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Zootechnical performance, biochemical response, and chromaticity in Pacific white shrimp (*Litopenaeus vannamei*) (Boone, 1931) after the inclusion of lyophilized açaí (*Euterpe oleracea*) in the diet

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Abstract
Functional foods have molecules that promote health benefits beyond their nutritional qualities. In this context, the zootechnical performance, color, and biochemical responses of Pacific white shrimp (*Litopenaeus vannamei*) fed with different levels of açaí (*Euterpe oleracea*), a fruit with unique antioxidant characteristics, were observed. Four diets with different levels of inclusion of lyophilized açaí (0.0%, 2.5%, 5.0%, and 10.0% W/W) were administered to shrimps reared in biofloc technology (BFT) systems. After 43 days, the zootechnical parameters (weight gain, feed conversion ratio, and specific growth rate) and coloration in fresh and cooked shrimps were measured, and the *L**, *a**, and *b** parameters were estimated with a digital colorimeter. Survival and growth performances were not affected by açaí inclusion in diets. Açaí inclusion modulates the flavonoid content in the gills of *L. vannamei* and in the

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bioflocs of the rearing BFT system. As in the diet of 10.0% açaí, performed with a total replacement of fish oil without affecting growth, we propose the use of açaí as a vegetal oil source for shrimp feeding in BFT systems. Açaí also induced the reddish color in fresh and cooked *L. vannamei*, an influential factor in their commercial value.

Keywords Sustainable aquaculture · Flavonoids · Polyphenols · Nutrition · Biofloc system

Introduction

Among shrimps, *Litopenaeus vannamei* is the species with the highest global production by virtue of its quick growth, high survival, and tolerance to salinity and temperature variations (Krummenauer et al. 2011; Kim et al. 2014; Liu et al. 2018). Its productivity has been benefited by the use of biofloc technology (BFT) systems, which allow the shrimps to be produced with low water exchange, the recycling of nitrogenous compounds, and high biosafety (Avnimelech 2006; Wasielesky et al. 2006; Lara et al. 2016). Also, bioflocs are a food supplement that improves the digestion, growth, and antioxidant capacity of *L. vannamei* (Xu and Pan 2012; Xu et al. 2013; Martins et al. 2015).

In the last few years, the improvement of rearing conditions has included the addition of antioxidant supplements (i.e., lipoic acid and quercetin) in the diets of aquatic organisms not only to improve their resilience against stressful environmental conditions but also to improve the growth and quality of the final product (muscle) since antioxidants can be accumulated in edible tissues (Yang et al. 2012; Yasin et al. 2012; Molina León et al. 2018; Martins et al. 2018). In line with this strategy, the use of native plants with high antioxidant content should be an interesting option to analyze. The fruit of the açaí (*Euterpe oleracea*) possesses a high concentration of phenolic compounds as flavonoids and anthocyanins that confer this Amazonian fruit's exceptional antioxidant properties, together with fibers, vitamin E, and minerals (Schauss et al. 2006; Dias et al. 2013; Schauss 2016). The antioxidant properties of açaí pulp have been analyzed extensively in the last few years, reporting several effects, including the induction of antioxidant enzymes, the reduction of reactive oxygen species generation, and the reduction of oxidative damage (De Lima Yamaguchi et al. 2015; Barbosa et al. 2016).

Other important molecules abundant in açaí pulp are lipids. According to Lubrano et al. (1994), the lipid profile of açaí pulp includes oleic acid, palmitic acid, linoleic acid, and palmitoleic acid. Ferreira et al. (2016) determined that this fruit contains 23.9%, 59.8%, and 11.9% of saturated, monounsaturated, and polyunsaturated fatty acids, respectively. The same authors reported that on a dry weight basis, the açaí fruit contains 33.49% of lipids. Usually, fish oil is added in the feed to supply the essential fatty acids that shrimps need in their diet. However, this lipidic source is being compromised because of overfishing as well as rising prices, implying the need for an alternative source of lipids (González-Félix et al. 2010; Ayisi et al. 2017). In this way, the use of vegetal oils can be a sustainable alternative (Soller et al. 2017, 2018), making açaí pulp a potential candidate for fish oil replacement and an antioxidant supplement.

Chromaticity analysis is valuable in shrimp culture since color is one of the most important criteria when consumers select fresh shrimps and cook them for consumption (Lucien-Brun and Vidal 2006; Erickson et al. 2007). The red/pink coloration of crustaceans is controlled by the concentration of astaxanthin (Ju et al. 2011), a carotenoid not synthesized by them but acquired through their diet. In this way, reddish color is considered an indicator of welfare and appropriate nutrition, impacting the commercial value of the organism (Martínez et al. 2014).

