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Growth Performance and Histomorphology of Intestine, Skin, Gills and Liver of Juvenile *Colossoma macropomum* Fed Diets Containing Different Levels of the Essential Oil of *Nectandra grandiflora*

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Abstract: The present study evaluated different levels of the essential oil of *Nectandra grandiflora* (EONG) in the diet of juvenile *Colossoma macropomum*. The juveniles (0.75 ± 0.05 g) were fed four experimental diets with differing levels of EONG (0, 0.5, 0.75, and 1.50 mL/kg). After 20 days, the weight, daily weight gain, specific growth rate, and daily feed consumption per fish were highest for the fish fed 0.5 mL EONG/kg, while after 30 days, only the weight and daily feed consumption per fish for that diet remained highest. The viscerosomatic index was highest for the fish fed 0.5 mL EONG/kg while the hepatosomatic index was higher for the groups that received dietary EONG for 30 days. The juveniles fed 1.5 mL EONG/kg showed a proliferative response of the mucous cells in the gills, but the proliferation of these lysozyme- and immunoglobulin-secreting mucosal cells was higher for the skin of the fish of all EONG levels, compared to that of the control fish. The fish fed 0.5 mL EONG/kg had the greatest height and width of intestinal villi. The two highest levels of dietary EONG supplementation (0.75 and 1.50 mL/kg) reduced the hepatocyte dimensions but did not modify the centrolobular vein area. Dietary supplementation with 0.5 mL EONG/kg improved the growth, gut health, and immune response of juvenile *C. macropomum*.

Keywords: phytogenic additive; growth promoter; tambaqui; immune modulation

Key Contribution: Dietary supplementation with 0.5 mL EONG/kg substantially enhanced the growth, gut health, and immune response of juvenile *C. macropomum*. Increased mucous cell proliferation in gills and improved development of intestinal villi were also observed for the fish fed this EONG level, suggesting potential advantages for the aquaculture industry.

1. Introduction

Studies with essential oils (EOs) of plant origin have shown that their main components have a wide spectrum of biological activity in various fields, from food chemistry

to pharmacology and pharmaceuticals [1]. EOs are a mixture of secondary compounds comprising active substances biosynthesized by plants, the properties of which are related to the presence of monoterpenoids, sesquiterpenoids and other volatile lipophilic compounds [2]. EOs can induce varied physiological, biochemical, and hemato-immunological responses in fish [3] and may improve fish growth and health [4]. The benefits of incorporating EOs into diets include an improved growth performance [5–16], enzyme activity and development of intestinal villi [7–10,15–17], morphological alterations of the liver [18–22], body composition [15], and muscle oxidant/antioxidant status [16].

Nectandra grandiflora Nees (Lauraceae), popularly known as "canela-amarela", is a heliophile tree endemic to Brazil [23]. The EO obtained from the leaves of this species (EONG) is composed mainly of sesquiterpenoids [24] and has shown in vitro antiparasitic activity against *Ichthyophthirius multifiliis* [25]. Sedative and anesthetic activities were also described for EONG and its major compound for juvenile tambaqui, *Colossoma macropomum*, and silver catfish, *Rhamdia quelen* [26–29]. Additionally, the anesthetic activity of EONG nanoemulsion showed enhanced activity with reduced side effects compared to free EONG in Nile tilapia, *Oreochromis niloticus* [25].

Tambaqui, *C. macropomum* (class Actinopteri, order Characiformes [29], family Serrasalmidae [30]) is a native fish species of northern South America, where it is widely distributed [29,31], mainly in the Amazon basin. It is currently the second-most produced fish species in Brazil [32,33], with its production spreading mainly in tropical countries due to its excellent zootechnical characteristics [34,35]. Various EOs have already been used in *C. macropomum* as anesthetics [36–43] and stress reducers for transportation [44,45], as well as for therapeutic effects [46–49], and incorporation into diets [18,50,51]. However, there are no reports in the literature on the use of EONG in the diets of juvenile *C. macropomum*.

Thus, the present study aimed to evaluate the growth performance and histomorphology of the intestine, skin, gills, and liver of juvenile *C. macropomum* fed diets containing different levels of EONG.

2. Materials and Methods

2.1. EO Extraction and Composition

Plant material was collected in southern Brazil (29°26′ S, 54°40′ W) and a voucher specimen was deposited at the Herbarium of the Forest Sciences Department, Universidade Federal de Santa Maria, Brazil (HDCF 13.162). EO was obtained by hydrodistillation of fresh *N. grandiflora* leaves in a Clevenger-type apparatus for 3 h (European Pharmacopeia, 2010).

The main compounds of EONG were determined using a gas chromatograph (GC) 7890A (Agilent) coupled to a 5975C mass selective detector (GC–MS), equipped with a DB5-MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Quantitative analysis was calculated based on GC peak areas produced by a GC coupled to a flame ionization detector (GC-FID). EONG compounds were identified by matching retention indexes and spectral fragmentation patterns with those of the National Institute of Standards and Technology Mass Spectral Library [24]. The major compounds of EONG were (+)-dehydrofukinone (23.34%), dehydrofukinone epoxide (8.69%), kaurene (5.58%), and selin-11-en-4-α-ol (4.71%).

