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Short-Term Feeding with *Hesperozygis ringens* Essential Oil Modulates Transportation-Induced Physiological Responses in *Colossoma macropomum*

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Abstract

Hesperozygis ringens essential oil (HREO), rich in pulegone and limonene, has potential application in aquaculture due to its beneficial properties. This study evaluated the effects of dietary supplementation with HREO (0.0, 0.75, 1.0, and 2.0 g HREO kg feed^{-1}) for 30 days on the physiological responses of *Colossoma macropomum* before and after a simulated 4 h transport. Fish were sampled at four time points: before transport (Basal), immediately after transport (IAT), and at 24 h (AT24) and 48 h (AT48) post-transport. Growth performance and survival (>96%) were not affected by HREO. Hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC), and intestinal coefficient showed significant responses to dietary HREO. After transport, survival was 100% for all treatments, and hematological and biochemical parameters varied according to dose and recovery time, with 2.0 g HREO kg feed^{-1} showing the most consistent benefits, such as stabilization of hemoglobin, MCHC, and plasma proteins during recovery at AT_{24} and AT_{48}. Water quality parameters did not differ between treatments at transportation end. These findings suggest that dietary HREO may contribute to enhance the physiological responses to transport in *C. macropomum*, supporting its potential use as a sustainable nutritional strategy.

Keywords: tropical fish; native fish; essential oil; transport

Key Contribution: Pre-transport feeding with *Hesperozygis ringens* essential oil improved the physiological stress responses of *Colossoma macropomum* during transportation, facilitating enhanced adaptation and recovery.

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1. Introduction

Fish farming has become increasingly intensive with the aim of increasing production [1,2], and the interest in more controlled systems has grown, mainly due to the advantages of smaller occupied area, greater biosecurity, and environmental benefits compared to conventional systems [3–5]. In intensive fish farming, factors like constant management, water parameter variation, and the high stocking density may lead to stress, which is a potentially harmful factor for fish growth and health [6]. Stressors cause behavioral changes such as increased aggressiveness, leading to greater metabolic demands and changes in fish feeding behavior [7]. Such conditions can generate significant levels of stress in aquatic organisms [8–10].

Another management that can generate stress in animals is the transportation of live fish, a fundamental step in the production chain. It is also a stressor since it alters the physical and chemical parameters of the water, while the duration of transportation and the loading density can also influence its success [11–13]. If transportation is conducted correctly and variations in water quality are minimized, stress can be reduced [14,15]. The preparation of fish for transportation is also a factor that can minimize stress and prevent mortalities after transport [16]. In this sense, the use of essential oils (EOs), complex natural mixtures of volatile and lipophilic substances, secondary metabolites of plants [17], commonly found in aromatic plants [18], can play a significant role in promoting the health, growth, and improvement in the metabolism of farmed fish [19]. Currently, the use of EOs has been a good alternative to mitigate the effects of stress by reducing the loss of ions and other physiological and biochemical responses [20–22].

The increase in research on the use of EOs in aquaculture is due to the growing need for alternatives to the chemicals used in intensive production, whether as anesthetics, antibiotics, antiparasitics, or performance enhancers, which may cause losses due to environmental contamination and bacterial drug resistance [23–25]. In addition, EOs are more accessible and sustainable, have low toxicity, and present fewer side effects [19]. EOs are composed of bioactive substances that may offer several beneficial properties to crops, acting as immunostimulants [26], including antibacterial and antifungal actions [17,25], stress-reducing effects [27,28], and animal growth stimulation [29].

Hesperozygis ringens (Lamiaceae) is a shrubby plant endemic to southern Brazil [25]. Its main active compounds are pulegone (95.18%) and limonene (1.28%) [30]. This EO has potential application in fish farming [25,31–33], being studied mainly as an anesthetic [27,31,32], antibacterial [23], antiparasitic, and antioxidant [25]. However, studies on the use of the EO of *H. ringens* (HREO) and its influences on hematological parameters and animal performance are still needed. Pulegone, the main compound of HREO, is also the main compound of the EOs of *Mentha pulegium* and *Mentha spicata*, whose dietary supplementation presented conflicting growth results in rainbow trout, *Oncorhynchus mykiss* [34], and common carp, *Cyprinus carpio* [35].

Tambaqui (*Colossoma macropomum*) belongs to the Characidae family, the most diverse among Neotropical fish [36]. It is endemic to the Amazon basin [37] and the main freshwater species produced in South America due to characteristics such as easy reproduction, domesticability [38], hardiness, and performance, in addition to a promising consumer market [39]. This species has also shown adaptation to more intensive cultivation in recirculating aquaculture systems (RASs) [40–43]. However, despite the species being quite resistant to cultivation conditions, excessive handling can impair its health [44].

Thus, the aim of the research was to investigate how the inclusion of the EO from *H. ringens* leaves in the diet can modulate the hematological (hemoglobin, erythrocytes, hematocrit, MCV, MCH, and MCHC), biochemical (triglycerides, glucose, cholesterol, and total proteins), and enzymatic (ALT and AST) responses of *C. macropomum* juveniles reared in RASs when subjected to transport.

2. Materials and Methods

2.1. Oil Extraction

The essential oil used in this study was collected in 2022, and fresh leaves yielded 3.85% oil. The EO used was extracted through 3 h hydrodistillation using a Clevenger-type apparatus [30]. After extraction and sample preparation for gas chromatography analysis, the essential oil is always transferred to a freezer and stored at subzero temperatures, resulting in little variation in chemical composition. We reanalyzed the chemical composition of the essential oil, using the analytical parameters described [30], and the components detected were pulegone (93.72%), menthone (3.46%), limonene (1.53%), and menthol (1.39%).

2.2. Study Location, Pre-Transport Conditions, and Animals

The research was conducted at the Federal University of Minas Gerais (UFMG, Brazil) at the Aquaculture Laboratory (LAQUA) and was approved by the UFMG Animal Use Ethics Committee (CEUA/UFMG-n° 193/2023).

Two hundred and twenty-four juveniles of *C. macropomum* (4.09 ± 0.007 g and 6.31 ± 0.06 cm) were randomly distributed at a density of 14 fish per tank (0.5 fish L^{-1}) in 16 circular tanks with 28 L of useful volume maintained in a recirculating aquaculture system (RAS). The RAS consisted of a mechanical and biological filter, supplementary aeration, and temperature control [45]. The feed used in the experiment contained 35% crude protein (Table 1) (Supplementary Table S1) and was supplied at 10% [46] of the biomass in two daily meals (8:00 a.m. and 4:00 p.m.). *H. ringens* essential oil (HREO) was mixed with canola oil during the elaboration of the feed, with the following treatments, with 4 replicates for each treatment distributed in a completely randomized design: $HR_{0.0}$—0.0 g (control); $HR_{0.75}$—0.75 g; $HR_{1.0}$—1 g; and $HR_{2.0}$—2 g HREO kg of feed^{-1}. As there are no studies regarding dietary supplementation with HREO, the doses chosen were based on a study with other essential oils and the same fish species [47] and within the range tested with other EOs in which pulegone was the main compound [34,35]. During the 30-day pre-transport feeding period, the tanks were cleaned three times a week to remove excess organic matter, and the water quality parameters were analyzed daily during the morning period before the first feeding of the day. Dissolved oxygen (4.92 ± 0.34 mg L^{-1}) was measured using portable HI9146-04 equipment (HANNA® Instruments, São Paulo, Brazil Exp. E Imp. LTDA). pH (6.98 ± 0.14), salinity (0.15 ± 0.01 g of salt L^{-1}), conductivity (0.29 ± 0.01 mS cm^{-1}), and temperature (28.32 ± 0.47 °C) were measured using a portable HI98130 equipment (HANNA® Instruments, São Paulo, Brazil Exp. E Imp. LTDA). Total ammonia was assessed using the colorimetric kit (Alcon/LabconTest, Irvine, CA, USA) and was kept below 0.25 mg L^{-1}.

Table 1. Composition of control diet.

Ingredients	(%)
Soybean meal [1]	35
Fish meal [2]	30
Rice bran [3]	12
Corn bran [4]	15
Canola oil [5]	3
Salt [6]	1
Vitamin and mineral premix *	3
Phosphate dicalcium [7]	1

Table 1. *Cont.*

Ingredients	(%)
Analyzed proximate composition	
Dry matter content	94.36
Crude protein	35.24
Crude fat	7.56
Mineral matter	13.5

* Vitamin and mineral mixture (minimum levels per kilogram of product according to manufacturer): folic acid: 250 mg; pantothenic acid: 5000 mg; biotin: 125 mg; antioxidant: 0.60 g; vitamin A: 1,000,000 UI; vitamin B1: 1250 mg; vitamin B2: 2500 mg; vitamin B6: 2485 mg; vitamin B12: 3750 mcg; vitamin C: 28,000 mg; vitamin D3: 500,000 UI; vitamin E: 20,000 UI; vitamin K: 500 mg; cobalt: 25 mg; copper: 2000 mg; iodine: 100 mg; iron: 820 mg; manganese: 3750 mg; niacin: 5000 mg; selenium: 75 mg; zinc: 17,500 mg. Superscript numbers are explained in Supplementary Table S1.