To sum up, first, açaí is considered a functional food (Wolf 2001) because of its huge amount of antioxidants (De Lima Yamaguchi et al. 2015), including flavonoids and anthocyanins, the last being natural colorants (He and Giust 2010) that can eventually influence not only the antioxidant status of *L. vannamei* but also its pigmentation. At the same time, a higher antioxidant status of reared organisms renders longer shelf life, as previously reported for *L. vannamei* and flatfish (*Paralichthys olivaceus*) (Li et al. 2016, 2017). Second, given its high lipid content, açaí pulp could be employed as a vegetal source of these macromolecules to substitute fish oil as a component of shrimp diet.

The goals of the study were to analyze the zootechnical and biochemical responses as well as the pigmentation parameters in *L. vannamei* feed with different inclusion levels of lyophilized açaí (*E. oleracea*). We hypothesized that lipids contained in açaí pulp should constitute a good alternative for fish oil without affecting the growth parameters of reared shrimps. Also, because of the high antioxidant content of this fruit, we expected to detect modulation in the shrimp antioxidant content as well as in their chromaticity as açaí contains anthocyanins.

Material and methods

Litopenaeus vannamei maintenance

Shrimps were maintained at the "Estação Marinha de Aquacultura" (EMA, Federal University of Rio Grande - FURG, RS, Brasil). Juvenile shrimps of 0.90 ± 0.15 g were randomly distributed in 16 tanks of 40 L of water ($n = 20$ per tank). Each tank was filled with 12.5 L of a biofloc inoculum from a superintensive rearing raceway of *L. vannamei* carried out during 90 days. Filtrated fresh and marine water were chlorinated with 10 ppm and dechlorinated with 1 ppm of ascorbic acid and added up to 40 L^{-1} in each experimental tank. Four different açaí inclusion levels (0.0, 2.5, 5.0, 10.0% W/W) in the feed were tested in quadruplicate during 43 days between May 13, 2017, and June 25, 2017. Shrimps were hand-fed twice a day (09:00 and 15:00 h), offering feed equal to 25% of total biomass (Jory 2001) in feeding trays as described by Wasielesky et al. (2006). Every week, 10 shrimps of each tank were randomly sampled and weighted individually in precision balance (± 0.01 g; Shimadzu) and then returned to the tanks. Temperature (°C), dissolved oxygen (mg/L), and pH (precision ± 0.3 °C, ± 0.3 mg/L, and ± 0.1%, respectively) were measured twice a day using a multiparameter equipment (YSI-550A) and a digital pH meter (YSI-pH 100). Salinity (ppt) was measured three times a week using an ATAGO refractometer. Analysis of total ammonia nitrogen levels (TAN: $NH_3 + NH_4^+$) was measured according to UNESCO (1983) and nitrite (NO_2^-–N) and nitrate (NO_3^-–N) following the methodologies described by Aminot and Chaussepied (1983). Alkalinity (mg $CaCO_3$ L^{-1}) was measured once a week following APHA (1998). Total suspended solids (mg TSS L^{-1}) were measured after collecting 20 mL of water of each tank every week and filtered, following Strickland and Parsons (1972), registering the weight of the withheld material in a filter of 0.45 μm pore.

Experimental diets

Lyophilized açaí (*E. oleracea*) was purchased from the company "Amazon Comércio de Açaí Liofilizado e Exportação LTDA," located in Belém, Pará, Brazil. The four diets with different

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, 2.5, 5.0, 10.0%, W/W)

Ingredients (g/100 g)	0% (control)	2.5%	5.0%	10.0%
Fish meal	27.00	27.00	27.00	27.00
Soybean meal[1]	25.00	25.00	24.00	23.40
Brewer's yeast[2]	5.00	5.00	5.00	5.00
Corn Starch[3]	23.00	22.00	22.05	19.70
Wheat meal[4]	5.60	5.60	5.60	5.60
Fish oil[5]	3.90	2.75	1.65	0.00
Mineral/vitamin mixture[6]	1.00	1.00	1.00	1.00
Cholesterol[7]	0.50	0.50	0.50	0.50
Calcium phosphate[8]	2.00	2.00	2.00	2.00
Cellulose[9]	7.00	6.65	6.20	5.80
Lyophilized açaí[10]	0.0	2.5	5.0	10.0

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[6] Vitamin A (500.000 Ul/kg), vitamin D3 (250.000 Ul/kg), vitamin E (5.000 mg/kg), vitamin K3 (500 mg/kg), vitamin B1 (1.000 mg/kg), vitamin B2 (1.000 mg/kg), vitamin B6 (1.000 mg/kg), vitamin B12 (2.000 mcg/kg), niacin (2.500 mg/kg), calcium pantothenate (4.000 mg/kg), folic acid (500 mg/kg), biotin (10 mg/kg), vitamin 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)

