DANIELLE ALVES DA SILVA

EFEITO DA DENSIDADE DE ADIÇÃO DE *Brachionus plicatilis* (Müller, 1786) NO CULTIVO DE PÓS-LARVAS DE *Litopenaeus vannamei* (Boone, 1931) EM SISTEMA DE BIOFLOCOS

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Danielle Alves da Silva

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RESUMO

A produção de juvenis de camarões marinhos tem sido utilizada com estratégias de convivência com os patógenos, reduzindo as perdas produtivas na carcinicultura. A aplicação de microrganismos capazes de melhorar o teor nutricional dos flocos microbianos nas fases iniciais de cultivo, contribuindo para o maior crescimento dos camarões e melhora do sistema imune é uma ferramenta bastante promissora nessa fase. Neste sentido, o presente trabalho avaliou o efeito da densidade de adição do rotífero *Brachionus plicatilis* em diferentes densidades no cultivo de pós-larvas de *Litopenaeus vannamei* em sistema de bioflocos incentivado por fermentação de carbono ogânico e à base de mix de bactérias. Para tal, o desenho experimental foi composto por quatro tratamentos e três repetições cada, totalizando 12 unidades experimentais em delineamento inteiramente casualisado durante 42 dias. Os tratamentos foram: BFT (Bioflocos); BFT-10 (Bioflocos com adição de *B. plicatilis* na densidade de 10 organismos mL^{-1}), BFT-20 (Bioflocos com adição de *B. plicatilis* na densidade de 20 organismos mL^{-1}) e BFT-30 (Bioflocos com adição de *B. plicatilis* na densidade de 30 organismos mL^{-1}). As pós-larvas de dez dias (PL_{10}) com peso médio de 3,4 ± 0,02 mg foram estocadas na densidade de 3.000 indivíduos m^{-3}em unidades experimentais de 40L de volume útil. A adição do rotífero *B. plicatilis* foi realizada no 1º, 10º, 20º e 30º dias. Os camarões foram alimentados com ração comercial com 45% de proteína bruta, quatro vezes ao dia com taxa de alimentação inicial de 35% da biomassa. Durante o experimento foi avaliado o desempenho zootécnico, qualidade da água, caracterização de bactérias do gênero *Vibrio*, comunidade planctônica, composição centesimal e contagem total de hemócitos. Sobre o desempenho zootécnico, podem-se destacar os maiores valores médios de peso final e produtividade nos tratamentos com adição de *B. plicatilis*, nos quais os camarões atingiram de 1,09 a 1,26g e 2,25 a 3,41 kg m^{-3}, respectivamente. As variáveis de qualidade de água estiveram dentro do recomendado para camarões marinhos e não foram observadas diferenças significativas entre os tratamentos. O efeito das densidades de adição foi significativo para o consumo de água por quilograma de juvenil produzido, além da redução da porcentagem de colônias de sacarose negativas do gênero *Vibrio* presentes na água e no camarão. Os gêneros mais frequentes observados para fitoplâncton foram: *Oscillatoria* (6,97 a 9,27%), *Aphanocapsa* (6,48 a 7,85%), e para e zooplâncton foram: *Brachionus* sp. (21,49 a 33,73%), *Daphnia* sp. (15,44 a 31,25%) e *Arcella* sp. (12,75 a 20,62%). Os tratamentos com maiores níveis de inclusão do rotífero (BFT-20 e BFT-30) obtiveram os melhores resultados em relação à quantidade de proteína e lipídios. Além disso, a adição de rotífero também proporcionou uma melhor resposta imunológica aos animais cultivados comprovada através da contagem total de hemócitos apresentando altas concentrações de hemócitos antes e após os animais serem submetidos ao teste de estresse salino. Portanto, a adição de rotífero em densidade de 20 a 30 organismos mL^{-1} demonstrou incrementar o desempenho zootécnico de juvenis de *Litopenaeus vannamei* na fase berçário em bioflocos.

Palavras-chave: *Litopenaeus vannamei*, *Brachionus plicatilis*, Berçário, Bioflocos, *Vibrios*, Crescimento, Composição centesimal

ABSTRACT

The production of juvenile marine shrimp has used strategies that involve coexistence with pathogens, reducing production losses in shrimp farming. One of these strategies is the application of microorganisms that can improve the nutritional content of microbial biofloc in the early stages of cultivation, contributing to greater growth of shrimp and stronger immune systems. This study evaluated the effect of addition of the rotifer *Brachionus plicatilis* at different densities to the cultivation of post-larvae *Litopenaeus vannamei* in a biofloc system supported by fermented base with a mix of bacteria. The experimental design consisted of four treatments with three replicates of each, generating a total of 12 experimental units in a completely randomized design for 42 days. The treatments iuncluded: BFT (Bioflocs); BFT - 10 (Bioflocs with the addition of *B. plicatilis* at a density of 10 organisms mL^{-1}), BFT - 20 (Bioflocs with the addition of *B. plicatilis* at a density of 20 organisms mL^{-1}) and BFT - 30 (Bioflocs with addition of *B. plicatilis* at a density of 30 mL^{-1} organisms). Ten-day-old post-larvae (PL_{10}) with an average weight of 3.4 ± 0.02 mg were stocked at a density of 3,000 individuals m^{-3} in experimental units of 40L of useful volume. The rotifer *B. plicatilis* were added on the 1st, 10th, 20th and 30th days. The shrimps were fed with a commercial ration with 45% crude protein, four times a day with an initial feed rate of 35% of the biomass. During the experiment, we evaluated the water quality, zootechnical performance, and monitored the zooplankton community, centesimal composition, total hemocyte count, and quantified and characterized the *Vibrio* bacteria. The water quality variables were within the recommendations for marine shrimp and no significant differences were observed between treatments. At the end, regarding zootechnical performance, the highest average values of final weight and productivity were in treatments with the addition of *B. plicatilis*, in which the shrimp reached 1.09 to 1.26 g and 2.25 to 3.41 kg m^{-3}. The most frequent phytoplankton genera observed were: *Oscillatoria* (6.97 to 9.27%), *Aphanocapsa* (6.48 to 7.85%), and for and zooplankton were: *Brachionus* sp. (21.49 to 33.73 %), *Daphnia* sp. (15.44 to 31.25%) and *Arcella* sp. (12.75 to 20.62%). The effect of the stocking densities was also significant for water consumption per kilogram of juveniles produced, and on the reduction in the percentage of negative sucrose colonies of the *Vibrio* genus in the water and the shrimp. The treatments with higher levels of rotifer (BFT-20 and BFT-30) had the best results for protein and lipids. The addition of rotifers also strengthened the immune system of the cultivated shrimp, as demonstrated by the total hemocyte count, with high concentrations of hemocytes before and after the animals were subjected to a salt stress test. Therefore, the addition of rotifer at densities of 20 to 30 mL^{-1} organisms coupled with an efficient fertilization strategy demonstrated excellent results in the production of *L. vannamei* juveniles.

Keywords: *Litopenaeus vannamei*, *Brachionus plicatilis*, Nursery, Bioflocs, *Vibrios,* Growth, Centesimal composition

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1. INTRODUÇÃO

Dentre os principais produtos da aquicultura mundial, está a produção de camarão, ao qual destaca-se em termos financeiros, representando o segundo segmento de maior faturamento com US$ 69,3 bilhões de dólares em 2018 (27,7%) e, o terceiro segmento em volume de produção, com 9,38 milhões de toneladas produzidas (FAO, 2020). Atualmente, *Litopenaeus vannamei* (Boone, 1931) é a espécie com maior representatividade mundial no setor do cultivo de crustáceos (FAO, 2020) e, no Brasil, sua produção atingiu, em 2018, o valor de 45,8 mil toneladas que corresponde a 20,6% de toda produção aquícola nacional, com R$ 1,1 bilhão de receita (IBGE, 2019). O principal polo produtor é a Região Nordeste, responsável por quase toda a produção nacional (99,4%), com os estados do Rio Grande do Norte e Ceará se destacando com os melhores índices de produtividade (IBGE, 2019).

O crescimento acelerado do setor nas últimas décadas ocorreu frente a graves problemas ambientais e disseminação de doenças (MOSS et al., 2012; NÓBREGA et al. 2013). Esses surtos de doenças causam a diminuição do consumo alimentar, crescimento reduzido e altas mortalidades, gerando uma acentuada queda na produtividade do setor (SOTO-RODRIGUEZ et al., 2012; TAW, 2012). As perdas econômicas geradas por essas quedas na produtividade foram de aproximadamente US$ 15,0 bilhões de dólares na produção em 2015, onde pode-se destacar os surtos do Vírus da Síndrome da Mancha Branca como o principal fator (VERBRUGGEN, et al., 2016, NUNES E FEIJÓ, 2016; YU et al., 2017). Além disso, bactérias patogênicas e oportunistas do gênero *Vibrio* encontradas na água também acometem os cultivos de camarão (KUMAR et al., 2014; ZHANG et al., 2014; BOWLER, et al., 2015).

Diante disso, pesquisadores e produtores buscam novas técnicas de manejo, mais biosseguras, que visam minimizar essas perdas produtivas (AVNIMELECH, 2012; PÉREZ-FUENTES et al., 2013). Dentre essas técnicas destaca-se o cultivo de camarões em sistema com mínima troca de água denominado de sistema de bioflocos (BFT), tecnologia alternativa que utiliza altas densidades de estocagem, com fertilizações orgânicas e probióticos para incentivar o crescimento da comunidade microbiana e manutenção da qualidade da água (KRUMMENAUER et al., 2012; EMERENCIANO et al., 2013; ROMANO et al., 2018). A fertilização consiste na adição de uma fonte de carbono orgânica que ajuda a aumentar a relação carbono: nitrogênio, estimulando o crescimento das bactérias heterotróficas no sistema (PANIGRAHI et al., 2018). Essa comunidade assimila os compostos nitrogenados tóxicos presentes na água transformando em proteína microbiana, fornecendo uma fonte de

suplementação alimentar e melhorando a qualidade de água (OTOSHI et al., 2011; FÓES et al., 2012; RAJKUMAR et al., 2016).

Esta adequada relação de C:N pode acelerar a ciclagem dos nutrientes (AHMAD et al., 2017). De acordo com Pérez-Fuentes et al. (2016) a relação C:N deve estar acima de 10:1. Porém relações muito elevadas resultam na predominância de bactérias heterotróficas em detrimento das bactérias nitrificantes que são as mais eficientes na remoção dos compostos nitrogenados como amônia e nitrito (RIOS DA SILVA, 2009; BALLESTER et al., 2010). Além disso, a longo prazo, as relações C:N elevadas propiciam o aumento substancial dos sólidos sedimentáveis que podem causar entupimento das brânquias do camarão e deterioração da qualidade da água (SCHVEITZER et al., 2013). Dessa forma, recomenda-se relações CHO:N (10-12:1) onde a distribuição de bactéria heterotrófica e nitrificantes sejam mais homogênea, também estimulando o crescimento do plâncton e proporcionando, redução na carga orgânica (ROMANO, 2017).

Os bioflocos são compostos por microalgas, bactérias, protozoários, resto de ração, exoesqueletos e fezes, entre outras partículas de matéria orgânica (MANAN et al., 2017). Além de contribuir para a manuntenção da qualidade da água, os flocos microbianos (bioflocos) são considerados com uma importante fonte de proteínas, lípidios, minerais e vitaminas (EKASARI et al., 2015). Apesar da presença de proteína e lípideos na sua composição nutricional, existe uma deficiência de metioniana e lisina, ácido linoleico, eicosapentaenóico (EPA) e docasahexanóico (DHA) (CRAB et al., 2010; DANTAS et al., 2016; MAGAÑA-GALLEGOS et al., 2018), que são nutrientes essenciais à sobrevivência e crescimento dos camarões (LIN et al, 2015; NESARA E PATURI, 2018).