2.3. Zootechnical Performance: Pre-Transport Period

The weight gain and growth of the fish were determined through biometric measurements performed after 10, 20, and 30 days of feeding. The weight was obtained using a Marte digital scale (Marte Científica, São Paulo, Brazil) with 0.001 g precision, and the length using a caliper with 0.01 mm precision. The weight gain (WG), daily weight gain (DWG), apparent feed conversion (FCR), daily specific growth rate (SGR), and survival were calculated with the obtained data:

- WG: Final weight − Initial weight;
- DWG (g): Weight gain (g)/experiment time (days);
- FCR: Total feed intake (g)/weight gain (g);
- SGR (% day^{-1}): 100 × (*ln*Pf − *ln*Pi)/interval between biometrics (days), where Pi is the initial weight, Pf is the final weight;
- Survival (%): (final number of fish/initial number of fish) × 100.

2.4. Blood Analysis

After 30 days, the animals were fasted for 24 h. Then, three animals from each tank (n = 12 animals per treatment) were anesthetized with 50 mg L^{-1} of eugenol (20 mL of concentrated clove oil diluted in 60 mL of absolute ethanol, Biodinâmica®, Ibiporã, Brazil) [48] for blood collection through venipuncture in the caudal vertebral artery. Between 300 and 500 µL of blood was collected in a heparinized syringe (HEPAMAX-S®, Blau Farmacêutica S/A, São Paulo, Brazil; 5000 IU/mL) and, subsequently, 10% heparin was added in relation to the volume of blood collected. Of the whole blood, 10 µL was used to determine the hemoglobin concentrations (LABTEST, Delta, BC, Canada (Bioclin®)), followed by reading in a spectrophotometer (Bioclin 100) and part was used to determine the hematocrit by the microhematocrit method [49] using capillary tubes. The number of erythrocytes was assessed by diluting 10 µL of whole blood with heparin in 2 mL of citrate formalin and then counting in a Neubauer chamber.

The remaining aliquots of whole blood were centrifuged (1792 g-force for 10 min) to separate the plasma. The plasma samples were used to evaluate cholesterol, triglycerides, glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) using commercial kinetic UV kits (Bioclin®, Belo Horizonte, Brazil; batch no. 0121/0124, respectively) (Bioclin®), followed by reading in a spectrophotometer (Bioclin 100). Total plasma protein was determined after breaking the microhematocrit tube, where the plasma was placed in an analog refractometer (0 to 90% Brix-RHB0-90) (Formis Instrumentos de Medição—LTDA, São Paulo, Brazil).

The hematimetric indices, i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated according to the following formulas [50]:

- MCV (fL) = (Hematocrit × 10)/(No. of erythrocytes ($\times 10^6\ \mu L^{-1}$));
- MCH (pg) = (Hemoglobin concentration × 10/(No. of erythrocytes ($\times 10^6\ \mu L^{-1}$));
- MCHC (g dL^{-1}) = (Hemoglobin concentration × 100)/Hematocrit.

2.5. Viscerosomatic and Hepatosomatic Indices

After blood collection, the fish were euthanized with a solution containing 285 mg L^{-1} of eugenol [51] to determine the hepatosomatic index (HSI), intestinal coefficient (IC), and viscerosomatic index (VSI) using the following formulas:

- HSI (%) = 100 × (liver weight (g)/body weight (g));
- IC = intestine length (cm)/total fish length (cm);
- VSI (%) = 100 × (visceral weight (g)/body weight (g)).

2.6. Transport Experiment

After biometrics, 160 juveniles were selected, 40 animals from each treatment (10 fish per tank). These animals were kept in the tanks fasting for an additional 24 h to recover from the biometric stress. Then, 10 fish from each treatment were anesthetized with 50 mg L^{-1} of eugenol [48] and submitted to blood collection, thus constituting the basal group. The remaining fish (n = 30 fish/treatment) were placed in plastic transport bags (40 × 60 cm) containing 8 L of water and oxygen added in the proportion of 3 parts of oxygen to 1 part of water, with each individual being considered a replicate. The transport simulation lasted 4 h, during which the plastic bags were placed inside a 1000 L empty tank and the tank was manually shaken for 10 s every 10 min to mimic vehicle movement. At the end of this period, the bags were opened and water quality parameters were measured. Then, 40 fish (n = 10 fish/treatment) underwent blood collection as previously described, constituting the immediately after transportation (IAT) group. The remaining 80 fish were returned to the same 28 L circular tanks in the RAS where they had been maintained throughout the experimental period, for recovery and kept for 48 h. These tanks presented the same water quality conditions as during the experimental phase. During this period, the animals were not fed and after 24 h (AT_{24}) (n = 10 fish/treatment) and 48 h (AT_{48}) (n = 10 fish/treatment), they were anesthetized with 50 mg L^{-1} of eugenol [48] and underwent blood collection. Each animal had its blood collected once.

2.7. Statistical Analysis

All data were checked for homogeneity of variances and normality of residuals using Levene's test and the Shapiro–Wilk test, respectively. If the assumptions were not met, the data were transformed, and the ANOVA requirements were assessed again. The growth variables, blood tests, intestinal coefficient, and viscerosomatic and hepatosomatic indices were subjected to ANOVA, followed by regression analysis. Data related to transport were subjected to two-way ANOVA to compare concentrations ($HR_{0.0}$, $HR_{0.75}$, $HR_{1.0}$, and $HR_{2.0}$) and collection times (Basal, IAT, AT_{24}, and AT_{48}) and the interaction of factors (concentrations and collection times), followed by Tukey's post hoc test with a significance level of 5% probability. All data were analyzed using SAS statistical software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Pre-Transport Period

No significant differences were recorded for W, TL, WG, DWG, SGR, FCR, biomass, or survival after 10, 20, and 30 days of cultivation ($p > 0.05$) (Table 2).

Table 2. Growth (mean ± standard error of mean) of juveniles of *Colossoma macropomum* fed for 30 days with diets containing different doses of essential oil of *Hesperozygis ringens* (HREO).

HREO Inclusion (g kg of Feed^{-1})	W (g)	TL (cm)	WG (g)	DWG (g Day^{-1})	SGR (% Day^{-1})	Biomass (kg m^{-3})	FCR	Survival (%)
1–10 Days								
$HR_{0.0}$	7.79 ± 0.19	7.89 ± 0.06	3.71 ± 0.19	0.37 ± 0.01	6.45 ± 0.24	3.89 ± 0.09	1.73 ± 0.22	100.00
$HR_{0.75}$	7.67 ± 0.31	7.86 ± 0.15	3.58 ± 0.31	0.35 ± 0.03	6.26 ± 0.41	3.83 ± 0.15	1.56 ± 0.11	100.00
$HR_{1.0}$	7.91 ± 0.31	8.20 ± 0.28	3.83 ± 0.32	0.38 ± 0.03	6.60 ± 0.42	3.95 ± 0.16	1.53 ± 0.13	100.00
$HR_{2.0}$	8.11 ± 0.18	7.99 ± 0.09	4.02 ± 0.18	0.40 ± 0.01	6.84 ± 0.22	4.05 ± 0.09	1.51 ± 0.07	100.00
p-value	0.6870	0.5137	0.6824	0.6824	0.6771	0.6870	0.7309	1.0000
CV (%)	6.34	4.18	13.19	13.19	9.89	6.34	17.53	0.00
11–20 days								
$HR_{0.0}$	13.73 ± 0.87	10.84 ± 0.18	5.94 ± 0.69	0.59 ± 0.06	5.61 ± 0.42	6.60 ± 0.29	1.24 ± 0.10	100.00
$HR_{0.75}$	13.49 ± 0.85	10.60 ± 0.18	5.81 ± 0.60	0.58 ± 0.06	5.60 ± 0.36	7.16 ± 0.34	1.04 ± 0.12	98.21 ± 1.78
$HR_{1.0}$	13.83 ± 0.80	10.60 ± 0.15	5.91 ± 0.56	0.59 ± 0.05	5.54 ± 0.31	6.68 ± 0.50	1.25 ± 0.24	98.21 ± 1.78
$HR_{2.0}$	14.50 ± 0.87	10.81 ± 0.13	6.38 ± 0.70	0.63 ± 0.07	5.75 ± 0.39	7.07 ± 0.46	1.29 ± 0.26	100.00
p-value	0.8565	0.6122	0.9249	0.9249	0.9822	0.7213	0.8049	0.5885
CV (%)	11.32	2.99	19.53	19.53	12.14	11.3	11.24	2.46
21–30 days								
$HR_{0.0}$	22.81 ± 1.45	11.39 ± 0.13	9.07 ± 0.64	0.90 ± 0.06	5.07 ± 0.16	11.40 ± 0.72	1.10 ± 0.11	100.00
$HR_{0.75}$	22.78 ± 1.81	11.47 ± 0.27	9.28 ± 1.19	0.92 ± 0.11	5.19 ± 0.46	10.95 ± 0.79	1.16 ± 0.19	96.42 ± 2.06
$HR_{1.0}$	22.52 ± 1.23	11.34 ± 0.15	8.69 ± 0.64	0.86 ± 0.06	4.88 ± 0.26	11.26 ± 0.61	1.15 ± 0.14	98.21 ± 1.78
$HR_{2.0}$	23.95 ± 1.48	11.47 ± 0.30	9.45 ± 0.66	0.94 ± 0.06	5.01 ± 0.15	11.97 ± 0.74	1.14 ± 0.13	100.00
p-value	0.9091	0.9694	0.9209	0.9209	0.9003	0.7898	0.9928	0.2476
CV (%)	12.01	3.62	16.45	16.45	10.53	11.86	21.85	2.91