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inclusion levels of açaí were formulated to be isoproteic (35%) and isolipidic (6%). Note that because of the high lipid content of açaí (see below), no fish oil was added at the maximum inclusion level of lyophilized *E. oleracea*. Dietary composition and proximate analysis are presented in Tables 1, 2, and 3. Dry ingredients were mixed and added 30% warm water to produce a mixture that was then pelletized, and dried in an oven at 65 °C for 24 h. Pellets were broken to obtain particles of 1.0–2.0 mm diameter and stored at − 20 °C until use. Analyses to determine the proximate composition of lyophilized açaí and diets followed the Association of Official Analytical Chemists (AOAC 2005) standard procedures. Dry matter was measured in an oven at 102 °C until constant weight; ashes were determined in a muffle furnace at 600 °C during 5 h; crude protein was determined with the Kjeldahl method after sample digestion and nitrogen (N) distillation and then calculated as $N \times 6.25$; the lipid content was determined by petroleum ether extraction using a Soxhlet extractor (AOAC 2005). The nitrogen-free extract (NFE) is calculated by subtracting the sum percentage of crude protein, crude fiber, ether

Table 2 Proximate matter of lyophilized açaí (g/100 g of dry weight)

Crude protein	Ether extracts	Crude fiber	Mineral content
10.54 ± 0.47	42.79 ± 0.31	13.02 ± 0.71	3.03 ± 0.11

Table 3 Proximate matter of diets (g/100 g of dry weight)

	0% inclusion (control)	2.5% inclusion	5.0% inclusion	10.0% inclusion
Crude protein	36.8 ± 1.37	36.4 ± 0.81	34.9 ± 0.12	35.3 ± 0.12
NFE[1]	38.7 ± 1.15	38.8 ± 1.79	38.8 ± 0.19	38.5 ± 0.40
Ether extract	6.4 ± 0.07	6.1 ± 0.10	6.0 ± 0.05	6.8 ± 0.10
Moisture	4.3 ± 0.06	3.5 ± 0.29	3.0 ± 0.29	3.7 ± 0.12
Ash	8.7 ± 0.12	8.0 ± 0.77	8.7 ± 0.00	8.8 ± 0.00

[1] Calculated value: NFE = 100 − (Crude protein + crude lipid + ash + moisture)

extract, and total ash from 100. Crude fiber (CF) was determined in acid (1.25% sulfuric acid) and neutral (1.25% NaOH solution) detergent according to Silva and Queiroz (2009) (Tables 2 and 3).

Zootechnical parameters

At the end of the experiment (43 days), shrimps from each tank were counted and weighed individually (± 0.01 g) and the following variables were determined:

1. Weight gain (WG) (g): final weight (g) − initial weight (g);
2. Feed conversion ratio (FCR): feed offered (g) / weight gain (g);
3. Specific growth rate (SGR): [ln (final weight (g)) − ln (initial weight (g))] / 43 (days) × 100;
4. Survival (%): (final shrimp number / initial shrimp number) × 100.

After measurements, organisms were killed in liquid nitrogen, dissected (muscle, hepatopancreas, and gills) and stored in an ultrafreezer (− 80 °C), for determination of total flavonoid content.

Bioflocs sampling

Water (1 L^{-1}) of each tank of the BFT system was sampled using Imhoff cones and then let to settle for 15 min (Avnimelech 2012). After decantation, water was siphoned and settled solids were transferred to 15-mL Falcon tubes. Samples were centrifuged at 10,000×*g* (4 °C) for 5 min and the supernatant water was discarded. Bioflocs were transferred to Eppendorf tubes and stored at − 80 °C in an ultrafreezer, for determination of total flavonoid content.

Antioxidant extraction from shrimp organs, bioflocs, and diets

The collected organs (muscle, hepatopancreas, and gills), bioflocs, and diet samples were homogenized in methanol 100% (HPLC grade) using a relation (W/V) of 1:2, 1:1, and 1:4, respectively. These relations were selected after conducting preliminary assays to analyze the proper W/V relationship between sample weight and the volume of methanol added that assured readings higher than the blank. Homogenates were transferred to Eppendorf covered with aluminum foils and shaken during 3 h. After that, the samples were centrifuged at 10,000×*g* for 10 min at 4 °C and supernatant was kept for total flavonoid measurements.

Measurement of total flavonoid content

Total flavonoid content was measured according to Gajula et al. (2009) and Pękal and Pyrzynska (2014) with modifications based on the work of Molina León et al. (2018). Methanolic extracts aliquots (25 μL) from shrimp organs, bioflocs, and diets were added to Eppendorf tubes and then added 125 μL of distilled water and after 5 μL of 5% $NaNO_2$. Samples were incubated for 5 min at room temperature in the dark and then 15 μL of 10% $AlCl_3$ was added and the mix was incubated for another 5 min at room temperature in the dark. Finally, it was added 50 μL of NaOH 1 M and 27.5 μL of distilled water and the absorbance read at 510 nm in a microplate reader (Biotek LX 800). A standard curve ($R^2 = 0.99$) was prepared with quercetin (Sigma) and data was expressed in terms of μg of quercetin/g of wet samples. All measurements were performed in duplicate.

