UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

MARCOS JOSUÉ SCHMITZ

INCLUSÃO DE AÇAÍ NA DIETA DE CAMARÃO *Litopenaeus vannamei* (BOONE 1931) REALIZADA EM SISTEMA DE BIOFLOCOS: EFEITOS NA MODULAÇÃO DA TOXICIDADE DA CIANOTOXINA NODULARINA.

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SUMÁRIO

DEDICATÓRIA

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"Não cruze os braços diante de uma dificuldade, pois o maior homem do mundo morreu de braços abertos.". (Bob Marley)

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RESUMO EXPANDIDO

Dentre as espécies cultivadas mundialmente, o camarão branco *Litopenaeus vannamei* tem sido o crustáceo mais cultivado. Nos últimos anos, a carcinocultura tem apresentado grande interesse na produção de camarões em Sistema de Bioflocos. Dentre os diversos benefícios que o sistema oferece, o principal motivo pode ser considerado o aumento da produtividade de camarões no cultivo. Esse aumento da produtividade é devido as elevadas densidades de camarões estocados nos viveiros de cultivo que o sistema permite. Em suma, os benefícios que este sistema oferece estão principalmente relacionados a presença de microorganismos nos bioflocos, como: Copépodos, Protozoários, Rotíferos e Bactérias. Estes organismos são principalmente relacionados a uma segunda fonte de alimento para o camarão, enquanto as bactérias também são relacionadas ao fato de possibilitarem a reciclagem de nutrientes dentro do sistema.

Dentro do reino monera, à qual as bactérias fazem parte, existem as cianobactérias. Estas cianobactérias são organismos procarióticos, ou seja, apresentam estrutura celular semelhante a estrutura celular bacteriana. Também são organismos fotossintetizantes, capazes de gerar sua própria energia dentro dos fotossistemas 1 e 2. Sua cor é devida a presença de alguns pigmentos, como: clorofila, ficocianinas e ficoeritrinas, estas refletem a cor verde, azul e vermelho, respectivamente. Algumas destas cianobactérias apresentam como mecanismos de defesa frente a predadores, a capacidade de produzir toxinas, como por exemplo hepatotoxinas, que atuam preferencialmente na região gastrointestinal do organismos predador; as Neurotoxinas, que atuam causando efeitos na região do sistema nervoso; e por último, as dermatotoxinas, que em contato com as membranas celulares, são capazes de causarem irritações na pele. As cianobactérias, ao encontrarem um ambiente, neste caso um viveiro de produção de camarões, com altas concentrações de nutrientes, elevadas temperaturas, e grandes períodos de luminosidades, tornam este ambiente propício para a sua proliferação, neste caso, formando um *bloom* de cianobactérias.

A *Nodularia spumigena,* é uma cianobactéria marinha, mas também encontrada em regiões estuarinas. Esta apresenta como mecanismo de defesa, a produção da cianotoxina Nodularina. Esta toxina faz parte de um grupo maior, chamado de hepatotoxinas. Uma característica curiosa deste grupo, é que estas toxinas apresentam resistência a degradação por temperaturas elevadas. Devido a isto, é de extremamente preocupante o consumo de alimentos aquáticos, devido ao fato destes organismos

(peixes, ostras, camarões, moluscos e etc.) possivelmente estarem contaminados com esta cianotoxina.

A nodularina é um pentapeptídeo cíclico, assim como a microcistina (toxina que também faz parte do grupo das hepatotoxinas). Devido sua estrutura cíclica, confere a esta molécula, maior resistência a degradação por proteínas dentro do organismo, o que aumenta as chances destas toxinas serem bioacumuladas pelo organismo predador. Para a nodularina, ainda não se tem descrito o mecanismo de detoxificação que os organismos apresentam, entretanto, devido a semelhança que a nodularina apresenta quando comparada a microcistina, sugere que a nodularina seja eliminada do organismo, pela mesma via que as microcistinas são eliminadas.

Estudos prévios já evidenciaram o efeito negativo que estas toxinas causam em organismos: geração de espécies reativas de oxigênio, diminuição dos teores de glutationa reduzida (GSH), aumento da atividade da enzima glutationa S- transferase (GST) e também a peroxidação lipídica (Bouaıcha, N., & Maatouk, I.,2004) (Pflugmacher, S., Olin, M., & Kankaanpää, H.,2010) (Pflugmacher, S., Olin, M., & Kankaanpää, H., 2007).

Entretanto, alguns trabalhos também já evidenciam o efeito de quimioproteção de algumas substâncias frente a exposição a estas toxinas (Amado *et al.* 2011). A quimioprevenção, pode ser denominada pelo uso de substâncias químicas visando a proteção do organismo contra efeitos tóxicos e doenças. Amado *et al.,* (2011), mostrou em seu trabalho, que os organismos expostos a microcistina, tratados com ácido lipóico, tiveram um aumento significativo na atividade da enzima glutationa S- transferase, sendo que esta enzima atua principalmente a desintoxicação do organismo.

Diante disto, surgiu-se a ideia de se trabalhar com o fruto *Euterpe oleracea*, mais conhecido como açaí. Um fruto amazônico, sua cor roxo escuro, é devido a presença de antocianinas, a qual juntamente com flavonoides e polifenóis conferem alta capacidade antioxidante (Schauss, 2016).

Deste modo, o objetivo do trabalho foi avaliar o efeito da adição do açaí na dieta do camarão como uma estratégia quimioprotetora no camarão *L. vannamei* exposto a cianotoxina nodularina (Nod), através das análises de determinação da concentração de toxina no músculo do camarão, também avaliar a concentração de glutationa reduzida (GSH), avaliar a atividade da enzima glutationa S- transferase, avaliar a peroxidação lipídica e também a oxidação de proteínas.

Previamente, foram elaboradas duas dietas experimentais de forma isoprotéica e isoenergética, com teor proteico de 35% de proteína bruta e 9% de lipídios (Tabela 1), uma sem inclusão de açaí (controle) e outra com inclusão da polpa do fruto açaí liofilizada (10% p/p).

Tabela 1. Formulação e composição proximal das dietas experimentais

Ingredientes (%)	**Dietas**	
	Controle	**Açaí**
Farinha de peixe[a]	28,50	28,50
Farelo de soja	23,90	21,90
Levedura de cerveja	5,00	5,00
Amido de milho	21,60	18,84
Farelo de trigo	5,60	5,60
Óleo de peixe[b]	4,90	0,66
Mistura mineral e vitamínica[c]	1,00	1,00
Colesterol	0,50	0,50
$Ca(H_2PO_4)_2$	2,00	2,00
Celulose	7,00	6,00
Polpa de açaí liofilizado	0,00	10,00
Composição proximal (%)		
Matéria Seca	97,16	96,79
Proteína Bruta	35,39	35,73
Extrato Etéreo	9,46	9,23
Cinza	10,36	10,54
Fibra Bruta	5,45	5,30
ENN[d]	39,34	39,20
Energia Bruta (kj g^{-1})[e]	16,05	15,99

[a] Valores analisados da Farinha de Peixe (como % da matéria seca): 93,59 de matéria seca; 71,46 de proteína bruta; 4,47 de lipídios; 16,28 de cinza; 0,71 de fibra bruta. Empresa (RS, Brasil).

[b] Campestre Ind. E Com. De Oleos Vegetais Ltda (São Paulo, SP, Brasil).

[c] Premix M. Cassab, SP, Brasil: Vit. A (500000 UI kg^{-1}), Vit. D3 (250000 UI kg^{-1}), Vit. E (5000 mg kg^{-1}), Vit. K3 (500 mg kg^{-1}), Vit. B1 (1000 mg kg^{-1}), Vit. B2 (1000 mg kg^{-1}), Vit. B6 (1000 mg kg^{-1}) Vit. B12 (2000 mcg kg^{-1}), niacin (2500 mg kg^{-1}), panteonato de cálcio (4000 mg kg^{-1}), ácido fólico (500 mg kg^{-1}), biotina (10 mg kg^{-1}), Vit. C (10000 mg kg^{-1}). Colina (100000mg kg^{-1}), inositol (1000 mg kg^{-1}). Elementos traços: selênio (30 mg kg^{-1}), ferro (5000 mg kg^{-1}), cobre (5000 mg kg^{-1}), manganês (5000 mg kg^{-1}), zinco (9000 mg kg^{-1}), cobalto (50 mg kg^{-1}), iodo (200 mg kg^{-1}).

[d] Calculado por diferença (100 – proteína bruta – extrato etéreo – cinza – fibra bruta).

