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Chemoprotection of amazonian *Mauritia flexuosa* fruit pulp against ammonia and nitrite toxicity to postlarvae shrimps *Litopenaeus vannamei*

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ABSTRACT

The chemoprotection of "buriti" *Mauritia flexuosa* (inclusion in diet: 0-control group; 1.25; 2.50; 5.00; and 10.00% W/W) to *Litopenaeus vannamei* postlarvae (PL) exposed to ammonia or nitrite was investigated. Analyzed variables include antioxidant (ACAP) and oxidative damage (TBARS) responses and levels of total carotenoids. The results indicated that there was no significant difference (p >0.05) in zootechnical variables between diets. The PL carotenoid content (R^2 = 0.86), ACAP (R^2 = 0.78), and TBARS (R^2 = 0.91) showed a dose-dependent relationship with the inclusion levels of "buriti" (p <0.05). After 43 days, juvenile shrimps were exposed for 96 h to ammonia (0.48 mg NH_3-N L^{-1}) or nitrite (40 mg NO_3 L^{-1}). Higher scavenging activity against peroxyl radicals was observed in PL fed with 2.50 and 5.00% of "buriti" (ammonia exposure), or 5.00 and 10.00% (nitrite exposure) of "buriti" inclusion. Content of reduced glutathione was higher in shrimps exposed to ammonia and fed with 10.00% of "buriti". Lipid peroxidation levels were lower in shrimps exposed to ammonia or nitrite and previously fed with inclusion higher than 2.50% of "buriti". The increased scavenging activity and lower lipid peroxidation in ammonia or nitrite-exposed organisms and previously supplemented with "buriti" point to a hormetic response that increases the resilience of *L. vannamei* to cope with nitrogenous compounds, pointing to the use of this fruit as a chemoprotectant agent.

Keywords: bioactive molecule; nitrogen compound; functional feed; sustainable aquaculture.

Quimioproteção da polpa da fruta amazônica *Mauritia flexuosa* contra a toxicidade da amônia e nitrito em pós-larvas do camarão *Litopenaeus vannamei*

RESUMO

Foi avaliada a quimioproteção do "buriti" *Mauritia flexuosa* (inclusão na dieta: 0-grupo controle; 1,25; 2,50; 5,00; e 10,00% P/P) em pós-larvas (PL) de *Litopenaeus vannamei* expostas à amônia ou nitrito. As variáveis analisadas incluíram respostas antioxidantes (ACAP) e de dano oxidativo (TBARS) e teores de carotenoides totais. Os resultados obtidos indicaram que não houve diferenças significativas (p >0,05) nas variáveis zootécnicas entre as dietas. O conteúdo de carotenoides nas PL (R^2 = 0,86), ACAP (R^2 = 0,78), e TBARS (R^2 = 0,91) mostraram um relação dose-resposta com os níveis de inclusão de "buriti" (p <0,05). Após 43 días, os camarões juvenis foram expostos por 96 h à amônia (0,48 mg NH_3-N L^{-1}) ou à nitrito (40 mg NO_3 L^{-1}). Uma elevada capacidade antioxidante contra radicais peroxil foi observada em PL alimentadas 2,50 e 5,00% de "buriti" (exposição à amônia), ou 5,00 e 10,00 % (exposição à nitrito) de inclusão de "buriti". O conteúdo de glutationa reduzida foi superior em camarões expostos à amônia e alimentados com 10.00% de "buriti". Os níveis de peroxidação lipídica foram inferiores em camarões expostos à amônia ou nitrito e previamente alimentados com níveis de inclusão de buriti superiores a 2,50%. O aumento de capacidade antioxidante e redução da peroxidação lipídica nos organismos expostos à amônia ou nitrito que previamente foram suplementados com "buriti" sugere uma resposta hormética, incrementando a resiliência de *L. vannamei* frente a compostos nitrogenados, recomendando o uso deste fruto como agente quimioprotetor.

Palavras-chave: molécula bioativa; composto nitrogenado; alimento funcional; aquicultura sustentável.

INTRODUCTION

In aquaculture, the nursery phase is fundamental in the superintensive shrimp production during the breeding phase. At this stage, the postlarvae (PL) are transferred from hatchery to intermediate rearing tanks in the nursery phase (Esparza-Leal et al., 2015). For better efficiency, the nursery phase uses high stocking densities and eventually heaters to enable rearing in cold months (Fóes et al., 2011). A high stocking densi-

ty during this phase may affect the zootechnical parameters and survival of PL because of cannibalism and water quality deterioration, a direct consequence of decrease space availability and food competition (Arnold et al., 2006; Wasielesky et al., 2013).