Color analysis

Color parameters were measured according to Hunt (1977) using a digital colorimeter (Minolta CR400). It was determined the following parameters: lightness (L^*), red/green chromaticity (a^*), and yellow/blue chromaticity (b^*) by placing the instrument in the cephalothorax and abdominal region of fresh and cooked (2 min at 70 °C) shrimps. From the a^* and b^* values, the chroma (C^*) and hue ($H°$) were calculated according to the following equations (Torres Rosa et al. 2019):

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2}$$

$$H° = \tan^{-1}\left(b^*/a^*\right),$$

where C^* expresses the intensity and clarity of the color and $H°$ is a relationship between redness and yellowness.

Statistical analysis

Zootechnical and color parameters were measured using a mixed model analysis of variance (ANOVA), where the açaí inclusion levels were the fixed factor and the different tanks where the shrimps were maintained were the random factor (Searle et al. 2006). Water quality data, organs, and bioflocs content of polyphenols and total flavonoids and muscle centesimal composition were analyzed using a one-way ANOVA (factor: açaí inclusion). In all cases, post hoc comparisons were performed using the Newman-Keuls test and orthogonal contrasts. Before each analysis, normality and variance homogeneity were verified using Shapiro-Wilks and Levene test, respectively. In all cases, the significance level was set in 0.05.

Results

Water quality parameters

The mean values of temperature, dissolved oxygen, pH, salinity, alkalinity, phosphate, ammonia, nitrites, nitrates, and total solids were similar among the experimental groups ($p > 0.05$; Table 4).

Table 4 Water quality parameters and total suspended solids (TSS) determined in the tanks of the BFT rearing system for *Litopenaus vannamei* fed during 43 days with different açaí inclusion levels (0.0, 2.5, 5.0, and 10.0%). Data are expressed as mean ± 1 standard error of the mean. Similar letters between different açaí inclusion levels indicate the absence of statistical differences ($p > 0.05$). DO stands for dissolved oxygen. $MS_{treatment}$ and MS_{error} refer for the treatment mean square and error mean square estimated for each variable

Variables	0.0% (control)	2.5%	5.0%	10.0%	$MS_{treatment}$	MS_{error}
DO (mg L^{-1})	5.01 ± 0.09[a]	5.13 ± 0.34[a]	5.07 ± 0.19[a]	5.04 ± 0.03[a]	0.038	0.040
Temperature (°C)	28.78 ± 0.23[a]	28.29 ± 0.38[a]	28.86 ± 1.52[a]	28.55 ± 0.56[a]	0.750	0.700
pH	7.99 ± 0.08[a]	8.06 ± 0.15[a]	8.04 ± 0.12[a]	8.01 ± 0.05[a]	0.001	0.012
Salinity	34.67 ± 0.32[a]	34.60 ± 1.46[a]	34.28 ± 1.23[a]	34.20 ± 0.66[a]	0.230	1.050
Alkalinity (mg $CaCO_3$ L^{-1})	125.71 ± 27.31[a]	153.53 ± 13.99[a]	156.01 ± 36.72[a]	128.57 ± 22.67[a]	363.6	700.8
Phosphate (mg L^{-1})	0.30 ± 0.05[a]	0.29 ± 0.06[a]	0.32 ± 0.09[a]	0.29 ± 0.06[a]	0.002	0.005
Ammonia-N (mg L^{-1})	0.07 ± 0.01[a]	0.06 ± 0.02[a]	0.08 ± 0.02[a]	0.09 ± 0.01[a]	$2.59.10^{-4}$	$2.71.10^{-4}$
Nitrite-N (mg L^{-1})	0.35 ± 0.22[a]	0.29 ± 0.29[a]	0.82 ± 1.01[a]	0.28 ± 0.29[a]	0.418	0.309
Nitrate-N (mg L^{-1})	46.87 ± 11.20[a]	49.66 ± 6.30[a]	50.77 ± 3.86[a]	48.56 ± 1.34[a]	18.61	45.41
TTS (mg L^{-1})	178.00 ± 27.87[a]	207.50 ± 87.23[a]	211.25 ± 65.14[a]	170.00 ± 83.00[a]	1404.6	4879.8

Zootechnical parameters

No statistical differences were registered for the different zootechnical parameters (weight gain, feed conversion ratio, and specific growth rate) among treatments ($p > 0.05$; Table 5).

