UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG

INSTITUTO DE OCEANOGRAFIA (IO)

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

Uso do açaí liofilizado (*Euterpe oleracea* Mart. 1824) no enriquecimento dos bioflocos: Potencial para a produção do camarão branco do Pacífico *Penaeus vannamei* (Bonne, 1931) cultivado em sistema BFT

GRECICA MARIANA COLOMBO

FURG

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GRECICA MARIANA COLOMBO

Tese apresentada como parte dos requisitos para obtenção do grau de doutora em Aquicultura no Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande.

Orientador: José María Monserrat

Co-orientador: Wilson Wasielesky Junior

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Pós Graduação em AQUICULTURA
Universidade Federal do Rio Grande - FURG

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DE DEFESA DA 84ª TESE DE DOUTORADO EM AQUICULTURA

No dia quatro de outubro de dois mil e vinte e três, às treze horas e trinta minutos, reuniu-se a Banca Examinadora de Tese de Doutorado em Aquicultura, da **GRECICA MARIANA COLOMBO**, orientado pelo Prof. Dr. José Maria Monserrat composta pelos seguintes membros: Prof. Dr. José Maria Monserrat (orientador – ICB/FURG), Prof. Dr. Dariano Krummenauer (IO/FURG), Dr. Mateus Tavares Kütter (UFPel) e o Prof. Dr. Ricardo Berteaux Robaldo (UFPel). Título da Tese: **"USO DO AÇAÍ LIOFILIZADO (*Euterpe oleracea* MART. 1824) NO ENRIQUECIMENTO DOS BIOFLOCOS: POTENCIAL PARA A PRODUÇÃO DO CAMARÃO BRANCO DO PACÍFICO *Penaeus vannamei* (BONNE, 1931) CULTIVADO EM SISTEMA BFT"** Dando início à defesa, o Coordenador do PPGAq Prof. Dr. Ricardo Vieira Rodrigues, passou a presidência da sessão ao Prof. Dr. José Maria Monserrat, que na qualidade de orientador, passou a palavra para a candidata apresentar a Tese. Após ampla discussão entre os membros da Banca e a candidata, a Banca se reuniu sob a presidência do Coordenador. Durante esse encontro ficou estabelecido que as sugestões dos membros da Banca Examinadora devem ser incorporadas na versão final da Tese, ficando a cargo do Orientador o cumprimento desta decisão. A candidata **GRECICA MARIANA COLOMBO** foi considerada **APROVADA**, devendo a versão definitiva da Tese ser entregue a Secretaria do PPGAq, no prazo estabelecido nas Normas Complementares do Programa. Nada mais havendo a tratar, foi lavrada a presente ata, que após lida e aprovada, será assinada pela Banca Examinadora, pela candidata e pelo Coordenador do PPGAq.

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SUMÁRIO

DEDICATÓRIA

Com o coração muito grato a Deus, dedico esta tese aos meus pais Luiz Colombo e Veranice Colombo e ao meu irmão Gabriel Colombo, meus maiores incentivadores! Amo vocês!

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Agradeço principalmente à Deus por ter cuidado, sustentado e me dado graça ao longo de todo esse período!

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À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de doutorado.

RESUMO GERAL

O açaizeiro (*Euterpe oleracea*) é uma palmeira endêmica da bacia amazônica, rica em proteínas, vitaminas, minerais, fibras, ácidos graxos e compostos fenólicos. Dentre os compostos fenólicos, os principais são os flavonóides, os quais conferem ao açaí ampla capacidade antioxidante. Um sistema de cultivo amplamente utilizado nos camarões é o sistema BFT (Biofloc Technology), onde os bioflocos possibilitam a remoção de compostos nitrogenados e também representam uma fonte suplementar de alimento para os camarões, tendo em sua composição compostos bioativos de natureza antioxidante. Desta maneira, o presente estudo teve como objetivo avaliar a capacidade dos bioflocos em assimilar e transferir os compostos bioativos presentes no açaí para pós-larvas (Capítulo 1) e juvenis (Capítulo 2) do camarão *Penaeus vannamei* cultivados em sistema BFT, além de aferir os efeitos da adição do açaí liofilizado na coloração dos camarões (Capítulo 3). No capítulo 1 foram realizados dois experimentos. No primeiro experimento foram testadas seis concentrações de açaí, além do controle (2,5; 5,0; 10,0; 20,0; 40,0 e 80,0 mg açaí L^{-1}) no sistema BFT sem a inclusão de pós-larvas. A cada 24 horas, durante sete dias, as respectivas concentrações de açaí foram adicionadas aos bioflocos. Com base nos resultados bioquímicos deste experimento foram definidas três concentrações de açaí (5, 20, e 80 mg açaí L^{-1}), além do tratamento controle, para serem testadas no cultivo com pós-larvas. No segundo experimento, as pós-larvas de todos os tratamentos com açaí (5, 20, e 80 mg açaí L^{-1}), apresentaram maior sobrevivência, no entanto a administração de 20 mg açaí L^{-1} incrementou o ganho de peso e conversão alimentar dos animais ($p < 0,05$). Os bioflocos e os camarões perderam capacidade antioxidante com o aumento da concentração de açaí ($p < 0,05$). Os níveis de TBARS dos bioflocos foram menores nas concentrações de 5 e 20 mg de açaí L^{-1} em comparação com 80 mg de açaí L^{-1} ($p < 0,05$). No segundo capítulo, foram utilizados camarões juvenis distribuídos em quatro tratamentos (controle, 5, 20 e 80 mg de açaí L^{-1}). Novamente a aplicação de açaí em sistema BFT agiu como alcalinizante natural na água de cultivo, assim como observado no Capítulo 1. Os bioflocos e o hepatopâncreas dos camarões perderam sua capacidade antioxidante com o aumento das concentrações de açaí, no entanto, o dano lipídico foi atenuado no tratamento com 20 mg açaí L^{-1} ($p < 0,05$). A aplicação de 20 mg açaí L^{-1} aumentou os valores médios da altura e área das microvilosidades do intestino médio ($p < 0,05$). A mortalidade e os danos proteicos e lipídicos no músculo dos camarões aumentaram com a administração diária de 80 mg açaí L^{-1} ($p < 0,05$). No terceiro capítulo, o teor de polifenóis aumentou em pós-larvas que receberam 80 mg açaí L^{-1} ($p < 0,05$). Não foram observadas diferenças significativas entre os tratamentos para a concentração de flavonóides totais nas pós-larvas ($p > 0,05$). Os dados de luminosidade (L*) apresentaram uma queda significativa no tratamento administrado com 80 mg açaí L^{-1} ($p < 0,05$), o que indica que as pós-larvas estavam mais escuras. Não foram observadas diferenças nos parâmetros a* e b* ($p > 0,05$). O valor médio do parâmetro Delta E para o tratamento de 80 mg açaí L^{-1} permaneceu acima de 5, valor este considerado como evidência de que as diferenças na cor são perceptíveis para o olho humano. De modo geral, a aplicação do açaí no sistema BFT demonstrou efeitos antioxidantes sobre os bioflocos e crustáceos. Os bioflocos foram capazes de assimilar parte dos compostos

antioxidantes presentes no açaí incrementando sua capacidade antioxidante. Em relação as concentrações de açaí, recomenda-se a administração de até 20 mg açaí L^{-1} no cultivo de camarões em sistemas BFT, uma vez que esta concentração melhorou índices zootécnicos, incrementou a sobrevivência, a absorção intestinal, e diminuiu o estresse oxidativo durante o cultivo.

Palavras-chave: alimentos funcionais, antioxidante, compostos bioativos, estresse oxidativo, fruta amazônica, polifenóis.

ABSTRACT

Açaí (*Euterpe oleracea*) is a palm tree endemic to the Amazon, rich in proteins, vitamins, minerals, fibers, fatty acids, and phenolic compounds. Among the phenolic compounds, the main are the flavonoids, which give açaí a wide antioxidant capacity. Biofloc Technology (BFT) is a culture system widely used in shrimp, where bioflocs make it possible to remove nitrogenous compounds and represent a source of food for shrimp, having in their composition bioactive compounds with antioxidant properties. Thus, the present study aimed to evaluate the ability of bioflocs to assimilate and transfer the bioactive compounds present in açaí to post-larvae (Chapter 1) and juveniles (Chapter 2) of the shrimp *Penaeus vannamei* cultivated in a BFT system, and to measue the effects of adding lyophilized açaí on shrimp color (Chapter 3). In Chapter 1, two experiments are conducted. In the first experiment, six concentrations of açaí were tested, in addition to the control (control; 2.5; 5.0; 10.0; 20.0; 40.0 and 80.0 mg açaí L^{-1}) in the BFT system without the inclusion of post-larvae. Every 24 h, for seven days, the respective concentrations of açaí were added to the bioflocs. Based on the biochemical results of this experiment, three concentrations of açaí (5, 20, and 80 mg açaí L^{-1}) were defined, in addition to the control treatment, for testing in the culture with post-larvae. In the second experiment, shrimp from all açaí treatments resulted in greater survival; however, the administration of 20 mg açaí L^{-1} increased weight gain and feed conversion of the animals ($p < 0.05$). Bioflocs and shrimp lost their antioxidant capacity with increasing açaí concentration ($p < 0.05$). The TBARS levels of the bioflocs were lower at concentrations of 5 and 20 mg of açaí L^{-1} than at 80 mg of açaí L^{-1} ($p < 0.05$). In Chapter 2, juvenile shrimps were distributed into four treatments (control, 5, 20 and 80 mg of açaí L^{-1}. Again, the application of açaí in the BFT system acted as a natural alkalinizer in culture water. Shrimp bioflocs and hepatopancreas lost their antioxidant capacity with increasing concentrations of açaí, however, lipid damage was attenuated in treatment with 20 mg açaí L^{-1} ($p < 0.05$). Application of 20 mg açaí L^{-1} increased the mean height and area of the midgut microvilli ($p < 0.05$). Mortality and protein and lipid damage in shrimp muscle increased with daily administration of 80 mg açaí L^{-1} ($p < 0.05$). In Chapter 3, the polyphenol content increased in the post-larvae that received 80 mg açaí L^{-1} ($p < 0.05$). No significant differences were observed between treatments for total flavonoid concentrations in the post-larvae ($p > 0.05$). The luminosity data (L*) showed a significant drop in the treatment with 80 mg açaí L^{-1} ($p < 0.05$), indicating that the post-larvae were darker. No significant differences were observed in parameters a* and b* ($p > 0.05$). The average value of the Delta E parameter for the 80 mg açaí L-1 treatment remained above 5, a value considered evidence that the differences in color are perceptible to the human eye. In general, the application of açaí in the BFT system demonstrated antioxidant effects on the bioflocs and crustaceans. The bioflocs were able to assimilate part of the antioxidant compounds present in açaí, thereby increasing their antioxidant capacity. Regarding açaí concentrations, it is recommended to administer up to 20 mg açaí L^{-1} in shrimp cultures in BFT systems, since this concentration improves zootechnical indices, increases intestinal absorption, and reduces oxidative stress during cultivation.

Key-words: amazonian fruit, antioxidant, bioactive compounds, functional foods, oxidative stress, polyphenols.

INTRODUÇÃO GERAL

Entre os anos de 1997 a 2017, a produção de pescado via aquacultura mais que triplicou em volume de peso vivo, partindo de 34 mil toneladas para 112 mil toneladas, respectivamente (Naylor et al., 2021). Em 2020, a produção aquícola mundial registrou record de 122,6 milhões de toneladas, gerando uma receita de US$ 281,5 bilhões. Este crescimento foi resultado da expansão da atividade no Chile, China e Noruega, influenciando positivamente todas as regiões, exceto o continente africano (FAO, 2022).

Dentre os setores da aquicultura o que mais se destaca em termos de produção de pescado é a piscicultura, representando 66% da aquicultura mundial. Em 2020, foram despescados cerca de 57,5 milhões de toneladas de peixes (US$ 146,1 bilhões). A nível de espécie, os peixes de águas interiores mais produzidos no mundo são, em ordem, a carpa capim *Ctenopharyngodon idellus*, a carpa prateada *Hypophthalmichthys molitrix* e a tilápia do Nilo *Oreochromis niloticus*. Já na piscicultura marinha e costeira, destacam-se os peixes salmão do atlântico *Salmo salar*, o milkfish *Chanos chanos* e as tainhas (Mugilidae) (FAO, 2022). A Ásia permanece sendo o continente com maior produção de animais aquáticos e algas do mundo, contribuindo com 91,6% do total (FAO, 2022). Em termos de exportações de peixes, a China ocupa o primeiro lugar no ranking (US$ 20 milhões), seguida da Noruega (US$ 10 milhões) e Vietnã (US$ 8 milhões), as quais aparecem ocupando o segundo e terceiro lugar, respectivamente, no ano de 2016 (FAO, 2018).

Os crustáceos também compõem importante parcela da produção aquícola global (9,2%), sendo as principais espécies produzidas o camarão marinho *Penaeus vannamei*, a lagosta vermelha (*Procambarus clarkii*) e o caranguejo, *Eriocheir sinensis* (FAO, 2022). A carcinocultura, ramo da aquicultura responsável pela produção de crustáceos, supre cerca de 60% da demanda atual por camarões e, a maior parte da produção (85%) é

oriunda de países tropicais e subtropicais, além da China, Indonesia, Vietnam, India, Equador e Tailândia. As duas principais espécies de camarão peneídeos mais produzidas mundialmente são o *P. vannamei* e *P. monodon,* gerando em 2014 uma receita anual de US$ 23,6 bilhões (Boyd e Jescovitch, 2020).

O *P. vannamei* pertence a família Penaeidae e, a nível de espécie, foram despescados um total de 5,8 milhões de toneladas – o equivalente a 51,7% do total de crustáceos produzidos (FAO, 2022). O sucesso produtivo deste crustáceo é resultado do seu potencial zootécnico, que inclui rápido crescimento, alto índice de sobrevivência, eficiente conversão alimentar, tolerância a amplas faixas de salinidade e temperatura, além do pacote tecnológico já bem estabelecido, como exigências nutricionais, métodos para indução da reprodução, larvicultura, engorda e faixas ideais de qualidade da água para manutenção em condições de cativeiro (Xu e Pan, 2012; Jia et al., 2015).

No Brasil, a prática da carcinocultura comercial iniciou na região nordeste na década de 70, com um histórico de insucesso com as espécies nativas *Farfantepenaeus brasiliensis*, *F. paulensis* e *Litopenaeus schmitti* (Damasceno et al., 1982; Machado, 1988). Em uma posterior tentativa, melhores resultados foram obtidos com as espécies exóticas *Marsupenaeus japonicus* e *P. monodon* (Damasceno et al., 1982). Em meados dos anos 80 os sistemas de produção de camarões eram principalmente caracterizados pela baixa densidade de estocagem. Nas décadas seguintes, com a introdução do *P. vannamei* e melhoramento das técnicas de cultivo, a produtividade das fazendas de carcinocultura aumentaram consideravelmente (Wasielesky et al., 2006, 2017). A carcinocultura, mesmo enfrentando entraves, alcançou importante papel social no país e, hoje, é responsável por 99,6% da produção nacional de camarões (majoritariamente *P. vannamei*) com pouco mais de 19.000 ha de unidades ativas em 2018 (de Lacerda et al., 2021; IBGE, 2021). Em 2021, a produção do camarão *P. vannamei* alcançou 78,6 mil

toneladas, somando uma receita de R$ 1,6 bilhão, correspondendo a um crescimento produtivo de 18,14% e econômico de 14,9% quando comparado ao ano anterior (IBGE, 2021).

A maioria das fazendas de camarão realizam o cultivo em sistema de monocultura semi-intensivo e intensivo, isto é, em viveiros escavados com elevada renovação de água, geralmente próxima a estuários ao longo da costa (BRASIL et al., 2013). Em consequência da intensificação das atividades aquícolas, a quantidade de efluentes, matéria orgânica e nutrientes gerados no cultivo e lançados nos corpos de água naturais é elevado, podendo representar um alto impacto ambiental. O acúmulo de compostos nitrogenados no sistema de cultivo é um dos principais problemas relacionados à deterioração da qualidade de água (Wasielesky et al., 2017). Em sistemas convencionais, altas taxas de renovação de água precisam ser realizadas para o controle dos níveis de amônia (Krummenauer et al., 2014). No ambiente aquático a amônia está presente nas formas ionizada (NH_4^+) e a não ionizada (NH_3), sendo essa última considerada a mais tóxica em função de sua natureza lipofílica (Baldisseroto, 2013).

Além dos efeitos tóxicos causados aos animais aquáticos pelo excesso de nutrientes, o bombeamento de água diretamente do ambiente possibilita a introdução de microrganismos patogênicos nos cultivos (Robles-Porchas et al., 2020). A ocorrência de doenças bacterianas e patógenos virais nas últimas décadas dissiparam cultivos de camarões peneídeos por todo o mundo, acarretando inúmeros prejuízos econômicos e sociais (Ogello et al., 2021). Para o desenvolvimento de uma aquicultura sustentável, fatores ambientais, econômicos e sociais precisam ser levados em consideração (Ogello et al., 2021). Nesse contexto, o interesse pela intensificação da produção em zonas interiores tem aumentado associado à busca por sistemas com baixo impacto ambiental e biosseguros (Khanjani e Sharifinia, 2020).

O sistema de bioflocos, também conhecido como sistema BFT (Biofloc Technology System), tem atraído considerável atenção nas últimas décadas por ser sustentável, ao se basear na mínima ou nenhuma troca de água dos sistemas de produção. A presença dos microorganismos que compõem os bioflocos apresentam basicamente quatro funções biológicas dentro da produção: manutenção da qualidade de água, redução da taxa de conversão alimentar, diminuição dos custos com alimentação e, biossegurança (Ebeling et al., 2006; Krummenauer et al., 2014). Além disso, estes microrganismos possuem compostos bioativos em sua composição, influenciando em uma série de benefícios bioquímicos (Martins et al., 2015; Leon et al., 2018; Colombo et al., 2020; Colombo et al., 2023), fisiológicos (Suita et al., 2016) e imunológicos (Xu e Pan, 2013) para os camarões cultivados. O conjunto destas funções convergem para o aumento das taxas de sobrevivência, alta produtividade, lucratividade e proteção ambiental do cultivo (Khanjani et al., 2020; Ogello et al., 2021; Colombo et al., 2023).

Dentre as funções dos bioflocos no sistema de cultivo, a manutenção da qualidade de água pela transformação dos compostos nitrogenados em proteína microbiana é uma das mais importantes (Santos et al., 2019). No sistema BFT, o acúmulo inicial de nitrogênio orgânico é controlado através da manipulação da relação carbono/nitrogênio na água de cultivo, onde a adição de carboidratos, como o melaço de cana, favorece o desenvolvimento das bactérias heterotróficas, as quais absorvem os compostos nitrogenados convertendo-os em biomassa microbiana (Ebeling et al., 2006). No processo quimio-autotrófico, as bactérias nitrificantes possuem maior eficiência na remoção de nitrogênio, porém apresentam lenta taxa de crescimento em relação às heterotróficas, demorando mais tempo para metabolizar a amônia à nitrato (Ebeling et al., 2006).

A nitrificação é crucial para cultivos em sistema BFT. A primeira fase da nitrificação envolve a presença de bactérias autotróficas nitrificantes, em sua maioria pertencentes

aos gêneros *Nitrosomonas, Nitrosococcus, Nitrospira, Nitrosolobus e Nitrosovibrio*, conhecidas como Amônia Oxidantes (AOB), que convertem a amônia à nitrito (NO_2). Na segunda fase de nitrificação estão as bactérias Nitrito Oxidantes (NOB), pertencentes principalmente aos genêros *Nitrobacter, Nitrococcus e Nitrospira*, as quais convertem o nitrito à nitrato (NO_3) (Figura 1; Reis et al., 2019; Santos et al., 2019). Como o processo de nitrificação consome parte da alcalinidade da água na forma de carbonatos e bicarbonatos, é comum que no sistema BFT ocorra uma diminuição do material carbonático ao longo do cultivo (Furtado et al., 2011). Correções dos níveis de alcalinidade podem ser realizadas através da aplicação de hidróxido de cálcio ou bicarbonato de sódio para manter os valores médios acima de 100 mg $CaCO_3$ L^{-1}, uma vez que concentrações abaixo desse valor podem prejudicar a qualidade da água e índices zootécnicos do camarão (Furtado et al., 2011).

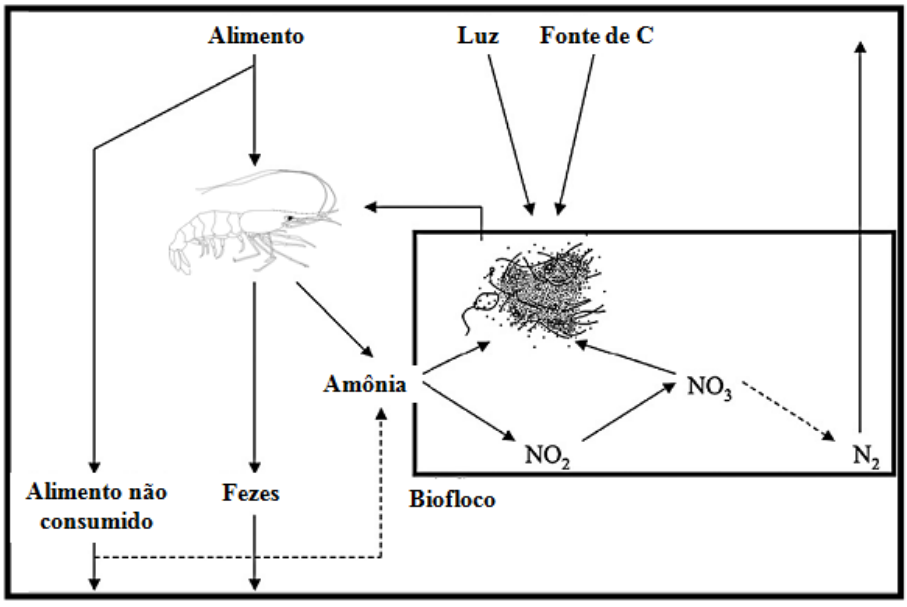


Figura 1. Ciclo do nitrogênio no cultivo de camarões em sistema de bioflocos. Fonte: Adaptado de Crab et al. (2007).

Outra vantagem do cultivo em sistema de bioflocos está na possibilidade da reutilização da água para iniciar novos ciclos de produção através da aplicação de inóculos (Krummenauer et al., 2014). Ao iniciar um cultivo, leva-se um período de tempo

para o aparecimento das bactérias heterotróficas até o desenvolvimento das bactérias nitrificantes na água. Enquanto a comunidade bacteriana não se estabiliza na água, nesse meio tempo ocorrem oscilações nas concentrações dos compostos nitrogenados, necessitando de uma maior atenção e controle dos parâmetros de qualidade de água (Reis et al., 2019; Robles-Porchas et al., 2020). Segundo Krummenauer et al. (2014), a aplicação de um inóculo maturo de um ciclo de produção anterior (mínimo de 25% do volume de água do tanque com bioflocos) pode ser inoculado para iniciar um novo ciclo produtivo, pois acelera o desenvolvimento das bactérias e consequentemente a rapidez na remoção de amônia e nitrito da água, além de fornecer melhores condições nutricionais para os camarões.

Os bioflocos são compostos por bactérias, microalgas, protozoários, zooplâncton, nematóides, fezes, e restos de ração, além de apresentar alto teor de proteínas, lipídios, minerais, vitaminas, enzimas digestivas e moléculas antioxidantes (Reis et al., 2019; Martins et al., 2015). O valor nutricional dos bioflocos é bem dinâmico, podendo variar em função do tamanho da partícula, comunidade bacteriana, compostos bioquímicos, fonte de carbono, luminosidade e concentração de antioxidantes (Ekasari et al., 2014; Reis et al., 2019; Khanjani e Sharifinia, 2020; Colombo et al., 2023). Devido ao seu teor proteico, a quantidade de ração fornecida durante o ciclo produtivo pode ser reduzida, pois a proteína formada por bactérias heterotróficas por meio da absorção de N inorgânico é utilizada como fonte de alimento suplementar pelos camarões (Robles-Porchas et al., 2020). A melhora das taxas de crescimento, conversão alimentar, parâmetros imunológicos e nutricionais em camarões juvenis e pós larvas é justificada pelo consumo dos bioflocos pelos camarões, no qual pode representar até 29% da dieta do *P. vannamei* (Burford et al., 2004; Wasielesky et al., 2006; Suita et al., 2014, 2016; Khanjani e Sharifinia, 2020). Além dos benefícios relacionados a qualidade de água e aspectos

nutricionais, diferentes estudos realizados com *P. vannamei* indicaram que os bioflocos possuem capacidade de aumentar o estado antioxidante e amenizar danos oxidativos nos camarões, justamente por apresentarem em sua composição diferentes microrganismos naturais e compostos bioativos de natureza antioxidante (Martins et al., 2015; Leon et al., 2018; Colombo et al., 2023).

Durante o cultivo os organismos aquáticos precisam constantemente enfrentar as variações de determinados fatores ambientais, incluindo operações de manejo, as quais podem atuar como agentes estressores e afetar sua homeostase. Em sistemas de produção fechado, situações corriqueiras do manejo produtivo, como alterações nos níveis dos compostos nitrogenados, disponibilidade de oxigênio dissolvido, variações de temperatura e salinidade, transporte, despesca, combinadas com altas densidades de estocagem, são mais intensas, e estimulam a produção excessiva de espécies reativas de oxigênio (ERO) através de reações de oxi-redução, causando um desbalanço fisiológico e bioquímico nos camarões (de Souza et al., 2014; Martins et al., 2014; Hostins et al., 2015; Martins et al., 2015; Colombo et al., 2020; Brol et al., 2021; König et al., 2021).

As ERO são caracterizadas como produtos intermediários da redução tetravalente do oxigênio (O_2), geradas durante o metabolismo celular e produção de energia (ATP) na mitocôndria, resultando em diferentes compostos altamente reativos a outras biomoléculas e com elevado potencial tóxico como o ânion superóxido ($O_2^{\bullet-}$), radical hidroxila ($OH^{\bullet}$), peróxido de hidrogênio (H_2O_2), e o oxigênio singlete ($^1O_2$) (Abele e Pintarulo, 2004; Halliwell e Gutteridge, 2015). A respiração celular consiste na principal fonte endógena da produção destes intermediários. Quando em condições fisiológicas normais há um equilíbrio entre a produção de ERO e sua neutralização pelos sistemas de defesas antioxidantes. Porém, a produção excessiva de ERO tende a perturbar o equilíbrio

entre os agentes oxidantes e a capacidade antioxidante do organismo, gerando uma condição de estresse oxidativo (Monserrat et al., 2008; Sies e Jones, 2020).