Estes valores nutricionais dos flocos microbianos são influenciados pela composição de microorganismos presentes, salinidade, temperatura, oxigênio dissolvido e a fonte de carbono utilizada para incentivar a comunidade bacteriana (JU et al., 2008; MAICA et al., 2012; PHULIA et al., 2012). Então essa deficiência de aminoácidos e ácidos graxos pode ser suplementada de outra forma, com a administração de microorganismos vivos que servirão como fonte de aminoácidos, ácidos graxos e minerais para as fases iniciais do cultivo (SCHAAL et al., 2016; KURMAR et al., 2017).

Brachionus plicatilis (MÜLLER, 1786) é uma das espécies de rotíferos mais conhecidas por ser considerada uma fonte rica de aminoácidos, ácidos graxos essenciais (EPA e DHA) e minerais, sendo de notável importância nutricional (JEEJA et al., 2011). A composição deste rotífero pode ser manipulada para se adequar aos requerimentos nutricionais do organismo alvo

do cultivo (LUBZENS et al., 1989). Nesse sentido, a composição bioquímica está relacionada com as dietas fornecidas durante o cultivo, podendo apresentar variações no conteúdo de proteína e ácidos graxos polinsaturados (FERREIRA et al., 2008; 2009).

Além da contribuição nutricional, essa espécie de rotífero apresenta características adequadas para a produção em larga escala como o crescimento rápido, baixa mobilidade na coluna d'água, fácil assimilação de substâncias enriquecedoras e elevada atratibilidade como alimento vivo às pós-larvas, quando comparado ao alimento inerte (CONCEIÇÃO et al., 2010). Porém, os aquicultores ao produzir alimento vivo devem buscar um equilíbrio entre a qualidade do alimento e o custo de produção. Os rotíferos são organismos filtradores não seletivos, dessa forma, o uso de microalgas, como *Nannochloropis* sp., *Isochrysis galbana* e *Chlorella vulgaris* e soluções comerciais como dietas de enriquecimento são frequentemente utilizadas, desde que seja economicamente viável e proporcione o fornecimento constante de alta qualidade (CARVALHO et al., 2014; FUENTES-GRÜNEWALD et al., 2015; TORZILLO E CHINI ZITELLI, 2015; FERREIRA et al., 2018).

A condição nutricional dos animais cultivados está diretamente relacionada com o seu estado imunológico, podendo determinar a capacidade de resistência a mudanças no ambiente e doenças (CUÉLLAR-ANJEL et al., 2010). Nesse cenário vem sendo proposto além do uso do sistema intensivo com mínima troca de água, a adição de microorganismos capazes de melhorar nutricionalmente a composição dos flocos microbianos, também possibilitando um aumento da resistência imune do animal (MARINHO et al., 2017; JAMALI et al., 2015; BRITO et al., 2016; ABREU et al., 2019).

2. OBJETIVO GERAL

Avaliar o efeito da densidade de adição de *Brachionus plicatilis* no cultivo de pós-larvas de *Litopenaeus vanammei* em sistema de bioflocos.

2.1 Objetivos Específicos

- Avaliar o efeito da densidade de adição de *B. plicatilis*no desempenho zootécnico de pós-larvas de *L. vanammei* cultivadas em sistema de bioflocos.
- Mensurar e avaliar as variáveis físico-químicas de água ao longo do cultivo de pós-larvas de *L. vanammei* com adição *B. plicatilis* em diferentes densidades;

- Analisar a composição centesimal dos bioflocos e camarões com adição *B. plicatilis* em diferentes densidades;
- Identificar e quantificar a comunidade planctônica ao longo do cultivo de pós-larvas de *L. vanammei* com adição *B. plicatilis* em diferentes densidades;
- Quantificar as Unidades Formadoras de Colônia (UFC) de *Vibrio* spp. ao longo do cultivo na água e no camarão;
- Avaliar a sobrevivência dos camarões a choque osmótico após o cultivo e o status imunológico através da contagem total de hemócitos.

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Effects of addition of *Brachionus plicatilis* (rotifer) in different densities to *Litopenaeus vannamei* reared in a nursery BFT system with anaerobic and aerobic process wheat bran as an organic carbon source: Growth, Water quality, *Vibrio*, plankton and proximal composition

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ABSTRACT

The objective of this study was to evaluate the effect of the addition of *Brachionus plicatilis* on the growth, *Vibrio* count, water quality, plankton composition, total hemocyte count and proximal composition of *Litopenaeus vannamei* raised in a biofloc system. Four treatments were used in the first phase: BFT (bioflocs); BFT-10 (addition of 10 org mL^{-1} of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL^{1-1} of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL^{1} of *Brachionus plicatilis*), all in triplicate. The shrimp ($PL_{10,}$ 3.4 ± 0.02 mg) were reared in a biofloc system (with inorganic fertilization - nitrogen, phosphorus and silicate and; anaerobic and aerobic process with wheat brain and a microbial mix with 7.7 X 10^{8} UFC g^{-1} of *Lactobacilus* sp., *Bacillus subtilis* and *B. Licheniformes* strains for 42 days at a stocking density of 3,000 shrimp m^{-3} fed with commercial feed (45% crude protein and 8% lipids) with an initial feed rate of 35% of shrimp biomass. The rotifers were added every 10 days (from day 1 to 30), and at the end of 42 days, shrimp performance, water quality, *Vibrio* count, and proximal composition were evaluated and a salinity stress test conducted. The BFT-20 and BFT-30 treatments had higher performance values, highlighted by final weight (1.18 and 1.26 g) and yield (3.14 and 3.41 kg m^{-3}). There were no significant differences ($p > 0.05$) in the water quality parameters among the four treatments. The percentage of positive sucrose colonies increased and of negative sucrose colonies decreased by the end of the experimental period, with the negative sucrose colonies dropping to 0% in the BFT-30 treatment. The THC was significantly higher ($p < 0.05$) at the end of culture and after the salinity stress test for shrimp submitted to treatments with the addition of rotifers than in the BFT. The proximal composition (g kg^{-1} dry weight basis) of crude protein, 203.11 to 298.25, and crude lipids of 66.19 to 130.01 in the bioflocs were within significant differences ($p < 0.05$) for BFT-20 and BFT-30 as compared to BFT and the initial value. The best shrimp performance was observed in treatments with BFT- 30 and 20, indicating the benefits of

Brachionus plicatilis on growth enhancement and microbial floc content of *L. vannamei* postlarvae grown in biofloc systems.

Keywords: juvenile; shrimp; zooplankton; yield; colonies bacterial

1. INTRODUCTION

Inadequate management practices in shrimp farming have contributed to the rapid spread of diseases, causing significant production losses in recent years (New et al., 2010; Vidal and Ximenes, 2016). In Brazil, shrimp production decreased by more than 50% between the years 2003 and 2018 (ABCC, 2013; IBGE, 2019). This reduction is linked to outbreaks of diseases, mainly of viral and bacterial origins, such as White Spot Syndrome Virus and Infectious Myonecrosis Virus and the presence *Vibrio* bacteria (Seiffert and Winckler, 2005; Guerrelhas and Teixeira, 2012; Soto-Rodriguez et al., 2014; Rebouças et al., 2017; Shinn et al., 2018).

In this scenario, new technologies are required to make shrimp culture more sustainable and increase its biosafety, minimizing the amount of exchange water by maintaining water quality in ponds (Brito et al., 2011). Among these shrimp farming technologies, biofloc systems with minimal water exchange stand out (Avnimelech, 2015; Bossier and Ekasari, 2017). The system promotes growth of the microbial community that assimilates toxic nitrogenous compounds in the water, transforming them into microbial proteins that serve as food to supplement shrimp nutrition (Crab et al., 2012; Souza et al., 2014; Avnimelech, 2015). Organic carbon sources such as molasses and sugar are applied to maintain the C:N ratio above 10:1 (Ray and Lotz. 2014; Pérez-Fuentes et al., 2016). However, high C:N ratios cause a substantial increase in sedimentable solids, which can deteriorate water quality and cause gills to clog (Schveitzer et al., 2013). Thus, C:N ratios are

recommended in which the distribution of heterotrophic bacteria and nitrifying bacteria are more homogeneous (C:N 10-12:1) (Xu et al., 2016; 2018; Romano, 2017).

Applications of organic carbon have been used after anaerobic and aerobic process of prebiotics (wheat, soybean and rice bran), associated with the use of probiotics, which can have synergistic effects on the growth environment (Hapsari, 2016; Romano, 2018). This type of strategy reduces problems related to excess suspended solids, improving the distribution of heterotrophic and nitrifying bacteria and stimulating plankton growth (Romano, 2017; Kawahigashi, 2018).

In general, microbial aggregates offer a supplemental source of proteins and lipids (Bakhshi et al., 2018). However, low levels of methionine and lysine, eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA) are observed (Zhou et al., 2007; Hu et al., 2011; Magaña-Gallegos et al., 2018), which are important nutrients for shrimp reared. Thus, some studies have shown that the addition to microorganisms to shrimp culture can improve zootechnical performance, probably due to the proximal composition of the microbial flocs (Marinho et al., 2014; Marinho et al., 2017; Jamali et al., 2015; Brito et al., 2016; Martins et al., 2016; Abreu et al., 2019).

The rotifer *Brachionus plicatilis* (Müller, 1786) has been used in the early stages of fish and shrimp hatcheries as a supplementary source of protein (amino acids) and lipids (fatty acids) (Dhert et al., 2001; Jeeja et al., 2011). The morphotypes of the rotifer can be classified as: “SS” (*Super small*), “S” (*Small*) and “L” (*Large*) based on the length of the lorica, which may be approximately 90-150, 100-210 or 130-340 (Hagiwara et al., 2001; Mills et al., 2017). According to Demir and Diken (2011a), *B. plicatilis* has approximately 480 - 590 g of crude protein and 61 -142 g of lipids per kilogram of dry matter. Jeeja et al. (2011) found amino acid levels between 241.4 to 411.8 g kg-1 in *B. plicatilis* fed with different types of microalgae. Regarding essential fatty acids, Kotani et al. (2017) found mean values between

101 2.52 to 4.83 mg g^{-1} dry weight of EPA and DHA in rotifers of the species *B. plicatilis* of the
102 strain "*Large*" fed with *Chlorella vulgaris*.

103 These organisms are capable of absorbing the nutritional value of the diet offered,
104 which must be chosen by considering production cost, nutritional quality and ease of large-
105 scale production (Torzillo and Chini Zitelli, 2015; Ferreira et al., 2018). To improve the
106 nutritional content of rotifers, microalgae are used in various forms (frozen, dry, concentrated
107 or fresh) (Tzovenis et al., 2004; Seychelles et al., 2009; Kotani et al., 2010). One widely used
108 microalgae is *Nannochloropsis* sp., which contains substantial amounts of EPA (Adissin et
109 al., 2019). Other forms of enrichment include the use of baker's yeast (*Saccharomyces*
110 *cerevisiae*) (Penglase et al., 2011), microcapsules (Langdon, 2003) and emulsions (Haché and
111 Plante, 2011) rich in lipids. The choice of the diet offered is directly related to the
112 biochemical composition of the rotifers being cultivated (Whyte et al., 1994).

