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Protective effect of hydroxy-selenomethionine supplementation in the diet of tambaqui (*Colossoma macropomum*) subjected to transportation stress

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ABSTRACT

Selenium (Se) is an antioxidant mineral and has been included in fish feed formulations in the organic form of hydroxy-selenomethionine (OH-SeMet). This study evaluated how different concentrations of this substance, supplemented in the diet, act on tambaqui (*Colossoma macropomum*) muscle before and after a stressor (transportation). Juvenile fishes were divided into five treatments receiving 0.0; 0.3; 0.6; 0.9; 1.2 mg kg^{-1} Se supplementation for 75 days. After that period, the fish were exposed to transportation for four hours. Sampling of muscle tissue for the measurement of biochemical parameters occurred on day 75, prior to transportation, and one week after transportation (day 83). The activity of enzymes superoxide dismutase and glutathione-*S*-transferase did not change. Supplementation with 1.2 mg kg^{-1} Se increased the level of reduced glutathione before transportation, and 0.9 and 1.2 mg kg^{-1} Se reduced the level of thiobarbituric acid reactive substances levels before and after transportation. After transportation, we observed reduced glutathione levels in fish treated with 0.3, 0.6 and 1.2 mg kg^{-1}, reduced ascorbic acid level in fish fed 0.6 mg kg^{-1} Se, and reduced total protein concentration in fish fed 0.3 mg kg^{-1} Se, as compared to the levels before transportation. In conclusion, the presence of different concentrations of Se in the fish diet promoted different patterns of response to redox status, minimizing oxidative damage generated by the stressor event.

KEYWORDS: Amazon fish; feed; micromineral; reactive oxygen species

Efeito protetor da suplementação de hidróxi-selenometionina na dieta de tambaqui (*Colossoma macropomum*) submetidos ao estresse pelo transporte

RESUMO

O selênio (Se) é um mineral antioxidante e tem sido incluído em formulações de rações para peixes na forma orgânica de hidróxi-selenometionina (OH-SeMet). Este estudo avaliou como diferentes concentrações dessa substância, suplementada na dieta, atuam no músculo do tambaqui (*Colossoma macropomum*) antes e após um estressor (transporte). Os peixes juvenis foram divididos em cinco tratamentos recebendo a suplementação de 0,0; 0,3; 0,6; 0,9; 1,2 mg kg^{-1} de Se por 75 dias. Após esse período, os peixes ficaram expostos ao transporte por quatro horas. A amostragem de tecido muscular para medição dos parâmetros bioquímicos ocorreu no dia 75, antes do transporte, e uma semana após o transporte (dia 83). A atividade das enzimas superóxido dismutase e glutationa-S-transferase não se alterou. A suplementação com 1,2 mg kg^{-1} de Se aumentou o nível de glutationa reduzida antes do transporte, e 0,9 e 1,2 mg kg^{-1} de Se reduziram os níveis de substâncias reativas ao ácido tiobarbitúrico antes e após o transporte. Após o transporte, observamos redução nos níveis de glutationa reduzida nos peixes tratados com 0,3, 0,6 e 1,2 mg kg^{-1}, redução nos níveis de ácido ascórbico nos peixes alimentados com 0,6 mg kg^{-1} de Se e redução na concentração de proteínas totais nos peixes alimentados com 0,3 mg kg^{-1} Se, em comparação com os níveis antes do transporte. Conclui-se que a presença de diferentes concentrações de Se na dieta dos peixes promoveu diferentes padrões de resposta ao estado redox, minimizando os danos oxidativos gerados pelo evento estressor.

PALAVRAS-CHAVE: peixe amazônico; ração; micromineral; espécies reativas de oxigênio

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INTRODUCTION

With a predominantly tropical climate and abundance of water resources, Brazil has favorable conditions for fish farming, which generates jobs and income for the population of different regions. In 2019, Brazil produced 758,006 tons of fish through aquaculture, with native fish contributing 287,930 tons (Medeiros 2020). Tambaqui (*Colossoma macropomum* Cuvier 1818) is one of the main fish produced in northern and midwestern Brazil, in the context of the Amazon basin, with Mato Grosso state leading production, yielding over 60 thousand tons (EMBRAPA 2017).