2.2. Animal Maintenance and Handling

All procedures were carried out in accordance with guidelines for the care and use of experimental animals in Brazil. The experimental procedure was approved by the Ethics Committee on Animal Use (CEUA/UFMG—nº 114/2021).

Juvenile *C. macropomum* (n = 448, 0.75 ± 0.05 g and 3.43 ± 0.11 cm) were distributed in a recirculating aquaculture system (RAS) consisting of 16 28-L tanks, with mechanical and biological filters and temperature control. Juveniles were stocked at a density of 1 juvenile/L water (28 juveniles per tank). Water quality parameters were measured daily in the morning: temperature 28.33 ± 0.89 °C, pH 7.26 ± 0.29, salinity 0.45 ± 0.11 g salt/L, and conductivity 0.79 ± 0.21 mS/cm by means of a probe (model HI9146 Hanna instruments);

dissolved oxygen 7.88 ± 0.27 mg/L by multiparameter probe (YSI 6920VZ2); and total ammonia 0.15 ± 0.20 mg/L by test kit (Labcon). The photoperiod was 12L:12D (Key West DNI group, digital timer).

2.3. Diets and Treatments

Four isoproteic and isoenergetic diets with differing levels of EONG (0, 0.5, 0.75, and 1.50 mL/kg of feed) (Table 1) were created based on the protocols described by [52]. All ingredients were mixed until completely homogeneous. EONG was mixed with canola oil and then all ingredients and water were joined to produce a hard dough. The mass was then pelleted and dried at 40 °C, and subsequently mashed and stored at −20 °C. Each treatment had four replicates in a completely randomized design. Analysis of the approximate composition of the diet was based on [53,54].

Table 1. Formulation of experimental diet.

Ingredients	(%)
Soybean meal	35.00
Fish meal	30.00
Rice bran	12.00
Corn	15.00
Canola oil	3.00
Salt	1.00
Vitamin and mineral premix [1]	3.00
Phosphate dicalcium	1.00
Analyzed proximate composition	
Dry matter content	93.68
Protein	35.33
Ether extract	7.13
Mineral matter	22.77
Acid detergent fiber	1.40
Neutral detergent fiber	19.62

[1] Vitamin and mineral mixture (security levels per kilogram of product)—Pantothenic acid: 5000 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 D3: 500,000 UI, vitamin E: 20,000 UI, vitamin C: 28,000 mg, vitamin K: 500 mg, folic acid: 250 mg, biotin: 125 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.

Juveniles were fed 10% of their biomass daily in three meals (08:00, 12:00, and 16:00 h), with the values being corrected every 10 days through biometry. Tanks were cleaned daily in the morning to remove feces and 30% of the water volume in each tank was replaced every 5 days.

2.4. Survival and Zootechnical Performance

Survival (direct individual count) and growth were determined by weight and length biometry after 10, 20, and 30 days of rearing using a precision digital balance (Ay-220–220 g × 0.001 g Mars–Brazil analytical scale) and digital caliper with 0.01 mm accuracy (Starrett Electronic, Athol, MA, USA).

The obtained data was used to calculate the following:

1. Weight (W) (g) = average weight;
2. Daily weight gain (DWG) (g) = average weight gain (g)/time (days);
3. Daily feed consumption per fish (DC) (g) = total feed consumption/experiment time (days)/number of animals per tank;
4. Feed conversion ratio (FCR) = apparent total feed intake (g)/weight gain (g);
5. Daily specific growth rate (SGR) (%) = 100 × (lnWf—lnWi)/interval between biometrics (days), where Wi is the initial weight, Wf is the final weight;
6. Protein efficiency rate (PER) (%) = 100 × (weight gain (g)/protein consumed (g)).

2.5. Hepatosomatic and Viscerosomatic Indices and Intestinal Coefficient

After 30 days of rearing, three animals from each replicate (n = 12 per treatment) were euthanized with a solution containing 285 mg/L of eugenol [55]. Visceral fat, liver, and intestine of each animal were subsequently collected to determine the following indices:

$$\text{Viscerosomatic Index (VSI)} = 100 \times (\text{Visceral weight (g)}/\text{Animal body weight (g)})$$

$$\text{Hepatosomatic Index (HSI)} = 100 \times (\text{Liver weight}/\text{Animal body weight})$$

$$\text{Intestinal Coefficient (IC)} = \text{Intestine length}/\text{Total fish length}$$

2.6. Histology

Intestine, liver, and skin samples on the left side and all gills were collected from the euthanized fish (n = 12 each treatment). Intestines were delicately washed with 0.9% NaCl solution and then all organs were placed individually in tubes with Bouin solution for 24 h, followed by storage in ethanol 70% for subsequent routine histotechnical processing. Tissues were embedded in paraffin to produce 5 µm-thick sections. Histological slides were made according to the histological protocols of hematoxylin-eosin, PAS and Alcian-blue, following the methodology described by [56] for the Masson–Goldner trichrome technique, and later observed under a Zeiss Axio Scope light microscope with an image capture system. The slides were observed and photomicrographed with the aid of an Axio Scope.A1 microscope and a digital image capture system using a coupled Axiocam 105 color camera (ZEISS®, Jena, Germany).