[e] Energia bruta (kj g^{-1} dieta) = (% proteína bruta x 16,7) + (% estrato etéreo x 37,7) + (% ENN x 16,7).

Foram estocados camarões juvenis da espécie *Litopenaeus vannamei* com peso médio (± erro padrão) inicial de 1,50 ± 0,39g em 6 tanques circulares de polietileno com volume útil de 100 litros cada, dispostos em 2 tratamentos em triplicata, contendo 50 camarões por tanque. O experimento foi realizado em sistema de bioflocos, com aeração constante e salinidade de 25 ppt. Os tratamentos foram designados por duas dietas

experimentais: dieta controle, sem inclusão do fruto do açaí liofilizado; e dieta com 10% de inclusão de açaí (p/p), baseado no trabalho de Silva (2018) para mesma espécie.

Os camarões foram alimentados durante 30 dias duas vezes ao dia. Após os 30 dias, 126 camarões com peso médio de 4,87 ± 0,51g, foram distribuídos entre 18 caixas com volume útil de 14 litros, onde estes animais seriam expostos durante 96 h a três concentrações subletais de nodularina (Controle, 0,25 e 1 μg de Nod/L), baseadas a partir da legislação brasileira para microcistina. A toxina era adicionada diretamente na água, e para a manutenção destas concentrações, a água das caixas eram renovadas a cada 24 horas e era novamente adicionados os respectivos conteúdos de toxina na água.

Após as 96 horas de exposição, os animais foram eutanasiados em nitrogênio líquido, dissecados e retirados os órgãos: Hepatopâncreas, músculo e brânquias.

No final do experimento foi avaliado a bioacumulação da toxina no músculo do camarão através de cromatografia líquida de alta eficiência. Também foi avaliada a concentração do antioxidante glutationa reduzida (GSH), no hepatopâncreas, músculo e nas brânquias, utilizando ácido 5,5-ditio-bis-(2-nitrobenzóico), DTNB, e avaliando a absorbância em 405nm. Com o mesmo método foi avaliação a concentração de grupos sulfidrilas associados à proteínas. Também foi dosada a peroxidação lipídica nos 3 tecidos, avaliado pelo método TBARS e, quantificada por fluorimetria. Finalmente, foi medida a atividade da enzima glutationa-S-transferase (GST) espectrofotometricamente. Os dados foram analisados estatisticamente utilizando um modelo linear com componentes de variância (fatores: pré tratamento com açaí e concentrações de nodularina) seguidos pelo teste de Newmann-Keuls.

A inclusão com açaí na dieta foi capaz de aumentar os níveis de glutationa reduzida (GSH), diminuir os níveis de peroxidação lipídica e diminuir os teores de grupos sulfidrila ($P<0,05$).

Na análise da peroxidação lipídica nas amostras de hepatopâncreas, observou-se redução significativa do conteúdo de TBARS nos organismos tratados com açaí (0,13 ± $5,1x10^{-4}$ nmol/mg de tecido) comparados com os que não receberam açaí (0,16 ± $5,2x10^{-4}$ nmol/mg de tecido) ($P <0,05$).

Na análise de determinação da atividade da enzima GST, não foi encontrada diferenças estatísticas em nenhum dos tratamentos ($P>0,05$).

Os dados da análise de GSH nas amostras de hepatopâncreas dos animais que receberam açaí em sua dieta apresentaram um aumento significativo (0,46 ± 0,03 nM GSH / mg de proteína) em relação ao grupo que não recebeu açaí na dieta (0,27 ± 0,03

nM GSH / mg de proteína) (P <0,05). Entretanto, a exposição a toxina não causou efeito na concentração de GSH no organismo (P>0,05).

Nas amostras de músculo, a presença de açaí na dieta reduziu a concentração de grupos sulfidrilas associados às proteínas (5,99 ± 0,24 μmol equiv GSH/mg de proteína), quando foram comparadas ao grupo que não recebeu açaí em sua dieta. (6,99 ± 0.32 μmol equiv GSH/mg de proteína) (P<0,05). Mas a exposição à toxina não causou diferenças na concentração dos grupos sulfidrila associados às proteínas (P>0,05).

É notório o fato de que organismos aquáticos quando expostos a florações de cianobactérias estão sujeitos a apresentar efeitos negativos em seus parâmetros bioquímicos e fisiológicos, podendo levar inclusive a morte. Estas florações podem acontecer naturalmente em ambientes oligotróficos, ou seja, em ambientes característicos por serem pobres em nutrientes, ou também podem acontecer em ambientes ricos em nutrientes e temperaturas elevadas. No caso em estudo, a toxina nodularina apesar desta toxina ser hepatotóxica (Zimba et al; 2006) também têm sido reportados efeitos em outros tecidos e órgãos (Žegura, Zajc, Lah & Filipič, 2008).

Neste estudo, foi verificada a presença da toxina no músculo do organismo, porém as concentrações evidenciadas nas amostras estão abaixo do limite para consumo humano estabelecido no Brasil. Este limite de consumo está baseado na legislação brasileira para microcistinas, visto que a nodularina assim como a microcistina são classificadas como hepatotoxinas. O valor limite de 1 $\mu g.L^{-1}$, adotado pela Portaria 518 de 2004, do Ministério da Saúde, foi estipulado pela Organização Mundial da Saúde com base na variante LR de microcistina. Em camundongos e porcos foi estabelecido o valor de 0,04 $\mu g.Kg^{-1}$ como a dose oral máxima diária aceitável (Chorus & Bartram, 1999), comparando com os resultados do presente trabalho, pode ser visto que os teores de nodularrina no músculo ficaram dentro do limite permitido (0,00475±0007 μg.g)para os organismos da dieta controle, E (0,00472±0047 μg.g) para os animais que receberam açaí na sua dieta. Tanto a inclusão de açaí quanto a exposição à toxina não foram capazes de induzir diferenças significativas (P>0,05) na concentração de toxina encontrada no músculo do organismo. Entretanto, um fator curioso, pode ser notado, onde nos organismos controles não expostos a nodularina, foi possível detectar a presença de toxina no músculo, sugerindo que estes organismos já estejam sendo expostos previamente nos tanques de berçário da Estação Marinha de Aquicultura.

Neste trabalho, pôde ser observado que a inclusão de biomoléculas antioxidantes fornecida através do fruto açaí na dieta dos organismos, permitiu ao camarão enfrentar o

estresse causado pela toxina, minimizando seus efeitos negativos sobre o estresse oxidativo, e trazendo qualidade na saúde do animal, isto pode ser afirmado com base nos dados de conteúdo de GSH no hepatopâncreas, levando em conta que as hepatotoxinas atuam preferencialmente na região gastrointestinal.

A detoxificação do organismo ocorre pela atividade de algumas enzimas, como por exemplo as enzimas do grupo das GSTs, que conjugam cianotoxinas como as microcistinas com o grupo SH (tiol) da molécula de GSH, facilitando a excreção (Jayaraj, Anand & Rao, 2006). Entretanto, no presente estudo, não foi verificado alterações na atividade da GST, o que pode ser consequência do tempo de exposição e/ou as concentrações de toxina utilizadas no trabalho. Também é importante salientar que até o momento não existem trabalhos que indiquem que a nodularina é substrato das GSTs. Uma alternativa rápida para ver se a nodularina é de fato substrato das GSTs seria avaliar através de ensaios de docagem molecular.

Estudo anterior demonstrou aumento significativo no dano lipídico em molusco *Perna viridis* expostos a *Nodularia spumigena* durante 3 dias (Davies *et al*., 2005). Entretanto, no presente estudo foi verificado a diminuição dos níveis de peroxidação lipídica no hepatopâncreas, evidenciando a atuação do açaí como fonte quimioprotetora frente a toxicidade da toxina.

Ao todo, o tratamento com açaí foi capaz de melhorar a capacidade antioxidante do camarão, restando, para trabalhos futuros desafiar ao organismo a concentrações mais elevadas de cianotoxinas e analisar mais apropriadamente a diminuição da concentração dos grupos sulfidrila associados à proteínas observado no músculo dos camarões que receberam açaí, visto que esta é uma análise de extrema importância quando observado o estado redox da célula, e que pode quantificar a concentração de proteínas oxidadas, o que acarretaria na perda de função destas proteínas.

PALAVRAS-CHAVE: Cianobactéria, Crustáceos, Antioxidantes, Quimioproteção, Fruto.

Capítulo 1

INCLUSÃO DE AÇAÍ (*Euterpe olaracea*) NA DIETA DE CAMARÃO *Litopenaeus vannamei* (BOONE 1931) REALIZADA EM SISTEMA DE BIOFLOCOS: EFEITOS NA MODULAÇÃO DA TOXICIDADE DA CIANOTOXINA NODULARINA

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Obs: Tabelas e Figuras foram dispostas no corpo do texto para facilitar a leitura.