Total flavonoid concentration

The diets showed a dose-response relationship between the total flavonoid levels and the percentage of açaí inclusion ($p < 0.05$; Fig. 1a). In the biofloc samples, the levels of total flavonoids did not differ, according to the results of the Newmann-Keuls test, although orthogonal contrast pointed to higher levels of bioflocs from tanks of shrimps fed with 5.0% or 10.0% açaí compared with those that received 0.0% (control group) or 2.5% ($p < 0.05$; Fig. 1b). The total flavonoid content showed no variation ($p > 0.05$) among treatments in the muscle and hepatopancreas (Fig. 2a and b). In the gills, higher levels of total flavonoids

Table 5 Zootechnical parameters measured in shrimp *Litopenaeus vannamei* reared in a BFT system and fed during 43 days with different açaí inclusion levels (0.0, 2.5, 5.0, and 10.0%). Data are expressed as mean ± 1 standard error of the mean ($n = 59–74$). Similar letters between different açaí inclusion levels indicate the absence of statistical differences ($p > 0.05$). *WG*, weight gain; *FCR*, feed conversion ratio; *SGR*, specific growth rate. $MS_{treatment}$ and MS_{error} refer for the treatment mean square and error mean square estimated for each variable

Açaí inclusion (W/W)	WG (g)	FCR	SGR	Survival (%)
0.0%	3.33 ± 0.08[a]	2.10 ± 0.10[a]	7.74 ± 0.80[a]	92.50 ± 4.70[a]
2.5%	3.36 ± 0.12[a]	2.00 ± 0.08[a]	7.81 ± 0.28[a]	97.5 ± 3.50[a]
5.0%	3.33 ± 0.09[a]	2.20 ± 0.20[a]	7.75 ± 0.21[a]	85.0 ± 8.60[a]
10.0%	3.30 ± 0.08[a]	1.80 ± 0.02[a]	7.68 ± 0.20[a]	100.0 ± 0.00[a]
$MS_{treatment}$	0.036	0.017	0.193	267.4
MS_{error}	1.043	0.022	5.638	208.3

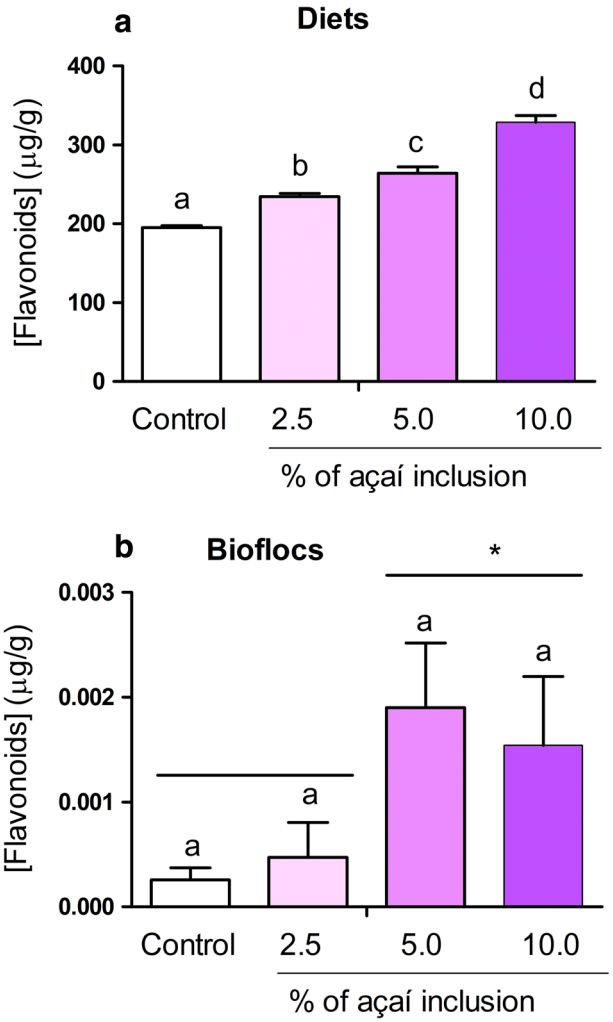

Fig. 1 Total flavonoid content (expressed in µg of quercetin equivalents/g of wet sample) in diets (**a**) and bioflocs (**b**) from tanks where shrimps *Litopenaeus vannamei* were fed with different inclusion levels of açaí in the diet (control, 2.5, 5.0, and 10.0%). Data are expressed as mean ± 1 standard error of the mean ($n = 3–5$). Different letters indicate statistical significance ($p < 0.05$) after Newmann-Keuls test. Asterisk indicates statistical significance ($p < 0.05$) after orthogonal contrast between flavonoid content in bioflocs from tanks where shrimps fed with 0 (control) or 2.5% of açaí versus tanks of shrimps fed with 5.0 or 10.0% of açaí. The treatment and error mean square values were 15,870 and 41.46 for **a**, and 0.158 and 0.071 for **b**

($p < 0.05$) were observed in shrimps fed with 5.0% or 10.0% açaí compared with the control group (Fig. 2c).

