R. Bras. Zootec., 54:e20240167, 2025
https://doi.org/10.37496/rbz5420240167

Aquaculture
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Morpho-adaptive and antioxidant response of silver catfish naturally infected by *Aeromonas hydrophila* fed diets with *Cymbopogon flexuosus* essential oil nanoemulsion

José Francisco Valério Júnior[1]*, Cristian Camilo Suarez-Barazeta[2], Isabela Andres Finamor[3], Maria Amália Pavanato[3], Samantha Nunes de Godoi[4], Aline Ferreira Ourique[4], Juliana Felipetto Cargnelutti[5], Roger Wagner[6], Marcelo Leite da Veiga[7], Bernardo Baldisserotto[3], Sílvio Teixeira da Costa[7]

[1] Universidade Federal de Santa Maria, Departamento de Zootecnia, Santa Maria, RS, Brasil.
[2] Mississippi State University, Department of Wildlife, Fisheries, and Aquaculture, Starkville, MS, USA.
[3] Universidade Federal de Santa Maria, Departamento de Fisiologia e Farmacologia, Santa Maria, RS, Brasil.
[4] Universidade Franciscana, Laboratório de Nanotecnologia, Santa Maria, RS, Brasil.
[5] Universidade Federal de Santa Maria, Departamento de Medicina Veterinária Preventiva, Laboratório de Bacteriologia, Santa Maria, RS, Brasil.
[6] Universidade Federal de Santa Maria, Departamento de Tecnologia e Ciência dos Alimentos, Santa Maria, RS, Brasil.
[7] Universidade Federal de Santa Maria, Departamento de Morfologia, Santa Maria, RS, Brasil.

***Corresponding author:**
jose.valerio@acad.ufsm.br

Received: November 5, 2024
Accepted: February 21, 2025

How to cite: Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Finamor, I. A.; Pavanato, M. A.; Godoi, S. N.; Ourique, A. F.; Cargnelutti, J. F.; Wagner, R.; Veiga, M. L.; Baldisserotto, B. and Costa, S. T. 2025. Morpho-adaptive and antioxidant response of silver catfish naturally infected by *Aeromonas hydrophila* fed diets with *Cymbopogon flexuosus* essential oil nanoemulsion. Revista Brasileira de Zootecnia 54:e20240167. https://doi.org/10.37496/rbz5420240167

Editors:
Leandro Cesar de Godoy
Fernando Yugo Yamamoto

ABSTRACT - This study evaluated the dietary supplementation of *Cymbopogon flexuosus* essential oil (CFEO) nanoemulsion in the cutaneous and branchial morpho-adaptive response and its antioxidant potential for silver catfish juveniles naturally infected by *Aeromonas hydrophila*. Four hundred silver catfish juveniles were randomly distributed in a water recirculation system and fed diets supplemented with CFEO nanoemulsion at concentrations of 0, 0.25, 0.5, 1.0, and 2.0 mL/kg of diet for 25 days. At the end of the experiment, skin, liver, and gill samples were collected for morphometric and oxidative status analyses. Our results showed that the highest concentrations of CFEO nanoemulsion (1.0 and 2.0 mL/kg) did not exert a structural preservation effect on the gill and cutaneous morphometry of animals naturally infected by *A. hydrophila.* However, the supplementation of 0.5 mL/kg of CFEO nanoemulsion demonstrated an antioxidant effect on their gills. In addition, dietary supplementation with CFEO nanoemulsion protected the liver of silver catfish against liver damage. Thus, due to these beneficial effects, CFEO nanoemulsion shows promising use in aquaculture.

Keywords: fish, food additive, oxidative stress, skin and gill morphometry

1. Introduction

Aeromonas hydrophila is a highly pathogenic infectious agent that causes lesions in different organs of fish, resulting in high mortality in aquaculture (Sun et al., 2022; Younis et al., 2023). Gills and skin are usually the first organs affected by aeromonosis because they are the first line of defense of the mucosal barrier, thus being constantly exposed to the external environment (Doyle et al., 2022).

Therefore, damage to the cutaneous and branchial epithelium, as well as changes in mucosal cells are often attributed to *A. hydrophila* infection (Marinho-Neto et al., 2019; Abdel-Latif and Khafaga, 2020). Another organ that is greatly affected by bacterial infections is the liver (Chen et al., 2018). Hepatic alterations (dilation and congestion of the sinusoid capillaries, as well as severe hepatic necrosis) have been described in the liver of *Piaractus mesopotamicus* and Nile tilapia after natural or experimental infection by *A. hydrophila* (AlYahya et al., 2018; Marinho-Neto et al., 2019; Abdel-Latif and Khafaga, 2020).

Infection by *A. hydrophila* can also have a negative impact on the defense mechanism of fish by promoting oxidative stress. Thus, due to the excessive production of oxygen-reactive species (ROS) or the inefficient scavenging of free radicals (Baldissera et al., 2017), the antioxidant system of animals can be impaired. The increase in ROS can result in elevated levels of malondialdehyde (MDA), consequently, causing lipid peroxidation, which causes oxidative damage to cellular constituents (Lushchak, 2016). Elevated levels of MDA in fish tissues have been linked to aeromonosis infection (Bandeira Junior and Baldisserotto, 2020).

Despite the harmful effects of bacterial infections, it has been reported that the use of natural plant-based ingredients in the diet may contribute to the improvement of the structure of the mucosal barrier in fish (Mousavi et al., 2021). According to Pittman et al. (2011), a preserved mucosal barrier is fundamental to maintain defense functions against pathogenic organisms. Similarly, the nutritional approach with the use of essential oils (EO) and/or compounds derived from medicinal plants has been shown to improve the antioxidant system of fish, inhibiting damage caused by oxidative stress, and reduce MDA levels in animals infected by pathogenic bacteria (Bandeira Junior et al., 2022; Abdel-Rahim et al., 2023; Viana et al., 2023; Mansour et al., 2024). When added to the diet, EO have been shown to improve antioxidant status, innate immunity, and resistance of different fish species to infectious diseases (Abdel-Latif et al., 2020b; Dawood et al., 2021; Shourbela et al., 2021; Das et al., 2023; Hajirezaee et al., 2024). Lemongrass (*Cymbopogon flexuosus*) is a plant native to East India widely used in traditional medicine (Kumar et al., 2021). Its EO has numerous pharmacological properties, including antifungal and antibacterial activities (Gao et al., 2020; Devi et al., 2021). In aquaculture, it has been shown to induce anesthesia and sedation in silver catfish (Santos et al., 2017), in addition to exert *in vitro* antibacterial activity against strains of fish pathogens (Pathirana et al., 2019).

In general, EO are volatile and light-sensitive compounds (Tyagi and Malik, 2010), which can hinder their use in fish farms. Therefore, they can deteriorate easily (by oxidation, volatilization, and heating), if they are not protected from external factors. One way to preserve the EO from these factors may be their formulation with nanoemulsions, which are recognized for protecting the EO against instability and decomposition, being widely used by the pharmaceutical industry due to its capacity to increase the bioavailability of the drug while keeping its release controlled (Devalapally et al., 2007). For these reasons, nanoemulsions have been studied in both *in vitro* and *in vivo* models, yielding several positive results against infectious agents (Gündel et al., 2018; Baccega et al., 2021; Özil et al., 2022). However, dietary supplementation of CFEO nanoemulsion in aquaculture has not been studied yet.

Silver catfish (*Rhamdia quelen*) is a native species of great economic relevance for aquaculture in the southern region of Brazil (Valenti et al., 2021). However, the species is susceptible to infection by *A. hydrophila*, which can cause great economic losses to fish farmers (Souza et al., 2016). Thus, the objective of this study was to evaluate the effect of dietary supplementation of CFEO nanoemulsion on the morphoadaptive and antioxidant responses of silver catfish juveniles naturally infected by *A. hydrophila*.

2. Material and methods

2.1. Ethics statement

The study complied with the Brazilian standards of care and use of animals for scientific and educational purposes and was approved by the Ethics Committee on the Use of Animals (CEUA) of Universidade Federal de Santa Maria (UFSM) under the number 6899161121.

2.2. Obtention and composition of the essential oil

The CFEO used in this study was commercially acquired (FERQUIMA Indústria e Comércio Ltda., São Paulo, Brazil). The determination of its major compounds was performed using a Varian Star 3400CX chromatograph (CA, USA) equipped with a flame ionization detector (GC-FID). The main compounds present in the CFEO were β-geranial (46%), Z-citral (34%), and geraniol (6%). Its full characterization is available in Gündel et al. (2018).

2.3. Preparation and characterization of nanoemulsions

The nanoemulsion was elabored by the homogenization techinique under high agitation, in an IKA® Ultra-Turrax® (Königswinter, Germany), composed of two phases: the oil phase, containing 5% CFEO and 2% sorbitan mooelate, and an aqueous phase, consisting of 2% polysorbate 80 and ultrapure water, according to the protocol developed by Gündel et al. (2018). For the physicochemical analysis of the nanoemulsion, the following parameters were considered: pH, zeta potential, polydispersity, and average droplet size. The pH was measured with a pHmeter DM-22, Digimed® (São Paulo, Brazil) directly in the formulations. Electrophoretic mobility (zeta potential) was determined using a zeta potential analyzer Nano-ZS Zetasizer® model ZEN 3600 (Malvern, United Kingdom). The polydispersity index and the average droplet size were analyzed using the dynamic light scattering method Nano-ZS Zetasizer® model ZEN 3600 (Malvern, United Kingdom). Further details regarding the preparation, composition, and characterization of the nanoemulsion can be found in Gündel et al. (2018).

2.4. Experimental diets

The experimental diets were formulated according to Zeppenfeld et al. (2016) and their compositions can be found in Table 1. The dietary treatments consisted of five diets: 0 (control diet) and 0.25, 0.5, 1.0, and 2.0 mL of CFEO nanoemulsion per kg of diet. The control diet was prepared based on the same isonutritional matrix as the other dietary treatments, containing 2.0 mL of nanoemulsion, but without the addition of CFEO. The ingredients of each diet were weighed and homogenized manually.