Açaí (*Euterpe olaracea*) inclusion in shrimp *Litopenaeus vannamei* (BOONE 1931) reared in biofloc system: Effects in the toxicity modulation of cyanotoxin nodularin

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ABSTRACT

The experiment considered the inclusion of 10% of açaí *Euterpe olaracea* in the diet of shrimp *Litopenaeus vannamei*, besides the control diet, without açaí. The feed was given twice a day for 30 days and, afterward, the shrimps were subdivided into another 3 treatments (Control, 0.25 and 1 μg/L of hepatotoxin nodularin), both in the group that received inclusion with açaí and those fed with the control diet. At the end of the experiment, it was evaluated the concentration of reduced glutathione (GSH), sulfhydryl groups associated with proteins (P-SH), lipid peroxidation (TBARS method), the activity of glutathione-S-transferase (GST) in muscle, hepatopâncreas and gills, and nodularin accumulation in muscle. The inclusion of açaí in the diet was able to increase the levels of GSH in the hepatopancreas and gills and to decrease the levels of lipid peroxidation in the muscle of nodularin-exposed shrimps in respect to the control group. Nodularin exposure did not affect P-SH levels in the analyzed organs, although açaí treatment reduced P-SH levels in muscle. TBARS muscle concentration was reduced in shrimps fed with açaí and exposed to nodularin. Finally, nodularin exposure was not followed by toxin accumulation in muscle but, notably, it was detected measurable levels in control groups (fed or not with açai). In general, the results showed that açaí was able to infuse the antioxidant effects of shrimp, and it was also able to reduce the levels of TBARS in the muscle when exposed to nodularina, suggesting the use of açaí as chemoprotector.

Keywords: Cyanobacterian, Crustaceans, Antioxidant, Quimioprotection, Acumulation, Fruit.

1.Introduction

Aquaculture has been one of the most efficient sources for human food generation. Carcinoculture accounts for about 55% of world production, being the main producers China, Thailand, Indonesia, India, Vietnam, and Brazil (FAO, 2016). The shrimp *Litopenaeus vannamei* (Boone 1931) is the most cultivated species because of its fast-growing, high survival rate, and tolerance to high stocking density (Krummenauer *et al.,* 2011).

Due to concerns about the use of large water volumes for food production (Gaona *et al.,* 2011; Wang *et al*., 2015), the development of zero-exchange systems, as the Biofloc Technology (BFT), has brought great benefits because of the reduction of water use with a parallel decrease in the introduction of pathogens that can enter in the production system with the water renewal (Emerenciano *et al.*, 2013).

BFT system relies on the use of carbon and nitrogen required for bacterial growth, transforming nitrogen into microbial biomass that represents a food source for the shrimp (Avnimelech, 1999). This system is based on the interaction between organic matter and a great variety of organisms such as phytoplankton, rotifers, ciliate, bacteria, and copepods (Ray *et al.,* 2010). The use of excreta and also of foods not consumed by nitrifying bacteria, results in the oxidation of ammonia, generating nitrite and nitrate, promoting the recycling of nutrients. This fact allows cultivating shrimps at high densities without the exchange of water, thus making it a sustainable activity when compared to traditional procedures (Krummenauer *et al.,* 2011).

However, biotic and abiotic factors can cause problems in these systems, because this environment is rich in nutrients and the high densities of animals can eventually favor the spread of diseases or pathogenic organisms, including cyanobacteria, that can affect the productivity. Pacheco *et al*. (2016) reported that

blooms of the cyanobacterium *Nodularia spumigena* affected the growth of reared *L. vannamei* white shrimp.

High temperature and nutrients concentration allow the exacerbated growth of cyanobacteria, negatively influencing the oxygen availability within the cultivation system and, in extreme cases, entail the death of all organisms present. Further lysis of cyanobacteria can release toxins such as microcystins (MCs) and nodularin (Nod), hepatotoxins that represent a threat to human and animal health (Amado and Monserrat, 2010; Pearson *et al.*, 2010). The genus *Nodularia, Microcystis, Lyngbya*, and *Oscillatoria* are known for the production of these hepatotoxins, which have been extensively studied (Yuan *et al.,* 2015; Pacheco *et al*., 2016).

Cyanobacteria blooms can generate reactive oxygen species (ROS) by promoting hyperoxia/anoxia associated with photosynthesis and respiration of these organisms (Amado and Monserrat, 2010). It is known that nodularins and microcystins are toxins that can affect not only liver (or hepatopancreas) but also other organs, inducing antioxidant responses in aquatic organisms, including antioxidant enzymes such as catalase (CAT), glutathione S-transferase (GST), superoxide dismutase (SOD) (Pinho *et al*., 2005) and reducing the levels of the antioxidant reduced glutathione (GSH) by the conjugation of this antioxidant with hepatotoxins (Gonçalves-Soares *et al.,* 2012). Other effects already reported in several experimental organisms are oxidative damage such as lipid peroxidation and DNA oxidation (Štern *et al.,* 2019).

In front of reactive oxygen species (ROS) generation, the organisms need to intercept and/or eliminate them using endogenous and exogenous antioxidants, being these last acquired through diet. The inclusion of antioxidants as lipoic acid in the diet of carp (*Cyprinus carpio*, Cyprinidae) provided a protective effect when exposed to

microcystins (Amado *et al.,* 2011) and in HT22 cellsthe same antioxidant mitigated the toxicity of saxitoxin (Ramos *et al*., 2018).

Currently, great attention is given to studies with the plant *Euterpe oleracea* Mart, popularly known as “açaí”. The fruit offers many several benefits to human health due to the high amount of antioxidant molecules that it possesses (Lichtenthäler *et al*., 2005; da Silva *et al.*, 2017). The fruit is rich in phenolic compounds, such as anthocyanins, quercetins and flavonoids (Del Pozo-Insfran *et al.*, 2004; Kang *et al*., 2010; Heinrich *et al.*, 2011).

Taking into account that, as mentioned above, the inclusion of antioxidants in diet of aquatic organism can ameliorate the toxic effects of cyanotoxins, we evaluated the effects of the inclusion of lyophilized açaí in *L. vannamei* diet prior to nodularin exposure. Antioxidant and oxidative damage responses was measured in gills, muscle, and hepatopancreas of this organism.

2.Material and Methods

Prior to storage, the animals were being kept in nursery tanks of the Aquaculture Marine Station. It was employed six water reservoirs with a capacity of 100 liters, where 20% of the volume corresponded to the inoculum of mature biofloc from a superintensive rearing system. Fifty juvenile shrimps (1.5 ± 0.39 g) were stored in each unit at a density of 500 organisms/m^3 of water. The total 300 shrimps were divided into two groups: juveniles fed with a ration containing 10% of açaí inclusion and juveniles fed only with ration (no açaí added) (see below the feed composition). Lyophilized açai (*E. oleracea*) was purchased from the “Company Amazon Comércio de Açaí Liofilizado e Exportação LTDA”, located in Belém do Pará, Brazil. The ration offer was made twice a day (9:00 e 17:00 h). After 30 days, 126 shrimps with a mean weight of

4.87 ± 0.51 g were relocated to another 18 different experimental units with 14 L of useful volume. The group of shrimp fed with açaí was subdivided into 3 different toxin treatments: Control (0 µg/L), 0.25 µg/L Nodularin and 1 µg/L Nodularin, in triplicate, totaling 9 experimental boxes. Concomitantly, the animals that did not receive açaí in the feeding were transferred to other experimental units and exposed to the same nodularin concentration already mentioned. Each experimental unit contained seven shrimps, totaling twenty-seven organisms per treatment. The exposure time was 96 h (Yuan *et al.*, 2015). After the exposure period, the animals were euthanized in liquid nitrogen, and their organs (hepatopancreas, muscle, and gills) dissected. After, all samples were kept in ultra-freezer at -80 °C for future analysis. The salinity and temperature were maintained at 25± 1.30 and 28 ±1.34ºC, respectively, with a photoperiod of 12 h light/12 h dark. The temperature, salinity, pH and dissolved oxygen were measured daily. The salinity was measured with an ATAGO® refractometer, temperature and dissolved oxygen with a digital multi-parameter oximeter (YSI®-550A) and pH with a digital pH meter (± 0.01, YSI®-pH100). Determinations of alkalinity, ammonia, nitrite were performed daily. The alkalinity was based and analyzed following the methodology proposed by APHA (1998). Concentrations of nitrate and phosphate were measured weekly during the experiment period (Aminot & Chaussepied, 1983).

