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Ocimum basilicum essential oil as an anesthetic for tambaqui *Colossoma macropomum*: Hematological, biochemical, non-specific immune parameters and energy metabolism

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ABSTRACT

The present study evaluated the efficacy of *Ocimum basilicum* (basil) essential oil in the anesthetic induction and recovery of juvenile tambaqui *Colossoma macropomum* and verified its physiometabolic effects after biometric handling. Juveniles (86.51 ± 7.82 g) were exposed to different concentrations of basil essential oil: 400, 500, 600, 700, 800, 900, and 1000 μL L^{-1}. Subsequently, the fish were divided into five groups: control (non-handled fish), handling without anesthetic (water only), handling with ethanol (720 μL L^{-1}), and handling with *Ocimum basilicum* essential oil (400 and 800 μL L^{-1}). The shortest ($p < 0.05$) time to induce anesthesia was at a concentration of 1000 μL L^{-1} (222 s). The recovery time from anesthesia did not differ significantly among different concentrations (400–1000 μL L^{-1}). The results of the present study confirm the essential oil of *O. basilicum* as a new and safe natural anesthetic for juvenile tambaqui. The use of this essential oil in biometrics handling procedures reduces or attenuates the secondary responses to handling stress without showing deleterious effects on the non-specific immune system or metabolism of energy, carbohydrates, and lipids. However, further studies are necessary to determine the exact mechanism of action of this essential oil.

1. Introduction

Tambaqui *Colossoma macropomum* (Cuvier, 1816), from the Amazon Basin, is one of the most important native fish in South America. In 2018, its production, at 102,554 tons (IBGE, 2020), was the second highest of all aquatic organisms in Brazil. Tambaqui aquaculture's success is due to its rusticity, high commercial value, high level of acceptance by consumers, fast growth, omnivorous feeding behavior, and adaptation to farming (Morais and O'Sullivan, 2017). Although tambaqui is highly tolerant of farming conditions, it can be adversely affected by excessive handling (Morais and O'Sullivan, 2017). Therefore, the use of anesthetics during routine aquaculture practices is necessary to mitigate the stress effects that directly affect fish physiology and welfare (Ross and Ross, 2008; Velisek et al., 2011).

Several studies have evaluated the sedative and anesthetic potential of essential oils from various medicinal plants for tambaqui handling (Boijink et al., 2016; Barbas et al., 2017a, 2017b; Saccol et al., 2017; Baldisserotto et al., 2018; Batista et al., 2018; Hoseini et al., 2019; da Silva et al., 2019; da Souza et al., 2019; Vilhena et al., 2019). In addition, other products of plant origin, such as an extract of *Spilanthes acmella* (Barbas et al., 2016) and *Lippia alba* hydrate (Maia et al., 2019), have also been evaluated. These products are presented as alternatives to synthetic drugs, such as MS-222, which are expensive and are often unavailable in Latin American countries (Popovic et al., 2012).

Biometric handling and transportation are routine practices in farms producing tambaqui and are likely to trigger secondary responses to

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stress, such as changes in osmoregulation, blood glucose, and hematological biochemical parameters (Barton, 2002). When not mitigated, these responses can evolve into tertiary and undesirable effects that compromise performance, cause behavior changes, and increase susceptibility to disease (Ortuño et al., 2001; Costas et al., 2011). The liver is the organ responsible for energy metabolism; so, the levels of blood glucose and liver glycogen indicate the efficiency of carbohydrate metabolism in fish (Mommsen et al., 1999). The hepatic metabolic changes associated with handling stress and transportation in anesthetized fish can indicate the impact of natural anesthetics on the target organisms (Becker et al., 2016; Saccol et al., 2017; Hoseini et al., 2019).

Plants of the genus *Ocimum* sp., belonging to the Lamiaceae family, are widely distributed globally and in tropical regions of South America (Simon et al., 1990). In fish, the sedative and anesthetic effects of the essential oil of *Ocimum basilicum,* commonly known as basil, has been reported in the hybrid tambacu (*Piaractus mesopotamicus* male × *Colossoma macropomum* female) (Lima-Netto et al., 2016), juvenile Nile tilapia (*Oreochromis niloticus*) (Lima-Netto et al., 2017), in the ornamental fish *Amphiprion clarkii* (Correia et al., 2018), and in the transport and modulation of the ventilatory frequency of adults of Nile tilapia (Ventura et al., 2020a, 2020b). However, no studies to date have assessed the physiometabolic impacts of this essential oil on the mitigation of secondary responses to the handling stress of tambaqui. Therefore, the objective of the present study was to evaluate anesthetic induction and recovery in juvenile tambaqui *Colossoma macropomum* exposed to different concentrations of the essential oil of *Ocimum basilicum* and verify its effectiveness in facilitating biometric handling.

2. Material and methods

2.1. Essential oil of Ocimum basilicum

The essential oil used in the present study was purchased commercially (Phytoterápica®, Nova Cantareira, Brazil). The chemical composition analyses were performed by gas chromatography coupled to mass spectrometry (GC–MS), as described by Ventura et al. (2019). The major constituents of the essential oil used in this study were methyl chavicol (66.51%) and linalool (20.90%), and 12.57% was composed of minor constituents (Ventura et al., 2020a).

2.2. Animals

Tambaqui juveniles with a weight of 86.51 ± 7.82 g and a total length of 4.62 ± 4.49 cm (n = 153) were maintained in a 1000-L fiberglass tank for 30 days of acclimation at the fish farming facilities of the National Research Institute of the Amazon (INPA), city of Manaus (3°5′26.55″S/59°59′37.84″O), Amazon, Brazil. Fish were fed until apparent satiety three times a day with a commercial diet (Multi Peixe 32, Multifós®) with a 4–6 mm pellet (32% crude protein, 6.0% crude fiber, 8.0% mineral matter, 4.5% ether extract, 9.2% moisture, and 1.9% calcium). The feeding was suspended for 24 h before the start of the experiment.