Table 1 - Formulation of the experimental diets

Ingredient (g/kg)	Diet (mL of CFEO nanoemulsion per kg of diet)				
	Control	0.25	0.5	1.0	2.0
Soybean meal	300	300	300	300	300
Meat flour	350	350	350	350	350
Rice bran	120	120	120	120	120
Corn bran	150	150	150	150	150
Canola oil	30	30	30	30	30
Salt	10	10	10	10	10
Mineral and vitamin (premix)[1]	30	30	30	30	30
CFEO nanoemulsion	0	0.25	0.5	1	2
Dicalcium phosphate	10	10	10	10	10
Analysed proximate composition (%)					
Dry matter content			94.96		
Crude protein			29.55		
Mineral matter			19.80		
Ether extract			9.95		
Neutral detergent fiber			24.00		
Acid detergent fiber			2.91		

CFEO - *Cymbopogon flexuosus* essential oil.
[1] Vitamin and mineral mixture (security levels per kilogram of product): pantothenic acid, 5000 mg; antioxidant, 0.60 g; vitamin B6, 2485 mg; vitamin B1, 1250 mg; vitamin B12, 3750 mcg; vitamin A, 1,000,000 IU; vitamin B2, 2500 mg; vitamin C, 28,000 mg; vitamin K, 500 mg; vitamin D3, 500,000 IU; vitamin E, 20,000 IU; zinc, 17,500 mg; biotin, 125 mg; cobalt, 25 mg; copper, 2000 mg; iron, 820 mg; iodine, 100 mg; manganese, 3750 mg; niacin, 5000 mg; selenium, 75 mg; folic acid, 250 mg.

The nanoemulsion was then diluted in canola oil and added to the diet. Then, the diet was moistened (water at 28 °C), pelleted and, finally, dried at 40 °C in a forced-air circulation oven for 24 h. After drying, the rations were packed in plastic bags and stored at – 20 °C.

2.5. Animals and experimental procedures

The silver catfish juveniles acquired from a local producer (Santa Maria, Rio Grande do Sul, Brazil) were transported to the Laboratório de Fisiologia de Peixes (LAFIPE), UFSM, Rio Grande do Sul, Brazil (29°43'30" S, 53°42'47"), and acclimatized for seven days to the experimental conditions. After acclimatization, fish were anesthetized in a 50 mg/L eugenol solution (Cunha et al., 2010) for measuring its individual length (cm) and weight (g).

Four hundred and twenty silver catfish juveniles (7.60 ± 3.29 g; 10.01 ± 1.40 cm) were randomly distributed in twenty polypropylene boxes, with a capacity of 60 L, 11 fish per box, in a recirculating aquaculture system (RAS). The RAS was composed of mechanical and biological filter (two 500L tanks), a circulation pump, and a system for maintaining the water temperature (heater with thermostat). Each box was equipped with a continuous individual aeration system. In addition, as environmental enrichment, each box had two shelters (brown PVC pipes) (Oliveira et al., 2019).

The animals were fed the experimental diets in excess (4% of the total biomass throughout all the experiment) for 25 days twice a day (09:00 and 19:00 h) (Zeppenfeld et al., 2016). The boxes were cleaned daily by siphonage 30 min after the last feeding to remove leftover feed and feces. The experimental design resulted in five treatments and four replications.

During the experimental period, daily occurrences of mortality were recorded. Skin lesions, fins, barbels, and hemorrhage were the most observed signs in dead and dying fish. Two dying fish that showed these signs were collected for complementary investigation. A bacteriological examination was carried out at the Laboratório de Bacteriologia (LABAC), UFSM, using the aerobic microbiological culture method. The analysis identified the presence of *A. hydrophila*.

The occurrence of natural breakout of infection by *A. hydrophila*, which contaminated the fish of the experimental units, resulted in the complete loss of the animals in the control group. Therefore, a second experimental stage was conducted later for obtaining a reference system – control group, which was not contaminated by *A. hydrophila* and was fed a diet without CFEO, which allowed morphometric and biochemical comparisons with infected groups (Figure 1). All fish were from the same cohort, were maintained in the same RAS with the same levels of water quality parameters, and were fed as described in the first experiment.

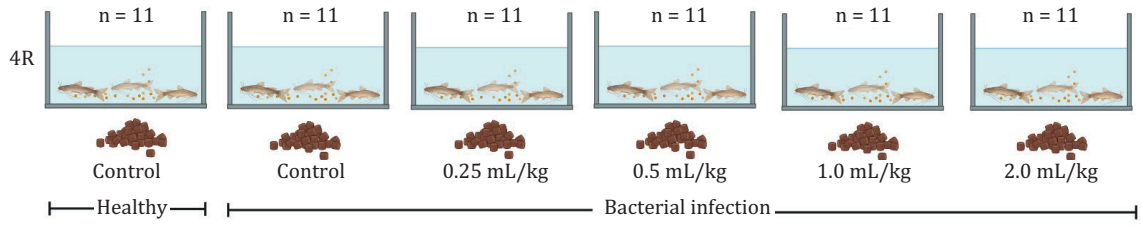


Figure 1 - Experimental desing for the cultivation of silver catfish fed increasing levels of *Cymbopogon flexuosus* essential oil nanoemulsion in the diet, and naturally infected by *A. hydrophila*.

2.6. Collection of biological materials

Ah the end the experiment, eight fish from each treatment were anesthetized with 50 mg/L eugenol (Cunha et al., 2010) and euthanized by spinal cord section to collect biological material. Then, samples

of liver, skin lining (ventral region), and gills (second branchial arch) were carefully collected, stored in sterile tubes, and fixed in 10% buffered formalin for further histological processing. Gills (second branchial arch) were also collected for analysis of oxidative stress markers; for this purpose, they were placed in sterile tubes, frozen in liquid nitrogen, and stored at −80 °C.

2.7. Histomorphometry

Histological processing was performed at the Laboratório de Morfofisiologia Experimental (LABITEX), UFSM. Tissue samples were subjected to routine histological processing for paraffin ingestion. The blocks were sectioned at a thickness of 6 µm a rotating microtome Easy path® (São Paulo, Brazil). Liver samples were stained with hematoxylin and eosin (HE) (Abdel-Latif et al., 2020a). The morphological analysis of the hepatic parenchyma was performed by the qualitative method.

For the analysis of mucus secreting cells of the cutaneous and branchial lining, the slides were stained using the PAS and Alcian Blue technique (Zheng et al., 2021). The slides were analyzed and photomicrographed under a microscope model Axio Scope.A1 ZEISS® (Oberkochen, Germany), equipped with an Axioncam 105 color image capture system ZEISS® (Oberkochen, Germany) interfaced to ZEN software. Ten photomicrographs of each section of the cutaneous epithelium and the second branchial arch were analyzed using ImageJ v. 1.49 (Schneider et al., 2012). The analyses of mean diameter of mucous cells, number of mucous cells and density mucous cells in the gill and cutaneous epithelium were calculated according to Dang et al. (2020) and resulted in three mucosal indices:

The area of mucous cells was the average size of the mucous cells in the tissue of the individuals.

$$\text{Mucous cell density} = \frac{(\text{area of mucous cells} \times \text{number of mucous cells})}{\text{area of epithelium}} \times 100 \quad (1)$$

$$\text{Barrier status} = \frac{1}{\text{mucous cell area/mucous cell density}} \times 1000 \quad (2)$$

2.8. Oxidative status markers

Gills were homogenized in ice-cold 50 mM Tris buffer pH 7.5, 2 mM EDTA, 150 mM NaCl, 0.5% Nonidet-P40, and 1 mM phenylmethylsulfonyl fluoride using a IKA® Ultra-Turrax® (City, Germany) and centrifuged at 1000 × *g* for 10 minutes at 4 °C. The resultant supernatants were used in the assays. Total antioxidant capacity (TAC) was determined according to protocol developed by Campos and Lissi (1997), and absorbance was recorded at 734 nm. Lipid peroxidation was evaluated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) (Hermes-Lima et al., 1995), and absorbance was measured at 532 nm. The TAC values and TBARS levels are shown as nmol/mg of protein. Proteins were detected using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, USA).

2.9. Water quality analysis

Water quality parameters were monitored daily. Temperature and dissolved oxygen were checked with an oximeter (YSI 50 ®, Yellow Springs, EUA). The pH of the water was monitored with a pH meter DMPH-2 (Digimed, São Paulo, Brazil). Total ammonia concentration was monitored using commercial kits (LabconTest, Alcon®, Camboriú, Brazil). The data obtained were: temperature (23.2 ± 2.24 °C), dissolved oxygen (4.6 ± 1.31 mg/L), pH (7.2 ± 0.44), and total ammonia (0.025 ± 0.04). Water quality variables remained in the ideal range for the species (Gomes et al., 2000).

2.10. Statistical analysis

Statistical analysis was performed using GraphPad Prism® version 8.0. The homogeneity of variances was analyzed using Levene's test. No significant relationship between the determined parameters and CFEO was found; then, for the analysis of oxidative stress markers one-way ANOVA followed by

Tukey's post-hoc test was used. The values of cell size, cell density, and barrier state of the branchial and cutaneous epithelium were compared using the non-parametric Kruskal-Wallis test followed by Dunn's test for comparing means. In addition, orthogonal polynomial contrasts were performed to verify the linear and quadratic effects of the CFEO dietary supplementation on the different parameters. Differences were considered significant when P<0.05.