Mucous cell percentage was later evaluated in relation to the epithelial lining of the intestinal, branchial, and skin mucosa, using ImageJ software and correction with the formula: (comparative area X % area/total area of the photo). Histological sections of the liver were used to evaluate centrolobular vein area and hepatocyte size.

2.7. Statistical Analysis

All data were submitted to the Shapiro–Wilk normality test and Levene's homoscedasticity test. Data were then evaluated by one-way ANOVA and Tukey's post hoc test with 95% probability ($p < 0.05$). Infostat and R software were used for data analysis.

3. Results

3.1. Performance

After 10 days of rearing, the W, DWG, DC, SGR, FCR, and PER did not differ among the treatments ($p > 0.05$), but after 20 days, the W, DWG, DC, and SGR were higher for the fish fed 0.5 mL EONG/kg than for the control fish ($p < 0.05$). After 30 days of rearing, only the W and DC remained higher for the fish fed 0.5 mL EONG/kg than for the control fish ($p < 0.05$). The other parameters did not differ among the treatments ($p > 0.05$) (Table 2).

After 30 days of culture, the VSI was higher for the fish fed 0.5 mL EONG/kg than for the control fish and the HSI was higher for all groups that received dietary EONG supplementation than for the control fish ($p < 0.05$) (Figure 1A,B). The intestinal coefficient did not differ among treatments ($p > 0.05$) (Figure 1C).

Table 2. Growth (mean ± standard deviation) of *Colossoma macropomum* juveniles fed with different dietary levels of the EO of *Nectandra grandiflora* (EONG) in a recirculating aquaculture system (RAS).

EONG (mL/kg Feed)	0.00	0.50	0.75	1.50	*p*-Value
			Day 10		
W (g)	2.26 ± 0.09	2.41 ± 0.02	2.22 ± 0.15	2.35 ± 0.08	0.0593
DWG (g)	0.15 ± 0.01	0.17 ± 0.00	0.15 ± 0.02	0.16 ± 0.01	0.0628
DC (g)	0.07 ± 0.0002	0.08 ± 0.0004	0.08 ± 0.0006	0.08 ± 0.0007	0.5524
FCR	0.5 ± 0.028	0.45 ± 0.008	0.52 ± 0.055	0.47 ± 0.021	0.0757
SGR (%)	11.05 ± 0.38	11.66 ± 0.12	10.8 ± 0.71	11.37 ± 0.30	0.0737
PER (%)	0.58 ± 0.03	0.63 ± 0.01	0.56 ± 0.06	0.60 ± 0.03	0.0730
Survival (%)	100	100	100	100	
			Day 20		
W (g)	3.88 ± 0.14 ^{b}	5.04 ± 0.40 ^{a}	4.35 ± 0.39 ^{ab}	4.32 ± 0.49 ^{ab}	0.0078
DWG (g)	0.16 ± 0.02 ^{b}	0.26 ± 0.04 ^{a}	0.21 ± 0.04 ^{ab}	0.20 ± 0.04 ^{ab}	0.0146
DC (g)	0.23 ± 0.01 ^{b}	0.26 ± 0.01 ^{a}	0.23 ± 0.02 ^{ab}	0.24 ± 0.01 ^{ab}	0.0424
FCR	1.44 ± 0.16	0.99 ± 0.17	1.12 ± 0.23	1.25 ± 0.29	0.0721
SGR (%)	5.41 ± 0.67 ^{b}	7.35 ± 0.84 ^{a}	6.72 ± 0.90 ^{ab}	6.04 ± 0.91 ^{ab}	0.0333
PER (%)	0.20 ± 0.02	0.29 ± 0.05	0.26 ± 0.06	0.24 ± 0.06	0.1283
Survival (%)	99.11 ± 1.79	100.00 ± 0.00	95.24 ± 5.46	99.1 ± 1.79	0.1665
			Day 30		
W (g)	5.64 ± 0.22 ^{b}	7.49 ± 0.52 ^{a}	6.58 ± 0.88 ^{ab}	6.63 ± 0.69 ^{ab}	0.0421
DWG (g)	0.18 ± 0.02	0.25 ± 0.03	0.22 ± 0.05	0.23 ± 0.11	0.4884
DC (g)	0.35 ± 0.01 ^{c}	0.46 ± 0.00 ^{a}	0.41 ± 0.01 ^{b}	0.42 ± 0.01 ^{b}	<0.001
FCR	1.99 ± 0.20	1.90 ± 0.20	1.94 ± 0.55	2.21 ± 1.10	0.8990
SGR (%)	3.73 ± 0.42	3.97 ± 0.40	4.12 ± 0.53	4.12 ± 1.36	0.8956
PER (%)	0.14 ± 0.01	0.15 ± 0.02	0.15 ± 0.04	0.16 ± 0.08	0.9802
Survival (%)	94.64 ± 4.61	94.64 ± 4.61	94.64 ± 4.61	94.64 ± 4.61	>0.999

