









Hydroalcoholic extract of *jabuticaba* peel in the diet of betta fish

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ABSTRACT

The objective of this study was to evaluate the effects of jabuticaba (*Plinia cauliflora*) peel extract on the diets of female blue *Betta splendens* concerning the level of digestive enzymes, liver metabolism, and antioxidant activity. The sample constituted 150 individuals subdivided into five groups in triplicate, totaling 10 fish per 20-liter experimental unit. Commercial diets (40.88% crude protein (CP) and 4374.8 Kcal•kg⁻¹v) were added to hydroalcoholic jabuticaba peel extract (EHJ) at concentrations of 0 (control), 0.5, 1.0, 1.5, and 2.0 g•kg⁻¹. The diets were provided twice a day, until apparent satiety, for 21 days. Fish mortality was not observed during the study. Growth indices did not show any significant differences, apart from feed conversion. The use of the extract promoted an increase in the luminosity of the fish; however, there was no statistical difference in chromaticity a* and b*. A decrease in the activity of SOD on the skin of fish fed with diets of 1.5 and 2 g•kg⁻¹EHJ was observed in comparison to the other diets. There was no change in catalase (CAT) activity among the experimental treatments. It is concluded that the use of 2 g•kg⁻¹EHJ has an antioxidant effect that reflects the greater luminosity of blue female *Betta splendens*.

Key words: antioxidant, feed additive, coloring, ornamental fish.

Extrato hidroalcoólico de casca de jabuticaba na dieta de peixe betta

RESUMO

O objetivo deste estudo foi avaliar os efeitos do extrato hidroalcoólico de casca de jabuticaba em dietas de *Bettasplendes* azuis em relação ao conteúdo de enzimas digestivas, ao metabolismo hepático e à atividade antioxidante. Foram utilizados 150 indivíduos divididos em cinco grupos em triplicatas, totalizando dez peixes por unidade experimental de 20 L. Às dietas comerciais (40,88% PB e 4.374,8 Kcal kg⁻¹), foi acrescido extrato hidroalcoólico de casca de jabuticaba (EHJ) nas seguintes concentrações: controle; 0,5; 1; 1,5; e 2 g•kg⁻¹). A ração foi fornecida aos exemplares duas vezes ao dia, até a saciedade aparente, por 21 dias. Não foi registrada a mortalidade de peixes durante o ensaio, bem como diferença significativa nos índices de crescimento, com exceção da conversão alimentar. A utilização do extrato aumentou a luminosidade dos peixes, porém não se verificou diferença estatística na cromaticidade a* e b*. Quanto ao superóxido dismutase, diminuiu-se sua atividade na pele do grupo alimentado com dietas acrescidas de 1,5 e 2 g•kg⁻¹EHJ em comparação com as demais. O último item avaliado foi a catalase, cuja atividade não se alterou no período estudado. Concluiu-se, portanto, que a utilização de 2 g•kg⁻¹EHJ proporciona efeito antioxidante, que se reflete em maior luminosidade de *Bettasplendes* azuis.

Palavras-chave: antioxidante, aditivo alimentar, coloração, peixes ornamentais.

INTRODUCTION

The ornamental fish betta (*Betta splendens*) of Asian origin has great commercial value in Brazilian aquaculture, where it is the fourth most numerous pet according to IBGE estimates (IBGE, 2013). Betta fish are popular because of their ease of handling and aerial breathing, which allow them to be grown in aquariums without oxygenation, and their pleasant appearance, as they display a wide variety of colors (Souza, 2019).

In ornamental species of high commercial value, it is important to emphasize the relevance of high levels of skin pigmentation, which, along with the size and shape of the body and fin, are the most important quality criteria in terms of market value (Gouveia et al., 2003). Fish, like other animals, are unable to perform de novo carotenoid synthesis (Goodwin, 1954); therefore, they depend on food supplementation to achieve their natural pigmentation. Under intensive farming conditions, fish are fed exclusively with compound food, which must therefore be supplemented with carotenoids such

as astaxanthin (3,3-dihydroxy-4,4'-diketo- β -carotene) and canthaxanthin (4,4'-diketo- β , β -carotene) (Gouveia et al., 2003). Because of the negative impact on the environment and high cost of synthetic compounds, some natural compounds have also been tested (Gouveia et al., 2003; Lopes et al., 2007; Eaton et al., 2016) as possible sources of carotenoids in the diet (Aydin and Barbas, 2020).