113 In addition to these nutritional aspects, *B. plicatilis* grows quickly, which facilitates
114 large-scale production, and is combined with its high tolerance to environmental changes that
115 occur during cultivation, low mobility in the water column and a size suitable for feeding the
116 postlarvae (Pousao-Ferreira et al., 2003; Seychelles et al., 2009; Jeeja et al., 2011; Dhont et
117 al., 2013; Yin et al., 2013).

118 The nutritional quality of food supplied to cultivated animals contributes to better
119 nutritional status and consequently to better immunological strength (Mohapatra et al., 2013;
120 Jin et al., 2013; Newaj-Fyzul et al., 2014). Healthy animals are more resistant to changes in
121 the culture system and to possible infections (Cuéllar-Anjel et al., 2010). Greater resistance
122 may allow them to survive longer when submitted to stress tests, such as osmotic tests
123 (Álvarez et al., 2004).

124 In this context, it is important to study the addition of microorganisms to improve the
125 nutritional floc and zootechnical performance (Crab et al., 2012; Shah et al., 2018). The

purpose of this study is to evaluate the effects of addition of *Brachionus plicatilis* (rotifer) at different densities to the production of *L. vannamei* juveniles on zootechnical performance, water quality; presence of *Vibrio*, plankton composition, proximal composition and total hemocyte count in a nursery BFT system with the addition of anaerobic and aerobic wheat bran as an organic carbon source.

2. MATERIALS AND METHODS

2.1 Experimental conditions

An indoor trial was conducted for 42 days and the experimental design was completely randomized with four treatments: BFT (biofloc); BFT-10 (biofloc with addition of 10 org mL^{-1} of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *Brachionus plicatilis*), all in triplicate.

A matrix tank with water salinity of 35g L^{-1} was chlorinated with 13 mg L^{-1} of chlorine and after 72 hours of aeration, it was applied inorganic fertilization (divided in two applications) composed of urea (4.5 N g m^{-3}), triple superphosphate (0.225 g P m^{-3}) and sodium silicate (3.0 g Si m^{-3}). After two days, organic fertilization was added through 10 applications of a product for 48h in an anaerobic phase followed by a 24h aerobic phase, with wheat bran (50 g m^{-3}), molasses (25 g m^{-3}), sodium bicarbonate (10 g m^{-3}) and a bacteria-based product (0.5 g m^{-3}), containing *Lactobacilus* sp., *Bacillus subitulis* e *B. Licheniformes* com 7.7 x 10^8 UFC g^{-1} (Kayros Ambiental e Agrícola, Brazil). Sea water (with 10 ppm of chlorine) was applied in a 10x proportion of wheat bran. The organic fertilizer was added at three-day intervals between applications. At the end of this organic fertilization process the water from a matrix tank (0.45 mg L^{-1} Total ammonia nitrogen, 0.3 mg L^{-1} $N\text{-}NO_2$, 1.44 mg L^{-1} $N\text{-}NO_3$, 140 mg alkalinity $CaCO_3$ L^{-1}, 20.51 mg L^{-1} de orthophosphate, pH 8.4 and C:N 11.2) was mixed and distributed equally to fill twelve black-plastic tanks (40 L). The organic

fertilizer was added to the experimental treatments every three days during culture until settleable solids reached 10 mL L^{-1}.

The experimental units were constantly aerated (> 5.0 mg L^{-1}), temperature maintained at (~31C) with a thermostat (100 W/40L) and light intensity was kept at 27 μmol m^{-2} s^{-1} with a natural photoperiod. The water was exchanged during the experimental period at a mean of 1% day^{-1}, with the addition of dechlorinated freshwater to compensate for evaporation losses. Sodium bicarbonate (relative total neutralization power of 56%) was added to maintain alkalinity >100 mg L^{-1} and pH >7.

2.2 Production and addition of *Navicula sp.* and *Brachionus plicatilis*

Rotifers (*Brachionus plicatilis*) strain "*L"* (lorica length 198 μm) were cultured in a transparent glass conical-cylinder with a volume of 2.0 L and 20L and light intensity was maintained at 37 μmol m^{-2} s^{-1} using a fluorescent lamp with an integral light photoperiod. Culture water with 35 g L^{-1} of salinity was initially chlorinated with 15 ppm chlorine and constant water aeration, pH 7.5-8.0, temperature 28 ± 1 ° C. Each week, 0.4 mL L^{-1} of B-complex vitamins (B_1, B_6 and B_{12}) and 0.15 g L^{-1} of fish oil (360 mg EPA and 240 mg DHA) were added, which was suspended 3 days before inoculation with rotifers in the experimental units. The rotifers were fed with microalgae in exponential growth phase *Nannochloropsis* sp. (50 x 10^4 cell mL^{-1}). The organisms were filtered using a 50 μm mesh (and had approximate body size between 150 and 250 μm) and their density was estimated with a Sedgewick-Rafter chamber, before being added to the experimental units. Rotifers were added on the 1^{st}, 10^{th}, 20^{th} and 30^{th} days to the BFT-10 (10 org mL^{-1}), BFT-20 (20 org mL^{-1}) and BFT-30 (30 org mL^{-1}) treatments, with an addition of approximately 0.5 L of rotifers to each experimental tank, based on Brito et al. (2016).

176 **2.3 Shrimp stocking, feeding and monitoring**

177 *L. vannamei* postlarvae (3.4 mg ± 0.02 mg) were obtained from a commercial shrimp
178 hatchery (Aquatec LTDA, RN, Brazil) and stocked at a density of 3,000 shrimp m^{-3} (120
179 shrimp by experimental units) for 42 days. The postlarvae were fed four times a day (at 08:00
180 a.m.,11:00 a.m., 02:00 p.m. and 04:00 p.m.), with a commercial shrimp feed with 45% crude
181 protein, 8% lipids, 13% moisture, 9.5% crude fiber, 4% mineral matter (In vivo Animal
182 Nutrition and Health). The daily feeding rate of 35% of body weight used at the start of the
183 culture was gradually reduced to 10% of body weight after 42 days based on the Van Wyk
184 (1999) table and adjusted daily according to estimated shrimp consumption and mortality rate.
185 Shrimp weight was monitored weekly after 21 days of culture to determine shrimp
186 growth and adjust the amount of feed offered. At the end of the experiment, biomass gain,
187 mean final weight, feed conversion ratio (FCR), survival, and yield and water consumption
188 (WC) were determined based on the following equations: Biomass gain (g) = *final biomass*
189 *(g) − initial biomass (g)*; Final weight (g) = *final biomass (g)/number of individuals at the end*
190 *of evaluation period*; FCR = *feed supplied/biomass gain*; Survival (%) = *(number of*
191 *individuals at the end of evaluation period/initial number of individuals) × 100*; Yield (kg m^{-3}) = *final biomass (kg)/volume of experimental unit (m^3)* and WC = *Total water consumed (L)/*
193 *Final biomass (kg).*
194
195 **2.4 Water quality**
196 Dissolved oxygen, temperature, salinity and pH were monitored (YSI model 100,
197 Yellow Springs, Ohio, USA) twice a day (at 08:00 hours and 16:00 hours). The settleable
198 solids (Imhoff cone) (Avnimelech, 2015) were monitored three times a week. Total ammonia
199 nitrogen (APHA, 2012), N-nitrite (APHA, 2012) and alkalinity (mg L^{-1} $CaCO_3$) (APHA,

2012) were monitored once a week. N-nitrate (APHA, 2012) and orthophosphate (APHA, 2012) were monitored every fifteen days.

2.5 C:N ratio

At the end of the experiment the C/N ratio was measured, based on the amount of organic carbon source (wheat bran, molasses and feed) and nitrogen (feed, urea 45% nitrogen and wheat bran) used in the matrix tank during the experiment. The total nitrogen feed was measured considering that 1 kg of the 45% crude protein feed with 6.25%-N has 72 g of nitrogen. The estimated organic carbon content was determined using the formula presented by Hart et al., (2007) as follows: Organic carbon (%) = *(0.53 x protein) + (0.80 x lipids) + (0.42 x nitrogen free extract)*.

2.6 Microbiological samples and Analyses in TCBS medium

Water samples were collected at the beginning and end of the trial from each treatment with sterile falcon tubes (50 mL) at aerated surface water. For this analysis, each 500 µL of the sample was diluted in 9.0 mL of alkaline peptone water (pH 8.6), thus making six successive serial dilutions. The shrimp samples were collected at the beginning (50 pL_{10} whole body) and end of the experiment (5 shrimp – hepatopancreas samples (0.14g) from each culture unit and were immersed in a 70% alcohol solution individually for 15 seconds, then immersed individually in a 1.5% sodium hypochlorite solution with 0.1% of tween-80 for 15 minutes and washed thoroughly using sterilized water to remove the remaining surface bacteria and disinfectant. After disinfection, the shrimp or shrimp hepatopancreas were weighed, macerated and diluted in 9.0 mL of alkaline peptone water (10^{-1}). After homogenization, water and shrimp samples were serially diluted 10^{-1} to 10^{-6} (100 µL). Samples were taken from water (0.1 ml) and shrimp with the help of a sterilized pipette and

were applied using the spread-plate method on Thiosulphate Citrate Bile Sucrose (TCBS) agar and incubated at 30°C for 24h in triplicates. After incubation, the bacterial colonies of negative sucrose (green *Vibrio* like bacteria) and positive sucrose (yellow *Vibrio* like bacteria) (CFU mL^{-1} and CFU g^{-1}) were counted between 30 and 300 colonies using a colony counter.

2.7 Phytoplankton and Zooplankton Community

Water sampling was performed at the 1st, 21st and 42nd day of culture (500 mL). The water was filtered through a cylindrical-conical 250, 125 and 70 μm net mesh to reduce the suspended solids in the sample and then filtered through a 50 μm mesh, to retain zooplankton, and a 15 μm mesh to retain phytoplankton, which were stored in 25 mL containers. Next, a 2.5 mL aliquot was fixed in 4% formalin and stored for further analysis. A Sedgewick-Rafter chamber and binocular optical microscope (Olympus CH30) with magnification of 800x (Pereira-Neto et al., 2008) were used for identification at the genus level, with the aid of identification keys for phytoplankton (Hoek et al., 1995; Bicudo and Menezes, 2006) and zooplankton (Bradford-Grieve et al*.,* 1999; Foissner et al., 1999). Phytoplankton was expressed in cells per milliliter (cell mL^{-1}) following the methodology described by Hötzel and Croome (1999) and zooplankton were expressed in individuals per liter (ind L^{-1}) following the methodology described in APHA (2012).

2.8 Proximal Composition

Analysis of crude protein, lipids, moisture, ash and fiber contents of whole-body shrimp, biofloc, wheat bran, molasses and rotifers were performed in triplicate using standard methods (AOAC, 2012). Biofloc samples were collected to determine their proximal composition at the beginning from matrix tank and end of the experiment from each tank using a 50-μm mesh. Shrimp samples were also collected post larvae (pL_{10}) at the beginning

and from each tank at the end of the experiment, and washed with distilled water to remove epiphytes and encrusting material. Rotifers were collected to determine their proximal composition at the end of production and separated using a 50 μm mesh. The biofloc, shrimp and rotifers were oven-dried at 60°C. For moisture content, the samples were oven-dried at 105°C until constant weight (315 SE model, Fanem). The difference in weight before and after sample drying was recorded and expressed in percentage. Protein content was determined by measuring nitrogen (N x 6.25) using the Kjeldahl method (TE 0363 model; Tecnal, São Paulo, Brazil). Total lipid content was determined by the Soxhlet extraction method using pure Hexane (98%) solvent (Ma 044/8/50 model, Marconi, São Paulo, Brazil). The crude fiber content was determined with the Enzymatic-gravimetric method by measuring the residue after acid and alkaline digestion (Vasconcelos et al., 2010). The ash was determined by oven incineration at 550°C (Q318 D24 model; Quimis, São Paulo, Brazil). The carbohydrate was determined by the equation: (% dry weight) = 100 - (%crude protein – %lipid – %ash- %fiber).