Thus, becomes essential to take care of these several factors that can affect the water quality during the nursery phase in clear-water (CW) systems. Toxic metabolites, such as ammonia and nitrite, that are formed from PL feces, uneaten food, and dead microorganisms can accelerate water quality degradation (Mishra et al., 2008). Ammonia accumulation can be harmful to structures as enzymes and other molecules present in cell membranes (Shan et al., 2019). In intensive production systems, ammonia concentrations might rise quickly and become toxic, representing a high risk for the reared organisms. Shrimps under increased ammonia-N concentration can present impaired antioxidant capacity, with loss in compensatory antioxidant mechanisms that can cause a severe oxidative stress condition (Romano and Zeng, 2013; Colombo et al., 2020; Wang et al., 2020). Also, nitrite is a toxic nitrogen waste that can induce not only lethal responses in the shrimp *Litopenaeus vannamei* (Ramírez-Rochin et al., 2017) but also affects its gut microbiota, favoring pathogens like *Pseudoalteromonadaceae* and *Vibrionaceae (Huang et al., 2020).*

The low capacity of the development of microorganisms able to degrade nitrogenous in a CW system is a limiting factor that needs to be compensated using water recirculation systems or water exchange (Wasielesky et al., 2013; Esparza-Leal et al., 2015). Postlarvae exposure to adverse conditions can affect the shrimp growth rate, feed conversion rate, and biomass production, which reflects directly on PL quality and production costs (Nga et al., 2005; Ray et al., 2011).

Stressful conditions are commonly observed in superintensive production systems (Gao et al., 2017; Souza et al., 2019) as elevated ammonia concentration can promote the generation of reactive oxygen species (ROS) (Ray et al., 2012; Colombo et al., 2020). ROS induction in aquatic animals occurs under unfavorable biochemical and physiological conditions, such as pH, temperature and toxins (Kütter et al., 2014; Birnie-Gauvin et al., 2017). Aerobic organisms possess an antioxidant network to cope with ROS (Kütter et al., 2014; Birnie-Gauvin et al., 2017), including antioxidant enzymes (Duan et al., 2015), and cellular non-enzymatic antioxidants (Souza et al., 2019). However, prolonged exposure to high ROS levels can affect the welfare of farmed shrimp (Zhang et al., 2006). The imbalance between ROS occurrence and antioxidant defenses may lead to impairment of cell membranes through lipid peroxidation and oxidative damage in proteins and DNA (De Jesus Raposo et al., 2015; Birnie-Gauvin et al., 2017).

The use of food supplementation with bioactive compounds, including pigments, antioxidants, and provitamins, improves several biochemical and physiological functions of reared organisms by neutralizing the excess ROS in tissues (De Jesus Raposo et al., 2015; Colombo et al., 2020). Commercial pelleted feeds are the main food source in aquaculture production but may not provide the nutrients needed for the optimal growth of shrimp (Craig and Helfrich, 2017), and the use of bioactive compounds obtained from natural sources can be a viable strategy. Studies have reported that the inclusion of fruits and plants rich in carotenoids and other bioactive compounds in shrimp diets is associated with increased antioxidant activity against peroxyl radicals (Chen et al., 2013; Pakravan et al., 2017; Niu et al., 2019; Colombo et al., 2020). Carotenoids can scavenge free radicals and induce positive effects on growth, immunological responses, and lipid preservation when used in shrimp diets (Zhang et al., 2013; Da Silva et al., 2015; Takeungwongtrakul and Benjakul, 2016).

Amazonian "buriti" *Mauritia flexuosa* fruit pulp is a rich source of bioactive and antioxidant molecules. Its oil is rich in carotenes (mainly in *trans*-β-carotene, 13-*cis*-β-carotene, and 9-*cis*-β-carotene), tocopherol (almost 90% is α-tocopherol), and unsaturated fatty acids (Albuquerque et al., 2005). Simião et al. (2020) reported that *M. flexuosa* fruit pulp has properties of a functional fed in juveniles of shrimp *L. vannamei*, improving their total antioxidant capacity and decreasing lipid peroxidation levels. According to this previous information, it should be important to consider its use for shrimp postlarvae nutrition, especially during the nursery phase and when exposed to toxic nitrogenous compounds. Thus, the objective of this study was to evaluate Amazonian *M. flexuosa* fruit pulp inclusion in diets employed during the nursery culture of *L. vannamei* PL and, subsequently, to evaluate the effect of variations in growth performance, total antioxidant capacity, levels of lipid peroxidation, and carotenoid content. Finally, the potential chemoprotection effects of *M. flexuosa* were evaluated after exposing *L. vannamei* to sub-lethal concentrations of ammonia or nitrite.

