = 24 h anaerobic and 24 h aerobic. The animals were fed four times a day (40% crude protein) and the main variables of water quality and animal growth were evaluated. The synbiotic preparation strategies used did not influence the stabilization time of nitrogen compounds in water. There were no differences in survival rates and the water quality variables remained adequate for the species. For the variables final average weight (mg) and yield (gm^{-3}), treatments T24|24 (221.3 ± 22 and 195.4 ± 14.6) and T12|24 (218.2 ± 27.6 and 196.2 ± 33.4) were higher than 24|00 (176.1 ± 24.5 and 151.3 ± 21.6). Thus, it is concluded that a longer preparation time of the fertilizer, especially contemplating the aerobic and anaerobic stages, can promote greater performance of the cultivated animals.

Keywords: Prawn, Anaerobic, Aerobic, Rice Bran, Production.

Introduction

Freshwater shrimp farming has *Macrobrachium rosenbergii* as one of its main protagonists, reaching a world production of 234 thousand tons in 2018 (FAO, 2021). Of this total, Brazil contributed with around 150 tons (FAO, 2021).

The productive potential of this species — high fecundity, rusticity and rapid growth — (New et al., 2010), has stimulated the development of research aimed at the intensification of crops, in order to make it possible to obtain greater productivity and profitability linked to the minimum use of water and environmental control (Crab et al. 2010; Perez-Fuentes et al. 2013; Ballester et al. 2017; Miao et al. 2017; Negrini et al. 2017; Ballester et al. 2018; Hosain et al. 2021; Santos et al. 2022). As a result, heterotrophic or mixotrophic farming, such as biofloc technology (BFT) and synbiotics, have gained prominence.

In these types of reared, the source of organic carbon as a promoter of microbial growth has become one of the most researched parameters, and its application tries to associate efficiency, low cost and availability in the local market (Dauda 2019; Romano et al. 2018; Abakari et al. 2020b). Thus, depending on the region, the most commonly used sources of organic carbon are carbohydrates - $(CH_2O)_n$ - from monosaccharides and disaccharides (glucose, molasses, sucrose, glycerol) to polysaccharides (rice bran, soy, wheat, starch, etc.) (De Schryver et al. 2008; Avnimelech et al. 2012; Crab et al. 2010; Emerenciano et al. 2017; Dauda et al. 2017; Kumar et al. 2017; Abakari et al. 2020b; Hosain et al. 2021; Santos et al. 2022).

In the case of polysaccharides, such as rice, wheat and soybean bran, for example, it is possible to find some works adopting fermentation and/or microbial respiration with *Bacillus* spp and other probiotic microorganisms (*Lactobacillus*, *Saccharomyces*, etc.) in order to achieve better results in terms of solubility; increase in soluble sugars, crude protein, and lipids content; and decrease in crude fiber and antinutritional factors (Romano et al. 2018; Dawood and Kashio 2019; Santos et al. 2022). This process has been known as synbiotic, as this terminology basically consists of the combination of a prebiotic source (e.g., bran, such as rice, wheat or soybean, used as a substrate) and the action of probiotic microorganisms (Romano 2017; Huynhtg et al. 2017; Romano et al. 2018; Andrade et al. 2020; Deepak et al. 2020; Liñan-Vidriales et al. 2021; Silva et al. 2021a, b; Santos et al. 2022). It is, therefore, the formulation of a biofertilizer using agro-industrial residues and probiotic microorganisms submitted to semi-solid and/or liquid fermentation. As a result, a nutritionally improved and rich in microorganisms beneficial to the reared environment product is obtained (Romano et al. 2018; Silva et al. 2021b).

Bacteria and yeasts are able to use a variety of agroindustry residues through fermentation processes and/or microbial respiration, such as rice bran and wheat bran. *B. licheniformis* and *B. subtilis*, for example, represent an attractive source of proteases when used in fermentation processes (Shim et al. 2010; Huynhtg et al. 2017; Cienfuegos-Martinez et al. 2020; Dawood and Kashio 2019). These processes generate transformations in carbohydrates (increase in soluble sugar, crude protein and lipids content; decrease in crude fiber, etc.), which result from the production of hydrolytic enzymes and secondary metabolites such as liposaccharides and peptidoglycans by these microorganisms (Romano et al. 2018; Huynh et al. 2017; Hofvendahl and Hagerdal 2000; Vassileva et al. 2021). Among the most commonly used vegetable bran, rice bran has shown advantages due to its low cost and low protein content (Romano et al., 2018), avoiding problems with nitrogen in the system, and also due to the reduction of solids, along with benefits to animal growth (Romano et al. 2018; Santos et al. 2022).

Fermentation of polysaccharide carbohydrates using probiotic microorganisms in the food and feed industry has been performed for quite some time (Huynh et al. 2017; Dawood and Koshio 2019). However, the application of this technique in the fertilization of aquaculture ponds is relatively recent, dating from the mid−1990s in Thailand, where it later became known as Aquamimicry (Romano 2017). In synbiotic systems, these fermentation and/or microbial respiration strategies have been useful to promote greater flexibility to shrimp farmers in terms of choosing carbon sources in the preparation of fertilizers in systems with minimal water exchange, in addition to generating beneficial effects on the reared environment, such as metabolization of nitrogen compounds, increase in beneficial bacteria, growth performance and use as food by reared aquatic animals (Romano et al. 2018; Dauda 2019; Andrade et al. 2020; Liñan-Vidriales et al. 2021; Lima et al. 2021; Silva et al. 2021a, b; Santos et al. 2022).

It is known, however, that for these fermentation processes to occur efficiently, it is necessary to know the environmental conditions that favor the metabolism of microorganisms, since variables such as temperature, aeration, pH and fermentation time have a great influence on microbial growth, production and enzyme activity (Potumarthi et al. 2007; Shim et al. 2010; Aguilar et al. 2019; Dawood and Koshio 2019; Sugiharto and Ranjitkar 2019). Sudden changes in pH or inappropriate values for the metabolism of microorganisms, for example, can lead to a reduction or even inhibition of the production of important metabolites, in addition to a decrease in microbial growth and enzymatic activity (Naidu and Devi 2005; Dawood and Kashio 2019). Most microorganisms prefer a pH between 4 and 7, especially lactic acid bacteria and yeasts (Hofvendahl and Hagerdal 2000; Peña et

al. 2015; Vassileva et al. 2021). To assist in this control, it is necessary to use alkalizing agents (limestones, carbonates, calcium and magnesium oxides or hydroxides, etc.) in adequate amounts.

Other authors have also reported the influence of fermentation time and/or microbial respiration in improving the nutritional value of the synbiotic fertilizer and the floc formed in the culture environment (increase in lipids and carbohydrate protein used as a substrate for the preparation of the synbiotic) (Romano et al. 2018; Santos et al. 2022).