Tambaqui shows good adaptation in captivity, readily accepts commercial feed, and demonstrates desirable growth and feed conversion rates (Lopera-Barrero et al. 2011; Tregidgo et al. 2021). To enhance their performance, it is advisable to use minerals with antioxidant properties, such as selenium (Se), instead of potentially harmful chemical products (Arthur et al. 2003). However, selenium supplementation in fish warrants careful monitoring, considering its narrow range of beneficial use and the risk of toxicity at high levels (Berntssen et al. 2018).

Selenium plays a crucial role in the immune system, being essential for its proper functioning. It forms an integral part of seleno-proteins, including enzymatic antioxidant systems like glutathione peroxidase (GPx), which eliminates harmful lipid hydroperoxides and hydrogen peroxides, thus preventing diseases (Biller-Takahashi et al. 2015). Furthermore, selenium is involved in maintaining skeletal development, influencing bone cell differentiation, and mineralization (Mechlaoui et al. 2019). Selenium has been demonstrated to be indispensable for fish fertility and growth (Kumar and Singh 2019), with dietary selenium levels for fish ranging from 0.15 to 1.85 mg kg^{-1} Se (Prabhu et al. 2016).

Iqbal et al. (2020) pointed out that a low selenium dose can lead to conditions like exudative diathesis and muscular dystrophy in fish. Selenium deficiency can also result in reduced growth, and diminished GPx activity, causing oxidative damage to cell membranes, a decline in antioxidant defenses, and an increased mortality rate (Mechlaoui et al. 2019).

Selenium is found in inorganic forms like sodium selenite (Na_2SO_3) and sodium selenate (Na_2SO_4), and in various organic forms such as seleno-glycinate, seleno-proteinate and selene-methionine (Wang and Lovell 1997). Sodium selenite (Na_2SeO_3) is the most common and traditional inorganic selenium source in animal feeds, including fish. However, over the past decade, alternative selenium sources have emerged, such as Se-yeast (yeast enriched with selenomethionine – SeMet, and dietary forms of selenocysteine – SeCys) as well as pure chemically synthetized SeMet forms like hydroxy-selenomethionine (OH-SeMet), also known as 2-hydroxy-4-methylselenobutanoic acid (HMSeBA). Feeding animals with SeMet, as opposed to inorganic or other organic Se compounds, enhances selenium deposition in tissues (Ferreira et al. 2022) and seleno-protein formation, thereby improving cellular antioxidant capacity and benefiting animals strengthened defense mechanisms during times of stress (Fontagné-Dicharry et al. 2020).

Fish transportation is a stressful procedure that impacts the animals' metabolic balance and can provoke diseases (Urbinati and Carneiro 2004). This management process may cause injuries to fish compromising their natural protective barriers (mucus and scales), making them susceptible to bacterial and fungal infections (Mendes et al. 2015). In light of these considerations, we aimed to assess the oxidative stress and metabolic effects resulting from fish transportation and evaluate the protective role of dietary hydroxy-selenomethionine supplementation.

To achieve this goal, we selected tambaqui muscle tissue as the focus of our study, as this tissue serves a storage function and represents the main consumed part of fish, therefore, influencing shelf life and potentially impacting human health (Abdelazim et al. 2018).