The values were expressed as the mean ± SEM. The statistical model used is as follows:

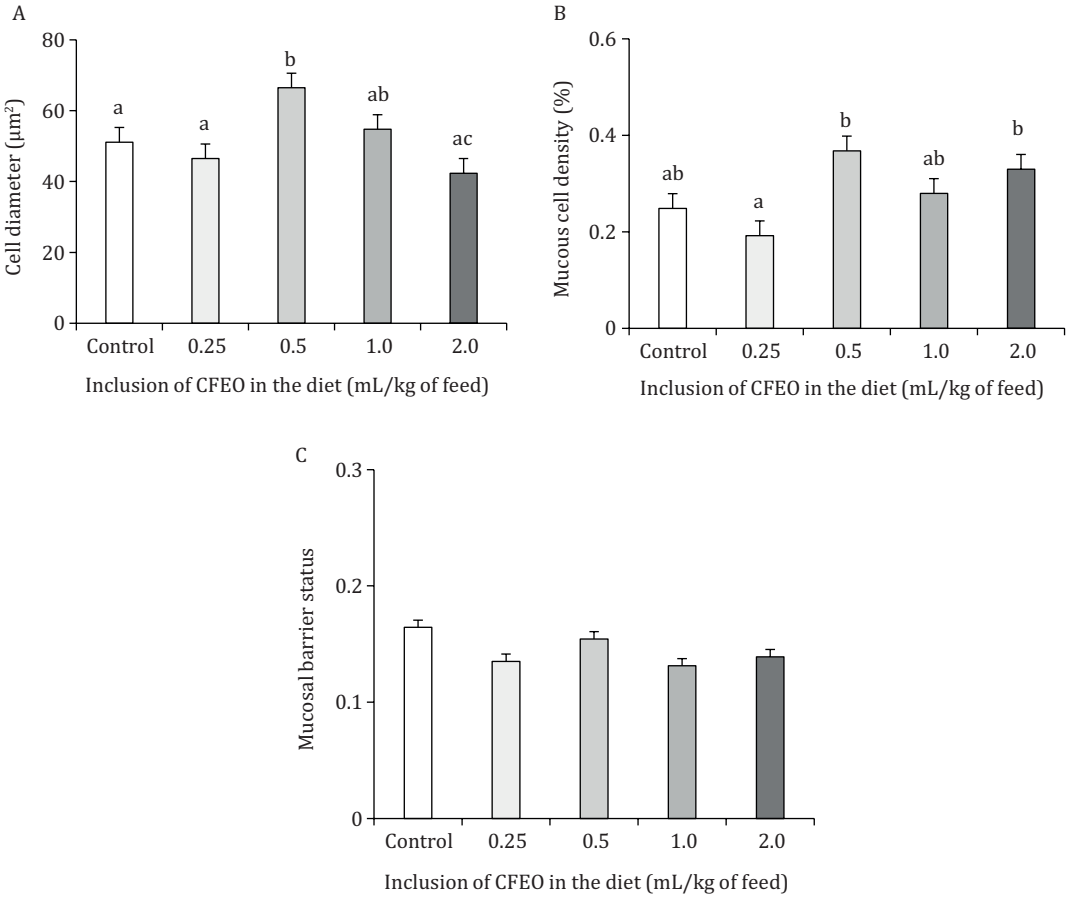
$$Y_{ij} = \mu + T_i + e_{ij}, \quad (3)$$

in which Y_{ij} = dependent variable referring to repetition j of treatment i; μ = overall average; T_i = effect of the i-th treatment (i = 1 to 5); and e_{ij} = experimental error.

3. Results

3.1. Gillial morphometry

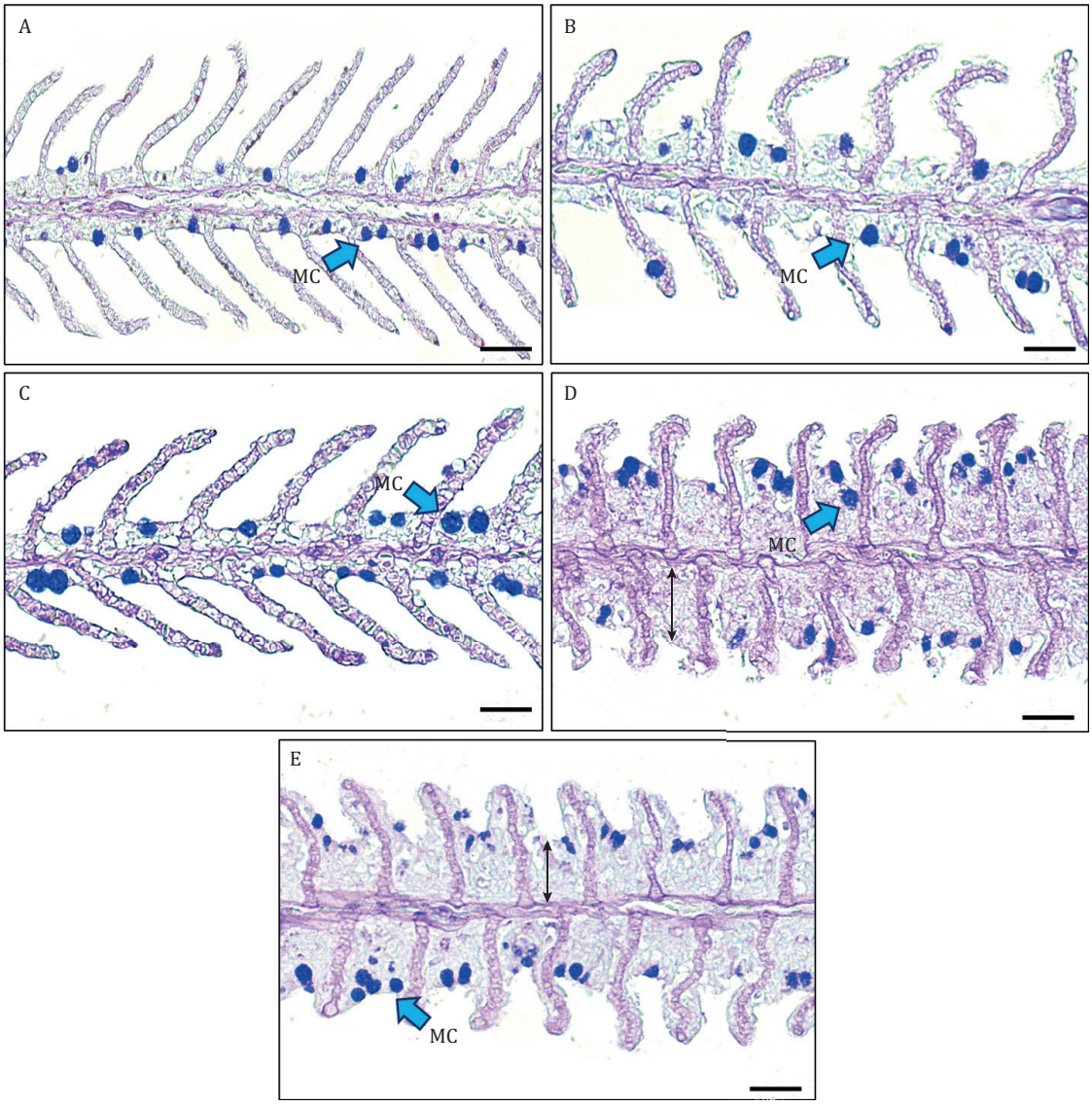
Fish fed 0.5 mL/kg of CFEO nanoemulsion in the diet showed significantly larger (P<0.05) mucous cells in the gill epithelium (66.47 μm²) (compared with the animals in the control group and those fed 0.25 and 2.0 mL/kg of CFEO nanoemulsion in the diet (51.13, 46.50, and 42.35 μm², respectively) (Figure 2A). However, no significant difference (P>0.05) in mucous cell diameter was observed between



A: Diameters of the mucous cells of the gill tissue; B: density of mucous cells of gill tissue; C: barrier status of the mucous cells of the gill filament.
Control - uninfected fish fed the control diet without CFEO.
Different letters indicate a statistically significant difference at P<0.05 among treatments. The values were expressed as mean ± SEM.

Figure 2 - Effect of different doses of *Cymbopogon flexuosus* essential oil (CFEO) nanoemulsion in the diet on the gill morphology of silver catfish naturally infected by *A. hidrophila*.

animals fed 0.5 and 1.0 mL/kg CFEO nanoemulsion (66.47 and 54.74 μm^2, respectively) (Figure 2A). Cell density (Figure 2B) was significantly higher (P<0.05) in animals fed 0.5 and 2.0 mL/kg of CFEO nanoemulsion compared with the group fed 0.25 mL/kg of CFEO nanoemulsion. Mucosal barrier status (Figure 2C) was not significantly affected by the treatments (P>0.05). The gills of silver catfish from the uninfected control group and from the animals fed 0.25 and 0.5 mL/kg CFEO nanoemulsion showed normal structure (Figures 3A-C). However, interlamellar hyperplasia was observed in the gills of animals in the groups fed 1.0 and 2.0 mL/kg of CFEO nanoemulsion (Figures 3D-E).