The popularization of these reared system around the world has been accompanied by protocol variations in the preparation of the synbiotic, which has generated different effects on its proximal composition, on the of microbial floc composition, and on the performance of cultured animals (Romano 2017; Romano et al. 2018; Andrade et al. 2020; Hussain et al. 2021; Lima et al. 2021; Liñan-Vidriales et al. 2021; Silva et al. 2021b; Santos et al. 2022). Therefore, a need to contribute with information that helps in the consolidation of a protocol for synbiotic preparation using rice bran arises. Thus, the objective of this study was to evaluate the quality of the synbiotic prepared with different fermentation and microbial respiration times, as well as its effect on the zootechnical performance and water quality of the freshwater shrimp *Macrobrachium rosenbergii* reared in the nursery stage.

Material and Methods

Experimental structure and facilities

The experimental tests were carried out at the Laboratory of Shrimp Culture (LabCarci) at the Federal University of Alagoas, Brazil. For this purpose, rectangular tanks with dimensions of 55x35x30 cm were used, totaling a bottom area equivalent to 0.19 m², a useful internal surface area of 0.64 m² and a useful volume of 48 L. The aeration in the experimental units was provided by a centrifugal compressor (0.28 hp) and two porous stones (2.5 cm Ø). This structure was used both to carry out the water maturation procedure with the symbiotic and to test the animals' growth.

Experiment 1: Monitoring pH in the preparation of synbiotic with different alkalizers

For the preparation of the synbiotic, alkalizing agents, microbiological mix, rice bran and water were used in the following proportion: for every 100 grams of rice bran, 10 grams of alkalizing agent (sodium bicarbonate, dolomitic limestone or *Lithothamnium*) were added, along with 0.2 grams of microbiological mix and 1 liter of water. The alkalizing agents used were sodium bicarbonate – $NaHCO_3$ 27% Na, PRNT (real neutralizing power) 55% and PN (neutralizing power) 59%; *Lithothamnium* – Lothar, a commercial product containing 33% Ca, 2.54% Mg, PRNT 92.6% and

PN 93%, extracted from the seaweed *Lithothamnium calcareum*; dolomitic limestone filler – PRNT 100%, PN 100%, MgO 18% and CaO 30%. The probiotic product (microbiological mix) used had a minimum concentration of 1.0 x 10^9 CFU g^{-1} (colony forming unit per gram) (Epicin G2 — Epicore Networks Eastampton, United States — containing *Bacillus subtilis, B. licheniformis, Lactobacillus acidophilus, B. pumilus* and *Saccharomyces cerevisiae*). The rice bran was sieved through a 300 µm mesh and the water used was filtered (50 µm).

After mixing the components, they were placed in a cylindrical-conical container (2 L – experimental unit) and kept according to the time established for the processes of fermentation and microbial respiration to occur. During this procedure, the pH of the solution was monitored at the beginning and every 12 hours using a pH meter (Instrutherm pH 2600 – Instrutherm, Brazil). Six replicates were used with the following pH preparation and measurement strategies for each alkalizing agent: 24|24 Treatment = initial pH measurement, after 12 h of fermentation and after 24 h of fermentation (aeration was added to the replicates of the experimental units and the pH was measured for the respiration phase – aerobic, that is, pH 36 h aerobic and 48 h aerobic); Treatment 12|24 = initial pH measurement, after 12 h of fermentation (aeration was added to the experimental units and the pH was measured for the respiration phase – aerobic, i.e., pH 24 h aerobic and 36 h aerobic). The tested alkalizers had the following nomenclature: sodium bicarbonate (SB), dolomitic limestone (CA) and *Lithothamnium* (LT).

Experiment 2: Preparation of water from bioreactors with the synbiotic

After Experiment 1 of monitoring the pH in the preparation of the synbiotic, sodium bicarbonate (SB) was chosen as an alkalizing agent for the maturation of the water that would later be used for the reared of the animals. This bioreactor water preparation process was monitored in order to assess the effect of different synbiotic preparation strategies (fermentation| and/or microbial respiration times) on the behavior of nitrogen compounds. To accomplish this, 15 tanks (dimensions of 55x35x30 cm) were used, divided into five treatments and three replicates. Treatment 12|12 = 12 h anaerobic and 12 h aerobic; Treatment 12|24 = 12 h anaerobic and 24 h aerobic; Treatment 24|0 = 24 h of anaerobic fermentation only; Treatment 24|12 = 24 h anaerobic and 12 h aerobic; 24|24 treatment = 24 h anaerobic and 24 h aerobic. The same proportions of the components of Experiment 1 were maintained for the preparation of the synbiotic, that is, for every 100 grams of rice bran, 10 grams of alkalizing agent (SB), 0.2 grams of microbiological mix and 1 liter of water were added.

Initially, the water used for the bioreactors was filtered (50 µm), chlorinated (10 mg L^{-1} of active chlorine) and dechlorinated (sodium thiosulfate – 5 mg L^{-1} + aeration). Afterwards, there was

the initial fertilization of this water with triple superphosphate (0.287g P m^{-3}) and ammonium sulfate (2.7g N m^{-3}). Then, the protocol of feed addition (40% of crude protein) was initiated with nitrogen input in the experimental units, and the rice bran (synbiotic) as source of organic carbon. Thus, twice a week, feed (equivalent to 1 mg N L^{-1} = 40% CP x 0.16 = 6.4% N) and synbiotic prepared with rice bran (43% C) (Santos et al., 2022) were added to obtain a C:N ratio equivalent to 15:1, as adapted by Crab et al. (2012) and Emerenciano et al. (2017).

This bioreactor preparation stage lasted 35 days, and during this period the following variables were monitored: total alkalinity (at the start and at the end), total ammonia nitrogen (TAN), N-NO_2 and N-NO_3 (weekly).

Centesimal composition of the symbiotic fertilizer

In order to understand the effects of fermentation, microbial respiration, or a combination of the two on the lipid and protein content of the synbiotic, three forms of preparation were evaluated: ANA (24 hours of fermentation only – anaerobic), AER (24 hours of respiration only – aerobic), and ANA + AER (48 hours – combining 24 hours anaerobic plus 24 hours aerobic). These analyzes were performed in triplicate using standard methods (AOAC, 2016) at the Laboratory of Small Ruminants, Department of Animal Science, Federal Rural University of Pernambuco (UFRPE), Recife, Brazil. For protein content, nitrogen (N x 6.25) was measured using the Kjeldahl method in a nitrogen still (TE 0363/180L model; 256 Tecnal, São Paulo, Brazil). The total lipid content, in turn, was obtained by the Soxhlet extraction method using Hexane (98%) as solvent (model TE 1881/6, Tecnal, São Paulo, Brazil).

Experiment 3: Evaluation of the animals' growth in the synbiotic culture system

The animals used in the experiment (*M. rosenbergii*) were acquired in a commercial freshwater shrimp laboratory (Acquamarão Laboratory, Goiana, PE, Brazil) and acclimated to local conditions for five days. After acclimatization and initial biometry (10.01 ± 2.0 mg), 48 animals were stored (1 PL L^{-1}) for 35 days in the experimental units, composing a completely randomized design with five treatments and four replications. The water matured in Experiment 2 was used as inoculum (20% of the volume) to prepare the experimental units of treatment 3, which followed the same nomenclature and preparation strategy of the synbiotic biofertilizer: Treatment 12|12 = 12 h anaerobic and 12 h aerobic; Treatment 12|24 = 12 h anaerobic and 24 h aerobic; Treatment 24|0 = 24 h of anaerobic fermentation only; Treatment 24|12 = 24 h anaerobic and 12 h aerobic; 24|24 treatment = 24 h anaerobic and 24 h aerobic.