Anthocyanins constitute the largest group of water-soluble pigments in the plant kingdom according to Bridle and Timberlake (1997) and Bendokas et al. (2020). They are studied globally as natural coloring agents in food and are responsible for shades ranging from red to blue in many fruits and vegetables (Mazza and Miniati, 1993). The use of these bioactive substances has increased due to their antioxidant activity, and other compounds that act as pigments, such as amino acids, organic acids, flavonoids, and alkaloids, produce an increase in color intensity (Lopes et al. 2007; Faria et al., 2016).

The Brazilian fruit *jaboticaba* (*Plinia cauliflora*) is among the plants that present these substances, containing phenolic compounds such as anthocyanins, which can contribute to intensifying fish skin pigmentation (Leite-Legatti et al., 2012; Silva et al., 2014). The peel of *jaboticaba* represents 50% of the fruit and has significant amounts of anthocyanins generating its purple color, allowing it to act as a natural dye (Ferreira et al., 2012).

Fruit residues have been proposed as a nutritional additive for incorporation into animal feed (Morales et al., 2016; Marquetti et al., 2018). In addition, the use of nutritional additives that are bioactive with antioxidant activity can promote wellness benefits, helping to intensify the skin color of fish (Eaton et al., 2016; Benvenuto et al., 2021). However, the use of *jaboticaba* peel as a source for the manufacture of diets is a challenge, as it can alter the texture by forming pectates (Sato and Cunha, 2007). This suggests that anthocyanins are derived from *jaboticaba* peel obtained through solid-liquid extraction, such as maceration (Celli and Brooks, 2017). However, dyes produced from fruit and vegetable sources can affect the quality of the products to which they are applied (Freidig and Goldman, 2014). The action of anthocyanins depends on the food matrix, which affects bioaccessibility and bioavailability (Charron et al., 2009).

In this regard, there are no studies on the pharmacokinetics (absorption, distribution, and metabolism) of anthocyanin extracts in ornamental fish. Therefore, the objective of this study was to evaluate the potential of the EHJ as a color and wellness promoter for blue *Betta splendens*.

MATERIAL AND METHODS

Hydroalcoholic extract of *jaboticaba* peel

The sample of *jaboticaba* (*Plinia cauliflora*) was acquired at Fazenda Flor do Campo, located at km 21 of Highway 153, Dourados-MS, Brazil, which is the location with the highest quantity of the species found in the region. *P. cauliflora* was identified by the

Universidade Federal da Grande Dourados (UFGD) and registered at the Herbarium DDMS under number 5025.

Jaboticaba peels previously dehydrated in an air circulation oven (65°C) were subjected to static maceration in a dark flask for a period of 7 days, shaking three times a day without renewing the extraction liquid, followed by filtration. The extract was characterized according to the anthocyanin content, according to Lees and Francis (1972).

Experimental diets

A commercial diet for *B. splendens* was used, which was analyzed for chemical composition (AOAC, 2000), and it was found that the diet had 31.05% CP; 3.21% Crude Fiber (CF); 4.60 Ether Extract (EE), and 4,125.29 kcal•kg⁻¹. According to the manufacturer, the ingredients of the diet were: Corn starch, garlic, beets, canthaxanthin, l-carnitine, vegetable extract, flaxseed bran, soybean meal, artemia flour, fish meal, soy protein isolate, L-lysine, corn, fish oil, soy oil, protenosis, rice chips, salt, yucca extract, premix mineral vitamin and preservative additives, antioxidant, mycotoxin adsorber, probiotic, prebiotic, and multienzyme complex.

For each concentration, the EHJ was properly aliquoted and added to diets. The diets were subjected to drying in an open and ventilated place using fans for 24 hours. Subsequently, the diets were packed in dark bottles and kept in the refrigerator. The final concentration of each diet was control; 0.5; 1.0; 1.5; and 2.0 g.kg⁻¹.

Fish feeding experiment and rearing conditions

Juveniles of female *B. splendens* (150 individuals, 0.149±0.066 g, and 5.82±0.40 cm) with blue color from the same litter were randomly distributed in 15 aquariums of 20 L each in a static system, with 30% of the water changed every five days, two hours after the last feeding, with a stocking density of 10 fish per aquarium. The delirium was completely randomized with five treatments and three replicates. Fish fed with the experimental diets twice a day until apparent satiety for 21 days. The feed was provided twice a day (at 8 am and 4 pm), until apparent satiety. The trial was approved by the Animal Research Ethics Committee of the Centro Universitário da Grande Dourados / CEUA, protocol: 034/17, before the feeding experiment began.