Color analysis

Tables 6 and 7 show the mean values (± 1 standard error of the mean) of color parameters measured in the cephalothorax and abdominal regions of fresh and cooked shrimps. In the cephalothorax region, a significant ($p < 0.05$) lack of lightness (darker color) was verified in

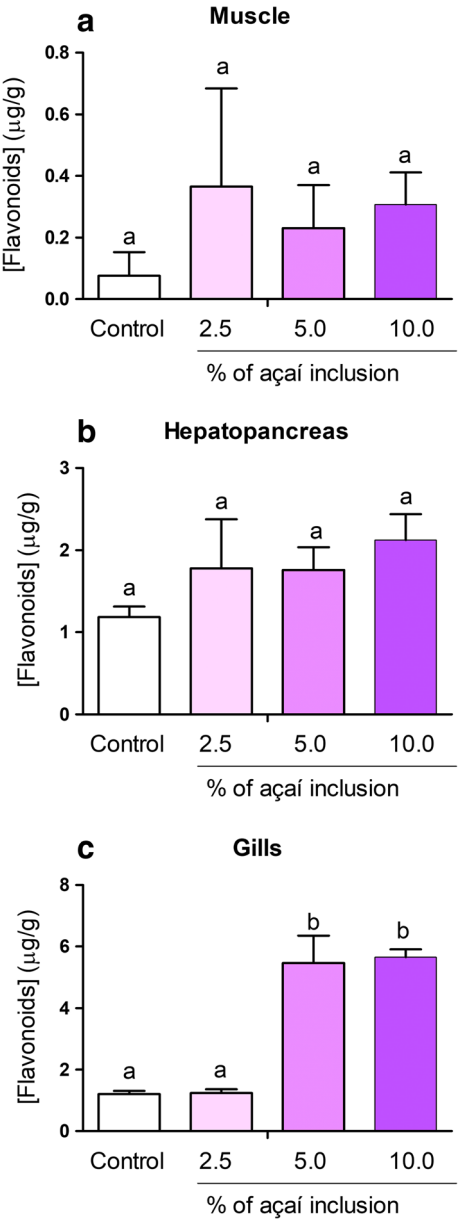

Fig. 2 Total flavonoid content (expressed in µg of quercetin equivalents/g of wet tissue) in muscle (**a**), hepatopancreas (**b**), and gills (**c**) of shrimps *Litopenaeus vannamei* fed with different inclusion levels of açaí in the diet (control, 2.5, 5.0, and 10.0%). Data are expressed as mean ± 1 standard error of the mean ($n = 4$). Different letters indicate statistical significance ($p < 0.05$) after Newmann-Keuls test. The treatment and error mean square values were 0.094 and 0.134 for **a**, 0.152 and 0.095 for **b**, and 1.356 and 0.021 for **c**

fresh shrimps fed with 10% açaí compared with the control group. No differences in chromaticity were verified in the cephalothorax region of cooked shrimps ($p > 0.05$; Table 6). In the abdominal region, no differences in lightness were verified among treatments ($p > 0.05$). A reddish color was evident in organisms fed with 10.0% açaí with respect to the control group in both fresh and cooked shrimps ($p < 0.05$; Table 7). Cooked shrimps fed with 10.0% açaí showed a reddish hue compared with shrimps from the control group ($p < 0.05$; Table 7).

Table 6 Color parameters (lightness L^*, red/green chromaticity a^*, yellow/blue chromaticity b^*, intensity and clarity of the color C, relationship between redness and yellowness $H°$) in the cephalothorax region of shrimp *Litopenaeus vannamei* fed with different inclusion levels of açaí in the diet (0.0, 2.5, 5.0, and 10.0%). Data are expressed as mean ± 1 standard error of the mean ($n = 8$). Similar letters between different açaí inclusion levels indicate the absence of statistical differences ($p > 0.05$). $MS_{treatment}$ and MS_{error} refer for the treatment mean square and error mean square estimated for each variable

Açaí inclusion (W/W)	L^*	a^*	b^*	C	$H°$
Fresh					
0.0%	42.87 ± 2.8^{a}	-0.11 ± 2.10^{a}	6.52 ± 2.40^{a}	6.24 ± 0.86^{a}	-0.39 ± 0.47^{a}
2.5.%	38.72 ± 4.00^{ab}	0.39 ± 1.70^{a}	4.51 ± 3.40^{a}	5.22 ± 1.21^{a}	0.29 ± 0.48^{a}
5.0%	39.61 ± 4.30^{ab}	0.24 ± 1.10^{a}	4.43 ± 1.40^{a}	4.25 ± 0.51^{a}	0.37 ± 0.49^{a}
10.0%	34.26 ± 2.70^{b}	1.04 ± 0.90^{a}	3.11 ± 2.40^{a}	3.39 ± 0.72^{a}	0.54 ± 0.40^{a}
$MS_{treatment}$	102.821	1.433	12.425	12.151	0.785
MS_{error}	8.931	2.623	8.939	8.783	1.181
Cooked					
0.0%	58.38 ± 5.90^{a}	6.36 ± 1.50^{a}	13.57 ± 1.50^{a}	14.98 ± 0.53^{a}	1.16 ± 0.04^{a}
2.5.%	54.34 ± 5.10^{a}	4.50 ± 1.50^{a}	10.91 ± 1.60^{a}	12.04 ± 0.70^{a}	1.18 ± 0.03^{a}
5.0%	52.04 ± 7.80^{a}	4.67 ± 2.10^{a}	11.07 ± 3.00^{a}	12.29 ± 1.19^{a}	1.15 ± 0.04^{a}
10.0%	47.09 ± 7.90^{a}	6.97 ± 1.60^{a}	10.65 ± 3.40^{a}	13.36 ± 1.15^{a}	1.05 ± 0.05^{a}
$MS_{treatment}$	93.664	5.767	0.018	14.305	0.0278
MS_{error}	57.871	3.819	0.005	4.180	0.022