2.9 Salinity stress test

At the end of the experiment, a salinity stress test was carried out by transferring juvenile shrimps from each treatment to three replicated units (30 shrimp per unit) containing freshwater, gently aerated by one air stone per recipient for 30 min. The shrimp were then transferred to water with salinity of 35 g L^{-1}. After an additional exposure of 30 min, all shrimp not responding to mechanical stimulus were considered dead. Experimental conditions were 29.0 ± 0.5°C and pH 7.8 ± 0.1, using twelve 2-L plastic bottles (Burbano-Gallardo et al., 2015).

2.10 Total haemocyte count

275 At the end of shrimp culture and after the final salinity stress test a hemolymph sample
276 was taken according Guertler et al. (2013) with a 1-mL syringe containing 200 µl of
277 precooled anticoagulant modified Alsever solution (MAS) (336 mmol/L NaCl, 115 mmol/L
278 glucose, 27 mmol/L sodium citrate, 9 mmol/L EDTA, pH 7.2) at a proportion of 1:2 (v:v). For
279 total hemocyte counting (THC), triplicates of 100 µl of diluted hemolymph were counted for
280 the number of hemocytes using a hemocytometer under a light microscope.
281
282 **2.11 Statistical analyses**
283 Statistical analyses of the data were performed using Statistica software version 10.0
284 (StatSoft). Data were checked for homogeneity of variance with the Cochran test (Cochran $p < 0.05$) and normality using the Shapiro-Wilk test ($p < 0.05$). One-way variance analysis
286 (ANOVA) was conducted to evaluate the zootechnical performance variables, proximal
287 composition and repeated ANOVAs were used to compare water quality data, followed by the
288 Tukey test ($p < 0.05$) to compare means. The *Vibrio* and plankton genera were evaluated with
289 the Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison test ($p < 0.05$).
291 The phytoplankton and zooplankton density were previously logarithmized, and
292 transformation ($\log (x + 1)$), and Cluster analysis (Bray–Curtis similarity) were used to
293 observe the community similarity on temporal and spatial scales and possible group
294 formation. The analysis of similarity (ANOSIM) ($p < 0.05$), with 999 permutations, was used
295 to identify difference within and between groups (Clarke and Warwick, 2001); and the
296 percentage similarity (SIMPER) to identify the main typifying species of the groups. Data
297 analyses were performed using PRIMER 6.0 software.
298

3. RESULTS

3.1 Shrimp zootechnical parameters

The zootechnical parameters of the shrimp during the 42-day experimental period are summarized in figure 1. The shrimp weight on days 21 and 42 ranged from 0.19 to 0.29 g and 1.09 g to 1.26 g respectively and the BFT-30 had a significant difference ($p < 0.05$) with the other treatments (Figure 1A). The shrimp survival rates were all above 77% during the 42-day experimental period and for BFT-30 were significantly higher ($p< 0.05$) than the BFT (Figure 1B). The yield was higher ($p < 0.05$) in BFT-20 (3.14 kg m^{-3}) and BFT-30 (3.42 kg m^{-3}) than in BFT (Figure 1 C). The FCR on days 21 and 42 ranged from 0.67 to 1.05 g and 1.12 g to 1.23 g, respectively with significant difference ($p < 0.05$) in the treatments (Figure 1D).

3.2 Water quality

There were no significant differences ($p > 0.05$) in the water quality parameters between the four treatments (Table 1). The water quality parameters were temperature ~31ºC; dissolved oxygen ~ 5 mg L^{-1}; salinity ~ 32 g L^{-1}; pH ~8.06; SS 7.49 ± 0.75 to 9.13 ± 0.87 mL L^{-1}; TAN 0.38 ± 0.03 to 0.47 ± 0.06 mg L^{-1}; N-nitrite 0.59 ± 0.07 to 0.75 ± 0.12 mg L^{-1}; N-nitrate 18.61 ± 6.71 to 23.68± 8.68; orthophosphate 27.08 ± 6.97 to 33.38 ± 8.10; and alkalinity 98.61 ± 7.92 to 106.67 ± 5.67 mg $CaCO_3$ L^{-1}. Water consumption ranged from 261.34 to 372.45 L kg^{-1} shrimp juvenile and the C:N ratio ranged from 10.57 to 10.73.

3.3 Microbiological samples and Analyses in TCBS medium

Microbiological organisms in TCBS (*Vibrio*-like bacteria colonies) in water (CFU mL^{1}) and shrimp (postlarvae and hepatopancreas) (CFU g^{-1}) were classified as yellow (positive sucrose) or green (negative sucrose). The *Vibrio*-type bacteria colonies in the water ranged from 1.58 to 5.66 x 10^4 CFU $mL^{-1,}$ without significant differences between treatments,

however, the higher the addition of rotifers, the higher the percentage of sucrose positive colonies. The *Vibrio*-like bacteria colonies in the shrimp ranged from 10.30 to 227.6 x 10^4 CFU $g^{-1,}$ without significant differences between the treatments, however, the percentage of positive sucrose colonies increased and of negative sucrose colonies decreased by the end of experimental period, with values ranging from 15 to 100% and 62 to 0%, respectively (Table 2).

3.4 Phytoplankton and Zooplankton Communities

A total of 26 genera at the beginning and 34 genera at the end were identified and distributed in the following groups: Cyanophyta (7 and 7), Heterokontophyta (8 and 12), Chlorophyta (6 and 10), Euglenophyta (2 and 2) and Dinophyta (3 and 3), respectively. The total cell density of phytoplankton ranged from 3.871 to 13.938 cells mL^{-1} (Table 3). The most frequent genera at the beginning were *Oscillatoria* (Cyanophyta), *Fragilaria* (Heterokonphyta) and *Ulothrix* (Chlorophyta). At the end, the most frequent were *Oscillatoria*, *Aphanocapsa* (Cyanophyta) and *Cylindrotheca* (Heterokonphyta) (Table 3). The analysis of similarity (ANOSIM) showed no significant differences in the phytoplankton communities among the treatments (R global = 0.019, p=0.29). However, it demonstrated that there were significant differences in the phytoplankton over the days of culture (R global = 0.767, p<0.001) from the 1^{st} to 21^{st} and 42 nd days (Table 3). In a 71,26% cut-off, the cluster analysis showed the formation of two groups, with group I composed by the beginning (day 0), and group II by days 21 and 42 (Figure 2). The results of the similarity percentage analysis (SIMPER) reflect the contribution rate of the main species to the similarity between treatments. The microalgae *Oscillatoria* (6.97 a 9.27%) and *Aphanocapsa* (6.48 a 7.85%) were the main species responsible for the similarities present in each treatment, without significant differences (p>0.05) between treatments.

A total of 9 genera were identified at the beginning and 12 genera at the end, in the following groups: Protozoa (2), Cladocera (2), Copepoda (3), Rotifera (4) and Cirripedia (1). The total density of zooplankton ranged from 2.06 to 4.37 org. mL^{-1} (Table 4). The most frequent genera at the beginning were *Arcella* sp. (Protozoa) and *Daphnia* sp. (Cladocera). At the end, the most frequent were *Arcella* sp. (Protozoa), *Bosmina* sp. (Cladocera) and *Brachionus* sp. (Rotifers) (Table 4). The analysis of similarity (ANOSIM) showed no significant differences in the zooplankton communities among the treatments (R global = 0.069, p=0.11). However, it demonstrated that there were significant dissimilarities in the phytoplankton during the culture (R global = 0.593, p=0.001) from the 1st to 21 st and 42nd days (Table 4). At a 70% cut-off, the cluster analysis showed the formation of two groups, with group I composed by the beginning (day 0), group II (by days 21 and 42) (Figure 3). The results of the similarity percentage analysis (SIMPER) reflect the contribution of the main species to the similarity between treatments. The zooplankton *Brachionus* sp. (21.49 to 33.73%), *Daphnia* sp. (15.44 to 31.25%) and *Arcella* sp. (12.75 to 20.62%) were the main species responsible for the similarity in each treatment, with significant differences ($p<0.05$) between treatments for Protozoa, Rotifera and Copepoda.

3.5 Proximal composition

There were significant differences ($p < 0.05$) in the proximal composition between the four treatments (Table 5). The shrimp proximal composition (g kg^{-1} dry weight basis) ranged from crude protein 212.10 to 283.07 and crude lipids 41.57 to 111.40, with significant differences ($p< 0.05$) between BFT-20 and BFT-30 and BFT and the initial value (postlarvae). The ash content ranged from 124.15 to 144.29, fiber from 30.04 to 33.27 and carbohydrate from 90.23 to 117.67 (Table 5). The microbial biofloc proximal composition (g kg^{-1} dry weight basis) of crude protein ranged from 203.11 to 298.25, of crude lipids from 66.19 to

130.01, ash from 243.62 to 61.94, fiber from 64.36 to 66.83, and carbohydrates from 31.17 to 48.55, with significant differences ($p < 0.05$) for crude protein and lipids between BFT-20 and BFT-30 and BFT and the initial amounts (post larvae) (Table 2). The proximal composition of wheat bran (g kg^{-1} dry weight basis) was crude protein 257.10 ± 4.84, lipids 14.07 ± 0.50, fiber 172.30 ± 2.42, ash 59.37 ± 2.47 and carbohydrates 439.45 ±13.96. The rotifers' proximal composition (g kg^{-1} dry weight basis) were crude protein 483.79 and 194.94 lipids (Table 5).

3.6 Salinity stress test and total haemocyte count (THC)

Shrimp survival after the salinity stress test was 100% without a significant difference ($p > 0.05$) between the treatments with and without the addition of rotifers. The THC was significantly higher ($p < 0.05$) at the end of culture and after a salinity stress test for shrimp submitted to treatments with the addition of rotifers (32.89 to 21.6 x 10^6 cells mL^{-1}) than in the BFT (11.15 × 10^6 cells mL^{-1}) (Figure 4). Moreover, there was higher reduction in the percentage of THC (47%) in BFT than BFT-30 (32%) after salinity stress test.

4. DISCUSSION

4.1 Shrimp zootechnical parameters

The analysis of zootechnical indexes demonstrated a relationship between the stocking density of the rotifer and the parameters evaluated, confirming the nutritional quality of the enriched rotifer linked to the biofloc.

