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Hepatoprotective Action of Including Açaí (*Euterpe oleracea* Mart.) in the Diet of Koi Carp (*Cyprinus carpio*)

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Abstract This study evaluated the hepatoprotective effect of açaí inclusion in the diets of juvenile Koi carp (*Cyprinus carpio*). For the experimental trial, 240 fish were distributed across 20 tanks (n = 12), using a completely randomized design that involved four treatments and one control group. The tested diets were: control (DC0.0%) with no açaí; 0.5% açaí (DA0.5%); 1.0% açaí (DA1.0%); 1.5% açaí (DA1.5%); and 2.0% açaí (DA2.0%) inclusion. The açaí-supplemented diets showed higher concentrations of phenolic compounds, tannins, and flavonoids, as well as superior antioxidant potential compared to the control group ($p<0.05$). After 30 days of feeding, four specimens from each tank were collected for histological analysis. The analysis of variance at 5% significance revealed a significant difference in the loss of the hepatic cord arrangement, with a reduction of 62.50 ± 13.06% in the control group (DC0.0%) compared to the other treatments. The DA2.0% diet exhibited greater congestion in the sinusoids (54.17 ± 20.87%) compared to the control (33.33 ± 11.68%). The control group also showed a higher number of mononuclear inflammatory infiltrates (72.92 ± 7.54%). Necrotic areas were more intense in the control group (64.58 ± 12.87%) and less pronounced in the DA1.5% diet (43.75 ± 24.13%). The results suggest that diets with intermediate levels of açaí can exert a hepatoprotective effect on Koi carp, indicating that diets with the inclusion of açaí for carp can help in growth and nutrient assimilation, since the liver tissue metabolizes a large amount of the substance, but additional studies are needed to determine the ideal dose and explore its application in other fish species.

Keywords Nutrition; Liver health; Ornamental aquaculture; Antioxidant

1 Introducion

Aquariology is constantly expanding, and it is estimated that, in 2020, more than 4 million tons of live ornamental fish were traded worldwide (Food and Agriculture Organization of the United Nations - FAO 2022). This sector is gaining increasing importance as restrictions related to extractivism are being implemented (Ladisa et al., 2017). In addition, fish hold the top position in the global ranking of pet groups, and in Brazil, they are the fourth group, behind only dogs, birds, and cats, in 2023 (Brazilian Association of the Pet Products Industry - ABINPET 2024).

Among ornamental species, the common carp, Koi variety (*Cyprinus carpio*), stands out. It is a freshwater species native to Asia, belonging to the order Cypriniformes and the family Cyprinidae (Shi et al., 2024). According to data from the FAO (2024), the production of *C. carpio* accounts for 4,012.6 tons of the global freshwater aquaculture production. The species has a high market value due to its vibrant colors, body shape, and its easy adaptation to different farming systems (Laksono et al., 2021; Yanuhar et al., 2021).

Although ornamental fish production has developed significantly with advances in infrastructure and technologies to meet market demand (Goswami et al., 2023), this growth has also contributed to the frequent increase in health issues in farming environments (Saengsitthisak et al., 2020). These problems are triggered by a variety of factors, such as poor water quality, inadequate management practices, and the provision of imbalanced diets, which impact the development of these animals and, consequently, make them more vulnerable to disease outbreaks (Debnath et al., 2024).

In light of this situation, some tools are commonly employed in an attempt to mitigate these effects, such as the use of chemotherapeutics in the prophylaxis and treatment of bacterial infections (Cardoso et al., 2021). However, when applied inappropriately, this practice has consequences for the environment and for animals, because, as well as leaving chemical residues in the environment, it can promote the selection of resistant bacterial strains (Hossain and Heo, 2021). Considering this scenario, other tools are being developed with the intention of reducing the use of chemotherapeutics, among which are phytochemicals. These have been investigated as nutritional strategies for use as modulators of immune, physiological, and antioxidant responses, acting as health promoters in animal production (Xu et al., 2020). An example of phytotherapeutics with great potential for use in aquaculture is açaí (*Euterpe oleracea*), an Amazonian fruit.