O estresse oxidativo é definido como uma sobrecarga oxidativa causado pelo desequilíbrio entre oxidantes e antioxidantes em favor dos pró-oxidantes e/ou na sinalização redox, com potencial capacidade de gerar danos oxidativos a lipídios, proteínas, DNA e RNA, afetando a homeostase celular (Jones, 2006; Liang et al., 2016; Sies e Jones, 2020). Tais danos causados nos camarões, bem como em qualquer organismo aeróbico, podem ter origem exógena ou endógena, quando gerados em função do meio ambiente ou pelo próprio organismo, respectivamente. A intensidade dos danos oxidativos promovidos pelas espécies reativas de oxigênio depende da natureza e quantidade das ERO as quais as células estão expostas, assim como as suas proteções antioxidantes (Sies e Jones, 2020). Não necessariamente a produção de ERO gera danos celulares, porém quando há um acúmulo além da capacidade de defesa dos sistemas antioxidantes, um quadro de estresse oxidativo é instaurado (Liang et al., 2016).

Em células aeróbicas, as mitocôndrias são as principais fontes endógenas da produção de ERO geradas durante o transporte de elétrons na cadeia respiratória (Banh et al., 2016). Na tentativa de proteger a integridade celular contra a toxicidade das ERO, no decorrer da evolução os organismos aeróbicos desenvolveram mecanismos de defesas antioxidantes, os quais são divididos em enzimáticos e não-enzimáticos (Halliwell e Gutteridge, 2015). O sistema de defesa enzimático atua como o primeiro mecanismo de defesa celular, destacando-se as enzimas superóxido dismutase (SOD), glutationa peroxidase (GPx) e a catalase (CAT), dentre outras. A SOD possui um importante papel nos sistemas de proteções antioxidantes, pois sua ação catalítica sobre o ânion superóxido compõe o passo inicial do processo de detoxificação das ERO produzidas durante o metabolismo celular (McCord e Fridovich, 1969; Halliwell e Gutteridge, 2015). Já no

sistema de defesa não-enzimáticos estão presentes as vitaminas C (ácido ascórbico) e E (α-tocoferol), bioflavonóides e a glutationa reduzida (GSH), sendo essa última considerada a primeira linha de combate contra as ERO (Ventura-Lima et al., 2009).

Para avaliar as condições do sistema antioxidante dos bioflocos e dos camarões *P. vannamei* no presente estudo, análises da capacidade antioxidante total contra radicais peroxil (ACAP), peroxidação lipídica (TBARS), concentração da glutationa reduzida (GSH), proteínas associadas a grupos sulfridrilas (P-SH), polifenóis e flavonóides totais foram realizadas. Por serem substratos ricos em elétrons, os lipídios são alvos constantes das ERO. A análise conhecida como TBARS permite estimar a intensidade do estresse oxidativo através dos níveis de peroxidação lipídica causados nos tecidos celulares (Oakes e Van Der Kraak, 2003). Durante o processo de lipoperoxidação um dos subprodutos formados são os radicais peroxil, porém, quando a capacidade antioxidante contra esses radicais (ACAP) é aumentada nos organismos aquáticos, a propagação deste processo é impedida ou pelo menos diminuída (Amado et al., 2009; Okpala et al., 2016). Também atuando como linha de defesa antioxidante contra a ação das ERO nos organismos, a glutationa reduzida (GSH) é um importante antioxidante na proteção do estado redox celular e detoxificação de xenobióticos. A GSH encontra-se presente nos organismos sob a forma reduzida (GSH) e oxidada (GSSG), contudo, devido a toxicidade da glutationa oxidada, as células tendem a manter a razão GSH/GSSG elevada em condições fisiológicas normais (Hellou et al., 2012). Quando em condições de intenso estresse oxidativo e na presença de toxinas, os níveis de glutationa dos organismos diminuem podendo ficar comprometidas a proteção contra os radicais livres e toxinas (Nuttall et al., 1998; Huber et al., 2008). A diminuição e oxidação dos grupos sulfidrilas (-SH), compreendendo amino ácidos e pontes dissulfetos, também estão associados aos danos provocados pela ação de ERO nas proteínas.

Danos oxidativos podem ser prevenidos e/ou amenizados na presença de compostos bioativos, que dentre suas principais ações biológicas estão as de natureza antioxidante (Kaur e Singh, 2017). Estas moléculas estão presentes naturalmente em pequenas quantidades em uma variedade de frutas e vegetais, sendo que os principais compostos bioativos encontrados são os polifenóis, flavonóides e carotenóides (Kaur e Singh, 2017; Septembre-Malaterre et al., 2018). O aumento do seu conteúdo nos tecidos auxilia na interceptação de ERO, como os radicais peroxil, pois devido a sua estrutura química estes antioxidantes apresentam alta capacidade de contenção e remoção de compostos radicalados, prevenindo a ação de agentes estressores e danos moleculares (Zamora & Hidalgo, 2016). Estudos direcionados para a inclusão de frutos e vegetais ricos em antioxidantes na dieta de organismos aquáticos tem apresentado diversos benefícios, desde a ação prebiótica na microbiota intestinal (Ahmadifar et al., 2021); alteração nos parâmetros de coloração do organismo (Long et al., 2017); e melhora de parâmetros zootécnicos e imunológicos (Macias-Sancho et al., 2017). Recentemente, um dos frutos que tem obtido destaque no setor aquícola é o açaí. A redução de danos histopatológicos (Colombo et al., 2020), efeitos quimioprotetor contra compostos nitrogenados (Colombo et al., 2020), e neuprotetor (da Silva et al., 2021) são alguns dos benefícios reportados pela inclusão deste fruto na dieta de peixes e crustáceos.

O açaizeiro (*Euterpe oleracea*) é uma palmeira endêmica da bacia amazônica encontrado naturalmente em áreas de inundação (Figura 2), conhecidas como várzea, ocupando uma área estimada em 11 milhões de hectares (Odendaal e Schauss, 2014). Esta espécie de açaí é uma das mais produzidas e de maior interesse agroindustrial, e seu fruto um dos principais produtos de extrativismo nacional brasileiro (CONAB 2022). O Brasil é o país que detém a maior produção, consumo e exportação do *Euterpe oleracea,* sendo os estados do Pará e Amazonas responsáveis por 94,41% e 4,85% da produção nacional,

respectivamente (Menezes et al., 2008; Oliveira et al., 2019; Tavares et al., 2020; Medina e Cruz, 2021).

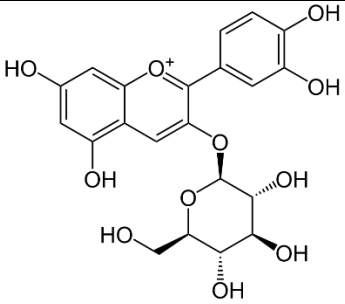
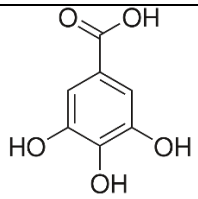
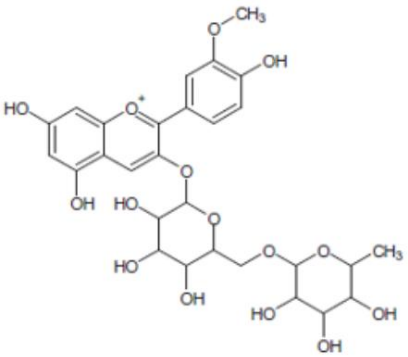
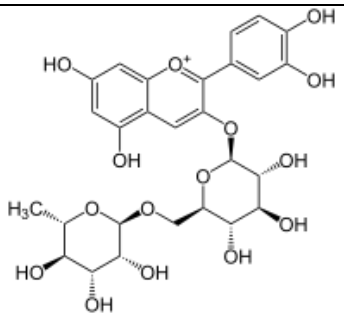
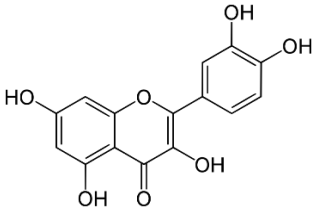
Os troncos do açaizeiro podem crescer até uma altura de 30 metros com capacidade de produção em sistema de extrativismo de 4,2 ton/ha em um período de 5 anos de safra (Odendaal e Schauss, 2014). Devido ao crescimento da demanda no mercado interno e externo, o cultivo do açaí tem migrado de sistemas extrativistas para sistemas de produção manejado e irrigado, alcançando produtividades médias de 6,6 ton/ha a 15 ton/ha, respectivamente (Medina e Cruz, 2021). Seu fruto é redondo, medindo de 1 a 2 cm de diâmetro com peso de 0,8 a 2,3 gramas (Figura 3), e sua semente chega a compor mais de 80% do fruto (Oliveira et al., 2019). O processo de amadurecimento do fruto leva aproximadamente 175 dias, atingindo coloração final preto-violeta (Odendaal e Schauss, 2014).

Figura 2. Açaizeiro (*Euterpe oleracea*). Fonte: de Oliveira e Schwartz, 2018.

Figura 3. Cacho de açaí. Fonte: Arquivo pessoal.

O fruto do açaí é a principal matéria prima para a fabricação diferentes subprodutos com alto valor comercial, desde a indústria alimentícia como bebidas energéticas, sorvete, geléia, a itens da indústria cosmética e farmacêutica (de Almeida Magalhães et al., 2020). No entanto, ainda é o fruto na forma de sucos e polpas o mais consumido entre a população (Tavares et al., 2020). Sua polpa destaca-se por ser rica em proteínas, vitaminas, minerais, fibras, ácidos graxos (principalmente ácido oleico, palmítico e linoleico) e compostos fenólicos (Alqurashi et al., 2016; Schauss, 2016; Lucas et al., 2018). Segundo Alqurashi et al. (2016), os principais compostos fenólicos encontrados no açaí são o ácido gálico, quercetina e antocianinas, com predominância de cianidina-3-O-glucosídeo, cianidina-3-O-rutinosídeo e peonidina-3-O-rutinosídeo (Tabela 1; Carvalho et al., 2016). As antocianinas são responsáveis pela coloração do fruto além de conferir ao açaí ampla capacidade antioxidante total (Odendaal e Schauss, 2014; Dos Santos et al., 2022).

Tabela 1. Principais compostos fenólicos presentes no açaí (*Euterpe oleracea*).

Composto fenólico	**Estrutura química**
Cianidina-3-O-glucosídeo	
Ácido gálico	
Peonidina-3-O-rutinosídeo	
Cianidina-3-O-rutinosídeo	
Quercetina	

Devido ao seu alto valor nutricional e capacidade antioxidante em comparação com outras frutas e legumes, a polpa do açaí tem sido objeto de diferentes estudos voltados para a saúde (Bichara e Rogez, 2011; Dos Santos et al., 2022). Nestes estudos foram comprovados que diversas propriedades do açaí estão associadas a presença de compostos fenólicos, os quais conferem a este fruto papel antinflamatório e anticancerígeno (Martino et al., 2016; Da Silva et al., 2023), neuroprotetor (Crespo-Lopez et al., 2019), cardiovascular (Cordeiro et al., 2017; de Moraes Arnoso et al., 2022) e antioxidante (Colombo et al., 2020; Dos Santos et al., 2022) em humanos e animais. Em camundongos, a suplementação do extrato de açaí aumentou a capacidade antioxidante total do sangue e diminuiu a peroxidação lipídica, além de ter reduzido o estresse oxidativo periodontal, prevenindo a perda óssea alveolar (Dos Santos et al., 2022). A suplementação com farinha de açaí na alimentação galinhas poedeiras em final de ciclo produtivo apresentou diversos efeitos positivos na produtividade e parâmetros bioquímicos, tais como: aumento da produtividade; melhora da resistência da casca dos ovos; incremento da capacidade antioxidante; e diminuição da peroxidação lipídica, tanto em ovos frescos como armazenados (Fortuoso et al., 2019).

Atualmente poucos estudos avaliando os efeitos do açaí na aquicultura foram publicados. A inclusão da polpa do açaí liofilizado em dietas para organismos aquáticos foi reportada para as espécies do peixe zebra (*Danio rerio*) (Kim et al., 2012), tambaqui (*Colossoma macropomum*) (Da Silva et al., 2023) e no camarão branco do Pacífico (*Penaeus vannamei*). No caso do camarão *P. vannamei*, os principais efeitos da inclusão do açaí na dieta estiveram relacionados a redução de danos oxidativos e efeito quimioprotetor. Colombo et al. (2020) observaram que o açaí contribuiu para diminuição do dano oxidativo e histológico em camarões expostos a concentrações subletais de amônia após 96 h. Efeitos positivos do açaí frente a toxicidade causada pela saxitoxina

(Ramos et al., 2022) e cianotoxina nodularina (Schmitz et al., 2020) foram reportados em camarões juvenis. Dentre os principais resultados encontrados pelos autores estão a diminuição do dano lipídico e proteico no músculo, e aumento dos níveis de GSH no hepatopâncreas e brânquias dos camarões quando exposto a nodularina. O aumento do conteúdo de polifenóis totais e flavonóides nos bioflocos e brânquias de camarões alimentados com 5% e 10% (p/p) de açaí, respectivamente, foram reportados por Silva et al. (2020). Além disso, a inclusão de açaí na dieta dos camarões também influenciou em parâmetros de coloração destes organismos. Silva et al. (2020) e Ramos et al. (2022) verificaram que a inclusão de açaí (10% p/p) na dieta diminuiu os valores de luminosidade da região do cefalotórax e também na região abdominal de camarões frescos, deixando os camarões mais escuros, e após o cozimento, colorações mais avermelhadas foram observadas.

Como mencionado anteriormente, estudos com diferentes modelos de animais já constataram que o consumo de açaí promove uma série de efeitos bioquímicos e fisiológicos positivos aos organismos, no entanto, pesquisas prévias voltadas para aquicultura ainda são escassas. Neste contexto, considerando a relevância nutricional e antioxidante do açaí, bem como a capacidade dos bioflocos em absorverem antioxidantes exógenos, o presente estudo teve como objetivo enriquecer os bioflocos com a polpa do açaí liofilizada em meio ao cultivo do camarão branco do Pacífico *P. vannamei* em sistema BFT, e analisar os possíveis efeitos antioxidantes causados nos camarões por meio da transferência dos compostos bioativos presentes no açaí para os bioflocos.

OBJETIVOS

Geral

Avaliar o efeito do uso do açaí liofilizado *Euterpe oleracea* para enriquecimento dos bioflocos durante o cultivo do camarão *Penaeus vannamei* em sistema BFT, bem como a assimilação dos compostos bioativos presentes no açaí pelos bioflocos e sua transferência para os camarões.

Específicos

- Testar diferentes concentrações de açaí liofilizado em um sistema de bioflocos sem camarões, para definir as melhores concentrações deste fruto a serem avaliadas no cultivo do camarão *P. vannamei* em sistema BFT (Capítulo 1).
- Avaliar o uso de diferentes concentrações de açaí no cultivo de pós-larvas do camarão *P. vannamei* cultivadas em sistema BFT, e seus feitos sobre parâmetros zootécnicos, de respostas antioxidantes e dano oxidativo nas pós-larvas (Capítulo 1).
- Explorar o uso de diferentes concentrações de açaí no cultivo de juvenis do camarão *P. vannamei* cultivados em sistema de bioflocos, analisando parâmetros de crescimento, histológicos e de respostas antioxidantes nos camarões (Capítulo 2).
- Desenvolver procedimentos simples e objetivos através de programas gratuitos para mensurar possíveis efeitos sobre a coloração do camarão *P. vannamei* cultivados em sistema BFT enriquecidos com açaí (Capítulo 3).

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CAPÍTULO 1

Bioflocs enriched with lyophilized açaí (*Euterpe oleracea*) improved the survival and weight gain of *Litopenaeus vannamei* post-larvae cultivated in the BFT system

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Bioflocs enriched with lyophilized açaí (*Euterpe oleracea*) improved the survival and weight gain of *Litopenaeus vannamei* post-larvae cultivated in the BFT system

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Abstract

This study evaluates the bioflocs capacity to assimilate and transfer the bioactive compounds present in açaí to post-larvae of *Litopenaeus vannamei* cultured in the BFT system. In the first experiment, 21 experimental units (1 liter) were randomly distributed among seven treatments (control, 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 mg açaí L^{-1}) in the BFT system without the inclusion of post-larvae and no water change. Every 24 hours, for seven days, the respective concentrations of açaí were added to bioflocs. Initial characteristics of the biofloc used were of 0.13 mg TAN L^{-1}, 0.51 mg $N\text{-}NO_2^-$ L^{-1}, 60.5 mg $N\text{-}NO_3^-$ L^{-1}, 245 mg $CaCO_3$ L^{-1} and 330 mg TSS L^{-1}. In the second experiment, 12 experimental units (20 liters), all in triplicate, were randomly distributed in four treatments (control, 5.0, 20.0, and 80.0 mg açaí L^{-1}) with 600 post-larvae (twenty-three day-old PL) per tank cultivated in the BFT system for 27 days. The biofloc inoculum used in the second experiment contained 0.52 mg TAN L^{-1}, 0.13 mg $N\text{-}NO_2^-$ L^{-1}, 35.70 mg $N\text{-}NO_3^-$ L^{-1}, 177.5 mg $CaCO_3$ L^{-1} and 330 mg TSS L^{-1}. Water changes during post-larvae cultivation were performed when the concentration of nitrite and total suspended solids in the water exceeded the safe levels of 1.38 mg/L and 500 mg/L, respectively. In the first experiment, an increase in the antioxidant capacity was observed against peroxyl radicals of bioflocs ($p<0.05$) at concentrations of 5.0 and 20.0 mg açaí L^{-1} and a decrease in lipid peroxidation (TBARS) at a concentration of 20.0 mg açaí L^{-1}. In the second experiment, the mean values of the physicochemical parameters of water remained within the range considered adequate for post-larval culture. Shrimp from treatments with açaí showed greater survival, weight gain, and FCR at the concentration of 20.0 mg açaí L^{-1} ($p<0.05$). Bioflocs and shrimp lost antioxidant capacity with increasing açaí concentration ($p<0.05$). The TBARS levels of the biofloc were lower at concentrations of 5.0 and 20.0 mg açaí L^{-1} compared with 80.0 mg açaí L^{-1} ($p<0.05$). It is concluded that bioflocs can assimilate part of the antioxidant compounds, allowing the addition of up to 80.0 mg of lyophilized açaí in BFT systems, and are an alternative to minimize oxidative stress during culture and influence the quality and growth success of post-larvae.

Keywords: antioxidant, bioactive compounds, polyphenols, nursery, shrimp

1. Introduction

According to FAO (2022) the Pacific White Shrimp, *Litopenaeus vannamei,* is the main cultivated species in the world, with an estimated production of 5,812 thousand tons in 2020, representing 51.7% of marine and coastal aquaculture production of all crustacean species. The various technological advances achieved in all shrimp culture stages contributed to the success of this activity (Avnimelech, 1999; Wasielesky et al., 2006; Gaona et al., 2011; Krummenauer et al., 2014; Suita et al., 2014; Khanjai and Sharifinia, 2022a, 2022b).

Shrimp post-larvae intensive production improves aquaculture production through the use of nurseries (Emerenciano et al., 2012; Schveitzer et al., 2017; Rezende et al., 2019). This process can be done in clear waters with high rates of daily water renewal and using a high-quality feed (Mishra et al., 2008; Suita et al., 2016; Schveitzer et al., 2017; Tierney and Ray, 2018). Nursery cultivation can also be done in a BFT system, however, few studies have documented successful use of BFT with minimal water renewal (Serra et al., 2015; Suita et al., 2014, 2016; Legarda et al., 2018; Rezende et al., 2019).

The BFT system is based on minimal or zero water exchange in the production systems (Ebeling et al., 2006). In addition to performing a fundamental role in maintaining water quality by absorbing toxic nitrogen compounds to shrimp, by converting them into microbial biomass, this can provide up to 29% of the *L. vannamei* diet (Burford et al., 2004). Due to the microbial composition of bioflocs, their consumption improves immunological parameters and nutritional aspects, both in post-larvae and in adult shrimp (Ekasari et al., 2014; Cardona et al., 2015; Suita et al., 2014, 2016; Xu and Pan, 2012, 2013). Such improvements are a consequence of the bioflocs composition because they are a natural source rich in proteins and lipids available as supplementary food in the culture tanks 24 hours a day (Wasielesky et al., 2006).

The benefits of consumption and ingestion of bioflocs by shrimp have been documented in several studies. Suita and co-workers (2016), in their stable isotopes, work with *L. vannamei* PL showed that bioflocs ingestion contributes to improving the quality of shrimp and increases the amount of enzyme-producing cells (B cells) in the tubules of the hepatopancreas, improving the nutritional conditions of this post-larvae (Suita et al., 2014). Xu and Pan (2013) observed that the increase in the cellular immune response and the antioxidant status of *L. vannamei* juveniles is related to the consumption of bioflocs, which present natural microorganisms and bioactive compounds in their composition. The consumption of bioflocs by *L. vannamei* post-larvae cultivated in the BFT system with high salinity (32 ppt) showed better growth and survival rates (Khanjani et al., 2020). Khanjani et al. (2016) observed that the cultivation of post-larval *L. vannamei* in a biofloc system with maximum feeding level improved water quality and increased the lipid and protein content of the bioflocs, resulting in better shrimp growth performance. Another positive factor in biofloc consumption is maintaining the balance of physiological functions, increasing the shrimp's antioxidant status by having bioactive compounds of an antioxidant nature in their composition (Martins et al., 2014).

Considering the benefits described above, enriching the bioflocs with alternative inputs rich in antioxidants could reinforce their action in aquaculture. Because they can incorporate antioxidants exogenously added to the BFT system, bioflocs become an important vector in the transfer of bioactive molecules to shrimp (León et al., 2018; Silva et al., 2020).

In the study by León et al. (2018), the enrichment of bioflocs with quercetin in the culture *L. vannamei* in a BFT system showed several positive results, including the increased bioflocs antioxidant capacity, increased the flavonoid content, decreased lipid peroxidation in the shrimp's hepatopancreas and muscle, and augmented the weight gain of animals.

101 The açaí (*Euterpe oleracea*) is a natural source of bioactive compounds with high
102 antioxidant capacity due to the presence of polyphenols and flavonoids, including
103 anthocyanins, and other components such as fatty acids, amino acids, quercetin, gallic acid,
104 and others (Odendal and Schauss, 2014; Schauss, 2016). Different studies have analyzed the
105 antioxidant properties of açaí with application in aquaculture to reduce the generation of
106 reactive oxygen species (ROS) and oxidative damage. Studies including açaí in the diet of *L.*
107 *vannamei* juveniles have demonstrated: (1) an increase in the content of total polyphenols
108 and flavonoids in the biofloc and gills of shrimp fed 5% and 10% (W/W) açaí, respectively
109 (Silva et al., 2020); (2) chemoprotective effect against exposure to sub-lethal ammonia
110 concentrations by decreasing oxidative and histological damage (Colombo et al., 2020); (3)
111 increased levels of reduced glutathione (GSH) in the hepatopancreas and gills, and decreased
112 lipid and protein damage in the muscle of shrimp exposed to cyanotoxin nodularin (Schmitz
113 et al., 2020); and (4) decreased levels of lipid peroxidation in shrimp exposed to saxitoxin
114 fed with the inclusion of 10% açaí (W/W) in the diet (Ramos et al., 2022).
115 In this context, when considering the relevance of bioflocs and açaí as to nutritional
116 aspects and antioxidant capacity in shrimp, the present study aimed to enrich bioflocs with
117 açaí in a BFT system. The hypotheses of the two experiments realized were that the bioflocs
118 would be incorporated the addition of açaí in the BFT system. Consequently, the enriched
119 bioflocs would serve as supplementary food in the cultivation of post-larvae of *L. vannamei*,
120 influencing the antioxidant responses of this crustacean against the conditions of cultivation.
121 Therefore, the study's objective was to evaluate the ability of bioflocs to assimilate and
122 transfer the bioactive compounds present in açaí to *L. vannamei* post-larvae cultured in a
123 BFT system through antioxidant responses and oxidative damage in bioflocs and shrimp.

2. Material and methods

2.1 Location and acquisition of shrimps and açaí

The present study was carried out at the Marine Station of Aquaculture (EMA), Institute of Oceanography (IO) of the Federal University of Rio Grande - FURG, located at Cassino beach, Rio Grande, RS, and Southern Brazil. The *L. vannamei* post-larvae (PL) were obtained from a laboratory Larvisul located in Santa Catarina, Brazil. The lyophilized açaí (*Euterpe oleracea*) used in these studies was obtained from the Amazônia Comércio de Açaí Liofilizado e Exportação LTDA, located in Belém, PA, Brazil.