Fertilization of the experimental units was performed three times a week with the synbiotic. For this fertilization in the experimental units, a C:N ratio of 5:1 was used, as recommended by Ebeling et al. (2006), who suggest that 82% of the total ammonia nitrogen will be converted by heterotrophic bacteria and 18% by nitrifying bacteria, reducing problems with nitrite accumulation in the water. This adopted ratio, however, did not take into account the amount of organic carbon present in the feed.

Water quality analysis

Water quality variables such as pH, temperature (°C), DO – dissolved oxygen (mg L^{-1}), TDS – total dissolved solids (mg L^{-1}), and electrical conductivity (µS cm^{-1}) were measured daily using a multiparameter probe (HANNA® Instruments, model 9828). The variables TAN – total ammonia nitrogen (mg L^{-1}), N-NO_2 (mg L^{-1}) and N-NO_3 (mg L^{-1}) were measured weekly with the aid of a HANNA® Instruments spectrophotometer, model HI 83399 (Belgium), using the Nessler methods (TAN), an adaptation of the diazotization method (N-NO_2), and an adaptation of the cadmium reduction method (N-NO_3).

Total alkalinity (mg $CaCO_3$ L^{-1}) and total hardness (mg $CaCO_3$ L^{-1}) were determined every 10 days by titration with sulfuric acid (H_2SO_4) and EDTA (Na_2H_2Y), respectively (Brazil, 2013). Settleable solids (SS) were determined at days 1, 12, 24 and 35 of culture, using 1,000 mL samples from each experimental unit transferred to Imhoff cones and kept at rest for sedimentation during 50 minutes.

Feeding and prawn zootechnical performance

The prawns were fed four times a day (8 am, 11 am, 2 pm and 4 pm) with commercial feed for marine shrimp (Purina, Camaronina CR2, 40% crude protein and 8% lipids). Weekly biometrics were performed to adjust the daily feeding rate, starting at 40% of body weight and gradually decreasing to 12% in the last seven days of the experiment.

At the end of the experimental period (35 days) all prawns from each experimental unit were counted and weighed individually, which made it possible to assess survival (S% = (number of surviving shrimp / number of shrimp stocked) x 100); yield (grams m^{-3}), average final weight, weekly weight gain (WWG = weight gain / number of weeks of cultivation), specific growth rate (SGR = (ln final weight – ln initial weight / number of days of cultivation) x 100) and feed conversion factor (FCF = feed supplied / increase in biomass).

Statistical analysis

Possible differences between treatments were verified after their assumptions of variances homogeneity and data distribution normality with the Cochran ($p < 0.05$) and Shapiro-Wilk ($p < 0.05$) tests, respectively. The analysis of variance (ANOVA one-way) was used for data on zootechnical performance and proximal composition of the synbiotic. When the means were significantly different between treatments ($p < 0.05$), the test of Duncan ($p < 0.05$) was applied. In the case of water quality variables, Friedman's non-parametric test was implemented. When the medians were significantly different between treatments ($p < 0.05$), the Multiple Conover test with Holm-Bonferroni correction ($p < 0.05$) was used. Statistical routines and graphic illustrations were performed in the R Project 3.1.0 software (Package Agricolae) and SigmaPlot 12.0 software, respectively.

Results

The results of pH monitoring tests during fermentation and microbial respiration in the preparation of the synbiotic with rice bran showed a greater variation between minimum and maximum pH values when using the alkalizing dolomitic limestone (CA) and *Lithothamnium* (LT). For LT, the lowest pH values were observed during the anaerobic phase at 24 h. As for CA, the lowest pH values were recorded during the aerobic phase at 36 h. In Fig**. 1** it is possible to visualize this variation for treatments SB, CA and LT. The SB treatment, in comparison with the others, presented higher pH value and lower variation between minimum and maximum in the fermentation and the microbial respiration processes.