Açaí (*E. oleracea*) is a species from the Arecaceae family, native to several countries in the Amazon region, such as Brazil, Venezuela, and Ecuador (Oliveira et al., 2020). This fruit stands out for its antioxidant, anti-inflammatory, and immunomodulatory properties, in addition to being rich in bioactive compounds such as polyphenols, essential fatty acids, and vitamins (Moura et al., 2022). Its main chemical constituents are anthocyanins, polyphenols, and flavonoids (Inácio et al., 2013). Furthermore, studies have shown that this fruit provides a wide range of therapeutic benefits and promotes health due to its nutritional value and phytochemical composition (Pacheco-Palencia et al., 2008). In consideration of the above, this research aimed to evaluate the impact of different levels of inclusion of freeze-dried açaí in the diet of koi carp on liver health through histological methods.

2 Materials and Methods

2.1 Biological materials

The juvenile koi carp (*C. carpio*) were acquired from the Girassol fish farm in Joinville, Brazil, with an average weight of (6.2 ± 1.2) g and an average length of (7.8 ± 1.4) cm. The açaí (*E. oleracea*) used was of commercial origin. The feeding trial was conducted at the Aquatic Organism Health Laboratory (AQUOS) at the Federal University of Santa Catarina (UFSC), and all procedures were approved by the Animal Ethics Committee (CEUA) of UFSC (CEUA/UFSC 5552130324).

2.2 Dietas experimentais

The procedure for preparing the feed was adapted from Heluy et al. (2023), using a commercial freeze-dried açaí product (Liomeal®), incorporated into a commercial feed that meets the nutritional requirements of the target species (Figure 1). For the formulation of the diets, 20 kg of feed was ground in a Willey knife mill. The feed was then divided into four treatments (5, 10, 15, and 20 g/kg) and a control group without açaí addition (0.0%), resulting in a total of five experimental diets.

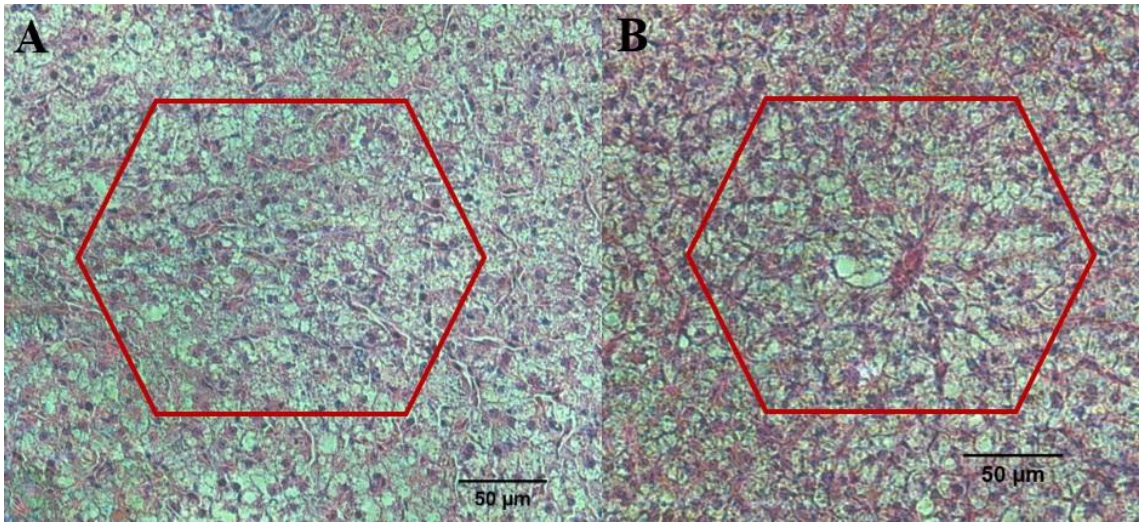

Figure 1 Hexagons indicate loss of the cord-like appearance of koi carp hepatocytes in the DC0.0% group (A); and maintenance of the cord-like appearance of koi carp hepatocytes in DA1.0% (B); Tukey (p<0.05)

The inclusion of açaí was achieved by adding distilled water at 55 °C to promote the gelatinization and agglomeration of the starch. After this process, the mixture was homogenized in a horizontal mixer until a semi-solid consistency was reached. The pelleting process was then carried out using a die with a 2.5 mm opening. The feed pellets were dried in an oven at 50 °C for 6 hours, broken, and sifted to separate the granules sized between 2 and 2.5 mm and stored at ~20 °C until use.