MATERIAL AND METHODS

The postlarvae (PL) were obtained from the shrimp hatchery system of the Marine Station of Aquaculture (EMA), Federal University of Rio Grande, Brazil. In the experiment, PL with initial weight (mean ± 1 standard deviation) of 5.0 ± 0.3 mg were assayed and placed in circular tanks of 100 L, with a stocking density of 1 PL L^{-1}, totaling 100 PL per tank. Five experimental groups: control (0.00), 1.25, 2.50, 5.00, and 10.00% of "buriti" inclusion were formed in triplicate. A one-week acclimation (seawater, 30 ppt, and 28°C) with the offer of the control experimental diet was performed. Before use, the seawater was treated with sodium hypochlorite (10 ppm). After 24 hours, the water was dechlorinated with ascorbic acid (1 mg L^{-1}). During this process, it was maintained constant aeration until later use in the experiment boxes. Temperature was maintained by means of an air conditioning.

During the trial period, seawater was used, and the photoperiod was fixed with a timer at 12 h L/12 h (light:dark), the parameters of seawater were kept within optimal values for *L. vannamei* PL (Lin and Chen, 2003; Zhang et al., 2006; Furtado et al., 2011). During the first two weeks, no water renewals were performed. After, 80% of water renewal was performed twice a week. Oxygen and temperature were measured with multiparameter digital oximeter (YSI®-550A, YSI, United States). pH was determined using a

digital pH meter (AT 315 SP, Alfakit, Brazil). Total ammonia (TAN) (NH_3+NH_4^+) and nitrite ($N\text{-}NO_2^-$) were measured according to protocols from UNESCO (1983) and alkalinity according to APHA (1998). Salinity was measured with a Refractometer (Alfakit, Brazil). The dissolved oxygen concentration (average ± 1 standard error) was 6.50 ± 0.04 mg L^{-1}, the pH was 8.21 ± 0.14, the salinity was 30 ± 2.75 ppt and the temperature was 26.75 ± 0.30°C. The concentrations of total ammonia-N, nitrite-N and nitrate-N were 0.34 ± 0.19 mg L^{-1}, 1.36 ± 0.25 mg L^{-1} and 1.58 ± 0.60 mg L^{-1}, respectively. The alkalinity was maintained at 147 ± 2.90 mg L^{-1} $CaCO_3$ with the use of calcium hydroxide (Furtado et al., 2011).

After 43 days of feeeding with the experimental diets, the PL were kept one day without food and then weighed in a precision balance (Marte ATY224 model, Brazil) and killed in liquid nitrogen. Afterwards, PL were stored in an ultrafreezer (-80°C) (Tectalmaq, 125H-85 model, Brazil) before the measurements being executed (see below)

Feeding with "buriti" inclusion

All diets were formulated with 37% protein and 9% lipids (Lee and Lee, 2018). The ingredients of the diets and proximate composition of the experimental diets have been described previously by Simião et al. (2020). The "buriti" used in the preparation of experimental diets had a content (expressed as g/100 g of dry matter) of 7.72 (protein), 34.07 (lipid), 2.70 (ash), 55.77 (carbohydrate), and 5.00 (moisture) (Simião et al., 2020). The same level of lipid content was maintained between the experimental diets.

All experimental diets were prepared individually. Initially, the ingredients were mixed from the lowest to the highest inclusion level, and oil was added at the end of the preparation. After that, distilled water (heated to 40°C) was added to approximately 30% of the diet volume. Complete homogenization of the blend was performed. Using a meat grinder, the blend of the diet was formed into pellets. The pellets were dried in an oven at 50°C for 12 hours. After drying, the diet was kept in plastic bags in a freezer at -20°C until use.

Each experimental group was fed three times daily (08:00, 13:00, and 18:00 h). Over the trial period, the amount of diet was supplied and regulated according to Jory et al. (2001). PL were fed with an amout of food equal to 20% of their weights and the supply was adjusted according to consumption daily and growth performance for each tank individually.

Growth performance

The PL of each tank were weighed individually and weighed at the end of the experimental period to determine the growth performance. The formulas used to determine the weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), the protein efficiency ratio (PER), and survival can be found in Simião et al (2020).

Total carotenoid analysis

The extraction of carotenoids from diets and whole PL was performed (n = 9, by treatment) in 0.5 g of samples (diets and whole PL) that were placed in Falcon tubes. After, the procedure and estimation of total carotenoids were the same that was described by Simião et al. (2020).

Exposure to ammonia or nitrite

At the end of the experiment, 40 juveniles (mean weight of 396.00 ± 0.35 mg) per tank of each treatment were separated, and they were randomly divided into two groups (20 shrimps): one exposed to nitrite and the other to ammonia using tanks with 30 L of useful volume, adding up to 15 tanks for exposure to each nitrogen compound.