MATERIAL AND METHODS

Animals

Juvenile tambaqui (*C. macropomum*) acquired from commercial fish farming were used. They were kept at the Fish Farming Sector of Universidade Federal do Mato Grosso (UFMT) - Campus Cuiabá (Mato Grosso state, Brazil) to carry out the experiment, following approval by the Ethics Committee on the Use of Animals (CEUA/UFMT) (Protocol No. 23108.961214/2018-99). A total of 195 young tambaqui (initial average weight 15.71 ± 1.90 g) were placed in 15 experimental 100-liter polyethylene boxes (13 fish/box), supplied with a continuous water flow recirculation system with biological filter and constant aeration. Water quality parameters (dissolved oxygen: 6.14 ± 2.01 mg L^{-1}; temperature: 26.58 ± 1.36 °C; pH: 8.17 ± 0.49; alkalinity: 221.40 ± 21.72 mg $CaCO_3$ L^{-1}; non-ionized ammonia - NH_3: 0.07 ± 0.06 mg L^{-1}, and nitrite - NO_2 - 1.00 ± 0.44 mg L^{-1}) were monitored and maintained within species-appropriate ranges, according to Araujo-Lima and Gomes (2005).

Experimental design

An isoproteic and isoenergetic basal diet was formulated using commonly used raw materials in the animal feed industry (Table 1). Additionally, a vitamin and mineral complex without a source of selenium (Premix Nutrepharm, Cuiabá, MT, Brazil) was included. The basal diet was supplemented with selenium in the form of hydroxy-selenomethionine (OH-SeMet) (Selisseo®, Adisseo France S.A.S., Antony, France) in concentrations of 0.0; 0.3; 0.6; 0.9; 1.2 mg kg^{-1} Se (Table 1), resulting in five treatments, each with three replicates. The mixture was moistened in distilled water and pelleted in a meat grinder.

Table 1. Composition of the experimental fish diets supplemented with selenium in the form of hydroxy-selenomethionine (OH-SeMet).

Ingredient (%)	Selenium content (mg kg^{-1})				
	0.0	0.3	0.6	0.9	1.2
Maize	19.6	19.6	19.6	19.6	19.6
Wheat bran	13	13	13	13	13
Soybean meal	41.8	41.8	41.8	41.8	41.8
Fish meal	22	22	22	22	22
Soybean oil	2.2	2.2	2.2	2.2	2.2
Selisseo® 2%	0	0.0015	0.0030	0.0045	0.0060
Dicalcium phosphate	0.9	0.9	0.9	0.9	0.9
Vitamin/mineral premix[a]	0.4	0.4	0.4	0.4	0.4
Excipient (kaolin)	0.1	0.0985	0.097	0.0955	0.094
TOTAL	100	100	100	100	100
Centesimal (%)[b]	0.0	0.3	0.6	0.9	1.2
Moisture	5.0	6.0	7.6	9.4	6.9
Crude protein	37.3	38.3	37.5	38.3	39.4
Lipids	5.9	6.1	6.0	5.8	3.6
Carbohydrates	47.0	46.0	47.0	46.2	47.0
Ash	9.8	9.6	9.5	9.7	10.0

[a]Folic acid; pantothenic acid; BHT; biotin; calcium; cobalt; copper; choline; iron; iodine; manganese; niacin; vit. B1; vit. B12; vit. B2; vit. B6; vit. C; vit. D3; vit. E; vit. K3; vit. A; zinc. [b]Percentage on a dry-matter basis.

Fish were offered the experimental diets twice daily (9 a.m. and 3 p.m.), until apparent satiety, for 75 days. After 75 days of feeding with the experimental diets, the fish were packed in plastic bags containing water and inflated with oxygen, and transported for 4 h without a utility vehicle. After transport, the fish were distributed into the tanks, from which they were taken before transport, for recovery. Sampling took place at day 75 of feeding and one week after transport. The one-week interval between samplings allowed for an assessment of whether the stress effects persisted or if recovery occurred.

At each sampling time, the fish (n = 3 per replicate, n = 9 per treatment) were captured, anesthetized using eugenol (30 mg L^{-1} water), and then sacrificed via spinal cord section to extract the white muscle (middle part) tissue (fillet), which was vacuum-packaged and frozen at -80 °C for further analysis at the Biochemistry Laboratory - LIPEQ, UFMT (campus Sinop, Mato Grosso state). The fish in this study are the same analyzed for the effect of Se supplementation on the liver by Ferreira et al. (2022).