During the acclimation and experimental period, the following water quality parameters were monitored: temperature (27.68 ± 0.33 °C), dissolved oxygen (7.15 ± 0.12 mg L^{-1}) with an AT 155 Microprocessor oximeter (Alfakit®, Florianópolis, Brazil), and pH (6.32 ± 0.28) with an AT 315 Microprocessor pHmeter (Alfakit®, Florianópolis, Brazil). In addition, total ammonia nitrogen (0.13 ± 0.07 mg L^{-1}), alkalinity (40.0 ± 0.01 mg L^{-1}), nitrite (0.10 ± 0.01 mg L^{-1}), and nitrate (1.90 ± 0.20 mg L^{-1}) were monitored with a commercial kit (Alfatecnoquimica®, Florianópolis, Brazil). The water quality parameters were maintained according to the requirements of the species, as reported by Boyd (1998).

2.3. Experiment I: Anesthetic induction and recovery

After the acclimation period, 63 juveniles (87.73 ± 6.04 g; 14.20 ± 0.45 cm) were evaluated for anesthesia induction and recovery. The fish were transferred to three aquariums (35 cm long; 15.20 cm wide; 22.25 cm high) with 4 L of water and *O. basilicum* essential oil at concentrations of 400, 500, 600, 700, 800, 900, and 1000 μL L^{-1}, equivalent to 362.27, 452.83, 543.40, 633.96, 724.53, 815.10, and 905.67 mg L^{-1}, respectively, considering that the density of the *O. basilicum* essential oil is 0.90 g mL^{-1}. The concentrations were diluted with ethanol 1:10.

To assess anesthetic induction time, nine juvenile fish were used per concentration, with three fish anesthetized per aquarium, one after the other (three fish × three repetitions per treatment). To avoid residual effects from glass adsorption, the trial began with the lowest concentration and progressed to the highest. Fish were considered to be anesthetized when they completely lost equilibrium and were totally unable to regain an upright position; this corresponds to stage 3 of anesthesia as described by Ross and Ross (2008), which was chosen for being compatible with the fish characteristics that are necessary when carrying out handling practices in commercial fish farms.

Anesthesia (total loss of equilibrium without response to stimuli) was evaluated for up to 10 min, with each fish being used only once. The fish were then transferred to an anesthetic-free aquarium (4 L) to assess the post-anesthetic recovery time. The recovery stage was characterized by the return to movement and normal swimming balance with reaction to external stimuli (Woody et al., 2002). After recovery, the fish were separated by treatment and observed for 96 h for survival evaluation. The methodology was approved by the Ethics and Animal Welfare Committee of the Federal University of Mato Grosso do Sul (n° 1.073/2019).

2.4. Experiment II – Fish response to anesthetic after biometric handling

Based on the anesthetic induction and recovery experiment, two concentrations of the basil essential oil were chosen according to the criteria for promoting light and deep anesthesia described by Woody et al. (2002) to evaluate the following effects in response to biometric handling: hematology, blood biochemistry, and muscle and liver metabolism. Tambaqui juveniles (85.29 ± 9.60 g; 15.04 ± 8.53 cm; n = 90) were divided into five groups (18 fish in each group): control (non-handled fish), handling without anesthetic (water only), handling with ethanol (720 μL L^{-1}), handling with essential oil of *O. basilicum* (400 and 800 μL L^{-1} equivalent to 362.27 and 724.53 mg L^{-1}, respectively, considering that the density of the *O. basilicum* essential oil was 0.90 g mL^{-1}). Fish were maintained in a system with 250-L tanks with oxygenation (Ibram - CJ2/1 blower of 1 HP coupled to a 5-cm air stone in each tank) installed in the laboratory and a 10% continuous renovation system for an acclimation period of seven days. The essential oil was first diluted in ethanol at a 1:10 dilution. The ethanol was evaluated to check whether it was capable of inducing sedation or anesthesia in tambaqui at the concentration of 720 μL L^{-1}, which was used to dilute the highest concentration of basil essential oil. Fish were netted and transferred to four 20-L aquariums (one aquarium for each treatment with 18 fish each) and maintained for 10 min in the aquariums according to the respective treatments. The fish from the control treatment (non-handled fish) were kept in the acclimation tank without exposure to handling.

After 10 min of maintenance in the aquariums with the different treatments, biometrics handling and exposure of fish to air were carried out for 1 min in all treatments (Ventura et al., 2019). Blood, liver, and white muscle samples were taken from nine fish per treatment. The remaining fish (n = 9 per treatment) were stored in 250-L tanks with an oxygenation system (Ibram lower CJ2/1 of 1 HP coupled to a 5-cm air stone in each tank) installed in the laboratory and a 10% continuous renovation system for handling recovery. After 24 h of handling and biometrics, the remaining fish (n = 9 per treatment) were netted and

sampled for blood, liver, and white muscle for the physiometabolic analyses of hematology, blood biochemistry, and muscle and liver metabolism. The non-handled fish were sampled at the same time and used as controls.

2.4.1. Blood sampling and analysis

Samples of venous-arterial blood were taken from the caudal vein using a syringe with 10% ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Using all the blood collected, the concentrations of hemoglobin, hematocrit, and the total number of erythrocytes were determined. From these data, the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the methodology described by Ranzani-Paiva et al. (2013). The samples were analyzed individually (nine per treatment at each time point evaluated, just after handling, and after 24 h).

A second aliquot of the blood was sampled without anticoagulant and stored at 4 °C for 24 h for coagulation. The coagulated blood was centrifuged (Merck, Eppendorf - Centrifuge 5415R, Hamburg, Germany) at 1400 ×*g* for 10 min. The serum was aliquoted and stored at −20 °C until further analysis. The samples were pooled for analysis (three fish per sample), with three samples analyzed per treatment at each evaluation time, just after handling, and after 24 h. The metabolites glucose and lactate, the electrolytes ionized calcium, chloride, sodium, and potassium, and osmolarity were measured using a multiparametric analyzer (Roche Gasometry Cobas B221, Group Biogene®, Basel, Switzerland). The levels of total proteins, albumin, and enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by an automatic biochemical analyzer (Roche Biochemistry Cobas C111, Group Biogene®, Basel, Switzerland). The globulin was determined by the result of the subtracting albumin from the serum total proteins. Ammonia was determined according to Gentzkow and Mazen (1942).