Ammonia and nitrite concentrations were selected using reference values derived from the LC_{50} levels of these compounds for juvenile *L. vannamei* shrimp. The organisms were exposed for 96 h to 0.48 mg NH_3-N L^{-1} of ammonia (Lin and Chen, 2001) or 40 mg NO_3 L^{-1} of nitrite (Lin and Chen, 2003). Ammonia and nitrite solutions were prepared by dissolving proper amounts of ammonium chloride and sodium nitrite (Chen et al., 1990). Two stock solutions of 250 mg L^{-1} (NH_4Cl - Synth, P.A.) and nitrite ($NaNO_2$ - Synth, P.A.) were prepared. Both solutions were diluted to the desired concentration, being pipetted directly into the water of the tanks. The proportions of non-ionized ammonia (NH_3) were calculated from the total ammonia concentration, based on equations described by Colt (2002).

For maintenance of the respective concentrations, 100% of the experimental water of the tanks was renewed daily and the solutions were added again. Water samples from all treatments were collected daily to determine the total ammonia and nitrite concentration. Therefore, the concentrations of non-ionized ammonia and nitrite-N reported in this study are the measured concentrations. After 96 h of exposure, the PL were collected and killed in liquid nitrogen. Posteriorly, PL were stored in an ultrafreezer (-80°C) and the estimated survival (Simião et al., 2020) and the biochemical analyzes performed.

Biochemical analyses

The whole PL (n = 9 for each treatment) were homogenized following the procedure of Simião et al. (2020). The analysis of scavenging activity against peroxyl radicals (ACAP) (n = 9, by treatment) was performed according to the method of Amado et al. (2009). Lipid peroxidation (n = 9 for each treatment) was determined in terms of concentration of thiobarbituric acid reactive substances (TBARS) according to the method of Oakes and Kraak (2003). Reduced glutathione (GSH) levels and the concentration of protein sulfhydryl groups (P-SH) followed the procedure of Sedlak and Lindsay (1968). P-SH was measured only in juvenile shrimps exposed to ammonia or nitrite due to the higher amount of tissue availability.

Statistical analysis

For the experimental feeding assay, data were analyzed statistically using a mixed model of variance components, with "buriti" inclusion levels as the fixed factor and the different aquariums employed for the same *M. flexuosa* fruit pulp inclusion levels as

the random factor (Searle et al., 2006). Data normality was verified with the Shapiro-Wilk test, and the homogeneity of the variances was verified with the Levene test. A polynomial second-order function was used to fit the levels of "buriti" inclusion in the diets with carotenoid content, scavenging activity, and TBARS levels. For the ammonia and nitrite exposure experiments, a two-way analysis of variance was applied (factor 1: "buriti" inclusion levels; factor 2: the treatments control, ammonia, and nitrite groups. Data normality was verified with the Shapiro-Wilk test, homogeneity of the variances was verified with the Levene test, and *post-hoc* tests were performed with SNK. The probability of error I type was set at 0.05.

RESULTS

There were no significant differences (p >0.05) in weight gain (g), specific growth rate (% day^{-1}), food conversion ratio, protein efficiency ratio, or survival between all treatments (p >0.05; Table 1). The use of diets supplemented with "buriti" presented higher carotenoid concentration in PL (R^2 = 0.86; p <0.05; Figure 1A). The maximum carotenoid levels were estimated at 7.67% of *M. flexuosa* inclusion. PL fed with 10% of "buriti", that showed a statistically higher carotenoid accumulation (p <0.05) when compared with the control group (Figure 1A). Means comparisons did not show a significant increase (p >0.05) in total antioxidant capacity (lower relative area) in the PL fed with "buriti"-enriched feed. However, a significant second-order function (R^2 = 0.78; p <0.05) was fitted, showing that 5.88% of "buriti" in the feed promoted the highest antioxidant capacity (lowest relative area) (Figure 1B). The inclusion of 5.00 and 10.00% of "buriti" induced a reduction of TBARS in PL when compared to shrimp fed with the control diet and the groups fed with 1.25 and 2.50% of "buriti" inclusion (p <0.05; Figure 1C). TBARS levels showed a second-order dose-response (R^2 = 0.91; p <0.05), and 9.17% of inclusion was estimated as the value where TBARS reached a minimum (Figure 1C).