2.2 First experiment

The study was conducted in 21 glass beakers with a working volume of 1 L randomly distributed in seven treatments (0-control, 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 mg açaí L^{-1}), all in triplicate, with constant aeration through porous stones and mean temperature (±1 standard error) of 26.38 °C ± 0.10. The bioflocs used in this experiment were collected from a superintensive culture system of Pacific White Shrimp *L. vannamei* from EMA (pH 8.03, 0.13 mg TAN L^{-1}, 0.51 mg $N\text{-}NO_2^-$ L^{-1}, 60.5 mg $N\text{-}NO_3^-$ L^{-1}, 245 mg $CaCO_3$ L^{-1} and 330 mg TSS L^{-1}). No water was added or exchanged throughout the trial of seven days, and every 24 hours, the respective concentrations of açaí were added directly to the water. Daily dissolved oxygen and temperature were measured through a multiparameter digital oxygen meter (YSI®-550A, Yellow Springs, OH) and pH with a digital pH meter (Alfakit, AT 315 SP, Florianópolis, SC). Total ammonia (TAN) ($NH_3 + NH_4^+$), nitrite ($N\text{-}NO_2^-$), and nitrate ($N\text{-}NO_3^-$) were monitored following the UNESCO (1983) protocols, alkalinity (APHA, 1998),

147 salinity (Refractometer Alfakit), and total suspended solids (TSS) (Gaona et al., 2011) were
148 measured three times during the experimental period. At the end of the seven days, bioflocs
149 samples were collected for biochemical analysis.
150
151 *2.3 Second experiment*
152 Based on the results of the first experiment, four treatments (0-control, 5.0, 20.0, and
153 80.0 mg açaí L^{-1}) were considered for the culture of post-larvae of the shrimp *L. vannamei.*
154 Twenty-three-day-old postlarvae (PL23) with an initial average weight (± 1 standard error)
155 of 0.0129 ± 0.0011 g were raised for 27 days until the stage PL50 and stored in 12 plastic
156 tanks at a density of 600 shrimps per tank, randomly distributed in four treatments, all in
157 triplicate. The biofloc inoculum was again collected from a superintensive shrimp culture
158 system (0.52 mg TAN L^{-1}, 0.13 mg $N\text{-}NO_2^-$ L^{-1}, 35.70 mg $N\text{-}NO_3^-$ L^{-1}, 177.5 mg $CaCO_3$ L^{-1}
159 and 330 mg TSS L^{-1}). In each tank were added 17 liters of treated seawater (chlorinated and
160 dechlorinated with vitamin C) and 3 liters of biofloc inoculum previously enriched with açaí
161 as in the first experiment, totaling a volume of 20 liters in each tank. Aeration was supplied
162 through porous stones. A targeted açaí level (5.0, 20.0, or 80.0 mg açaí L^{-1}) was added daily
163 into each experimental tank.
164 The experiment was realized inside the laboratory with artificial light and a photoperiod
165 fixed at 12 hr L/12 hr D. The post-larvae were counted one by one for storage in the
166 experimental units. The initial and final biometrics were performed to estimate the average
167 weight and length of the post-larvae. The average weight was estimated from the individual
168 weighing of post-larvae. The final biomass was measured by collectively weighing all shrimp

169 in each tank. The shrimp were individually measured with a graduated ruler to obtain the
170 average length.

171 Post larvae were fed with two commercial diets (INVE®/StressPak and Guabi XL®/PL
172 40) administered three times a day during the experimental period. In the first five days of
173 the study, PL were fed only the INVE StressPak diet. From the sixth to the tenth day, GUABI
174 PL 40 started to be introduced into the diet together with the INVE StressPak ration. From
175 the eleventh day until the end of the trial, only GUABI PL 40 ration was administered. Live
176 food was not offered in the culture tanks. The rest of the feed was removed from the
177 experimental units. The amount of feed provided was based on the recommendations of the
178 feed manufacturers (INVE® and GUABI®) and also on a protocol adapted from FAO (2004).

179 Daily dissolved oxygen, temperature, pH, total ammonia and nitrite were measured in
180 the morning. Weekly nitrate, phosphorus, alkalinity, salinity and total suspended solids (TSS)
181 were analyzed. Cane molasses was added as a carbon source, in proportion of 6 g of carbon
182 for each 1 g of total ammonium nitrogen in the water, to adjust the C:N ratio in treatments
183 when ammonia levels exceeded 1 mg L^{-1} (Ebeling et al., 2006; Avnimelech, 2009). Water
184 changes of up to 20% of the total volume of each tank were performed when the concentration
185 of nitrite in the water exceeded the safe level of 1.38 mg/L (Cheng and Chen, 1994). The
186 excess total suspended solids were removed by clarification to keep them close to 500 mg/L
187 as recommended by Gaona et al. (2011). For water alkalinity values below 100 mg $CaCO_3$
188 L^{-1}, corrections were performed with sodium bicarbonate ($NaHCO_3$) to increase alkalinity to
189 150 mg $CaCO_3$ L^{-1} according to the methodology described by Furtado et al. (2011).
190

191 *2.4 Proximal analysis of bioflocs*

192 Dry matter analysis was performed by drying the biofloc samples in an oven at 102 ºC
193 for 5 h. For ash analysis, the samples were pre-calcined and then taken to a muffle furnace at
194 600 °C for 5 h (AOAC, 1999). Crude protein content was determined after sample digestion
195 and nitrogen distillation (AOAC, 1999). Ethereal extract content was evaluated using the cold
196 extraction method (Bligh & Dyer, 1959). The nitrogen-free extract (NFE) is calculated by
197 subtracting the sum percentages of dry matter, protein, ether extract and ash.

198

199 *2.5 Biochemical analysis*

200 *2.5.1 Homogenization of the samples*

201 For the collection of biofloc samples, all the volume of water from each experimental
202 unit in the first experiment and 1 liter of water from each tank in the second experiment were
203 collected, respectively, and then added to Imhoff cones, letting them decant for 20 minutes.
204 The sedimentable solids were centrifuged at 1.500 x g for 15 minutes at 10 °C to remove
205 excess water and subsequently stored in an ultra-freezer at -80 °C, following the methodology
206 of Leon et al. (2018). To obtain the homogenates, the bioflocs samples were sonicated at 50%
207 of their maximum power (QSonica Sonicators) for 1 minute in artificial salt water at 30 ppt
208 (Sal Veromix, 95%) at a ratio of 1:1 (W/V), agitated for 3 hours in an orbital shaker at 80
209 rpm and later centrifuged at 10.000 x g for 5 minutes at 4 °C. The supernatants were kept at
210 -80 ºC for further biochemical analysis.

211 At the end of experiment 2, the shrimps were euthanized in liquid nitrogen and whole
212 homogenized (1:4 W/V) in crustacean buffer (pH 7.2) containing Tris-base (20 mM), EDTA
213 (1 mM), $MgCl_2$ (0.05 mM), sucrose (5 mM), and KCl (1 mM), all dissolved in distilled water

(Colombo et al., 2020). The samples were centrifuged at 20.000 x g at 4 °C for 30 minutes and the supernatants were placed in an ultra-freezer (-80 °C) for biochemical analysis. The total protein content of bioflocs and shrimp homogenized samples was determined in a microplate reader (Biotek Synergy HT, *Winooski, VT*) using the Doles Total Protein Kit, based on the Biuret method (550 nm) (Amado et al., 2009).

2.5.2 Total flavonoids and polyphenols analysis

To measure the total polyphenol content in the bioflcs, 25 µl of methanolic extract (1:1.2 W/V) and 625 µl of 0.1 M Folin-Ciocalteau were added to a transparent microplate. After 5 min, 500 µl of Na_2CO_3 at 7.5% were added (W/V), being the standard curve prepared with quercetin previously diluted in 100% methanol. The samples were incubated in the dark at room temperature for 60 min and the absorbance was read at 740 nm in a spectrofluorimeter (Biotek Synergy HT, Winooski, VT). The content of total polyphenols was expressed in µg of quercetin per g of bioflocs and the analysis was carried out according to Dias et al. (2013).

For the analysis of flavonoids total, aliquots of methanolic extract (160 µl) of the bioflocs (1:1.2 W/V), 110 µl of methanol and 80 µl of $AlCl_3$ (5% W/V) were added in transparent microplates. A standard curve was prepared with quercetin (Sigma), previously diluted in 100% methanol, and the data was expressed in terms of µg of quercetin per g of $bioflocs^{-1}$. The microplate with samples and quercetin standards was incubated in the dark for 30 min at room temperature and the absorbance (450 nm) was read in a spectrofluorimeter (Biotek Synergy HT, Winooski, VT). The analysis was carried out according to Gajula et al. (2009), with modifications based on León et al. (2018).

238 *2.5.3. Total antioxidant capacity against peroxyl radicals (ACAP)*

239 This analysis was performed according to the method described by Amado et al. (2009),
240 which is based on the detection of reactive oxygen species (ROS) in samples treated or not
241 with a peroxyl radical generator. The bioflocs and shrimp samples were previously diluted to
242 2 mg of mL^{-1} protein with artificially saline water (*Sal Veromix,* 95%) and homogenization
243 buffer, respectively, and then exposed to peroxyl radicals generated by the thermal
244 decomposition at 37 °C of ABAP (2,2-azobis-2-methylpropionamidine dihydrochloride)
245 (Sigma Aldrich). The peroxyl radicals reacted with H_2DCF-DA (2', 7' dichlorofluorescein
246 diacetate) (Sigma) at 40 µM to form a fluorescent compound (DCF), detected in a
247 spectrofluorimeter (Biotek Synergy HT, Winooski, VT) at excitation and emission
248 wavelengths of 485 and 535 nm, respectively, with readings in the microplate every 5 minutes
249 for 45 minutes. The results were quantified by the relative area with and without ABAP,
250 where the larger the relative area, the smaller the total antioxidant capacity against peroxyl
251 radicals.

252

253 *2.5.4 Lipid peroxidation*

254 The lipid peroxidation levels were determined according to the method described by
255 Oakes and Van Der (2003) through the measurement of thiobarbituric acid reactive
256 substances (TBARS). In glass tubes, the following solutions were added to the samples
257 (bioflocs: 50 µl; shrimp: 100 µl), 20 µl of BHT solution (butylated hydroxytoluene, 67 µM),
258 150 µl of acetic acid solution 20%, 150 µl of 0.8% TBA solution (thiorbarbituric acid), 50 µl
259 of distilled water, 20 µl of 8.1% SDS (sodium dodecyl sulfate) and then incubated at 95 °C
260 for 30 min. After cooling, 100 µl of distilled water and 500 µl of n-butanol were added to the

final solution, which was centrifuged at 3,000 × g for 10 min at 15 °C. The supernatant (150 µl) was used to determine the fluorescence (excitation: 520 nm; emission: 580 nm) in a white microplate reader (Biotek Synergy HT, Winooski, VT). The compound tetramethoxypropane (TMP, Across Organics) was used as a standard, and results were expressed in TMP equivalents per mg of wet tissue.

2.5.5 Reduced glutathione (GSH) concentration

To determine GSH concentrations, 240 µl of the bioflocs and shrimp samples (previously diluted to 2 mg of mL^{-1} protein with artificially saline water or homogenization buffer, respectively) were placed in eppendorfs together with 28 µl of trichloroacetic acid (TCA 50% W/V) (Sedlak and Lindsay, 1968). The samples were centrifuged at 20.000 x g at 4 °C for 10 minutes for protein precipitation. Then, 100 µl of the supernatant was added to a transparent microplate together with 200 µl of 0.4 M Tris-Base at pH 8.9 and 10 µl of DTNB (5.5 '-dithiobis (2-nitrobenzoic acid)) (Sigma Aldrich). The microplate was incubated for 15 minutes in the dark at room temperature and absorbance readings (405 nm) were performed in a spectrofluorimeter (Biotek Synergy HT, Winooski, VT), and the concentration was expressed in µmoles of GSH per mg of protein.

2.5.6. Sulfhydryl groups associated with protein (P-SH) concentration

The protein precipitate formed after centrifugation for GSH analysis was used to determine P-SH based on the method of Sedlak and Lindsay (1968). The pellets of bioflocs and shrimp samples were resuspended in 240 µl of artificially saline water or

284 homogenization buffer, respectively. In transparent microplates, 100 µl of the extract
285 (from bioflocs or shrimp samples), 160 µl of 0.2 M Tris-base at pH 8.2 and 10 µl DTNB
286 were added. The mix was incubated for 15 minutes in the dark at room temperature, and
287 after the absorbance readings (405 nm) were performed in a spectrofluorimeter (Biotek
288 Synergy HT, Winooski, VT), the concentration was expressed in µmoles of P-SH
289 equivalent per mg of protein.
290
291 *2.6. Statistical analysis*
292 For the statistical analysis of the two experiments, all data were submitted to a mixed
293 model of analysis of variance (ANOVA), with the açaí concentrations added to *L. vannamei*
294 culture in BFT the fixed factor and the different experimental units used as random factors
295 (Searle et al., 2006). The homogeneity of the variances and the data distribution's normality
296 was evaluated using the Levene and Shapiro-Wilk tests, respectively ($\alpha = 0.05$). The
297 Newman-Keuls test was used to determine statistically significant differences between
298 treatments and the level of significance was set at 0.05 in all cases.
299 **3. Results**
300 *3.1. First experiment*
301 *3.1.1. Water quality*
302 Table 1 describes the physical-chemical parameters of water from the first experiment
303 are in. During the seven days of experiment 1, there were no statistical differences between
304 treatments for the mean values of temperature, nitrite, and TSS ($p>0.05$). The tanks that
305 received 5.0 mg açaí L^{-1} showed an increase in dissolved oxygen compared to the 10.0 mg
306 açaí L^{-1} treatment ($p<0.05$); however, both treatments did not differ from the rest of the

307 groups, including the control ($p>0.05$). Statistical differences in the mean values of pH were
308 observed only between the treatments of 2.5 and 5.0 mg açaí L^{-1} ($p<0.05$), which did not
309 differ from the control group either ($p>0.05$).

310 Ammonia mean values were higher in the treatments of 2.5 and 80.0 mg açaí L^{-1}
311 compared to the 10.0 and 40.0 mg açaí L^{-1} treatments ($p<0.05$); however, none of the açaí
312 treatments were different from the control group ($p>0.05$). Nitrate's mean value was
313 significantly lower at the 80.0 mg açaí L^{-1} concentration than the rest of the treatments
314 ($p<0.05$). The alkalinity values increased significantly from the 40.0 mg açaí L^{-1} compared
315 to the control treatment ($p<0.05$). Salinity was significantly lower in the treatment of 40.0
316 mg açaí L^{-1} ($p<0.05$) than in the rest of the treatments (Table 1).

317 *3.1.2. Biochemical analysis*

318 The mean values of the total polyphenols and flavonoids in the bioflocs corresponding
319 to the first experiment are presented in Figure 1. A significant increase in the total polyphenol
320 content was observed in the treatments of 40.0 and 80.0 mg açaí L^{-1} compared to the control
321 ($p<0.05$). For the average values of flavonoids in the bioflocs, all treatments at a
322 concentration of 5.0 mg açaí L^{-1} decreased significantly ($p<0.05$) compared to the control
323 treatment at the end of the experiment (Figure 1).

324 Bioflocs total antioxidant capacity was higher (lower relative area) in treatments of 5.0
325 and 20.0 mg açaí L^{-1} than the control, 2.5, 40.0, and 80.0 mg açaí L^{-1} ($p<0.05$). Bioflocs
326 enriched with 20.0 mg açaí L^{-1} showed lower levels ($p<0.05$) of lipid peroxidation than the
327 control (Figure 2). An increase in GSH levels in bioflocs was observed in treatments of 10.0
328 and 20.0 mg açaí L^{-1} compared to the control ($p<0.05$). No significant differences ($p> 0.05$)
329 were observed for the mean values of P-SH (Figure 3).

330 *3.2. Second experiment*

331 *3.2.1. Water quality and proximal analysis*

332 Data on water quality parameters and the proximal composition of bioflocs are shown
333 in Table 2. In the second experiment, the mean values of temperature, nitrite, nitrate,
334 phosphorus, and salinity did not show significant differences ($p>0.05$) between treatments.
335 In the enriched treatment with 80.0 mg açaí L^{-1} there was a decrease in dissolved oxygen
336 levels ($p<0.05$). The 20.0 and 80.0 mg açaí L^{-1} treatments showed a significant increase
337 compared to the control and the 5.0 mg açaí L^{-1} enrichment treatments ($p<0.05$). The 80.0
338 mg açaí L^{-1} treatment increased ammonia and TSS levels compared to the other treatments
339 ($p<0.05$). The mean values of alkalinity started to increase significantly ($p<0.05$) after the
340 treatment of 20.0 mg açaí L^{-1} related to the control. An increase in protein levels in the
341 bioflocs was observed in the 80.0 mg açaí L^{-1} treatment ($p<0.05$). The amount of NFE
342 increased significantly in the 2.00 and 80.0 mg açaí L^{-1} treatments ($p<0.05$), however, in
343 these treatments there was a reduction in the ash content in relation to the control treatment
344 ($p<0.05$).

345 *3.2.2. Zootechnical parameters*

346 Concerning the zootechnical parameters, the administration of 20.0 mg açaí L^{-1} for 27
347 days resulted in significantly higher final shrimp weight than all other treatments ($p<0.05$),
348 however, significantly higher final biomass was noticed between this treatment and the 80
349 mg açaí L^{-1} treatment ($p<0.05$). Lower feed conversion was observed in the 20.0 mg açaí L^{-1}
350 treatment compared to the control ($p<0.05$). Part of the lyophilized açaí added daily into the
351 culture water was ingested directly by the post-larvae in all treatments except in control

352 (visual observation). All treatments that received açaí showed a significant increase in shrimp
353 survival ($p<0.05$) compared to the control treatment (Table 3).

354 *3.2.3. Biochemical analysis*

355 The antioxidant capacity of the bioflocs decreased significantly (larger relative area)
356 according to the increment of the açaí concentrations in the treatments, showing dose-
357 response behavior ($p<0.05$). Similar to what was observed in bioflocs, the antioxidant
358 capacity of shrimp has also decreased (larger relative area) with the increase in the amount
359 of açaí ($p<0.05$) (Figure 4A). In terms of lipid peroxidation, no significant differences were
360 detected in shrimp ($p>0.05$), however, in the bioflocs enriched treatments, there was a
361 decrease in lipid damage at concentrations of 5.0 and 20.0 mg açaí L^{-1} ($p<0.05$) compared to
362 the 80.0 mg açaí L^{-1} treatment (Figure 4B).

363 The biofloc GSH levels increased significantly in the 80.0 mg açaí L^{-1} treatment
364 compared to the 20.0 mg açaí L^{-1} treatment ($p<0.05$); however, they did not differ from the
365 control ($p>0.05$). In the case of shrimp, GSH levels decreased significantly ($p<0.05$) in the
366 80.0 mg açaí L^{-1} treatment compared to the control and 20 mg açaí L^{-1} (Figure 5A). The
367 addition of 80.0 mg açaí L^{-1} in *L. vannamei* culture significantly increased the P-SH levels
368 of the bioflocs when compared to the other treatments ($p<0.05$). Similar behavior was also
369 observed in the treatment of 80.0 mg açaí L^{-1} for shrimp samples, where there was an increase
370 in P-SH levels ($p<0.05$) compared to the control and 20.0 mg açaí L^{-1} (Figure 5B).

371

372 **4. Discussion**

373 *4.1. Water quality and proximal analysis*

374 BFT systems are sustainable alternatives in aquaculture as they result in increased
375 productivity and allow the reuse of water for several cultivation cycles, reducing waste and
376 the spread of diseases (Krummenauer et al., 2014; Avnimelech, 2015). The reuse of water is
377 mainly attributed to the bacterial community that composes the bioflocs, which remove toxic
378 nitrogenous compounds generated during cultivation (Wasielesky et al., 2006; Santos et al.,
379 2019).

380 Although differences were observed in water quality parameters such as dissolved
381 oxygen, temperature, and pH, the mean values of these parameters remained within the
382 appropriate range for the growth and survival of *L. vannamei* (Van Wyk et al., 1999). The
383 administration of the highest açaí concentration (80.0 mg L^{-1}) significantly reduced nitrate
384 concentrations in the first experiment (Table 1). The nitrification processes performed by
385 bacteria are crucial to the development of the BFT system by converting the ammonia
386 generated in the culture medium into nitrite (ammonia-oxidizing bacteria, AOB) and then
387 into nitrate (nitrite-oxidizing bacteria, NOB) (Ebeling et al., 2006; Reis et al., 2019). In
388 agricultural soils, studies aimed at increasing the efficiency of nitrogen use have shown that
389 the application of plant extracts with antioxidant capacity rich in phenolic acids derived
390 mainly from compounds such as tannic acid, chlorogenic acid, gallic acid, caffeic acid,
391 quercetin, and others have the potential to inhibit the first nitrification stage by blocking the
392 ammonia monooxygenase pathway (Erickson et al., 2000; Suescun et al., 2012).

393 From the point of view of the BFT system functioning, açaí bioactive compounds
394 present at high concentrations could slow down the growth of AOB and indirectly of NOB,
395 resulting in higher ammonia levels for a longer period during culture and, consequently,
396 lower nitrate levels. In the second experiment, no significant differences were observed in

nitrate values. However, in the treatment of 80.0 mg açaí L^{-1}**,** the mean ammonia value was higher than the others (Table 2), supporting the hypothesis of the potential effects of açaí at high concentrations on AOB bacteria. Importantly, a mature biofloc inoculum may have mitigated a possible inhibition of the ammonia monooxygenase pathway. The biofloc inoculum used in the study presented nitrate levels, indicating that the nitrifying bacterial community had developed in the system. However, such inhibition in aquaculture processes needs further investigation, particularly during biofloc maturation. The concentrations of the nitrogen compounds during the second study are summarized in Table 2. These concentrations remained within the recommended range for this species (Chen and Lin, 1991; Cheng and Chen, 1994; Cobo et al., 2014).

The mean values of alkalinity and TSS in the first experiment were higher than in the second test due to the absence of shrimp (Tables 1 and 2), corroborating the results observed by Léon et al. (2018) since, as mentioned previously, these crustaceans consume part of the bioflocs as a supplementary food and absorb large amounts of calcium carbonate present in the water for mineralization of the exoskeleton (Wasielesky et al., 2006; González-Vera and Brown, 2017). BFT systems have little or no water renewal throughout the culture. Nevertheless, since nitrification consumes inorganic carbon in the form of carbonates and bicarbonates by heterotrophic and nitrifying bacteria present in bioflocs, it requires the permanent use of alkalizing compounds to maintain water buffering capacity (Ebeling et al., 2006). During the second experiment, to correct the water alkalinity when it was lower than 100 mg $CaCO_3$ L^{-1}, the addition of sodium bicarbonate ($NaHCO_3$) was necessary for the control and 5.0 mg açaí L^{-1} treatments (Furtado et al., 2011), with 9.3 g and 12.0 g of $NaHCO_3$ added in all experimental period, respectively (Table 2). However, the treatments of 20.0 and 80.0 mg açaí L^{-1} did not require alkalinity corrections because the alkalinity values always

421 remained above 100 and 140 mg $CaCO_3$ L^{-1}, respectively. In this case, adding lyophilized
422 açaí to the BFT system would act as a natural alkalizer, assisting in the alkalinity preservation
423 of the *L. vannamei*. Foods such as fruits and vegetables have these characteristics, and the
424 lower their degree of processing, the greater their alkalizing potential (Fardet and Richonnet,
425 2020).

426 An increase in TSS concentrations was noticed in the 80.0 mg açaí L^{-1} treatment in the
427 presence of shrimp reaching a concentration of 535 mg/L (Table 2). Nevertheless, this level
428 is much lower than the 1,000 mg/L reported by Suita et al. (2014). In the study by Suita et al.
429 (2014) in the biofloc system, the mean TSS levels found when PL16 was raised to PL30 were
430 above 1,000 mg/L without affecting survival. It should be noted that, unlike the study by
431 Suita et al. (2014), in our trial, the TSS was maintained at about 500 mg/L, as recommended
432 by Gaona et al. (2011). Therefore, we can assume that TSS levels in the 80.0 mg açaí L^{-1}
433 concentration did not adversely affect the final biomass and the survival in this treatment.

434 The daily addition of açaí in the BFT system contributed to the accumulation and
435 assimilation of this fruit by the bioflocs, influencing the protein, NFE and ash contents after
436 27 days of enrichment. These results can be attributed to the fact that bioflocs and açaí are
437 each considered important natural sources of antioxidant molecules, minerals, vitamins, fatty
438 acids and proteins (Silva et al., 2020; Khanjani and Sharifinia, 2020; Schauss, 2016;
439 Wasielesky et al., 2006). Authors such as Reis et al. (2019), Khanjani et al. (2016) and
440 Emerenciano et al. (2011) found protein levels in bioflocs of 25%, 28% and 30%,
441 respectively. The use of antioxidant supplements in a BFT system, such as açaí, demonstrated
442 in the present study to be able to significantly increase the protein content of bioflocs by up
443 to 29.4%, which can further contribute to the nutritional quality of farmed shrimp and reduce
444 feed costs. Possibly the decrease in the ash content in the bioflocs from the 20.0 mg açaí L^{-1}

445 treatment is due to the low ash content (3.03%–3.80%) found in the freeze-dried açaí pulp
446 (Silva et al., 2020; Odendal and Schauss, 2014; Menezes et al., 2008).
447
448 *4.2. Biochemical analysis*
449 As mentioned earlier, bioflocs can assimilate antioxidants added externally to the
450 culture medium through supplementation or the inclusion of fruits rich in phenolic
451 compounds in diets (Silva et al., 2020) or antioxidants added directly to the culture water in
452 the BFT system (Léon et al., 2018). When analyzing Figure 1A, the levels of total flavonoids
453 in the bioflocs in the control treatment at the end of the seven days remained higher than the
454 açaí treatments. Although this fruit is rich in phenolic compounds, the reason for the drop in
455 flavonoid concentrations in bioflocs enriched with açaí is not clear, and it may suggest that
456 the antioxidant compounds and quercetin were assimilated and metabolized to molecules not
457 detected by the protocol of Gajula et al. (2009), corroborating the results obtained by Leon
458 et al. (2018) in bioflocs enriched with quercetin for 30 days. For analyzing total polyphenols,
459 at the end of the first experiment, the addition of 40.0 and 80.0 mg açaí L^{-1} significantly
460 increased the levels of these compounds in the bioflocs (Figure 1B). However, even the
461 increase in phenolic compounds being able to act in preventing lipid damage by intercepting
462 reactive oxygen species (ROS), unlike Leon et al. (2018) finding the increase in polyphenols
463 did not reduce the levels of lipid peroxidation in the bioflocs (Figure 2B).
464 The addition of 5.0 and 20.0 mg açaí L^{-1} in the BFT system induced a larger antioxidant
465 capacity (low relative area) against peroxyl radicals in the bioflocs, reflecting lower lipid
466 peroxidation levels for the same treatments (Figure 2). This result can be explained by
467 considering that the by-products formed during the lipid peroxidation, such as peroxyl

radicals, should be intercepted or degraded in the presence of a larger antioxidant capacity against these radicals and the peroxidation process interrupted, resulting in less lipid damage (Zamora and Hidalgo, 2016). Besides the increase in antioxidant capacity that has influenced the lipid damage reduction of bioflocs, the increase in GSH levels observed in the 5.0, 10.0, and 20.0 mg açaí L^{-1} treatments (Figure 3A) have also potentiated the defense lines of the bioflocs against the action and the excessive production of ROS (Monserrat et al., 2008; Ventura-Lima et al., 2009). Also, Xu and Pan (2013) reported an increase in the GSH/GSSG ratio in plasma and hepatopancreas of *L. vannamei*, suggesting an improvement in shrimp redox homeostasis in the presence of bioflocs.