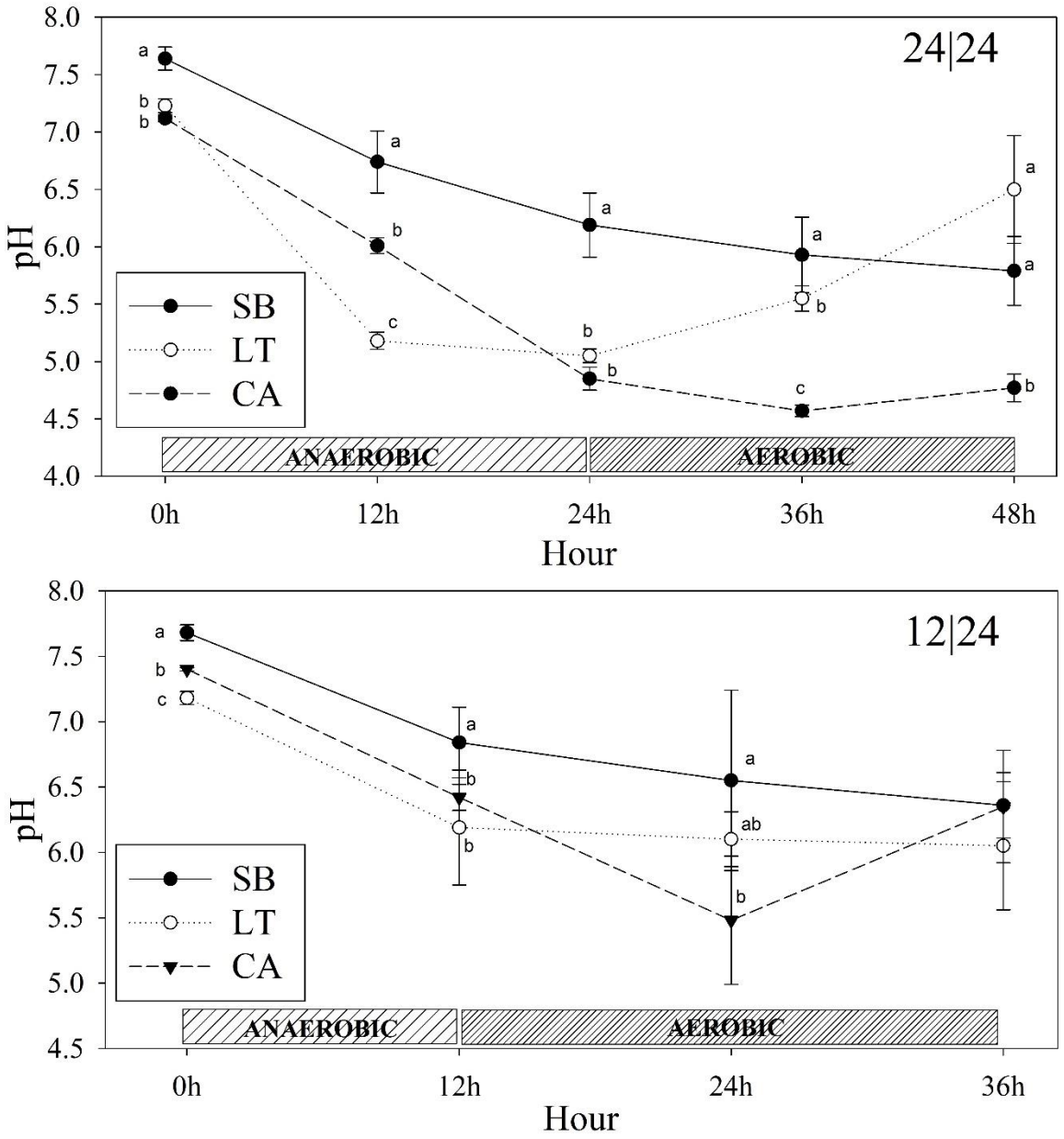

Fig. 1 Fluctuation of pH during the synbiotic preparation process. [abc*]Different letters between columns represent significant differences ($p < 0.05$) between treatments by Conover 's Multiple test with Holm-Bonferroni correction. T24|24 = 24h anaerobic and 24h aerobic; T12|24 = 12h anaerobic and 24h aerobic. SB = sodium bicarbonate; CA = dolomitic limestone; LT = *Lithothamnium*.

In the preparation of the bioreactors water (Experiment 2), the variables total alkalinity (start 110.44 ± 9.8 and end 200.27±7.8 mg $CaCO_3$ L^{-1}) and pH (start 7.9 ± 0.03 and end 8.2 ± 0.05) did not differ significantly. Settleable solids also did not show significant differences ($p>0.05$) between treatments. TAN peaked at the end of the first week of preparation for all treatments, with means varying between 1.25 ± 0.66 and 1.83 ± 0.28 mg L^{-1} (Fig. 2). N-NO_2, in turn, had a different behavior, with peaks in the second week (T12|24 = 2.2 ± 1.03; 24|0 = 1.85 ± 0.9; T12|12 = 1.74 ± 0.04 mg L^{-1}) and in the third maturation week (T24|24 = 2.03 ± 1.32; T24|12 = 2.2 ± 1.03 mg L^{-1}) (Fig. 2). From

the fourth week of maturation onwards, TAN and N-NO_2 did not show significant differences ($p>0.05$) between treatments. As for N-NO_3, peaks occurred in the third week for all treatments, with a decrease in the subsequent week (Fig. 2).

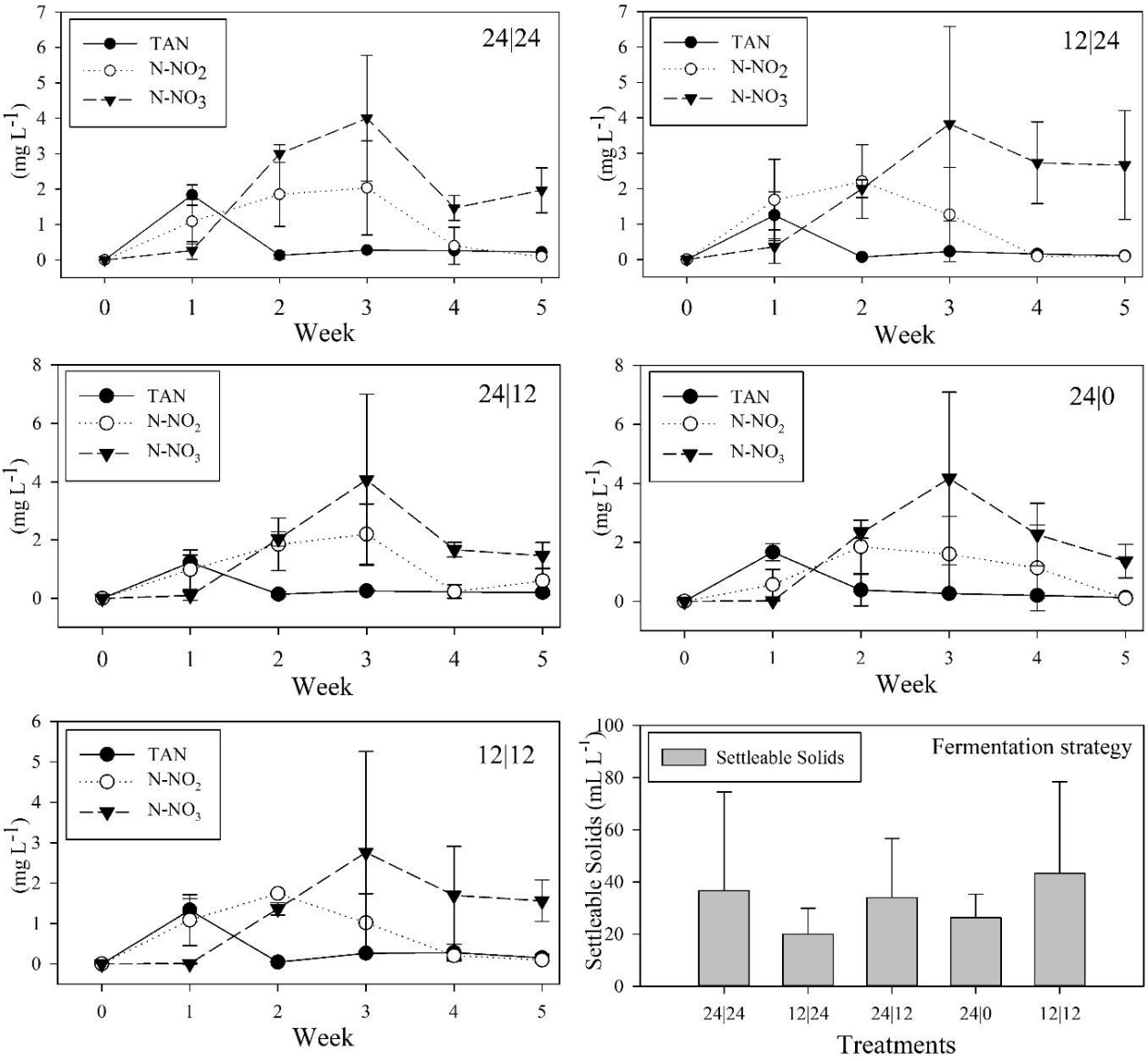

Fig. 2 Concentrations of nitrogen compounds during the preparation process of bioreactors with a synbiotic system over five weeks. T12|12 = 12 h anaerobic and 12 h aerobic; T12|24 = 12 h anaerobic and 24 h aerobic; T24|0 = 24 h anaerobic; T24|12 = 24 h anaerobic and 12 h aerobic; T24|24 = 24 h anaerobic and 24 h aerobic. TAN = total ammonia nitrogen.

The results of the prawns' reared environment water quality variables are shown in Table 1.

Table 1 Water quality variables during of *M. rosenbergii* reared with synbiotic in the nursery phase.