Different letters indicate significant differences between treatments by one-way ANOVA and Tukey's test ($p < 0.05$). W—weight; DWG—daily weight gain; SGR—specific growth rate; DC—daily feed consumption per fish; FCR—feed conversion rate; PER—protein efficiency rate.

3.2. Histomorphology

Histological sections of the liver, intestine, and skin from each EONG level are depicted in Figures 2–4. The fish fed 1.5 mL EONG/kg showed a proliferative response of the mucous cells of the gills ($p < 0.05$) (Figure 5A), but the proliferation of the lysozyme- and immunoglobulin-secreting mucosal cells was higher in the skin of the fish that received any of the levels of EONG dietary supplementation compared to that in the skin of the control fish ($p < 0.05$) (Figure 5B). The fish that received a diet supplemented with 0.5 mL EONG/kg had a greater height and width of intestinal villi ($p < 0.05$) (Figure 6A,B). The two highest dietary EONG supplementations (0.75 and 1.50 mL/kg of feed) progressively reduced the hepatocyte dimensions compared to the control diet (Figure 6C), but EONG supplementation did not modify the centrolobular center vein area (Figure 6D).

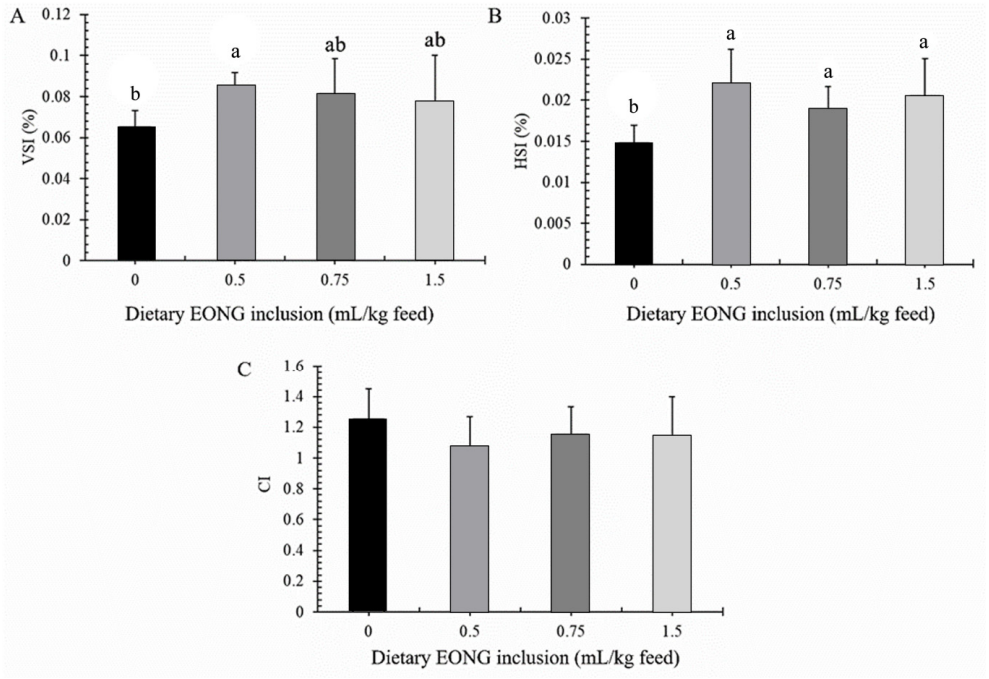

Figure 1. (**A**)—Viscerosomatic index (VSI), (**B**)—hepatosomatic index (HSI) and (**C**)—intestinal coefficient (IC) for *Colossoma macropomum* juveniles fed for 30 days with different dietary levels of the EO of *Nectandra grandiflora* (EONG) in a recirculating aquaculture system (RAS). Different letters in rows indicate significant differences between treatments by one-way ANOVA and Tukey's test ($p < 0.05$).