W = weight; TL = total length; WG = weight gain; DWG = daily weight gain; SGR = daily specific growth rate; FCR = feed conversion ratio. The results showed no difference by ANOVA ($p > 0.05$).

After 30 days of feeding, the hemoglobin concentration showed a direct relationship with the increase in HREO in the diet ($p = 0.0367$) (Figure 1A), while the MCHC showed a quadratic response with a lower value estimated by the derivative of the equation at 0.90 g HREO kg feed^{-1} (Figure 1B). The other hematological and biochemical parameters were similar between treatments ($p > 0.05$) (Table 3).

Table 3. Hematological and blood biochemical parameters (mean ± standard error of mean) of juvenile *C. macropomum* fed for 30 days with diets containing different concentrations of essential oil of *H. ringens* (HREO).

Parameters	HREO Inclusion (g kg of Feed^{-1})				*p*-Value	CV (%)
	$HR_{0.0}$	$HR_{0.75}$	$HR_{1.0}$	$HR_{2.0}$		
Hematocrit (%)	22.87 ± 0.47	22.33 ± 0.83	22.60 ± 0.49	23.20 ± 0.46	0.7818	8.68
Erythrocyte (×10^6 μL^{-1})	0.41 ± 0.05	0.49 ± 0.05	0.45 ± 0.05	0.51 ± 0.05	0.6178	37.24
MCV (fL)	645.34 ± 93.10	461.99 ± 51.00	551.65 ± 73.10	524.48 ± 60.10	0.3143	37.67
MCH (pg)	182.46 ± 24.82	144.48 ± 14.57	170.72 ± 21.07	174.80 ± 18.89	0.5494	37.71
Total proteins (g dL^{-1})	4.07 ± 0.05	4.11 ± 0.09	4.12 ± 0.09	4.12 ± 0.06	0.9821	6.32
Glucose (mg dL^{-1})	49.79 ± 3.54	56.38 ± 3.43	53.50 ± 3.23	56.28 ± 5.05	0.5948	24.62
Cholesterol (mg dL^{-1})	69.78 ±3.51	65.71 ± 4.37	70.69 ± 4.42	78.68 ± 3.99	0.1972	19.67
Triglycerides (mg dL^{-1})	158.27 ± 11.16	184.24 ± 9.83	171.90 ± 13.85	191.54 ± 16.26	0.3569	23.79
ALT (U L^{-1})	11.83 ± 2.50	8.63 ± 1.40	8.00 ± 1.02	7.33 ± 1.21	0.6550	28.09
AST (U L^{-1})	244.66 ± 28.86	195.41 ± 9.82	220.41 ± 20.57	181.10 ± 26.58	0.2203	36.50

The variable ALT was transformed using the logarithmic function, expressed by the function: Y = log (x). The results showed no difference by ANOVA ($p > 0.05$). ALT: alanine aminotransferase; AST: aspartate aminotransferase; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin.

HSI and VSI were similar between treatments ($p > 0.05$) (Table 4). The IC showed a direct relationship with the increase in HREO in the diets ($p = 0.0330$) (Figure 2).

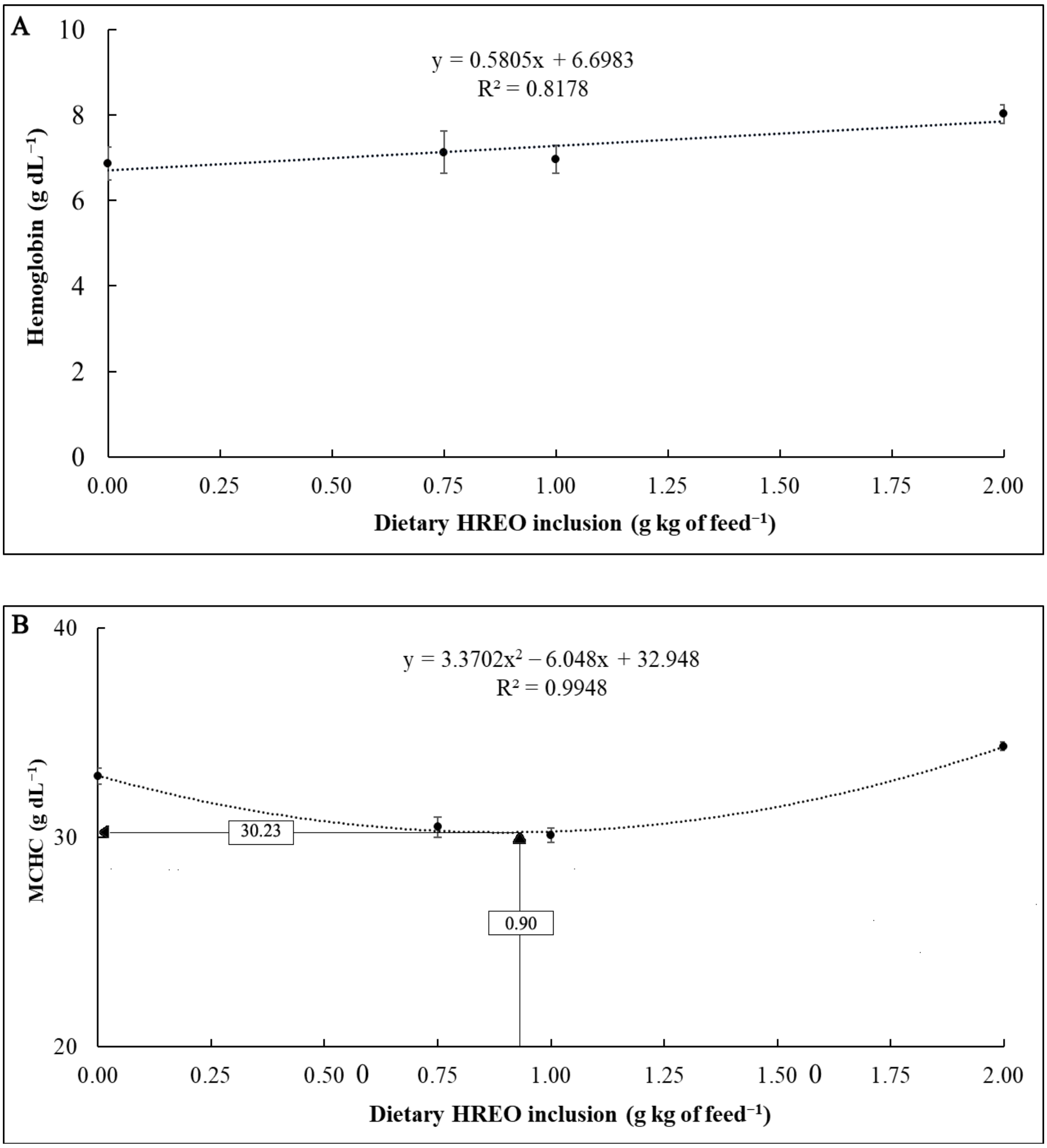

Figure 1. Hemoglobin (**A**) and MCHC (**B**) (mean ± standard error of mean) of *C. macropomum* juveniles after 30 days of feeding with diets containing different concentrations of *H. ringens* essential oil. MCHC: mean corpuscular hemoglobin concentration.

Table 4. Somatic indices (mean ± standard error of mean) of *C. macropomum* juveniles after 30 days of feeding with diets containing different concentrations of essential oil of *H. ringens* (HREO).