Studies with BFT (fertilized with fresh molasses at an initial density of 2,500 pL m^{-3} and an initial weight of 16.2 mg), and the addition of rotifers (30 org mL^{-1}) and an average temperature of 26.8 ^oC in shrimp nurseries have demonstrated good zootechnical performance (1.76 ± 0.27 kg m^{-3}, 0.82 g ± 0.09 g and FCA 1.32 ± 0.20) over 35 days (Brito et al., 2016). In

399 addition, the use of BFT with the addition of *Navicula* in tanks with molasses at a temperature
400 of 28.3ºC (initial density of 3,000 pL m^{-3} and initial weight of 1.0 mg) also demonstrated
401 good zootechnical results (2.19 to 2.42 kg m^{-3}, 0.80 to 0.86 g and FCA 0.77 to 0.82) over 42
402 days (Abreu et al., 2019). However, in this study, organic fertilization from anaerobic and
403 aerobic process of wheat bran and rotifers addition led to higher zootechnical performance
404 values (2.52 to 3.42 kg m^{-3}, final weight of 1.09 to 1.26 g and FCA of 1.23 to 1.12) in the
405 same laboratory conditions as the studies cited with the addition of molasses as an organic
406 carbon source. The addition of different densities of the rotifer improved the zootechnical
407 performance of the treatments. The addition of the microorganisms provides important
408 nutritional compounds, enriches the biofloc composition and improves digestive enzyme
409 activity (Anand et al., 2013; Martins et al., 2016, Shah et al., 2018).

410 The control treatment also had good results, probably due to the fertilization protocol
411 adopted. Studies have found that the anaerobic and aerobic process of bran improves the
412 availability of nutrients such as proteins and amino acids. Some enzymes produced by
413 microorganisms during anaerobic and aerobic process improve the digestibility of bran,
414 indicating transforms complex organic compounds into simpler compounds (Al-Mashhadani,
415 2011; Kraler et al., 2014; Al-Mashhadani, 2019).

416 Three factors were fundamental to this productive improvement, the maintenance of
417 temperature close to 31ºC, which is one of the environmental factors that affect the growth
418 and physiological performance of the species (Madeira et al., 2015; Zhang et al., 2019), the
419 use of a mixture of bacteria, and the fertilization adopted, which provides a beneficial
420 bacterial community and favors the primary community (Romano, 2018).

421

422 **4.2 Water quality**

The water quality variables found in this study remained in an ideal range for intensive farming according to Samocha (2019). The pH dropped from the middle to the end of the experiment, which was related to an increase in organic matter during the culture and consumption of inorganic carbon by autotrophic bacteria (Silva et al., 2013; Furtado et al., 2015; Samocha, 2019). The decrease in pH was not detrimental to shrimp development in any of the treatments, since this reduction was controlled through weekly additions of sodium bicarbonate to maintain alkalinity above 100 mg $CaCO_3^{-1}$. Van Wyk and Scarpa (1999) found that alkalinities $\geq$ 100 mg $CaCO_3^{-1}$ favor the stabilization of pH levels and autotrophic bacteria growth.

In intensive systems, high stocking densities are used and the concentrations of nitrogen compounds tend to increase as a result of the remains of uneaten feed, high biomass and accumulation of organic matter during culture (Wasielesky et al., 2006; Furtado et al., 2011). In all treatments, the TAN and N-nitrite levels were constant during the first five weeks, however, there was a small increase at the end, although the amounts remained at the ideal levels (TAN < 3 mg L^{-1} and N-nitrite < 10 mg L^{-1}) recommended by Samocha (2019). The increase in N-nitrate concentrations observed at the end of the experiment indicates the presence of nitrifying bacteria (Zhao et al., 2012; Luo et al., 2013), which was also observed in other studies with the addition of microalgae and rotifers to shrimp nurseries (Marinho et al., 2014; Brito et al., 2016; Abreu et al., 2019). Maintaining the levels of nitrogenous compounds within the ideal ranges for culture of the species is extremely important, since higher values of nitrogen compounds are a limiting factor for growth and survival (Ebeling et al., 2006). Thus, the protocol of inorganic and organic fertilization (anaerobic and aerobic process of wheat bran and a bacterial mix) proved to be an efficient strategy for controlling nitrogen compounds in a shrimp nursery phase.

The minimal exchange of water and high densities practiced in intensive systems increase the solids in the system (Van Wyk, 2006). Sedimentable solids must be monitored during culture to avoid problems such as gill clogging and excessive consumption of dissolved oxygen due to the degradation of this organic matter (Gaona et al., 2011). The mean values of sedimentable solids in this study remained below the critical limit of 14 mL L^{-1} for the nursery phase (Samocha et al., 2019), ranging from 7.49 to 9.13 mL L^{-1}.

During culture, freshwater was replaced to compensate for evaporation losses, reaching a daily replacement rate between 1.05 and 1.17% of the total volume of the experimental unit at the end of the experiment. In this context, we can measure the effectiveness of water use by the amount used (replacement + exchange) for the production of 1 kg of shrimp juveniles (Krummenauer et al., 2014). The values obtained in this study were between 372.45 and 261.34 L kg^{-1}, which is close to 352 L kg^{-1} reported by Cohen et al. (2005) and the 200 to 400 L kg^{-1} by Hargreaves (2013). This result emphasizes that the addition of rotifers at the different densities used in this study, does not negatively influence water quality.

4.3 Microbiological samples and Analyses in TCBS medium

Minimal water exchange favors the development of pathogenic and opportunistic bacteria such as the *Vibrio* genus, due to the large amount of organic matter accumulated during the culture (Ferreira et al., 2011; Yanong e Erlacher-Reid, 2012). However, it was observed that at the beginning of culture, 92.77% of these were negative sucrose colonies and at the end 55.59 to 98.61% were composed of positive sucrose colonies. Concerning the CFU found in the shrimp, a change in the proportion of negative and positive sucrose from the beginning to the end of the experiment was also observed, reaching zero negative sucrose concentration in the BFT-30 treatment.

The amount of rotifers added was directly related to increases in the percentage of positive sucrose. The main species of *Vibrio* reported as pathogenic to shrimp are sucrose negative, such as *V. parahaemolyticus*, a microorganism that causes mortality in intensive shrimp systems (Leaño and Mohan, 2012; Gomez-Gil et al., 2014; Hostins et al., 2017). This inversion of negative and positive sucrose associated with the increased stocking density may indicate a competition for nutrients in the growing environment or the presence of antibacterial compounds found in rotifers, which can inhibit the negative sucrose *Vibrio* (Rimper, 2014; Farisa et al., 2019).

The BFT treatment also had a negative to positive inversion of sucrose concentration in both water and shrimp. This may suggest the influence of the fertilization protocol adopted (anaerobic and aerobic process of wheat bran with a bacterial mix) used in all treatments, which reduced the load of this type of bacteria through the addition of beneficial species that compete for nutrients (Lakshmi et al., 2013). Therefore, the use of a fertilization method that encourages the increase of beneficial bacterial communities can help minimize impacts resulting from pathogenic bacteria of the genus *Vibrio* spp. (Costa et al., 2008, Romano et al., 2018).

4.4 Phytoplankton and Zooplankton Community

The planktonic community varies according to the culture system, stocking biomass and nutrient input (Muangkeow et al., 2007; Casé et al., 2008; Melo et al., 2010). In intensive systems, nutrients accumulate, mainly inorganic phosphorus and nitrogen (in the form of nitrate), which are not incorporated into the shrimp biomass and thus do not induce their growth (Franceschiniet al., 2010; Wang et al., 2015).

There was an increase in the density and diversity of the planktonic community along the culture period, which is correlated to this gradual accumulation of nutrients in the system,

497 as also reported by Marinho et al. (2014) and Campos et al. (2019). These differences were
498 emphasized in the Cluster and ANOSIN cluster analysis.

499 A wide variety of phytoplankton communities are found in culture water according to
500 the literature. In this study, there was a predominance of the Cyanophyta group, but according
501 to the SIMPER analysis, the contribution of phytoplankton to the treatments was not due to
502 just one genus. The results showed a low contribution of each genus (<10%), which indicates
503 that there was a wide diversity of genera demonstrating an ecological balance.

504 The relative abundance revealed that about 67% of the phytoplankton was composed
505 by the Cyanophyta group, as reported by other studies (Green et al., 2014; Marinho et al.,
506 2014; Campos et al., 2019), however, the densities in this study were lower than those found
507 in other studies. This predominance of Cyanophyta is related to their capacity to fix nitrogen
508 present in the environment, favoring their growth in relation to other phytoplankton groups.
509 They are also well adapted to low light, and can grow at higher temperatures and with stand
510 the accumulation of phosphorus in the system (Emerenciano et al., 2011; Almanza et al.,
511 2016). In this study, the genera *Aphanoscapsa* and *Oscillatoria* were those most abundant and
512 commonly found in environments rich in organic matter, such as aquaculture systems (Rosini
513 et al., 2016).

514 At the end of the experiment there was a significant difference in the average densities
515 of zooplankton composition, which were lower in the treatments to which *B. plicatilis* was
516 added than in the control, with an approximately 60% reduction of protozoa and a near 40%
517 increase in Rotifera, which suggests that the presence of *B. plicatilis* influenced these
518 communities. Rotifera was observed with greater relative abundance even in the BFT
519 treatment, which may be related to the adaptation of these organisms to nutrient rich
520 environments. Similar results were reported by Anand et al. (2013); Marinho et al. (2014);
521 Gálvez et al. (2015) and Brito et al. (2017). High concentrations of rotifers and protozoa as

seen in this study can benefit shrimp performance (Thompson et al., 2002). Loureiro et al. (2012), analyzed the intestinal content of *L. vannamei* grown in BFT and found protozoa and rotifers, indicating a preference for these microorganisms that are considered an important nutritional source.

4.5 Proximal composition

In terms of proximal composition, it was observed that the addition of rotifers contributed to an increase in the levels of protein and lipids in *L. vannamei* juveniles. The protein and lipid levels increased according to the level of inclusion of rotifers, this increase is linked to the better nutritional composition of microbial flocs with the addition of rotifers. Although there is no significant difference between the initial values and the BFT treatment, we can observe an increase in the lipid and protein concentrations. A study using *L. vannamei* grown in bioflocs and clear water found better nutritional properties in shrimp grown in bioflocs (Rajkumar et al., 2016). However, we observed improved proximal composition (protein and lipids) of whole juvenile shrimp when rotifers were added to the BFT system than to BFT. This improvement increased with additional rotifer density and is probably linked to the better nutritional composition of microbial flocs with additional rotifers.

The values of protein (244 to 298 g kg^{-1}) and lipids (87 to 130 g kg^{-1}) in microbial flocs were higher than those reported by Abreu et al. (2019) for protein (172 to 206 g kg^{-1}) and lipids (63 to 98 g Kg^{-1}) in a nursery to which *Navicula* sp. were added at different densities and with molasses fertilization. These differences in the bioflocs' nutritional characteristics may be related to the carbon source, system maturation time, level of total suspended solids, the planktonic and bacterial communities (Emerenciano et al., 2013) and the nutritional quality of the rotifer added.