Parameters of oxidative stress

Superoxide dismutase enzyme (SOD) activity was measured based on the adrenaline detection principle (adrenochrome), following the method described by Misra and Fridovich (1972). Changes in absorbance in 60 seconds were measured at 480 nm and expressed in UI SOD mg protein^{-1}.

Glutathione-*S*-transferase (GST) activity was determined according to the method developed by Habig et al. (1974), by adding the reactive 1-chloro-2,4 dinitrobenzene (CDNB), which in the presence of glutathione forms GS-dinitrobenzene (GS-DNB) at 340 nm. Results were presented in µmol GS-DNB min^{-1} mg protein^{-1}.

Reduced glutathione (GSH) levels were determined according to Sedlack and Lindsay (1968). Absorbance readings at 412 nm were compared to a standard GSH curve and expressed in µmol GSH mg protein^{-1}. Ascorbic acid (ASA) levels (vitamin C) were determined based on the Roe model (Roe 1954) with absorbance readings at 520 nm compared to a standard ASA curve. The results were presented in µmol ASA g tissue^{-1}.

Lipoperoxidation levels (thiobarbituric acid reactive substances, TBARS) were measured following the protocol by Buege and Aust (1978), whose principle results from the formation of a malondialdehyde complex [MDA - thiobarbituric acid (TBA)], after boiling. The absorbance was determined at 535 nm and the concentration of MDA in the sample was expressed as nmol of MDA mg of protein^{-1} and compared to a standard MDA curve.

Protein content for the analysis of oxidative stress parameters, except for ASA, was determined using Bradford's method (Bradford 1976) with a bovine albumin standard curve for comparison.

Metabolic parameters

Lactate levels were determined according to the Harrower and Brown (1972) protocol, with readings at 570 nm, and results expressed in µmol lactate g tissue^{-1}. Glucose measurements were performed according to Dubois et al. (1956), at 480 nm and the results were compared to a standard glucose curve, and presented in µmol glucose g tissue^{-1}. Total amino acids (AA) were measured according to Spies (1957), using 0.5% Ninhydrin (diluted in isopropyl alcohol), and the absorbance of the data was obtained at 570 nm and compared to a standard amino acid curve, the results being expressed in mmol AA g tissue^{-1}. Total protein content was determined following the methodology of Bradford (1976), with a standard curve based on bovine serum albumin and results presented as mg protein g tissue^{-1}.

Statistical analysis

The Kolmogorov-Smirnov normality test was applied to verify data distribution conformity to a normal Gaussian distribution. The Bartlett's test was applied to verify whether data variance was homogeneous. Two variables that did not meet the requirements for parametric analysis (SOD, GSH) were normalised through log transformation. Normal variables were analyzed with parametric one-way analysis of variance (ANOVA) followed by a *post hoc* Tukey's test, and the results are expressed as mean ± standard deviation (SD). Variables with a non-parametric distribution were analysed with a Kruskal-Wallis (KW) test, followed by Dunn's test, with results presented as median and total amplitude. In all

cases, a significance level of 5% was established ($p < 0.05$) was established for rejecting the null hypothesis.

RESULTS

Oxidative stress biomarkers

There were no significant changes in the activity of SOD and GST among the groups before and after transportation, indicating that it did not affect the activity of these enzymes (Table 2). Conversely, the non-enzymatic antioxidant GSH exhibited a significant increase in fish fed with 1.2 mg kg^{-1} Se compared to the control and fish fed with 0.9 mg kg^{-1} Se. However, fish fed 0.3, 0.6 and 1.2 mg kg^{-1} showed a significant decline in the levels of GSH after transportation compared to the levels before transportation (Table 2).

Table 2. Enzymatic and non-enzymatic biomarkers of oxidative stress and TBARS in tambaqui muscle after being fed with diets containing different concentrations of selenium in the form of hydroxy-selenomethionine and subjected or not to transport stress.