2.4.2. Metabolic parameters, sampling, and determinations

After blood sampling, the fish ($n = 3$ per treatment) were euthanized by rapid brain concussion, and fragments of the liver and white muscle were collected and frozen at −20 °C. The tissue fragments were homogenized with 100 mg mL^{-1} (liver) and 250 mg mL^{-1} (muscle) of 20% TCA (trichloroacetic acid), using a Potter-Elvejhem (Sigma Aldrich, St. Louis, United States) homogenizer. Subsequently, the homogenates were centrifuged at 1000 ×*g* for 10 min (Centribio 80-2B, Curitiba, Brazil), and the supernatants were used to determine the metabolic parameters lactate, total proteins, albumin, glucose, triglycerides, and cholesterol. Lactate levels were measured using the method described by Harrower and Brown (1972). Total ammonia was analyzed according to the method of Verdouw et al. (1978). Total protein levels were assessed according to Lowry et al. (1951). The analyses of glucose, albumin, triglycerides, and total cholesterol were performed using a commercial kit Labtest®, which is based on the colorimetric-enzymatic method (Trinder, 1969).

2.5. Statistical analysis

The pre-assumptions of the analysis of variance (ANOVA) were verified using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variances. The assumptions of the ANOVA were not verified for all dependent variables, because of the lack of normality or heteroscedasticity. Therefore, the Kruskal-Wallis test was used to compare the different doses used at the time of anesthetic induction and recovery, followed by Dunn's test to compare the medians. For the experiment that tested anesthesia and handling at different evaluation times, the Kruskal-Wallis test was used in a 5 × 2 (handlings × times) factorial scheme, followed by Dunn's test to compare the medians. A significance level of 5% was used for both analyses. The analyses were performed using the SAS software (Statistical Analysis System, version 9.0).

3. Results

3.1. Anesthesia induction and recovery

All concentrations of the *O. basilicum* essential oil induced the tambaqui juveniles to anesthesia and recovery. Mortality was not observed during and after 96 h from the end of the experiment. The shortest ($p < 0.05$) time of anesthesia induction was at a concentration of 1000 μL L^{-1}, which did not significantly differ from concentrations between 600 and 900 μL L^{-1}. The recovery time from anesthesia did not differ significantly among concentrations of basil essential oil (Table 1).

3.2. Effects of handling, sedation, and anesthesia

3.2.1. Blood

Immediately after handling, a higher ($p < 0.05$) hemoglobin concentration was obtained in fish handled after anesthesia with 800 μL L^{-1} *O. basilicum* essential oil compared to fish handled after maintenance in water only and ethanol only. The highest ($p < 0.05$) MCV was obtained in fish handled after maintenance in ethanol only compared to fish handled after maintenance in water only and handled with anesthetic at concentrations of 400 and 800 μL L^{-1} of *O. basilicum* essential oil. However, after 24 h, only the number of erythrocytes differed significantly ($p < 0.05$), with higher values in fish handled after maintenance with ethanol compared to fish from the control group (non-handled) (Fig. 1).

In the comparison of treatments at the different evaluated times (right after handling and after 24 h) the following trends were found: the percentage of hematocrit was lower ($p < 0.05$) in fish from the control group (non-handled) after 24 h; the MCHC was higher ($p < 0.05$) in fish from the control group, fish handled without anesthesia, and fish handled after maintenance in ethanol solution after 24 h; the number of erythrocytes was higher ($p < 0.05$) in fish handled after maintenance in ethanol solution and after anesthesia with 800 μL L^{-1} of *O. basilicum* essential oil after 24 h; and the MCV was lower ($p < 0.05$) in fish handled after maintenance in ethanol solution after 24 h. The hemoglobin concentration did not differ significantly ($p > 0.05$) between times (Fig. 1).

The biochemical and osmoregulatory parameters and serum ALT and AST levels did not differ ($p > 0.05$) among the different treatments immediately after handling and after 24 h, with the exception of the serum chloride concentration, which after handling was higher ($p < 0.05$) in fish managed after anesthesia with 800 μL L^{-1} of essential oil of basil compared to fish managed without anesthesia (kept only in water). Likewise, at the different times evaluated (just after handling and after 24 h), these parameters were not different ($p > 0.05$) (Table 2).

3.2.2. Metabolites in the muscle and liver

In the muscle, a higher concentration of glucose was observed ($p < 0.05$) in fish managed after anesthesia with 400 μL L^{-1} than in fish not

Table 1
Median values (mean rank) of induction and recovery times with the use of the essential oil of *Ocimum basilicum* as an anesthetic for tambaqui (*Colossoma macropomum*).

Concentration* μl L^{-1}	**Induction (seconds)	Recovery (seconds)
400	606 (56.67)^{a}	266 (41.11)^{a}
500	574 (51.22)^{ab}	220 (25.17)^{a}
600	449 (41.0)^{abc}	332 (46.94)^{a}
700	311 (26.22)^{bcd}	181 (23.00)^{a}
800	271 (13.00)^{d}	205 (26.83)^{a}
900	242 (16.78)^{cd}	223 (34.44)^{a}
1000	222 (19.11)^{cd}	221 (26.50)^{a}

Medians followed by equal letters in the same column do not differ statistically by Dunn's test ($p < 0.05$).

* Concentration of the essential oil of *Ocimum basilicum*.