The two types of rations used in the work were isoproteic and isoenergetic, with 38% of crude protein (CP) and 8% of lipids, respectively. Previously, the bromatological composition of the ingredients was analyzed, according to the methodology described by AOAC (1999). Dry matter (DM) analysis was performed in an oven. For ash (MM), the samples were pre-calcined and then transferred to the muffle. PB was performed according to the Kjeldahl methodology. The ethereal extract

(EE) was obtained employing a Soxhlet extractor. For the crude fiber (FB), acid and basic digestion of the sample was used. For non-nitrogenous EE were calculated by the difference of the values added of PB, EE, MM, and FB.

The rations were prepared by mixing the ingredients fish meal, soybean meal, brewer's yeast, corn starch, wheat bran, fish oil, vitamin and mineral blend, cholesterol and lyophilized acai berry at the end of the preparation and subsequent pelleting (Table 1). Then, it was transferred to oven drying at 50 °C. The final rations were stored at -20 ° C until use.

Table 1. Dietary composition (g/100 g) of ingredients employed in the experimental diets offered to shrimp *Litopenaeus. vannamei* with different inclusion levels of lyophilized açaí *Euterpe oleracea* (0.0; and 10;0 %, W/W)

Ingredientes (%)	**Dietas**	
	Control	**Açaí (10%)**
Fish meal[a]	28.50	28.50
Soybean meal	23.90	21.90
Brewer's yeast	5.00	5.00
Corn Starch	21.60	18.84
Wheat mea	5.60	5.60
Fish oil[b]	4.90	0.66
Mineral/Vitamin Mixture[c]	1.00	1.00
Cholesterol	0.50	0.50
$Ca(H_2PO_4)_2$	2.00	2.00
Cellulose	7.00	6.00
Lyophilized açaí	0.00	10.00
Proximal composition (%)		
Dry matter	97.16	96.79
Crude protein	35.39	35.73
Ether extract	9.46	9.23
Ashes	10.36	10.54
Fiber	5.45	5.30
NFE[d]	39.34	39.20
Gross energy (kj g^{-1})[e]	16.05	15.99

[a] Analyzed values of fish meal (as % of dry matter): 93.59 of dry matter; 71.46 of crude protein; 4.47 of lipids; 16.28 of ashes; 0.71 of crude fiber. Empresa (RS, Brasil).
[b] Campestre Ind. E Com. De Oleos Vegetais Ltda (São Paulo, SP, Brasil).
[c] Premix M. Cassab, SP, Brasil: Vitamin A (500.000 Ul/kg), Vit. D3 (250.000 Ul/kg), Vit. E (5.000 mg/kg), Vit. K3 (500 mg/kg), Vit. B1 (1.000 mg/kg), Vit. B2 (1.000 mg/kg), Vit. B6 (1.000 mg/kg), Vit. B12 (2.000 mcg/kg), Niacin (2.500 mg/kg), Calcium pantothenate (4.000 mg/kg), Folic acid (500 mg/kg), Biotin (10mg/kg), Vit. C (10.000 mg/kg), Choline (100.000 mg/kg), Inositol (1.000 mg/kg), Selenium (30 mg/kg), Iron (5.000 mg/kg), Copper (1.000 mg/kg), Manganese (5.000 mg/kg), Zinc (9.000 mg/kg), Cobalt (50 mg/kg), Iodine (200 mg/kg).

[d] Calculated value. NFE = 100 – (Crude protein + crude lipid + ash +moisture).
[e] Gross energy (kj g^{-1} diet) = (% crude protein x 16,7) + (% ether extract x 37,7) + (% NFE x 16,7).

2.1.Organs homogenization

Organs were weighed and homogenized (1:5, P/V) in crustacean buffer consisting of Tris-HCl (100 mM, pH 7.75) plus EDTA (2 mM) and Mg^{2+} (5 mM), all dissolved in MilliQ water (Pinho *et al.,* 2005), without the use of fluoreto de fenilmetano sulfonil (PMSF), since the use would harm the methodology of some protocols (serine protease inhibitor). Thereafter, the homogenized organs were centrifuged at 10.000 x g for 20 minutes at 4 °C and the supernatant kept for all measurements described below. The total protein content was determined by the Biuret method (λ = 550 nm; Total Protein Doles Kit) in triplicate using a microtiter reader (BioTek LX 800) (Amado *et al*., 2009).

2.2.Biochemical analysis

2.2.1.Determination of reduced glutathione (GSH) and protein- sulfhydryl groups

Prior to the analysis, the extracts had their protein concentrations set at 2 mg/mL. For this methodology (Sedlak & Lindsay, 1968), the following solutions were previously prepared: 0.4M Tris-Base buffer adjusted at pH 8.9 and DTNB (5,5-dithio-bis-(2-nitrobenzoic acid)) solution, which was diluted in methanol 100%. The procedure is based on the addition of 240 µL of sample and then 28 µL of TCA (trichloroacetic acid). After mechanical stirring, the samples were centrifuged for 10 minutes using a force of 20,000 x g at a temperature of 4 °C. After centrifugation, for measurement of GSH, 200 µL of 0.4 M Tris-Base pH 8.9 was added to each well of a transparent microplate; 100 µl of the supernatant; and 10 µL of the DTNB solution. The microplate

was incubated at room temperature in the dark for 15 minutes and immediately read in duplicate with the aid of a spectrofluorometer Synergy™ HT at a wavelength of 405 nm.

For determination of the concentration of proteins-sulfhydryl groups (Sedlak & Lindsay, 1968), the pellet (obtained from the centrifugation in the GSH analysis) of the sample was resuspended in 240 µl of crustacean buffer. The reaction was conducted in a transparent microplate, adding in each well 20 µl of the sample extract, 160 µl of 0.2 M Tris-Base at pH 8.2 and 10 µl of DTNB. The plate was incubated at room temperature in the dark for 15 minutes and immediately read in a spectrofluorometer at a wavelength of 405 nm.

2.2.2.Lipid peroxidation

Lipoperoxidation was determined by the fluorometric method described by Oakes and Van Der Kraak (2003), where malondialdehyde (MDA) -a by-product of lipid peroxidation- reacts with thiobarbituric acid (TBA) which, under conditions of high temperatures and acidity, generates a chromogen that can be quantified by fluorimetry. The methodology measured the fluorescence of the samples and a standard curve made of 1,1,3,3-tetramethoxypropane (TMP) (range: 0.0121- 25 nmol of TMP).

The assembly of the glass tube sequences was done as follows: in duplicate were added 41.2 µL of TMP standards and 30, 50 or 100 µL of the homogenized extract (hepatopancreas, gills, and muscle, respectively. Then 20 µl of 35 mM of butylated hydroxytoluene (BHT) was added only to tubes containing samples, including blank. Subsequently, 150 µL of 20% acetic acid solution, 150 µL of 0.8% thiobarbituric acid solution, 50 µL MilliQ water and 20 µL of 8.1% sodium dodecyl sulfate solution was added. Thereafter, the tubes were vortexed and covered with foil and heated in a water bath at 95 °C for thirty minutes. After, the tubes were withdrawn to reach room

temperature (10 minutes), and then added 100 µl of MilliQ water in each tube and then the content transferred to 1.5 ml Eppendorff tubes. Finally, it was added 500 µl of n-butanol, vortexed and after centrifuged at 3000 x g for ten minutes at 15 °C, in order to separate the aqueous and the organic phases. Then, 150 µL of the organic phase was transferred to wells of a white microplate and read in a spectrofluorimeter (excitation and emission lengths of 520 and 580 nm, respectively). The results were expressed in nmol of TMP equivalents per mg of fresh tissue.

2.2.3.Glutathione-S-transferase

Glutathione-S-transferase (GST) activity was determined following the conjugation of 1 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm, as described by Habig *et al.* (1974) and Habig and Jakoby (1981).

2.2.4.Determination concentration of nodularin by High Performace Liquid Chromatography

For the extraction of nodularin in the shrimp muscle, an adaptation of the methodology of Magalhaes and Azevedo (1998) was performed. The shrimps were dissected and the muscle was macerated in 25 mL of absolute methanol and placed in an orbital shaker for 2 hours, and in the refrigerator by at least 15 hours. The material was centrifuged for 20 min at a speed of 5000 rpm. The supernatant was transferred to the separatory funnel, with the addition of 25 mL of 100% n-hexane. The material was agitated and after 5 min the methanolic fraction was removed to an Erlenmeyer, evaporated in a rotary evaporator at 55-60 ºC, and the dry fraction was resuspended in 1 mL of Milli Q water. All solvents were PA grade (Merck).