In the second study, the daily addition of lyophilized açaí for 27 days resulted in a loss of antioxidant capacity (a larger relative area) in bioflocs and shrimp as the concentrations of açaí increased (Figure 4A); however, it did not induce lipid or protein damage in bioflocs and shrimp (Figures 4B and 5B). Colombo et al. (2020) observed a decrease in antioxidant capacity in the gills of *L. vannamei* juveniles fed a diet containing açaí and exposed to non-ionized ammonia, which influenced larger lipid damage; however, regarding the proteins' sulfhydryl groups' concentration, the exposure to ammonia did not induce protein damage.

In the present study, when analyzing the relationship between GSH and P-SH levels in shrimp (Figure 5), specifically at the concentration of 80.0 mg açaí L^{-1}, it is noted that the consumption of GSH resulted in an increase in the degree of SH groups in the proteins, a mechanism known in the repair of glutathionylated proteins. This mechanism protects the proteins, maintaining their integrity and function during severe pro-oxidant events by preventing the irreversible oxidation of proteins such as carbonylation. The reduction of disulfide bridges through the catalytic action of glutaredoxin and thioredoxin should restore the P-SH levels, expected under a reduced cellular state (Hill et al., 2010).

492 The loss of antioxidant capacity in bioflocs and shrimp (Figure 4A) may be related to
493 the composition of açaí, which is rich in antioxidant molecules whose excessive absorption
494 and metabolization can cause pro-oxidant conditions that do not necessarily imply oxidative
495 damage (Léon et al., 2018; Colombo et al., 2020). Phenolic compounds can modulate AMPK,
496 stimulating mitochondrial biosynthesis and energy bioavailability. Activation of AMPK
497 increases catabolic activity and is associated with varied stimuli caused by oxidative stress,
498 such as an excessive increase in ROS (Zou et al., 2019). In this way, the decrease in
499 antioxidant competence (larger relative area) observed in shrimp could also be linked to
500 greater survival of post-larvae that received açaí (Table 3) and possible activation of AMPK
501 because the growth would be inducing a greater energy investment and increased
502 metabolism, consequently generating more ROS that should be intercepted or degraded, and
503 thus reducing the total antioxidant capacity (Marin et al., 2017; Zou et al., 2019).

504 *4.3. Zootechnical parameters*

505 Due to the high nutritional value of bioflocs, post-larvae cultivation in the BFT system
506 provides increased survival and productivity, improves the feed conversion rate, and
507 enhances nutritional quality, which allows the use of diets with lower protein contents
508 without compromising shrimp survival (Xu and Pan, 2012; Xu et al., 2013; Correia et al.,
509 2014; Krummenauer et al., 2014; Suita et al., 2014, 2016). Furthermore, saturated and
510 unsaturated fatty acids in açaí act as important substrates for mitochondrial β-oxidation
511 processes, promoting a pro-oxidant condition related to the energy production directed to
512 shrimp growth, as observed when using lipoic acid as a supplement (Shi et al., 2018).

513 A hypothesis for the improvement in survival observed in all treatments using açaí is
514 not necessarily due to the composition and action of the bioactive compounds present in this

515 fruit on bioflocs and shrimp but also to a greater food supply in the culture medium because,
516 besides the ration, we also observed consumption of açaí by the post-larvae. The excess açaí
517 consumed directly by post-larvae and incorporated into the bioflocs contributed to the
518 microbial biomass increase. It served as a source of supplementary food in the culture, which
519 incremented the final weight and weight gain as observed in the 20.0.0 mg açaí L^{-1} treatment,
520 consequently improving the feed conversion values in this treatment. Khanjai et al. (2022)
521 found that post-larvae of banana shrimp *Fenneropenaeus merguiensis* fed with 25% wet
522 biofloc showed significantly higher total biomass and survival than the other treatments.
523 Better survival and feed conversion indices in *Litopenaeus vannamei* post-larvae cultivated
524 in a BFT system compared to a clear water system were verified in the studies by Suita et al.
525 (2014) and Tierney and Ray (2018), evidencing the importance of bioflocs as supplementary
526 food in the culture.
527

528 **5. Conclusion**

529 The application of 20.0 and 80.0 açaí mg L^{-1} in shrimp cultivation in the BFT system
530 supplemented the alkalinity levels, keeping them above 100 mg $CaCO_3$ L^{-1}, acting as a natural
531 alkalizing agent in the culture medium. Although some variations were observed in the
532 physical-chemical parameters, the mean values remained within the acceptable range for *L.*
533 *vannamei* rearing, indicating the possibility of adding up to 80 mg L-1 of lyophilized açaí
534 (*Euterpe oleracea*) in BFT systems with no adverse impact on water quality and shrimp
535 survival. However, the use of 20.0 mg L^{-1} of açaí presented a better cost-benefit ratio for
536 post-larvae cultivation in a biofloc system. The bioflocs, after enrichment with açaí, increased
537 their protein content and were able to incorporate the bioactive compounds present in açaí,

which probably acted as a vector in the transfer of these compounds via food, influencing the redox state and zootechnical parameters of shrimp. Açaí inclusion improved survival in all treatments, which is critical in the production of post-larvae in the nursery phase. Thus, the use of açaí proves to be an alternative to minimize oxidative stress during the *L. vannamei* culture, which can influence the quality of the post-larvae and their growth rate.

Author's Contributions

Grecica Mariana Colombo: Designed and performed the study, measured the biochemical variables, and drafted the paper.

Cleber dos Santos Simião: Performed the study and measured biochemical variables.

Juan Rafael Buitrago Ramírez: Performed the study and measured biochemical variables.

Alan Carvalho de Sousa: Performed the study and measured biochemical variables.

Robson Matheus Marreiro Gomes: Performed the study and measured biochemical variables.

Sonia Astrid Munõz Buitagro: Performed the study and measured biochemical variables.

Wilson Wasielesky Jr: Designed the study and drafted the paper.

José M. Monserrat: Designed the study, performed the statistical analysis, and drafted the paper.

Conflicts of Interest

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

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565

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826 **Table 1 –** Physico-chemical parameters in of water of a Biofloc Technology System (BFT) without shrimps measured during the seven-
827 day experimental period with the addition of açaí *Euterpe oleracea* (first experiment).

Parameters	Treatments						
	Control	**2.5 mg L^{-1}**	**5 mg L^{-1}**	**10 mg L^{-1}**	**20 mg L^{-1}**	**40 mg L^{-1}**	**80 mg L^{-1}**
Dissolved oxygen (mg L^{-1})	6.36 ± 0.04^{ab}	6.30 ± 0.05^{ab}	6.47 ± 0.05^{b}	6.26 ± 0.05^{a}	6.35 ± 0.05^{ab}	6.34 ± 0.06^{ab}	6.42 ± 0.09^{ab}
Temperature	26.17 ± 0.23^{a}	26.32 ± 0.29^{a}	26.68 ± 0.26^{a}	26.46 ± 0.29^{a}	26.65 ± 0.26^{a}	26.19 ± 0.27^{a}	26.23 ± 0.29^{a}
pH	8.13 ± 0.02^{ab}	8.17 ± 0.02^{b}	8.06 ± 0.02^{a}	8.10 ± 0.03^{ab}	8.10 ± 0.02^{ab}	8.12 ± 0.02^{ab}	8.13 ± 0.03^{ab}
Ammonia (mg TAN L^{-1})	0.12 ± 0.005^{ab}	0.13 ± 0.005^{b}	0.11 ± 0.005^{ab}	0.10 ± 0.004^{a}	0.12 ± 0.007^{ab}	0.10 ± 0.006^{a}	0.13 ± 0.008^{b}
Nitrite-N (mg L^{-1})	0.13 ± 0.04^{a}	0.16 ± 0.04^{a}	0.11 ± 0.03^{a}	0.10 ± 0.03^{a}	0.10 ± 0.02^{a}	0.14 ± 0.03^{a}	0.20 ± 0.03^{a}
Nitrate-N (mg L^{-1})	71.84 ± 2.14^{c}	70.42 ± 1.42^{c}	67.35 ± 2.24^{bc}	69.47 ± 1.40^{c}	66.06 ± 1.65^{bc}	62.46 ± 2.02^{b}	56.94 ± 3.23^{a}
Alkalinity (mg $CaCO_3$ L^{-1})	241.11 ± 2.61^{a}	238.89 ± 4.84^{a}	236.67 ± 5.77^{a}	237.78 ± 3.24^{a}	244.44 ± 3.38^{a}	264.44 ± 4.44^{b}	280.00 ± 7.99^{c}
Salinity	30.59 ± 0.43^{b}	30.88 ± 0.39^{b}	30.34 ± 0.45^{b}	30.81 ± 0.34^{b}	30.54 ± 0.46^{b}	29.5 ± 0.63^{a}	30.38 ± 0.55^{b}
TSS (mg L^{-1})	381.88 ± 25.86^{a}	376.25 ± 17.49^{a}	357.22 ± 15.9^{a}	422.22 ± 33.25^{a}	338.75 ± 22.03^{a}	360.56 ± 28.02^{a}	456.67 ± 41.34^{a}

828 Values expressed as mean and ±1 standard error (n=3). Different letters on the same line indicate significant differences between treatments according
829 to the Newman–Keuls test at the significance level at 0.05.

Table 2 – Physical-chemical parameters of water during the culture of post-larvae of shrimp *L. vannamei* and proximal composition of the biofloc in second experiment.

Parameters	Treatments			
	Control	**5 mg L^{-1}**	**20 mg L^{-1}**	**80 mg L^{-1}**
Dissolved oxygen (mg L^{-1})	6.12 ± 0.06[b]	6.09 ± 0.06[b]	6.05 ± 0.05[ab]	5.97 ± 0.05[a]
Temperature	29.18 ± 0.04[a]	29.21 ± 0.04[a]	29.20 ± 0.05[a]	29.29 ± 0.08[a]
pH	8.00 ± 0.02[a]	7.98 ± 0.02[a]	8.05 ± 0.01[b]	8.08 ± 0.02[b]
Ammonia (mg TAN L^{-1})	0.25 ± 0.02[a]	0.28 ± 0.03[a]	0.29 ± 0.03[a]	0.73 ± 0.06[b]
Nitrite-N (mg L^{-1})	3.02 ± 0.31[a]	2.05 ± 0.18[a]	4.72 ± 0.32[a]	2.32 ± 0.17[a]
Nitrate-N (mg L^{-1})	37.13 ± 5.54[a]	38.91 ± 3.12[a]	20.80 ± 3.54[a]	19.88 ± 3.32[a]
Phosphorus-P (mg L^{-1})	2.67 ± 0.55[a]	2.39 ± 0.37[a]	1.89 ± 0.53[a]	1.47 ± 0.28[a]
Alkalinity (mg $CaCO_3$ L^{-1})[a]	123.33 ± 6.22[a]	126.09 ± 7.08[a]	146.09 ± 4.98[b]	205.45 ± 7.35[c]
Salinity	28.92 ± 0.72[a]	28.31 ± 0.71[a]	27.04 ± 0.74[a]	27.00 ± 0.90[a]
TSS (mg L^{-1})	277.05 ± 31.84[a]	321.52 ± 29.31[a]	300.87 ± 22.57[a]	535.68 ± 31.54[b]
Proximal composition				
Dry matter (%)	14.41 ± 0.45[a]	14.80 ± 0.22[a]	15.91 ± 0.57[a]	13.05 ± 0.76[a]
Protein (%)	23.77 ± 0.73[a]	22.91 ± 0.63[a]	21.85 ± 0.45[a]	29.43 ± 1.08[b]
Ether extract (%)	2.35 ± 0.47[a]	1.66 ± 0.42[a]	2.12 ± 0.50[a]	4.83 ± 1.06[a]
Ash (%)	47.40 ± 0.60[b]	47.19 ± 0.21[b]	39.68 ± 0.41[a]	31.14 ± 2.29[a]
NFE[b] (%)	12.07 ± 0.80[a]	13.44 ± 0.56[a]	20.45 ± 0.59[b]	22.36 ± 0.97[b]

Values expressed as mean and ±1 standard (n=3). Different letters on the same line indicate significant differences between treatments according to the Newman–Keuls test at the significance level at 0.05. [a]Amount of sodium bicarbonate ($NaHCO_3$) added in each treatment for alkalinity correction:

834 Control - 9.3 g; 5 mg açaí L^{-1} – 12.0 g; 20 mg açaí L^{-1} – 0.0 g; 80 mg açaí L^{-1} – 0.0 g. [b]Calculated value: NFE =100 - (Dry matter + Protein + Ether
835 extract + Ash). All analyses were performed with dry matter from the bioflocs.
836

837 **Table 3 –** Zootechnical parameters of *Litopenaeus vannamei* shrimp post-larvae (stocking density of 600 shrimp per tank) reared in
838 Biofloc Technology System (BFT) with different concentrations of açaí *Euterpe oleracea* added every day during 27 days.

Parameters	Treatments			
	Control	**5 mg L^{-1}**	**20 mg L^{-1}**	**80 mg L^{-1}**
Initial weight (g)	0.013 ± 0.001^{a}	0.013 ± 0.001^{a}	0.013 ± 0.001^{a}	0.013 ± 0.001^{a}
Final weight (g)	0.065 ± 0.004^{a}	0.072 ± 0.004^{a}	0.087 ± 0.005^{b}	0.072 ± 0.004^{a}
Initial length (cm)	1.354 ± 0.039^{a}	1.354 ± 0.039^{a}	1.354 ± 0.039^{a}	1.354 ± 0.039^{a}
Final length (cm)	2.261 ± 0.052^{a}	2.289 ± 0.049^{a}	2.424 ± 0.048^{a}	2.216 ± 0.040^{a}
Weight gain (g)	0.053 ± 0.004^{a}	0.059 ± 0.004^{ab}	0.074 ± 0.005^{b}	0.059 ± 0.004^{ab}
FCR[a]	1.16 ± 0.08^{b}	1.04 ± 0.06^{ab}	0.82 ± 0.04^{a}	1.04 ± 0.07^{ab}
Length gain (cm)	0.95 ± 0.05^{a}	0.94 ± 0.05^{a}	1.05 ± 0.04^{a}	0.89 ± 0.04^{a}
Final biomass (g)	34.12 ± 1.17^{ab}	35.89 ± 2.56^{ab}	39.90 ± 1.18^{b}	26.51 ± 3.77^{a}
Survival (%)	65.94 ± 1.83^{a}	81.89 ± 0.28^{b}	81.22 ± 2.56^{b}	78.89 ± 3.24^{b}

839 Values expressed as mean and ±1 standard error (n = 40 in initial weight and length; n = 30 in final weight and length). Different letters on the same
840 line indicate significant differences between treatments according to the Newman–Keuls test at the significance level at 0.05. [a]Feed conversion ratio.

841 **Figures Captions**

842

843 **Figure 1**. Concentration of total flavonoids (µg of quercetin/g of bioflocs) (A) and
844 polyphenols (µg of quercetin/g of bioflocs) (B) in bioflocs from a BFT system without
845 shrimps after addition of different concentration of lyophilized açaí for seven days. Values
846 expressed as mean ± 1 standard error (n = 9). Different letters indicate statistical differences
847 between the concentrations of lyophilized açaí according to the Newman–Keuls test at
848 significance level at 0.05.

849

850 **Figure 2**. Values of total antioxidant capacity against peroxyl radicals (expressed in relative
851 area) (A) and content of substances reactive to thiobarbituric acid (nmol of TMP/mg per
852 bioflocs) (B) in bioflocs from a BFT system without shrimps after addition of different
853 concentration of lyophilized açaí for seven days. Values expressed as mean ± 1 standard error
854 (n = 12 in A; n = 9 in B). Different letters indicate statistical differences between treatments
855 according to the Newman–Keuls test at significance level at 0.05. TMP stands for
856 tetramethoxypropane, used as standard.

857

858 **Figure 3**. Concentration of reduced glutathione (µmol/mg protein) (A) and concentration of
859 protein sulfhydryl groups (µmol/mg protein) (B) in bioflocs from a BFT system without
860 shrimps after addition of different concentration of lyophilized açaí for seven days. Values
861 expressed as mean ± 1 standard error (n = 12). Different letters indicate statistical differences
862 between treatments according to the Newman–Keuls test at significance level at 0.05.

863

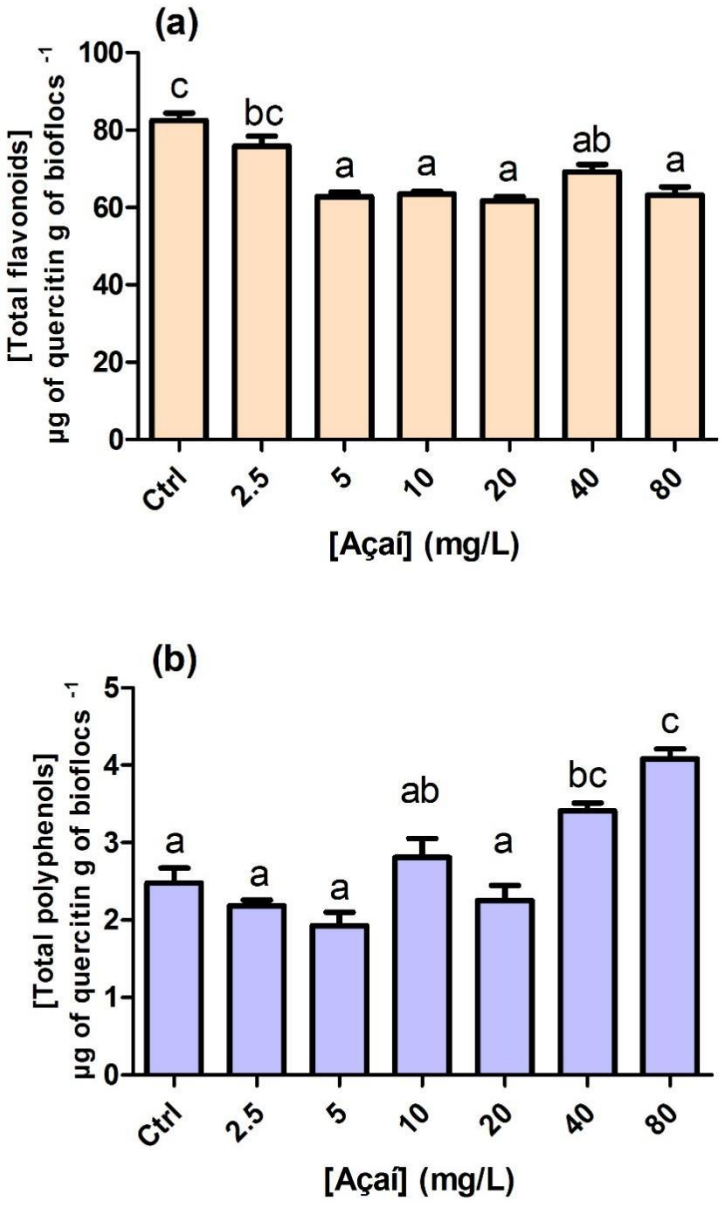
864 **Figure 4**. Values of total antioxidant capacity against peroxyl radicals (expressed in relative
865 area) (A) and content of substances reactive to thiobarbituric acid (nmol of TMP/mg per
866 tissue) (B) in bioflocs and of L. vannamei shrimps post-larvae after addition of different
867 concentration of lyophilized açaí for 27 days. Values expressed as mean ± 1 standard error (n
868 = 9 in bioflocs; n = 12 in shrimp). Different letters indicate statistical differences between
869 treatments according to the Newman–Keuls test at significance level at 0.05. TMP stands for
870 tetramethoxypropane, used as standard.

871

872 **Figure 5**. Concentration of reduced glutathione (μmol/mg protein) (A) and concentration of
873 protein sulfhydryl groups (μmol/mg protein) (B) in bioflocs and of L. vannamei shrimps post-
874 larvae after addition of different concentration of lyophilized açaí for 27 days. Values
875 expressed as mean and ± 1 standard error (n = 9 in bioflocs; n = 12 in shrimp). Different
876 letters indicate statistical differences between treatments according to the Newman–Keuls
877 test at significance level at 0.05.
878
879

880 **Figure 1**

881



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884 **Figure 2**

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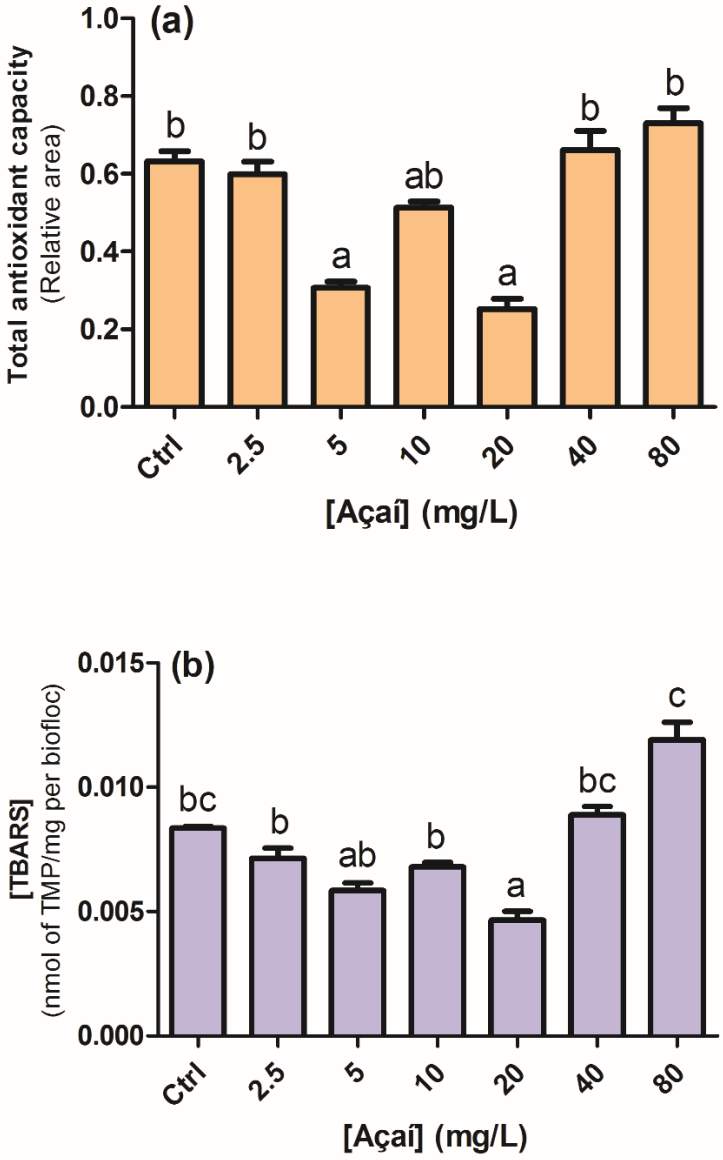

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888 **Figure 3**

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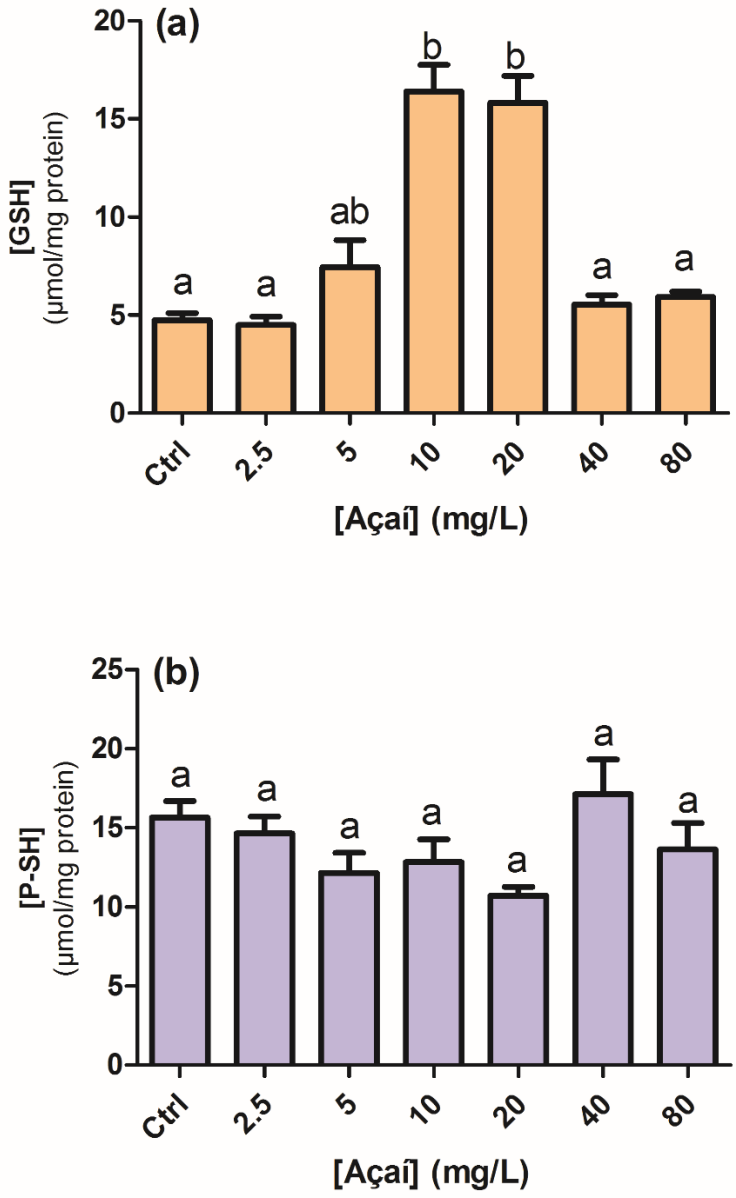