** Stage 3 anesthesia.

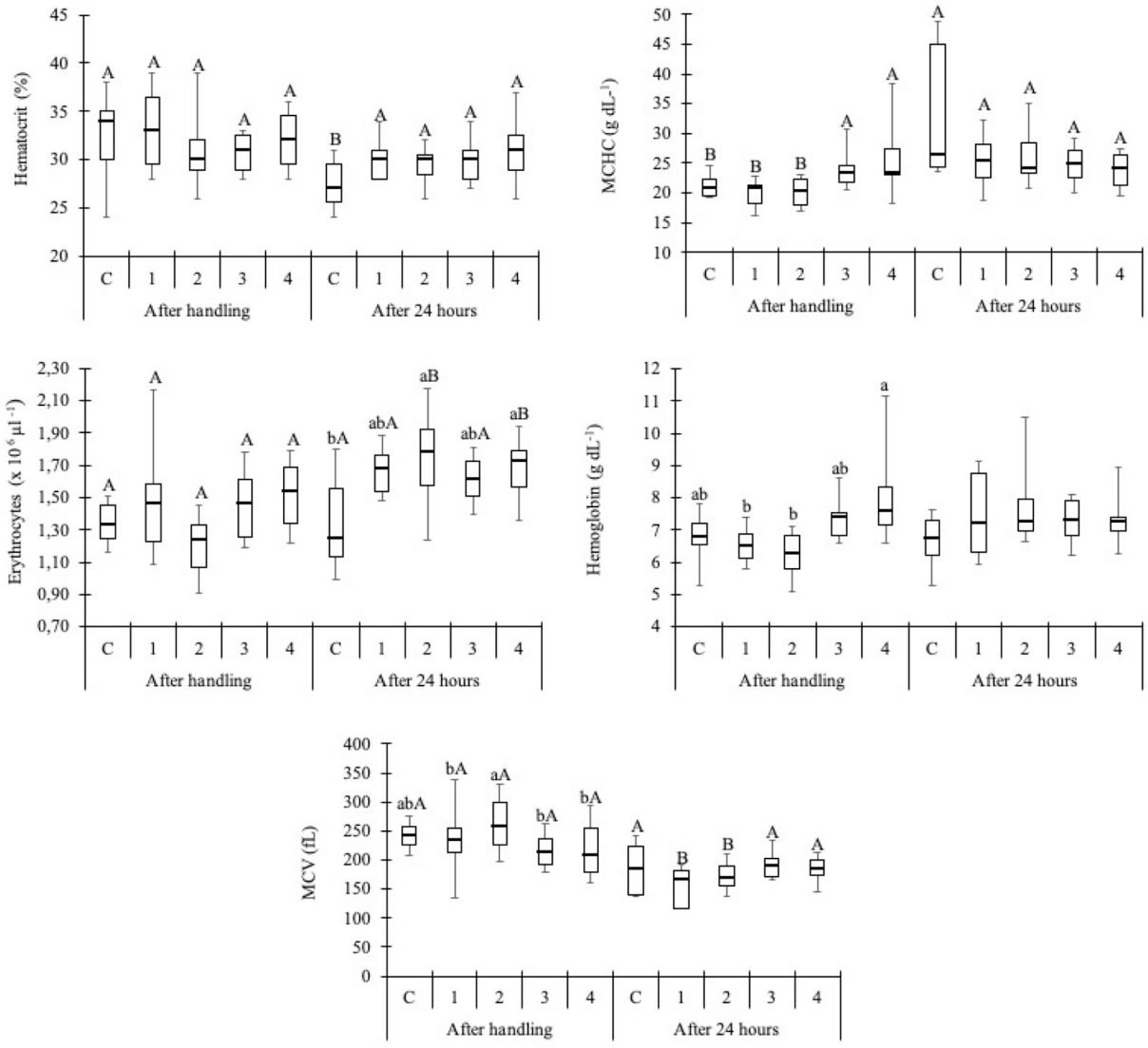

Fig. 1. Median values (maximum–minimum) of hematological values of tambaqui (*Colossoma macropomum*) submitted to anesthesia with essential oil of *Ocimum basilicum* and to biometrics at different times of evaluation. Capital letters are used to compare the two times (right after handling and after 24 h) for each treatment, and lower-case letters are used to compare the treatments at the same time. Medians followed by the same lower-case or capital letter did not differ statistically by Dunn's Test ($p < 0.05$). C, Control group of non-handled fish; 1, Fish handled without anesthesia after 10 min maintained in water only; 2, Fish handled after 10 min maintained in ethanol solution; 3, Fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil at 400 µL L^{-1} concentration; 4, Fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil at 800 µL L^{-1} concentration.

managed immediately after anesthesia. After 24 h, only the total protein concentration was higher ($p < 0.05$) in fish managed after anesthesia with 400 µL L^{-1} of *O. basilicum* essential oil in comparison to that in non-handled fish (Fig. 2).

In liver tissue, after 24 h, glucose concentration was higher ($p < 0.05$) in fish handled after anesthesia with 800 µL L^{-1} of essential oil of *O. basilicum* than in fish handled without anesthesia and in fish handled after maintenance in solution with ethanol. The total ammonia concentration was higher ($p < 0.05$) in fish handled after anesthesia with 400 and 800 µL L^{-1} of essential oil of *O. basilicum* and handled without anesthesia than in fish handled after maintenance in solution with ethanol. Regarding the evaluation of treatments at different times (immediately after handling and after 24 h), the concentration of triglycerides was lower in fish anesthetized with 800 µL L^{-1} of *O. basilicum* essential oil after 24 h than in other treatments, and the concentration of ammonia was lower ($p < 0.05$) in fish handled after maintenance with ethanol solution after 24 h than in other treatments (Fig. 3).

The other metabolic parameters evaluated in muscle and liver tissues did not differ ($p > 0.05$) between treatments. There was no difference ($p > 0.05$) in treatments at different times (immediately after handling and after 24 h) (Table 3).

4. Discussion

In this study, the essential oil of *O. basilicum* met the ideal requirements of anesthetic induction and recovery time for fish by inducing anesthesia in less than 10 min with the total loss of equilibrium (Ross and Ross, 2008) and allowing rapid recovery in less than 5 min without deleterious effects on survival (Marking and Meyer, 1985). In addition, mortality was not observed during and after 96 h from the end of the experiment, which implies that the animals recovered well from anesthesia and handling. The lowest concentration of basil essential oil (400 µL L^{-1}) induced stage 3 anesthesia in 606 s, with a recovery time of 266 s. A shorter time to induce deep anesthesia at a concentration of 400 µL L^{-1} of *O. basilicum* essential oil was observed for Nile tilapia (about 135.2 s), with a recovery time of 199.1 s (Lima-Netto et al., 2017).

Table 2
Median values (mean rank) for the biochemical parameters and serum tambaqui (*Colossoma macropomum*) enzymes submitted to different anesthesia treatments and handled with *Ocimum basilicum* essential oil at different evaluation times.