2.3.Statistical analysis

The data were analyzed using a linear model of variance components (fixed factors: açaí pretreatment and nodularin concentrations; random factor: experimental units) followed by the Newmann-Keuls comparison test. Previously, the pre-requisites of normality and homoscedasticity were evaluated. In all cases, a significance level of 5% was used. Data are presented as a mean ± 1 standard error of the mean.

3.Results

During the exposure period, no mortality was observed in any of the treatments.

3.1.Reduced glutathione (GSH)

In the hepatopancreas, the shrimps that received açaí in their diet presented a significant increase (0.46 ± 0.03 nM GSH/mg of protein) in relation to the group that did not receive açaí in the diet (0.27 ± 0.03 nM GSH/mg of protein) ($P < 0.05$). Nodularin exposure did not cause significant differences in the concentration of reduced glutathione within the organism ($P > 0.05$) (Fig. 1A).

In gills, the animals that received açaí in their diet had a significant increase in GSH content (0.36 ± 0.07 nM GSH/mg of protein) when compared to the group that did not receive açaí supplementation in the diet (0.13 ± 0.02 nM GSH/mg of protein) ($P < 0.05$). However, exposure to nodularin was not able to cause statistical differences compared to control ($P > 0.05$) (Fig. 1B).

In the muscle, similar GSH values were found in shrimps that received açaí in the diet (0.73 ± 0.05 nM GSH/mg of protein) when compared to the group treated with no açaí in the diet (1.02 ± 0.09 nM GSH/mg of protein) ($P > 0.05$). However, exposure to nodularin was not able to cause statistical differences compared to control ($P > 0.05$) (Fig.1).

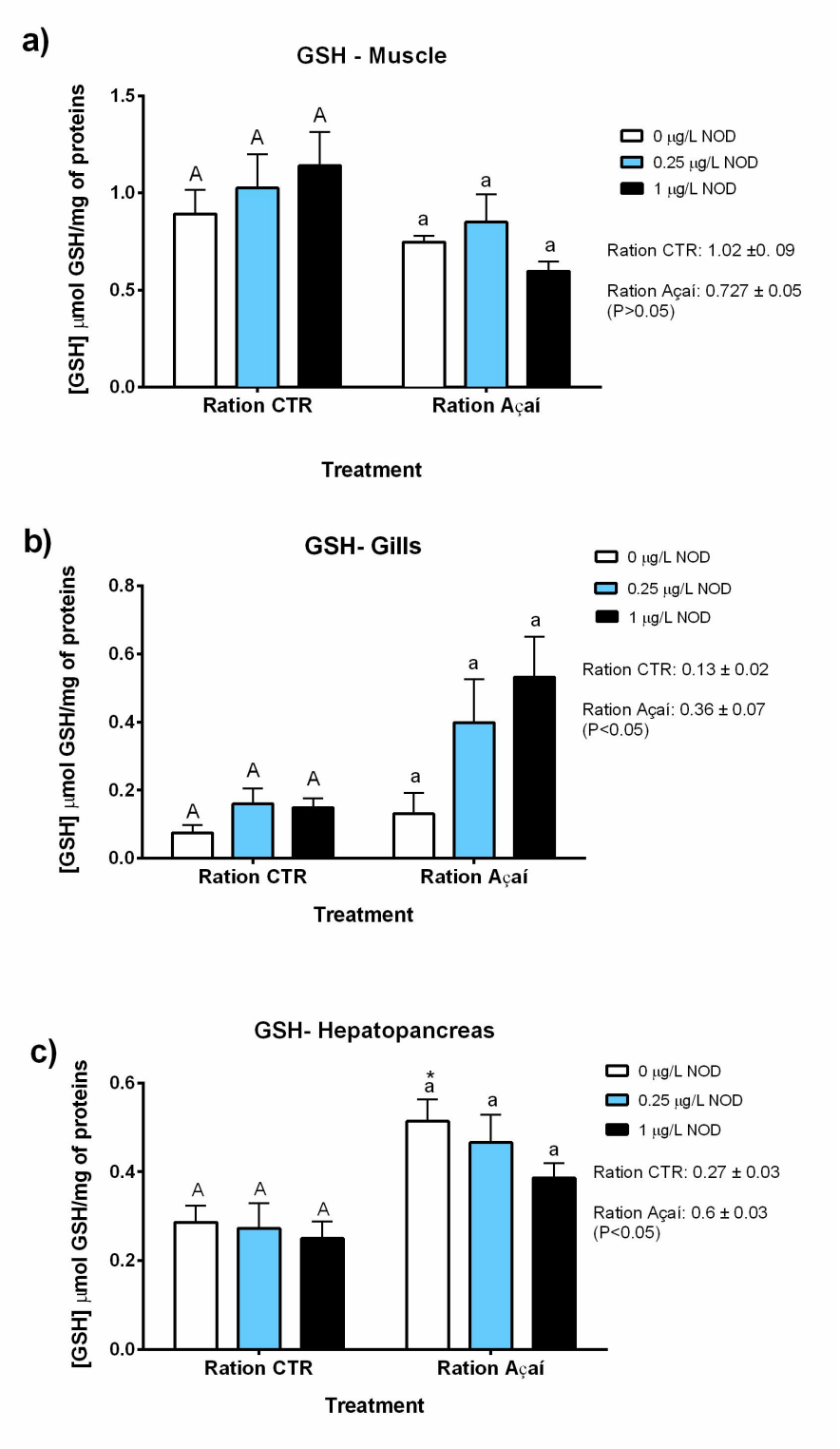

Fig. 1. Determination of reduced glutathione (GSH) content in hepatopancreas (A), gills (B), and (C) muscle in shrimp *L. vannamei* exposed at different concentrations of Nodularin (NOD). Data are expressed as mean ± standard error. Lower case letters (a) and upper case letters (A) in the bars show significant differences within the group fed

with or without açaí. Treatments followed by different letters indicate significant differences by the Newmann-Keuls test ($P < 0.05$).

3.2Glutathione S-transferase (GST) activity

In hepatopancreas, similar GST activity was observed in the group that received açaí (0.29 ± 0.08 nmoles/mg of protein/min) when compared to the group that did not receive açaí in the diet (0.35 ± 0.04 nmoles/mg of protein/min) ($P > 0.05$) (Fig. 2A).

The same was found in gills, where the group fed with açaí (10.70 ± 0.49 nmoles/mg of protein/min) showed similar GST activity that in the organisms that did not receive açaí in the diet (10.13 ± 0.60 nmoles/mg of protein/min) ($P > 0.05$) (Fig. 2B).

Similarly, in the muscle, the açaí treated organisms (8.48 ± 0.63 nmoles/mg of protein/min) did not show a significant difference in the activity of this enzyme when compared to the organisms that were not fed açaí (8.09 ± 0.68 nmoles/mg of protein/min) ($P > 0.05$) (Fig. 2C).