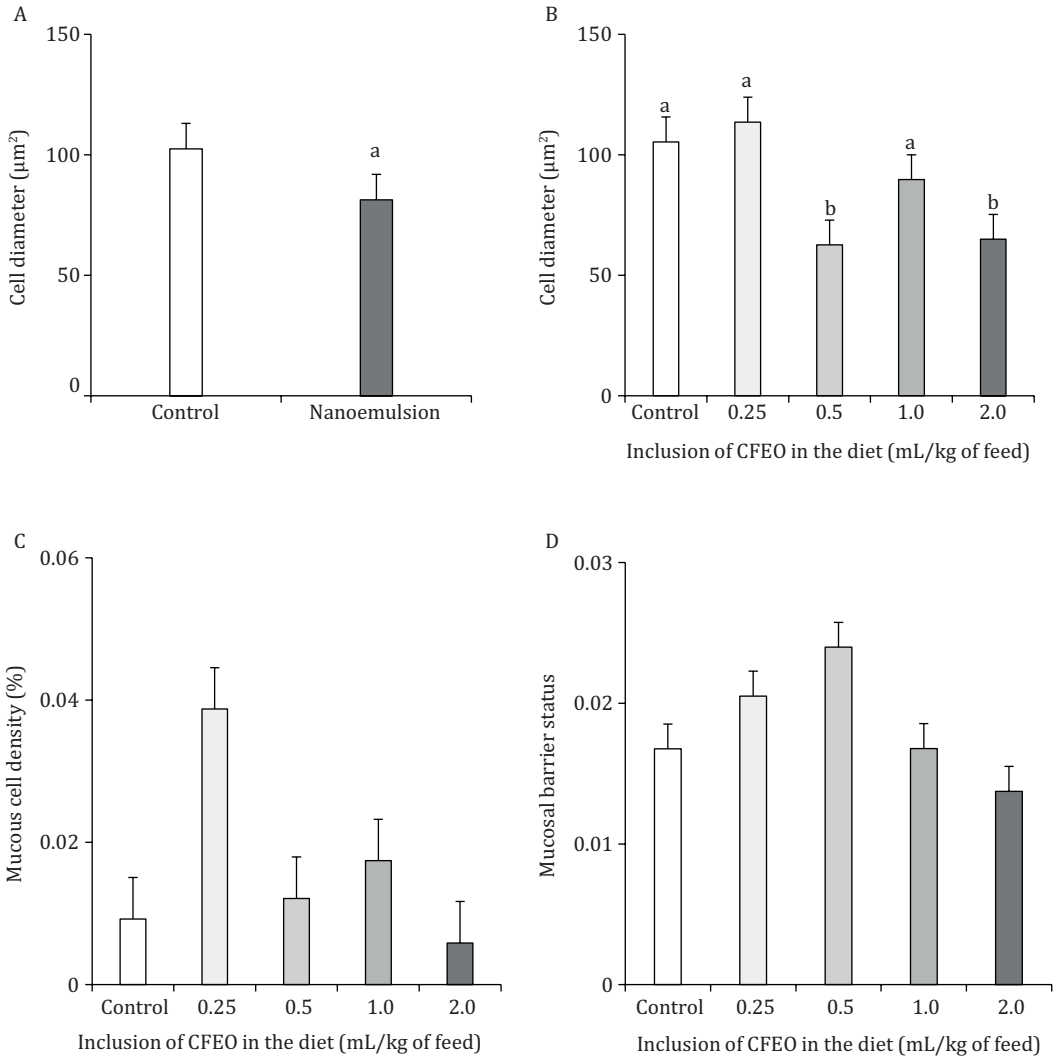


A: Uninfected fish fed the control diet without CFEO nanoemulsion; B: infected fish fed 0.25 mL CFEO/kg diet; C: infected fish fed 0.5 mL CFEO/kg diet; D: infected fish fed 1.0 mL CFEO/kg diet; E: infected fish fed 2.0 mL CFEO/kg diet.
Arrows: mucous cells (MC); double-pointed arrows: interlamellar hyperplasia.
PAS and Alcian Blue coloring. Scale bar = 50 μm. 20X magnification.

Figure 3 - Photomicrographs of histological sections of silver catfish gills naturally infected by *A. hydrophila* and fed diets supplemented with different doses of *C. flexuosus* essential oil (CFEO) nanoemulsion.

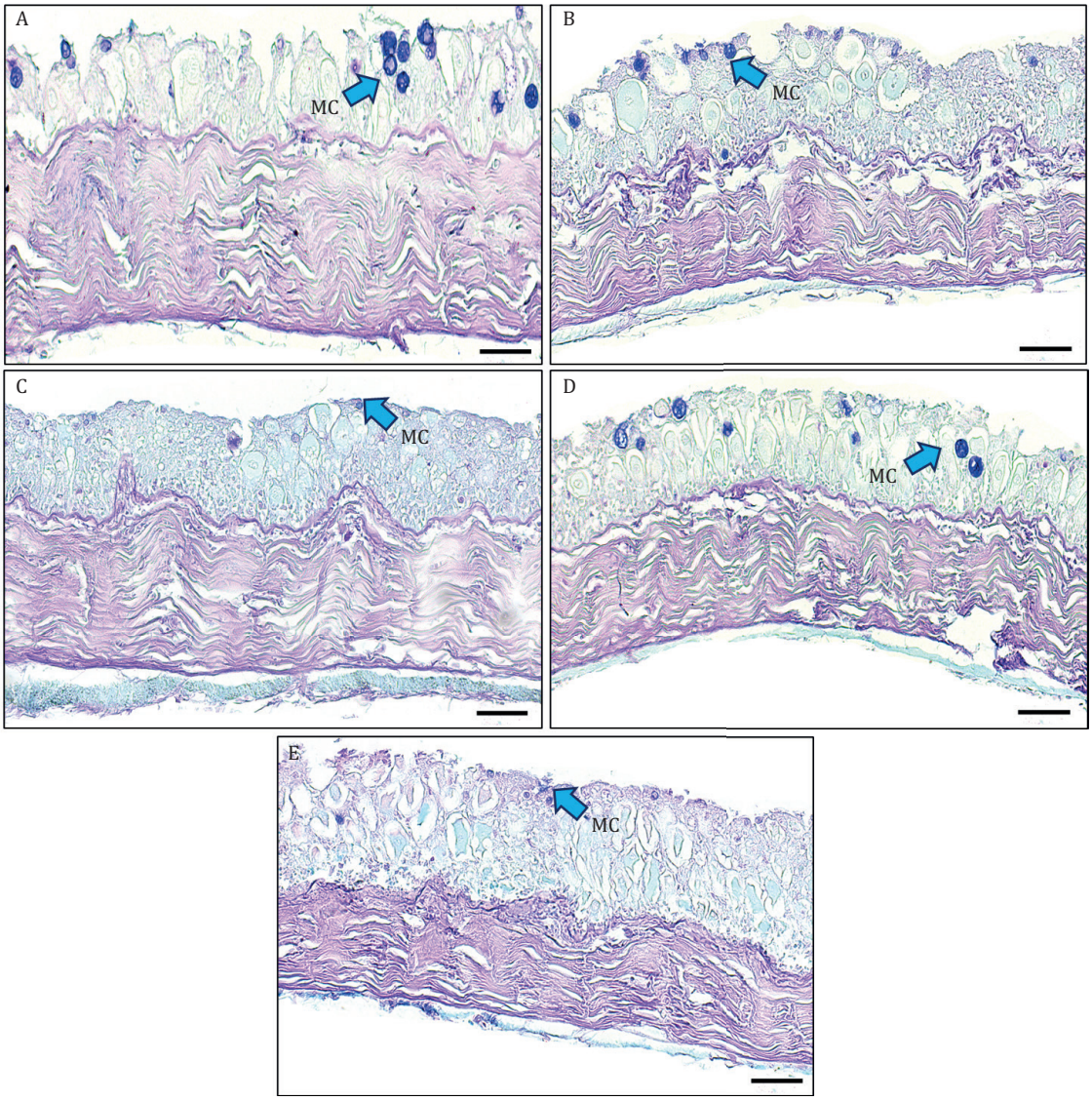
3.2. Cutaneous morphometry

Orthogonal contrast revealed that fish naturally infected by *A. hydrophila* and fed diets supplemented with CFEO nanoemulsion had significantly smaller mucous cells compared with fish in the control group (Figure 4A). This test did not reveal any significant difference in the other morphological gill parameters. The Kruskal-Wallis test followed by Dunn's test demonstrated that fish fed 0.5 and 2.0 mL/kg of CFEO nanoemulsion (62.36 and 64.94 μm^2) were significantly smaller (P<0.05) than those of the uninfected control group and those of the infected group fed 0.25 and 1.0 mL/kg of CFEO nanoemulsion (105.37, 113.58, and 89.69 μm^2, respectively) (Figure 4B). Mucosal cell density was not significantly affected by the treatments (P>0.05) (Figure 4C). No significant difference (P>0.05) was observed in the skin barrier status of the animals fed the experimental diets (Figure 4D). The histological sections of skin (Figure 5) showed normal structure.



A: Orthogonal contrast of the diameters of the mucous cells; B: diameters of the mucous cells; C: density of mucous cells; D: barrier status of mucous cells.
Control - uninfected fish fed the control diet without CFEO; nanoemulsion - infected fish fed a diet supplemented with CFEO nanoemulsion.
Different letters indicate a statistically significant difference at P<0.05 among treatments. The values were expressed as mean ± SEM.

Figure 4 - Effect of different doses of *Cymbopogon flexuosus* essential oil (CFEO) nanoemulsion in the diet on the skin morphology of silver catfish naturally infected by *A. hidrophila*.

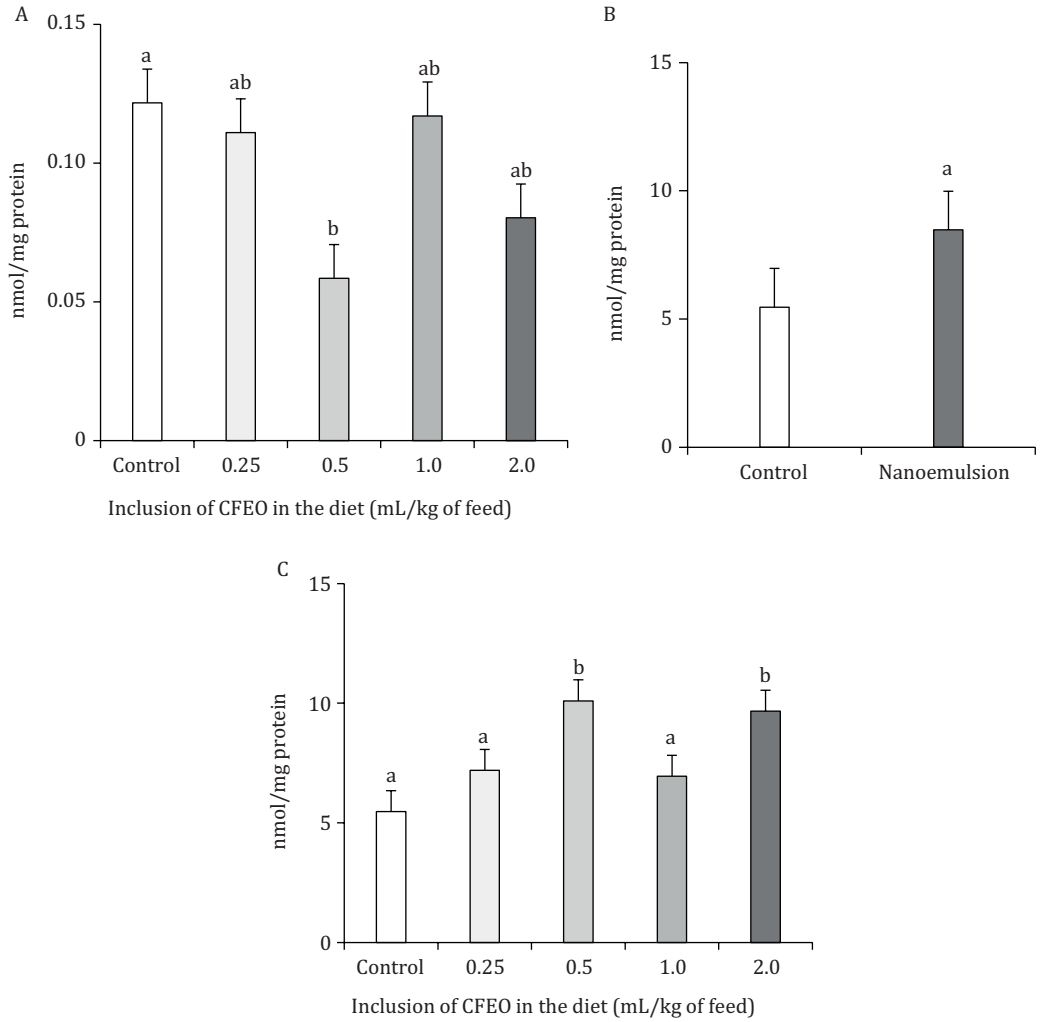


A: Uninfected fish fed the control diet without CFEO nanoemulsion; B: infected fish fed 0.25 mL CFEO/kg diet; C: infected fish fed 0.5 mL CFEO/kg diet; D: infected fish fed 1.0 mL CFEO/kg diet; E: infected fish fed 2.0 mL CFEO/kg diet.
Arrows: mucous cells (MC). PAS and Alcian Blue coloring. Scale bar = 50 µm. 20X magnification.