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892 **Figure 4**

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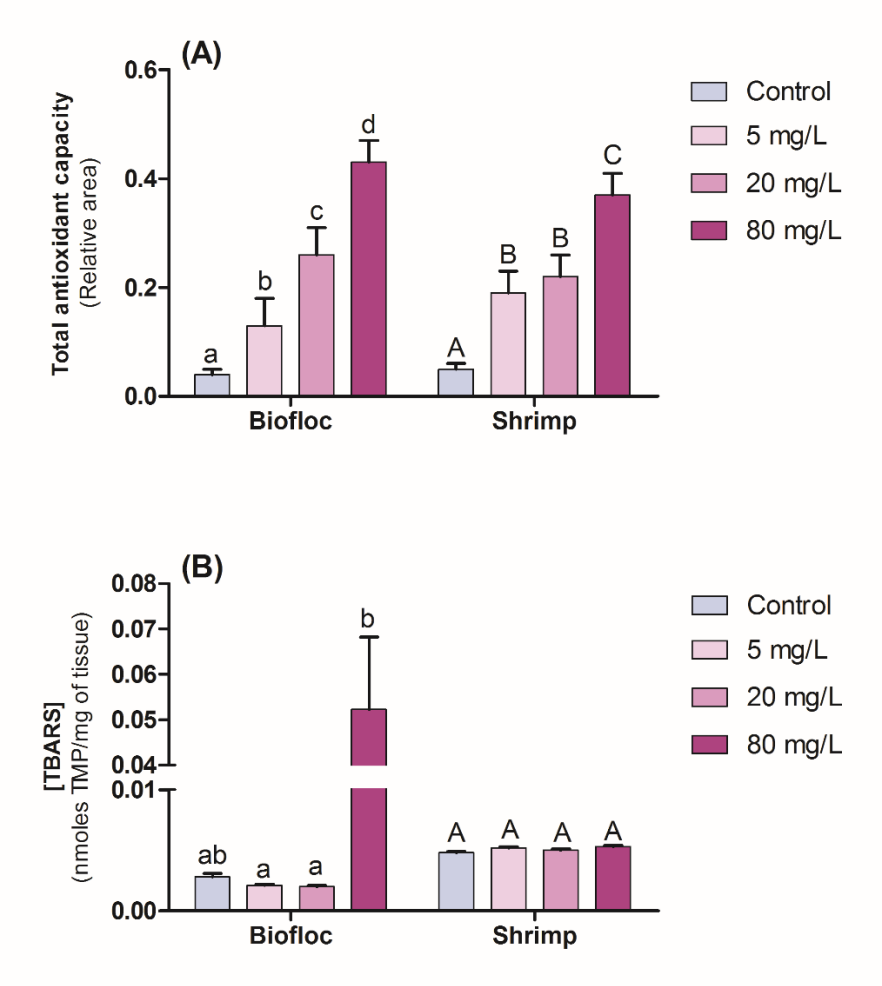

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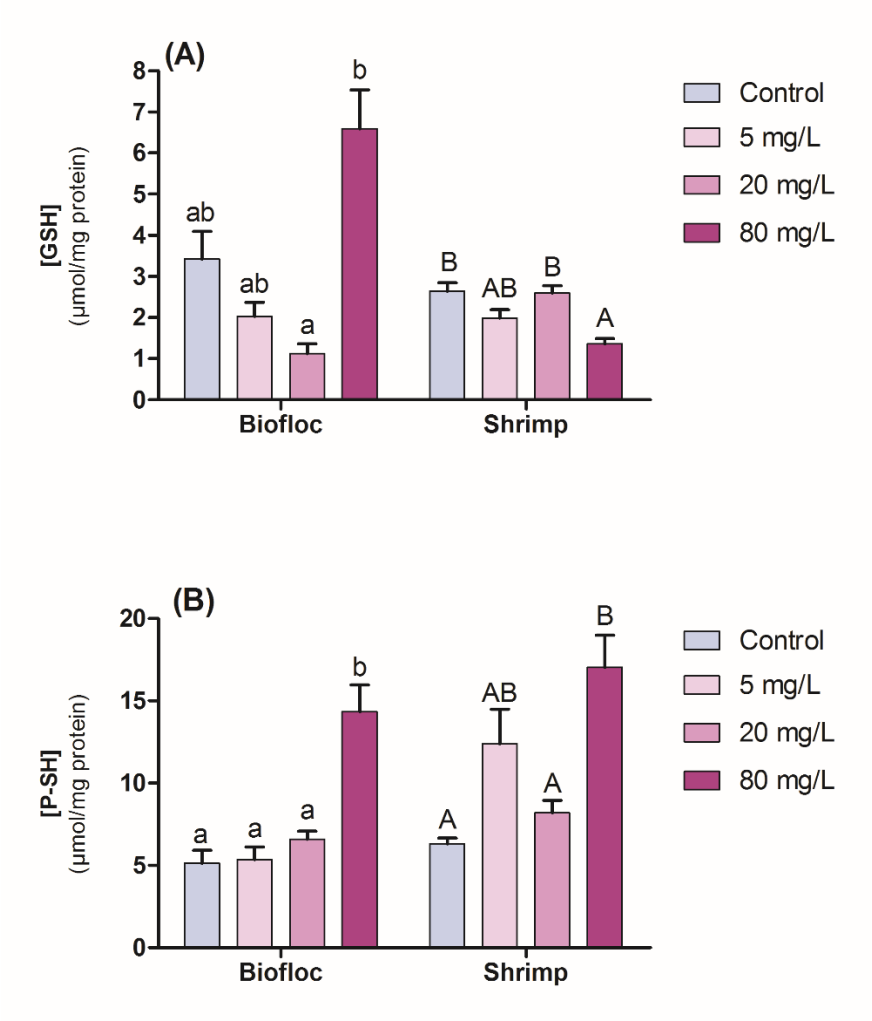
Figure 5


(A)
[GSH]
(µmol/mg protein)
8
7
6
5
4
3
2
1
0
ab
ab
a
b
B
AB
B
A
Biofloc
Shrimp
Control
5 mg/L
20 mg/L
80 mg/L
(B)
[P-SH]
(µmol/mg protein)
20
15
10
5
0
a
a
a
b
A
AB
A
B
Biofloc
Shrimp
Control
5 mg/L
20 mg/L
80 mg/L

CAPÍTULO 2

Effects of lyophilized açaí (*Euterpe oleracea*) supplementation on oxidative damage and intestinal histology in juvenile shrimp *Penaeus vannamei* reared in biofloc systems

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925 Effects of lyophilized açaí (*Euterpe oleracea*) supplementation on oxidative damage and
926 intestinal histology in juvenile shrimp *Penaeus vannamei* reared in biofloc systems

927

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Abstract

The objective of the present study was to evaluate the ability of bioflocs to assimilate and transfer antioxidant compounds present in açaí *Euterpe oleracea* to juvenile *Penaeus vannamei* shrimps grown in a biofloc system. Juvenile shrimp were distributed into four treatments (control, 5, 20, and 80 mg açaí L^{-1}), containing 31 shrimp/tank (90 L), and cultivated for 30 days. Every 24 h throughout the experimental period, the respective açaí concentrations were added directly to the cultivation water. The bioflocs and hepatopancreas lost their antioxidant capacity with increasing concentrations of açaí, however, lipid damage was mitigated after treatment with 20 mg of açaí L^{-1} ($p < 0.05$). The application of 20 mg açaí L^{-1} increased the mean values of the height and area of the anterior intestinal microvilli ($p < 0.05$). Mortality and protein and lipid damage in shrimp muscle increased with daily administration of 80 mg açaí L^{-1} ($p < 0.05$). It is concluded that the bioflocs were able to assimilate the antioxidants present in the açaí and transfer them to the shrimp, the administration of 20 mg açaí L^{-1} presented the best performance demonstrating the possibility of its application in the cultivation of *P. vannamei* in a biofloc system.

Key words: antioxidant, bioactive compounds, polyphenols

1. Introduction

Açaí (*Euterpe oleracea*) is a palm tree endemic to the Amazon region, in the states of Pará and Amazonas, where the world's largest production of açaí is concentrated, with a culture corresponding to 87.5% of the Brazilian national market (CONAB 2020; Menezes et al., 2008). Among the different types of açaí processing, the most consumed is the fruit, which is the main raw material for the production of juices and pulps (CONAB 2022; Lucas et al., 2018). Owing to its nutritional composition, açaí has gained notoriety in financial and consumer markets, becoming the object of different studies because of its high content of proteins, lipids, fibers, and bioactive compounds with high antioxidant capacity (Odendaal and Schauss, 2014; Martino et al., 2016; Schauss, 2016;).

The antioxidant activity observed in the fruit is directly related to the phenolic compounds, mainly from the flavonoid group. Among these, anthocyanins, phenolic acids, flavonols, and carotenoids are the most common, with anthocyanins being the main pigment responsible for the purple color of açaí and its significant antioxidant capacity (Schauss, 2016). These compounds are associated with nutritional, anti-inflammatory, antimicrobial, anticancer, cardiovascular, neurological, and antioxidant effects in humans and animals (da Costa et al., 2012; Schauss, 2016; Aranha et al., 2019; Colombo et al., 2020).

The production of aquatic organisms is subject to variations in different environmental factors common to cultivation, which can generate stress conditions in animals and contribute to the production of reactive oxygen species (ROS) and reduction of antioxidant activity (Monserrat et al., 2008; Martins et al., 2014; da Silva et al., 2021; Brol et al., 2021). Although animals possess endogenous antioxidant defenses to cope with oxidative stress, a considerable amount of metabolic energy is required to improve or prevent this imbalance (Sokolova et al., 2012). In this context, supplementation or inclusion of food additives with

high antioxidant capacity in the diet of fish and shrimp serves as a strategy to increase the tolerance of these organisms to stressful conditions (da Silva et al., 2021; Brol et al., 2021; de Moura et al., 2022; dos Santos Simião et al., 2022).

Although scarce, studies on the application of açaí in shrimp diets have shown promising results in relation to chemoprevention. The inclusion of 10% (w/w) açaí in the diet of *P. vannamei* shrimp cultivated in clear water showed a chemoprotective effect against ammonia exposure, resulting in an improvement in the antioxidant defense system and attenuation of histopathological damage in the hepatopancreas (Colombo et al., 2020), in addition to conferring protection against lipid damage in this same organ in shrimp challenged with saxitoxin (Ramos et al., 2022).

In contrast to conventional production systems, the biofloc technology system (BFT) is characterized by minimal or no water exchange, reduced effluent release, high productivity, and biosafety (Ebeling et al., 2006; Krummenauer et al., 2014). Bioflocs have 18–43% protein in their composition and are responsible for contributing up to 29% of shrimp feed, collaborating to reduce artificial feeding rates and improve feed conversion rates (Wasielesky et al., 2006; Khanjani and Sharifinia, 2020). Because they have proteins, fatty acids, minerals, vitamins, digestive enzymes and antioxidant molecules, bioflocs not only contribute as supplementary food for the growth of farmed shrimp, but are also natural and abundant sources of bioactive compounds that contribute to the physiological health of the crustaceans (Xu and Pan, 2012; da Silva Martins et al. 2015; León et al., 2018; Khanjani and Sharifnia, 2020).

Recently, studies on the application of lyophilized açaí in biofloc systems have been conducted. In a study by Silva et al. (2020) açaí was included directly in the diet composition of juveniles of *P. vannamei*, with four concentrations tested (control, 2.5%, 5.0%, and 10.0%

w/w). Although there were no effects on growth, the accumulation of flavonoids in the bioflocs and gills of the 5.0% açaí treatment group demonstrated the ability of the bioflocs to absorb the antioxidants present in this fruit. Balancing and elaborating a diet that includes supplements require considerable effort. Therefore, the direct addition of açaí to cultivation appears to be more practical, as reported by Colombo et al. (2023). The authors tested the daily addition of four concentrations of lyophilized açaí (control, 5, 20, and 80 mg of açaí L^{-1}) to the culture of *P. vannamei* post-larvae. In addition to the improvement in the antioxidant status of the bioflocs, shrimp survival significantly increased in all treatments that received açaí.

Therefore, due to the high antioxidant content and protection conferred by açaí to crustaceans, and based on the same principle as Colombo et al. (2023) with post larvae, the present study aimed to continue the application of lyophilized açaí in a biofloc system to rearing juveniles of *P. vannamei*. Through biochemical and histological analyses and zootechnical parameters, we sought to evaluate the ability of shrimp to absorb the antioxidants present in açaí, as well as the role of bioflocs in the assimilation and transfer of such bioactive compounds via food.

2. Material and methods

2.1 Shrimp maintenance and experimental design

Penaeus vannamei shrimps were obtained from a shrimp farming system at the Marine Aquaculture Station (EMA) of the Federal University of Rio Grande - FURG, located at Cassino Beach, Rio Grande, RS, Brazil. Lyophilized açaí (*Euterpe oleracea*) used in the

1043 present study was obtained from Amazônia Comércio de Açaí Liofilizado e Exportação
1044 LTDA, Belém, PA, Brazil.

1045 The biofloc inoculum used in the experiment was collected from a super-intensive
1046 cultivation system of Pacific White Shrimp *P. vannamei* and distributed in four tanks (90
1047 liters of usable volume) without the presence of shrimp (pH 7.94, total ammonia 0.21 mg/L,
1048 nitrite 0.07 mg/L, nitrate 80.00 mg/L, phosphorus 4.30 mg/L, alkalinity 137.50 mg/L, SST
1049 310 mg/L and salinity of 30 ppt). For seven days the bioflocs were enriched with three
1050 concentrations of açaí, beyond the control treatment, added directly to the water in each tank
1051 every 24 h. These açaí concentrations were previously tested by Colombo et al. (2023) for
1052 the cultivation of *P. vannamei* post-larvae in a biofloc system.

1053 Juvenile shrimp with medium initial weight of 0.96 to 1.10 g were stored in 12 circular
1054 polyethylene tanks with a useful volume of 90 liters each, disposed in four treatments
1055 (control, 5, 20 and 80 mg of açaí L^{-1}) in triplicate, containing 31 shrimp per tank. In each
1056 experimental unit, 67.5 liters of seawater treated with a chlorine solution (10 ppm) and
1057 dechlorinated with ascorbic acid (1:1 w/w) and 22.5 liters of biofloc inoculum previously
1058 enriched with açaí were used. The inoculum volume in each tank corresponded to 25% of the
1059 useful volume of the experimental units according to the methodology of Krummenauer et
1060 al. (2014). The experiment lasted 30 days and every 24 hours, during the experimental period,
1061 the respective concentrations of lyophilized açaí were added directly to the cultivation water.
1062 Shrimps were fed twice a day with commercial feed GUABI® 35% PB, according to the
1063 protocol described by Jory (2001). Weekly biometrics (n = 12) were performed to adjust the
1064 ration provided. Aeration used during the enrichment of the inoculum with açaí and in the
1065 cultivation of shrimp was provided through aerotubes. The study was carried out in the

laboratory with a photoperiod fixed at 12 h L/12 h D, temperature, and mean salinity of 29.8 °C and 26.7, respectively.

Daily dissolved oxygen and temperature were measured using a multiparameter digital oximeter (YSI®-550A), and pH was measured using a digital pH meter (Alfakit, AT 315 SP). Total ammonia (TAN) ($NH_3 + NH_4^+$), nitrite ($N\text{-}NO_2^-$), and nitrate ($N\text{-}NO_3^-$) were monitored following the protocols of UNESCO (1983), and alkalinity (APHA, 1998), salinity (Alfakit refractometer), and total suspended solids (TSS) (Gaona et al., 2011) were measured weekly during the study. For ammonia levels above 1 mg L^{-1}, cane molasses was added to the experimental units as a carbon source in a proportion of 6 g of carbon for each 1 g of total ammoniacal nitrogen in the water, with the objective of adjusting the C:N ratio of the system (Ebeling et al., 2006; Avnimelec, 2015). When water alkalinity values were below 100 mg $CaCO_3$ L^{-1}, corrections with sodium bicarbonate ($NaHCO_3$) were performed to increase alkalinity to 200 mg $CaCO_3$ L^{-1} (Furtado et al., 2011). Treatments in which TSS levels exceeded 500 mg/L were used to remove excess total suspended solids, according to the methodology described by Gaona et al. (2011).

2.2 Zootechnical performance and proximal analysis of shrimp bioflocs and muscle

At the start and end of the experiment, individual shrimp weighing was performed to estimate the following zootechnical parameters:

1. Weight gain (g) = final weight – initial weight
2. Specific growth ratio = [100% × (Ln final weight – Ln initial weight) / trial duration]
3. Feed conversion ratio = dry feed intake / weight gain
4. Protein efficiency ratio = weight gain / dry protein intake

5. Survival (%) = (final number of shrimps / initial number of shrimps) × 100.

Dry matter analysis was performed by drying the bioflocs and muscle samples in an oven at 102 ºC for 5 h. For the ash analysis, the samples were pre-calcined and then placed in a muffle furnace at 600 °C for 5 h (AOAC, 1999). The crude protein content was determined after sample digestion and nitrogen distillation according to Kjeldahl's protocol (AOAC, 1995). The lipid content was evaluated using the cold extraction method (Bligh and Dyer, 1959).

2.3 Biochemical analysis

2.3.1 Homogenization of the samples

At the end of the experiment, to collect the bioflocs, water from each experimental unit was collected and added to the Imhoff cones, which were allowed to settle for 20 min. The supernatant was discarded, and the sedimentable solids were centrifuged at 1.500 x g for 15 min at 10 °C to remove excess water and then stored in an ultrafreezer at -80 °C (Leon et al., 2018). The homogenate of the bioflocs was obtained by sonicating the samples (QSonica Sonicators) for 1 min in 30 ppt artificial salt water (Salt Veromix, 95% and 1:1 w/v), stirring in an orbital shaker at 80 rpm for 3 h, centrifugation at 10.000 x g for 5 min at 4 °C (Colombo et al., 2023). The shrimps were weighed individually, euthanized in liquid nitrogen and the organs (gills, hepatopancreas and muscle) were collected and homogenized (1:5 w/v) in a buffer solution for crustaceans (pH 7.2; Tris-base 20 mM; EDTA 1 mM; 0.05 mM $MgCl_2$; 5 mM sucrose; and 1 mM KCl). The samples were centrifuged at 20.000 x g for 30 min at 4°C, and the supernatants were stored at -80°C for biochemical analyses. The total protein content

of the biofloc and shrimp samples was determined by the biuret method (550 nm) using a microplate reader (Biotek Synergy HT) (Amado et al., 2009).

2.3.2 Total antioxidant capacity against peroxyl radicals (ACAP)

Analysis of total antioxidant capacity against peroxyl radicals (ACAP) was performed as described by Amado et al. (2009). Samples of bioflocs and shrimp organs were exposed to peroxyl radicals generated by the thermal decomposition of ABAP (2, 2-azobis-2-methylpropionamidine dihydrochloride) (Sigma Aldrich) at 37 °C. When reacting with 2',7'-dichlorofluorescein diacetate (H_2DCF), peroxyl radicals formed a fluorescent compound (DCF), which was detected using a spectrofluorometer (Biotek Synergy HT) (excitation:485 nm; emission:535 nm). ACAP was quantified using the relative area with and without ABAP, where a low relative area indicated a higher antioxidant capacity.

2.3.3 Lipid peroxidation (TBARS)

Lipid peroxidation was measured using thiobarbituric acid reactive substances (TBARS) as described by Oakes and Van Der (2003). The homogenates (bioflocs: 50 µl; gills: 50 µl; hepatopancreas: 30 µl; muscle: 100 µl) were added in glass tubes, along with 20 µl of BHT solution (butylated hydroxytoluene, 67 µM), 150 µl of solution of acetic acid 20%, 150 µl of TBA solution 0.8% (thiobarbituric acid), 50 µl of distilled water, 20 µl of SDS 8.1% (sodium dodecyl sulfate), and subsequently heated for 30 min in 95 °C. After cooling, 100 µl distilled water and 500 µl n-butanol were added to the final solution and centrifuged at 3.000 × g for 10 min at 15 °C. The supernatant was transferred to a microplate, and readings (excitation: 520 nm; emission: 580 nm) were performed using a microplate spectrofluorometer (Biotek Synergy HT). Tetramethoxypropane (TMP, Across Organics)

1134 was used as a standard, and the results were expressed in TMP equivalents per mg of wet
1135 tissue.

1136 *2.3.4 Reduced glutathione (GSH)*

1137 To measure the concentration of reduced glutathione (GSH), 240 µl of extract of
1138 bioflocs, gills, hepatopancreas, muscle and 28 µl of trichloroacetic acid (TCA 50% w/v) were
1139 added to Eppendorf tubes. After centrifugation at 20.000 x g at 4°C for 10 min, 100 µl of the
1140 supernatant was added to a transparent microplate, together with 200 µl Tris-Base 0.4 M at
1141 pH 8.9, and 10 µl of DTNB (5.5 '- dithiobis (2-nitrobenzoic acid)) (Sigma Aldrich), and
1142 incubated in the dark at room temperature for 15 min (Sedlak and Lindsay, 1968).
1143 Absorbance readings (405 nm) were measured using a spectrofluorometer (Biotek Synergy
1144 HT) and the concentration was expressed as µmol of GSH per mg of protein.

1145 *2.3.5 Protein-associated sulfhydryl groups (P-SH)*

1146 The concentration of sulfhydryl groups associated with the protein (P-SH) also
1147 followed the protocol described by Sedlak and Lindsay (1968). The protein precipitate from
1148 the bioflocs and shrimp organ samples formed after GSH centrifugation was resuspended in
1149 240 µl of artificial saline water and homogenization buffer for crustaceans, respectively, and
1150 100 µl of the extract, 160 µl of Tris-Base 0.2 M) at pH 8.2, and 10 µl of DTNB were added
1151 to a transparent microplate. The microplate was incubated in the dark at room temperature
1152 for 15 min, and absorbance readings (405 nm) were measured using a spectrofluorometer
1153 (Biotek Synergy HT). The final concentration was expressed as µmol of P-SH per mg of
1154 protein.

1155 *2.4 Histological analysis*

Three shrimps were collected per treatment and anesthetized with ice to collect the intestine, which was fixed in 10% formalin (NBF) for 24 h and later transferred to 70% alcohol. Samples of the anterior and middle intestines were processed in LEICA TP1020 and included in the Paraplast. Intestines were sectioned into cross-sections at a thickness of 5 µm using a LEICA RM2245 rotary microtome and stained with hematoxylin-eosin. Histological slides were viewed under a Zeiss Primo Star microscope. The height, width, and area of intestinal microvilli were measured. Histological analysis was performed according to the protocol of Bullerwell et al. (2016), in which four photos were taken per intestine (middle and posterior) of the shrimp, totaling 12 photos per treatment of each intestine. In each photograph, three microvilli were randomly selected to remove the established parameters. All measurements were performed using the ImageJ Software 1.53.

2.5 Statistical analysis

The homogeneity of the variances of the different treatments was evaluated using the Levene test, and the normality of the data distribution was evaluated using the Shapiro-Wilk test. After verifying the assumptions, all data were subjected to a mixed model analysis of variance (ANOVA), with the concentration of lyophilized açaí added to the cultivation of *L. vannamei* in the BFT system as the fixed factor and the experimental units of each treatment as the random factor (Searle et al., 2006). Possible significant differences between treatments were detected using the Newman-Keuls test, and the significance level was set at 0.05.

3. Results

The results referring to the water quality parameters are presented in Table 1. During the experimental period, the physicochemical parameters of the water, such as dissolved

oxygen, temperature, nitrite, and phosphorus, did not show statistical differences between the treatments ($p > 0.05$). The tanks that received 20 and 80 mg açaí L^{-1} showed an increase in the mean pH values in relation to the control treatments and 5 mg açaí L^{-1} ($p < 0.05$). The ammonia concentration increased in the 80 mg açaí L^{-1} treatment compared to that in the other treatments ($p < 0.05$); however, the nitrate concentration decreased significantly in the same treatment ($p < 0.05$). An increase in alkalinity was observed at concentrations above 20 mg açaí L^{-1} in relation to the control treatment and 5 mg açaí L^{-1} ($p < 0.05$). The mean values of total suspended solids increased proportionally with the concentration of lyophilized açaí added to the BFT system ($p < 0.05$).

The results of the proximal analysis of the bioflocs and shrimp muscles are presented in Table 2. The ash content of the bioflocs decreased proportionally with an increase in açaí concentration ($p < 0.05$). For shrimp muscle, no changes were observed in the content of dry matter, lipids and ash ($p > 0.05$), but the protein content of the 5 mg açaí L^{-1} treatment was higher than that of 20 and 80 mg açaí L^{-1} ($p < 0.05$). Regarding the zootechnical parameters, only survival rates showed a statistically significant difference (Table 3). Daily administration of 80 mg açaí L^{-1} resulted in higher shrimp mortality than the other treatments ($p < 0.05$).

The total antioxidant capacity (ACAP) of the bioflocs and hepatopancreas was lower (larger relative area) in treatments with 20 and 80 mg açaí L^{-1} and 5 and 20 mg açaí L^{-1}, respectively, compared to the control group ($p < 0.05$) (Figure 1A and 1C). No statistically significant differences were observed in the ACAP of the gills and muscles ($p > 0.05$) (Figure 1B and 1D). In relation to lipid peroxidation, bioflocs, gills, and hepatopancreas showed a reduction in lipid damage (Figure 2). In bioflocs and gills, a decrease in lipid damage was observed at 20 and 80 mg of açaí L^{-1} compared to the other treatments ($p < 0.05$). In the

hepatopancreas, a gradual decrease in lipid peroxidation was observed with increasing concentrations of lyophilized açaí ($p < 0.05$). There was an increase in lipid damage in the muscle in the treatment with 80 mg açaí L^{-1} when compared to the control treatments and 20 mg açaí L^{-1} ($p < 0.05$).

The GSH levels of the bioflocs increased significantly in the treatments with 5 and 80 mg açaí L^{-1}, which differed from the control group and 20 mg açaí L^{-1} ($p < 0.05$) (Figure 3A). In the gills, GSH levels differed only between treatments that received açaí ($p < 0.05$); however, when compared with the control treatment there were no significant differences (Figure 3B). No significant differences were observed in the hepatopancreas or muscle ($p > 0.05$) (Figure 3C and 3D). The P-SH levels of the bioflocs increased at a concentration of 80 mg açaí L^{-1} ($p < 0.05$); however, in the muscle tissue at the same concentration, there was a decrease in the mean values of P-SH compared to the control treatment ($p < 0.05$) (Figure 4A and 4D). No statistical differences were observed in the gill and hepatopancreas ($p > 0.05$) (Figures 4B and 4C).

The results of the histological analysis are presented in Table 4, Figure 5 and 6. When evaluating the data from the middle intestine of the shrimp, the addition of 5 and 20 mg açaí L^{-1} contributed to an increase in the mean values of the height and area of the intestinal microvilli ($p < 0.05$). However, in the posterior intestine, the application of 20 and 80 mg açaí L^{-1} in the BFT system decreased the mean size of the height and area of the microvilli compared to the control treatment ($p < 0.05$).