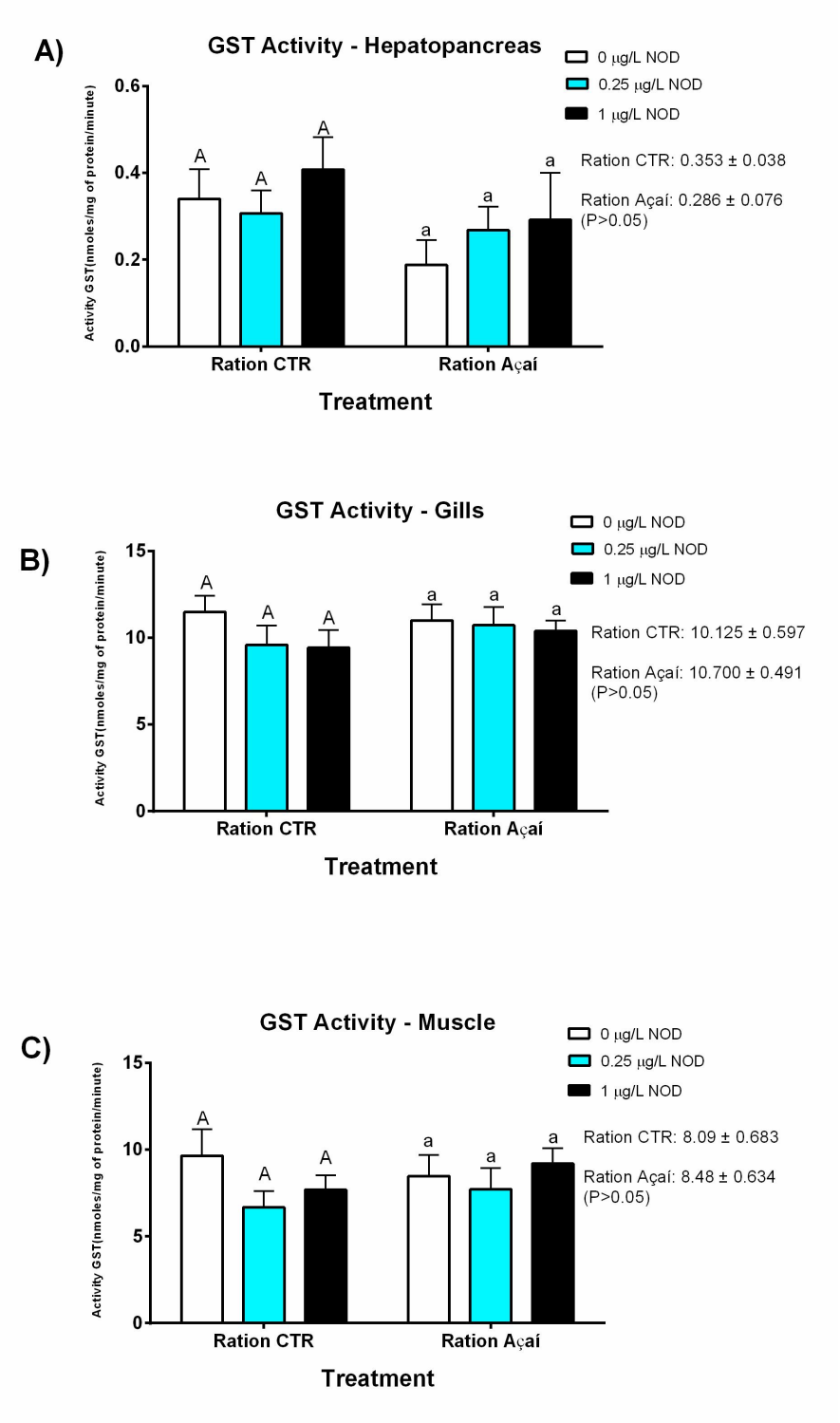

Fig. 2. Glutathione-S-transferase (GST) enzyme activity in hepatopancreas (A), gills (B), and (C) muscle in shrimp *L. vannamei* exposed at different concentrations of nodularin (NOD). Data are expressed as mean ± standard error. Lower case letters (a) and upper case letters (A) in the bars show significant differences within the group fed

with or without açaí. Treatments followed by different letters indicate significant differences by the Newmann-Keuls test ($p < 0.05$).

3.3.Lipid peroxidation (TBARS)

A significant reduction of the hepatopancreas MDA (indicative of lipid peroxidation) content was observed in the açaí-treated organisms ($0.13 \pm 5.1x10^{-4}$ nmol TMP equivalents/mg of fresh tissue) than in control animals ($0.16 \pm 5.2x10^{-4}$ nmol TMP equivalents/mg of tissue) ($P<0.05$). However, nodularin exposure did not affect TBARS levels ($P>0.05$) (Fig. 3A).

In the gills, no statistical differences were observed between the group that received açai in the diet ($0.0012 \pm 1.44x10^{-4}$ nmol TMP equivalents/mg of tissue) versus the group not treated with açaí ($0.0014 \pm 1.11x10^{-4}$ nmol TMP equivalents/mg of tissue) ($P>0.05$) (Fig. 3B).

In the muscle, the group of açaí-fed organisms presented similar TBARS levels ($3.74x10^{-4} \pm 3.60x10^{-5}$ nmol TMP equivalents/mg of tissue) when compared to the group that was not fed açaí in their diet ($5.21x10^{-4} \pm 8.60x10^{-5}$ nmol TMP equivalents/mg of tissue) ($P>0.05$). However, within the group treated with acai, there was a significant decrease in TBARS content in organisms exposed to 0.25 μg/L Nod ($3.29x10^{-4} \pm 5.80x10^{-5}$ nmol TMP equivalents/mg of tissue) and 1 μg/L Nod ($3.57x10^{-4} \pm 6.00x10^{-5}$ nmol TMP equivalents/mg of tissue) when compared to control organisms ($4.38x10^{-4} \pm 6.90x10^{-5}$ nmol TMP equivalents/mg of tissue) ($P<0.05$) (Fig. 3C).

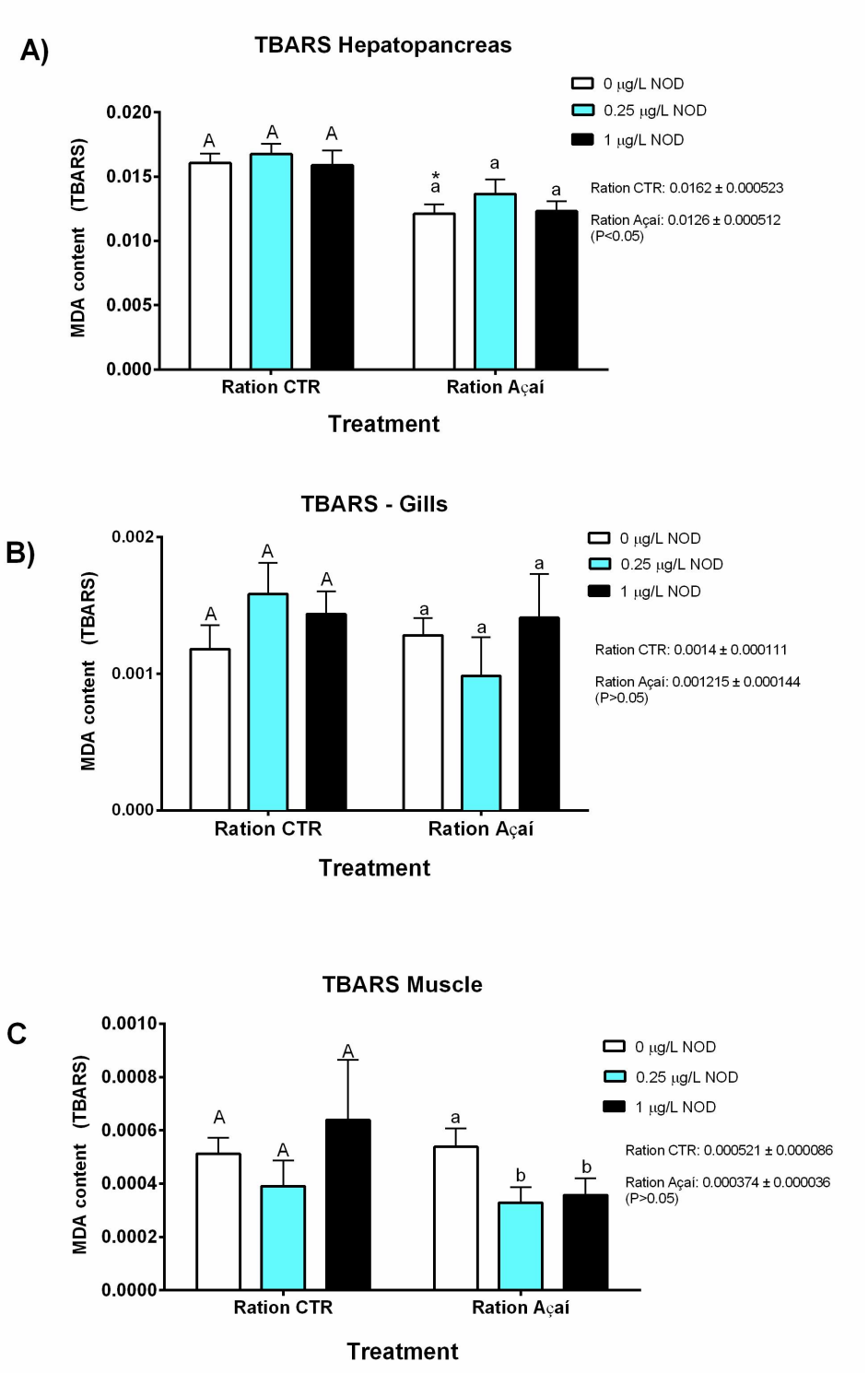

Fig. 3. Evaluation of lipid peroxidation (TBARS method) in hepatopancreas (A), gills (B), and (C) muscle in shrimp *L. vannamei* exposed at different concentrations of Nodularin (NOD). Data are expressed as mean ± standard error. Lower case letters (a) and upper case letters (A) in the bars show significant differences within the group fed

with or without açaí. Treatments followed by different letters indicate significant differences by the Newmann-Keuls test ($p < 0.05$).