	HREO Inclusion (g kg of Feed^{-1})					
	$HR_{0.0}$	**$HR_{0.75}$**	**$HR_{1.0}$**	**$HR_{2.0}$**	***p*-Value**	**CV (%)**
Hepatosomatic index (%)	1.30 ± 0.10	1.35 ± 0.07	1.44 ± 0.09	1.51 ± 0.06	0.3360	21.41
Viscerosomatic index (%)	6.94 ± 0.41	6.90 ± 0.30	7.33 ± 0.31	7.63 ± 0.24	0.3483	15.72

The results showed no difference by ANOVA ($p > 0.05$).

3.2. Transport Experiment Results

The water parameters did not show significant differences ($p > 0.05$) between the different doses of HREO immediately after transport (Table 5).

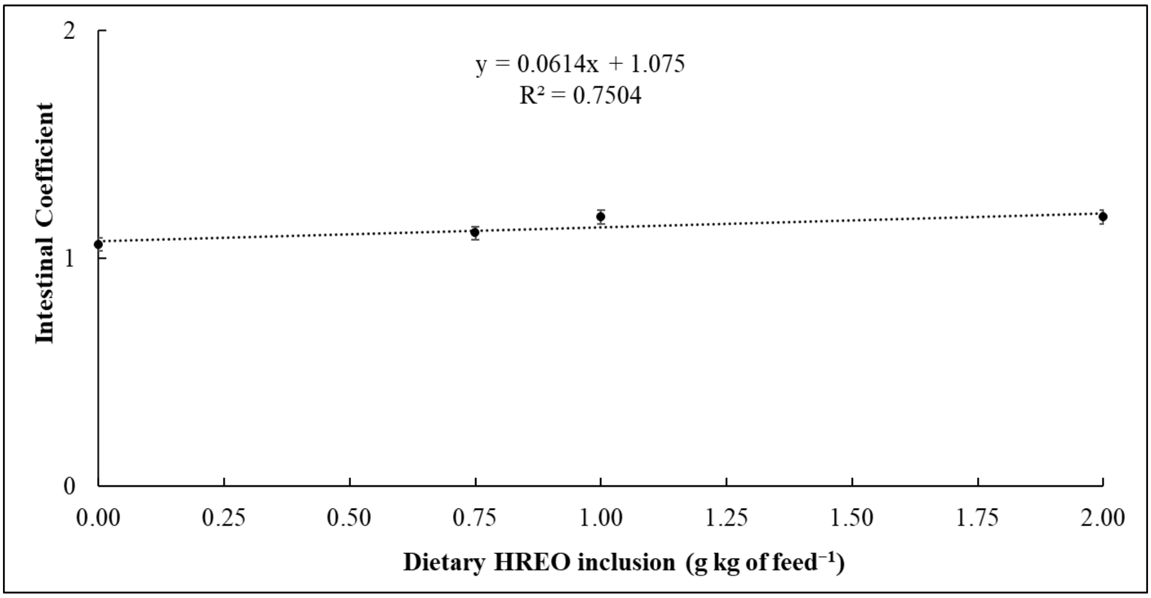

Figure 2. Intestinal coefficient (mean ± standard error of mean) of *C. macropomum* juveniles after 30 days of feeding with diets containing different concentrations of *H. ringens* essential oil.

Table 5. Water quality parameters (mean ± standard error of mean) immediately after simulated 4 h transport of *C. macropomum* juveniles after 30 days of feeding with diets containing different concentrations of essential oil of *H. ringens* (HREO).

	Temperature (°C)	**Dissolved Oxygen (mg L^{-1})**	**pH**	**Non-Ionized Ammonia (NH_3) (mg L^{-1})**
HREO inclusion (g kg of feed^{-1})				
$HR_{0.0}$	27.20 ± 0.34	7.55 ± 1.29	6.05 ± 0.18	0.020
$HR_{0.75}$	27.46 ± 0.08	7.91 ± 0.61	5.92 ± 0.02	0.020
$HR_{1.0}$	27.53 ± 0.03	7.38 ± 0.73	5.91 ± 0.01	0.020
$HR_{2.0}$	27.56 ± 0.03	6.48 ± 0.53	5.93 ± 0.01	0.020
p-value	0.1640	0.6837	0.6764	1.0000
CV (%)	0.81	18.64	2.50	0.00

The results showed no difference by ANOVA ($p > 0.05$).

Hemoglobin, hematocrit, erythrocyte count, MCV, MCH, and MCHC were all affected by the interaction between HREO concentrations and collection times ($p < 0.05$) (Table 6). At the $HR_{0.0}$ concentration, hemoglobin concentration was reduced at AT_{48} ($p < 0.05$). In the $HR_{0.75}$ group, transport caused a reduction at AT_{24}, with no recovery to basal levels ($p < 0.05$). For $HR_{1.0}$, hemoglobin values at AT_{24} and AT_{48} were lower than IAT but did not differ from basal ($p < 0.05$). In the $HR_{2.0}$ group, hemoglobin decreased at AT_{24} compared to IAT, but at AT_{48}, values did not differ from either basal or IAT ($p < 0.05$). No differences among HREO concentrations were observed at basal and IAT ($p > 0.05$). At AT_{24}, the highest hemoglobin concentration was recorded in $HR_{0.0}$, whereas the lowest values occurred in $HR_{0.75}$ and $HR_{2.0}$ ($p < 0.05$). At AT_{48}, the highest concentration was observed in fish fed $HR_{0.75}$ ($p < 0.05$). The concentrations $HR_{0.0}$, $HR_{1.0}$, and $HR_{2.0}$ provided more stability throughout the collection times, with hemoglobin levels not showing significant differences from basal values (Table 6).

Table 6. Interaction of factors for hematological parameters (mean ± standard error of mean) of *C. macropomum* juveniles subjected to simulated transport after 30 days of feeding with diets containing different doses of essential oil of *H. ringens* (HREO).

HREO Inclusion (g kg of Feed^{-1})	Collection Times (Hours)	Hemoglobin (g dL^{-1})	Hematocrit (%)	Erythrocytes ($\times 10^6$ µL^{-1})	MCV (fL)	MCH (pg)	MCHC (g dL^{-1})
$HR_{0.0}$	Basal	6.80 ± 0.38 ^{ABa}	24.90 ± 0.79 ^{ABb}	0.50 ± 0.05 ^{Ca}	547.72 ± 51.09 ^{Aa}	138.84 ± 13.42 ^{Aa}	27.20 ± 1.03 ^{Aa}
	IAT	7.64 ± 0.28 ^{Aa}	25.80 ± 0.64 ^{Aa}	0.41 ± 0.03 ^{Ca}	605.78 ± 42.79 ^{Aa}	165.16 ± 8.99 ^{Aa}	29.76 ± 1.29 ^{Aa}
	AT_{24}	7.78 ± 0.47 ^{Aa}	26.55 ± 0.70 ^{Aa}	1.29 ± 0.05 ^{Aa}	208.97 ± 11.52 ^{Cc}	61.47 ± 5.61 ^{Bc}	29.09 ± 1.44 ^{Ab}
	AT_{48}	5.92 ± 0.72 ^{Bb}	23.00 ± 1.25 ^{Bb}	0.89 ± 0.09 ^{Bb}	289.18 ± 17.96 ^{Ba}	67.74 ± 6.43 ^{Bab}	22.19 ± 1.57 ^{Bc}
$HR_{0.75}$	Basal	6.83 ± 0.11 ^{Ba}	27.20 ± 0.41 ^{Aa}	0.56 ± 0.08 ^{Ba}	558.38 ± 66.21 ^{Aa}	139.62 ± 15.86 ^{Aa}	25.16 ± 0.51 ^{Ca}
	IAT	7.68 ± 0.41 ^{ABa}	25.60 ± 0.70 ^{Aa}	0.54 ± 0.04 ^{Ba}	500.72 ± 44.20 ^{Aa}	140.93 ± 14.40 ^{Aa}	28.91 ± 1.38 ^{ABa}
	AT_{24}	5.41 ± 0.46 ^{Cb}	22.14 ± 1.18 ^{Bbc}	0.73 ± 0.12 ^{Bb}	229.89 ± 19.43 ^{Bbc}	68.56 ± 11.56 ^{Cbc}	25.47 ± 1.14 ^{BCb}
	AT_{48}	8.88 ± 0.56 ^{Aa}	25.70 ± 1.14 ^{Aa}	1.20 ± 0.09 ^{Aa}	226.09 ± 22.19 ^{Bb}	74.44 ± 1.80 ^{Ba}	31.79 ± 2.25 ^{Aa}
$HR_{1.0}$	Basal	7.19 ± 0.14 ^{ABa}	28.40 ± 0.54 ^{Aa}	0.60 ± 0.03 ^{Ba}	480.37 ± 27.04 ^{Aa}	131.39 ± 11.52 ^{Aa}	25.47 ± 0.73 ^{Ba}
	IAT	7.97 ± 0.19 ^{Aa}	24.50 ± 0.65 ^{Ba}	0.59 ± 0.07 ^{Ba}	455.43 ± 52.64 ^{Aa}	131.40 ± 14.82 ^{Aa}	32.56 ± 1.40 ^{Aa}
	AT_{24}	6.60 ± 0.38 ^{Bab}	23.87 ± 0.83 ^{Bb}	0.70 ± 0.08 ^{Bb}	318.71 ± 26.50 ^{Ba}	96.06 ± 9.76 ^{Ba}	28.13 ± 0.87 ^{Bb}
	AT_{48}	6.68 ± 0.48 ^{Bb}	24.37 ± 1.25 ^{Bab}	1.09 ± 0.10 ^{Aa}	226.11 ± 22.00 ^{Cb}	63.57 ± 5.28 ^{Cbc}	28.04 ± 0.69 ^{Bb}
$HR_{2.0}$	Basal	6.58 ± 0.30 ^{ABa}	24.37 ± 0.82 ^{Ab}	0.47 ± 0.04 ^{Ca}	492.84 ± 46.22 ^{Aa}	131.40 ± 12.44 ^{Aa}	28.61 ± 1.83 ^{BCa}
	IAT	7.59 ± 0.56 ^{Aa}	25.44 ± 0.85 ^{Aa}	0.50 ± 0.01 ^{Ca}	498.38 ± 30.42 ^{Aa}	161.30 ± 14.02 ^{Aa}	31.46 ± 1.69 ^{Ba}
	AT_{24}	5.75 ± 0.68 ^{Bb}	20.42 ± 0.99 ^{Bc}	0.78 ± 0.06 ^{Bb}	301.87 ± 57.36 ^{Bab}	73.25 ± 6.42 ^{Bb}	41.54 ± 2.15 ^{Aa}
	AT_{48}	6.70 ± 0.32 ^{ABb}	23.90 ± 0.86 ^{Aab}	1.26 ± 0.05 ^{Aa}	190.69 ± 6.85 ^{Cb}	53.09 ± 1.48 ^{Cc}	28.03 ± 0.92 ^{Cb}