Figure 5 - Photomicrographs of histological sections of silver catfish skin naturally infected by *A. hydrophila* and fed diets supplemented with different doses of *C. flexuosus* essential oil (CFEO) nanoemulsion.

3.3. Oxidative status markers in the gills

Fish fed 0.5 mL/kg of CFEO nanoemulsion showed a reduction in TBARS levels ($P<0.05$) when compared with uninfected fish fed the control diet (Figure 6A). There was no significant difference ($P>0.05$) in the TBARS levels of fish fed 0.25, 1.0, and 2.0 mL/kg of CFEO nanoemulsion in relation to the non-infected animals fed the control diet (Figure 6A). The orthogonal contrast demonstrated that TAC was significantly higher in fish naturally infected by *A. hydrophila* and fed CFEO nanoemulsion than in control fish (Figure 6B). Fish fed 0.5 and 2.0 mL/kg CFEO nanoemulsion showed significantly higher TAC levels ($P<0.05$) than fish fed the other CFEO nanoemulsion levels (Figure 6C). The TAC values showed no statistical difference ($P>0.05$) among the fish of the uninfected control group and those fed 0.25 and 1.0 mL/kg CFEO nanoemulsion (Figure 6C).

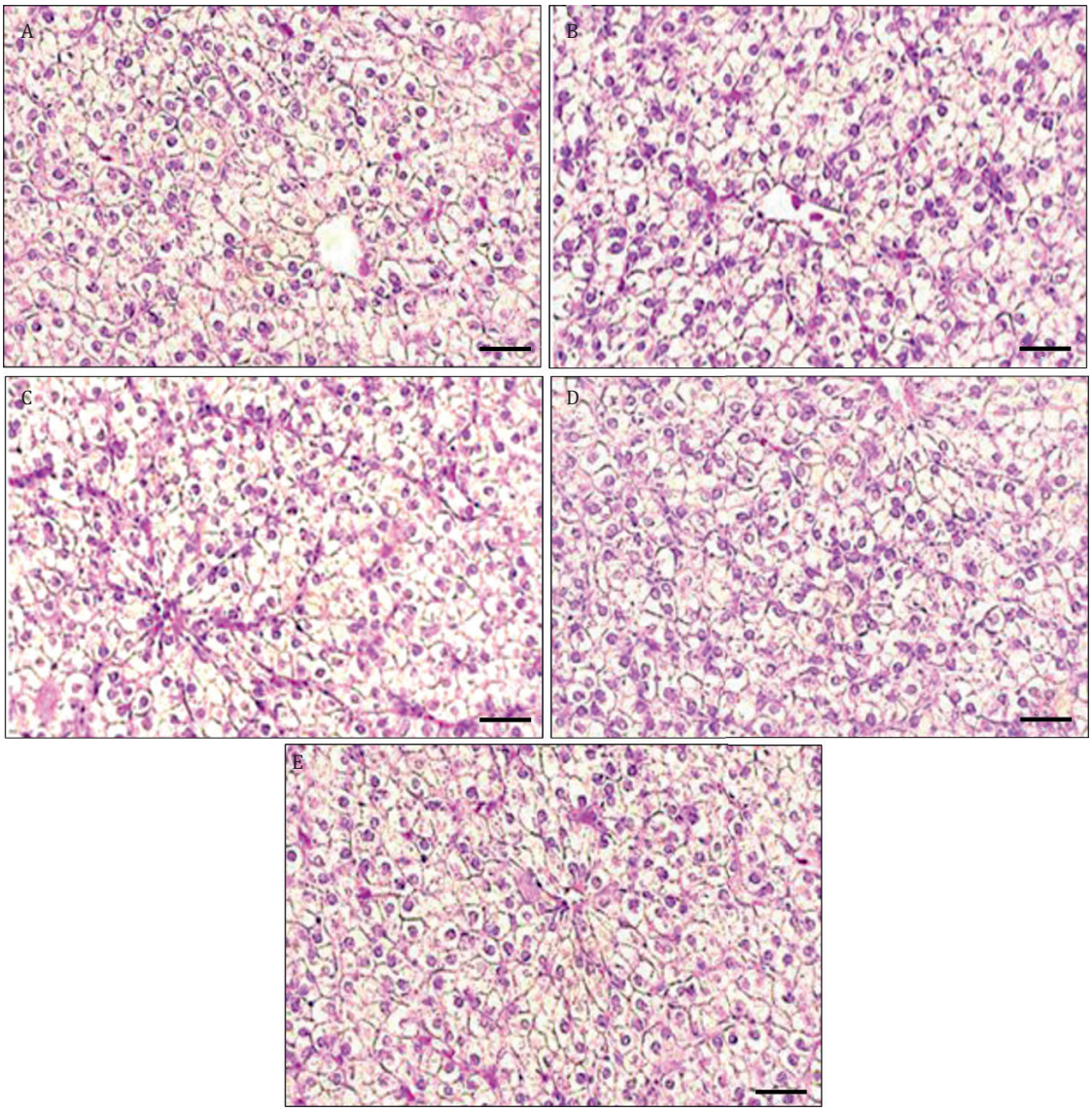


A: Thiobarbituric acid reactive substances (TBARS); B: orthogonal contrast of the total antioxidant capacity; C: total antioxidant capacity. Control - uninfected fish fed the control diet without CFEO; nanoemulsion - infected fish fed a diet supplemented with CFEO nanoemulsion. Different letters indicate a statistically significant difference at P<0.05 among treatments. The values were expressed as mean ± SEM.

Figure 6 - Effect of different doses of *Cymbopogon flexuosus* essential oil (CFEO) nanoemulsion in the diet on the antioxidant status in the gills of silver catfish naturally infected by *A. hidrophila*.

3.4. Hepatic histology

The hepatic tissue of the uninfected control group (Figure 7A) showed normal macroscopic parenchyma, with normal hepatocytes, homogeneous cytoplasm, and rounded nucleus. In addition, the central lobular vein and sinusoid capillaries showed normal structure. The other groups exhibited livers with a similar structure of the control group with no pathological lesion observed (Figures 7B-E).



A: Uninfected fish fed the control diet without CFEO nanoemulsion; B: infected fish fed 0.25 mL CFEO/kg diet; C: infected fish fed 0.5 mL CFEO/kg diet; D: infected fish fed 1.0 mL CFEO/kg diet; E: infected fish fed 2.0 mL CFEO/kg diet.
Hematoxylin and Eosin (HE) staining. Scale bar = 50 µm. 20X magnification.

Figure 7 - Photomicrographs of histological sections of silver catfish liver naturally infected by *A. hydrophila* and fed diets supplemented with different doses of *C. flexuosus* essential oil (CFEO) nanoemulsion.

4. Discussion

In the present work, silver catfish naturally infected by *A. hydrophila* and fed concentrations of 1.0 and 2.0 mL/kg of CFEO nanoemulsion showed interlamellar hyperplasia. Histopathological changes in fish gills have been related to *A. hydrophila* infection, and interlamellar hyperplasia was observed in experimentally infected blue tilapia (*Oreochromis aureus*) and pacu (*Piaractus mesopotamicus*) (AlYahya et al., 2018; Marinho-Neto et al., 2019). Additionally, morphologic alterations were also seen in the gills of Nile tilapia (*Oreochromis niloticus*) challenged with *Streptococcus agalactiae* and fed high doses of the essential oils (1.0 and 1.5%), including those of ginger and clove basil, being excessive concentrations related to such observations (Brum et al., 2018). Thus, like the previous study, the

current research shows that the lowest concentrations of CFEO nanoemulsion are the therapeutic ones for protecting the silver catfish gills against these modifications.

The mucosal surface of the gills and skin offers protection, being fundamental for fish health (Nimalan et al., 2022). A healthy mucosal structure has been attributed to the presence of immunostimulant ingredients in the diet, like proanthocyanidins, which can be found in grape fruit seed extract (Nichols and Katiyar, 2010). Mousavi et al. (2021) stated that the inclusion of this extract into the diet of rainbow trout (*Oncorhynchus mykiss*) improved its immunity, increasing the size and density of mucous cells in the gills and skin. Overall, dietary CFEO nanoemulsion reduced mucous cell diameter in the skin of silver catfish, but at 0.5 mL/kg, it triggered an increase in the diameter of mucosal cells in the branchial epithelium of silver catfish. This may be related to the presence of citral monoterpene and its β geranial and isomer (α-citral), the major components of CFEO (Gündel et al., 2018), which are recognized for their immunomodulatory properties (Bachiega and Sforcin, 2011). Besides, CFEO nanoemulsion at 0.25 mL/kg caused a reduction in mucosal cell density of silver catfish compared with 0.5 and 2.0 mL/kg. This result suggests that the supplementation of the diet with 0.25 mL/kg of CFEO nanoemulsion was not able to increase cell density; however, it may have minimized the interlamellar hyperplasia caused by microbial infection. The same concentration of CFEO (0.25 mL/kg diet) did not improve the biochemical and physiological responses of Nile tilapia challenged by *A. hydrophila* (Souza et al., 2020). The lack of a dose-response relationship, as observed in the current study, is common in the effects of dietary supplementation with essential oils. High doses may increase the amount of compounds that can have detrimental effects (Souza et al., 2019).