4. Discussion

1224 The water quality results are presented in Table 1. Although the daily addition of
1225 lyophilized açaí to the BFT system increased the TSS levels, the mean values of total
1226 suspended solids remained below 500 mg/L, as recommended by Gaona et al. (2011). The
1227 increase in açaí concentration from the 20 mg açaí L^{-1} treatment resulted in an increase in the
1228 pH and alkalinity of the water. Corrections with sodium bicarbonate ($NaHCO_3$) to maintain
1229 alkalinity levels during the experiment were only necessary in the control, 5 and 20 mg of
1230 açaí L^{-1} treatments, corroborating the recent study by Colombo et al. (2023), where
1231 corrections were performed only in the control and 5 mg of açaí L^{-1} treatments during the
1232 cultivation of post-larvae of *P. vannamei* in a BFT system. Açaí, as well as other fruits and
1233 vegetables, has antioxidant and alkalizing potential, intensifying according to the lower
1234 degree of food processing (Schwalfenberg, 2012; Fardet and Richonnet, 2020). Therefore,
1235 the inclusion of 80 mg açaí L^{-1} in the water for the cultivation of *P. vannamei* juveniles in the
1236 BFT system maintained alkalinity levels during the experimental period.
1237 The action of nitrifying bacteria in the BFT system is of fundamental importance for
1238 the success of their activity because they efficiently cycle nitrogenous compounds generated
1239 during the cultivation (Ebeling et al., 2006). The results of the present study demonstrated
1240 that the daily addition of 80 mg açaí L^{-1} to the cultivation of *P. vannamei* in a BFT system
1241 increased ammonia levels and decreased nitrate concentrations (Table 1), corroborating the
1242 results observed by Colombo et al. (2023). Considering the protein content of açaí – 8.1% to
1243 10.5% (Odendal and Schauss, 2014; Silva et al., 2020) - ally to the ration provided, it is
1244 possible that the ingestion of both by shrimp in the 80 mg açaí L^{-1} treatment contributed to
1245 an increase in ammonia excretion (Rosas et al., 2001; Zhao et al., 2020; Sui et al., 2023).
1246 Another hypothesis is the possible inhibition of the action of nitrifying bacteria due to the
1247 action of antioxidants present in the fruit. Studies have shown that the application of plant

extracts with antioxidant capacity in agricultural soils can produce a toxic effect on nitrifying bacteria of the genus *Nitrosomonas* spp. by blocking the ammonia monooxygenase pathway, an enzyme that acts in the first stage of the conversion of ammonia to nitrite (Erickson et al., 2000; Cesco et al., 2012; Tang et al., 2021). In aquaculture, this reflects higher levels of ammonia in the culture water and, consequently, lower concentrations of nitrite and nitrate. However, the results of the present study and those of Colombo et al. (2023) only identified changes in the ammonia and nitrate concentrations. The presence of AOB (ammonia-oxidizing bacteria) and NOB (nitrite-oxidizing bacteria) nitrifying bacteria in the biofloc inoculum used in the experiment may have attenuated the possible inhibition of the ammonia monooxygenase pathway because ammonia was already converted to nitrate in the system. The addition of antioxidants during bioflocs formation should be explored in future studies.

The ash levels in the bioflocs of the control treatments up to 20 mg açaí L^{-1} ranged from 45.61% to 40.47%, corroborating the results of Colombo et al. (2023), who verified levels from 47.40% to 39.68% in the same treatments (Table 2). Similar values have been reported by Wasielesky et al. (2006), Fernandes Da Silva et al. (2008), and Reis et al. (2019) that reported 44.85%, 45.50%, and 53.68% of ash in bioflocs from the cultivation of *P. vannamei* juveniles, respectively. According to studies by Silva et al. (2020) and Odendaal and Schauss (2014) the pulp of lyophilized açaí has ash content around 3.03% to 3.80%. In this context, the gradual decrease in the ash content in the bioflocs from the 20 mg açaí L^{-1} treatment was due to the accumulation of lyophilized açaí in the BFT system, which was incorporated into the biomass of the bioflocs.

Owing to their high protein content, bioflocs consumed by shrimps can replace a fraction of the required protein demand, complementing the artificial feed provided (Wasielesky et al., 2006; Suita et al., 2014; Yun et al., 2016). Considering the protein and

lipid content of lyophilized açaí pulp (Menezes et al., 2008; Odendaal and Schaus, 2014), combined with the daily addition of this fruit to the artificial diet and the simultaneous production of bioflocs during the experimental period, we expected to verify an increase in the levels of protein and/or lipids in the bioflocs, as observed by Colombo et al. (2023), but there were no statistically differences. The proximal composition of the muscle of juvenile *P. vannamei* also seemed to have been unaffected by the experimental treatments, although the protein content recorded was significantly higher in the 5 mg of açaí L^{-1} treatment compared to other treatments enriched with açaí, did not differ from the control (Table 2).

In relation to bioflocs, the increase in açaí concentration influenced the loss of its antioxidant capacity (larger relative area) (Figure 1A). Similar results were observed by Colombo et al. (2023), where the enrichment of bioflocs with açaí for 27 days showed a dose-response behavior by decreasing the antioxidant capacity as the concentrations of this fruit increased. Quercetin, a phenolic compound with antioxidant properties, when applied to a culture of *P. vannamei* in a BFT system for 30 days also influenced the gradual loss of the antioxidant capacity of the bioflocs (Leon et al., 2018). In the present study, the loss of ACAP from the bioflocs may have influenced the decrease in the antioxidant capacity of the hepatopancreas (greater relative area) with the increase in açaí concentrations, mainly in the 5 and 20 mg açaí L^{-1} treatments (Figure 1A and 1C), because this organ is linked to the digestive process, secretion, and transport of several enzymes necessary for metabolism (Suita et al., 2014).

Although it lost antioxidant capacity, the addition of 20 mg açaí L^{-1} prevented lipid peroxidation in the bioflocs, corroborating the results of Colombo et al. (2023); however it was not sufficient to prevent oxidative damage to proteins in the same treatment (Figure 2A and 4A). Peroxyl radicals are by-products formed during lipid peroxidation; however, owing

to their high antioxidant capacity, phenolic compounds can act in the interception of ROS, preventing the propagation of the lipoperoxidation process (Habotta et al., 2022; Naiel et al., 2023). The protein damage observed in the bioflocs is related to the oxidation and decrease in the sulfhydryl groups (-SH) caused by the action of ROS (Figure 4A) (Halliwell and Gutteridge, 2015).

The increase in height and area of the microvilli in the middle intestine of the shrimp was stimulated by the application of 20 mg açaí L^{-1}, suggesting better intestinal absorption of the nutrients present in the açaí and compensating for their absorption in the posterior intestine (Table 4). The function of the posterior intestine is associated to the compaction of the feces and the formation of the fecal cord than the absorption of nutrients (Pinn et al., 1999; Garibay-Valdez et al., 2021). The increasing the length and area of intestinal microvilli, in addition to expanding the nutrient absorption surface, can also influence the improvement of osmoregulation and the immune system of shrimp (Talbot et al., 1972; Duan et al., 2020; Klahan et al., 2023). The degree of intestinal absorption of flavonoids is related to their chemical structures. According to Lipinski et al. (2001), compounds that are more easily absorbed by the intestine contain up to five H-bond donors, ten H-bond acceptors, molecular weights greater than 500 Da and lipophilicity indices greater than 5. However, molecules that have many hydroxyls, glycosidic groups, and galloyl groups in their structure are difficult to absorb in the intestine Gonzales et al. (2015). Among the main flavonoids found in açaí are the orientin, homoorientin, vitexin, luteolin, chrysoeriol, quercetin, and dihydrokaempferol (Kang et al., 2010). Therefore, the intestinal absorption capacity of açaí depends on the chemical structure of the main flavonoids present in the fruit.

Regarding the zootechnical results, the mortality registered in the treatment with 80 mg açaí L^{-1} can be attributed to the effects provoked by the high administration of açaí, which

may have exerted a pro-oxidant condition for the cultivation of *P. vannamei* juveniles and to the low absorption of nutrients in the middle intestine (Tables 3 and 4). Impairment of zootechnial parameters has been reported by Xiong et al. (2022), where high levels of the antioxidant lipoic acid in diets with low levels of carbohydrates (15%) resulted in lower weight gain and lower specific growth rate. Açaí pulp has a high content and diversity of molecules that, when metabolized, can act as pro-oxidants in different shrimp organs (Colombo et al., 2020). The growth phases of *P. vannamei* cultivated in a BFT system enriched with açaí also seem to influence its oxidant state in different ways, since the survival of post-larvae of the same species was affected by the addition of 80 mg açaí L^{-1} (Colombo et al., 2023).

When analyzing biochemical data from the muscle of shrimp cultivated in the 80 mg açaí L^{-1} treatment, no differences were observed in the ACAP and GSH levels (Figure 1D and 3D). In the muscles of juvenile *P. vannamei* exposed to nanoencapsulated lipoic acid in a BFT system, there were also no statistical differences in the levels of GSH (da Silva Martins et al., 2015). The lack of response regarding antioxidant defenses may have aggravated the occurrence of protein and lipid oxidative damage in the muscle, contributing to low survival (Figure 2D and 4D). Despite the high mortality that occurred in the 80 mg açaí L^{-1} treatment, the antioxidant effects of lyophilized açaí in terms of reduction of lipid peroxidation were evident in the gills and hepatopancreas (Figure 2B and 2C).

5. Conclusion

The 20 and 80 mg açaí L^{-1} concentrations acted as natural alkalinizers in the BFT system by maintaining water alkalinity levels above 100 mg $CaCO_3$ L^{-1}. In relation to

nitrogenous compounds, more studies are needed to clearly demonstrate whether high concentrations of açaí in a biofloc system can interfere with nitrification processes. The application of up to 20 mg açaí L^{-1} was able to minimize oxidative damage in the bioflocs and organs of the shrimp, as well as to stimulate an increase in the height and area of the intestinal microvilli. The results demonstrated that the high mortality in the treatment of 80 mg açaí L^{-1} in the BFT system was due to the pro-oxidant action of açaí and the low intestinal absorption capacity of the shrimp. It is concluded that the bioflocs were able to assimilate the antioxidants present in the açaí and transfer them to the shrimp, and among the different concentrations of açaí, the administration of 20 mg açaí L^{-1} presented the best performance in terms of biochemical parameters and histological studies, demonstrating the possibility of its application in the cultivation of *P. vannamei* in a biofloc system.

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Table 1. Physicochemical parameters of water during shrimp *Penaeus vannamei* culture for 30 days. Values are expressed as the mean ±1 standard error ($n = 3$). Different letters on the same line indicate significant differences between treatments according to the Newman–Keuls test at a significance level of 0.05.

Parameters	Treatments			
	Control	**5 mg L^{-1}**	**20 mg L^{-1}**	**80 mg L^{-1}**
Dissolved oxygen (mg L^{-1})	6.13 ± 0.03^{a}	6.25 ± 0.04^{a}	6.19 ± 0.04^{a}	6.16 ± 0.03^{a}
Temperature	29.85 ± 0.06^{a}	29.67 ± 0.07^{a}	29.84 ± 0.07^{a}	29.84 ± 0.05^{a}
pH	7.93 ± 0.009^{a}	7.92 ± 0.008^{a}	7.97 ± 0.008^{b}	8.01 ± 0.005^{c}
Ammonia (mg TAN L^{-1})	0.31 ± 0.05^{a}	0.30 ± 0.04^{a}	0.32 ± 0.04^{a}	0.71 ± 0.06^{b}
Nitrite-N (mg L^{-1})	0.16 ± 0.02^{a}	0.16 ± 0.02^{a}	0.21 ± 0.04^{a}	0.27 ± 0.05^{a}
Nitrate-N (mg L^{-1})	44.95 ± 4.61^{a}	40.78 ± 3.68^{a}	32.51 ± 2.72^{a}	17.63 ± 1.48^{b}
Phosphorus-P (mg L^{-1})	2.06 ± 0.35^{a}	2.16 ± 0.37^{a}	1.86 ± 0.28^{a}	1.70 ± 0.28^{a}
Alkalinity (mg $CaCO_3$ L^{-1})	113.13 ± 5.09^{a}	135.63 ± 4.77^{a}	159.69 ± 6.02^{b}	197.50 ± 5.31^{c}
Salinity	26.48 ± 0.31^{a}	26.65 ± 0.27^{a}	27.05 ± 0.36^{a}	26.55 ± 0.34^{a}
TSS (mg L^{-1})	213.89 ± 16.98^{a}	266.85 ± 24.88^{b}	323.52 ± 30.05^{c}	341.11 ± 25.38^{c}

Table 2. Proximal composition of the biofloc and muscle of *Penaeus vannamei* shrimp reared in Biofloc Technology System (BFT) with different concentrations of açaí *Euterpe oleracea*. Values are expressed as the mean ±1 standard error (n = 6). Different letters on the same line indicate significant differences between treatments according to the Newman–Keuls test at a significance level of 0.05.

Parameters	Treatments			
	Control	5 mg L^{-1}	20 mg L^{-1}	80 mg L^{-1}
(A) Biofloc				
Dry matter (%)	14.64 ± 0.82^{a}	13.16 ± 0.86^{a}	12.09 ± 0.19^{a}	13.91 ± 0.21^{a}
Protein (%)	30.02 ± 0.57^{a}	29.56 ± 0.42^{a}	27.73 ± 0.36^{a}	32.27 ± 1.58^{a}
Ether extract (%)	2.62 ± 0.65^{a}	2.70 ± 0.49^{a}	4.01 ± 0.95^{a}	4.27 ± 0.33^{a}
Ash (%)	45.61 ± 0.26^{c}	44.92 ± 0.30^{c}	40.47 ± 0.69^{b}	27.74 ± 0.88^{a}
(B) Muscle				
Dry matter (%)	23.59 ± 0.27^{a}	23.47 ± 0.24^{a}	24.04 ± 0.33^{a}	25.13 ± 0.59^{a}
Protein (%)	76.23 ± 1.12^{ab}	77.75 ± 0.44^{b}	74.44 ± 1.35^{a}	74.80 ± 0.92^{a}
Ether extract (%)	2.58 ± 0.34^{a}	1.95 ± 0.39^{a}	3.68 ± 0.85^{a}	3.33 ± 0.21^{a}
Ash (%)	6.26 ± 0.09^{a}	6.42 ± 0.07^{a}	6.37 ± 0.08^{a}	5.59 ± 0.34^{a}

Table 3. Zootechnical parameters of *Penaeus vannamei* shrimp reared in Biofloc Technology System (BFT) for 30 days with different concentrations of açaí *Euterpe oleracea.* Values are expressed as the mean ±1 standard error (n = 31). Different letters on the same line indicate significant differences between treatments according to the Newman–Keuls test at a significance level of 0.05. Abbreviations: SGR - Specific Growth Ratio; FCR - Feed Conversion Ratio; PER - Protein Efficiency Ratio.

Parameters	**Treatments**			
	Control	**5 mg L^{-1}**	**20 mg L^{-1}**	**80 mg L^{-1}**
Initial weight (g)	1.09 ± 0.04[a]	1.10 ± 0.04[a]	1.06 ± 0.05[a]	0.96 ± 0.04[a]
Final weight (g)	3.95 ± 0.16[a]	4.14 ± 0.17[a]	3.80 ± 0.16[a]	3.56 ± 0.30[a]
Weight gain (g)	2.83 ± 0.15[a]	2.97 ± 0.17[a]	2.78 ± 0.16[a]	2.65 ± 0.30[a]
SGR (%/day)	4.05 ± 0.13[a]	4.06 ± 0.15[a]	4.03 ± 0.13[a]	3.98 ± 0.32[a]
FCR	2.00 ± 0.10[a]	2.27 ± 0.15[a]	2.27 ± 0.13[a]	1.90 ± 0.26[a]
PER	1.52 ± 0.08[a]	1.47 ± 0.08[a]	1.47 ± 0.09[a]	1.91 ± 0.21[a]
Survival (%)	79.57 ± 18.84[b]	92.47 ± 7.53[b]	88.45 ± 3.61[b]	38.71 ± 8.53[a]

Table 4. Histological analysis of middle and posterior intestine the shrimp *Penaeus vannamei* reared in a Biofloc Technology System (BFT) with different concentrations of açaí *Euterpe oleracea.* Values are expressed as the mean ±1 standard error (n = 12). Different letters on the same line indicate significant differences between treatments according to the Newman–Keuls test at a significance level of 0.05.

Treatments	Middle intestine			Posterior intestine		
	Width (µm)	Height (µm)	Area (µm²)	Width (µm)	Height (µm)	Area (µm²)
Control	32.70 ± 2.15^{a}	24.10 ± 2.91^{a}	674.34 ± 101.13^{a}	34.86 ± 3.51^{a}	29.49 ± 1.73^{a}	903.32 ± 127.90^{c}
5 mg L^{-1}	36.50 ± 3.45^{a}	36.99 ± 4.31^{b}	1311.42 ± 223.48^{b}	35.92 ± 3.91^{a}	27.26 ± 2.88^{a}	819.45 ± 111.33^{bc}
20 mg L^{-1}	40.89 ± 3.05^{a}	50.64 ± 3.70^{c}	1981.28 ± 212.50^{c}	26.77 ± 2.76^{a}	19.59 ± 1.84^{b}	532.58 ± 98.49^{ab}
80 mg L^{-1}	31.64 ± 2.06^{a}	28.31 ± 2.70^{ab}	921.03 ± 149.19^{ab}	28.66 ± 1.65^{a}	16.18 ± 0.58^{b}	443.39 ± 33.26^{a}

Figures Captions

Figure 1. Values of total antioxidant capacity against peroxyl radicals (expressed in relative area) in the bioflocs (a), gills (b), hepatopancreas (c), and muscle (d) of *Penaeus vannamei* shrimps after the addition of different concentrations of lyophilized açaí for 30 days. Values are expressed as the mean ± 1 standard error (n = 12). Different letters indicate statistical differences between the concentrations of lyophilized açaí according to the Newman–Keuls test at a significance level of 0.05.

Figure 2. Levels of lipid peroxidation (nmol TMP/mg per tissue) in the bioflocs (a), gills (b), hepatopancreas (c), and muscle (d) of *Penaeus vannamei* shrimps after the addition of different concentration of lyophilized açaí for 30 days. Values are expressed as the mean ± 1 standard error (n = 12). Different letters indicate statistical differences between treatments according to the Newman–Keuls test at a significance level of 0.05. TMP is tetramethoxypropane, which was used as the standard.

Figure 3. Concentration of reduced glutathione (GSH, µmol/mg protein) in the bioflocs (a), gills (b), hepatopancreas (c), and muscle (d) of *Penaeus vannamei* shrimps after the addition of different concentrations of lyophilized açaí for 30 days Values are expressed as the mean ± 1 standard error (n = 12). Different letters indicate statistical differences between treatments according to the Newman–Keuls test at a significance level of 0.05.

Figure 4. Concentration of protein sulfhydryl groups (µmol/mg protein) in in the bioflocs (a), gills (b), hepatopancreas (c), and muscle (d) of *Penaeus vannamei* shrimps after the addition of different concentrations of lyophilized açaí for 30 days. Values expressed as the mean ± 1 standard error (n = 12). Different letters indicate statistical differences between treatments according to the Newman–Keuls test at a significance level of 0.05.

Figure 5. Middle intestine of juvenile shrimp *Penaeus vannamei* reared in a Biofloc Technology System (BFT) with different concentrations of açaí *Euterpe oleracea* for 30 days. A - Control; B - 5 mg açaí L^{-1}; C - 20 mg açaí L^{-1}; D - 80 mg açaí L^{-1}. H-E. BAR: 10 µm.

Figure 6. Posterior intestine of juvenile shrimp *Penaeus vannamei* reared in a Biofloc Technology System (BFT) with different concentrations of açaí *Euterpe oleracea* for 30 days. A - Control; B - 5 mg açaí L^{-1}; C - 20 mg açaí L^{-1}; D - 80 mg açaí L^{-1}. H-E. BAR: 10 µm.

Figure 1

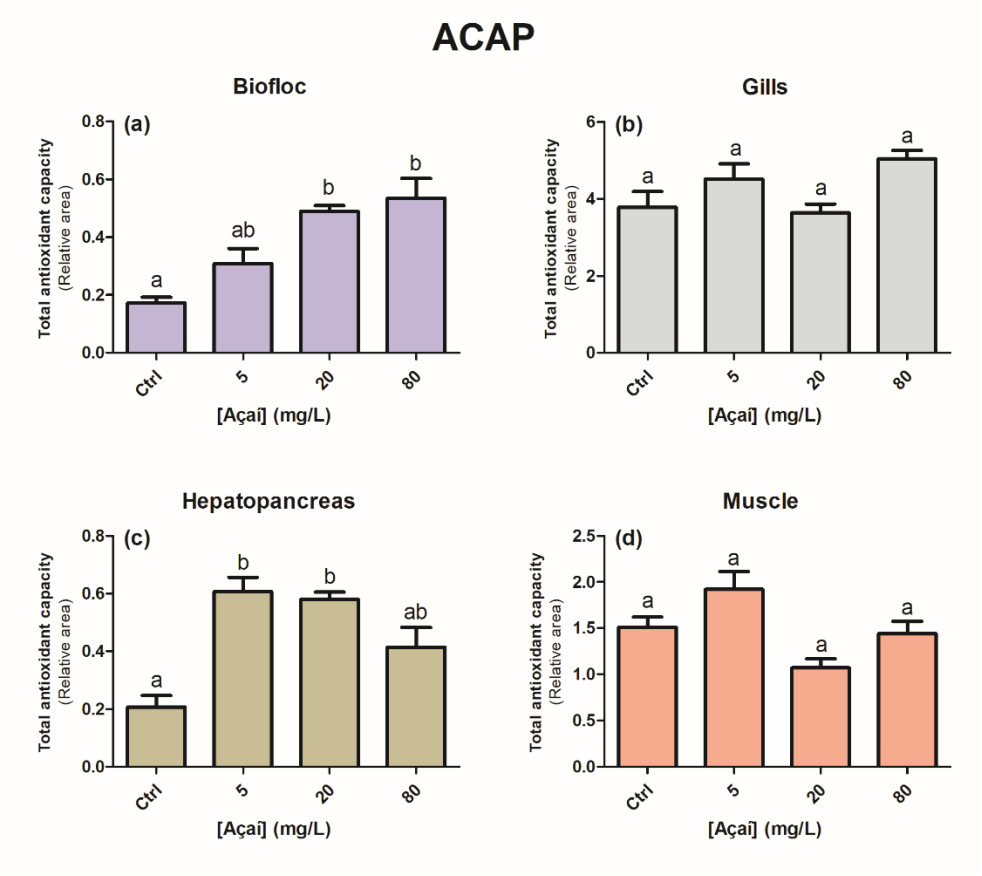

ACAP
Biofloc
(a)
Total antioxidant capacity (Relative area)
0.0
0.2
0.4
0.6
0.8
a
ab
b
b
Ctrl
5
20
80
[Açaí] (mg/L)
Gills
(b)
Total antioxidant capacity (Relative area)
0
2
4
6
a
a
a
a
Ctrl
5
20
80
[Açaí] (mg/L)
Hepatopancreas
(c)
Total antioxidant capacity (Relative area)
0.0
0.2
0.4
0.6
0.8
a
b
b
ab
Ctrl
5
20
80
[Açaí] (mg/L)
Muscle
(d)
Total antioxidant capacity (Relative area)
0.0
0.5
1.0
1.5
2.0
2.5
a
a
a
a
Ctrl
5
20
80
[Açaí] (mg/L)

Figure 2

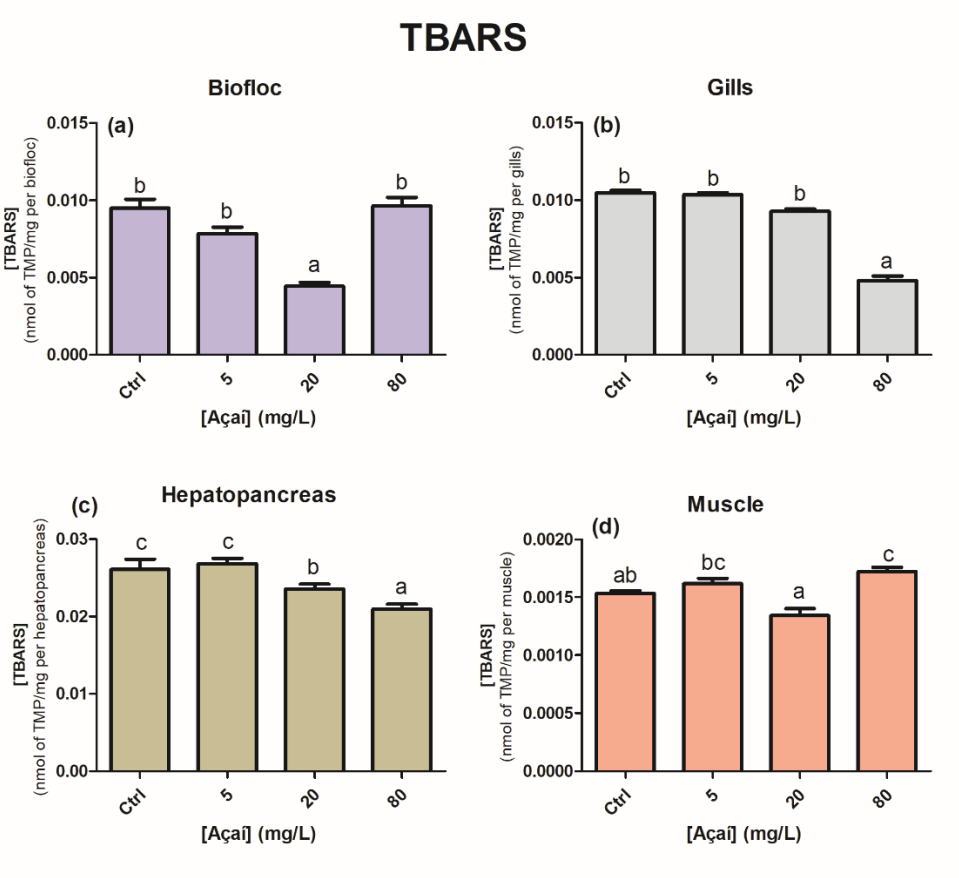

TBARS
Biofloc
(a)
[TBARS] (nmol of TMP/mg per biofloc)
0.015
0.010
0.005
0.000
b
b
a
b
Ctrl
5
20
80
[Açaí] (mg/L)
Gills
(b)
[TBARS] (nmol of TMP/mg per gills)
0.015
0.010
0.005
0.000
b
b
b
a
Ctrl
5
20
80
[Açaí] (mg/L)
Hepatopancreas
(c)
[TBARS] (nmol of TMP/mg per hepatopancreas)
0.03
0.02
0.01
0.00
c
c
b
a
Ctrl
5
20
80
[Açaí] (mg/L)
Muscle
(d)
[TBARS] (nmol of TMP/mg per muscle)
0.0020
0.0015
0.0010
0.0005
0.0000
ab
bc
a
c
Ctrl
5
20
80
[Açaí] (mg/L)