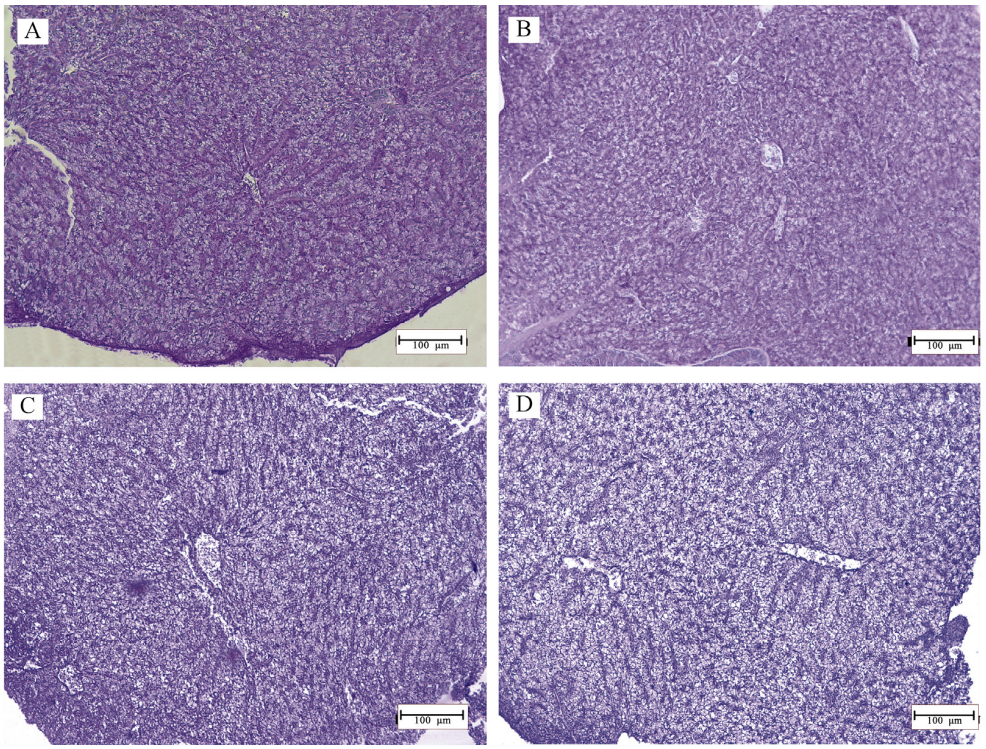

Figure 2. Histological sections of the liver of *Colossoma macropomum* juveniles fed for 30 days with different dietary levels of the EO of *Nectandra grandiflora* (EONG) in a recirculating aquaculture system (RAS). (**A**)—0 mL/kg, (**B**)—0.5 mL/kg, (**C**)—0.75 mL/kg, (**D**)—1.5 mL/kg.

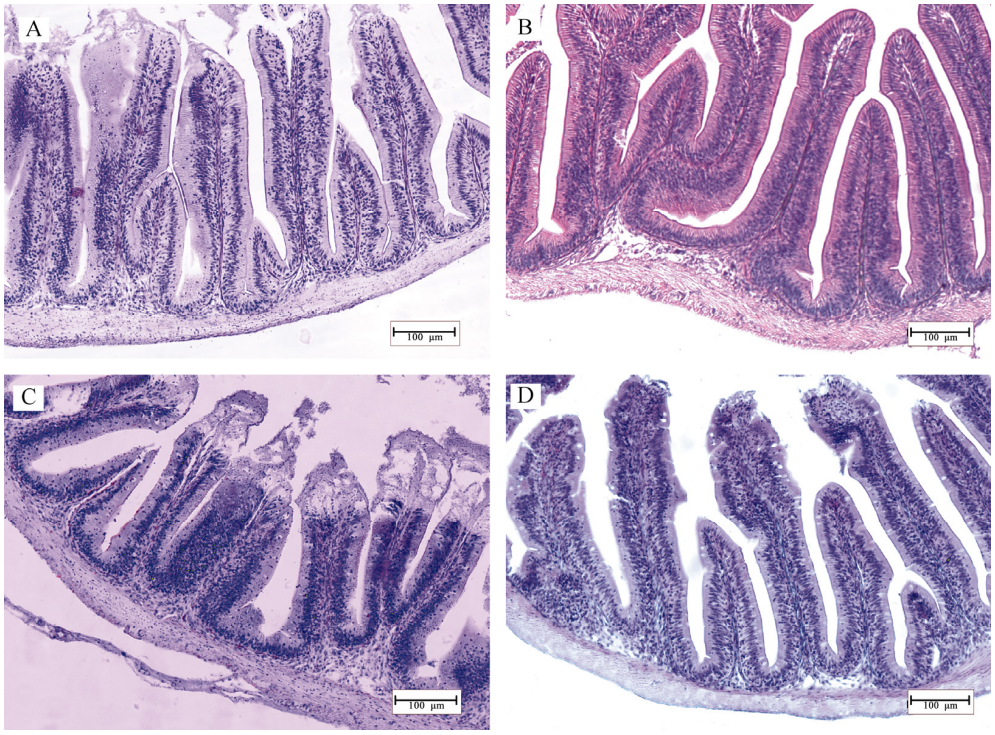

Figure 3. Histological sections of the intestine of *Colossoma macropomum* juveniles fed for 30 days with different dietary levels of the EO of *Nectandra grandiflora* (EONG) in a recirculating aquaculture system (RAS). (**A**)—0 mL/kg, (**B**)—0.5 mL/kg, (**C**)—0.75 mL/kg, (**D**)—1.5 mL/kg.