The proximal composition of the formulated diets (Table 1) was determined following the methods established by AOAC (2016). Crude protein (N × 6.25) was determined by the Kjeldahl method. The crude lipid content was evaluated using a Soxhlet extractor with solvent extraction (petroleum ether). Crude fiber was analyzed through acid and alkaline digestion of the samples, as described by Silva and Queiroz (2002).

Table 1 Ingredients of the experimental diets and analysis of the centesimal composition before and after the inclusion of açaí

Ingredients (%)	Diets				
	DC0.0%	DA0.5%	DA1.0%	DA1.5%	DA2.0%
Fish meal	30.00	30.00	30.00	30.00	30.00
Soybean meal	25.00	25.00	25.00	25.00	25.00
Wheat flour	15.00	15.00	15.00	15.00	15.00
Soybean hulls	15.00	15.00	15.00	15.00	15.00
Fish oil	≤5.00	≤5.00	≤5.00	≤5.00	≤5.00
Corn bran	15.00	15.00	15.00	15.00	15.00
Premix [1]	≤4.00	≤4.00	≤4.00	≤4.00	≤4.00
Crude fiber	4.50	4.50	4.50	4.50	4.50
Moisture	12.00	12.00	12.00	12.00	12.00
Proximal composition before the inclusion of açaí (g/kg)					
Moisture	100.0~120.0	100.0~120.0	100.0~120.0	100.0~120.0	100.0~120.0
Crude protein	360.00	360.00	360.00	360.00	360.00
Ether extract	50.0~80.0	50.0~80.0	50.0~80.0	50.0~80.0	50.0~80.0
Crude fiber	20.0~40.0	20.0~40.0	20.0~40.0	20.0~40.0	20.0~40.0
Mineral matter	70.0~100.0	70.0~100.0	70.0~100.0	70.0~100.0	70.0~100.0
Total carbohydrates	350.0~400.0	350.0~400.0	350.0~400.0	350.0~400.0	350.0~400.0
Proximal composition after the inclusion of açaí (g/kg)					
Moisture	55.20	57.97	62.23	68.93	59.57
Crude protein	359.60	360.30	358.60	348.20	356.75
Ether extract	80.65	84.50	82.55	86.50	88.75
Crude fiber	15.70	16.65	26.50	14.50	24.50
Mineral matter	97.33	97.03	97.27	95.40	98.20
Total carbohydrates	392.00	385.00	370.00	391.00	372.00
Sodium	2.90	2.95	2.90	2.90	2.90

Note: [1] Vitamin A (10 000~15 000 IU/kg). Vitamin C (500~1 000 mg/kg). Vitamin D (2 000~3 000 IU/kg). Vitamin E (100~200 mg/kg). Vitamin K (5~10 mg/kg). B1-Thiamine (20~30 mg/kg). B2-Riboflavin (30~50 mg/kg). B3-Niacin (100~200 mg/kg). B5-Pantothenic acid (50~100 mg/kg). B6-Pyridoxine (20~30 mg/kg). B7-Biotin (1~3 mg/kg). B9-Folic acid (10~20 mg/kg). B12-Cobalamin (0.02~0.05 mg/kg). Choline (1 000~2 000 mg/kg)

2.3 Experimental design

A total of 240 juvenile koi carp were distributed in 20 tanks with 80 L of water at stocking density of 12 fish per tank and 4 replicates each treatment. The animals were acclimated for 10 days and were fed with the feed recommended by the producer during this period. The tanks, containing the fish, were connected to a recirculating aquaculture system (RAS) consisting of a clarifier, mechanical and biological filters, and a UV reactor, with a

photoperiod of 12 hours. The experiment was conducted in a completely randomized design (CRD), with the animals being fed for 30 days with five distinct experimental diets and four repetitions, as follows:

(1) Control diet without açaí inclusion: DC0.0%;
(2) Diet with 0.5% açaí inclusion: DA0.5%;
(3) Diet with 1.0% açaí inclusion: DA1.0%;
(4) Diet with 1.5% açaí inclusion: DA1.5%;
(5) Diet with 2.0% açaí inclusion: DA2.0%.