Selenium content (mg kg^{-1})	**Before stress**	**After stress**
Superoxide dismutase (SOD) (UI SOD mg $protein^{-1}$)		
0.0	1.12 ± 0.09	1.26 ±0.09
0.3	1.07 ± 0.13	1.17 ±0.14
0.6	1.12 ± 0.09	1.27 ±0.09
0.9	1.04 ± 0.09	1.07 ± 0.11
1.2	1.14 ± 0.07	1.25 ± 0.19
Glutathione S-transferase (GST) (µmol GS-DNB min^{-1} mg $protein^{-1}$)		
0.0	0.08, 0.06 – 0.16	0.08, 0.05 – 0.11
0.3	0.09, 0.05 – 0.17	0.06, 0.01 – 0.11
0.6	0.08, 0.61 – 0.11	0.09, 0.05 – 0.18
0.9	0.08, 0.05 – 0.10	0.07, 0.05 – 0.09
1.2	0.08, 0.05 – 0.09	0.09, 0.05 – 0.14
TBARS (nmol MDA mg $protein^{-1}$)		
0.0	46.19, 18.48 – 84.79^{a}	96.42, 52.42 – 168.80^{ac}
0.3	37.21, 21.58 – 54.00^{abA}	158.80, 69.08 – 211.80^{abB}
0.6	33.46, 10.24 – 72.86^{ab}	54.75, 21.73 – 73.86^{c}
0.9	20.26, 5.42 – 41.12^{bA}	92.79, 65.78 – 128.60^{acB}
1.2	53.96, 9.74 – 106.60^{ac}	48.78, 35.24 – 61.61^{c}
Reduced glutathione (GSH) (µmol GSH mg $protein^{-1}$)		
0.0	1.03 ± 0.16^{a}	0.81 ± 0.13
0.3	1.29 ± 0.27^{abA}	0.89 ± 0.12^{B}
0.6	1.21 ± 0.21^{abA}	0.88 ± 0.08^{B}
0.9	1.12 ± 0.11^{a}	0.87 ± 0.10
1.2	1.43 ± 0.15^{bA}	0.94 ± 0.15^{B}
Ascorbic acid (µmol ASA g $tissue^{-1}$)		
0.0	1.65, 1.11 – 1.74	0.95, 0.35 – 1.62
0.3	1.48, 0.72 – 1.81	1.03, 0.87 – 1.31
0.6	1.65, 1.19 – 2.22^{A}	0.81, 0.36 – 1.32^{B}
0.9	1.25, 0.97 – 1.80	0.92, 0.36 – 1.59
1.2	1.31, 0.94 – 1.80	0.97, 0.67 – 1.67

Values for SOD and GSH are the mean ± standard deviation according to a one-way ANOVA followed by a Tukey test. Values for GST, TBARS and ASA are the median followed by the total amplitude according to a Kruskal-Wallis test followed by Dunn's test. Different lowercase letters (columns) indicate significant pairwise differences between treatments. Different uppercase letters (lines) indicate significant differences between measurements before and after stress.

Fish fed 0.9 mg kg^{-1} Se showed significantly lower TBARS levels before transportation than the control group and those fed 1.2 mg kg^{-1}. After transportation, this parameter was significantly lower for fish fed 0.6 and 1.2 mg kg^{-1} compared to the 0.3 mg kg^{-1} treatment. The comparison of measures before and after transportation showed that TBARS levels increased significantly in fish fed 0.3 and 0.9 mg kg^{-1} Se. Selenium supplementation for 75 days did not affect the concentration of vitamin C in the tambaqui muscle, except for a significant reduction in the levels of fish fed with 0.6 mg kg^{-1} Se after transportation compared to the levels before transportation (Table 2).