3.4.Protein-sulfhydryl groups (P-SH)

The analysis of sulfhydryl groups associated to proteins (P-SH), reflects in the redox state of the cell, that is, the higher the concentration of sulfhydryl groups associated to proteins, the more reduced the cell. Differently, if there is a decrease in the levels of P-SH, it can be said that there is a higher concentration of oxidized proteins, indicating a situation of oxidative stress.

Regarding the analysis of sulfhydryl groups associated with proteins, in the hepatopancreas, similar levels were found in the group that received açai in the diet (1.00 ± 0.21 μmol GSH equivalents/mg of protein) when compared with shrimps that were fed with the control diet (0.58 ± 0.04 μmol GSH equivalents/mg of proteins) ($P > 0.05$). However, exposure to nodularin was not able to cause statistical differences compared to control ($P > 0.05$) (Fig. 4A).

In gills, similar P-SH levels were found in shrimps from the açaí group (2.83 ± 0.20 μmol GSH equivalents/mg of proteins) versus the açaí group (2.50 ± 0.19 μmol GSH equivalents/mg of proteins) ($P > 0.05$). However, exposure to nodularin was not able to cause statistical differences compared to control ($P > 0.05$). (Fig. 4B).

In muscle, the presence of açaí in the diet caused a reduction in the P-SH levels (5.99 ± 0.24 μmol GSH equivalents/mg of proteins) when compared with the group that did not receive açaí in their diet (6.99 ± 0.32 μmol GSH equivalents/mg of proteins) ($P<0.05$). Nodularin exposure did not cause differences in the concentration of P-SH in both experimental groups ($P>0.05$) (Fig. 4C).

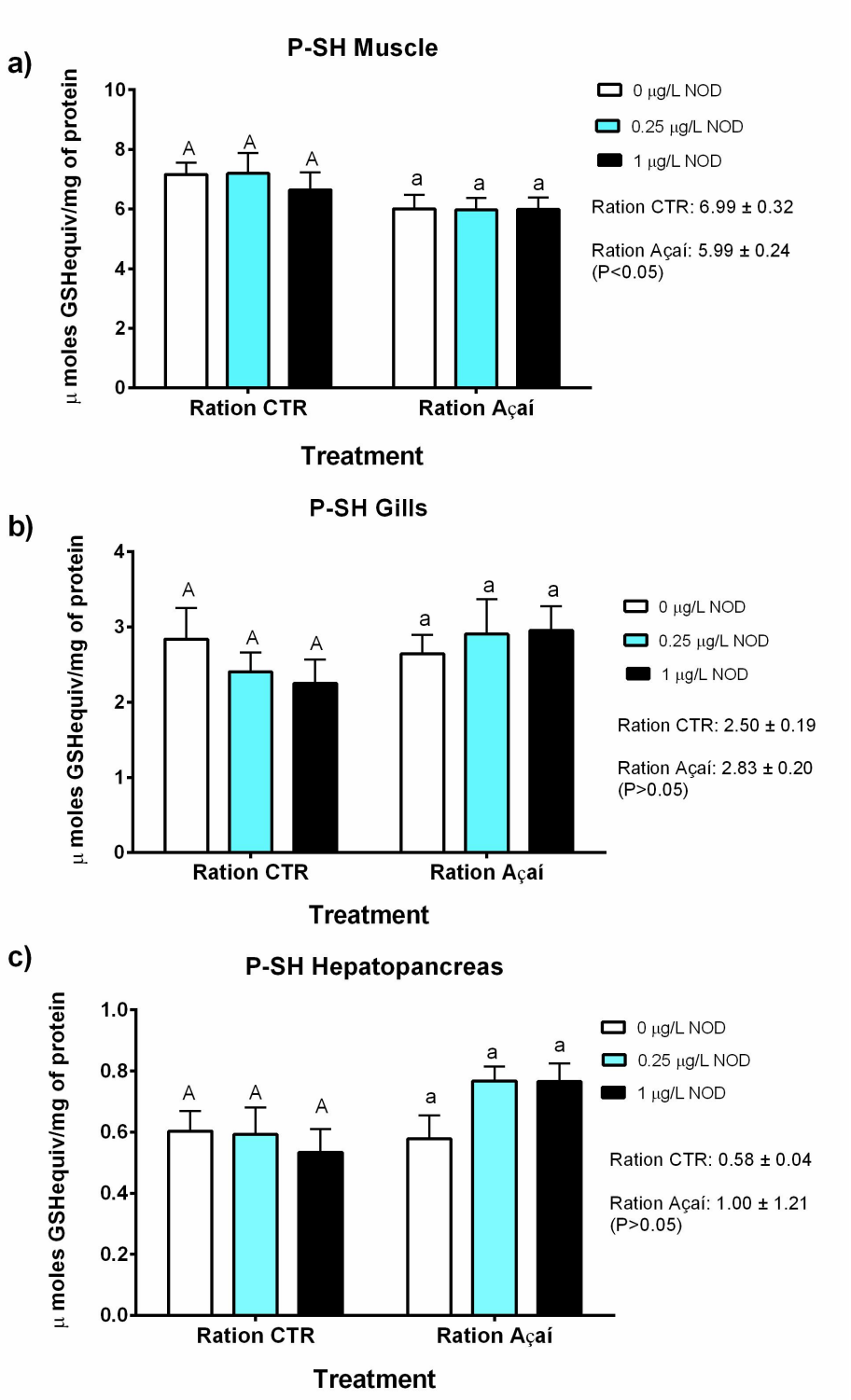

Fig. 4. Concentration of sulfhydryl groups associated with proteins (P-SH) in hepatopancreas (A), gills (B), and (C) muscle in shrimp *L. vannamei* exposed at different concentrations of Nodularin (NOD). Data are expressed as mean ± standard error. Lower case letters (a) and upper case letters (A) in the bars show significant

differences within the group fed with or without açaí. Treatments followed by different letters indicate significant differences by the Newmann-Keuls test ($p < 0.05$).

3.5. Nodularin concentration in shrimp muscle

In the muscle samples, no significant differences were detected in the açaí-fed group compared to the non-acai fed group ($P > 0.05$). However, exposure to different nodularin concentrations was not able to cause statistical differences compared to control ($P > 0.05$) (Fig. 5).

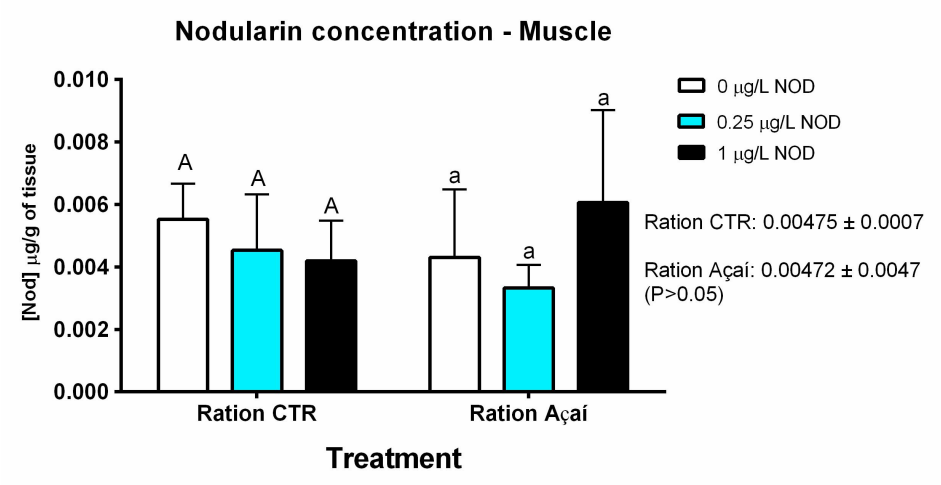

Fig. 5. Determination of nodularin muscle concentration in shrimp *L. vannamei* exposed at different concentrations of Nodularin. Data are expressed as mean ± standard error. Lower case letters (a) and upper case letters (A) in the bars show significant differences within the group fed with or without açaí. Treatments followed by different letters indicate significant differences by the Newmann-Keuls test ($p < 0.05$).

4. Discussion

It is noteworthy that aquatic organisms exposed to cyanobacterial blooms are subject to several toxicological effects, and may even lead to death (Zimba *et al.,* 2006).

These blooms can occur naturally in oligotrophic environments by some species that absorbs atmospheric nitrogen from the environment. However, most cyanobacterial

species do not present this ability to fix atmospheric nitrogen and thus require a nutrient-rich environment for their massive development (Czerny, Barcelos, Ramos & Riebesell, 2009).