The inclusion of *M. flexuosa* in the PL diet did not significantly influence the survival among all treatments (Table 2; p >0.05). In the second experiment, PL exposed to ammonia and previously no feed with *M. flexuosa* showed higher antioxidant competence than shrimps from the first experiment (p <0.05). Shrimps fed with 2.50 or 5.00% of *M. flexuosa* showed a significant increment in their antioxidant capacity when exposed to ammonia (p <0.05; Figure 2A, orange bars). Nitrite-exposed shrimps showed higher antioxidant competence against peroxyl radicals when fed with the inclusion of *M. flexuosa* fruit pulp higher than 5.00% (p <0.05; Figure 2A, red bars). The concentration of reduced glutathione was higher in ammonia-exposed shrimps previously fed with 10.00% of *M. flexuosa* (p <0.05; Figure 2B, orange bars). No significant differences (p >0.05) in the concentration of protein sulfhydryl groups (P-SH) were observed between the different *M. flexuosa* inclusion levels when shrimps were exposed to ammonia or nitrite (Figure 2C). However, higher P-SH levels were registered in shrimps previously fed with 2.50 or 5.00% of *M. flexuosa* and exposed to nitrite when compared with the ammonia-exposed shrimps and fed with the same inclusion levels (p <0.05; Figure 2C, compare orange and red bars). In respect to lipid peroxidation, ammonia-exposed shrimps showed statistical reduction (p <0.05) in TBARS levels when previously fed with inclusion levels of *M. flexuosa* higher than 2.50% (Figure 3, orange bars) and the same response was observed in nitrite-exposed (p <0.05; Figure 3, red bars).

DISCUSSION

Most of the previous studies on the use of supplements of aquatic organisms have focused on fruit and plant inclusion in diets (Yin et al., 2014; Elizondo-González et al., 2018; Niu et al., 2018; Xie et al., 2018; Shi et al., 2019; Srichaiyo et al., 2020). Both plants and fruits can be rich in essential nutrients for nutrition, such as vitamins and minerals and bioactive compounds as carotenoids, unsaturated fatty acids, and polyphenols, which can

Table 1. Effects on the growth performance of *Litopenaeus vannamei* postlarvae (PL) fed with diets containing different inclusion levels of "buriti" for 43 days. Data are expressed as the mean values ± standard error (n = 9). No differences (p >0.05) between treatments were verified for all analyzed variables. SGR: Specific growth rate; FCR: Food conversion rate. PER: Protein efficiency ratio.

Parameters	*Mauritia flexuosa* inclusion levels (%)				
	Control	1.25	2.50	5.00	10.00
Weight gain (mg)	393±16.9	420±6.7	411±8.2	447±22.7	405±7.9
Feed intake (mg)	516±8.9	539±20.1	563±21.6	539±32.2	560±53.4
SGR (% day^{-1})	10.14±0.10	10.30±0.04	10.25±0.05	10.44±0.12	10.22±0.05
FCR	1.30±0.05	1.21±0.02	1.23±0.03	1.14±0.06	1.25±0.02
PER	2.15±0.09	2.30±0.04	2.25±0.04	2.45±0.12	2.22±0.04
Survival (%)	98.33±1.67	94.33±3.48	90.33±3.53	94.67±5.33	92.00±8.00