Lightness (L^*) ranges between 0 (black) and 100 (white). Red/green chromaticity (a^*) ranges from negative (green) to positive values (red). Yellow/blue chromaticity (b^*) ranges from negative (blue) to positive values (yellow). The chroma (C^*) measures the clarity and intensity of the color. Hue ($H°$) parameter is the relationship between redness and yellowness

Table 7 Color parameters (lightness L^*, red/green chromaticity a^*, yellow/blue chromaticity b^*, intensity and clarity of the color C, relationship between redness and yellowness $H°$) in the abdominal region of shrimp *Litopenaeus vannamei* fed with different inclusion levels of açaí in the diet (0.0, 2.5, 5.0, and 10.0%). Data are expressed as mean ± 1 standard error of the mean ($n = 8$). Different letters indicate statistical significance ($p < 0.05$) after the Newmann-Keuls test. $MS_{treatment}$ and MS_{error} refer for the treatment mean square and error mean square estimated for each variable

Açaí inclusion (W/W)	L^*	a^*	b^*	C	$H°$
Fresh					
0.0%	42.87 ± 1.30^{a}	-1.57 ± 0.30^{b}	2.44 ± 0.70^{a}	2.86 ± 0.23^{a}	-0.95 ± 0.06^{a}
2.5.%	44.06 ± 1.90^{a}	-1.10 ± 0.30^{ab}	1.89 ± 1.40^{a}	2.29 ± 0.36^{a}	-0.68 ± 0.20^{a}
5.0%	42.25 ± 1.60^{a}	-0.97 ± 0.60^{ab}	2.40 ± 1.60^{a}	2.52 ± 0.38^{a}	-0.89 ± 0.29^{a}
10.0%	41.39 ± 2.00^{a}	-0.85 ± 0.60^{a}	2.41 ± 1.90^{a}	2.50 ± 0.56^{a}	-1.06 ± 0.22^{a}
$MS_{treatment}$	8.370	0.876	0.729	0.433	0.208
MS_{error}	4.267	0.225	2.476	1.381	0.390
Cooked					
0.0%	68.90 ± 4.10^{a}	3.70 ± 1.60^{b}	42.87 ± 2.70^{a}	12.56 ± 1.04^{a}	1.27 ± 0.03^{a}
2.5.%	70.29 ± 3.20^{a}	3.19 ± 1.80^{ab}	44.06 ± 1.70^{a}	10.97 ± 0.73^{a}	1.29 ± 0.05^{a}
5.0%	62.83 ± 3.70^{a}	4.28 ± 2.60^{ab}	42.25 ± 2.20^{a}	10.99 ± 1.01^{a}	1.19 ± 0.06^{ab}
10.0%	65.82 ± 6.40^{a}	6.28 ± 1.30^{a}	41.39 ± 2.90^{a}	13.94 ± 0.93^{a}	1.11 ± 0.04^{b}
$MS_{treatment}$	53.994	12.612	11.344	16.265	0.058
MS_{error}	16.561	2.843	6.702	6.581	0.021

Lightness (L^*) ranges between 0 (black) and 100 (white). Red/green chromaticity (a^*) ranges from negative (green) to positive values (red). Yellow/blue chromaticity (b^*) ranges from negative (blue) to positive values (yellow). The chroma (C^*) measures the clarity and intensity of the color. Hue ($H°$) parameter is the relationship between redness and yellowness

Discussion

The use of BFT in aquaculture is considered an effective alternative in reducing the emission of effluents from water reuse. Indeed, in rearing systems such as BFT, the maintenance of water quality parameters is of paramount importance (Avnimelech 2012). In our study, all water quality parameters were within the optimal range for *L. vannamei*, including temperature and salinity (Ponce-Palafox et al. 1997), dissolved oxygen, pH, and alkalinity (Van Wyk and Scarpa 1999) as well as levels of nitrogenous compounds (ammonia and nitrite levels) (Lin and Chen 2001, 2003). The values of total suspended solids registered in our study (170.00–207.50 mg L^{-1}) were in the range (100–300 mg L^{-1}) recommended by Gaona et al. (2015). Thus, the observed results should be ascribed to direct or indirect effects elicited by açaí administration and not to the effects related to suboptimal water quality parameters.