Variables	**Treatments**				
	24|24	**12|24**	**24|12**	**24|00**	**12|12**
DO (mg L^{-1})	6.36±0.36	6.43±0.31	6.50±0.25	6.38±0.27	6.55±0.46

T (°C)	28.97±0.37	28.81±22	28.43±0.68	28.74±0.32	28.85±0.44
pH	8.15±0.13	8.14±0.14	8.16±0.19	8.15±0.20	8.20±0.19
Total alkalinity (mg $CaCO_3$ L^{-1})	124.47±7.2	123.10±6.9	126.27±11.03	127.28±6.0	129.98±6.9
Total hardness (mg $CaCO_3$ L^{-1})	52.87±4.1	51.37±3.6	50.5±4.1	50.87±4.3	51.87±5.2
TAN (mg L^{-1})	0.15±0.14	0.13±0.17	0.09±0.11	0.09±0.07	0.16±0.25
N-NO_2 (mg L^{-1})	0.024±0.02	0.027±0.038	0.03±0.017	0.024±0.017	0.025±0.02
N-NO_3 (mg L^{-1})	3.5±2.62	3.52±2.1	3.42±2.46	3.84±2.63	3.44±2.57
Conductivity (µS cm^{-1})	457.7±53.6	447.1±49.9	444.5±46.5	450.7±47.9	450.2±53.5
TDS (mg L^{-1})	275.9±30.9	270.5±28.8	271.0±27.0	272.9±28.4	270.2±36.9
SS (mL L^{-1})	15.8±13.2	10.7±9.9	8.4±6.1	13.3±12.6	18.1±11.9

Data correspond to mean ± standard deviation per treatment. [abc*]Different letters between columns represent significant differences ($p < 0.05$) between treatments by Conover's Multiple test with Holm-Bonferroni correction. Treatment 24|24 = 24 h anaerobic fermentation and 24 h aerobic fermentation; Treatment 12|24 = 12 h anaerobic and 24 h aerobic; Treatment 24|12 = 24 h anaerobic and 12 h aerobic; Treatment 24|00 = 24 h anaerobic; Treatment 12|12 = 12 h anaerobic and 12 h aerobic. DO = dissolved oxygen, T °C = temperature in degrees Celsius, TDS = total dissolved solids, SS = settleable solids.

During the nursery phase in the experimental units, TAN fluctuated with maximum values in Treatment 12|12 (0.58 ± 0.44 mg L^{-1}) in the fourth week (Fig. 3A). Regarding N-NO_2, variations were also observed during the reared cycle with maximum values (0.06 ± 0.08 mg L^{-1}) in Treatment 12|24 (Fig. 3B). N-NO_3, in turn, showed a tendency to accumulate throughout the experimental period (Fig. 3C), with an average between 5.9 and 8.2 mg L^{-1} at the end of the reared. Settleable solids (SS) showed values above 15 mL L^{-1} at the end of the experiment for all treatments (Fig. 3D), with averages ranging from 17.25 ± 2.21 mL L^{-1} (24|12) and from 30.7 ± 13.32 mL L^{-1} (24|0) in the last week of reared.

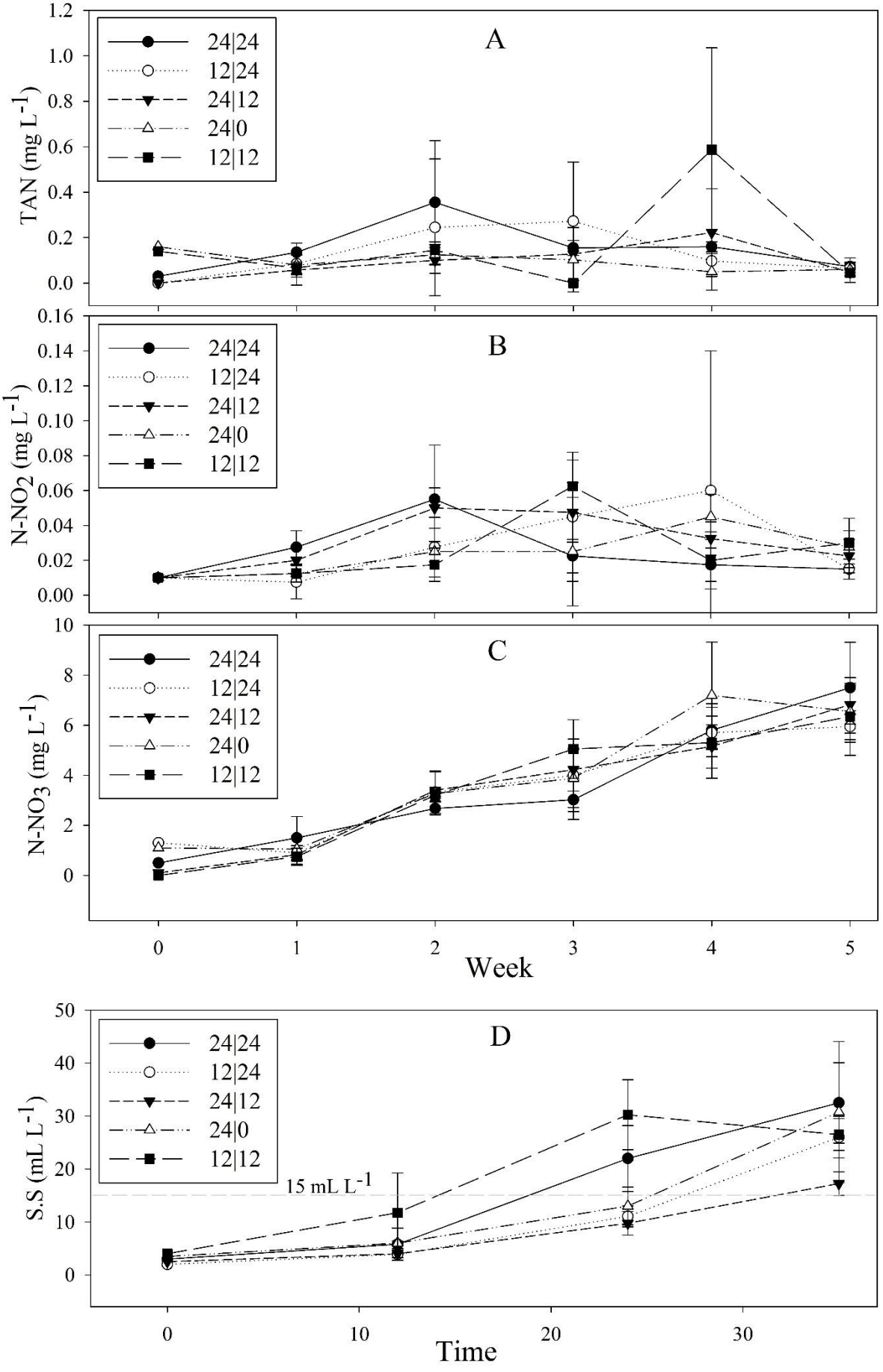
A
TAN (mg L-1)
24|24
12|24
24|12
24|0
12|12
B
N-NO2 (mg L-1)
C
N-NO3 (mg L-1)
Week
D
S.S (mL L-1)
15 mL L-1
Time

Fig. 3 Values of variables TAN (A), N-NO_2 (B), N-NO_3 (C), and settleable solids – SS (D) throughout the experimental period. Treatment 24|24 = 24 h anaerobic fermentation and 24 h aerobic fermentation; Treatment 12|24 = 12 h anaerobic and 24 h aerobic; Treatment 24|12 = 24 h anaerobic and 12 h aerobic; Treatment 24|0 = 24 h anaerobic; Treatment 12|12 = 12 h anaerobic and 12 h aerobic.