The water quality was monitored during the feeding experiment and daily temperature was measured (29.6±0.1°C) using the HANNA multiparameter equipment, model HI929828-13. Ammonia levels (0.002±0.001 mg•L⁻¹) were measured weekly with the Labcon Test Kit (Industry and Commerce of Dehydrated Food Alcon Ltda., Balneário Camboriú, SC, Brazil).

Calculations

Survival was assessed daily during the feeding experiment. On the 21st day, the fish were euthanized, and the weight, length, and individual color were measured (Equations 1, 2, 3, 4 and 5).

Weight gain (WG) = final weight - initial weight (1)

Standard length gain (SLG) = final length - initial length (2)

Feed Conversion Ratio (FCR) = feed intake / weight gain (3)

Survival (S) = (final number of fish / initial number of fish) × 100 (4)

condition factor: $K = W / L^b$ (5)

Where:

W = total weight;

L = length;

b = slope of the weight / length ratio.

Analysis

The color of the fish was measured individually using a portable photocolometry, Chroma Meter CR-400 (Konica Minolta®), using the Hunter L*, a*, and b* coordinate system, (Hunt, 1977; Rezende et al., 2012; Gumuş et al., 2017). Referential A standard white tile with reflectance values of luminosity (-100, black and +100, white); the chromaticity of a*, shades of green (-100) and red (+100); and the chromaticity of b*, shades of blue (-100) and yellow (+100) were used as reference.

To evaluate the gastric and hepatic activity of the EHJ in blue *B. splendens*, activity analyses of digestive enzymes, hepatic metabolic enzymes, and antioxidants were performed. For these analyses, the test fish were desiccated, and the intestine, liver, and muscle with skin were separated, and the samples were frozen in liquid nitrogen and stored at -20°C until analysis.

Enzyme analysis

For the analysis of hepatic metabolic enzymes, the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was measured. Liver samples (100 mg) were homogenized with sodium phosphate buffer (glycerol v/v in 20 mM sodium phosphate buffer and 10 mM Tris - pH 7) in a Potter-Elvehjem homogenizer. Subsequently, this sample was centrifuged at 4°C for three minutes at 600 x g and the supernatant subjected to new centrifugation for eight minutes at 6,000 x g. The supernatant was used for the ALT and AST enzyme assays. Measurements of the activity of these enzymes were determined by a modification of the method of Reitman and Frankel (1957). Sample readings were performed by spectrophotometry (Bioplus S-200 semiautomatic spectrophotometer), with an appropriate wavelength for each test.

For digestive enzyme analysis, intestine samples (100 mg) were homogenized with sodium phosphate buffer (glycerol v/v in 20mM sodium phosphate buffer and 10 mM Tris - pH 7) in a Potter-Elvehjem homogenizer. Subsequently, this sample was centrifuged at 4°C for three minutes at 600 x g and the supernatant subjected to new centrifugation for eight minutes at

6,000 x g. The supernatant was used for the enzymatic assays of amylase, lipase, non-specific protease, and alkaline phosphatase. Sample readings were performed by spectrophotometry (Bioplus S-200 semiautomatic spectrophotometer), with an appropriate wavelength for each test.

The antioxidant activity on the skin was measured through SOD activity tested by pyrogallol auto-oxidation, which is inhibited in the presence of SOD (Beutler, 1984, modified). Absorbance readings were performed at 420 nm, considering that 1 IU inhibits pyrogallol auto-oxidation by 50%. CAT activity was assessed by reading the H₂O₂ decay at 230 nm (Beutler, 1984). A CAT unit was defined as the amount of enzyme required in 1 µmol of H₂O₂ min⁻¹ of oxidation, and the molar absorptivity used was (H₂O₂) ε_{λ230}=0.071 mM cm⁻¹. The protein was determined with Bradford reagent against a standard BSA solution (Kruger, 2009).

Statistical analysis

The experiment was carried out in a completely randomized design containing five concentrations of EHJ (control, 0.5, 1, 1.5, and 2 g•kg⁻¹) and three replicates. The normality and homogeneity of the data variance were verified by the Levene and Shapiro-Wilk tests, using the Bioestat program (version 5.0). Analysis of variance (ANOVA) was performed; and when a significant difference was detected (p<0.05), the means were compared using the Tukey test.

RESULTS

No fish mortality was observed during the experimental feeding test with the inclusion of the EHJ, demonstrating the safety of this product for ornamental fish feeding. Growth rates did not show significant differences among the treatments. It is noteworthy that there was a change in feed conversion. The use of EHJ promoted an increase in the luminosity of fish fed with diets containing 2 g•kg⁻¹ EHJ; however, there was no statistical difference in chromaticity a* and chromaticity b* (Table 1).