The histological analysis of the cutaneous epithelium revealed that the diameters of the mucosal cells of silver catfish were significantly smaller when the fish were treated with 0.5 and 2.0 mL/kg of CFEO nanoemulsion. One hypothesis for this reduction may be the physiological state of the mucous cells of these animals at the time of sample collection. Mucous cells originate in the middle layer of the epidermis, where they develop. As they mature, they accumulate mucus, increase in size, and are located on the surface of the epidermis, where mucus is secreted (Yamamoto et al., 2011). Thus, the smaller cell diameter observed here could be a consequence of the increase in mucus secretion, which may have caused a reduction in cell volume after mucus elimination.

The density of mucous cells can change as a result of bacterial infection (Alkan and Oğuz, 2021). It has been reported that if the challenge posed by the bacterial load may exceed the ability of the mucosal barrier to defend itself, the density of mucous cells would decrease indicating a state of depletion (Ledy et al., 2003; Thomas et al., 2013). However, the current research demonstrates that the density of mucosal cells in the cutaneous epithelium did not differ between treatments, indicating that the density of mucous cells in the skin of silver catfish naturally infected by *A. hydrophila* remained stable, suggesting a balance between challenge and response. Dietary addition of 5-10 g/kg ginger (*Zingiber officinale*) powder and ginger extract nanoparticles and 2.0 mL/kg EO of *Melaleucae aetheroleum* improved survival of Nile tilapia infected by *A. hydrophila* and *A. sobria*, respectively, and did not change histology of liver, kidney or intestine, and spleen (Ahmed et al., 2023; Shaalan et al., 2025).

The barrier status can be understood as a mathematical measure of mucosal barrier quality as a function of mucous cell diameter and volumetric density (Lazado et al., 2020). The present study revealed that there is no difference in the barrier status of the branchial and cutaneous epithelium of catfish juveniles naturally infected by *A. hydrophila*, suggesting that the barrier state was not impaired by the infection.

A. hydrophila infection has been linked to oxidative stress, being the lipid peroxidation, measured as TBARS, identified in several fish tissues affected by aeromonosis (Bandeira Junior and Baldisserotto, 2020). We found in the current research that after 25 days of feeding, dietary CFEO nanoemulsion increased TAC levels, and the diet containing 0.5 mL/kg of CFEO nanoemulsion reduced TBARS levels, linked to the elevation of TAC in the gills of silver catfish naturally infected by *A. hydrophila*. The CFEO nanoemulsion at 2.0 mL/kg also increased TAC levels in this condition. Total antioxidant capacity is a method that permits to estimate the power of total nonenzymatic antioxidants to counteract oxidative

stress induced cell damage (Apak et al., 2016). Therefore, we believe that the CFEO nanoemulsion stimulated the nonenzymatic antioxidant system of the silver catfish through its antioxidant features, since it is known that this essential oil has been strongly associated with citral monoterpene, which has remarkable antioxidant properties (Souza et al., 2018; Ling et al., 2022). However, citral, when used in its free form is hydrophobic; besides, it is unstable and can be oxidized if not stored properly, compromising its antioxidant activity (Lu et al., 2018). Thus, nanoemulsion, like this one used in the current investigation, has been shown to be an excellent protection tool for citral (Lu et al., 2018). Thus, we consider that nanoemulsion protects the antioxidant compounds of CFEO from possible oxidation, preserving their characteristics; and, therefore, we ascribe the reduction of TBARS to the presence of several antioxidant compounds, including citral.

In the present research, all the CFEO nanoemulsion levels added to the diet protected the liver of silver catfish naturally infected by *A. hydrophila*, since these animals exhibited histological characteristics similar to those found in the noninfected control fish. These results may be related to the antibacterial potential of CFEO in inhibiting bacterial infection by *A. hydrophila in vitro* and *in vivo* (Souza et al., 2016; Souza et al., 2020). The antibacterial activity of CFEO has been associated with the presence of monoterpenes, such as citral, constituent that is present in the essential oil used in this study. In a simplified way, it has been shown that citral is able to disrupt the lipid structure and penetrate the bacterial cell wall, causing cell lysis and death (Saddiq and Khayyat, 2010).

Citral nanoemulsion has shown antibacterial activity against different types of bacteria such as *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis*, and *Salmonella typhimurium* (Lu et al., 2018). Recently, it has been found that nanoemulsion was capable to increase the *in vitro* antibacterial activity of cinnamon (*Cinnamomum cassia*) essential oil against *A. hydrophila* (Bandeira Junior et al., 2022). According to the same authors, nanoemulsion can be an adequate mechanism to protect this compound. Thus, we believe that nanoemulsion protected the antibacterial compounds of CFEO against possible oxidation, preserving its characteristics.

5. Conclusions

The highest CFEO nanoemulsion levels in the diet (1.0 and 2.0 mL/kg) do not preserve the structure of the gill and cutaneous morphometry of silver catfish naturally infected by *A. hydrophila*. On the other hand, the supplementation of 0.5 mL/kg of CFEO nanoemulsion in the diet stimulates the antioxidant responses, since it reduces TBARS levels and increases TAC in the gills of infected silver catfish. All the CFEO nanoemulsion levels evoke a protective effect on the liver of infected silver catfish. Finally, the inclusion of CFEO nanoemulsion at 0.5 mL/kg of diet is highly advisable to protected silver catfish against *A. hydrophila* infection.

Data availability

The entire dataset supporting the results of this study is available upon reasonable request to the corresponding author.

Author contributions

Conceptualization: Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Baldisserotto, B. and Costa, S. T. **Data curation:** Valério Júnior, J. F. and Suarez-Barazeta, C. C. **Formal analysis:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Finamor, I. A.; Baldisserotto, B. and Costa, S. T. **Funding acquisition:** Baldisserotto, B. and Costa, S. T. **Investigation:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Finamor, I. A.; Pavanato, M. A.; Godoi, S. N.; Ourique, A. F.; Cargnelutti, J. F.; Wagner, R.; Veiga, M. L.; Baldisserotto, B. and Costa, S. T. **Methodology:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Finamor, I. A.; Pavanato, M. A.; Godoi, S. N.; Ourique, A. F.; Cargnelutti, J. F.; Wagner, R.; Veiga, M. L.; Baldisserotto, B. and Costa, S. T. **Project administration:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Baldisserotto, B. and Costa, S. T.

Resources: Finamor, I. A.; Pavanato, M. A.; Godoi, S. N.; Ourique, A. F.; Cargnelutti, J. F.; Wagner, R.; Veiga, M. L.; Baldisserotto, B. and Costa, S. T. **Supervision:** Baldisserotto, B. and Costa, S. T. **Validation:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Baldisserotto, B. and Costa, S. T. **Visualization:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Baldisserotto, B. and Costa, S. T. **Writing – original draft:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Finamor, I. A.; Pavanato, M. A.; Godoi, S. N.; Ourique, A. F.; Cargnelutti, J. F.; Wagner, R.; Veiga, M. L.; Baldisserotto, B. and Costa, S. T. **Writing – review & editing:** Valério Júnior, J. F.; Suarez-Barazeta, C. C.; Finamor, I. A.; Pavanato, M. A.; Godoi, S. N.; Ourique, A. F.; Cargnelutti, J. F.; Wagner, R.; Veiga, M. L.; Baldisserotto, B. and Costa, S. T.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brasil – process n. 301816/2022-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brasil – process n. 88887.604746/2021-00).

References

Abdel-Latif, H. M. R.; Abdel-Tawwab, M.; Khafaga, A. F. and Dawood, M. A. O. 2020a. Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (*Cyprinus carpio* L.) fingerlings. Aquaculture 526:1-10. https://doi.org/10.1016/j.aquaculture.2020.735432

Abdel-Latif, H. M. R.; Abdel-Tawwab, M.; Khafaga, A. F. and Dawood, M. A. O. 2020b. Dietary origanum essential oil improved antioxidative status, immune-related genes, and resistance of common carp (*Cyprinus carpio* L.) to *Aeromonas hydrophila* infection. Fish and Shellfish Immunology 104:1-7. https://doi.org/10.1016/j.fsi.2020.05.056

Abdel-Latif, H. M. R. and Khafaga, A. F. 2020. Natural co-infection of cultured Nile tilapia *Oreochromis niloticus* with *Aeromonas hydrophila* and *Gyrodactylus cichlidarum* experiencing high mortality during summer. Aquaculture Research 51:1880-1892. https://doi.org/10.1111/are.14538

Abdel-Rahim, M. M.; Elhetawy, A. I. G.; Mansour, A. T.; Mohamed, R. A.; Lotfy, A. M.; Sallam, A. E. and Shahin, S. A. 2023. Effect of long-term dietary supplementation with lavender, *Lavandula angustifolia*, oil on European seabass growth performance, innate immunity, antioxidant status, and organ histomorphometry. Aquaculture International 32:3275-3293. https://doi.org/10.1007/s10499-023-01322-1

Ahmed, A. A. S.; El-Murr, A.; Elhakim, Y. A.; Metwally, M. M.; Gharib, A. A. A.; Amer, S. A.; Younis, E. M.; Abdel-Warith, A. A.; Davies, S. J. and Khalil, E. N. M. 2023. Comparative study on ginger powder and ginger extract nanoparticles: Effects on growth, immune–antioxidant status, tissue histoarchitecture, and resistance to *Aeromonas hydrophila* and *Pseudomonas putida* infection in *Oreochromis niloticus*. Fishes 8:259. https://doi.org/10.3390/fishes8050259

Alkan, Z. and Oğuz, A. R. 2021. Investigation of gill mucus cells of Lake Van fish (*Alburnus tarichi*) during reproductive migration. Fish Physiology Biochemistry 47:409-419. https://doi.org/10.1007/s10695-020-00921-6