Figure 3

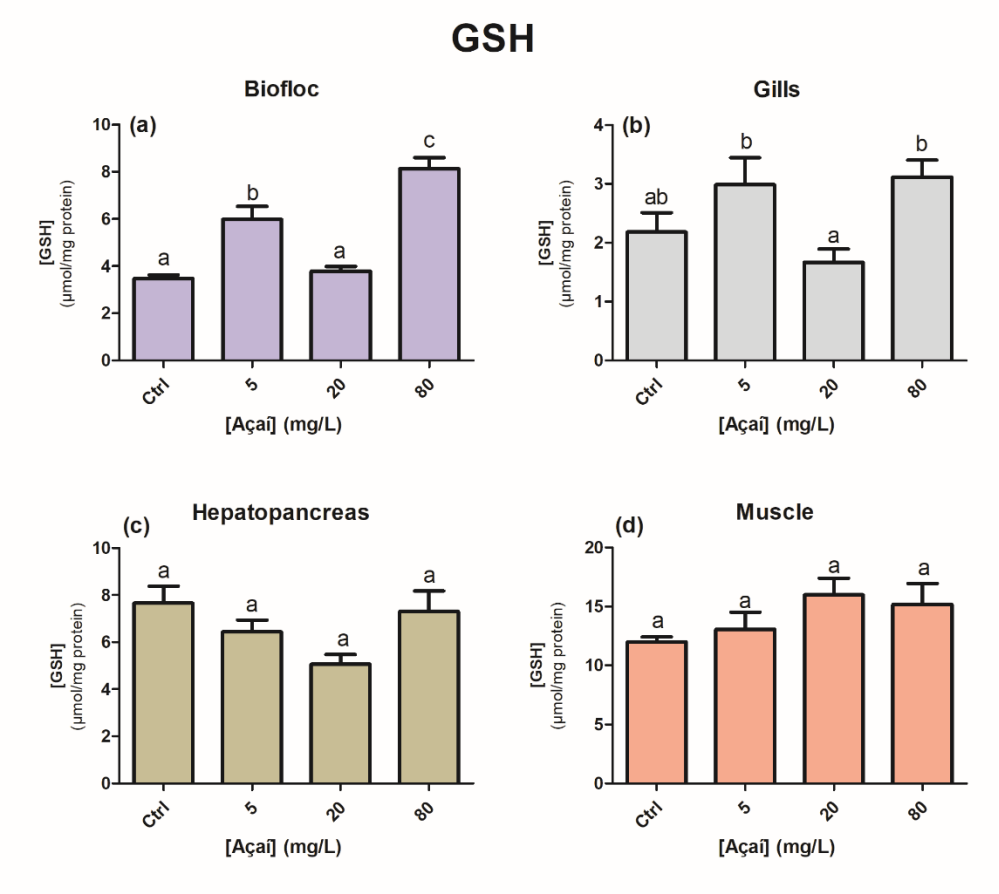

GSH
Biofloc
(a)
[GSH] (µmol/mg protein)
a
b
a
c
Ctrl
5
20
80
[Açaí] (mg/L)
Gills
(b)
[GSH] (µmol/mg protein)
ab
b
a
b
Ctrl
5
20
80
[Açaí] (mg/L)
Hepatopancreas
(c)
[GSH] (µmol/mg protein)
a
a
a
a
Ctrl
5
20
80
[Açaí] (mg/L)
Muscle
(d)
[GSH] (µmol/mg protein)
a
a
a
a
Ctrl
5
20
80
[Açaí] (mg/L)

Figure 4

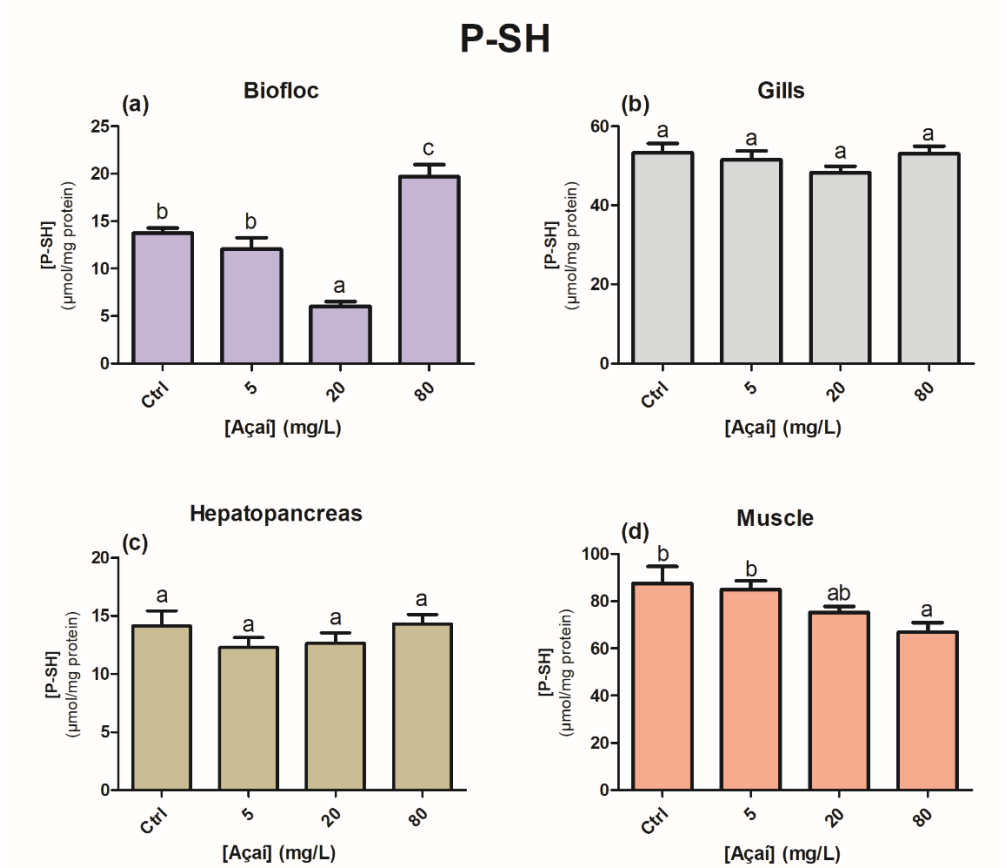
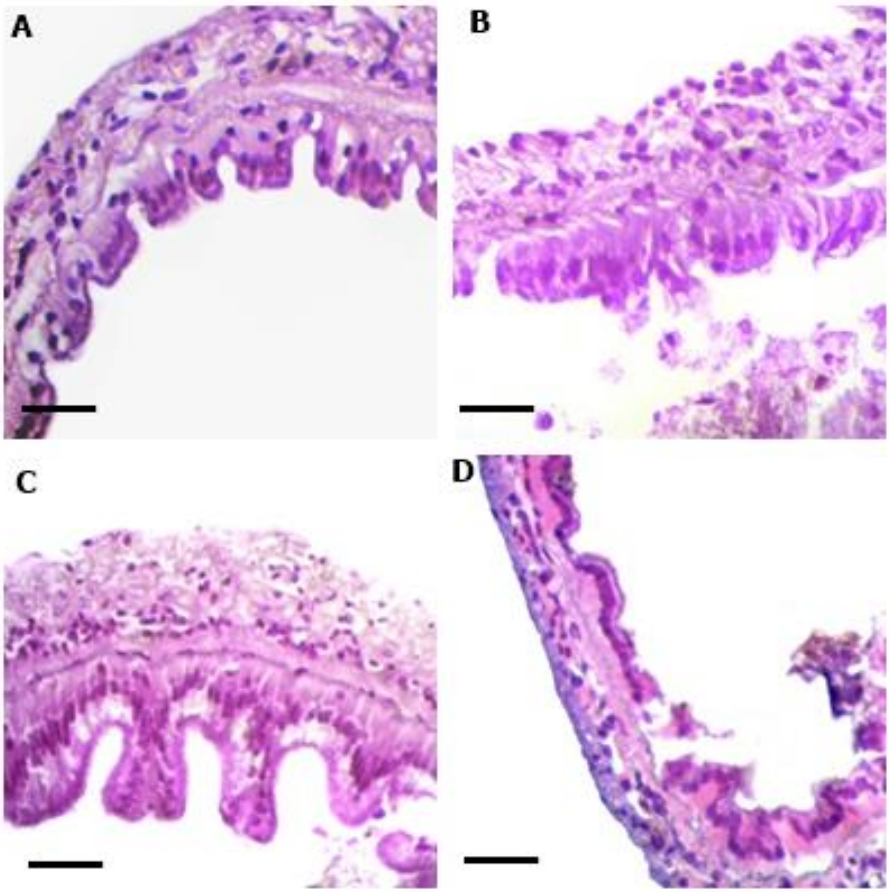
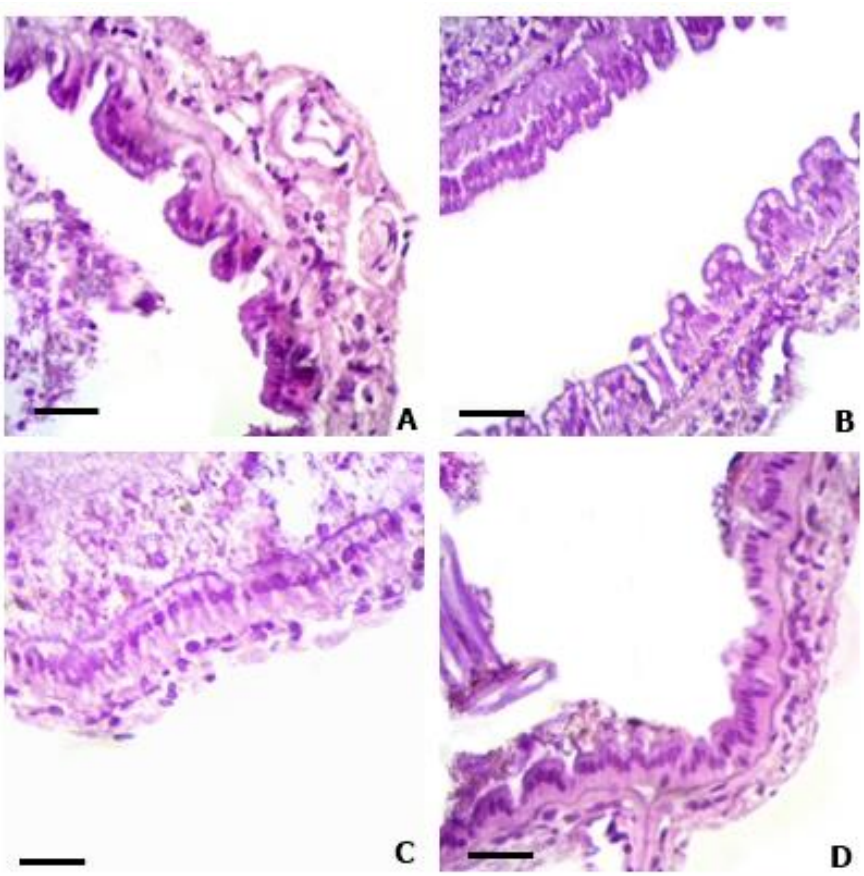

P-SH
Biofloc
(a)
[P-SH] (µmol/mg protein)
[Açaí] (mg/L)
Ctrl
5
20
80
Gills
(b)
Hepatopancreas
(c)
Muscle
(d)

Figure 5



1672 **Figure 6**

1673



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CAPÍTULO 3

Procedimentos para análise de cromaticidade no camarão *Penaeus vannamei* (Boone, 1931) cultivado em sistema de bioflocos enriquecido com açaí (*Euterpe oleracea* Mart. 1824)

Procedimentos para análise de cromaticidade no camarão *Penaeus vannamei* (Boone, 1931) cultivado em sistema de bioflocos enriquecido com açaí (*Euterpe oleracea*)

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Resumo

O presente estudo teve como objetivo desenvolver procedimentos simples, através de programas gratuitos, para quantificar a coloração de pós-larvas do camarão *Penaeus vannamei*, expressando de forma numérica as possíveis mudanças obtidas após a adição da polpa liofilizada de açaí em um sistema de cultivo de bioflocos. Camarões do estádio PL10 foram cultivados durante 30 dias em um sistema BFT, distribuídos em quatro tratamentos (controle, 5, 20 e 80 mg de açaí L^{-1}). Durante o período experimental as respectivas concentrações de açaí liofilizado foram adicionadas diariamente à água de cultivo. Foram analisados parâmetros de cromaticidade (L*, a*, b* e Delta E), níveis de polifenóis e flavonoides totais, capacidade antioxidante total (ACAP) e a peroxidação lipídica (TBARS). Observou-se uma diminuição significativa nos níveis de peroxidação lipídica nos bioflocos enriquecidos com 20 e 80 mg açaí L^{-1} ($p < 0.05$). As diferentes concentrações de açaí não afetaram estatisticamente os níveis de TBARS das pós-larvas ($p > 0.05$). Não foram observadas diferenças nos parâmetros a* e b* ($p > 0.05$). A administração de 80 mg açaí L^{-1} incrementou o conteúdo de polifenóis totais nos camarões ($p < 0.05$), possivelmente contribuindo para a diminuição dos valores de luminosidade (L*) observados nos organismos. Os valores de Delta E indicaram que a coloração dos camarões do tratamento de 80 mg açaí L^{-1} se diferenciaram dos demais tratamentos ($p < 0.05$), sendo perceptível ao olho humano. Baixos níveis de luminosidade servem como indicativo que os pigmentos presentes no fruto do açaí podem ter deixado o organismo mais escuro. Portanto, conclui-se que o açaí aumentou a concentração de pigmentos nos cromatóforos das pós-larvas, resultando na perda de luminosidade no corpo destes organismos.

Palavras-chave: coloração, estresse oxidativo, sistema antioxidante, polifenóis, pós-larvas

1. Introdução

As diferentes cores apresentadas pelos animais aquáticos são determinadas pelas substâncias pigmentares presentes em seu organismo, tais como carotenóides e compostos fenólicos (Pereira da Costa e Miranda-Filho, 2020). Estas substâncias atuam como importantes antioxidantes e estão presentes na natureza conferindo pigmentação a animais e plantas. Em crustáceos, a intensidade da pigmentação avermelhada é controlada particularmente pela concentração de astaxantina, um tipo de carotenóide (Long et al., 2017). No entanto, como os crustáceos não são capazes de sintetizar carotenoides, o acúmulo de pigmentos em seu organismo depende diretamente da ingestão de alimentos ou substâncias que contenha tais compostos (Maoka, 2011; Bernal Rodríguez et al., 2017). Consequentemente, na indústria aquícola, o uso de corantes tem sido aplicado principalmente na alimentação de salmonídeos e crustáceos para intensificar a pigmentação dos animais cultivados (Stahl, 2012; Daniel et al, 2017), uma vez que a coloração mais intensa de organismos aquáticos apresenta valor de mercado superior ao de animais com cores mais claras (Long et al., 2017; da Costa et al., 2021).

Devido ao seu potencial de coloração, maior estabilidade a processos de oxidação e redução, e o baixo custo em comparação com corantes naturais, os corantes sintéticos são majoritariamente utilizados pela indústria de alimentos (Alburqueque et al., 2020). O consumo excessivo de corantes sintéticos pode representar sérios riscos à saúde, desde efeitos tóxicos, alérgicos, cancerígenos a problemas ecológicos, por possuírem estrutura molecular complexa e baixa capacidade de biodegradação, se acumulando no meio aquático e/ou terrestre (Attokaram, 2017; Manzoor et al., 2021). Entretanto, o consumo de alimentos que contenha pigmentos naturais diminuí a incidência de estresse, problemas cardiovasculares, imunológicos, cancerígenos, ao conter vitaminas, minerais e compostos

bioativos de natureza antioxidante, conferindo agilidade no sequestro de radicais livres (Martínez et al., 2014; Long et al., 2017)

Na busca por práticas sustentáveis, a demanda pelo uso de pigmentos naturais como corantes para diferentes produtos tem atraído atenção de pesquisadores e direcionado a pesquisa para o uso de suplementos naturais ricos em compostos fenólicos, que possam outorgar cores mais atraentes aos organismos destinados ao consumo humano, e ao mesmo tempo melhorar a capacidade antioxidante prevenindo danos às membranas celulares (Hatta e Ottaman, 2020). Deste modo, destacam-se os frutos amazônicos, como o açaí (*Euterpe oleracea*), que apresenta elevado potencial nutricional (Menezes et al., 2008; Schauss, 2016). O açaí é uma importante fonte de antioxidantes, tais como polifenóis, flavonóides, carotenóides e antocianina, sendo esta última principal responsável pela coloração roxa do fruto (Odendaal e Schauss, 2014). Embora existam poucos estudos sobre a aplicação do açaí na aquicultura, alguns trabalhos descreveram a inclusão deste fruto na alimentação do *Colossoma macropommum* e *Penaueus vannamei* e seus efeitos na capacidade antioxidante (Colombo et al., 2020; Schmitz et al., 2020; Colombo et al., 2023) e cromaticidade (Silva et al., 2020; Ramos et al., 2022; Da Silva et al., 2023).

Contudo, um dos impasses para determinados experimentos está na maneira em que os dados são obtidos e processados, a fim de comprovar de forma objetiva os parâmetros de cromaticidade nestes. Tendo em vista o impasse apresentado, este estudo teve como objetivo desenvolver procedimentos simples para caracterizar uma forma de averiguar a coloração de pós-larvas do camarão *Penaeus vannamei*, expressando de forma numérica as possíveis mudanças obtidas após a adição da polpa liofilizada de açaí em um sistema de cultivo de bioflocos. Além disso, os efeitos das diferentes concentrações de açaí sobre o conteúdo de polifenóis e flavonóides totais, capacidade antioxidante e peroxidação

lipídica nos bioflocos e camarões também foram investigados, uma vez que os bioflocos podem servir como um importante vetor na transferência de compostos fenólicos aos camarões via ingestão, e assim melhorar a qualidade dos camarões cultivados em sistema de BFT (Colombo et al., 2023).

2. Materiais e Métodos

2.1. Inóculos de bioflocos e delineamento experimental

O estudo foi realizado na Estação Marinha de Aquicultura (EMA) do Instituto de Oceanografia (IO) da Universidade Federal do Rio Grande - FURG, localizada na praia do Cassino, Rio Grande, RS, e Sul do Brasil. O açaí liofilizado (*Euterpe oleracea*) foi obtido da Amazônia Comércio de Açaí Liofilizado e Exportação LTDA, Belém, PA, Brasil.

Os inóculos de bioflocos utilizados no experimento foram coletados de um cultivo superintensivo de camarão branco do Pacífico *P. vannamei* na Estação de Aquicultura Marinha (EMA). Antes da adição dos bioflocos às respectivas unidades experimentais, os inóculos foram distribuídos em diferentes tanques sem a presença de camarão e enriquecidos por sete dias com três concentrações de açaí liofilizado (5, 20 e 80 mg L^{-1}), seguindo o protocolo descrito por Colombo et al. (2023). Após o enriquecimento, os inóculos foram adicionados aos tanques experimentais para início dos estudos com pós-larvas do camarão *P. vannamei*.

Camarões do estádio PL10 (décimo dia do estágio pós-larval) foram cultivados por um período experimental de 30 dias com densidade de 30 camarões por L em 12 tanques plásticos com volume útil de 18 L cada, distribuídos aleatoriamente em quatro tratamentos (controle, 5, 20 e 80 mg de açaí L^{-1}), todos em triplicata. Em cada tanque foram adicionados 17 litros de água do mar tratada (clorada e desclorada com vitamina

C) e um litro de inóculo de bioflocos coletados em sistema superintensivo de cultivo e previamente enriquecidos com açaí. Durante o período experimental as concentrações de açaí liofilizado foram adicionadas diariamente à água de cultivo. As pós-larvas foram alimentadas com duas dietas comerciais (INVE®/StressPak e Guabi XL®/PL 40) administradas três vezes ao dia durante o período experimental de acordo com recomendações dos fabricantes de rações e protocolo adaptado da FAO (2004).

2.2. Análises bioquímicas

2.2.1. Coleta e homogeneização das amostras

Ao final do experimento, amostras de água (1 litro) de cada tanque foram coletadas e adicionadas em cones Imhoff para obtenção de bioflocos, conforme protocolo descrito por León et al. (2018). Os sólidos sedimentáveis foram centrifugados 1**.**500 x g durante 15 minutos a 10°C para remover o excesso de água. Para homogeneização, as amostras de bioflocos foram sonicadas a 50% da potência máxima (QSonica Sonicators) por 1 minuto em água salgada artificial a 30 ppt (Sal Veromar, 1:1 p/v), agitadas por 3 horas em agitador orbital (80 rpm) e posteriormente centrifugad**as** a 10.000 x g por 5 minutos a 4°C. As pós-larvas foram homogeneizadas inteiras (1:4 P/V) em solução tampão para crustáceos (pH 7,2; Tris-base 20 mM; EDTA 1 mM; $MgCl_2$ 0,05 mM; sacarose 5 mM; e KCl 1 mM). Todas as amostras foram centrifugadas a 20.000 x g por 30 minutos a 4 °C e os sobrenadantes colocados em ultrafreezer (-80 °C) para as análises bioquímicas.

2.2.2. Análises do conteúdo de polifenóis e flavonóides totais

Para a análise de polifenóis e flavonóides totais, amostras de bioflocos (1:1 P/V) e pós-larvas (1:3 P/V) foram homogeneizadas em metanol 100% e centrifugadas a 10.000

x g por 5 minutos a 4 °C (bioflocos) e 20.000 x g por 30 minutos a 4 °C (camarão) para remoção do extrato metanólico.

O teor de polifenóis totais foi medido de acordo com Dias et al. (2013). Em placas transparentes foram adicionados 25 µl do extrato, 625 µl de Folin-Ciocalteau 0,1 M e 500 µl de Na_2CO_3 a 7,5%. Uma curva padrão foi preparada com quercetina previamente diluída em metanol 100%. As placas foram incubadas no escuro à temperatura ambiente durante 60 min e a absorvância lida a 740 nm num espectrofluorímetro (Biotek Synergy HT).

A análise do teor de flavonóides totais foi baseada na metodologia descrita por Gajula et al. (2009), modificado por León et al. (2018). Após adição de 50 µl de extrato metanólico, 50 µl de água destilada, 37,5 µl de $NaNO_2$ 5% em eppendorfs, estes foram incubados no escuro em temperatura ambiente por 6 minutos. Em seguida, 75 µl de $AlCl_3$ a 10% foram adicionados aos eppendorfs e incubados por 5 minutos. Por fim, 250 µl de NaOH 1M foram acrescentados aos eppendorfs e incubados por um período de 30 minutos. Uma reta padrão foi preparada com quercetina previamente diluída em metanol 100%. Alíquotas (200 µl) do conteúdo dos eppendorfs foram transferidas em duplicata para microplacas transparentes e lidas a uma absorbância de 510 nm em espectrofluorímetro (Biotek Synergy HT).

2.2.3. Analise da capacidade antioxidante total (ACAP) e peroxidação lipídica (TBARS)

A análise de capacidade antioxidante total foi realizada segundo protocolo descrito por Amado et al. (2009). As amostras foram expostas aos radicais peroxil gerados pela decomposição térmica a 37 ◦C do ABAP (dicloridrato de 2,2-azobis-2-metilpropionamidina) (SigmaAldrich) e reagiram com 2′,7′ diacetato de diclorofluoresceína (H_2DCF), resultando em um composto fluorescente (DCF) detectado

através de um espectrofluorímetro de microplaca (excitação: 485 nm e emissão: 535 nm; Synergy™ HT). A ACAP foi quantificada por meio da área relativa com e sem ABAP. O valor da área relativa é inversamente proporcional a capacidade antioxidante, logo, quanto menor a área relativa, maior a capacidade antioxidante total contra radicais peroxil.

Para a análise da peroxidação lipídica, foram adicionados em tubos de vidro os homogeneizados de bioflocos (50 µl) e camarão (100 µl), 20 µl de solução de BHT (hidroxitolueno butilado, 67 µM), 150 µl de solução de acético ácido 20%, 150 µl de solução de TBA 0,8% (ácido tiobarbitúrico), 50 µl de água destilada, 20 µl de SDS 8,1% (dodecilsulfato de sódio) e aquecidos por 30 min em banho-maria a 95 °C. Adicionou-se à solução 100 µl de água destilada e 500 µl de n-butanol e em seguida as amostras foram centrifugadas a 3.000 × g por 10 min a 15 °C. O sobrenadante foi transferido para microplaca e lido a 520 nm (excitação) e 580 nm (emissão) em espectrofluorômetro (Biotek Synergy HT). Os resultados foram expressos em equivalentes de TMP por mg de amostra. A metodologia usada na análise foi baseada no protocolo descrito por Oakes e Van Der Kraak (2003).

2.3. Análise de cromaticidade

Ao final do experimento as pós-larvas foram eutanasiadas em choque térmico com gelo e mantidos em ultra-freezer a – 80°C até o momento da análise de cromaticidade. Fotografou-se 60 animais distintos, sendo 15 de cada tratamento avaliado. As fotos das pós-larvas foram tiradas com auxílio de uma lupa LEICA DFC295 em aumento de 1x. Em todo o processamento das fotos, até chegar à análise de parâmetros de cor, foram utilizados programas exclusivamente de uso gratuito.

Todas as etapas referente a análise de cromaticidade foram descritas na Tabela 1. O primeiro passo para o processamento das fotos foi realizar um balanço de branco das imagens, a fim de impedir a interferência de sombras e cores distintas nas análises das fotografias. Um cartão de branco de referência foi incluído na hora da tomada de cada fotografia. O balanço de branco foi efetuado através do programa de uso livre e gratuito Picasa 3, o qual permitiu padronizar o branco das imagens com base no cartão de branco de referência inserido nas fotos. Devido as fotos apresentarem sombras ao fundo, o que poderia afetar os parâmetros de luminosidade, todo o fundo das imagens foi retirado e substituído por um fundo branco inserido por software, permanecendo apenas os camarões. Para essa etapa foi utilizado o site de livre acesso "PhotoRoom" (https://www.photoroom.com/remover-fundo-de-imagem), no qual, após realizar o "upload" das fotos, o próprio site realizou automaticamente o contorno dos animais por meio da ferramenta de remoção de fundo.