Parameters	After handling					After 24 h				
	C(1)	1	2	3	4	C(1)	1	2	3	4
Lactate (mmol L^{-1})	2.57 (17.3)	4.44 (23.3)	3.57 (20.3)	5.74 (26.0)	6.47 (28.0)	0.46 (6.3)	0.61 (8.8)	0.56 (7.5)	0.67 (10.7)	0.5 (6.7)
Glucose (mg dL^{-1})	67.2 (13.7)	123.1 (25.3)	115.2 (23.7)	94 (22.0)	146.1 (26.3)	55.2 (5.8)	72.7 (16.3)	56.6 (6.3)	64.6 (10.5)	51.8 (5.0)
Ammonia (μmols mL^{-1})	37.42 (20.0)	19.68 (15.0)	40.65(27.0)	25.48 (16.3)	30.03 (15.7)	16.77 (9.7)	23.23 (12.3)	23.87 (14.7)	21.29 (12.3)	20.97 (12.0)
Total protein (g L^{-1})	2.91 (24.7)	2.71 (21.5)	2.36 (5.0)	3.01 (23.7)	2.58 (13.0)	2.39 (10.50)	2.42 (13.0)	2.49(11.7)	2.72 (18.7)	2.53 (13.3)
Albumin (g L^{-1})	0.7 (21.2)	0.7 (17.0)	0.6 (3.0)	0.7 (16.2)	0.7 (17.0)	0.7 (17.0)	0.7 (17.0)	0.7 (12.7)	0.7 (17.0)	0.7 (17.0)
Globulin (g L^{-1})	2.21 (23.3)	2.0 (20.8)	1.8 (12.3)	2.3 (26.3)	1.9 (11.3)	1.7 (8.7)	1.7 (12.0)	1.8 (11.2)	2.0 (17.0)	1.8 (12.0)
*ALT (μL^{-1})	6.4 (27.7)	6.3 (22.3)	4.1 (13.3)	4.4 (14.8)	5.1 (14.2)	5.6 (22.8)	3.5 (10.0)	3.1 (8.7)	4.3 (15.0)	3.5 (6.2)
**AST (μL^{-1})	145.3 (22.3)	159.7 (27.0)	121.5 (21.3)	110 (15.0)	97.7 (10.7)	146.3 (24.3)	101.5 (10.3)	88.2 (4.3)	99.5 (11.7)	101.1 (8.0)
Chloride (mmol L^{-1})	110.4 (9.0) ab	107.3 (2.7)b	116.7 (24.0) ab	115.4 (20.0) ab	119.1 (28.7)a	113.4 (14.2)	109.5 (4.7)	113.8 (14.5)	114.6 (16.7)	115.8 (20.7)
Ionized Calcium (mmol L^{-1})	1.41 (18.7)	1.38 (12.5)	1.4 (15.7)	1.4 (18.8)	1.54 (28.8)	1.37 (10.7)	1.34 (7.3)	1.39 (10.8)	1.37 (13.7)	1.42 (18.0)
Sodium (mmol L^{-1})	132.2 (14.8)	131.8 (10.5)	140.7 (24.0)	141.8 (25.0)	144.8 (28.3)	131.6 (12.3)	127.9 (4.0)	134.3 (12.7)	131.5 (10.0)	131.7 (13.3)
Potassium (mmol L^{-1})	7.41 (15.0)	4.66 (6.3)	5.39 (10.0)	4.4 (4.0)	5.92 (11.0)	7.26 (18.8)	6.46 (16.3)	7.46 (21.2)	8.99 (26.2)	8.83 (26.2)
Osmolarity (mOsm kg^{-1})	264 (14.2)	264 (13.2)	281 (24.3)	282 (24.5)	291 (28.7)	262 (12.2)	256 (3.5)	267 (11.8)	262 (10.5)	261 (12.2)

Medians followed by the same lower-case letters do not differ statistically by Dunn's test ($p < 0.05$). (1) Handling: C, Control group of non-handled fish; 1, Fish handled without anesthesia after 10 min kept in water only; 2, fish handled after 10 min in ethanol solution; 3, fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil 400 μL L^{-1}; 4, fish handled after 10 min anesthesia with *Ocimum basilicum* essential oil 800 μL L^{-1}.

* ALT, alanine aminotransferase.

** AST, aspartate aminotransferase.

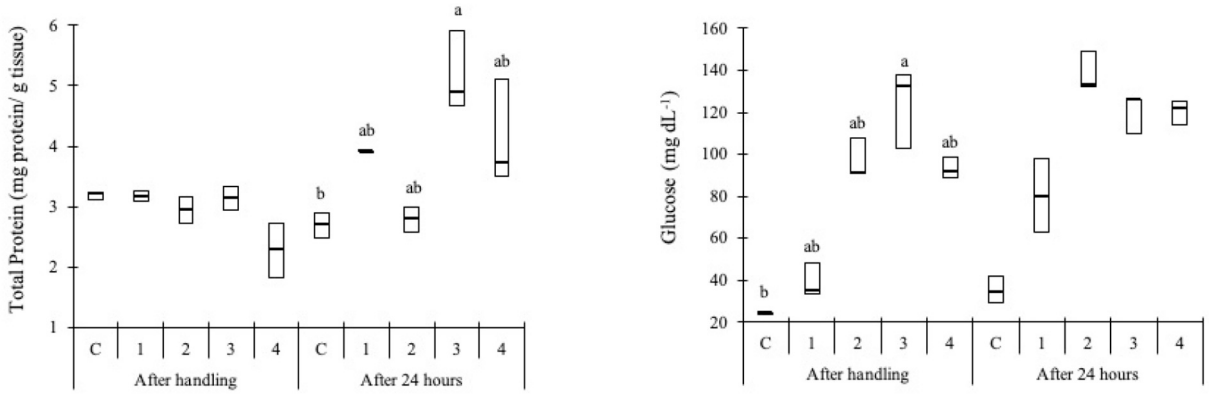

Fig. 2. Median values (maximum–minimum) of metabolites in tambaqui muscle (*Colossoma macropomum*) submitted to anesthesia management with *Ocimum basilicum* essential oil and biometrics at different evaluation times. Medians followed by the same lower-case letters do not differ statistically by Dunn's Test ($p < 0.05$). C, Control group of non-handled fish; 1, Fish handled without anesthesia after 10 min in water only; 2, fish handled after 10 min in ethanol solution; 3, fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil 400 μL L^{-1}; 4, fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil 800 μL L^{-1}.