AlYahya, S. A.; Ameen, F.; Al-Niaeem, K. S.; Al-Saadi, B. A.; Hadi, S. and Mostafa, A. A. 2018. Histopathological studies of experimental *Aeromonas hydrophila* infection in blue tilapia, *Oreochromis aureus*. Saudi Journal of Biological Sciences 25:182-185. https://doi.org/10.1016/j.sjbs.2017.10.019

Apak, R.; Özyürek, M.; Güçlü, K. and Çapanoglu, E. 2016. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. Journal of Agricultural and Food Chemistry 64:997-1027. https://doi.org/10.1021/acs.jafc.5b04739

Baccega, B.; Islabão, Y. W.; Mello, A. B.; Martins, F. O.; Santos, C. C.; Ourique, A. F.; Gündel, S. S.; Macedo, M. R. P.; Vianna, E. E. S.; Farias, N. A. R. and Oliveira, C. B. 2021. *In vitro* and *in vivo* activity of the essential oil and nanoemulsion of *Cymbopogon flexuosus* against *Trichomonas gallinae*. Avicenna Journal of Phytomedicine 11:32-43. https://doi.org/10.22038/AJP.2020.15662

Bachiega, T. F. and Sforcin, J. M. 2011. Lemongrass and citral effect on cytokines production by murine macrophages. Journal of Ethnopharmacology 137:909-913. https://doi.org/10.1016/j.jep.2011.07.021

Baldissera, M. D.; Souza, C. F.; Júnior, G. B.; Vargas, A. C.; Boligon, A. A.; Campos, M. M. A.; Stefani, L. M. and Baldisserotto, B. 2017. *Melaleuca alternifolia* essential oil enhances the non-specific immune system and prevents oxidative damage in *Rhamdia quelen* experimentally infected by *Aeromonas hydrophila*: Effects on cholinergic and purinergic systems in liver tissue. Fish and Shellfish Immunology 61:1-8. https://doi.org/10.1016/j.fsi.2016.12.016

Bandeira Junior, G. and Baldisserotto, B. 2020. Fish infections associated with the genus *Aeromonas*: a review of the effects on oxidative status. Journal of Applied Microbiology 131:1083-1101. https://doi.org/10.1111/jam.14986

Bandeira Junior, G.; Bianchini, A. E.; Souza, C. F.; Descovi, S. N.; Fernandes, L. S.; Silva, L. L.; Cargnelutti, J. F. and Baldisserotto, B. 2022. The use of cinnamon essential oils in aquaculture: Antibacterial, anesthetic, growth-promoting, and antioxidant effects. Fishes 7:133. https://doi.org/10.3390/fishes7030133

Brum, A.; Cardoso, L.; Chagas, E. C.; Chaves, F. C. M.; Mouriño, J. L. P. and Martins, M. L. 2018. Histological changes in Nile tilapia fed essential oils of clove basil and ginger after challenge with *Streptococcus agalactiae*. Aquaculture 490:98-107. https://doi.org/10.1016/j.aquaculture.2018.02.040

Campos, A. M. and Lissi, E. A. 1997. Kinetics of the reaction between 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) derived radical cations and phenols. International Journal of Chemical Kinetics 29:219-224.

Chen, N.; Jiang, J.; Gao, X.; Li, X.; Zhang, Y.; Liu, X.; Yang, H.; Bing, X. and Zhang, X. 2018. Histopathological analysis and the immune related gene expression profiles of mandarin fish (*Siniperca chuatsi*) infected with *Aeromonas hydrophila*. Fish and Shellfish Immunology 83:410-415. https://doi.org/10.1016/j.fsi.2018.09.023

Cunha, M. A.; Zeppenfeld, C. C.; Garcia, L. O.; Loro, V. L.; Fonseca, M. B.; Emanuelli, T.; Veeck, A. P.; Copatti, C. E. and Baldisserotto, B. 2010. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet. Ciência Rural 40:2107-2114.

Dang, M.; Pittman, K.; Sonne, C.; Hansson, S.; Bach, L.; Søndergaard, J.; Stride, M. and Nowak, B. 2020. Histological mucous cell quantification and mucosal mapping reveal different aspects of mucous cell responses in gills and skin of shorthorn sculpins (*Myoxocephalus scorpius*). Fish and Shellfish Immunology 100:334-344. https://doi.org/10.1016/j.fsi.2020.03.020

Das, S.; Pradhan, C. and Pillai, D. 2023. Dietary coriander (*Coriandrum sativum* L) oil improves antioxidant and anti-inflammatory activity, innate immune responses and resistance to *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). Fish and Shellfish Immunology 132:108486. https://doi.org/10.1016/j.fsi.2022.108486

Dawood, M. A. O.; El Basuini, M. F.; Zaineldin, A. I.; Yilmaz, S.; Hasan, M. T.; Ahmadifar, E.; El Asely, A. M.; Abdel-Latif, H. M. R.; Alagawany, M.; Abu-Elala, N. M.; Van Doan, H. and Sewilam, H. 2021. Antiparasitic and antibacterial functionality of essential oils: An alternative approach for sustainable aquaculture. Pathogens 10:185. https://doi.org/10.3390/pathogens10020185

Devalapally, H.; Chakilam, A. and Amiji, M. M. 2007. Role of nanotechnology in pharmaceutical product development. Journal of Pharmaceutical Sciences 96:2547-2565. https://doi.org/10.1002/jps.20875

Devi, M. A.; Sahoo, D.; Singh, T. B. and Rajashekar, Y. 2021. Antifungal activity and volatile organic compounds analysis of essential oils from *Cymbopogon species* using solid-phase microextraction-gas chromatography-mass spectrometry. Journal of Agriculture and Food Research 3:100110. https://doi.org/10.1016/j.jafr.2021.100110

Doyle, D.; Carney Almroth, B.; Sundell, K.; Simopoulou, N. and Sundh, H. 2022. Transport and barrier functions in rainbow trout trunk skin are regulated by environmental salinity. Frontiers in Physiology 13:882973. https://doi.org/10.3389/fphys.2022.882973

Gao, S.; Liu, G.; Li, J.; Chen, J.; Li, L.; Li, Z.; Zhang, X.; Zhang, S.; Thorne, R. F. and Zhang, S. 2020. Antimicrobial activity of lemongrass essential oil (*Cymbopogon flexuosus*) and its active component citral against dual-species biofilms of *Staphylococcus aureus* and *Candida* Species. Frontiers in Cellular and Infection Microbiology 10:603858. https://doi.org/10.3389/fcimb.2020.603858

Gomes, L. C.; Golombieski, J. I.; Gomes, A. R. C. and Baldisserotto, B. 2000. Biologia do jundiá *Rhamdia quelen* (Teleostei, Pimelodidae). Ciência Rural 30:179-185. https://doi.org/10.1590/s0103-84782000000100029

Gündel, S. S.; Souza, M. E.; Quatrin, P. M.; Klein, B.; Wagner, R.; Gündel, A.; Vaucher, R. A.; Santos, R. C. V. and Ourique, A. F. 2018. Nanoemulsions containing *Cymbopogon flexuosus* essential oil: Development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. Microbial Pathogenesis 118:268-276. https://doi.org/10.1016/j.micpath.2018.03.043

Hajirezaee, S.; Rohanizadehghadikolaei, F.; Afzali-Kordmahalleh, A. and Khanjani, M. H. 2024. Effects of dietary common juniper (*Juniperus communis*) essential oil on growth, immunity, antioxidant status, and disease resistance in rainbow trout, *Oncorhynchus mykiss*. Aquaculture Reports 34:101895. https://doi.org/10.1016/j.aqrep.2023.101895

Hermes-Lima, M.; Willmore, W. G. and Storey, K. B. 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. Free Radical Biology and Medicine 19:271-280. https://doi.org/10.1016/0891-5849(95)00020-X

Kumar, S.; Prasad, R.; Kumar, V. and Krishna, A. K. 2021. Organic source on productivity of pomegranate–lemongrass-based agroforestry system in central India. Agroforestry Systems 95:615-624. https://doi.org/10.1007/s10457-021-00605-x

Lazado, C. C.; Haddeland, S.; Timmerhaus, G.; Berg, R. S.; Merkin, G.; Pittman, K. and Pedersen, L. F. 2020. Morphomolecular alterations in the skin mucosa of Atlantic salmon (*Salmo salar*) after exposure to peracetic acid-based disinfectant. Aquaculture Reports 17:100368. https://doi.org/10.1016/j.aqrep.2020.100368

Ledy, K.; Giambérini, L. and Pihan, J. C. 2003. Mucous cell responses in gill and skin of brown trout *Salmo trutta fario* in acidic, aluminium-containing stream water. Diseases of Aquatic Organisms 56:235-240. https://doi.org/10.3354/dao056235

Ling, Q.; Zhang, B.; Wang, Y.; Xiao, Z.; Hou, J.; Xiao, C.; Liu, Y. and Jin, Z. 2022. Chemical composition and antioxidant activity of the essential oils of citral-rich chemotype *Cinnamomum camphora* and *Cinnamomum bodinieri*. Molecules 27:7356. https://doi.org/10.3390/molecules27217356

Lu, W. C.; Huang, D. W.; Wang, C. C. R.; Yeh, C. H.; Tsai, J. C.; Huang, Y. T. and Li, P. H. 2018. Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. Journal of Food and Drug Analysis 26:82-89. https://doi.org/10.1016/j.jfda.2016.12.018

Lushchak, V. I. 2016. Contaminant-induced oxidative stress in fish: a mechanistic approach. Fish Physiology and Biochemistry 42:711-747. https://doi.org/10.1007/s10695-015-0171-5

Mansour, A. T.; Arisha, A. H.; Abdelaziz, R.; Alwutayd, K. M.; Van Doan, H.; El-Murr, A. E. and El-Houseiny, W. 2024. Effects of extended dietary supplementation with *Santalum album* essential oil on hemato-biochemical changes, innate immune response, antioxidant status, and expression of related gene in Nile tilapia (*Oreochromis niloticus*). Fish Physiology and Biochemistry 50:955-971. https://doi.org/10.1007/s10695-024-01309-6

Marinho-Neto, F. A.; Claudiano, G. S.; Yunis-Aguinaga, J.; Cueva-Quiroz, V. A.; Kobashigawa, K. K.; Cruz, N. R. N.; Moraes, F. R. and Moraes, J. R. E. 2019. Morphological, microbiological and ultrastructural aspects of sepsis by *Aeromonas hydrophila* in *Piaractus mesopotamicus*. PLoS ONE 14:e0222626. https://doi.org/10.1371/journal.pone.0222626