Bioflocs rearing systems cause concerns in this issue because the production units have high temperatures and large amounts of nutrients, providing a favorable environment for the growth of target organisms as shrimps but also for secondary organisms and cyanobacteria. The study of Pacheco *et al*. (2016) reported an exacerbated proliferation of *Nodularia sp* in open-air nurseries, affecting the growth of *L. vannamei* reared in shrimp farm at Southern Brazil.

Several studies have reported some negative health effects observed in aquatic organisms, both in fish and in crustaceans, caused by exposure to hepatotoxins (Sotton *et al.,* 2015). Although several cyanotoxins are hepatotoxic (Zimba *et al.,* 2006), they can damage and accumulate in other tissues and organs (Žegura *et al.,* 2008). Persson *et al.* (2009) reported the accumulation of nodularin in the liver of the fish species *Platichthys flesus* but found no toxin in the muscle. Magalhães *et al.* (2003) also found bioaccumulated toxins in crustaceans and fish found in Sepetiba Bay (Brazil, RJ), reporting the risk of intoxication for human populations. Likewise, Sipiä *et al*. (2002) detected this nodularin toxin in flounder (*Platichthys flesus*), mussels (*Mytilus edulis, Dreissena polymorpha*), and clams (*Macoma balthica*) from the Northern Baltic Sea.

In the present study, the accumulation of nodularin in the muscle was evaluated, since it is the edible tissue consumed by humans. Kankaanpää *et al.* (2005) verified the nodularin bioaccumulation in the hepatopancreas of shrimp. In spite of having found great concentrations of this toxin in the liver, in the muscle was found concentrations below the limit allowed for consumption here in Brazil (0.04 $\mu g.Kg^{-1}$ for the hepatotoxin microcystins) The analogies between nodularin and microcystin made in

the work are due to their similar structure and the same mechanism of action. The presence of nodularin in the control organisms employed in this study suggests that they were already being exposed to this toxin in culture tanks. The presence of nodularin in the tissue can indicate worrying situations in the consumption of contaminated foods, since the presence of this toxin in large concentrations in the human organism and other animals can lead to the death of the individual due to toxic hepatitis as occurred in the city of Caruaru PE in the year 1996, where 126 patients from a hemodialysis clinic were infected while undergoing treatment.

As the first line of antioxidant defense, GSH can be conjugated to several toxic agents, in order to generate a more polar molecule, facilitating its excretion and thus favoring the organisms detoxification organism (Amado *et al.*, 2011; Yuan *et al*., 2016).

Several authors have already shown some toxics effects on crustaceans of the species *Chasmagnathus granulatus* in the hepatopancreas and gills in form of exposure (injected with *Microcystis aeruginosa* extracts), showing higher Total antioxidant capacity assay (TOSC) and GST in later gills (Vinagre *et al*., 2003), higher GST activity in crabs exposed to 860 μg MCs/kg for 12 hours, and higher LPO levels in crabs exposed to all doses after 72 hours of exposure (Dewes *et al*., 2006).

In this work, it was possible to observe the inclusion of antioxidant biomolecules supplied through açaí fruit in the diet of the organisms, enabling the shrimp to face the stress caused by the toxin, minimizing its negative effects, and bringing quality to the health of the animal, as can be seen in GSH content in hepatopancreas and gills.

The detoxification of the organism occurs by the activity of some enzymes, including those from GST family, known to catalyze microcystins conjugation with the group SH (thiol) of the molecule of GSH, facilitating its excretion (Jayaraj *et al*., 2006). The dysfunction (or low activity) of these enzymes make the organism more susceptible

to accumulation of this cyanotoxin in the body. It can also accumulate in front of the food chain, where animals or even humans can be depleted by eating food contaminated with nodularin (Lee, Lee & Jiang, 2017).

Several authors reported in their work the decrease of the enzymatic activity of GST in relation to the control group in relation to organisms exposed to cyanotoxins (Davies, Siu, Jack, Wu, Lam & Nugegoda, 2005) (Beattie *et al.*, 2003). However, Kankaanpää et al. (2007) did not show a significant decrease in the activity of glutathione S-transferase (GST). In the present study, the açaí did not alter the activity of the GST enzyme compared to the group not fed with açaí. Thus, the exposure time and the toxin concentrations used in the study were not able to negatively affect the activity of the enzyme, as well as the açaí factor also did not influence the activity of the enzyme.

Lipid peroxidation is considered lipid damage, where free radicals act in the process by capturing electrons from unsaturated lipids found in biological membranes. This damage is normally after exposure to cyanotoxins. Previous studies have demonstrated significant increases in lipid damage in several experimental organisms (Davies et al., 2005; Pflugmacher, Olin & Kankaanpää, 2007). However, Persson, Legrand & Olsson (2009) did not find significant differences in fish *P. flesus* exposed to nodularin. Similarly, in the present study, açaí showed no significant differences in MDA content in the gills and in the *L. vannamei* shrimp muscle; however, shrimps treated with açaí and then exposed to nodularin showed a significant decrease in lipid peroxidation in the muscle.

In mammals, MDA can be metabolized in the cells by the enzyme aldehyde dehydrogenases (ALDH) cytosolic (Hjelle & Petersen, 1983). MDA can be oxidized by ALDH to Acetyl-CoA and, after passing the Krebs cycle, to CO_2. It is known that *in*

vivo MDA can react with lysine residues of the proteins forming the N-ε- (2-propenal) lysine (NPL) adduct, which has also been detected in mammalian tissues, fluids, and urine as indicative of damage from lipid peroxidation. (Girón-Calle, Alaiz, Millán, Ruiz-Gutiérrez & Vioque, 2002). Another biochemical route responsible for decreasing the content of MDA is via carnosine. Carnosine (Carnosine (b-alanyl-L-histidine)) is present in aquatic organisms as fish (Geda, *et al*., 2015), possessing antioxidant capacity. Since the synthesis of carnosine in the organism is directly related to the presence or oral administration of the amino acid alanine (Harris *et al*., 2006), which is present in the açaí fruit in significant amounts (Schauss, 2010). A recent study reported a decrease in the amount of MDA in rats treated with carnosine (Hasanein, Kazemian-Mahtaj & Khodadadi, 2016), which may be a route of elimination of MDA.

However, in the the hepatopancreas, treatment with açaí was able to promote a significant decrease in the contents of MDA, suggesting that açaí has facilitated the metabolism of the MDA molecule, showing to be useful in the chemoprevention against the exposure to nodularin.

The measurement of P-SH allows to estimate the amount of non-oxidized sulfhydryl (SH) groups, which are present in the aminoacids residues (Aksenov & Markesbery, 2001), indicating the redox state of the tissue. The SH groups can be oxidized by free radicals and, eventually, compromising normal functions of proteins. In the present work, in the gills and hepatopancreas, neither the açaí treatment nor the toxin exposure was able to significantly modify P-SH levels. However, in the muscle, the P-SH concentration showed a significant decrease in shrimps fed with açaí, representing protein damage. One of the possible reasons for this protein damage is due to the large amount and diversity of antioxidant biomolecules present in the açaí fruit, which can, through the metabolism of these biomolecules, become a pro-oxidant, as Kütter *et al*.

(2013) reported in a study with pompano fish *Trachinotus marginatus* exposed with antioxidant lipoic acid via intraperitoneal injection. Other possible explanation for this result is that, as nodularin is a well-known inhibitor of protein phosphatases 1 (PP-1), 2A (PP-2A) and 3 (PP-3) (Dawson, 1998; Ohta *et al*., 1994). The inhibition of some phosphatases may regulate the activity of some heat shock proteins (Hsp) as, for example, Hsp72 (Yaglom, O'Callaghan-Sunol, Gabai & Sherman, 2003), extremely important in maintaining the cellular homeostasis that they promote to the tissue.

5.Conclusion

The inclusion of açaí in the diet diet of animals exposed to nodularin was able to: (a) increase the levels of reduced glutathione (GSH) in hepatopancreas and gills; (b) to diminish the levels of lipid peroxidation (TBARS) in muscle of nodularin-exposed shrimps; (c) to reduce P-SH in muscle. Points (a) and (b) should increase the defense mechanisms against reactive species of oxygen but point (c) deserves further investigation since, a pro-oxidant condition in muscle is negative for aquaculture practices. Accumulated nodularin in muscle of control shrimps is a case of concern, indicating a previous exposure to this toxin in the rearing tanks, a condition that should influence in the subsequent nodularin exposure performed in present study.

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