Different capital letters indicate differences between collection times within a given HREO dose by Tukey's test ($p < 0.05$). Different lowercase letters indicate differences between the same collection time at different HREO doses by Tukey's test ($p < 0.05$). IAT—immediately after transport. Sample size = 10 fishes per treatment in each collection time.

The highest hematocrit values at the $HR_{0.0}$ dose were found at IAT and AT_{24} and the lowest at AT_{48} ($p < 0.05$) (Table 6). Transport caused a decrease in hematocrit at AT_{24}, but with recovery of the basal level at AT_{48} at the doses of $H_{0.75}$ and $HR_{2.0}$. There was a reduction in hematocrit at IAT, without reestablishment of the initial basal condition at the other collection times at the $HR_{1.0}$ dose ($p < 0.05$). When comparing the same collection time for the different HREO doses, basal had the highest hematocrit value at $HR_{0.75}$ and $HR_{1.0}$ and the lowest at $HR_{0.0}$ and $HR_{2.0}$ ($p < 0.05$). There was no difference between the concentrations for IAT ($p > 0.05$). The highest hematocrit value was at $HR_{0.0}$ and the lowest at $HR_{2.0}$ at AT_{24} ($p < 0.05$). In AT_{48}, the highest value was found at $HR_{0.75}$ and the lowest at $HR_{0.0}$ ($p < 0.05$). In 48 h, the animals of the treatment $HR_{1.0}$ were not able to make necessary physiological adjustments to return to basal conditions.

The number of erythrocytes increased in fish fed $HR_{0.0}$ at AT_{24}, without recovery to the basal condition at AT_{48} ($p < 0.05$). In fish fed $HR_{0.75}$ and $HR_{1.0}$, the highest value was observed at AT_{48}, with the other times being similar to each other ($p < 0.05$) (Table 6). At these concentrations, the animal's response to increase circulating erythrocytes was delayed when compared to the $HR_{0.0}$ and $HR_{2.0}$ treatments. At the $HR_{2.0}$ dose, there was an increase in values at AT_{24}, with the peak of the response observed at AT_{48} ($p < 0.05$). There was no difference between the doses at the basal and IAT collection times ($p > 0.05$). At AT_{24}, the highest value was in fish fed $HR_{0.0}$ ($p < 0.05$). At AT_{48}, the lowest value was found at $HR_{0.0}$ ($p < 0.05$).

The MCV in fish fed the $HR_{0.0}$ dose presented the lowest value at AT_{24}, with no recovery of the basal level at AT_{48} ($p < 0.05$) (Table 6). There was a reduction in the values at AT_{24} for $HR_{0.75}$, remaining the same at AT_{48} ($p < 0.05$). There was also a reduction in the values for $HR_{1.0}$ and $HR_{2.0}$ at AT_{24}, with the lowest value observed at AT_{48} ($p < 0.05$). At the basal and IAT collection times, there was no difference between the HREO doses ($p > 0.05$). For the AT_{24} time, the highest value was found at $HR_{1.0}$ and the lowest at $HR_{0.0}$ ($p < 0.05$). At this time, the $HR_{0.0}$ concentration showed a more abrupt decrease in this parameter when compared to the treatments containing HREO. At AT_{48}, the highest value was at $HR_{0.0}$, with the other doses being similar to each other ($p < 0.05$).

There was a reduction in the MCH values for fish fed at $HR_{0.0}$ at AT_{24} and AT_{48}, with no recovery to the basal level ($p < 0.05$) (Table 6). Transport resulted in a lower MCH value for $HR_{0.75}$ at AT_{24}, followed by an increase at AT_{48}, but was not able to reestablish the initial condition ($p < 0.05$). There was also a reduction in values for $HR_{1.0}$ and $HR_{2.0}$ at AT24, but the lowest response was observed at AT_{48} ($p < 0.05$). At basal and IAT times, there was no difference between the HREO doses ($p > 0.05$). At AT_{24}, the highest value was at $HR_{1.0}$ and the lowest at $HR_{0.0}$, while at AT_{48}, the highest value was found at $HR_{0.75}$ and the lowest at $HR_{2.0}$ ($p < 0.05$).

The MCHC values of fish fed $HR_{0.0}$ remained stable until AT_{24}, but at AT_{48}, there was a reduction in values ($p < 0.05$) (Table 6). At $HR_{0.75}$, the highest value was at AT_{48} and the lowest at basal ($p < 0.05$). For $HR_{1.0}$, the highest value was observed in IAT with recovery to basal levels at AT_{24} and remaining stable at AT_{48} ($p < 0.05$). The values of fish fed $HR_{2.0}$ remained stable in IAT compared to basal ($p > 0.05$); however, in AT_{24}, there was an increase in values ($p < 0.05$), followed by the reestablishment of the initial condition in AT_{48} ($p > 0.05$). In basal and IAT times, there was no difference between the doses ($p > 0.05$). For AT_{24}, the highest value was observed in $HR_{2.0}$ ($p < 0.05$). In AT_{48}, the highest value was in $HR_{0.75}$ and the lowest in $HR_{0.0}$ ($p < 0.05$).

Only the effect of collecting time affected blood glucose, with the highest value for IAT and the lowest for AT_{24} and AT_{48} ($p < 0.05$) (Table 7). Total protein and triglycerides showed an effect of concentration, collection time, and interaction of the factors ($p < 0.05$). There was an effect of collecting time and interaction between the factors for cholesterol, ALT, and AST ($p < 0.05$).

Table 7. Blood biochemical parameters (mean ± standard error of mean) of *C. macropomum* juveniles subjected to simulated transport after 30 days of feeding with diets containing different doses of essential oil of *H. ringens* (HREO).