The digestive activity showed adaptations in the secretion of the non-specific protease enzymes, amylase, and lipase. The use of 2 g•kg⁻¹ EHJ promoted an increase in amylase secretion. The inclusion of EHJ did not alter the activity of intestinal alkaline phosphatase. The inclusion of EHJ did not promote changes in the enzymes ALT and AST, indicating that there was no liver overload.

There was a decrease in SOD activity on the skin of fish fed with diets containing 1.5 and 2 g•kg⁻¹ EHJ compared to other diets. There was no change in the CAT activity (Table 2).

DISCUSSION

The addition of natural pigments that act as food additives with an emphasis on the development of ornamental fish has not been reported for several species (Amar et al., 2001; Xu et al.,

2006; Gomes et al., 2007; Yi et al., 2015). The lack of response in development may be associated with the inhibitory effect of anthocyanins on the activity of digestive enzymes (Sales and Janssens, 2003). The inclusion of 450 µg of anthocyanin from rosella (*Hibiscus sabdariffa*) promoted undesirable changes in the activities of the digestive enzymes of goldfish (*Carassius auratus*). There are also reports of anthocyanin interacting with dietary compounds that decrease the digestibility of nutrients (Bordenave and Huc, 1995). In our study, digestive lipase was inhibited in fish fed with diets supplemented with EHJ. Digestive enzymes in fish are susceptible to induction by specific substrates (Dorce et al., 2020); however, the improvement in

digestive enzyme activity compared to that of a natural additive depends on the composition of the product (Carmo-Ota et al., 2019). Plant extracts in aquatic organisms are poorly explored, and knowledge of the influence of plants on fish health is relevant (Awad and Awaad, 2017).

The inclusion of EHJ did not result in changes in the chromaticity a^* and b^* of blue *B. splendens*. The use of pigments from natural sources has been shown to be effective in coloring the skin of fish, such as those of tomatoes (*Solanum lycopersicum*), carrots (*Daucus carota*) (Mirzaee et al., 2012), and beets (*Beta vulgaris*) (Xu et al., 2006; Singh et al., 2016), which showed an increase in the deposition of carotenoids in

Table 1. Performance parameters of blue *Betta splendens* females fed with diets containing hydroalcoholic jaboticaba peel extract (EHJ).

Parameters	Diets (EHJ g.kg ⁻¹)					p-value
	Control	0.5	1	1.5	2	
WG (mg)	1.37±0.13	1.70±0.0	1.60±0.00	1.39±0.12	1.38±0.16	0.11
SLG (cm) ²	35.47±1.16	36.36±1.01	35.13±0.84	34.98±0.68	35.43±1.69	0.92
FCR (g)	0.53±0.10 ^b	0.98±0.15 ^a	0.52±0.01 ^b	0.54±0.09 ^b	0.95±0.21 ^a	0.0012
K	0.031	0.031	0.037	0.032	0.031	--
Skin coloring						
L^5	28.76±2.31 ^b	26.61±2.05 ^c	24.24±0.85 ^c	26.6±1.20 ^b	34.53±2.33 ^a	0.0097
a^*	1.52±1.79	0.75±3.39	2.64±1.78	-2.76±1.70	0.83±1.80	0.5107
b^*	14.74±2.28	-13.68±3.13	-13.92±2.17	-11.34±1.63	-11.16±1.18	0.7006

Means followed by different letters indicate a difference by Tukey's test ($p > 0.05$); WG: Weight Gain; SLG: Standard Length Gain; FCR: Feed Conversion Ratio; K: Condition Factor; L : brightness (-100, black and +100, white); a^* : chromaticity of a^* , represented by the shades of green (-100) and red (+100); b^* : chromaticity of b^* , represented by the shades of blue (-100) and yellow (+100).

Table 2. Enzymatic parameters of *Betta splendens* fed with increasing levels of jaboticaba extract.