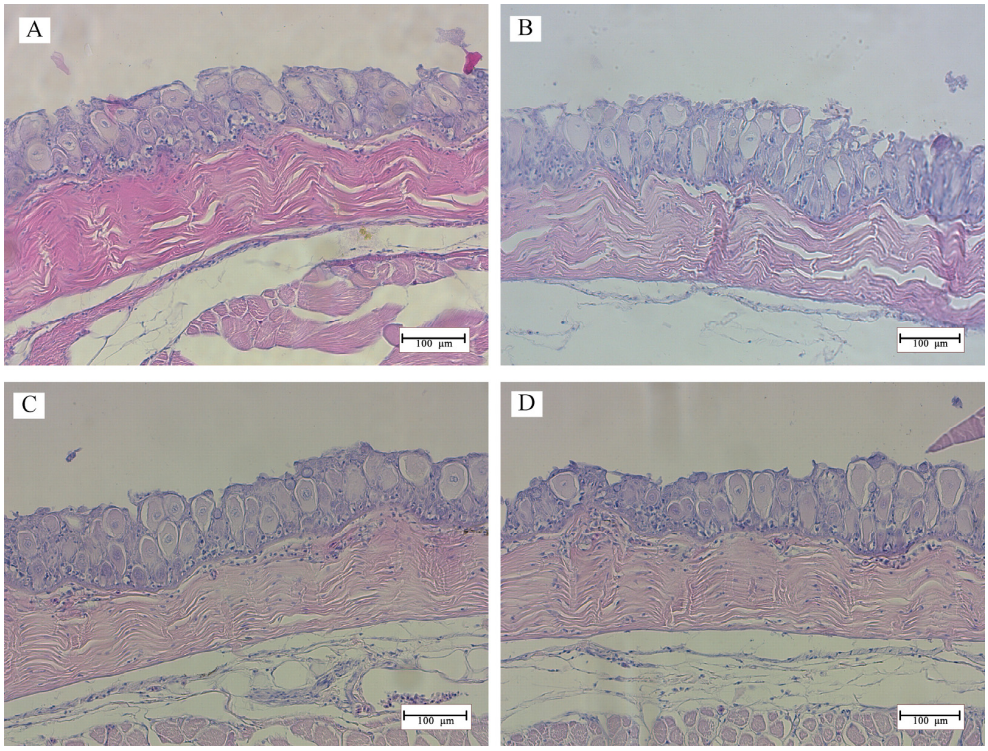

Figure 4. Histological sections of the skin of *Colossoma macropomum* juveniles fed for 30 days with different dietary levels of the EO of *Nectandra grandiflora* (EONG) in a recirculating aquaculture system (RAS). (**A**)—0 mL/kg, (**B**)—0.5 mL/kg, (**C**)—0.75 mL/kg, (**D**)—1.5 mL/kg.

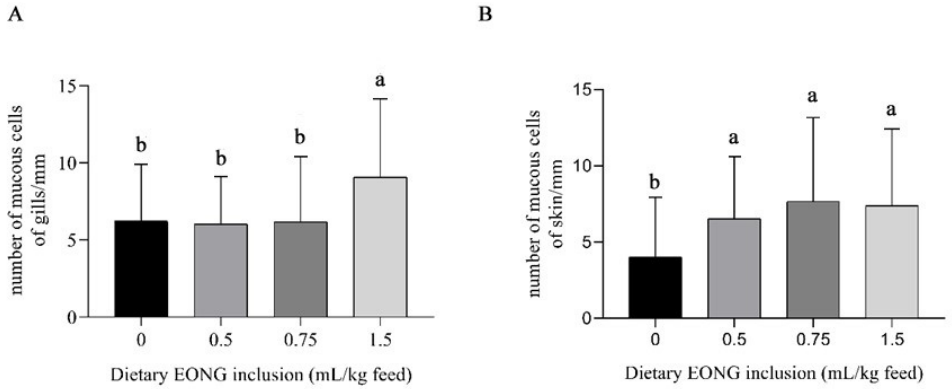

Figure 5. (**A**)—Number of mucous cells in gills and (**B**)—in skin of *Colossoma macropomum* juveniles fed for 30 days with different dietary levels of the EO of *Nectandra grandiflora* (EONG) in a recirculating aquaculture system (RAS). Different letters in rows indicate significant differences between treatments by one-way ANOVA and Tukey's test ($p < 0.05$).