	Total Proteins (g dL^{-1})	Glucose (mg dL^{-1})	Triglycerides (mg dL^{-1})	Cholesterol (mg dL^{-1})	ALT (U L^{-1})	AST (U L^{-1})
HREO inclusion (g kg of feed^{-1})						
$HR_{0.0}$	4.09 ± 0.08 ^{a}	67.16 ± 3.32	136.48 ± 5.45 ^{ab}	79.00 ± 2.16	7.93 ± 0.57	188.37 ± 9.87
$HR_{0.75}$	4.08 ± 0.08 ^{a}	69.99 ± 3.87	145.39 ± 6.82 ^{a}	81.08 ± 2.72	8.41 ± 0.71	199.14 ± 10.18
$HR_{1.0}$	3.92 ± 0.07 ^{ab}	66.26 ± 3.61	134.00 ± 5.88 ^{ab}	79.61 ± 2.29	7.17 ± 0.43	187.91 ± 8.85
$HR_{2.0}$	3.78 ± 0.07 ^{b}	63.44 ± 3.64	129.64 ± 6.19 ^{b}	79.09 ± 2.48	7.38 ± 0.53	209.48 ± 13.22
Collecting times (hours)						
Basal	4.21 ± 0.04 ^{a}	65.70 ± 1.77 ^{b}	189.45 ± 5.59 ^{a}	77.16 ± 2.29 ^{b}	6.92 ± 0.35 ^{bc}	193.77 ± 10.94 ^{ab}
IAT	4.12 ± 0.05 ^{a}	96.85 ± 2.21 ^{a}	130.59 ± 3.23 ^{b}	86.47 ± 1.26 ^{a}	5.97 ± 0.33 ^{c}	168.65 ± 7.53 ^{b}
AT_{24}	3.81 ± 0.11 ^{b}	53.35 ± 2.37 ^{c}	119.71 ± 2.83 ^{b}	74.72 ± 2.69 ^{b}	9.73 ± 0.83 ^{a}	224.10 ± 11.10 ^{a}
AT_{48}	3.73 ± 0.08 ^{b}	50.94 ± 2.15 ^{c}	105.77 ± 2.74 ^{c}	80.43 ± 2.72 ^{ab}	8.27 ± 0.56 ^{ab}	198.38 ± 11.54 ^{ab}
p-value HREO inclusion	0.0038	0.1836	0.0119	0.8954	0.6347	0.8305
p-value Collecting times	<0.0001	<0.0001	<0.0001	0.0011	<0.0001	0.0035
p-value Interaction	<0.0001	0.1770	0.0086	<0.0001	0.0078	0.0036
CV (%)	12.81	34.08	28.23	18.97	18.52	16.62

The ALT and AST variables were transformed using the inverse square root function (expressed by the function: $X = 1/\sqrt{x}$). Means followed by different letters in the same column indicate differences by Tukey's test ($p < 0.05$). IAT—immediately after transport. Sample size = 10 fishes per treatment in each collection time.

The transport caused a reduction in plasma protein levels at $HR_{0.0}$ and $HR_{1.0}$, with the lowest values observed at AT_{48} ($p < 0.05$). At $HR_{0.75}$ and $HR_{2.0}$, there was a reduction in values only at AT_{24} ($p < 0.05$), reestablishing the initial condition at AT_{48} ($p > 0.05$); these concentrations supported the return to homeostasis. When comparing the same collection times for the different doses of HREO, basal and IAT showed no differences between the different doses of HREO ($p > 0.05$). The highest value of total proteins was observed at $HR_{0.0}$ and the lowest at $HR_{0.75}$ and $HR_{2.0}$ at AT_{24} ($p < 0.05$). For AT_{48}, the highest value was at $HR_{0.75}$, with the other doses of HREO being similar to each other ($p < 0.05$) (Table 8).

Table 8. Interaction of factors for blood biochemical parameters (mean ± standard error of mean) of *C. macropomum* juveniles subjected to simulated transport after 30 days of feeding with diets containing different doses of essential oil of *H. ringens* (HREO).

HREO Inclusion (g kg of feed^{-1})	Collection Times (Hours)	Total Proteins (g dL^{-1})	Triglycerides (mg dL^{-1})	Cholesterol (mg dL^{-1})	ALT (U L^{-1})	AST (U L^{-1})
$HR_{0.0}$	Basal	4.24 ± 0.08 ^{Aa}	175.59 ± 10.82 ^{Ab}	70.79 ± 3.74 ^{Ca}	5.80 ± 0.57 ^{Ba}	170.80 ± 13.03 ^{Bb}
	IAT	4.19 ± 0.06 ^{Aa}	145.30 ± 6.09 ^{Ba}	82.84 ± 2.06 ^{ABa}	5.90 ± 0.84 ^{Ba}	151.80 ± 10.89 ^{Ba}
	AT_{24}	4.48 ± 0.10 ^{Aa}	126.15 ± 4.41 ^{Cab}	89.59 ± 1.77 ^{Aa}	8.62 ± 0.62 ^{Aa}	198.67 ± 21.73 ^{Aba}
	AT_{48}	3.43 ± 0.21 ^{Bb}	98.88 ± 4.80 ^{Db}	72.79 ± 5.85 ^{BCb}	11.40 ± 1.23 ^{Aa}	232.22 ± 24.31 ^{Aa}
$HR_{0.75}$	Basal	4.42 ± 0.05 ^{Aa}	208.79 ± 12.47 ^{Aa}	77.58 ± 5.08 ^{Ba}	7.00 ± 0.47 ^{ABa}	187.78 ± 12.81 ^{ABab}
	IAT	4.18 ± 0.12 ^{Aa}	135.90 ± 6.16 ^{Bab}	88.55 ± 2.05 ^{ABa}	5.50 ± 0.37 ^{Ba}	164.00 ± 16.27 ^{Ba}
	AT_{24}	3.46 ± 0.23 ^{Bc}	116.01 ± 6.50 ^{Cab}	63.94 ± 5.12 ^{Cb}	12.57 ± 2.77 ^{Aa}	230.78 ± 22.40 ^{Aa}
	AT_{48}	4.27 ± 0.11 ^{Aa}	120.86 ± 4.97 ^{BCa}	94.24 ± 3.54 ^{Aa}	8.60 ± 1.05 ^{Aab}	214.00 ± 23.40 ^{ABa}
$HR_{1.0}$	Basal	4.11 ± 0.07 ^{Aa}	185.36 ± 8.09 ^{Ab}	79.55 ± 4.40 ^{ABa}	6.90 ± 0.58 ^{ABa}	169.90 ± 17.63 ^{Ab}
	IAT	4.15 ± 0.07 ^{Aa}	121.53 ± 5.67 ^{Bb}	86.35 ± 1.32 ^{Aa}	5.60 ± 0.22 ^{Ba}	168.20 ± 10.10 ^{Aa}
	AT_{24}	3.95 ± 0.20 ^{Ab}	128.82 ± 6.01 ^{Ba}	73.45 ± 4.62 ^{Bb}	9.11 ± 1.37 ^{Aa}	214.25 ± 20.54 ^{Aa}
	AT_{48}	3.50 ± 0.13 ^{Bb}	100.31 ± 5.18 ^{Cb}	79.09 ± 6.26 ^{ABb}	7.10 ± 0.72 ^{ABbc}	199.30 ± 19.84 ^{Aa}
$HR_{2.0}$	Basal	4.07 ± 0.13 ^{Aa}	188.07 ± 12.81 ^{Ab}	80.72 ± 5.16 ^{ABa}	8.00 ± 1.08 ^{Aa}	246.60 ± 30.38 ^{Aa}
	IAT	3.99 ± 0.12 ^{Aa}	119.64 ± 4.94 ^{Bb}	88.13 ± 3.88 ^{Aa}	6.90 ± 0.92 ^{Aa}	190.60 ± 20.27 ^{Aa}
	AT_{24}	3.34 ± 0.14 ^{Bc}	107.86 ± 3.19 ^{Bb}	71.90 ± 6.01 ^{Bb}	8.62 ± 1.54 ^{Aa}	252.70 ± 22.73 ^{Aa}
	AT_{48}	3.73 ± 0.11 ^{ABb}	103.01 ± 4.62 ^{Bab}	75.61 ± 3.50 ^{Bb}	6.00 ± 0.71 ^{Ac}	148.00 ± 18.92 ^{Bb}

Different capital letters indicate differences between collection times within a single HREO dose by Tukey's test ($p < 0.05$). Different lowercase letters indicate differences between the same collection time at different HREO doses by Tukey's test ($p < 0.05$). IAT: immediately after transport. Sample size = 10 fishes per treatment in each collection time.

At the $HR_{0.0}$ dose, the lowest triglyceride value was observed at AT_{48} ($p < 0.05$). Transportation also caused a reduction in triglyceride levels in fish fed $HR_{0.75}$, with the lowest level observed at AT_{24}, without recovery to basal conditions ($p < 0.05$). For $HR_{1.0}$, there was also a reduction in levels after transportation, with the lowest value observed at AT_{48} ($p < 0.05$). For $HR_{2.0}$, transportation resulted in a decrease in levels at IAT, which remained the same until AT_{48} ($p < 0.05$), indicating a more stabilized response in this treatment. The highest value found at basal was $HR_{0.75}$ ($p < 0.05$). For IAT, the highest value was at $HR_{0.0}$ and the lowest at $HR_{1.0}$ and $HR_{2.0}$ ($p < 0.05$). The highest value at AT_{24} was found at $HR_{1.0}$ and the lowest at $HR_{2.0}$ ($p < 0.05$). The highest triglyceride value at AT_{48} was found at $HR_{0.75}$ and the lowest at $HR_{0.0}$ and $HR_{1.0}$ ($p < 0.05$) (Table 8).