Metabolic evaluation

The inclusion of selenium in the diet, as well as the application of the physical stressor, did not affect the concentration of glucose or the levels of amino acids in tambaqui muscle (Table 3). Lactate levels increased in all treatments after transportation, though not significantly. There was no statistical difference in lactate levels among treatments before transportation, but after transportation levels were significantly higher in fish fed with 0.6 mg kg^{-1} Se compared to

Table 3. Metabolic parameters in muscle of tambaqui after being fed with diets containing different concentrations of selenium in the form of hydroxy-selenomethionine and subjected to transportation stress.

Selenium content (mg kg^{-1})	**Before stress**	**After stress**
Glucose (µmol g $tissue^{-1}$)		
0.0	1.54 ± 0.46	1.97 ± 0.46
0.3	1.75 ± 0.63	1.48 ± 0.82
0.6	1.65 ± 0.59	1.34 ± 0.36
0.9	1.57 ± 0.82	1.58 ± 0.49
1.2	1.56 ± 0.61	1.94 ± 0.64
Lactate (µmol g $tissue^{-1}$)		
0.0	1.34 ± 0.27	1.89 ± 0.51^{ab}
0.3	1.21 ± 0.50	1.75 ± 0.54^{ab}
0.6	1.62 ± 0.57	2.40 ± 0.58^{a}
0.9	1.29 ± 0.61	1.70 ± 0.29^{b}
1.2	1.46 ± 0.39	1.82 ± 0.19^{b}
Aminoacids (µmol g $tissue^{-1}$)		
0.0	52.11, 41.25 – 94.52	63.48, 22.35 – 87.11
0.3	62.73, 51.93 – 82.87	75.02, 41.87 – 105.70
0.6	54.88, 39.53 – 66.68	77.78, 62.42 – 124.00
0.9	56.18, 47.49 – 77.79	70.90, 47.60 – 94.05
1.2	53.42, 31.20 – 77.35	66.28, 51.39 – 89.07
Total proteins (mg protein g $tissue^{-1}$)		
0.0	1.11, 0.85 – 1.35	0.91, 0.68 – 1.25
0.3	1.31, 1.06 – 1.61^{A}	0.86, 0.65 – 0.93^{B}
0.6	1.14, 0.69 – 1.18	0.94, 0.62 – 1.00
0.9	1.21, 1.05 – 1.27	0.99, 0.86 – 1.33
1.2	1.17, 0.90 – 1.31	0.94, 0.48 – 1.25

Values for glucose and lactate are the mean ± standard deviation according to a one-way ANOVA followed by a Tukey test. Values for aminoacids and total proteins are the median followed by the total amplitude according to a Kruskal-Wallis test followed by Dunn's test. Different lowercase letters (columns) indicate significant pairwise differences between treatments. Different uppercase letters (lines) indicate significant differences between measurements before and after stress.

those fed with higher selenium concentrations (0.9 and 1.2 mg kg^{-1}). The concentration of total proteins in tambaqui muscle remained unaffected by selenium inclusion in the diet both before and after transport. Notably, a reduction in protein levels was observed in all groups after transportation, with a significant decrease in fish fed with 0.3 mg kg^{-1} Se compared to the level before transportation (Table 3).

DISCUSSION

Selenium is a fundamental micronutrient for fish growth, acting as an antioxidant that can counteract reactive oxygen species (ROS) (Iqbal et al. 2020), thus mitigating or preventing damage caused by a stressor such as transportation. The initial phase of transportation, involving the use of plastic bags with pure oxygen, increases the gas concentration in the water, potentially inducing ROS formation in cells (Boaventura et al. 2021). The handling of live fish subjected to transportation, as carried out in this study, has the ability to cause the same reaction (Lushchak 2011). In our study, the highest Se concentration in the diet led to increased GSH levels at 75 days, but levels of this antioxidant decreased in all groups post-transportation, albeit not always significantly compared to the levels before transportation (Table 2). This observation aligns with Durigon et al. (2019), who reported increased levels of GSH (non-protein thiols) in the same tissue. According to Li et al. (2020) selenium offers beneficial effects in terms of antioxidant and redox reactions, as well as immune system support against oxidative stress. Selenium deficiency can reduce the activity of enzymes like glutathione oxidase (GO) and GPx, as GPx relies on Se as a coenzyme (Huber et al. 2008; Mechlaoui et al. 2019). While GPx activity was not assessed in our study, we hypothesize that at a concentration of 1.2 mg kg^{-1}, we might have observed improved GPx performance, given the significant selenium deposition in fish muscle observed by Ferreira et al. (2022).