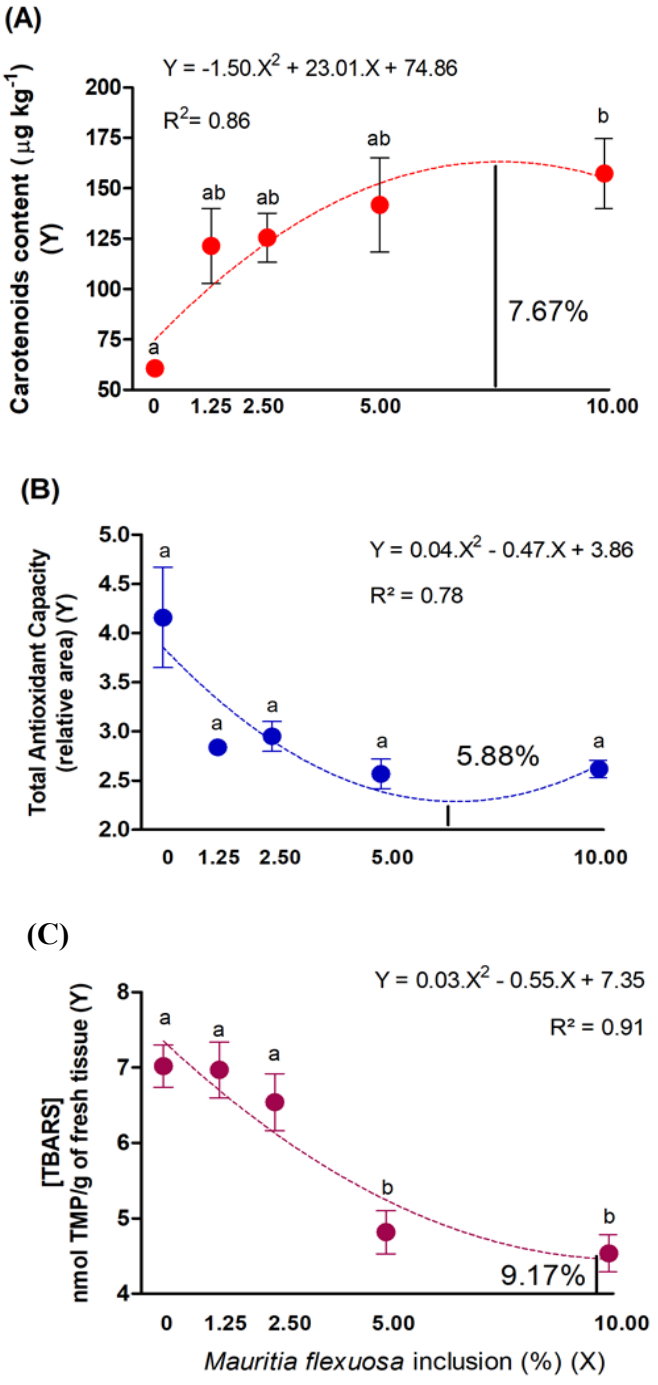

Figure 1. (A) Second-order polynomial fit of carotenoid content measured in postlarvae (PL) of *Litopenaeus vannamei* fed with diets with different inclusion levels of "buriti" (0.00 (control); 1.25; 2.50; 5.00; and 10.00 %) for 43 days. (B) Same as (A) for scavenging activity against peroxyl radicals (expressed as relative area). (C) same as (A) for lipid peroxidation (TBARS). Data are expressed as the mean values ± standard error (n = 9). The same letters indicate the absence of significant differences (p >0.05) between the different inclusion levels of "buriti" fruit pulp.

improve the health and resilience of aquatic organisms, including oxidative stress (He et al., 2017; León et al., 2018; Colombo et al., 2020; Schmitz et al., 2020). However, few previous studies have focused on the nutritive value of fruits on the growth performance and oxidative stress status of *L. vannamei* PL. Thus, this experimental design based on fruit pulp inclusion for shrimp *L. vannamei* postlarvae diets allowed us to evaluate the importance of bioactive compounds in the shrimp nursery phase and to analyze if this procedure was effective to organism's cope with nitrogenous compounds toxicity.

It was observed in this study that the inclusion of "buriti" does not influence PL production aspects, including survival, WG, FCR, PER, and SGR parameters, as previously observed with the same fruit in juvenile *L. vannamei* (Simião et al., 2020). These findings demonstrate that the inclusion of "buriti" in the diet of shrimp *L. vannamei* has no negative effects, as far as antinutritional factors are concerned, regardless of their stage of life. The inclusion of "buriti" showed a theoretical maximum at 7.67% of fruit pulp inclusion, being only significantly different at 10.00% inclusion (Figure 1A). The molecular structure of carotenoids has great relevance and influences their absorption, metabolism, and binding affinity in different organs (Yi et al., 2014). The amount and lipid composition in the diets and the number of lipid receptors in absorptive cells are factors that determine the maximum carotenoid uptake in the organs (Van Bennekum et al., 2005; Failla et al., 2014). Even when the mechanisms involved in these processes are not yet described in shrimp, the previous study of Simião et al. (2020) showed an increment of carotenoids concentration in the hepatopancreas and muscle of juvenile *L. vannamei* fed with 10.00% of *M. flexuosa* inclusion, indicating that carotenoid absorption in PL and juvenile shrimp do not change over the course of these two life stages.

The dose-response relationship between inclusion levels of "buriti" and scavenging activity against peroxyl radicals observed in this study can be associated with the augmented carotenoids concentration registered in PL. Several studies have shown ample evidence of carotenoid capacity as ROS scavengers, acting as a very effective quencher of singlet oxygen and free radicals (Sowmya and Sachindra, 2012; Da Silva et al., 2015). The positive effects of the total antioxidant capacity of the shrimp *L. vannamei* PL fed with *M. flexuosa* dietary inclusion corroborate the findings of previous studies carried out on shrimp fed with carotenoids-enriched the diets (Sowmya and Sachindra, 2012; Zhang et al., 2013; Da Silva et al., 2015).

Concomitant with the increase in scavenging activity against peroxyl radicals, a decrease in lipid peroxidation levels was observed in the shrimp PL fed with 5.00 and 10.00% of "buriti". The greater scavenging activity against peroxyl radicals should render less oxidative damage, including lipids (Yang et al., 2010). Authors like Takeungwongtrakul and Benjakul (2016) and Shi et al. (2019) also showed that lipid peroxidation in *L. vannamei* can be attenuated by using plants with high antioxidant capacity as dietary supplements.