Different fish species may present different responses to the same anesthetic. For example, a concentration of 200 μL L^{-1} of the essential oil of *Lippia alba* induced anesthesia in approximately 120 s in jundiá *Rhamdia quelen* (Becker et al., 2018). In contrast, for tambaqui a longer time (about 300 s) for anesthetic induction was reported with the same concentration of L. *alba* essential oil (Batista et al., 2018). Nevertheless, the anesthetic effect of the essential oil of *O. basilicum* in juvenile tambaqui was similar to that of other natural anesthetics evaluated for this species, such as *Myrcia sylvatica* essential oil (100–300 μL L^{-1}), which induced anesthesia within 570.00–119.50 s and promoted recovery from 464.22 to 33.03 s, and *Curcuma longa* essential oil (200–500 μL L^{-1}), which induced anesthesia within 1485.00–372.40 s and promoted recovery in 360.0 to 319.0 s (Saccol et al., 2017).

Although the shortest time of stage 3 anesthesia was achieved with the highest concentration of *O. basilicum* (1000 μL L^{-1}), it is possible to have a higher or lower stage of anesthesia with this concentration depending on the water quality; however, we were unable to evaluate this in the present study. The recovery time was similar among concentrations, indicating that different concentrations did not influence fish recovery. Similar results were obtained when evaluating the anesthetic effects of the *C. longa* and *L. alba* essential oils at concentration of 200–500 μL L^{-1} (Saccol et al., 2017) and 50–300 μL L^{-1} (Batista et al., 2018), respectively, for the same fish species. However, for the essential oil of *Nectandra grandiflora* at concentrations of 25 and 50 μL L^{-1} and an extract of *Spilanthes acmella* at a concentration of 10 mg L^{-1}, the lowest concentrations promoted a return from the anesthetic in a short time in

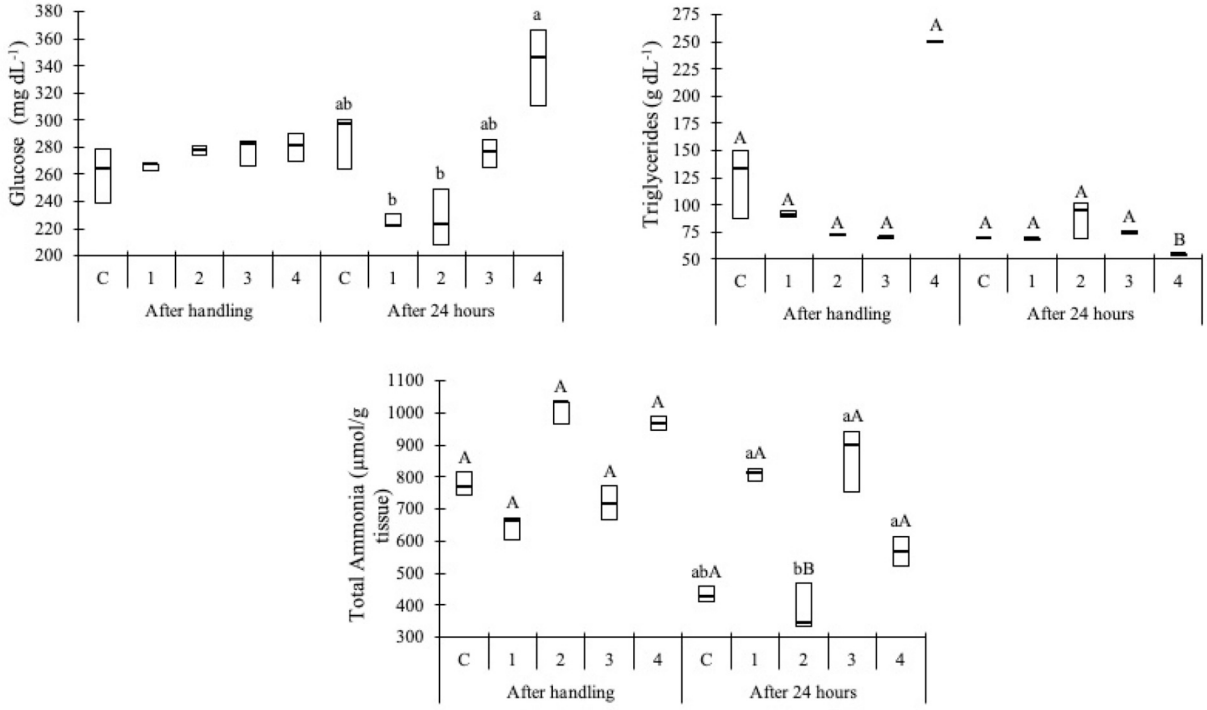

Fig. 3. Median values (maximum–minimum) of the hepatic metabolites of tambaqui (*Colossoma macropomum*) submitted to anesthesia management with *Ocimum basilicum* essential oil and biometrics at different evaluation times. Capital letters show differences between the two evaluation times (right after handling and after 24 h) for each treatment, and lower-case letters compare the treatments at the same time. Medians followed by lower- or upper-case letters do not differ statistically by Dunn's Test ($p < 0.05$). C, Control group of non-handled fish; 1, Fish handled without anesthesia after 10 min in water only; 2, fish handled after 10 min in ethanol solution; 3, fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil 400 µL L^{-1}; 4, fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil 800 µL L^{-1}.

Table 3
Median values (mean rank) of the metabolites in the muscle and liver of tambaqui (*Colossoma macropomum*) submitted to anesthesia management with *Ocimum basilicum* essential oil and biometrics at different evaluation times.

Metabolites [2]	After handling					After 24 h				
	C[1]	1	2	3	4	C[1]	1	2	3	4
Muscle										
Lactate	27.80 (24.5)	27.33 (17.5)	26.71 (23.0)	21.19 (16.7)	13.79 (5.3)	14.42 (9.0)	16.46 (8.0)	23.51 (19.7)	21.50 (19.7)	20.00 (11.7)
Total ammonia	69.55 (20.8)	59.23 (11.5)	53.42 (11.0)	58.19 (13.5)	65.94 (16.8)	54.97 (14.3)	73.55 (25.3)	69.42 (19.7)	63.23 (18.7)	47.48 (3.3)
Liver										
Total protein	6.12 (28.0)	3.65 (12.5)	5.00 (24.0)	4.71 (21.3)	4.90 (22.2)	4.18 (10.3)	4.43 (16.0)	4.31 (13.0)	2.23 (5.7)	1.13 (2.0)
Albumin	1.26 (27.3)	0.94 (24.7)	1.02 (22.2)	0.77 (18.5)	0.23 (5.7)	0.72 (19.0)	0.31 (5.8)	0.37 (7.2)	0.37 (9.3)	0.59 (15.3)
Lactate	6.59 (25.3)	6.22 (22.5)	6.33 (22.5)	6.10 (20.7)	3.18 (5.0)	3.00 (4.7)	5.99 (16.5)	6.07 (21.2)	3.45 (5.3)	4.94 (11.3)
Cholesterol	359.93 (20.7)	393.37 (24.3)	416.08 (27.0)	413.74 (26.0)	345.22 (16.7)	331.65 (13.0)	279.96 (8.3)	245.89 (6.0)	317.97 (10.7)	156.78 (2.3)