During the experimental period, water quality variables, such as pH, dissolved oxygen (DO), and temperature, were measured daily using a multiparameter probe (HI9829 Hanna). Total ammonia, toxic ammonia, and nitrite were measured using colorimetric tests (Alfakit®). These variables remained within safe standards for the species, according to Qur'ania and Verananda (2017): pH (6.2 ± 0.1), DO (6.6 ± 1.4 mg/L), temperature (25.4 ± 0.9 ℃), total ammonia (0.13 ± 0.14 mg/L), and nitrite (0.12 ± 0.14 mg/L).

2.4 Chemical analysis of the diets

2.4.1 Phenolic compounds, flavonoids, tannin content and antioxidant potential

A sample of 1 g of feed was used to determine the content of phenolic compounds and flavonoids. The compounds were extracted using 10 ml of ethanol for 28 minutes in an ultrasonic bath, followed by filtration and analysis. This procedure was performed for all experimental diets and for pure freeze-dried açaí.

To quantify the phenolic compounds, 0.1 mL of the extract was added to 0.5 mL of Folin-Ciocalteu reagent and 1 mL of water, allowing for 1 minute of incubation. Then, 1.5 mL of sodium carbonate (20%) was added and analyzed using a spectrophotometer (Global Trade Technology, Brazil) at 430 nm (Djeridane et al., 2006). The quantification was performed using a standard curve of gallic acid, and the results were expressed in mg of gallic acid equivalent (GAE) per g of sample.

For the determination of flavonoids, 1 mL of 2% aluminum chloride in methanol and 1 mL of the sample were added. After a 15 minute reaction, the reading was performed using a spectrophotometer at 430 nm (Djeridane et al., 2006). The quantification was carried out using a standard curve, expressing the results in mg of rutin equivalent (RE) per g of sample.

The tannin content was determined in all experimental diets using the Folin-Denis spectrophotometric method (Pansera et al., 2003) with adaptations to the reagent volumes while maintaining the proportions. To this end, 0.5 mL of Folin-Denis reagent was added to 0.5 mL of the sample, shaken, and allowed to stand for 3 minutes. Then, 0.5 mL of sodium carbonate (0.75 M) was added, homogenized, and kept in the dark for 2 hours. Absorbance was measured at 725 nm. The concentration of tannins was calculated using a standard curve of tannic acid, with results expressed in mg of tannic acid equivalent (TAE) per g of sample.

The antioxidant activity of the samples was evaluated using the DPPH free radical method (1.1-diphenyl-2-picrylhydrazyl), as described by Capanoglu et al. (2008). For each 100 µL of sample, 2 000 µL of 0.004% DPPH was added, and the reaction was allowed to proceed for 30 minutes in the dark before measuring the absorbance at 517 nm using a spectrophotometer. The percentage of inhibition was calculated using the equation: $Q=(A0-Ac)/A0\times100$, where Q is the percentage of inhibition, A0 is the absorbance of the control, and Ac is the absorbance of the sample after the reaction.

2.5 Histological analysis

For the histological analysis of the liver, 4 fish from each tank were anesthetized with eugenol (75 mg/L) and then euthanized by brain sectioning. Liver fragments were fixed for 24 hours in 10% buffered formalin and subsequently dehydrated through a graded series of ethanol, followed by embedding in paraffin. These tissues were then sectioned into 5 µm thick slices and stained with hematoxylin and eosin (HE), as described by Martins et al. (2018). The slides were analyzed using a light microscope and the Zeiss 12pro software to obtain microphotographs and identify any histological alterations.

For the analysis of lesions, corresponding values were assigned to the histological alterations: 0 (no alteration), 1 (mild alteration, corresponding to up to 25% of the analyzed tissue area), 2 (moderate alteration, 26%~50% of the tissue area), and 3 (severe alteration, >50% of the tissue area). The intensity grades were used solely to measure the severity of the lesion and were subsequently converted into percentages (%) according to Cardoso et al. (2024).

2.6 Statistical analysis

The data was subjected to the Shapiro-Wilk and Levene tests to assess normal distribution and homoscedasticity, respectively. If the data was declared non-parametric, it was transformed. Subsequently, in compliance with the assumptions, the data was analyzed using ANOVA (analysis of variance) and, when appropriate, the means were separated using Tukey's post-hoc test. All tests were carried out at a 5% significance level using Statistica 12.0 software.