The effects of nitrogenous compounds influence the physiology of shrimps (Geng et al., 2020; Xiao et al., 2020), causing productivity loss and leading to the non-viability of the crop (Han et al., 2017; Huang et al., 2020). Several studies have shown that dietary manipulation using supplements is an alternative pathway that can reduce the negative effects on shrimp exposed to ammonia and nitrite stress (Colombo et al., 2020; Geng et al., 2020). Thus, it was considered the use of *M. flexuosa* fruit pulp as a chemoprotectant against nitrogenous compounds toxicity as ammonia and nitrite.

It was shown that the use of *M. flexuosa* influenced the antioxidant capacity of *L. vannamei* when exposed to ammonia and nitrite (Figure 2A). However, the antioxidant capacity of shrimps fed 10.00% of *M. flexuosa* inclusion and exposed to ammonia toxicity decreased and there was no significant difference when compared with the control group. Thus, the inclusion of *M. flexuosa* showed a biphasic response, where the use of 2.50 and 5.00% of *M. flexuosa* inclusion triggered stimulatory effects, probably enhancing the resilience against ammonia toxicity. At lower and higher inclusion values that the range 2.50-5.00%, the scavenging activity against peroxyl radicals equals the control group (Figure 2A). These results can be considered under the view of hormetic responses (Calabrese and Agathokleous, 2020). In the context of the present study, it is interesting to mention the comment of Agathokleous (2018) about the need of more studies dealing with the accumulation of antioxidants (as we observed, A) as stress-induced compounds in the context of environmental hormesis. It was observed in the antioxidant response of ammonia-exposed shrimps, that the reduced glutathione (GSH) seems to play a minor role, since their induction was observed only in shrimps fed with the highest inclusion level of *M. flexuosa* (Figure 2B). As mentioned previously, carotenoids are ROS scavengers that should drive the observed responses, at least in part. Also, it is important to note that the scavenging activity against peroxyl radicals in nitrite-exposed shrimps showed a different inclusion range of *M flexuosa* since significant induction effects were observed in shrimps fed with 5.00 and 10.00% of *M. flexuosa*, without observing the biphasic response (Figure 2A).

For lipid peroxidation, the TBARS levels showed a significant reduction both in ammonia and nitrite-exposed shrimps when the organisms were previously fed with inclusion levels of *M. flexuosa* higher than 2.50% (Figure 3). Previous studies from our

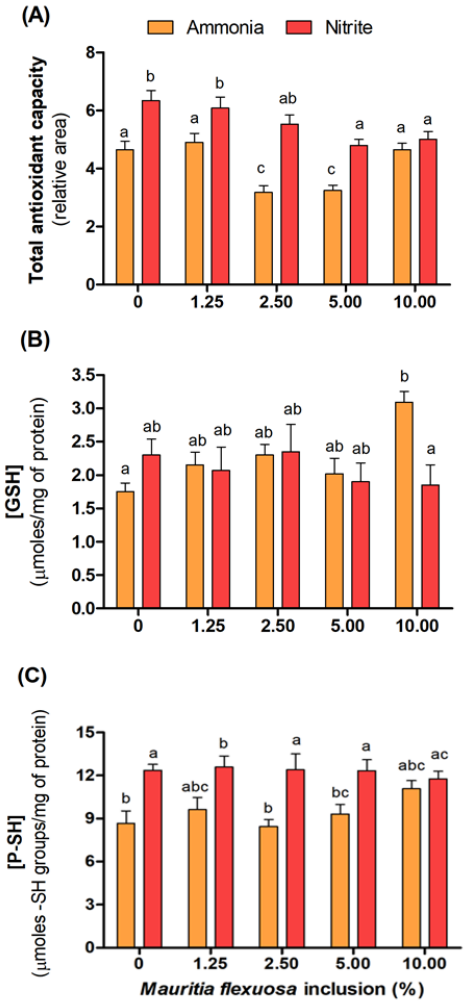

Figure 2. (A) Scavenging activity against peroxyl radicals in postlarvae (PL) of *Litopenaeus vannamei* fed with diets with different inclusion levels of "buriti" (0.00 (control); 1.25; 2.50; 5.00; and 10.00 %) for 43 days and then exposed to ammonia (orange bars) or nitrite (red bars) during 96 h. **(B)** Same as **(A)** for reduced glutathione (GSH) concentration. **(C)** Same as **(A)** for the concentration of protein sulfhydryl groups (P-SH). Data are expressed as the mean values ± 1 standard error (n = 9). The same letters indicate the absence of significant differences (p>0.05) between the different inclusion levels of "buriti" fruit pulp.

Table 2. Effects on survival of *Litopenaeus vannamei* postlarvae (PL) fed with diets containing different inclusion levels of "buriti" for 43 days and then exposed during 96 hours to ammonia and nitrite toxicity. No significant differences were found (p >0.05) in the survival of shrimps exposed to ammonia or nitrite between the different levels of "buriti" included in the diet were observed. Data expressed as mean values ± standard error (n = 3).