546 The proximate composition of *B. plicatilis* may change according to the type of food
547 used during its culture, such as microalgae cultures or commercial enrichment products
548 (Srivastava et al., 2006). Some authors have found values between 482.40 to 592.10 g kg^{-1} for
549 protein and 61.80 to 142.60 g kg^{-1} for lipids in rotifers cultivated with different commercial
550 enrichment products (Demir and Diken, 2011b), while in rotifers fed with microalgae the
551 reported values were 291.90 to 460.20 g kg^{-1} for protein and 159.10 to 361.00 g kg^{-1} for lipids
552 (Jeeja et al., 2011). The results were similar to those observed in this study 483.79 g kg^{-1} for
553 protein and 194.94 g kg^{-1} for lipids.
554
555 **4.6 Salinity stress test and total haemocyte count (THC)**
556 Salinity stress tests are used to measure the resistance of the cultured shrimp, and it
557 consists of an abrupt decrease in salinity over a given time and then a recovery to the original
558 salinity level (Burbano-Gallardo et al., 2015). The results obtained were 100% for all
559 treatments, which suggests that the resistance was not only related to the nutritional
560 supplementation with the rotifer, but also to the BFT system.
561 Although *L. vannamei* tolerates a wide range of salinity, sudden variations require
562 energy expenditure to maintain the animal's homeostasis and can be considered a stressor (Li
563 et al. 2007; 2008). Crustaceans, unlike vertebrates, have only innate or non-specific immune
564 systems, and therefore have no immune memory (Wang and Wang, 2013). The cellular
565 immune response of crustaceans is mediated by hemocytes, which constitute the first line of
566 defense and trigger a series of immunological reactions to situations of stress and infections
567 (Van de Braak et al., 2002; Ekasari et al., 2014; Niu et al. 2018).
568 In this study, we performed a total hemocyte count before the stress test, and the
569 highest mean values, 36.53 to 48.42 cells mL^{-1} x 10^6, were obtained in treatments with the
570 addition of rotifers, while the control treatment had 21.3 cells ml^{-1} x 10^6. The results were

similar for BFT with the addition of *Navicula,* where values of 41.25 cells mL^{-1} x 10^6 were reported for microalgae addition and 22.84 cells mL^{-1} x 10^6 for the BFT treatment (Abreu et al., 2019).

After salinity stress tests, treatments with the addition of rotifer showed concentrations of 32.89 to 21.60 cell mL^{-1} x 10^6, while in the BFT treatment it was 11.15 cell mL^{-1} x 10^6. Several authors have observed a decrease in the concentration of hemocytes in *L. vannamei* due to a decrease in salinity, and suggest that it is due to an increase in the volume of hemolymph in these conditions (Lu-Qing et al., 2005; Wang and Chen et al., 2005).

In reaction to an infection or stressful situation, hemocytes migrate to the affected regions, decreasing the levels of circulating hemocytes (Barracco et al., 2008). This can be seen in this experiment, where all hemocyte levels decreased after the salt stress test. However, it was observed that even after the stress test, treatments with the addition of rotifers had higher hemocyte concentrations under normal conditions than the BFT treatment. This indicates the importance of the nutritional conditions on shrimp health, through good immunological indicators such as the hemocyte count (Yeh et al., 2006). According to Jiravanichpaisal et al. (2006) the increase in THC may indicate better ability to fight infections.

5. CONCLUSION

The addition of rotifer (*B. plicatilis*) at a density of 20-30 org mL^{-1} every 10 days enriched the proximal composition of the bioflocs (protein and lipids), which contributes to better zootechnical performance. This was accompanied by a better immune response in *L. vannamei* juveniles and a decrease in the bacteria concentration of the negative sucrose *Vibrio* genus in water and shrimp. The cost of large-scale production must be considered, with alternatives such as frozen rotifers or the use of commercial emulsions for enrichment.

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8. APPENDICES

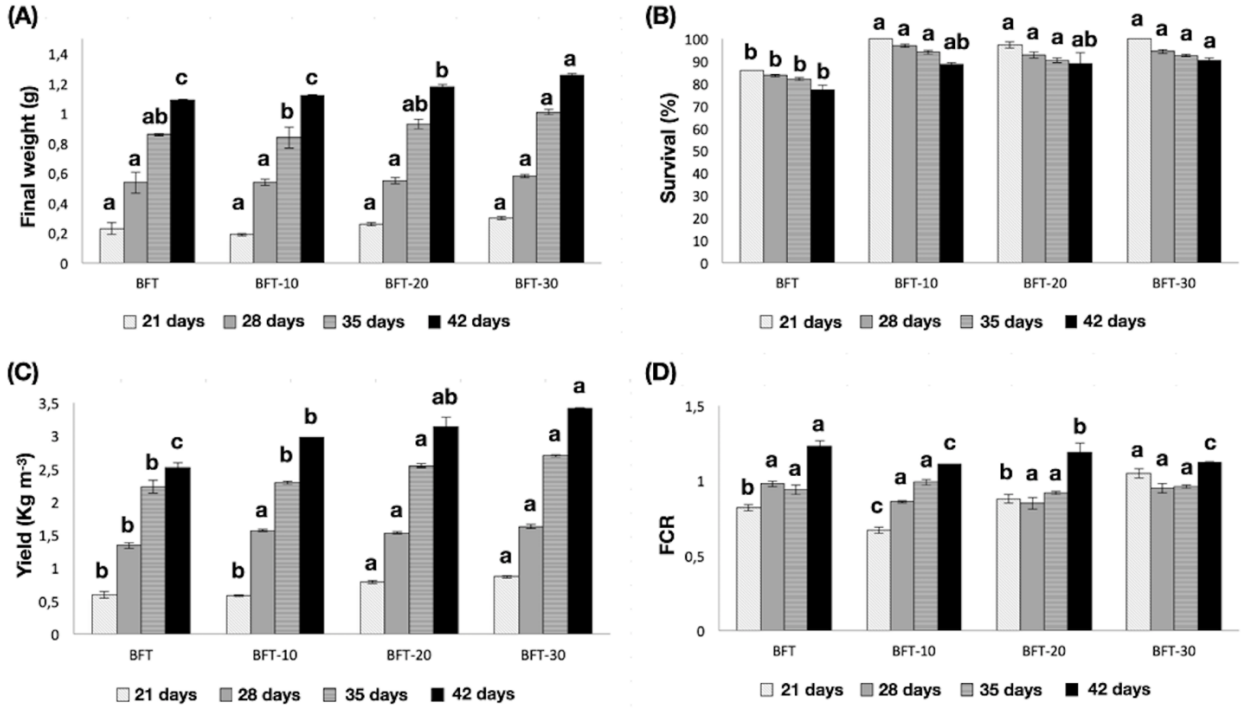

Figure 1. Nursery shrimp zootechnical parameters (final weight -A, survival -B, yield- C and FCR -D) with rotifers added at different densities during a 42-day experimental period. The data correspond to the mean ± SD. Results were analyzed by performing ANOVA one way and the Tukey's test. Mean values (column and time) with different superscripts differ significantly ($P < 0.05$). BFT (biofloc); BFT-10 (addition of 10 org mL^{-1} of *B. plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *B. plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *B. plicatilis*).

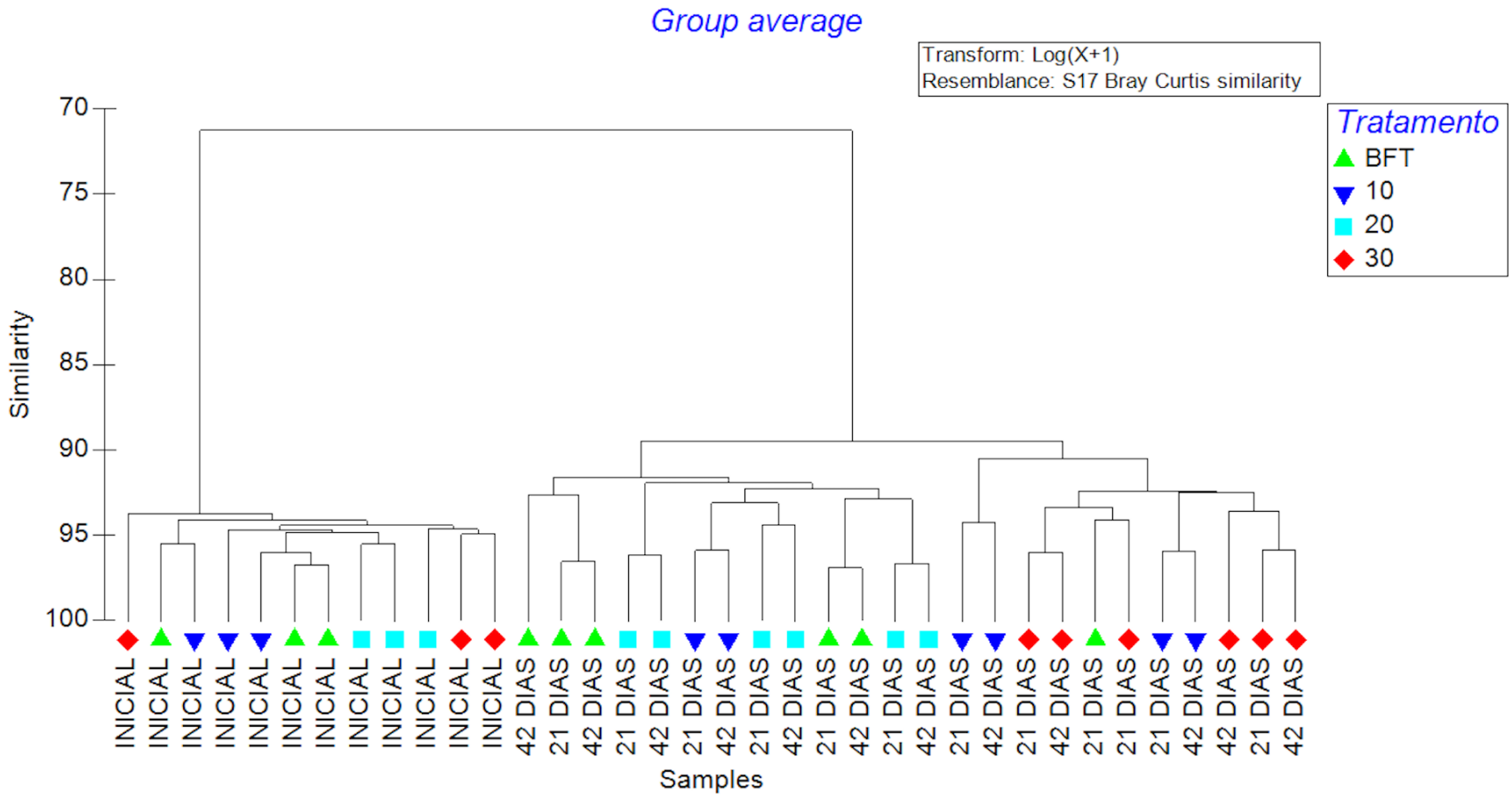

Figure 2. Cluster analysis of the phytoplankton community found of shrimp *L. vannamei* reared with and without different densities rotifers addition.