Oxidative stress induced by ROS leads to lipid peroxidation (LPO) with the production of malondialdehyde (MDA), a substance capable of reacting with thiobarbituric acid (Bhattacharya and Bhattacharya 2007; Paskerova et al. 2012). Thiobarbituric acid-reactive substances are indicative of LPO and, therefore, serve as biomarkers of oxidative stress (Cohen et al. 2007). Dietary selenium may modulate these substances. Mechlaoui et al. (2019) and Durigon et al. (2019) observed a reduction in muscle LPO in *Sparus aurata* (Linnaeus 1758) and *Oreochromis niloticus* (Linnaeus 1758), respectively, with selenium supplementation. In our study, an apparent increase in TBARS was observed post-transportation, except in fish fed with the highest concentration of selenium (1.2 mg kg^{-1} Se). This increase was significant in the groups with dietary supplementation of 0.3 and 0.9 mg kg^{-1} Se when compared to animals before transportation. On the other hand, the 0.9 mg kg^{-1} Se concentration promoted a reduction of physiological LPO, as evidenced by values lower than the control, suggesting a protective role that may be linked to dietary selenium deposits in tissue (Ferreira et al. 2022). Rocha et al. (2017) also observed a trend towards reduced lipoperoxidation in the liver and muscle of jundiá (*Rhamdia quelen* (Quoy & Gaimard 1824) fish receiving 3.0 ppm (mg kg^{-1}) of selenium in their diet.

Vitamin C, similar to selenium, has properties that can trigger responses to stressors (Arthur et al. 2003; Darias et al. 2011), such as transportation. Interestingly, across all selenium dosages, there was a reduction in ascorbic acid levels after transportation, though not always significant when compared to the levels before transportation (except for 0.6 mg kg^{-1} Se). Thus, we can suggest that vitamin C may have been used by the fish muscles to combat the ROS-induced damage generated during transportation. Regarding the metabolic profile, the tendency to increase in muscle lactate levels (though not significant) suggests anaerobic glycolysis activity in the fish. This increase may be related to the stress caused by transportation, which prompts glucose mobilization to provide extra energy for the fish to cope with the imposed disturbance (Bonga 1997).

As for proteins, they comprise a set of amino acids, and their degradation can generate free amino acids for the organism (Nelson and Cox 2014). Thus, the decrease in total proteins, accompanied by the apparent increase in amino acid concentration, implies that protein degradation occurred due to the animals' energy needs, resulting in muscle proteolysis (Nelson and Cox 2014). This proteolysis was likely exacerbated by transportation stress. The muscular demand for amino acids may lead to the generation of hepatic glucose (via glucose-alanine cycle), contributing to lactic fermentation in the muscle. This may explain the tendency to an increase in lactate levels observed after transportation, which was likely non significant due to the small sample size.

The biochemical characteristics of the tambaqui muscle were positively influenced by dietary supplementation with selenium for 75 days. After the stress caused by transportation, higher selenium concentrations in the diet delayed the onset of oxidative stress and subtly minimized metabolic changes. Our data suggest that adequate selenium supplementation, particularly at 0.9 and 1.2 mg kg^{-1}, may be valuable for preserving the quality of fillets for human consumption.

CONCLUSIONS

This study demonstrated that different concentrations of selenium in the fish diet promoted different responses to markers of redox status, but that supplementation was valid in minimizing the stress generated by transport.

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DATA AVAILABILITY

The data that support the findings of this study are available, upon reasonable request, from the corresponding author Valéria Dornelles Gindri Sinhorin.