3 Results

The chemical analysis of the diets (Table 2) revealed that phenolic compounds were more pronounced ($p > 0.05$) in the diets with higher levels of açaí inclusion, DA1.5% and DA2.0% (30.50 ± 0.35 mg/g and 33.07 ± 0.07 mg/g, respectively), compared to the diet without inclusion, DC0.0% (23.40 ± 0.10 mg/g). A similar pattern was observed for flavonoids, with DA1.5% (14.90 ± 0.10 mg/g), DA2.0% (16.60 ± 0.10 mg/g), and DC0.0% (12.00 ± 0.10 mg/g); for tannin content, DA1.5% (1.53 ± 0.06 mg/g), DA2.0% (1.73 ± 0.06 mg/g), and DC0.0% (1.07 ± 0.06 mg/g); and for antioxidant potential concentration, DA1.5% (3.33 ± 0.07%), DA2.0% (3.77 ± 0.06%), and DC0.0% (1.13 ± 0.05%).

Table 2 Content of phenolic compounds, flavonoids, tannins and antioxidant potential of the experimental diets. Different letters (a, b, c) in the same line indicate significant difference between treatments by Tukey's post-hoc test ($p < 0.05$)

Parameters (mg/g)	DA0.0%	DA0.5%	DA1.0%	DA1.5%	DA2.0%	p value
Phenolic compounds	23.40±0.10^{b}	24.23±0.25^{b}	26.40±0.10^{ab}	30.50±0.35^{a}	33.07±0.07^{a}	<0.01
Flavonoids	12.00±0.10^{b}	12.47±0.15^{b}	13.03±0.06^{ab}	14.90±0.10^{a}	16.60±0.10^{a}	<0.01
Tannins	1.07±0.06^{c}	1.17±0.06^{c}	1.37±0.06^{b}	1.53±0.06^{a}	1.73±0.06^{a}	<0.01
Antioxidant potential (%)	1.13±0.05^{c}	2.43±0.06^{b}	2.87±0.06^{b}	3.33±0.07^{a}	3.77±0.06^{a}	<0.01

Regarding the histological alterations in the hepatic tissue of koi carp (Table 3), a significant difference ($p < 0.05$) was observed in the loss of cord-like appearance, which was more pronounced in DC0.0% (62.50 ± 13.06%) (Figure 1A) compared to the other treatments: DA0.5% (50.00 ± 10.66%), DA1.0% (45.45 ± 15.08%) (Figure 1B), DA1.5% (56.82 ± 11.68%), and DA2.0% (54.55 ± 15.08%). The diet DA2.0% resulted in greater congestion in the sinusoidal cords, with 54.17 ± 20.87% (Figure 2A) compared to DC0.0% (33.33 ± 11.68%) and DA1.5% (35.42 ± 12.87%), while DA0.5% and DA1.0% did not differ ($p > 0.05$) from the other diets, showing 46.88 ± 8.84% and 43.75 ± 15.54%, respectively. The control group exhibited a higher concentration ($p < 0.05$) of mononuclear inflammatory infiltrates, at 72.92 ± 7.54% (Figure 2B), compared to DA1.0% (54.17 ± 20.87%) and DA1.5% (50.00 ± 21.32%), but no significant difference ($p > 0.05$) was found concerning the other inclusion levels.

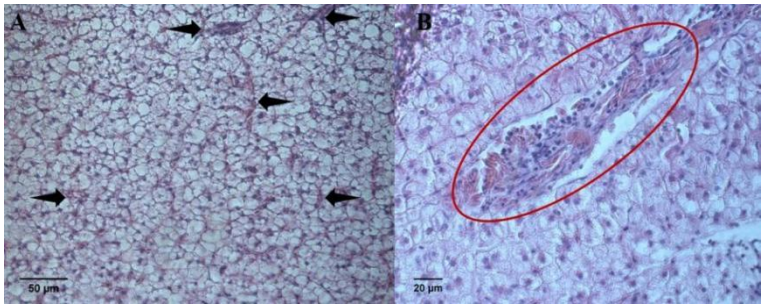

Figure 2 Arrows indicate sinusoidal congestion in the liver tissue of koi carp at DA2.0% (A); Ellipse shows mononuclear inflammatory infiltrate (purple dots) in a large vessel in the liver tissue of koi carp at DC0.0% (B); Tukey ($p<0.05$)