The proximal composition of the synbiotic after the anaerobic and aerobic phases are presented in Table 2, and it is possible to identify a greater contribution of the aerobic phase in the increase of the lipid content.

Table 2 Proximal composition of the synbiotic used as fertilizer in the water of shrimp *M. rosenbergii* reared in the nursery phase.

Variable	Phases			
	RB	**ANA**	**AER**	**ANA+AER**
Lipids (%)	17.99 ± 0.73^{d}	19.62 ± 0.48^{c}	26.32 ± 0.21^{b}	28.91 ± 0.57^{a}
Proteins (%)	17.41 ± 0.15^{b}	17.18 ± 0.15^{b}	18.27 ± 0.21^{a}	17.32 ± 0.19^{b}

The values expressed are in terms of dry matter weight. Data correspond to the mean of three replicates ± standard deviation per treatment. ^{abc*}Different letters between columns represent significant differences ($p < 0.05$) between treatments by Duncan's test. RB = protein and lipid content of raw rice bran; ANA = after 24 hours anaerobic; AER = after 24 hours aerobic; ANA+AER = after 48 hours combining the anaerobic (24 h) plus aerobic (24 h) steps.

Among the prawn zootechnical performance variables, only survival did not show significant differences ($p>0.05$) between treatments. In addition, according to the results, the importance of the aerobic phase in the preparation of the synbiotic is highlighted, since the treatments with longer time in this phase (24|24 and 12|24) showed better results than those without the aerobic phase (24|00), especially for the variables final average weight and yield (Table 3).

Table 3 Zootechnical performance variables of *M. rosenbergii* reared with synbiotic in the nursery phase.

Variables	Treatments				
	24|24	**12|24**	**24|12**	**24|00**	**12|12**
Survival (%)	88.53±3.6	89.58±4.81	77.60±5.47	85.93±3.55	86.97±6.88
Final average weight (mg)	221.3 ± 22.9^{a}	218.2 ± 27.6^{a}	183.8 ± 31.8^{ab}	176.1 ± 24.5^{b}	187.9 ± 14.5^{ab}
Weekly weight gain (mg)	40.25 ± 4.57^{a}	39.65 ± 5.52^{ab}	32.77 ± 6.37^{ab}	31.22 ± 4.9^{b}	33.59 ± 2.91^{ab}
SGR (% day^{-1})	6.85 ± 0.28^{a}	6.81 ± 0.35^{ab}	6.30 ± 0.47^{ab}	6.19 ± 0.38^{b}	6.39 ± 0.22^{ab}
FCR	2.58 ± 0.26^{b}	2.72 ± 0.49^{b}	3.62 ± 0.57^{a}	3.38 ± 0.54^{ab}	3.08 ± 0.36^{ab}
Yield (gm^{-3})	195.4 ± 14.6^{a}	196.2 ± 33.4^{a}	142.2 ± 23.04^{b}	151.3 ± 21.6^{b}	162.2 ± 16.2^{ab}

Data correspond to the mean of four replicates ± standard deviation per treatment. ^{abc*}Different letters between columns represent significant differences ($p < 0.05$) between treatments by Duncan's test. Treatment 24|24 =

24 h anaerobic fermentation and 24 h aerobic fermentation; Treatment 12|24 = 12 h anaerobic and 24 h aerobic; Treatment 24|12 = 24 h anaerobic and 12 h aerobic; Treatment 24|00 = 24 h anaerobic; Treatment 12|12 = 12 h anaerobic and 12 h aerobic. SGR = specific growth rate; FCR = feed conversion rate.

Discussion

pH monitoring in synbiotic preparation

The SB treatment showed higher pH values than the LT and CA treatments for the different phases (anaerobic and aerobic). However, all were able to maintain the pH within the range considered adequate for most of the probiotic microorganisms used (4.5 and 6.5) (Hofvendahl and Hagerdal 2000; Vassileva et al. 2021). However, the smaller difference between minima and maxima observed in the SB treatment promoted greater stability of this variable during the evaluation time. Sudden variations in this parameter can lead to a reduction or even inhibition of the production of important metabolites, microbial growth and enzymatic activity by some microorganisms (Naidu and Devi 2005; Dawood and Kashio 2019).

Most microorganisms prefer neutral or slightly acidic pH, which enables a higher biomass production, in the range of 4.5 to 6.5 (Hofvendahl and Hagerdal 2000; Vassileva et al. 2021). Lactic acid bacteria, such as those of the genus *Lactobacillus*, are fermentable with oxygen tolerance, grow at temperatures ranging from 30–40°C and have an ideal pH for the production of organic acids between 5.0 and 7.0 (Hofvendahl and Hagerdal 2000). Yeasts such as *Saccharomyces cerevisiae* show satisfactory growth in the pH range between 4.0 and 7.0. pH values above 8.0 can affect the fermentation process (Peña et al. 2015; Vassileva et al. 2021). Despite growing under extreme temperature and pH conditions, most *Bacillus* species express high growth at pH between 6.0 and 9.0 (Naidu and Devi 2005; Naraian and Kumari 2018). These results, associated with a smaller effect on water hardness, when compared to other alkalizers, contributed to the choice of sodium bicarbonate (SB) in the preparation of synbiotics in the growth experiments of the shrimp species *Macrobrachium rosenbergii*. However, it was possible to keep the pH within the appropriate range for the microorganisms used in the synbiotic with all the alkalizers evaluated, which increases the ability to select the product with better economic cost or greater availability in the local market.

Between the LT and CA alkalizers there were no significant differences for most of the times measured during the preparation of the synbiotic, but the times 36 h and 48 h of the 24|24 strategy stood out, in which the CA treatment presented pH values close to 5.0. This value is tolerated for several *Bacillus* spp species, but it is slightly below the range that optimizes their growth and protease production (Naidu and Devi 2005; Dawood and Kashio 2019).

During the anaerobic fermentation process, a decrease in the pH of the solution was observed, which is caused by the metabolism of microorganisms, especially the production of organic acids by lactic acid bacteria and yeasts (Hofvendahl and Hagerdal 2000; Vassileva et al. 2021). *Bacillus* spp species present in the microbiological mix are preferentially aerobic and, given their bioremediation character, the introduction of aeration (aerobic phase) in the solution probably contributes to the increase of their microbial biomass.