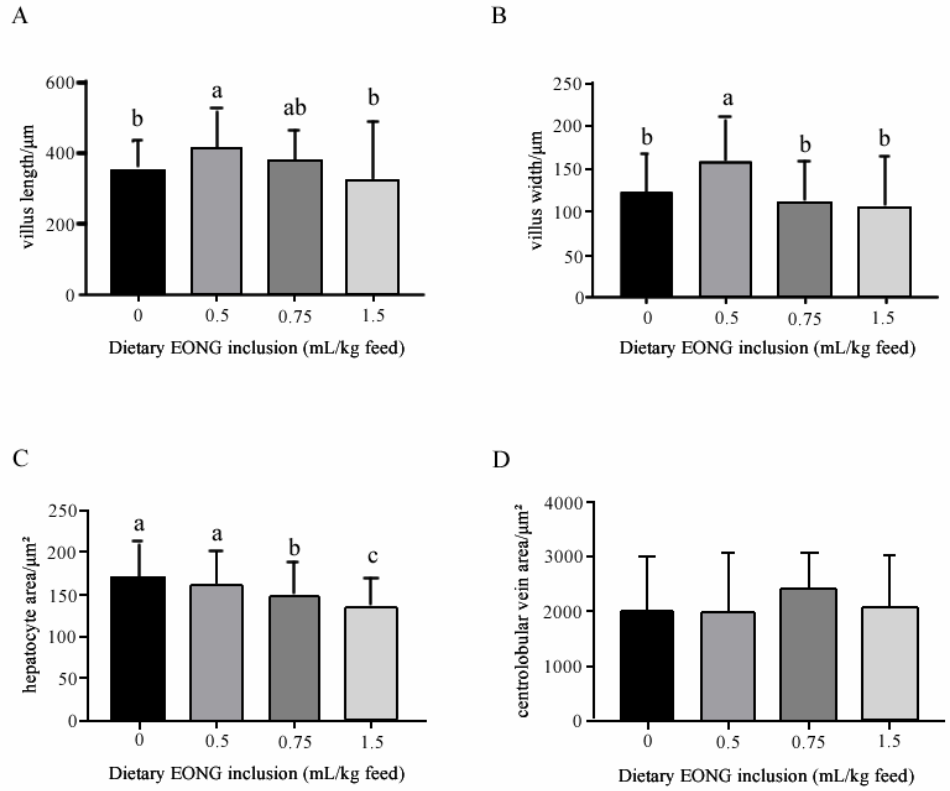

Figure 6. (**A**)—Length of intestinal villi, (**B**)—width of intestinal villi, (**C**)—hepatocyte area and (**D**)–centrolobular vein area of *Colossoma macropomum* juveniles fed for 30 days with different dietary levels of the EO of *Nectandra grandiflora* (EONG) in a recirculating aquaculture system (RAS). Different letters in rows indicate significant differences between treatments by one-way ANOVA and Tukey's test ($p < 0.05$).

4. Discussion

The juvenile *C. macropomum* that were fed diets containing different levels of EONG showed relevant and optimistic results for the growth performance and histomorphology of the intestine, skin, gills, and liver, allowing for its incorporation into diets for this species. The use of EONG in fish diets is unprecedented. The survival was not affected by the EONG levels and remained high (94.64%) during the 30 days of feeding. The use of EOs in fish diets has shown high survival rates with no differences in performance among the

tested inclusion levels, as demonstrated for the following: *Cyprinus carpio* L. using different levels of oregano EO (*Origanum vulgare*) [9]; *Cichlasoma nigrofasciatum* using fennel EO (*Foeniculum vulgare*) [57]; *O. niloticus* using basil EO (*Ocimum basilicum*) [58], menthol [10], oregano (*O. vulgare*) [11,59] and peppermint EO (*Mentha x piperita*) [15]; *Huso huso* using rosemary EO (*Rosmarinus officinalis*) [60]; and *Rhamdia quelen* using Chinese cinnamon EO (*Cinnamomum cassia*) [16]. However, worse survival rates were found when supplementing rainbow trout (*Oncorhynchus mykiss*) diets with mint EO (*Mentha spicata*) [61]. The negative effects of EOs on the survival may be related to toxic constituents, excessive doses, or allergic conditions, but they generally have no effect on these parameters when used in appropriate doses and applications [62].