Dunn's test ($p < 0.05$) showed no statistical difference between treatments immediately after handling and after 24 h, and treatments at different times. (1) Handling: C, Control group of non-handled fish; 1, Fish handled without anesthesia after 10 min in water only; 2, Fish handled after 10 min in ethanol solution; 3, Fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil 400 µL L^{-1}; 4, Fish handled after 10 min of anesthesia with *Ocimum basilicum* essential oil 800 µL L^{-1}. (2) Lactate (µmol/g tissue); total ammonia (µmol/g tissue); total protein (mg protein/g tissue); albumin (mg protein/g tissue); lactate (µmol/g tissue); and cholesterol (mg dL^{-1}).

tambaqui (Barbas et al., 2017a). This is possibly because of the composition of natural anesthetics as different essential oils have different chemical compositions, which influence the pharmacological response.

The chemical composition of essential oils can vary according to the season, location, type of cultivation, and circadian rhythm (Ribeiro et al., 2018). Furthermore, different methodologies for obtaining natural compounds from the same plant species can produce different levels of certain compounds (Dias et al., 2012). Moreover, the pharmacological presentation and route of exposure can influence the anesthesia results (Ross and Ross, 2008). Therefore, comparisons among anesthetic induction and recovery times of different essential oils should be analyzed carefully when evaluating the use of natural anesthetics.

The percentage of hematocrit and MCHC did not change between the different treatments at the same time, neither immediately after handling nor after 24 h, suggesting that the anesthetic concentrations of basil essential oil did not affect the hematological pattern. Similar results were observed in tambaqui anesthetized with clove oil (Pádua et al., 2013) and in juveniles of matrinxã *Brycon amazonicus* anesthetized with *Ocimum gratissimum* essential oil (Ribeiro et al., 2016). The decrease in hematocrit and increase in MCHC when comparing each treatment at different times may have been an adaptive response

without physiological significance, especially as the number of erythrocytes and hemoglobin remained unchanged in these treatments.

The increase in the hemoglobin levels in fish anesthetized with 800 μL L^{-1} of *O. basilicum* essential oil and evaluated immediately after handling indicated a greater capacity of oxygen transport by the erythrocytes. Studies have shown that these alterations occur in response to the increased physiological demand imposed by an adverse situation (Wojtaszek et al., 2002; Ventura et al., 2020a). Therefore, the observed hematological alterations indicate that the concentration of 800 μL L^{-1} of *O. basilicum* essential oil was less effective in preventing stress during handling situations, requiring adaptive response mechanisms from fish immediately after handling. However, after 24 h, the hemoglobin levels had returned to homeostasis.

The higher number of erythrocytes after 24 h in fish handled after bathing in ethanol solution and fish anesthetized with 800 μL L^{-1} of *O. basilicum* essential oil could be due to a greater need for oxygen transport because of the increase in energy demand, which was triggered by stress imposed by the exposure of the fish to these solutions. Similar results were obtained for tambaqui anesthetized with 50 mg L^{-1} and 100 mg L^{-1} clove oil (Pádua et al., 2013). However, Ribeiro et al. (2016) evaluated the essential oil of *O. gratissimum* at concentrations of 20 and 60 mg L^{-1} in juvenile matrinxã *B. amazonicus* and observed a decrease in the number of erythrocytes after 24 h. This finding demonstrates that erythrocyte responses related to defensive competence vary widely among fish species, even among related species.

Changes in globular volume can be related to an increase in the osmotic fragility of erythrocytes (Pádua et al., 2013), hemoconcentration, or hemodilution due to a membrane permeability disorder caused by the use of anesthetics (Inoue et al., 2011; Ribeiro et al., 2016). The increase in MCV in fish immediately handled after bathing in an ethanol solution and the greater number of erythrocytes after 24 h for fish in this same group can be explained as a response to acute stress. Thus, it is possible to infer that the alterations observed in the erythrogram of fish exposed to 800 μL L^{-1} of *O. basilicum* essential oil are triggered by the amount of ethanol used as a vehicle in the dilution of this essential oil. However, after 24 h, we observed that the MCV was reduced for the same treatment and also for fish handled without the addition of anesthetic; thus, further studies are needed to fully understand the effects of ethanol on the physiological characteristics of fish.

In stressful situations, the fish activate the hypothalamus-pituitary-interrenal axis, which triggers a change in blood hormone levels (Barton and Iwama, 1991). As a result of this activation, metabolic alterations can occur, such as an increase in glucose, lactate, and osmoregulatory disorders (Barton, 2002). The stimulus of anesthesia and handling with basil essential oil did not alter the blood metabolites lactate, glucose, ammonia, ALT, and AST enzymes in tambaqui. Similar results were obtained for this same species after sedation with *C. longa* essential oil, during which the serum glucose levels did not change (Saccol et al., 2017). This finding may indicate that there was no mobilization of these metabolites in fish anesthetized and subjected to biometrics. In addition, the increase in the enzymatic activity of ALT and AST can be related to hepatotoxicity and liver damage (El-Sayed et al., 2007). In our study, no increase in the enzymatic activity in the blood of tambaqui was observed, which suggests that the physiological state of homeostasis was maintained, thus avoiding hyperactivity and liver damage in tambaqui anesthetized with basil essential oil.

Total protein in the serum reflects the level of non-specific immunity (Ortuño et al., 2001). Globulins include proteins that act in the immune system, coagulation factors, enzymes, and transport proteins (McDonald and Milligan, 1992). In stressful situations, several immune responses are reduced, such as an increase in the level of total serum proteins and a decrease in the production of antibodies (Carlson et al., 1993). In our study, fish under anesthesia and biometric handling did not present alterations in the total serum protein levels. Therefore, it is possible to infer that non-specific immune responses were not changed in the present study.