Transport triggered an increase in cholesterol levels for fish fed $HR_{0.0}$ at IAT and AT_{24} ($p < 0.05$), returning to the initial condition at AT_{48} ($p > 0.05$). There was a reduction in cholesterol levels for $HR_{0.75}$ at AT_{24} ($p < 0.05$), with no recovery to basal values at AT_{48} ($p < 0.05$). There was a reduction in levels for $HR_{1.0}$ at AT_{24} compared to IAT, but when compared to baseline, all times were similar ($p > 0.05$). There was a similar response for $HR_{2.0}$, with a reduction in levels at AT_{24} and AT_{48} compared to IAT ($p < 0.05$), but all times were similar to baseline ($p > 0.05$). No significant difference was identified between the doses at basal and IAT ($p > 0.05$), but at AT_{24}, the highest value was recorded for $HR_{0.0}$, while at AT_{48}, the highest value was for $HR_{0.75}$ ($p < 0.05$). Fish fed $HR_{1.0}$ and $HR_{2.0}$ showed fluctuations in cholesterol levels, but the values did not differ from the basal levels (Table 8).

There was an increase in ALT levels for $HR_{0.0}$ at AT_{24} and AT_{48} compared to baseline and IAT ($p < 0.05$). Transport caused an increase in ALT levels for $HR_{0.75}$ at AT_{24} compared to IAT ($p < 0.05$). There was an increase in ALT levels for $HR_{1.0}$ at AT_{24} compared to IAT, but AT_{48} was similar to all sampling times ($p < 0.05$). There was no difference between the times analyzed at the $HR_{2.0}$ dose ($p > 0.05$), indicating that this treatment maintained consistent values during the collection period. The basal, IAT, and AT_{24} times did not show any difference between the HREO doses ($p > 0.05$). At AT_{48}, the highest ALT value was found in $HR_{0.0}$ and the lowest in $HR_{2.0}$ ($p < 0.05$) (Table 8).

There was an increase in AST levels for $HR_{0.0}$ at AT_{24} and AT_{48} compared to baseline and IAT ($p < 0.05$). There was an increase in AST levels for $HR_{0.75}$ at AT_{24} compared to IAT, with recovery to basal values at AT_{48} ($p < 0.05$). There was no difference between the times analyzed at the $HR_{1.0}$ dose ($p > 0.05$). Transportation caused a reduction in AST levels for $HR_{2.0}$ at AT_{48} ($p < 0.05$). At baseline, $HR_{2.0}$ showed the highest AST value compared to $HR_{0.0}$ and $HR_{1.0}$ ($p < 0.05$). IAT and AT_{24} times did not show any difference between the HREO doses ($p > 0.05$). At AT_{48}, the lowest value was found at $HR_{2.0}$ ($p < 0.05$) (Table 8).

4. Discussion

The present study evaluated for the first time dietary supplementation with HREO for *C. macropomum* in the pre-transport period. Although the different inclusion levels did not influence growth, survival, and most hematological and biochemical parameters, differences were observed for hemoglobin, MCHC, and intestinal coefficient. However, after transport, different physiological responses were observed between HREO doses.

4.1. Pre-Transport Period Performance

During the feeding period, the different doses of HREO did not affect survival, with values above 96%. Similar results were found with survival above 98% in *C. macropomum* fed diets containing different doses of the EO from *Lippia sidoides* [52]. The same result was observed with survival above 98% in juvenile *C. macropomum* fed diets containing the EO from ginger (*Zingiber officinale*) [47]. Dietary supplementation with three EOs (*L. grata*, *L. origanoides*, and *Ocimum gratissimum*) did not induce any mortality for *C. macropomum* [53]. The authors also obtained 100% survival for *C. macropomum* fed EO from *Croton conduplicatus* [54]. Considering the results obtained, the incorporation of small doses of EOs into the diet of *C. macropomum* for up to 30 days does not seem to affect animal survival.

Supplementation with different doses of HREO also did not influence the evaluated zootechnical performance parameters in *C. macropomum*. This response corroborates previous findings [47], which also showed no growth differences in the same species fed with EO from *Z. officinale*. Common carp (*Cyprinus carpio*) fed with menthol for 30 days showed no increase in growth [55]. However, some EOs improved fish growth, such as 0.5 mL EO *Nectandra grandiflora* kg feed^{-1} for 30 days in *C. macropomum* [46]. Similarly, positive effects were observed on the performance of *Oncorhynchus mykiss* fed diets supplemented with EO from *Myrtus communis* and *Satureja khuzistanica* [56], and the EO from *Mentha piperita* increased the growth of *Rutilus frisii* [57]. The variability in the observed responses may be due to factors such as physiological differences between the species studied, doses used, and the specific chemical composition of each EO, suggesting that the efficacy of EOs as growth promoters depends on the species and composition of the EO used.

The FCR observed in this study remained between 1.04 and 1.73 during the experimental period. In *O. niloticus* (average weight of 3.04 g), higher FCR values (1.75 and 1.89) were observed using EOs from *Cymbopogon citratus* and *Pelargonium graveolens* [58]. However, the values decreased proportionally to the increase in EO incorporated. In studies with *C. macropomum* (average weight of 24.16 g), researchers reported FCR values between 1.28 and 1.38 when using *Z. officinale* [47], similar to those found in the present study. On the other hand, working with larger *C. macropomum* (173.5 g) and different EOs (*L. grata*, *L. origanoides*, and *O. gratissimum*) resulted in higher FCR values (1.89–2.86) [53]. These results may indicate that the variation in FCR can be attributed to factors such as the concentration and chemical composition of EO, as well as the different developmental stages of the fish as recorded for *C. macropomum* fed with *N. grandiflora* [46].

Dietary treatments with HREO did not result in significant differences in hematocrit, erythrocyte, MCV, MCH, total proteins, glucose, cholesterol, triglycerides, ALT, or AST. However, changes in hemoglobin and MCHC levels were observed. Hemoglobin was directly related to the increase in HREO in the diet, corroborating previous findings of increased hemoglobin in juvenile *C. macropomum* in response to the increase in dietary *Z. officinale* EO [47]. Similarly, higher hemoglobin values were observed in treatments containing EO from *Allium sativum* compared to the control group in Asian sea bass (*Lates calcarifer*) [59]. Recent findings [54] reported increased hemoglobin and MCHC in *C. macropomum* in the treatment containing 1.0 mL *C. conduplicatus* EO kg feed^{-1}, although this was not the highest dose tested. Regarding MCHC, a quadratic response was observed with a lower value estimated by the derivative of the equation at 0.90 g kg feed^{-1} of HREO. This result is similar to previous observations [60] of lower MCHC values at intermediate doses of *Z. officinale* (5.0 g kg feed^{-1}) and *O. gratissimum* (5.0 and 10.0 g kg feed^{-1}) EOs in Nile tilapia (*O. niloticus*), although without changes in hemoglobin values. Different responses were previously reported [61], with no differences found for hemoglobin and MCHC when testing different concentrations of *O. basilicum* EO in *O. niloticus* diet. Similarly, no effects of dietary *M. piperita* EO on MCHC were detected in *C. macropomum* [62]. The increase in hemoglobin and MCHC associated with the elevation of HREO suggests a potential improvement in oxygen transport capacity, considering that these parameters are directly responsible for the transport of this molecule. These results indicate that HREO may exert a positive influence on these specific hematological parameters, although its mechanism of action has to be clarified.

HSI and VSI did not show differences between treatments, suggesting that the inclusion of HREO in the diets did not interfere with the development of the liver and viscera. Similar results were reported [63], with no changes in HSI observed in *Sciaenops ocellatus* fed different doses of *O. americanum* EO. Similarly, no differences in HSI and VSI were found [64] in *O. mykiss* fed diets supplemented with laurel seed (*Laurus nobilis*) EO. The absence of variations in VSI and HSI observed in the present study suggests that regardless of the doses of HREO used in the diet of *C. macropomum*, they provided adequate conditions for the metabolism of the animals, without causing impairment of liver functions or changes in the visceral proportion. Even though pulegone is recognized as a hepatotoxic compound that acts through glutathione depletion, potentially causing hepatomegaly and alterations in hepatic perfusion [65], liver size was not altered in the present study.

In contrast, the IC was directly related to the increase in HREO in the diet. Consequently, there is evidence that this EO may be able to increase the absorption and assimilation of nutrients as observed in *Brycon amazonicus* fed diets supplemented with *Minthostachys mollis* EO [66], since a more developed intestine has a larger absorption surface area, in agreement with that recorded in *C. macropomum* [52] and other species such as *C. carpio* [67,68] and *O. niloticus* [69–71].

4.2. Transport Experiment Discussion

Handling during fish transportation represents one of the most critical moments in aquaculture, being recognized as an important stressor capable of triggering several physiological changes [15,72]. In the present study, no mortality was recorded during the experiment. However, changes were recorded in hematological and biochemical parameters that may reflect an adaptive response of *C. macropomum* juveniles to transportation stress when fed with different doses of HREO. The evaluation of the physical and chemical indicators of the water is a fundamental aspect to ensure the effectiveness of fish transportation [72]. The water quality parameters remained within the optimal levels for *C. macropomum* [73].