The different levels of EONG evaluated here did not affect the juvenile performance in the first 10 days of feeding. However, after 20 days, the W, DWG, DC, and SGR were higher for the fish fed with 0.5 EONG mL/kg compared to the control, while after 30 days only the W and DC for these fish remained higher. These results show that a minimum feeding time is necessary to verify the benefits of using EONG. The intestinal microbiota can be influenced by several factors, such as the following: host species, genetics, developmental stage, diet, environment, and gender [63]. The main way by which EOs work appears to be through the regulation of the intestinal microflora [64]. These compounds may exert a prebiotic effect and modulate the intestinal bacterial composition [65]. This modulation can lead to the formation of bacterial communities that are complex and dynamic, and with the potential to positively influence host metabolism, immune system, and health maintenance [66–68]. Juvenile *O. niloticus* fed for 15 days with levels of *O. basilicum* EO of between 0 and 2.0 mL/kg showed a direct relationship between the performance and increasing inclusion level [59]. Juvenile *Oreochromis mossambicus* fed for 90 days using EO extracted from sweet orange peel (*Citrus sinensis*) at levels between 0 and 5 mL/kg, showed better performance results when fed with the addition of 1 mL/kg [69]. *Cichlasoma nigrofasciatum* juveniles fed with fennel EO (*F. vulgare*) at levels between 0 and 150 mg/kg in the diet showed a better feed conversion rate when compared to the control and other supplemented groups [57]. Juvenile *O. mykiss* fed diets supplemented with Turkish oregano EO (*Origanum onites* L.) at levels between 0 and 3 mL/kg showed better growth performance than the group fed without supplementation. Furthermore, feed conversion was better for fish fed with 1.5 and 3 mL/kg of *O. onites* EO [70]. Juvenile *R. quelen* fed diets supplemented with Chinese cinnamon EO (*C. cassia*) at levels between 0 and 1 mL/kg, showed better final weight, weight gain, and specific growth rate when fed 0.5 mL/kg [16]. Dietary supplementation with peppermint EO (*M. piperita*) at levels between 0 and 1 g/kg for juvenile *O. niloticus* showed better weight gain (0.52 g/kg), feed intake (0.51 g /kg), and feed conversion (0.51 g/kg) in a quadratic pattern [15]. In this way, the advantages of using EOs are evident, as is the need to evaluate different EOs for each different fish species.

The immune system is complex and encompasses both innate and adaptive branches to combat threats and maintain health [71,72]. The first lines of defense are the mucosal barriers present in the skin, gills, and intestine, which are lined with mucus secreted by mucous cells [73–75]. Mucus contains various innate immune molecules, such as mucins, proteases, lysozymes, esterases, complement proteins, antibodies, and antimicrobial peptides [76–80]. These chemical substances can inactivate pathogens or prevent their proliferation, protecting the organism against infections [81–86]. The fish fed 1.5 mL of EONG/kg of feed showed a proliferative response of the mucous cells in the gills, but the proliferation of these lysozyme- and immunoglobulin-secreting cells was greater in the skin of the fish that received any of the levels of EONG dietary supplementation compared to the fish fed the control diet. These results agree with those found for juvenile *Sparus aurata* fed diets containing 5 g/kg microencapsulated additive composed of a mixture of EOs of *Allium sativum* (garlic), carvacrol (3-isopropyl-2-methyl-phenol), and thymol (2-isopropyl-5-methyl-phenol). These fish experienced an improved immune response, protein strengthening in gills, and promoted anti-inflammatory control [87], as well as an

induced immune response in the skin, with an increase in immune molecules in the mucus and a reduction of pathogenic bacterial growth [88].

The use of 0.5 mL of EONG/kg of feed led to a greater height and width of intestinal villi, suggesting development of the absorptive area of the intestine. Long intestinal villi in fish are generally associated with improved intestinal health, greater nutrient absorption efficiency and, consequently, better performance [89,90]. This explains why this same level of EO inclusion had the best performance. The use of oregano EO (*O. vulgare*) resulted in an increase in the length and width of intestinal villi, thereby improving the intestinal health of *O. niloticus* [8,11] and *C. carpio* [9]. Juvenile *C. macropomum* fed with EO from the fruit peel of the lemon *Citrus* × *latifolia* at levels between 0 and 2 mL/kg in the diet had a greater height and length of intestinal villi than the control group [91]. Tests with juvenile *C. macropomum* fed ginger EO (*Zingiber officinale*) at levels between 0 and 2 mL/kg demonstrated that the dose should be limited to 0.5 mL/kg, as it improves intestinal health without causing villi reduction [18]. A reduction in the size of the intestinal villi can trigger a reduction in nutrient absorption [92] by reducing the integrity of the intestinal mucosa and decreasing the contact between absorptive cells and nutrients [93].

The liver is a key organ in the metabolism of nutrients, and so any histological changes to it may indicate compromised nutritional status [94]. HSI was higher for all EONG supplementation levels, compared to the control. Furthermore, the two highest dietary EONG levels (0.75 and 1.50 mL/kg of feed) progressively reduced hepatocyte dimensions, but EONG supplementation did not change the centrolobular vein area. Juveniles of the yellow tail tetra, *Astyanax altiparanae*, fed diets supplemented with *O. vulgare* EO at levels up to 2.5 g/kg showed an increasing linear effect on the cytoplasmic percentage of hepatocytes associated with the accumulation of hepatic glycogen [95], which could explain the higher HIS observed in the present study.

5. Conclusions

Dietary supplementation with 0.5 mL of EONG/kg of feed promoted improvements in the growth performance, intestinal health, and the immune response of *C. macropomum* juveniles, with a greater weight, growth rate, number of mucous cells in the gills and skin, and intestinal villi development, and a progressive reduction of the hepatocyte dimensions, suggesting a potential benefit for fish performance and general health.

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