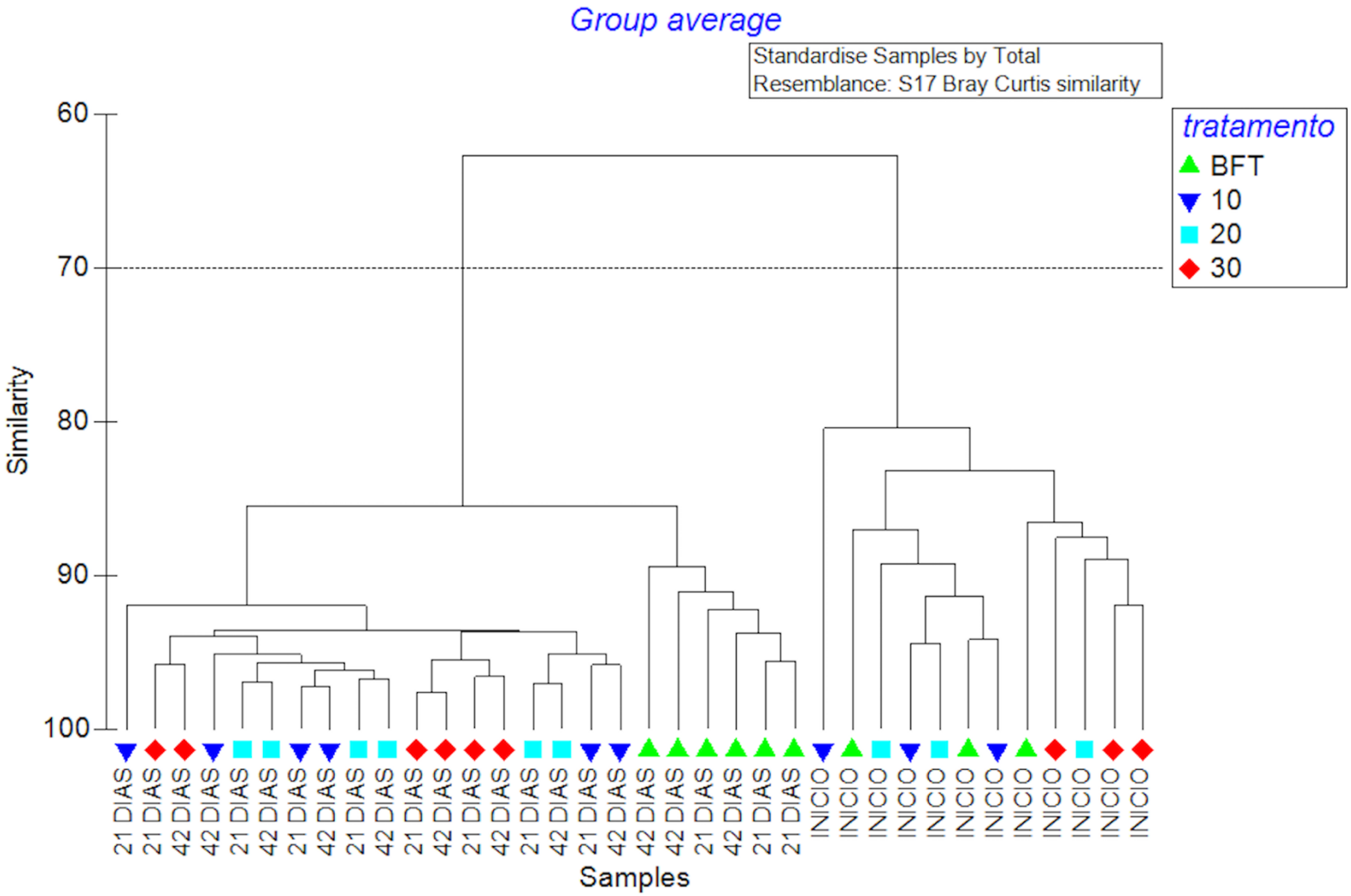

Figure 3. Cluster analysis of the zooplankton community found of shrimp *L. vannamei* reared with and without different densities rotifers addition.

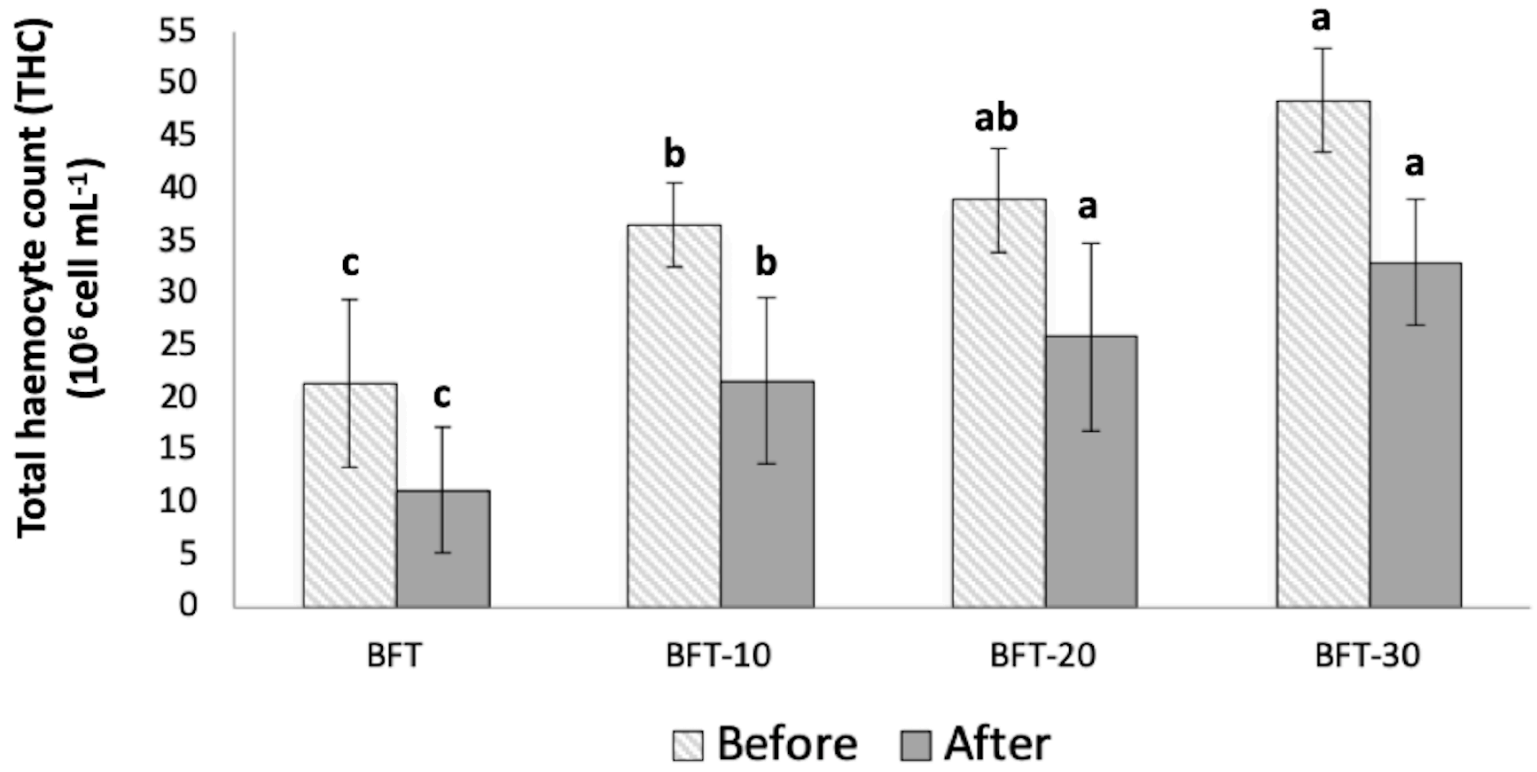

Figure 4. Total haemocyte count (THC) before and after salinity stress test in *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities. The data correspond to the mean ± SD. Results were analyzed by performing ANOVA one way and the Tukey's test. Mean values in the same color column with different superscripts differ significantly ($P < 0.05$). BFT (biofloc); BFT-10 (addition of 10 org mL^{-1} of *B. plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *B. plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *B. plicatilis*).

Table 1. Water quality parameters in the culture of *Litopenaeus vannamei* under nursery biofloc system with and *Brachionus plicatilis* added at different densities during a 42-day experimental period.

	Treatments			
Parameters	**Controle**	**BFT-10**	**BFT-20**	**BFT-30**
Temperature (M) (C°)	31.44±0.14[a]	30.97±0.13[a]	31.22±0.14[a]	31.05±0.13[a]
Temperature (A) (C°)	31.90±0.09[a]	31.59±0.08[a]	31.71±0.12[a]	31.57±0.11[a]
Dissolved oxygen (M) (mg L^{-1})	5.25±0.04[a]	5.34±0.04[a]	5.29±0.04[a]	5.32±0.05[a]
Dissolved oxygen (A) (mg L^{-1})	5.16±0.05[a]	5.23±0.05[a]	5.18±0.07[a]	5.21±0.07[a]
Salinity (g L^{-1})	32.90±0.25[a]	32.74±0.30[a]	32.65±0.31[a]	32.47±0.32[a]
pH (M)	8.31±0.06[a]	8.29±0.05[a]	8.26±0.06[a]	8.28±0.06[a]
pH (T)	8.27±0.06[a]	8.24 ± 0.06[a]	8.21±0.07[a]	8.21±0.07[a]
TAN (mg L^{-1})	0.38±0.03[a]	0.38±0.04[a]	0.47±0.06[a]	0.43±0.04[a]
N-Nitrite (mg L^{-1})	0.75±0.12[a]	0.66±0.09[a]	0.59±0.07[a]	0.65±0.14[a]
N-Nitrate (mg L^{-1})	23.68±8.68[a]	20.31±8.29[a]	18.61±6.71[a]	22.61±7.78[a]
Ortofosfato (mg L^{-1})	27.75±6.98[a]	30.04±7.68[a]	33.38±8.10[a]	27.08±6.97[a]
Alkalinity (mg $CaCO_3$ L^{-1})	106.67±5.67[a]	103.89±6.41[a]	98.61±7.92[a]	100.56±6.38[a]
SS (mL L^{-1})	9.13±0.87[a]	7.49±0.75[a]	8.26±0.77[a]	8.08±1.07[a]
Water consumption (L Kg^{-1})	372.45±11.17[a]	309.21±12.27[a]	297.58±16.82[a]	261.34±13.27[a]
C:N ratio	10.68±0.05[a]	10.73±0.13[a]	10.59±0.05[a]	10.57±0.05[a]

The data correspond to the mean ± SD. Results were analyzed by performing repeated ANOVA measures and the Tukey's test. The C:N ratio were analyzed by performing ANOVA one way and the Tukey's test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). BFT (biofloc); BFT-10 (addition of 10 org mL^{-1} of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *Brachionus plicatilis*). M -Morning; A -afternoon; DO - dissolved oxygen; TAN - total ammonia nitrogen; SS - Settleable solids.

Table 2. *Vibrio* density in the culture of *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities initial and at the end (42-day experimental period).

	Initial	**BFT**	**BFT-10**	**BFT-20**	**BFT-30**
Water (10^4 UFC mL^{-1})					
Positive Sucrose	0.04^b	2.50^a	1.09^a	3.94^a	5.58^a
(%)	(7.23)	(55.59)	(68.84)	(82.49)	(98.61)
Negative Sucrose	0.534^a	1.99^a	0.49^a	0.84^a	0.08^a
(%)	(92.77)	(44.41)	(31.16)	(17.51)	(1.39)
Total	0.58^a	4.49^a	1.58^a	4.77^a	5.66^a
Shrimp (10^4 UFC g^{-1})					
Positive Sucrose	0.01^b	12.60^a	34.70^a	81.30^a	10.30^a
(%)	(1.23)	(37.05)	(15.25)	(54.64)	(100)
Negative Sucrose	1.00^a	21.50^a	192.90^a	67.50^a	0^b
(%)	(98.77)	(62.95)	(84.75)	(45.36)	0
Total	1.01^a	34.10^a	227.60^a	148.80^a	10.30^a

The data correspond to the mean. Results were analyzed by performing Kruskal-Wallis and the Dunn test. Means in the same row with different superscripts differ significantly ($p< 0.05$). BFT (biofloc); BFT-10 (addition of 10 org mL^{-1} of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *Brachionus plicatilis*).

Table 3. Phytoplankton composition (initial and final) of *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities initial and at the end (42-day experimental period period).