Recent studies have demonstrated that preventive nutrition in aquaculture through the use of supplements with nutrients and functional additives has had beneficial effects on the physiology of the animal, resulting in improved zootechnical performance and health (Kiron 2012). The use of supplements can afford multiple benefits, including their role as fish oil substitutes and antioxidant effects, and influence the color of the reared organisms. No zootechnical parameters were altered by açaí included in the diet. Particularly, for the diet formulated with 10.0% açaí, a complete replacement of the fish oil was made (Table 1) given the high lipid levels determined in the lyophilized açaí (Table 2). Soller et al. (2018) used palm oil to replace fish oil in *L. vannamei* without affecting survival or weight gain, although the feed conversion ratio was impaired. Soller et al. (2017) also evaluated the partial substitution of menhaden fish oil with alternative lipid sources (soybean oil, poultry grease, and flax seed oil) in *L. vannamei* and found no differences in terms of fatty acid composition in shrimp muscle and the sensory ratings of the final product. In our study, this last variable was not affected by the different inclusion levels of açaí (Table 4), pointing to this fruit as a potential alternative for fish oil, a goal widely analyzed in aquaculture because of present overfishing and associated costs (Tacon and Metian 2008; Torres Rosa et al. 2019).

Fresh and cooked shrimps fed with the inclusion of 10.0% açaí developed more reddish abdominal regions (Table 7). The altered chromaticity of shrimp fed with açaí should be associated with the incorporation of anthocyanins, responsible for the purple color of the fruit, which is green before it ripens (Lichtenthäler et al. 2005). The antioxidant content found in bioflocs is relevant since they represent 29% of ingested food by *L. vannamei* (Burford et al. 2003), being an important source of minerals, vitamins, fatty acids, proteins, and antioxidant molecules (Moss et al. 2006; Martins et al. 2015; Magaña-Gallegos et al. 2018). Molina León et al. (2018) discovered that exogenous quercetin added to a BFT resulted in higher flavonoid content both in the bioflocs and in the muscle and hepatopancreas of *L. vannamei*. These authors postulated that antioxidant-enriched bioflocs should constitute an interesting vector for the transfer of bioactive molecules to shrimps. In the bioflocs, an alternative flow of nutrients can be observed, described as a "microbial loop," in which the primary producers are consumed by rotifers, copepods, and nematodes, bio-accumulating the nutrients and performing a transfer via the food chain (Emerenciano et al. 2013). In this study, the highest açaí levels (5.0% and 10.0%) included in the diets were shown to increase the total flavonoid levels in the bioflocs. The higher antioxidant levels in bioflocs did not alter the water quality parameters (Table 4), suggesting that BFT functionality was not altered by the açaí treatment. Although how flavonoids present in the diets were transferred to the bioflocs is unclear, we can consider that antioxidants present in the açaí pulp and included in the diets go through a

complex food web that includes the incorporation of and probably transference from bioflocs to shrimps. Antioxidant metabolization by microorganisms should generate chemically more diverse metabolites to shrimp when they are fed with bioflocs, an aspect that requires further study.

A significant flavonoid accumulation in shrimps fed with 5.0% or 10.0% açaí was observed only in the gills (Fig. 2c). The high antioxidant content in these organs should be important in coping with pro-oxidant conditions that occur in aquaculture, such as hypoxia followed by re-oxygenation. In *L. vannamei*, exposure to moderate hypoxia (3 $mg.L^{-1}$) followed by 4 hours of re-oxygenation resulted in antioxidant capacity lowering in the gills (Martins et al. 2014). The absorption of different antioxidants present in açaí depends on their chemical structure. Kang et al. (2010) found seven major flavonoids present in açaí pulp: orientin, homoorientin, vitexin, luteolin, chrysoeriol, quercetin, and dihydrokaempferol. According to Gonzales et al. (2015), compounds that are likely absorbable through the intestine should contain, at most, five H-bond donors, ten H-bond acceptors, a molecular weight > 500 Da, and a lipophilicity index > 5. For the group of flavonoids, molecules with many hydroxyl, glycosidic, and galloyl moieties are less likely to be absorbed through the intestines. Thus, the success of açaí inclusion depends on the prevalent molecules of flavonoids, as indicated above, that, in turn, rely on several factors. For instance, Malcher and Carvalho (2011) determined that the highest anthocyanin concentration in açaí was in the summer crop, from July to October. In this way, the functional properties of the fruit are, at least in part, determined by environmental factors and should influence the success of absorbing antioxidant molecules from an açaí-enriched diet.

The main results in this study are as follows: (a) the potential use of açaí oils for shrimp diets as 100% fish oil substitution by açaí lipids did not affect zootechnical parameters; (b) açaí inclusion inducing a reddish color in *L. vannamei*, an influential factor in their commercial value; (c) the accumulation of flavonoids in the gills, a response that should help minimize injuries in this organ under stressful conditions, such as hypoxia followed by re-oxygenation; (d) and the accumulation of flavonoids in the bioflocs, which points to their potential role as antioxidant vectors for reared shrimps.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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