Mousavi, S.; Sheikhzadeh, N.; Hamidian, G.; Mardani, K.; Oushani, A. K.; Firouzamandi, M.; Esteban, M. and Shohreh, P. 2021. Changes in rainbow trout (*Oncorhynchus mykiss*) growth and mucosal immune parameters after dietary administration of grape (*Vitis vinifera*) seed extract. Fish Physiology and Biochemistry 47:547-563. https://doi.org/10.1007/s10695-021-00930-z

Nichols, J. A. and Katiyar, S. K. 2010. Skin photoprotection by natural polyphenols: Anti-inflammatory, antioxidant and DNA repair mechanisms. Archives of Dermatological Research 302:71-83. https://doi.org/10.1007/s00403-009-1001-3

Nimalan, N.; Sørensen, S. L.; Fečkaninová, A.; Koščová, J.; Mudroňová, D.; Gancarčíková, S.; Vatsos, I. N.; Bisa, S.; Kiron, V. and Sørensen, M. 2022. Mucosal barrier status in Atlantic salmon fed marine or plant-based diets supplemented with probiotics. Aquaculture 547:737516. https://doi.org/10.1016/j.aquaculture.2021.737516

Oliveira, N. Y.; Sousa, O. M.; Kuhnen, V. V. and Sanches, E. G. 2019. Shelters influence in dusky grouper culture. Boletim do Instituto de Pesca 45:e473. https://doi.org/10.20950/1678-2305.2019.45.3.473

Özil, Ö.; Diler, Ö. and Nazıroğlu, M. 2022. Antifungal activity of some essential oil nanoemulsions against Saprolegniasis in rainbow trout (*Oncorhynchus mykiss*) eggs: Antifungal activity of essential oil nanoemulsions. Aquaculture International 30:2201-2212. https://doi.org/10.1007/s10499-022-00897-5

Pathirana, H. N. K. S.; Wimalasena, S. H. M. P.; Silva, B. C. J.; Hossain, S. and Heo, G. J. 2019. Determination of the in vitro effect of lemongrass (*Cymbopogon flexuosus*) oil against fish pathogenic bacteria isolated from cultured olive flounder (*Paralichthys olivaceus*). Slovenian Veterinary Research 56:125-131. https://doi.org/10.26873/SVR-687-2019

Pittman, K.; Sourd, P.; Ravnøy, B.; Espeland, Ø; Fiksdal, I. U.; Oen, T.; Pittman, A.; Redmond, K. and Sweetman, J. 2011. Novel method for quantifying salmonid mucous cells. Journal of Fish Diseases 34:931-936. https://doi.org/10.1111/j.1365-2761.2011.01308.x

Saddiq, A. A. and Khayyat, S. A. 2010. Chemical and antimicrobial studies of monoterpene: citral. Pesticide Biochemistry and Physiology 98:89-93. https://doi.org/10.1016/j.pestbp.2010.05.004

Santos, A. C.; Junior, G. B.; Zago, D. C.; Zeppenfeld, C. C.; Silva, D. T.; Heinzmann, B. M.; Baldisserotto, B. and Cunha, M. A. 2017. Anesthesia and anesthetic action mechanism of essential oils of *Aloysia triphylla* and *Cymbopogon flexuosus* in silver catfish (*Rhamdia quelen*). Veterinary Anaesthesia and Analgesia 44:106-113. https://doi.org/10.1111/vaa.12386

Schneider, C. A.; Rasband, W. S. and Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671-675. https://doi.org/10.1038/nmeth.2089

Shaalan, M.; Mahboub, H. H.; Abdelgawad, A. H.; Abdelwarith, A. A.; Younis, E. M.; Elnegiry, A. A.; Basher, A. W.; El-Houseiny, W.; Shawky, S. M.; Orabi, S. H.; Davies, S. J. and Mahmoud, Y. K. 2025. Dietary tea tree (*Melaleucae Aetheroleum*) oil fortifies growth, biochemical, immune-antioxidant trait, gene function, tissue reaction, and *Aeromonas sobria* resistance in Nile tilapia (*Oreochromis niloticus*). BMC Veterinary Research 21:1. https://doi.org/10.1186/s12917-024-04369-1

Shourbela, R. M.; El-Hawarry, W. N.; Elfadadny, M. R. and Dawood, M. A. O. 2021. Oregano essential oil enhanced the growth performance, immunity, and antioxidative status of Nile tilapia (*Oreochromis niloticus*) reared under intensive systems. Aquaculture 542:736868. https://doi.org/10.1016/j.aquaculture.2021.736868

Souza, C. F.; Baldissera, M. D.; Vaucher, A. R.; Lopes, L. Q. S.; Vizzotto, B. S.; Raffin, R. P.; Santos, R. C. V.; Veiga, M. L.; Rocha, M. I. U. M.; Stefani, L. M. and Baldisserotto, B. 2016. *In vivo* bactericidal effect of *Melaleuca alternifolia* essential oil against *Aeromonas hydrophila*: Silver catfish (*Rhamdia quelen*) as an experimental model. Microbial Pathogenesis 98:82-87. https://doi.org/10.1016/j.micpath.2016.07.002

Souza, C. F.; Baldissera, M. D.; Baldisserotto, B.; Heinzmann, B. M.; Martos-Sitcha, J. A. and Mancera, J. M. 2019. Essential oils as stress-reducing agents for fish aquaculture: A review. Frontiers in Physiology 10:785. https://doi.org/10.3389/fphys.2019.00785

Souza, E. M.; Souza, R. C.; Melo, J. F. B.; Costa, M. M.; Seldon, S. A.; Souza, A. M. and Copatti, C. E. 2020. *Cymbopogon flexuosus* essential oil as an additive improves growth, biochemical and physiological responses and survival against

Aeromonas hydrophila infection in Nile tilapia. Anais da Academia Brasileira de Ciências 92:e20190140. https://doi.org/10.1590/0001-3765202020190140

Souza, F. C.; Rampelotto, C.; Loureiro, B. B.; Pereira, F. A.; Bianchini, A. E.; Corcini, C. D.; Varela Junior, A. S.; Emanuelli, T.; Silva, L. P.; Costa, S. T.; Bertolin, K.; Rovani, M. T.; Gonçalves, P. B. D.; Heinzmann, B. M. and Baldisserotto, B. 2018. Effects of dietary microencapsulated *Cymbopogon flexuosus* essential oil on reproductive-related parameters in male *Rhamdia quelen*. Fish Physiology and Biochemistry 44:1253-1264. https://doi.org/10.1007/s10695-018-0518-9

Sun, B. Y.; He, W.; Yang, H. X.; Tian, D. Y.; Jian, P. Y.; Wu, K.; Yang, C. G. and Song, X. H. 2022. Increased susceptibility to *Aeromonas hydrophila* infection in grass carp with antibiotic-induced intestinal dysbiosis. Aquaculture 552:737969. https://doi.org/10.1016/j.aquaculture.2022.737969

Thomas, J.; Jerobin, J.; Seelan, T. S. J.; Thanigaivel, S.; Vijayakumar, S.; Mukherjee, A. and Chandrasekaran, N. 2013. Studies on pathogenecity of *Aeromonas salmonicida* in catfish *Clarias batrachus* and control measures by neem nanoemulsion. Aquaculture 396-399:71-75. https://doi.org/10.1016/j.aquaculture.2013.02.024

Tyagi, A. K. and Malik, A. 2010. Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: microscopic observations and chemical characterization of *Cymbopogon citratus*. BMC Complementary and Alternative Medicine 10:65. https://doi.org/10.1186/1472-6882-10-65

Valenti, W. C.; Barros, H. P.; Moraes-Valenti, P.; Bueno, G. W. and Cavalli, R. O. 2021. Aquaculture in Brazil: past, present and future. Aquaculture Reports 19:100611. https://doi.org/10.1016/j.aqrep.2021.100611

Viana, A. R.; Fortes, C. R.; Rodrigues, P.; Ribeiro, S.; Heinzmann, B. M.; Cunha, M. A.; Zeppenfeld, C. C.; Fernandes, L. S.; Wagner, R.; Baldisserotto, B.; Figueiredo, H. X.; Gonçalves, N. N.; Chitolina, A. B. M.; Justen, C. E. and Baldissera, M. D. 2023. Impact of dietary supplementation with *Cinnamomum cassia* essential oil on silver catfish (*Rhamdia quelen*): Toxicological effects and muscle antioxidant and fatty acid profiles. Journal of Toxicology and Environmental Health - Part A 86:313-325. https://doi.org/10.1080/15287394.2023.2198564

Yamamoto, T.; Kawai, K. and Oshima, S. 2011. Distribution of mucous cells on the body surface of Japanese flounder *Paralichthys olivaceus*. Journal of Fish Biology 78:848-859. https://doi.org/10.1111/j.1095-8649.2010.02898.x

Younis, N. A.; Elgendy, M. Y.; El-Samannoudy, S. I.; Abdelsalam, M. and Attia, M. M. 2023. *Cyathocotylidae* spp and motile aeromonads co-infections in farmed Nile tilapia (*Oreochromis niloticus*) causing mass mortality. Microbial Pathogenesis 174:105897. https://doi.org/10.1016/j.micpath.2022.105897

Zeppenfeld, C. C.; Hernández, D. R.; Santinón, J. J.; Heinzmann, B. M.; Cunha, M. A.; Schmidt, D. and Baldisserotto, B. 2016. Essential oil of *Aloysia triphylla* as feed additive promotes growth of silver catfish (*Rhamdia quelen*). Aquaculture Nutrition 22:933-940. https://doi.org/10.1111/anu.12311

Zheng, T.; Song, Z.; Qiang, J.; Tao, Y.; Zhu, H.; Ma, J. and Xu, P. 2021. Transport stress induces skin innate immunity response in hybrid yellow catfish (*Tachysurus fulvidraco*♀ × *P. vachellii*♂) through TLR/NLR signaling pathways and regulation of mucus secretion. Frontiers in Immunology 12:740359. https://doi.org/10.3389/fimmu.2021.740359