Parameters	***Mauritia flexuosa* inclusion levels (%)**				
	Control	**1.25**	**2.50**	**5.00**	**10.00**
Ammonia survival (%)	86.67±0.07	96.67±0.03	96.67±0.01	100.00±0.00	100.00±0.00
Nitrite survival (%)	65.00±0.03	65.00±0.08	58.00±0.06	68.00±0.11	80.00±0.00

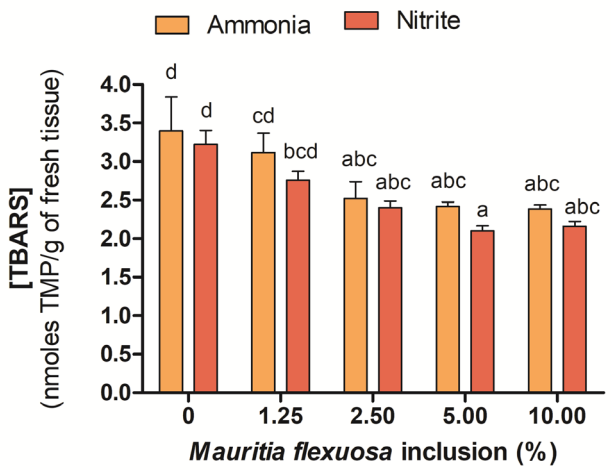

Figure 3. Lipid peroxidation levels, measured as thiobarbituric reactive substances (TBARS) concentrations (expressed in nmoles of TMP g^{-1} of fresh tissue) in postlarvae (PL) of *Litopenaeus vannamei* fed with diets with different inclusion levels of "buriti" (0 (control); 1.25; 2.50; 5.00; and 10.00%) for 43 days and then exposed to ammonia (orange bars) or nitrite (red bars) during 96 h. TMP stands for 1,1,3,3-tetramethoxypropane, employed as standard. Data are expressed as the mean values ± standard error (n = 9). The same letters indicate the absence of significant differences (p >0.05) between the different inclusion levels of "buriti" fruit pulp.

research group showed that *L. vannamei* fed with an açaí-enriched diet promoted a muscle TBARS reduction when exposed to the toxin nodularin (Schmitz et al., 2020), a result that can be interpreted, as the present results, in light of exposure to mild stressor factors than can improve the animal performance (Berry and López-Martínez, 2020) through hormetic responses. This theoretical framework has not been extensively considered in aquaculture, but these kinds of responses deserve more attention soon since scenarios with high ammonia (or nitrite) levels that can lead to a reduction in lipid peroxidation in organisms previously pre-conditioned with fruit or plants bioactive molecules could be a very interesting management practice.

Overall, the obtained results indicate a promising perspective about the use of "buriti" in PL shrimp diets, with quality improvement in the production of *L. vannamei* and of the development of sustainable aquaculture strategies. Also, as none of the growth parameters were affected (Table 1) in shrimps fed with the inclusion of *M. flexuosa,* it is possible to reduce the use of fish oil for diet formulations. The higher antioxidant capacity against peroxyl radicals after ammonia exposure in shrimps fed with 2.50-5.00% fruit inclusion and the reduction in TBARS levels in shrimps exposed to ammonia or nitrite and previously fed with inclusion levels higher than 2.50% points to the triggering of hormetic responses that should improve the capacity of organisms to cope with nitrogenous compounds toxicity.

CONCLUSIONS

Litopennaeus vannamei PL feed with different inclusion levels of the Amazonian fruit *M. flexuosa* showed a dose-response relationship in terms of total carotenoid and total antioxidant scavenging capacity (incresing trends in both cases) and lipid peroxidation (decreasing trend).

PL previously fed with *M. flexuosa* presented improvement in the total antioxidant scavenging capacity when exposed to ammonia or nitrite and reduced lipid peroxidation.

The result of this study indicates a chemoprotective role of *M. flexuosa* against the toxicity of nitrogen compound without affecting the growth parameters of the PL.

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Ethics Statement

According to Brazilian legislation, the use of invertebrate species does not require ethical approval from the institution where the study was conducted.

Conflict of interests

Nothing to declare.

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Authors' Contributions

Dos Santos Simião, C.: Conceptualization, Investigation, Visualization, Methodology, Writing – original draft Colombo, G.M.: Investigation, Methodology Marreiro Gomes, R.M.: Investigation, Methodology Ramos, P.B.: Investigation, Methodology, Supervision, Writing – original draft Tesser, M.B.: Conceptualization, Investigation, Methodology, Supervision, Writing – original draft Wasielesky Jr., W.: Conceptualization, Project administration, Resources, Writing – original draft Monserrat, J.M.: Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing

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