Parameters	Diets (g.kg ⁻¹)					p-value
	Control	0.5	1.0	1.5	2.0	
Digestive enzymes (Umg⁻¹prot)						
Protease	1.13±0.13 ^{ab}	0.33±0.26 ^b	1.24±0.11 ^a	1.15±0.16 ^{ab}	0.93±0.28 ^b	0.0245
Amylase	1.42±0.17 ^{ab}	0.96±0.06 ^b	0.97±0.04 ^b	1.03±0.03 ^b	1.69±0.04 ^a	0.0001
Lipase	0.26±0.25	0.09±0.02	0.04±0.01	0.01±0.00	0.24±0.25	0.7323
Alkaline phosphatase	0.51±0.51	0±0.000	1.12±0.74	1.22±0.75	1.91±0.51	0.198
Enzymes of hepatic metabolism (U/mg prot)						
ALT ¹	0.53±0.033	0.55±0.05	0.53±0.03	0.74±0.22	0.59±0.09	0.637
AST ²	0.44±0.03	0.39±0.01	0.49±0.04	0.42±0.01	1.14±0.48	0.112
Oxidative enzymes from the skin (U/mg prot)						
SOD ³	5.32±0.38 ^a	4.09±0.9 ^a	5.22±0.85 ^a	1.77±0.73 ^b	1.05±0.29 ^b	<0.0001
CAT ⁴	1.97±0.90 ^b	2.79±0.7 ^a	2.17±0.29 ^{ab}	1.86±0.90 ^b	1.41±0.20 ^c	0.0007

Means followed by different letters indicate a difference by Tukey's test ($p > 0.05$); ALT: alanine aminotransferase; AST: aspartate aminotransferase; SOD: superoxide dismutase; CAT: catalase.

quinguios fed for 15 days with 80 mg•kg⁻¹ from 0.118 to 0.339. However, some products, despite the presence of pigments in their composition, do not alter the color of the skin when added to fish diets. We can highlight the use of pink pepper essential oil for jewel tetra (*Hyphessobrycon eques*) (Porto et al., 2020a) and bacuri (*Attalea phalerata* Mart. Ex Spreng) pulp oil (Porto et al., 2020b). Fish pigmentation is associated with the fish species, the ability of the dye to pigment, and the dye concentration in the diet (Yi et al., 2015; Aydın et al. 2017; Li et al., 2017), particularly the form of inclusion (Dethlefsen et al., 2016). We must be aware that the search for dyes capable of conferring an increase in blue color is still incipient. However, it is noteworthy that anthocyanins at neutral pH tend to show a blue-violet color (Guimarães et al., 2012).

The inclusion of EHJ did not alter the activities of liver metabolism enzymes, which should be considered favorable for aquaculture. The increased activity of AST and ALT is the body's response to stressors and nutritional metabolism and is considered indicative of an injured or damaged organ (Menga and Swyngedouw, 2018; Kesbiç, 2019; Jahromi et al., 2021). This reminds us that EHJ is not hepatotoxic and can be safely used up to the level tested in this study.

The use of diets with 1.5-and 2 g•kg⁻¹ EHJ promoted a decrease in SOD activity. SOD catalyzes the destruction of superoxide radicals via the formation of H₂O₂. GSH-Px catalyzes the conversion of H₂O₂ into water (Ekin et al., 2018). Some plants have been reported to be efficient in improving antioxidant responses (Wang et al., 2018; Yılmaz, 2020; Yousefi et al., 2021). What differentiates ornamental fish from other species concerning a balanced diet are the different metabolic routes for carotenoids (Fries et al., 2014). In addition to pigmentation, the use of natural additives increases immunity, thereby increasing animal welfare (Yousefi et al., 2021). Studies have shown that fruits, in addition to providing nutrients, contain significant levels of carotenoids, tocopherols, and phenolics in their pulp (Nunes et al., 2019). Therefore, products originating from fruits are effective for implementation in the diet of ornamental fish. There is experimental evidence that animal coloring may be reduced if there is good antioxidant activity (Perez et al., 2008). In other words, there is a hypothesis that individuals who have strong antioxidant defenses may incur deviations in the carotenoid function in metabolism. A study in which a mixed diet was used, consisting of a formulated diet supplemented with microalgae and *A. gracilis* biomass, showed an influence on the skin color of fish (Morais et al., 2015). Therefore, the use of natural and live additives can positively complement the diet, thus being a viable option in ornamental nutrition, although further studies are required.

CONCLUSION

The use of 2 g•kg⁻¹ EHJ has an antioxidant effect that reflects the greater luminosity of blue female *Betta splendens*. The same

concentration of EHJ promoted an increase in amylase secretion and there was a decrease in SOD activity in the fish skin.

CONFLICT OF INTERESTS

Nothing to declare.

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AUTHOR'S CONTRIBUTIONS

França, G.B.: Conceptualization, Investigation, Data curation, Project administration, Writing — original draft. Siqueira, M.S.: Formal Analysis, Writing — review & editing. Melo, J.C.S.: Conceptualization, Investigation, Methodology. Albuquerque, D.T.: Conceptualization, Investigation, Methodology. Venturini, F.P.: Conceptualization, Investigation, Methodology. Honorato, C.A.: Project administration, Supervision, Validation, Formal Analysis, Investigation, Writing — review & editing.

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