Table 3 Histological changes in the liver tissue of koi carp, *Cyprinus carpio*, fed different levels of açaí *Euterpe oleracea* in the diet for 30 days. Data presented as mean ± standard deviation. Different letters (a, b, c) in the same line indicate significant difference between the diets using Tukey's post-hoc test (p<0.05)

Alterations	DC0.0%	DA0.5%	DA1.0%	DA1.5%	DA2.0%	p value
LCA	62.50±13.06^{a}	50.00±10.66^{b}	45.45±15.08^{c}	56.82±11.68^{b}	54.55±15.08^{b}	0.035
LU	31.25±11.68	31.25±11.31	31.25±11.31	35.42±12.87	35.42±16.71	0.824
PAG	33.33±12.61	31.25±11.31	31.25±15.54	31.25±11.31	37.50±16.85	0.754
BA	39.58±17.19	47.92±12.87	50.0018.46	58.33±16.28	50.00±15.08	0.093
CLV	43.75±18.77	43.75±15.54	45.45±15.08	43.75±24.13	45.83±25.75	0.998
PC	56.25±18.77	70.83±9.73	62.50±13.06	60.42±12.87	50.00±30.15	0.052
SC	33.33±11.68^{b}	46.88±8.84^{ab}	43.75±15.54^{ab}	35.42±12.87^{b}	54.17±20.87^{a}	0.003
PVC	8.33±7.54	2.08±7.22	0.00±0.00	4.17±9.73	7.50±12.08	0.515
DHN	56.25±16.17	52.08±7.22	52.08±19.82	58.33±12.31	56.25±11.31	0.485
SD	29.17±7.54	36.11±13.18	29.17±9.73	31.25±15.54	39.58±16.17	0.067
EI	25.00±0.00	29.17±9.73	18.75±11.31	18.18±11.68	22.92±12.87	0.067
MI	72.92±7.54^{a}	64.58±12.87^{ab}	54.17±20.87^{b}	50.00±21.32^{b}	64.58±16.17^{ab}	0.011
HH	47.92±13.48	43.75±15.54	54.17±17.94	54.17±17.94	58.33±12.31	0.171
HNH	25.00±0.00	25.00±0.00	25.00±0.00	25.00±0.00	22.92±7.22	0.416
MA	66.67±12.61	65.91±12.61	56.82±16.17	52.08±19.82	55.56±20.83	0.147
CV	72.92±7.54^{a}	64.58±12.87^{ab}	47.92±22.51^{b}	35.42±16.17^{c}	70.83±9.73^{a}	<0.001
NE	64.58±12.87^{a}	62.50 ± 13.06^{a}	45.83±20.87^{b}	43.75±24.13^{b}	56.82±11.68^{ab}	0.011
PN	41.67±19.66	43.75±15.54	37.50±16.85	33.33±19.03	52.08±19.82	0.098
KN	50.00±19.36	41.67±12.31	41.67±19.46	43.75±15.54	39.58±12.87	0.56
KCN	35.42±13.06	39.58±12.87	35.42±16.17	35.42±12.87	33.33±12.31	0.852
LHN	41.67±16.17	37.50±13.06	35.42±16.17	41.67±22.19	23.71±19.82	0.892
LPA	29.17±10.11	29.17±9.73	25.00±0.00	27.08±7.22	31.82±11.68	0.408
MCC	6.25±11.68	11.25±16.25	6.25±15.54	9.17±19.29	0.42±1.44	0.419

Note: LCA = Loss of cord-like appearance; LU = Loss of uniformity in cell and nucleus size; LAG = Loss of intact acini and visible eosinophilic zymogen granules; BA = Ballooning appearance; CLV = Congestion in large vessels; PC = Pancreatic congestion; SC = Sinusoidal congestion; PVC= Portal vein congestion; DHN = Displacement of hepatocyte nuclei; SD = Sinusoidal dilation; EI = Eosinophilic inflammatory infiltrate; MI = Mononuclear inflammatory infiltrate; HH = Hepatocyte hypertrophy; HNH = Hepatocyte nuclear hypotrophy; MA = Macrosteatosis; CV = Cytoplasmic vacuolization; NE = Necrosis; PN = Pyknotic nucleus; KN = Karyolytic nucleus; KCN = Karyorrhectic nucleus; LHN = Loss of hepatocyte nuclei; LPA = Loss of nuclei in pancreatic acini; MCC = Melanomacrophage centers