Handling procedures during fish farming are likely to cause osmoregulatory disorders in response to stress (Inoue et al., 2011). However, the concentrations of ionized calcium, sodium, potassium, and osmolarity in tambaqui were not altered as a result of anesthesia and handling with essential oil of *O. basilicum*. The decrease in serum chloride levels results from the increased stress-induced renal loss in fish handled without anesthesia. However, *O. basilicum* essential oil at 800 μL L^{-1} prevented changes in the osmoregulatory response in tambaqui juveniles. In a study with the addition of this same essential oil during transport of Nile tilapia *O. niloticus*, it was observed that the essential oil at a concentration of 20 μL L^{-1} did not prevent the drop in chloride levels (Ventura et al., 2020a). This can be attributed to the concentration of the essential oil used, which was approximately 40 times lower than the concentration used in the present study. Furthermore, studies have shown that the biochemical parameters in fish under anesthetic effect are variable (Velisek et al., 2011; Gressler et al., 2015) and that physiological or environmental manipulation can result in a small electrolyte imbalance (Stoskopf, 1993).

Cortisol at high levels stimulates the catabolism of muscle proteins generating energy in stressful situations (Aluru and Vijayan, 2009). However, shortly after handling, no alteration at the muscle protein level was observed. Unhandled fish supposedly have lower energy waste with higher levels of muscle protein than the handled ones. Nevertheless, in the present study the level of muscle protein in non-handled fish remained constant right after handling and 24 h after handling. The observed increase in the level of muscle proteins in anesthetized fish suggests that the essential oil of *O. basilicum* inhibited the catabolism of muscle proteins due to stress from handling and promoted the synthesis of proteins; this resulted in an increase in the level of muscle proteins 24 h after handling and anesthesia compared to non-handled fish. This metabolic adaptation favors the use of proteins for the organism's other vital functions (Becker et al., 2016).

Under stress conditions, fish use glycogen reserves through glycogenolysis to provide energy for the body to escape or adjust to new physiological conditions imposed by the environment (Iwama et al., 2004). This metabolic adaptation was observed from an increase in muscular glucose in the fish anesthetized with essential oil of *O. basilicum* at a concentration of 400 μL L^{-1} immediately after handling as compared to non-handled fish. However, after 24 h the level of muscle glucose had already returned to homeostasis. A similar result was observed in jundiás *R. quelen* with essential oil of *L. alba.* Here, the fish presented lower glycogen levels in the liver and muscle tissues due to the activation of the glycogenolysis pathway (Becker et al., 2016).

Under conditions of hypoxia caused by deepening in the anesthetic stage, the lactate resulting from the anaerobic glucose metabolism is elevated (Omlin and Weber, 2010). However, in the present study, the level of lactate in muscle tissue did not differ among the different treatments and times evaluated, suggesting that anesthesia with essential oil of basil did not change anaerobic metabolism. This finding may be due to adaptations of tambaqui under hypoxic conditions (Morais and O'Sullivan, 2017), or it could be attributed to reduced muscle activity in anesthetized fish. The altered blood flow makes nitrogen excretion difficult, a reaction that apparently does not occur in fish anesthetized with essential oil of *O. basilicum* as the level of muscle ammonia did not change. Therefore, it is possible to infer that the use of basil essential oil was efficient in immobilizing the fish without impairing the cardiorespiratory functions during the experimental procedures.

The increase in glucose and lactate in the liver tissue is indicative of glycogen mobilization and degradation (Larsson et al., 2014). In the present study, the liver lactate level did not differ among the different treatments and times evaluated. However, the higher liver glucose in fish anesthetized with essential oil of *O. basilicum* at 800 μL L^{-1} compared to fish handled without anesthesia after 24 h may have been caused by an increase in the release of cortisol triggered by the essential oil of basil at this concentration. Nevertheless, these glucose levels are similar to those of liver glucose in fish from the control group that were

not subjected to handling. Similar results were obtained when evaluating sedation and anesthesia with the essential oils of *M. sylvatica* and *C. longa* in tambaqui, when no alterations in the levels of liver glucose and lactate were detected (Saccol et al., 2017).

Albumin synthesis can be influenced by the general state of liver and stress (McDonald and Milligan, 1992). In the present study, the levels of total protein and albumin did not differ among the evaluated treatments and times, demonstrating that the hepatic metabolism of the fish was not altered by the treatments. After 24 h, total liver ammonia was lower in fish handled after kept in ethanol solution than in fish handled without anesthesia and those anesthetized with essential oil of *O. basilicum* at 400 and 800 μL L^{-1}. High levels of ammonia are associated with transaminase activity (Inoue et al., 2011; Gressler et al., 2015), but it is not possible to affirm that the essential oil of *O. basilicum* promoted transamination of the liver tissue as these results are similar to the liver ammonia level in fish from the control group that was not handled. Thus, these results allow us to infer that the use of the essential oil of basil maintained homeostasis in the liver metabolism of juvenile tambaqui.

The lowest triglyceride concentration was found in the liver tissue after 24 h in fish anesthetized with essential oil of basil (800 μL L^{-1}). This may be attributed to a change in the mobilization and circulation of lipids between the liver and other tissues related to the synthesis of phospholipids and cholesterol (Jun et al., 2015). However, these results are similar to those of the control group of non-handled fish. This helps to explain why the levels of total cholesterol in the liver tissue did not diverge among the different treatments and times evaluated. Thus, it is possible to state that the handling associated with anesthesia with basil essential oil had no effect on the metabolism of energy, carbohydrates, and lipids, indicating that the responses to stress were prevented, attenuated, or not modified.

5. Conclusion

The essential oil of basil can be safely used as an anesthetic and sedative for tambaqui *C. macropomum* to minimize the stress associated with handling in aquaculture. In addition to reduction or attenuation in the stress response, this essential oil had no effect on the non-specific immune parameters, metabolism of energy, carbohydrates, and lipids. However, further studies are needed to confirm the exact mechanism by which this essential oil exerts its anesthetic and sedative properties. Furthermore, it is necessary to determine whether the observed bioactivity results from the presence of its main compounds or from a synergistic effect among the various constituents of the essential oil.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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