Por fim, os dados de parâmetros de cor foram obtidos com o auxílio do site (também livre e gratuito) Image Color Summarizer (http://mkweb.bcgsc.ca/color-summarizer/?analyze). Na guia intitulada "Analyze", alterou-se apenas a configuração "precision" (precisão) que estava padronizado em "vlow" (50 pixels), sendo modificado para "high" (150 pixels). O programa fornece o parâmetro de luminosidade (L*), variando em uma escala de 0 (preto) a 100 (branco); o parâmetro a* (eixo verde/vermelho); e o parâmetro b* (eixo azul/amarelo). Após a obtenção dos parâmetros de L*, a*, e b*, foi calculado o parâmetro Delta E (Δ E*; Equação 1) o qual considera as diferenças totais de cor destes três parâmetros, analisando um grupo de referência que, neste caso, foi o grupo controle.

Equação 1:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

2.4. Analises estatísticas

Os diferentes parâmetros de cromaticidade (L*, a* e b*) foram avaliados por ANOVA de fatores mistos, onde o fator fixo foram as concentrações de açaí e o fator aleatório as diferentes unidades experimentais utilizadas para cada tratamento (Searle et al., 2006). Previamente, os pressupostos de homogeneidade das variâncias e normalidade dos diferentes tratamentos foram avaliados pelos testes de Levene e Shapiro-Wilk, respectivamente. Possíveis diferenças significativas entre os tratamentos foram detectadas por meio do teste de Newman-Keuls. Em todos os casos o nível de significação foi de 0,05.

3. Resultados

Os resultados bioquímicos do experimento 1 foram apresentados na Figura 3 e 4. Uma queda na concentração total de polifenóis nos bioflocos foi observada nos tratamentos de 5 e 20 mg açaí L^{-1} em relação ao controle e a concentração de 80 mg açaí L^{-1} ($p < 0.05$). A administração de 80 mg açaí L^{-1} no cultivo de pós-larvas em sistema BFT incrementou o teor de polifenóis no corpo das pós-larvas em relação aos demais tratamentos ($p < 0.05$; Figura 3A). O conteúdo de flavonóides nos bioflocos foi menor na concentração de 20 mg açaí L^{-1} ($p < 0.05$). Não foram observadas diferenças significativas entre os tratamentos para a concentração de flavonóides totais nas pós-larvas ($p > 0.05$; Figura 3B). Ao final do período experimental, a capacidade antioxidante total dos bioflocos e das pós-larvas não apresentaram diferenças ($p > 0.05$; Figura 4A). A administração de 20 e 80 mg açaí L^{-1} diminuiu significativamente os níveis de

peroxidação lipídica nos bioflocos ($p < 0.05$). As diferentes concentrações de açaí não afetaram estatisticamente os níveis de TBARS das pós-larvas ($p > 0.05$; Figura 4B).

Na Tabela 2 foram apresentados os valores médios dos parâmetros de cor (L*, a* e b*) das pós-larvas, os quais consideraram a coloração do animal como um todo (cefalotórax e abdômen). Nas pós-larvas os dados de luminosidade (L*) apresentaram uma queda significativa no tratamento administrado com 80 mg açaí L^{-1} ($p < 0.05$). Não foram observadas diferenças nos parâmetros a* e b* ($p > 0.05$). Os valores médios do parâmetro Delta E, calculado tendo como grupo de comparação o tratamento controle, foram maiores na concentração de 80 mg açaí L^{-1}, diferenciando-se estatisticamente dos demais tratamentos ($p < 0.05$).

4. Discussão

A coloração de crustáceos geralmente está relacionada à quantidade de pigmentos presentes no exoesqueleto ou dentro da camada hipodérmica subjacente, estrutura conhecida como cromatóforos (Ertl et al., 2013; Pereira da Costa e Miranda-Filho, 2020). Diferentes fatores podem influenciar na pigmentação dos camarões, tais como a cor do substrato de fundo, fotoperíodo, temperatura, presença de metais pesados, mas principalmente é através da alimentação que a coloração destes organismos é intensificada (Laohavisuti e Ruangdej, 2014; Martínez et al., 2014; Wade et al., 2015; Bernal Rodríguez et al., 2017; Long et al., 2017; Pereira da Costa e Miranda-Filho, 2020)

Os bioflocos possuem compostos fenólicos de propriedades antioxidantes e a capacidade para absorver outros antioxidantes adicionados ao cultivo, como por exemplo quercetina e açaí (León et al., 2018; Colombo et al., 2023). Via alimentação, tais compostos podem ser transferidos aos camarões, uma vez que os bioflocos representam uma fonte de alimento, correspondendo em até 29% da dieta destes crustáceos (Burford

et al., 2004). No presente estudo, apesar dos conteúdos de polifenóis e flavonóides totais dos bioflocos não terem aumentado com a administração de açaí no cultivo, como observado por Colombo et al. (2023), o enriquecimento dos bioflocos com 20 e 80 mg açaí L^{-1} amenizou os danos lipídicos nos bioflocos e incrementou o conteúdo de polifenóis nas pós-larvas do tratamento de 80 mg açaí L^{-1} (Figura 3A e 4B). Quando em níveis adequados, o aumento do conteúdo de compostos antioxidantes pode prevenir danos oxidativos ao interceptar espécies reativas de oxigênio (ROS) (Monserrat et al., 2008; Amado et al., 2011; Da Silva Martins et al., 2018; Colombo et al., 2020; Dos Santos Simião et al., 2022).

Os parâmetros cor (L*, a* e b*) são índices importantes para avaliar a coloração de crustáceos, a qual está diretamente relacionada ao conteúdo e concentração de compostos fenólicos e carotenóides em seus tecidos (Gong et al., 2014). A diminuição dos valores de luminosidade (L*) observado na concentração de 80 mg açaí L^{-1} sugerem que os pigmentos presentes no fruto do açaí podem ter deixado o organismo mais escuro (Tabela 1), já que neste mesmo tratamento houve um aumento significativo no conteúdo de polifenóis nas pós-larvas. Após a inclusão de açaí na dieta de juvenis do *Penaeus vannamei,* Silva et al. (2020) e Ramos et al. (2022) analisaram os efeitos do açaí na coloração dos camarões. Corroborando com o presente estudo, os autores citados anteriormente reportaram que a maior taxa de inclusão de açaí (10% p/p) na dieta diminuiu os valores de luminosidade da região do cefalotórax (Silva et al., 2020) e na região abdominal (Ramos et al., 2022) de camarões frescos, deixando-os mais escuros. Os valores de L* dos ovários e carapaça da fêmea do caranguejo *Eriocheir sinensis* apresentaram tendência decrescente de acordo com o aumento dos níveis de inclusão da alga de água doce *Haematococcus pluvialis* na dieta (Long et al., 2017).

Apesar de não ter sido observadas diferenças estatísticas nos dados de b*, percebe-se que com o aumento gradual das concentrações de açaí os valores médios deste parâmetro foram diminuindo, direcionando a coloração para tons azulados. No estudo de Ramos et al. (2022) os efeitos da inclusão de 10% de açaí na dieta foram notórios no cefalotórax de camarões frescos, ao diminuir significativamente os valores de b*. Além disso, uma coloração mais avermelhada no cefalotórax e músculo do *P. vannamei* após o cozimento (Ramos et al., 2022) e na região abdominal de camarões frescos e cozidos (Silva et al., 2020) também foram reportadas. A coloração avermelhada dos crustáceos é uma das mais buscadas entre os consumidores ao sugerir melhor qualidade e sabor, por isso tendem a ter o preço de mercado mais elevado quando comparadas com cores mais claras (Martínez et al., 2014; Long et al., 2017). Além de crustáceos, o efeito do açaí na pigmentação de organismos aquáticos também pode ser verificado no tambaqui *Colossoma macropomum* após 30 dias de alimentação com este fruto (Da Silva et al., 2023). Possivelmente a inclusão de 5 e 10% de açaí na dieta do tambaqui colaborou para o aumento dos pigmentos nos melanóforos e cromatóforos, fazendo com que a região dorsal dos animais adquirisse coloração ciano.

O Delta E (Δ E*) representa a diferença de cor entre os parâmetros L*, a* e b*. Para valores de Δ E* < 1,5 considera-se que a coloração das amostras é relativamente igual à amostra de referência. Diferenças de cor podem ser distinguidas a olho humano quando os valores permanecem entre a $1,5 \leq \Delta E^* \leq 5$. No entanto, tais diferenças na pigmentação são mais evidentes para valores $\Delta E^* > 5$ (Altemio et al., 2023). Considerando os resultados para Delta E das pós-larvas do tratamento de 80 mg açaí L^{-1} (7,13 ± 0,70), a coloração apresentada por estes organismos, de fato, foi perceptível e diferente dos camarões dos outros tratamentos (Tabela 1). Logo, pode-se dizer que a baixa luminosidade e os tons mais escuros apresentados pelas pós-larvas, identificados pelo

programa online “Image Color Summarizer”, foram resultados do enriquecimento dos bioflocos com o açaí.

5. Conclusão

A administração de 80 mg açaí L^{-1} diminuiu os níveis de peroxidação lipídica nos bioflocos e aumentou o conteúdo de polifenóis totais nas pós-larvas. Portanto, através da utilização do programa online e gratuito “Image Color Summarizer “, conclui-se que o açaí aumentou a concentração de pigmentos nos cromatófaros de pós-larvas do camarão *P. vannamei*, cultivadas em sistema BFT após o enriquecimento dos bioflocos com açaí, resultando na perda de luminosidade no corpo destes organismos.

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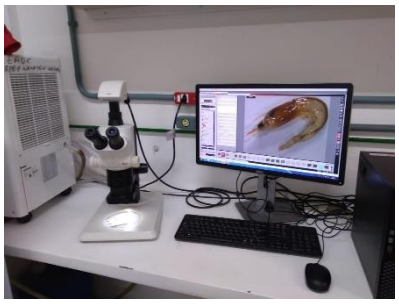
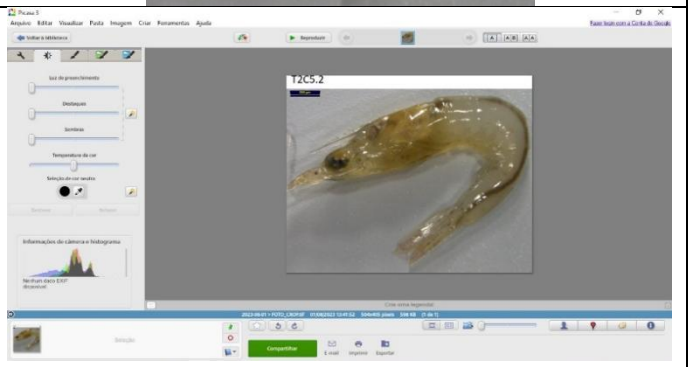
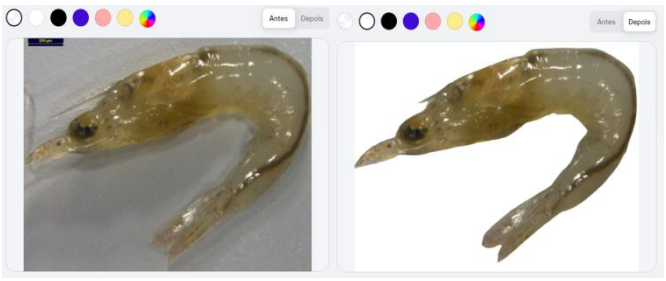
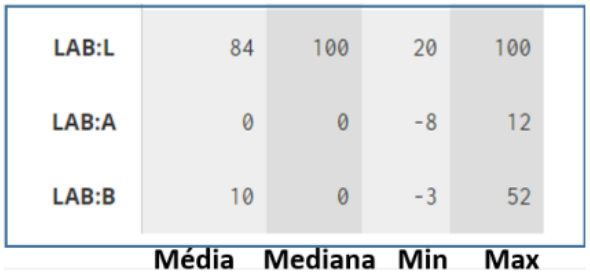
Tabela 1. Etapas para a realização da análise de cromaticidade em pós-larvas do *P. vannamei.*

Etapa		Ferramenta	
Passo 1	Obtenção das fotos	Lupa LEICA DFC295 (Obs: Outros dispositivos fotográficos também podem ser utilizados. A lupa foi usada devido ao tamanho das pós-larvas)	 
Passo 2	Balanço de branco	Programa de edição de imagens (Picasa3)	
Passo 3	Remoção do fundo	Site: "PhotoRoom" https://www.photoroom.com/remover-fundo-de-imagem	
Passo 4	Obtenção dos dados de cromaticidade	Site: "Image Color Summarizer" http://mkweb.bcgsc.ca/color-summarizer/?analyze	

	Média	Mediana	Min	Max
LAB:L	84	100	20	100
LAB:A	0	0	-8	12
LAB:B	10	0	-3	52

Tabela 2. Valores médio dos parâmetros de cor (L*, a*, b* e Delta E) considerando todo o corpo das pós-larvas (*Penaues vannamei*) cultivadas em sistema BFT com a adição de diferentes concentrações de açaí liofilizado (*Euterpe oleracea*).

Tratamentos	**Parâmetros**			
	L*	**a***	**b***	**Delta E**
Controle	$80,40 \pm 0,90^{a}$	$0,40 \pm 0,13^{a}$	$7,80 \pm 0,37^{a}$	**-**
5 mg L^{-1}	$78,27 \pm 0,71^{a}$	$0,13 \pm 0,09^{a}$	$7,33 \pm 0,34^{a}$	$3,19 \pm 0,49^{a}$
20 mg L^{-1}	$77,13 \pm 0,70^{ab}$	$0,53 \pm 0,13^{a}$	$7,47 \pm 0,27^{a}$	$3,87 \pm 0,54^{a}$
80 mg L^{-1}	$73,60 \pm 0,77^{b}$	$0,40 \pm 0,13^{a}$	$6,20 \pm 0,17^{a}$	$7,13 \pm 0,70^{b}$

Luminosidade L* - varia entre 0 (preto) e 100 (branco); cromaticidade a* - eixo verde/vermelho, varia de valores negativos (verde) a positivos (vermelho); cromaticidade b* - eixo azul/amarelo, varia de valores negativos (azul) a positivos (amarelo). Os valores são expressos como média $\pm$ 1 erro padrão (n = 15). Letras diferentes na mesma linha indicam diferenças significativas entre os tratamentos de acordo com o teste de Newman-Keuls ao nível de significância de 0,05.

Legendas das Figuras

Figura 1. Concentração de polifenóis (a) e flavonóides totais (b) nos bioflocos e pós-larvas de camarões *P. vannamei* após adição de diferentes concentrações de açaí liofilizado por 30 dias. Os valores são expressos como média ± 1 erro padrão (n = 12). Letras diferentes indicam diferenças estatísticas entre as concentrações de açaí liofilizado pelo teste de Newman-Keuls ao nível de significância de 0,05.

Figura 2. Capacidade antioxidante total (a) e peroxidação lipídica - TBARS (b) nos bioflocos e pós-larvas de camarões *P. vannamei* após adição de diferentes concentrações de açaí liofilizado por 30 dias. Os valores são expressos como média ± 1 erro padrão (n = 12). Letras diferentes indicam diferenças estatísticas entre as concentrações de açaí liofilizado pelo teste de Newman-Keuls ao nível de significância de 0,05.

2210 **Figura 1**

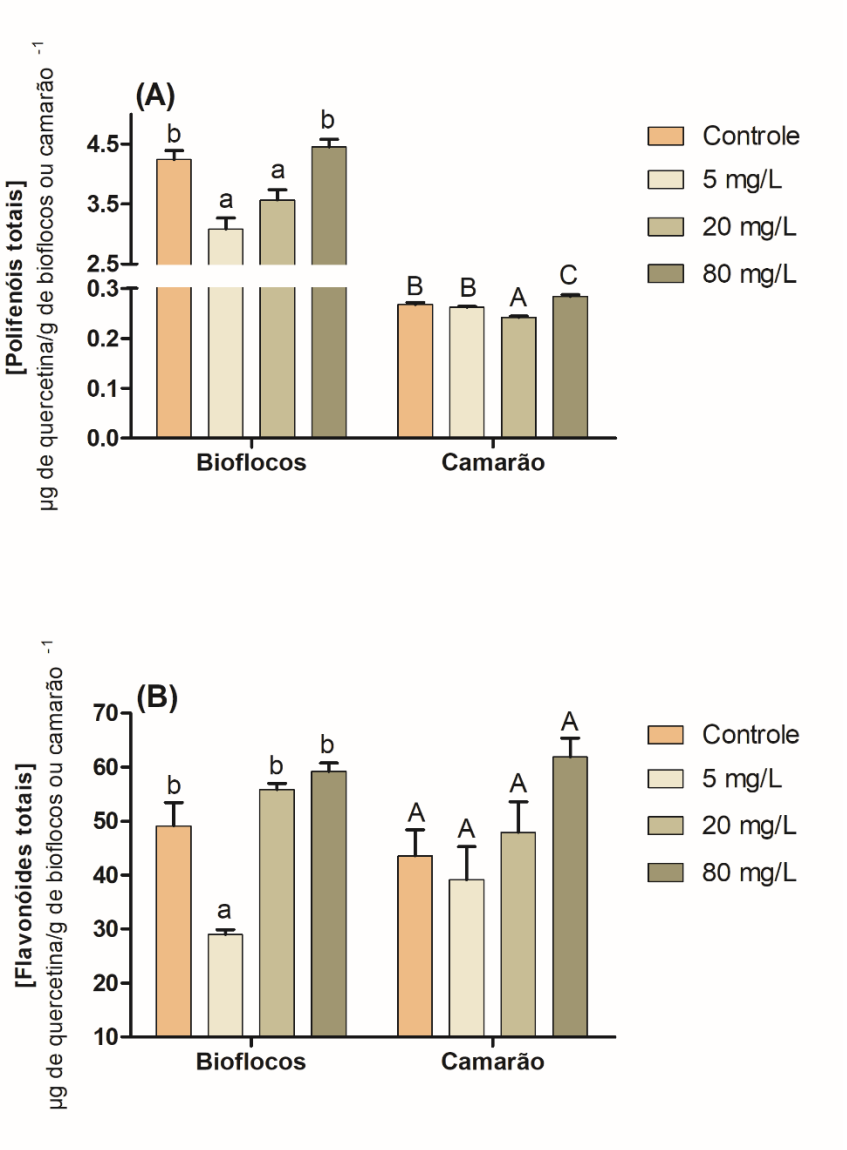


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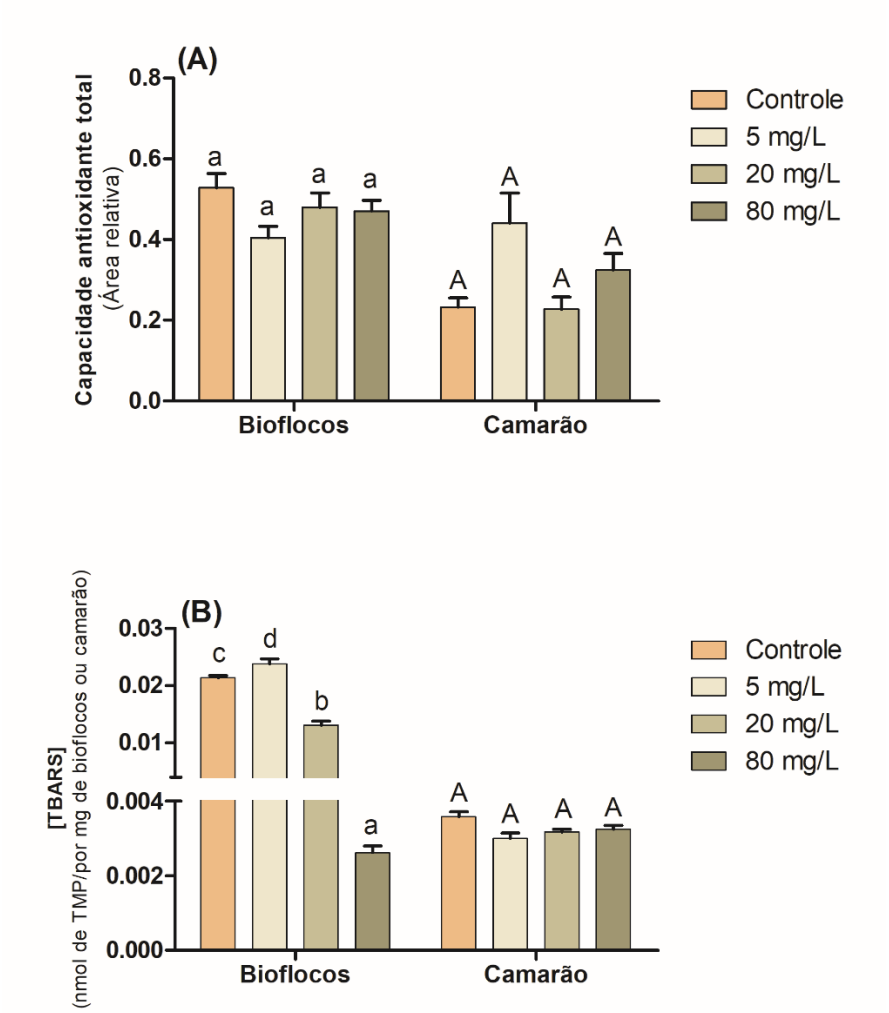
Figura 2


(A)
Capacidade antioxidante total
(Área relativa)
0.8
0.6
0.4
0.2
0.0
a
a
a
a
A
A
A
A
Bioflocos
Camarão
Controle
5 mg/L
20 mg/L
80 mg/L
(B)
[TBARS]
(nmol de TMP/por mg de bioflocos ou camarão)
0.03
0.02
0.01
0.004
0.002
0.000
c
d
b
a
A
A
A
A
Bioflocos
Camarão
Controle
5 mg/L
20 mg/L
80 mg/L

CONCLUSÃO GERAL

Nos experimentos realizados no Capítulo 1 e 2, os valores médios dos parâmetros de qualidade de água permaneceram dentro da faixa recomendada para a espécie em cultivo após a adição de diferentes níveis de açaí liofilizado. Em ambos os capítulos verificou-se comportamento semelhante quanto a alcalinização da água de cultivo, pH e concentração de amônia total. O açaí atuou como alcalinizante natural ao manter os valores médios acima de 100 mg $CaCO_3$ L^{-1}, sem a necessidade de correções com bicarbonato de sódio a partir da concentração de 20 mg açaí L^{-1}. A ausência de correções com bicarbonato de sódio ou outro produto representa uma economia dos custos de produção inerentes do cultivo, além de manter constantemente os níveis de alcalinidade de água propícios para o crescimento do camarão. Com a elevação dos valores de alcalinidade, consequentemente os valores de pH também ficaram mais elevados a partir do tratamento de 20 mg açaí L^{-1}.

A administração de 80 mg açaí L^{-1} causou um aumento nos níveis de amônia em relação aos demais tratamentos. A causa da elevação da concentração de amônia ainda não está claro, mas duas hipóteses foram levantadas: (a) que a alta concentração de antioxidantes poder induzir a inibição da primeira fase da nitrificação; (b) e que o consumo direto do açaí pelos camarões pode ter aumentado a excreção de amônia, uma vez que o açaí aliado a ração ofertada teria incrementado os níveis de ingestão proteica. Contudo, mais estudos são necessários para averiguar a possível interferência do açaí nos processos de nitrificação.

Os resultados obtidos na presente tese demonstraram a capacidade dos bioflocos em assimilarem e transferirem os antioxidantes presentes na polpa do açaí liofilizado para os camarões via alimentação. A administração de açaí favoreceu o aumento da capacidade antioxidante e reduziu danos oxidativos nos bioflocos. Nos camarões, minimizou o estresse

oxidativo, aumentou o conteúdo de polifenóis nos tecidos e além disso aumentou a concentração de pigmentos nos cromatófaros dos camarões (capítulo 3), deixando-os mais escuros devido a perda de luminosidade no corpo destes organismos. Outra importante evidência de que os bioflocos serviram como vetor na transferência dos compostos bioativos para os camarões, é o fato da área e altura das microvilosidades intestinais de juvenis terem aumentado significantemente na oferta de 20 mg açaí L^{-1}. O incremento da área e altura das microvilosidades está diretamente relacionado com uma maior absorção e aproveitamento dos nutrientes presentes da dieta. Logo, conclui-se que os compostos bioativos presentes no açaí estimularam o desenvolvimento da estrutura intestinal dos camarões juvenis.

Em relação aos parâmetros zootécnicos, foi observado que dependendo do estágio de vida dos camarões (pós-larvas/juvenis), os diferentes níveis de açaí liofilizado adicionados ao cultivo implicaram em diferentes respostas quanto a sobrevivência. Em pós-larvas, um aspecto de grande relevância desse estudo foi o aumento da sobrevivência em todos os tratamentos que receberam açaí, uma vez que na fase de larvicultura este é um dos objetivos mais buscados na produção (Capítulo 1). Entretanto, para camarões juvenis, a concentração de 80 mg açaí L^{-1} apresentou alta mortalidade (Capítulo 2). A hipótese inicial do presente estudo era que o açaí liofilizado adicionado diretamente à água de cultivo seria absorvido e metabolizado pelos bioflocos, servindo de alimento indireto para os camarões. Entretanto, segundo observações visuais durante o período experimental do Capítulo 1, parte do açaí era consumido diretamente pelas pós-larvas. Logo, pode-se considerar que o aumento da taxa de sobrevivência está relacionado não apenas a ação dos compostos bioativos deste fruto, mas também a uma maior oferta de alimento ministrada aos camarões dos tratamentos com açaí. Já no caso dos camarões juvenis, devido à ausência de observações visuais, não se pode afirmar que os camarões se alimentavam diretamente de grande parte do açaí ofertado, como

observado no Capítulo 1. Contudo, ainda não está claro a razão da alta mortalidade no tratamento de 80 mg açaí L^{-1}. A hipótese sugerida é que possivelmente tal concentração tenha exercido ação pró-oxidante no cultivo.

De modo geral, é possível concluir que o uso do açaí liofilizado em sistema BFT é capaz de favorecer a produção do camarão, principalmente na fase de berçário, através da melhora das taxas de sobrevivência. Com base nos resultados obtidos, a administração de 20 mg de açaí L^{-1} é a mais recomendada para o cultivo do *P. vannamei* em sistema de bioflocos, ao apresentar melhor desempenho em termos de parâmetros bioquímicos e histológicos.