Additionally, in the control diet (DC0.0%) and the diet with the highest level of açaí inclusion (DA2.0%), a greater intensity ($p < 0.05$) of hepatic cytoplasmic vacuolization was observed, with concentrations of 72.92 ± 7.54% (Figure 3A) and 70.83 ± 9.73%, respectively, compared to diets DA1.0% (47.92 ± 22.51%) and DA1.5% (35.42 ± 16.17%), with the latter showing the lowest intensity of this alteration. Areas of necrotic tissue were more prevalent ($p < 0.05$) in the control group (64.58 ± 12.87%) (Figure 3B) and in the DA0.5% diet (62.50 ± 13.06%) compared to DA1.0% and DA1.5% (45.83 ± 20.87% and 43.75 ± 24.13%, respectively). The DA2.0% group (56.82 ± 11.68%) did not differ ($p > 0.05$) between the treatments and the control.

4 Discussion

The highest concentrations of phenolic compounds, tannins, flavonoids, and antioxidant potential in diets with higher inclusion of açaí are understandable, considering that this Amazonian fruit, according to Laurindo et al. (2023), is rich in various bioactive compounds found not only in the pulp but also in the leaves, seeds, and skin. According to Pacheco-Palencia et al. (2008) and Laurindo et al. (2023), the bioactives found in freeze-dried pulps

range from phenolic acids, such as protocatechuic, p-hydroxybenzoic, vanillic, syringic, and ferulic acids, to flavonoids, including catechin, quercetin, homoorientin, orientin, taxifolin, deoxyhexose, isovitexin, and scopoletin.

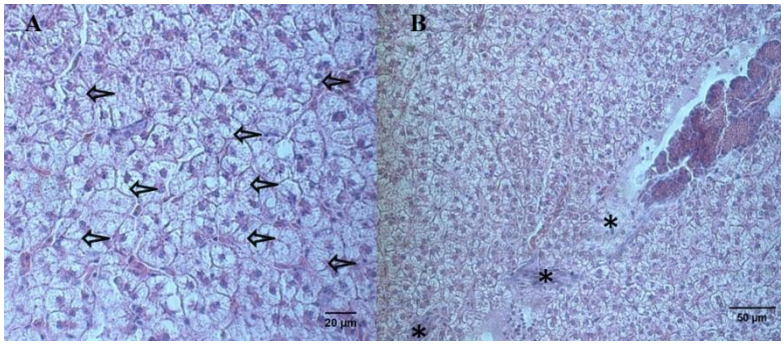

Figure 3 Open arrows show cytoplasmic vacuolization in the liver tissue of koi carp at 0.0% CD (A); asterisks show necrotic tissue in the liver of koi carp at 0.0% CD (B); Tukey (p<0.05)

In simplified terms, diets supplemented with açaí, due to the higher concentration of phytochemicals, contribute to the high antioxidant capacity observed in this study. According to Abbate et al. (2021) and Akbari et al. (2022), phenolic compounds and polyphenols, such as flavonoids, are effective in combating oxidative stress, promoting cell health, and increasing resistance to diseases and stress. The antioxidant process possibly involved is the neutralization of reactive molecules by non-enzymatic substances. Li et al. (2022) describe that these substances play an important role in reducing the activity of free radicals, leading to a decrease in the chain reactions mediated by these radicals and, consequently, protecting cells from oxidative damage. Among the non-enzymatic molecules present in açaí, flavonoids stand out, which may be associated with this antioxidant action, as evidenced by Rudenko et al. (2023) and Hu et al. (2025). The research by Carvalho et al. (2017) on the antioxidant capacity of genotypes of *Euterpe oleracea* pulp supports the results of this study, as it showed that açaí pulp exhibited high values in 2,2-diphenyl-1-picrylhydrazyl (DPPH) tests, as well as an increase in Oxygen Radical Absorbance Capacity (ORAC) and Trolox Equivalent Antioxidant Capacity (TEAC), confirming the exceptional effectiveness of açaí pulp in eliminating free radicals.