Division/Genera	Initial	Final			
		BFT	BFT-10	BFT-20	BFT-30
Chlorophyta (cells mL^{-1})	**1,316.79**	**1,603.12^{a}**	**1,654.22^{a}**	**1,367.94^{a}**	**1,459.90^{a}**
(%)	**34.01**	**11.50**	**12.57**	**10.18**	**11.67**
Botryococcus	0.00	159.92^{a}	177.31^{a}	239.36^{a}	200.15^{a}
Chlorella	0.00	1.49^{a}	2.82^{a}	1.37^{a}	1.70^{a}
Dunaliella	0.15	0.00^{a}	1.33^{a}	0.95^{a}	0.86^{a}
Haematococcus	0.70	0.41^{a}	1.04^{a}	2.26^{a}	1.33^{a}
Mychonastes	286.00	755.23^{a}	486.78^{a}	451.59^{a}	659.76^{a}
Planctonema	342.40	327.54^{a}	455.62^{a}	259.46^{a}	248.71^{a}
Pyramimonas	0.00	2.09^{a}	1.94^{a}	0.90^{a}	1.96^{a}
Spirogyra	223.20	3.53^{b}	11.38^{a}	4.28^{b}	1.91^{b}
Tretadesmus	0.00	0.00^{a}	0.47^{a}	0.48^{a}	0.82^{a}
Ulothrix	464.34	352.90^{a}	525.01^{a}	400.20^{a}	340.33^{a}
Dinophyta (cells mL^{-1})	**0.71**	**7.25^{a}**	**6.30^{a}**	**8.06^{a}**	**7.04^{a}**
(%)	**0.02**	**0.05**	**0.05**	**0.06**	**0.06**
Gymnodinium	0.18	2.85^{a}	2.34^{a}	3.49^{a}	2.74^{a}
Peridinium	0.20	2.40^{a}	1.63^{a}	1.82^{a}	1.91^{a}
Pyrophacus	0.33	2.01^{a}	2.34^{a}	2.75^{a}	2.40^{a}
Euglenophyta (cells mL^{-1})	**5.35**	**6.56^{a}**	**4.07^{a}**	**4.52^{a}**	**4.08^{a}**
(%)	**0.14**	**0.05**	**0.03**	**0.03**	**0.03**
Euglena	0.04	1.42^{a}	0.79^{a}	0.77^{a}	0.67^{a}
Trachelomonas	0.14	5.14^{a}	3.28^{a}	3.76^{a}	3.41^{a}
Heterokonphyta (cells mL^{-1})	**1,282.85**	**3,481.44^{a}**	**2,754.39^{a}**	**2,721.13^{a}**	**2,328.33^{a}**
(%)	**33.13**	**24.98**	**20.93**	**20.26**	**18.63**
Chaetoceros	0.00	4.13^{a}	3.46^{a}	3.32^{a}	2.53^{a}
Chloridella	21.41	1.72^{b}	2.07^{a}	2.93^{a}	2.33^{a}
Cylindrotheca	21.70	1,478.70^{a}	1,343.98^{a}	1,357.02^{a}	1,349.65^{a}
Diatoma	0.00	15.99^{a}	15.39^{a}	29.17^{a}	23.56^{a}
Fragilaria	489.18	20.42^{a}	12.28^{a}	11.01^{a}	22.20^{a}
Hemiaulus	33.11	598.82^{a}	699.86^{a}	661.44^{a}	467.16^{a}

Navicula	28.04	17.58[a]	27.09[a]	30.28[a]	24.54[a]
Orthoseira	188.37	9.35[a]	8.12[a]	16.22[a]	8.93[a]
Phaeodactylum	0.00	1.08[a]	1.63[a]	1.53[a]	2.07[a]
Rhabdonema	188.41	0.00	0.00	0.00	0.00
Skeletonema	312.65	1,333.63[a]	640.46[a]	608.18[a]	425.32[a]
Thalassiosira	0.00	0.00	0.06[a]	0.05[a]	0.03[a]
Cyanophyta (cells mL^{-1})	**1,266.18**	**8,840.49[a]**	**8,741.12[a]**	**9,332.47[a]**	**8,700.76[a]**
(%)	**32.70**	**63.42**	**66.45**	**69.47**	**69.61**
Anabaena	24.79	206.40[a]	212.03[a]	247.99[a]	252.41[a]
Aphanocapsa	433.17	2,153.81[a]	1,665.09[a]	2,077.72[a]	2,399.53[a]
Merismopedia	35.42	0.00[b]	168.45[ab]	0.00[b]	348.79[a]
Oscillatoria	614.93	6,169.55[a]	6,388.07[a]	6,789.62[a]	5,399.92[a]
Plectonema	64.57	26.64[a]	86.59[a]	61.62[a]	32.97[a]
Pseudanabaena	58.36	257.33[a]	183.63[a]	126.74[a]	231.93[a]
Spirulina	34.94	26.75[a]	37.27[a]	28.77[a]	35.14[a]
Total Phytoplankton (cells mL^{-1})	**3,871.89**	**13,938.85[a]**	**13,160.13[a]**	**13,434.12[a]**	**12,500.11[a]**

The data correspond to the mean ± SD. Results were analyzed by performing Kruskal-Wallis test. Mean values in the same color column with different superscripts differ significantly ($P < 0.05$). BFT (biofloc); BFT-10 (addition of 10 org mL^{-1} of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *Brachionus plicatilis*).

Table 4. Zooplankton composition (initial and final) of *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities initial and at the end (42-day experimental period period).

Division/Genera	Initial	Final			
		BFT	BFT-10	BFT-20	BFT-30
Protozoa (org mL^{-1})	**0.58**	**1.05^{a}**	**0.67^{b}**	**0.59^{b}**	**0.66^{b}**
(%)	**28.15**	**29.33**	**16.63**	**15.53**	**15.10**
Arcella sp.	0.46	0.83^{a}	0.45^{b}	0.42^{b}	0.44^{b}
Leprotintinnus sp.	0.12	0.23^{a}	0.22^{a}	0.16^{a}	0.22^{a}
Cladocera (org mL^{-1})	**0.62**	**0.77^{a}**	**1.11^{a}**	**0.97^{a}**	**0.86^{a}**
(%)	**30.10**	**21.51**	**27.54**	**25.53**	**19.68**
Bosmina sp.	0.08	0.38^{a}	0.51^{a}	0.41^{a}	0.47^{a}
Daphnia sp.	0.54	0.39^{a}	0.60^{a}	0.55^{a}	0.72^{a}
Copepoda (org mL^{-1})	**0.19**	**0.40^{b}**	**0.43^{ab}**	**0.39^{b}**	**0.56^{a}**
(%)	**9.22**	**11.17**	**10.67**	**10.26**	**12.81**
Clausocalanus sp.	0.04	0.16^{ab}	0.24^{a}	0.13^{b}	0.19^{ab}
*Euterpina*sp.	0.14	0.15^{a}	0.08^{a}	0.17^{a}	0.21^{a}
*Harpacticus*sp.	0	0.07^{b}	0.11^{ab}	0.09^{ab}	0.15^{a}
Rotifera (org mL^{-1})	**0.67**	**1.22^{b}**	**1.64^{a}**	**1.71^{a}**	**1.77^{a}**
(%)	**32.52**	**34.08**	**40.69**	**45.00**	**40.50**
*Asplanchna*sp.	0.04	0.25^{a}	0.31^{a}	0.34^{a}	0.28^{a}
*Brachionus*sp.	0.61	0.60^{b}	0.89^{a}	0.91^{a}	1.04^{a}
*Filinia*sp.	0	0.19^{a}	0.10^{b}	0.17^{ab}	0.08^{ab}
*Keratella*sp.	0.02	0.15^{b}	0.34^{a}	0.28^{a}	0.35^{a}
Cirripedia (ind. mL^{-1})	**0**	**0.14^{a}**	**0.19^{a}**	**0.15^{a}**	**0.20^{a}**
(%)	**0**	**3.91**	**4.71**	**3.95**	**4.58**
Nauplios	0	0.14^{a}	0.19^{a}	0.15^{a}	0.20^{a}
Total Zooplankton (org mL^{-1})	**2.06**	**3.58^{b}**	**4.03^{ab}**	**3.80^{b}**	**4.37^{a}**

The data correspond to the mean ± SD. Results were analyzed by performing Kruskal-Wallis test. Mean values in the same column with different superscripts differ significantly ($P < 0.05$). BFT (biofloc); BFT-10 (addition of 10 org mL^{-1} of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *Brachionus plicatilis*).

Table 5. Proximal composition of shrimp and biofloc in the culture of *Litopenaeus vannamei* under nursery biofloc system with and *Brachionus plicatilis* added at different densities during a 42-day experimental period.

	Treatments				
Proximal composition[1]	**Initial**	**BFT**	**BFT-10**	**BFT-20**	**BFT-30**
Shrimp					
Moisture (%)	89.43±0.55^{a}	79.56±0.34^{b}	78.05±0.20^{b}	76.58±0.77^{b}	79.10±0.67^{b}
Crude Protein	172.21±5.33^{b}	212.10±6.11^{b}	252.52±1.27^{ab}	274.45±1.60^{a}	283.07±2.82^{a}
Lipids	30.99±1.97^{b}	41.57±0.50^{b}	63.44±3.09^{ab}	89.04±2.10^{a}	111.40±1.77^{a}
Ash	-	144.29±1.35^{a}	124.15±1.46^{b}	138.26±0.42^{a}	139.09±1.71^{a}
Fiber	-	30.04±1.74^{a}	31.59±0.78^{a}	33.27±0.35^{a}	32.14±2.56^{a}
Carbohydrate	-	117.67±0.48^{a}	110.92±1.24^{a}	108.77±3.73^{a}	90.23±4.90^{b}
Biofloc					
Moisture (%)	93.84±0.10^{a}	90.22±1.80^{ab}	89.78±0.15^{ab}	88.87±0.17^{b}	88.20±1.31^{b}
Crude Protein	179.23±3.19^{c}	203.11±9.28^{b}	244.32±9.25^{b}	279.65±8.98^{ab}	298.25±5.38^{a}
Lipids	43.60±1.81^{c}	66.19±1.08^{b}	87.64±2.25^{ab}	117.39±12.52^{a}	130.01±4.62^{a}
Ash	191.51±21.9^{a}	243.62±18.50^{a}	261.94±14.90^{a}	258.48±9.05^{a}	253.03±7.94^{a}
Fiber	55.49±1.22^{a}	66.83±1.44^{a}	66.55±3.07^{a}	65.71±0.96^{a}	64.36±0.36^{a}
Carbohydrate	32.84±1.26	48.55±6.72^{a}	34.67±1.25^{b}	31.30±6.81^{b}	31.17±8.37^{b}

[1] Except for moisture (%), the other values are in terms of dry weight (g 100 g^{-1} dry weight). The data correspond to the mean ± SD. Results were analyzed by performing ANOVA one way and a Tukey's test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). BFT (biofloc); BFT-10 (addition of 10 org mL^{-1} of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL^{-1} of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL^{-1} of *Brachionus plicatilis*).

4. CONSIDERAÇÕES FINAIS

No presente estudo foi constatado que a adição do rotífero *Brachionus plicatilis* contribui positivamente no cultivo de *Litopenaeus vannamei* na fase berçário em sistema de bioflocos. As densidades de adição de 20-30 org mL^{-1} proporcionaram os maiores valores desempenho zootécnico, melhor composição centesimal e melhores concentrações de hemócitos após um teste de estresse salino. Atrelado a adição do rotífero, podemos atrelar o sucesso do cultivo a eficiente estratégia de fertilização adotada, o que possibilitou a manutenção da qualidade de água durante todo o cultivo. Contudo, outros estudos devem ser conduzidos para testar novas formas de ofertar os rotíferos.

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