Hemoglobin was higher and similar to basal at IAT for $HR_{0.0}$, $HR_{1.0}$, and $HR_{2.0}$, indicating a physiological imbalance in response to transport, probably associated with greater oxygen demand during this period [74,75]. This initial increase reflects the attempt to meet the high metabolic needs, aiming to restore physiological balance and ensure adequate oxygen supply to the organs [76]. At the same doses at AT_{48}, the values are like basal levels. However, the contrary was observed in *C. macropomum* supplemented with β-glucan and transported for 3 h, which showed a tendency to return to basal values after 24 h [77]; in the present study, with transport for 4 h, hemoglobin levels decreased at AT_{24} in fish fed $HR_{0.75}$, remaining below the basal value. This reduction in hemoglobin concentration associated with the decrease in MCV may reflect the osmoregulatory imbalance caused by transport, while the increase at AT_{48} may be related to the increase in the number of erythrocytes.

There was a reduction in hematocrit at AT_{24} in the $HR_{0.75}$ and $HR_{2.0}$ treatments. Lower hematocrit values after transportation were also found [78] in *B. amazonicus*. The decrease in hematocrit may be related to hemodilution, a phenomenon also described in previous studies [79] with tambacus (*C. macropomum* × *Piaractus mesopotamicus*) exposed to stress from repetitive fishing. This response may be a mechanism to regulate blood viscosity and maintain adequate flow in the tissues in the face of stress. The same may have occurred in the $HR_{1.0}$ treatment, since after transportation, at all collection times, hematocrit values were below basal values. However, at the $HR_{0.75}$ and $HR_{2.0}$ doses, at AT_{48}, hematocrit values returned to basal values. This response may indicate that these doses of HREO may have had a beneficial effect, helping to return to homeostasis. Since there are still no specific studies on the incorporation of HREO in the diet for comparison with the data from the present study, the variations observed in the hematocrit results may be attributed to the different types of EO used and their doses and the different species of fish evaluated.

The increase in the number of erythrocytes was more significant at AT_{48} in treatments with HREO. This result corroborates the study [80], where there was an increase in erythrocytes after the use of *Aloysia triphylla* EO in *O. niloticus* for 45 days. This increase may be due to the release of reserve cells from the spleen or the increased production of new erythrocytes to improve oxygen transport capacity [81,82]. These results suggest a compensatory mechanism, since hemoglobin levels decreased across all treatments during the collection times, despite the differences already discussed. In the $HR_{0.0}$ group, however, erythrocyte values increased at AT_{24} but decreased at AT_{48}, without returning to basal levels. This oscillation may indicate physiological adjustments in search of a return to homeostasis.

The hematometric indices (MCV, MCH, and MCHC) revealed variations that evidence adaptations in the morphology and function of erythrocytes during the transport. The reduction in MCV and MCH observed at AT_{24} and AT_{48} at all HREO concentrations and in the control treatment corroborates previous studies [83] with *Mugil cephalus*, which identified a decrease in MCV and MCH, associated with high levels of erythrocytes and hematocrit. This change reflects the influence of environmental conditions on blood parameters, suggesting that fish develop adaptive physiological responses to maintain homeostasis during stressful situations, such as transport, which can compromise their hematological integrity [84]. An increase in MCHC at IAT and a return to basal values at AT_{24} and AT_{48} was observed in fish fed $HR_{1.0}$. At the $HR_{2.0}$ concentration, the increase was recorded at AT_{24}, along with the tendency to return to basal values at AT_{48}. In contrast, no differences in MCHC values were recorded [85] for *O. niloticus* fed with *M. piperita*. These results may indicate that HREO and higher levels of it favored a faster return to homeostasis. The increase in this parameter demonstrates the need for physiological adjustment to improve oxygen circulation in the body in the face of transport [53].

Glucose showed differences only for collection times. The transport process may be responsible for inducing gluconeogenesis and glycogenolysis [72]. In the present study, an increase in plasma glucose was observed at IAT. This increase may be the organism's initial response to short-term acute stress, in the search for energy for the animal's "fight or flight" reaction [86]. A decrease in plasma glucose was observed at AT_{24} and AT_{48}. The decrease in glycemia may occur when there is a greater demand for energy to maintain biochemical and blood homeostasis [54]. This behavior was also observed [87] in *O. mossambicus* fed with *Citrus sinensis* when exposed to *Streptococcus iniae*. Similar responses were found [88] using *Thymus vulgaris* and *Foeniculum vulgare* EOs in *O. mykiss* exposed to *Yersinia ruckeri*. Furthermore, according to the authors cited above, this response can be attributed to the ability of EO to reduce the effects of stress.

Variations in total proteins revealed important changes at different times and concentrations of HREO. Total plasma protein is considered an essential component of nonspecific immunity [89]. Changes in plasma volume, especially under stress conditions, can modify total protein levels in plasma [90]. The doses of $HR_{0.75}$ and $HR_{2.0}$ demonstrated greater recovery capacity at AT_{48}, with a return to basal values. These results indicate the potential of these doses of HREO to perform a more efficient modulation of stress processes. Protein values decreased at AT_{48} in fish fed $HR_{0.0}$ and $HR_{1.0}$, demonstrating that these treatments would possibly require more time for the animals to recover. The authors of [85] recorded higher averages of total plasma proteins in *O. niloticus* fed with *M. x piperita* compared to the treatment with the control diet (diet + alcohol) when exposed to *S. agalactiae*. The decrease in plasma protein concentration may be associated with the hemodilution effect, resulting from the ionic disturbance due to dealing with the changes caused by transport [91].

There was a reduction in plasma triglyceride levels over time for all treatments. There was a tendency for recovery at AT_{48} for the $HR_{0.75}$ dose, while at the other doses, values did not show signs of returning to basal values. Unlike triglycerides, cholesterol levels showed more variable behavior. An initial increasing tendency was observed in most doses, followed by oscillations. Similar behavior was described before [92] in a study with *Z. officinale* EO incorporated into the diet of *O. mossambicus*, as well using *C. sinensis* EO for *O. mossambicus* [87], which revealed that the use of EO tended to reduce triglyceride and cholesterol levels in the fish studied. Similarly, a reduction in plasma cholesterol levels in *O. niloticus* when *Z. officinale* EO was added to the diet was observed [93]. Increased gill permeability can modify plasma volume, resulting in changes in total protein concentration [91]. These responses involve the degradation of body reserves, including liver glycogen, muscle lipids, and proteins [94]. As a direct consequence of these metabolic processes, biochemical markers such as cholesterol and triglycerides can present significant variations, being considered important physiological indicators of stress in fish [95].

The results of this study revealed a dynamic profile of plasma ALT levels in *C. macropomum* juveniles subjected to transport. At AT_{48}, the levels of this parameter tended to decrease, suggesting metabolic adaptation of the fish to the initial stress of transport. There was an increase in the levels of this enzyme at AT_{24} and AT_{48} in fish fed $HR_{0.0}$ and $HR_{0.75}$, which may indicate liver damage. There was a tendency to return to basal levels at AT_{48} at the $HR_{1.0}$ dose, and at $HR_{2.0}$, no differences were observed in ALT levels regardless of the collection time. In a study with *O. niloticus* fed diets containing *A. sativum* [96], a decrease in serum levels of plasma ALT was reported. In contrast, researchers [97] stated that the increase in plasma ALT indicates organ dysfunction, corroborating results of higher levels of ALT in *Oplegnathus punctatus* when subjected to transport [98], in addition to finding that this activity is responsible for affecting liver functions through changes in the structure of hepatocytes and inducing oxidative damage. This response indicates an interaction between transport and EO supplementation, demonstrating physiological adaptation of juveniles to stress. Based on these results, it is assumed that higher levels of HREO in the diet over the 30 days of feeding promoted greater liver protection in animals when subjected to transport. Therefore, it is likely that the 30-day feeding period with HREO was not long enough for potential hepatotoxic effects of pulegone to manifest as well as the use of low dietary inclusion levels ($\leq$2 g HREO kg feed^{-1}).

5. Conclusions

Dietary supplementation with *H. ringens* essential oil did not affect the growth or survival of juvenile *C. macropomum* during the 30-day pre-transport period, although higher IC, hemoglobin, and MCHC values were observed. Nevertheless, supplementation at 2.0 g HREO kg^{-1} feed improved the recovery of key hematological and biochemical parameters after transport, indicating enhanced resilience. These results highlight the potential of HREO as a nutritional strategy to support fish performance during transport in aquaculture. Further studies are recommended to validate these findings under commercial transport conditions and to include endocrine biomarkers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes10100532/s1. Table S1: Data from some of the feed ingredients (according to manufacturers).

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Data Availability Statement: The data supporting the results of this study are available from the author Thamara Bentivole Magalhães upon reasonable request.

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