The results obtained indicated attenuated histological changes as the inclusion of açaí in the fish diet increased. The hepatic tissue plays a key role in the metabolism process, including nutrient absorption and detoxification (Popović et al., 2023). Therefore, monitoring this tissue is crucial for assessing the animals' health status. The loss of the cord-like structure was more intense in the control group, possibly related to the larger area of necrotic tissue, which was also more pronounced in this group. As a result, tissue degeneration hindered the formation and organization of the sinusoidal cords.

These findings suggest that açaí supplementation exerts a protective action against hepatic tissue degeneration, possibly due to its antioxidant and anti-inflammatory compounds. The high antioxidant capacity of açaí neutralizes free radicals and reduces oxidative stress in hepatic tissues, as observed by Laurindo et al. (2023), which likely contributed to a hepatoprotective effect in the supplemented animals. These results are significant as they support observations made by Colombo et al. (2020), who identified antioxidant and hepatoprotective effects in *Litopenaeus vannamei* after the inclusion of açaí (10%) in the diet, and by Moura et al. (2022), who obtained promising results regarding the inclusion of açaí in the diet on mortality rates and batch uniformity, at concentrations of 2.48% for *Pterophyllum scalare* and 0.88% for *Heros severus*.

However, in the group that received 2.0% açaí in the diet, the highest index of sinusoidal congestion was observed compared to the other treatments. This condition may be associated with an increase in blood flow to the liver or a reduction in the organ's ability to adequately drain blood, potentially affecting liver function and leading to more severe complications (Téllez et al., 2022). According to Pereira et al. (2016), an increase in sinusoidal congestion was also observed in rats fed fat-rich diets supplemented with açaí. Furthermore, the congestion observed may

indicate an adverse effect of the high concentration of açaí, highlighting the need for more detailed analyses to determine the appropriate dosage of this fruit supplementation in fish diets.

It was observed that the animals receiving diets supplemented with açaí at concentrations of 1.0% and 1.5% had lower indices of Mononuclear Inflammatory Infiltrates (MI) compared to the control group and the other inclusion levels. The high presence of MI in the control group may be associated with a cellular inflammatory immune response in the hepatic tissue. The same effect may have occurred in the fish fed the 2.0% diet, suggesting that this concentration may be harmful to the organ's integrity. Our results support the findings of Leite et al. (2021), who reported in their trials with tilapia that açaí oil (3% and 6%) stimulates antioxidant effects in the liver, increasing the activity of glutathione peroxidase (GSH-Px) and Superoxide Dismutase (SOD). Meanwhile, in the study by Silva et al. (2023), juveniles of *Colossoma macropomum* that received 5% açaí in their diet showed positive results related to growth, metabolic biomarkers, and antioxidant capacity.

Additionally, cytoplasmic vacuolization was significant in the control group and the group with 2.0% açaí inclusion. This histological change is characterized by the presence of small fat accumulations within the hepatocytes. In the other diets, this effect was minimized, suggesting that açaí at lower concentrations may reduce the accumulation of vacuoles in hepatocytes, while higher concentrations may enhance the presence of these vacuoles. This effect was also observed by Zheng et al. (2017) in tilapia supplemented with rutin, one of the antioxidants found in açaí, at a dosage of 0.3 g/kg in the diet.

5 Conclusion

As far as we know, this is the first report on the impact of diets containing freeze-dried açaí on the histopathology of koi carp liver tissue. The results indicate that açaí has considerable potential as a dietary supplement for koi carp, provided it is administered in adequate quantities, taking advantage of its antioxidant benefits, and improving the fish's liver health. In this study, the inclusion of 1.5% açaí showed the best results. However, we stress the need for future studies that analyze oxidative stress in the liver, with the aim of determining the ideal dose of açaí and carrying out experiments with other fish species, to gain a comprehensive understanding of the effects of açaí in the diet of ornamental fish.

Authors' contributions

Fernandes M.C. and Costa D.S. contributed to the conceptualization, methodology, software development, validation, formal analysis, investigation, data curation, and the writing of the original manuscript, as well as visualization. Silva A.V. was responsible for designing the experimental trial structure and methodology. Lopes E.M. participated in the investigation and contributed to the original writing. Ventura A.S. and Cardoso C.A.L. were involved in methodology development, conducting chemical analysis of the feed, and reviewing and editing the manuscript. Tedesco M. played a key role in methodology development and performed the histological analysis. Mouriño J.L.P. and Martins M.L. provided support in visualization, supervision, and project administration.

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