In submerged microbial respiration (aerobic), as at this stage, dissolved oxygen is the main parameter for successful microbial growth, which directly affects metabolic activity and the type of end product (Vassileva et al. 2021). This aerobic phase may also have been responsible for changes in the downward trend of pH values, especially for the LT and CA treatments, which tended to increase or stabilize. The oxygenation inside the container and the stirring of the mixture may have stimulated the solubility of these alkalizers (LT and CA), in addition to producing effects on alkalinity and pH, since they have a lower solubility and reaction time than SB, which is known for its efficiency in elevation of alkalinity and maintenance of pH stability (Loyless and Malone 1997). Furthermore, in the process of microbial respiration, the presence of CO_2 contributes to the dissolution of $CaCO_3$ and may have resulted in an increase in alkalinity and pH (Van Wyk and Scarpa 1999).

Preparation of bioreactors with synbiotic

The toxicity of nitrogen compounds, especially TAN and N-NO_2 for intensive systems with minimal water exchange, can be considered critical, and may cause animal mortality. Thus, some studies demonstrate the need for initial water preparation to ensure the establishment of the nitrification process and reduce the risks of animal mortality (Crab et al. 2012; Emerenciano et al. 2017; Abakari et al. 2020a).

Knowing the dynamics of nitrogen compounds within a cropping system is essential to conduct the stabilization process of heterotrophic and nitrifying bacteria that contribute to water quality management in crop environments (Ebeling et al. 2006; De Schryver et al. 2008; Abakari et al. 2020a). In this sense, and given the results observed in Experiment 2, it is noted that the strategies used in relation to the time of the anaerobic and aerobic phases were efficient in providing a suitable environment for the fixation of the bacterial community (heterotrophic and nitrifying) capable of metabolizing nitrogenous compounds in the bioreactors' water (Ebeling et al. 2006; De Schryver et al. 2008; Romano et al. 2018; Santos et al. 2022).

The heterotrophic and ammonia-oxidizing bacteria started the nitrogen transformation processes from the first week of preparation for all treatments. This reduction in the concentration of

ammonia and the appearance of nitrite is indicative of adequate entry of organic and inorganic carbon into the system (Ebeling et al. 2006; De Schryver et al. 2008). The results of nitrite-oxidizing bacteria metabolism, in turn, began to be evidenced from the second week of preparation, when an increase in $N\text{-}NO_3$ concentrations was observed. For this water preparation process, it is common for $N\text{-}NO_3$ concentrations to present higher values, since this is the end product of TAN metabolism within the system (Ebeling et al. 2006; Avnimelech et al. 2012; Emerenciano et al. 2017; Abakari et al. 2020a). Thus, it was noted that from the fourth week of preparation onwards, the water from all treatments was ready to be used as an inoculum in prawn farming units for the variables TAN (<1.0 mg L^{-1}) and $N\text{-}NO_2$ (<1.0 mg L^{-1}) (New et al. 2010).

The adoption of a C:N ratio of 15:1 during the water preparation process stimulated the rapid accumulation of settleable solids. The high C:N ratio (10–20:1) is also a factor that helps to promote and stabilize the heterotrophic bacterial community, responsible for the removal of TAN through assimilation and conversion into microbial biomass without producing high values of $N\text{-}NO_2$ and $N\text{-}NO_3$ (Ebeling et al. 2006; Avnimelech et al. 2012; Abakari et al. 2020a).

Water quality

The aeration and temperature control system was efficient in keeping the variables temperature (> 25°C) and dissolved oxygen (> 5mg L^{-1}) at adequate levels for the species, in addition to optimizing bacterial growth and the formation of high nutritional quality floc (De Schryver et al. 2008; New et al. 2010; Crab et al. 2012; Emerenciano et al. 2017; Dauda 2019). The pH also presented values within the range considered ideal for the species (7.0 to 8.5) (New 2002).

Total alkalinity presented averages above 120 mg $CaCO_3$ L^{-1} in all treatments. These concentrations are in accordance with those recommended by Adhikari et al. (2007) (± 100 mg $CaCO_3$ L^{-1}) and Coyle et al. (2010) (> 50 mg $CaCO_3$ L^{-1}) for *M. rosenbergii*. Furthermore, it is worth noting that total alkalinity values above 100 mg $CaCO_3$ L^{-1} are recommended for systems with minimal water exchange due to their role in controlling pH fluctuations and in the metabolism of nitrifying bacteria (Ebeling et al. 2006; Furtado et al. 2011; Avnimelech et al. 2012; Emerenciano et al. 2017; Samocha et al. 2017).

Mean values of total hardness greater than 50 mg $CaCO_3$ L^{-1} found in the present experiment are in agreement with the reports by New et al. (2010) (50 to 150 mg $CaCO_3$ L^{-1}) and the works by Vasquez et al. (1989) (20 to 200 mg $CaCO_3$ L^{-1}). High levels of water hardness can affect both growth and survival of *M. rosenbergii*, but the results of the present study and those presented by these authors demonstrate the wide tolerance of this species for this variable in the culture environment.

The different synbiotic preparation strategies, as well as the adoption of a C:N ratio of 5:1, were effective in controlling nitrogen compounds (TAN, $N\text{-}NO_2$ and $N\text{-}NO_3$) within the culture environment by colonizing heterotrophic and nitrifying bacteria (Ebeling et al. 2006; Abakari et al. 2020a) after water maturation. This is evident when we observe that the concentrations of these variables found in the prawn growth experiment were in accordance with those recommended by several authors – TAN < 1.0 mg L^{-1}, $N\text{-}NO_2$ < 1.0 mg L^{-1}, and $N\text{-}NO_3$ < 10.0 mg L^{-1} (New 2002; New et al. 2010; Pérez-Fuentes et al. 2013; Dutra et al. 2020; Dong et al. 2020). In addition, the efficiency in controlling these variables in a reared environment using carbohydrates with anaerobic and aerobic (synbiotic) processes has been reported by other authors (Romano et al. 2018; Abdel-Tawwab et al. 2020; Andrade et al. 2021; Hussain et al. 2021; Silva et al. 2021b; Santos et al. 2022).

The different preparation strategies of the synbiotic (anaerobic and aerobic) with rice bran had no effect on the TDS and electrical conductivity variables. Values above 270 mg L^{-1} and 444 µS cm^{-1} for these variables, respectively, recorded in the present experiment, indicate high availability of nutrients in the culture system (Sipauba-Tavares 1995; Samocha et al. 2017), which has been notably associated with synbiotic or heterotrophic systems (Crab et al. 2012; Romano 2017; Romano et al. 2018; Abdel-Tawwab et al. 2020; Deepak et al. 2020; Andrade et al. 2021; Hussain et al. 2021; Santos et al. 2022). In the same way, settleable solids (SS) are another strong indication of the availability of nutrients and natural food within the system, although values above 15 ml L^{-1} (recorded in the second half of the experiment) may incur damage to the growth and survival of the animals (Emerenciano et al. 2017; Samocha et al. 2017).

Growth of prawn reared

The different synbiotic preparation strategies (anaerobic and aerobic) did not influence the survival rate, which maintained averages above 77% and were similar to those found by other authors reared the same species in a heterotrophic system (Crab et al. 2010; Ballester et al. 2017; Frozza et al. 2021 Hosain et al. 2021; Santos et al. 2022). According to New (2002), at the end of the nursery phase, a minimum survival of 75% is expected. Several factors may be associated with obtaining survivals similar to those found in the present experiment, such as maintenance of water quality, improvement of immune status due to probiotic supplementation, and adequate feeding and stocking density (Adhikari et al. 2007; New et al. 2010; Dutra et al. 2020; Dauda 2019; Dong et al. 2020; Miao et al. 2017).

Probiotic supplementation with yeasts and bacteria of the genus *Bacillus* spp and *Lactobacillus* spp using fermentation processes and microbial respiration (synbiotic) or bioflocs has

shown efficiency in increasing the immunity of animals and reducing the count of pathogenic bacteria, whose growth was probably prevented due to competition for nutrients (Romano et al. 2018; Abdel-Tawwab et al. 2020; Miao et al. 2017; Andrade et al. 2021; Silva et al. 2021a). In this sense, the use of these microorganisms in the synbiotic preparation process, regardless of the strategy adopted, contributed to the maintenance of a culture environment favorable to the survival of the animals.

The treatments with longer preparation time with aerobic phase (24|24 and 12|24) showed better growth and yield results when compared to the treatment that did not include the aerobic phase (24|0). Despite evidence of beneficial effects of synbiotic preparation with only the anaerobic phase of rice bran for the growing environment (Romano et al. 2018; Abdel-Tawwab et al. 2020; Liñan-Vidriales et al. 2021), the results of the present experiment point to the aerobic step as an important component in the synbiotic preparation process.

Liñan-Vidriales et al. (2021) reared *Peaneus vannamei* in tanks fertilized with rice bran and fermented rice bran for 24 hours (consortium of *Bacillus* and *Lysinibacillus* species or commercial probiotic containing *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans, Lactobacillus acidophilus* and *Saccharomyces cerevisiae*) and found no significant differences related to final mean weight, survival, SGR and FCR. Abdel-Tawwab et al. (2020), however, reared *L. vannamei* using rice bran fermented with *B. subtilis* (24h anaerobic, pH 6-7, 28°C – 30°C) and observed greater growth compared to the control (commercial diet and water exchange). These differences may be linked to the forms of preparation and application of the synbiotic in water.

Within this context, the work carried out by Romano et al. (2018) provide important information on the effects of pre-treatment (24 h fermentation or 24 h respiration) of rice bran on its nutritional value and on the nutritional value of the flocs. The results of these authors pointed to an increase in lipids (14.79%) and proteins (11.12%) resulting from pre-treatment, with the highest increase observed for microbial respiration (24 h aerobic). These results are compatible with those found in the present experiment, as the mean values of lipids in the AER and ANA+AER replicates were higher than those using only anaerobic (ANA).

The increase in microbial respiration time in the pre-treatment of rice bran stimulates the growth of bacteria of the genus *Bacillus* spp (Vassileva et al. 2021), which play an important role not only in increasing the protein and lipid levels of the final product, but also in stimulating immunity and balance of the gastrointestinal microbiota and digestive enzyme activities in cultured animals

(Romano et al. 2018; Cienfuegos-Martínez et al. 2020; Liñan-Vidriales et al. 2021; Andrade et al. 2021).

Most cultivable aquatic organisms satisfy the majority of their energy needs through protein and lipid metabolism. Lipid metabolism produces several essential fatty acids to the growth and metabolic functions of these animals (Tseng and Hwang 2008; New et al. 2010; Li et al. 2017). Furthermore, adequate levels of lipids in the diet of *M. rosenbergii* (< 10%) (New et al. 2010) and other aquatic organisms are associated with weight gain, reducing the need for dietary protein and their use for membrane synthesis and energy reserve (Santos et al. 2007; Tseng and Hwang 2008; New et al. 2010). It can be seen, therefore, that a longer time dedicated to microbial metabolism with rice bran as a substrate (24|24 and 12|24) may have contributed to the production of a synbiotic that generated better nutritional value of the floc within the reared environment.

Even under similar conditions in terms of feeding, stocking density and synbiotic preparation strategy (24 h anaerobic and 24 h aerobic), the results found for the variables final average weight, weekly weight gain, and yield were higher than those found by Santos et al. (2022) (122.85 ± 12.5 mg, 22.26 ± 2.97 mg and 85.02 ± 2.15 g m^{-3}, respectively). This difference may be related to the composition of the microbiological mix used in the preparation of the synbiotic, denoting the importance of lactic acid bacteria (*Lactobacillus acidophilus*) and yeasts (*Saccharomyces cerevisiae*) in the preparation of rice bran. The FCR and SGR results obtained in this experiment were similar to those found by Hasain et al. (2021), who, reared *M. rosenbergii* with different carbon sources (wheat bran, rice flour, molasses and corn starch) in a biofloc system, found averages of FCR between 2.21 and 4.47 and of SGR between 6.02% day^{-1} to 6.98% day^{-1}.

Conclusion

The proportion of alkalizing agents used (10% in relation to the amount of rice bran) was efficient in maintaining the fermentation pH and microbial respiration adequate for most microorganisms (> 4.5). The different synbiotic preparation strategies did not influence the water preparation time of the bioreactors. The results of this research indicate that a longer preparation time of the synbiotic including the aerobic and anaerobic stages can promote better performance of the prawn reared, since the treatments T24|24 and T12|24 presented results of growth and yield superior to those of the treatment T24|00.

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Authors' contribution

Robson Batista dos Santos: Research, conceptualization, methodology, development of experiments, tabulation, data analysis and writing (original version); Tais Nunes dos Santos, Josefa Honorio da Silva, Chaiane Santos Assunção and Gênison Carneiro Silva: Experimental management, water quality analysis and biometrics; Petrônio Alves Coelho Filho: Methodology and textual review; Luis Otavio Brito: Guidance, methodology, conceptualization and textual review.

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Data availability

The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethical approval

The authors followed international and institutional animal management guidelines for the experiments.

Interest conflicts

The authors declare no competing interests.

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4. CONSIDERAÇÕES FINAIS

As condições experimentais utilizadas nessa pesquisa permitiram identificar maior vantagem no uso do simbiótico produzido com farelo de arroz para o crescimento do *Macrobrachium rosenbergii* na fase de berçário. A proporção dos alcalinizantes utilizados – Bicarbonato de Sódio, Calcário e *Lithothamnium* (10% em relação a quantidade do farelo de arroz) na preparação do simbiótico conseguiu manter o pH da fermentação adequado para a maioria dos microrganismos (> 4.5), o que possibilita escolher aquele de melhor custo benefício ou de maior disponibilidade no mercado local. Além disso, foi possível identificar que a combinação do tempo de fermentação e respiração microbiana no processo de preparação do simbiótico tem efeitos na sua qualidade como fertilizante, podento afetar os indicadores de crescimento dos animais. À parte disso, recomenda-se a realização de pesquisas adicionais que auxiliem a compreender os efeitos do pré-tratamento com microorganismos probióticos nas alterações bioquímicas dos carboidratos, crescimento de bactérias benéficas e patogênicas, além da avaliação de outras combinações de fermentação e respiração microbiana na preparação dos simbióticos.

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