Ciência Rural, Santa Maria, v.56:5, e20250271, 2026
http://doi.org/10.1590/0103-8478cr20250271
ISSNe 1678-4596
MICROBIOLOGY

Cymbopogon flexuosus essential oil nanoemulsion added to the diet of silver catfish naturally infected by *Aeromonas hydrophila*: survival, biomarkers of oxidative stress, and intestinal morphology

Cristian Camilo Suarez-Barazeta[1] José Francisco Valério Júnior[1] Isabela Maraschin Vieira[2] Silvio Teixeira da Costa[2] Isabela Andres Finamor[3] Maria Amália Pavanato[3] Carla Cristina Zeppenfeld[3] Aline Ferreira Ourique[3] Juliana Felipetto Cargnelutti[4] Samantha Nunes de Godoi[5] Bernardo Baldisserotto[1, 3*]

[1]Programa de Pós-graduação em Zootecnia, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[2]Departamento de Morfologia, Centro de Ciências da Saúde (CCS), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[3]Departamento de Fisiologia e Farmacologia, Centro de Ciências da Saúde (CCS),Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E -mail: bernardo.baldisserotto@ufsm.br. *Corresponding author.
[4]Laboratório de Bacteriologia, Departamento de Medicina Vaterinária Preventiva, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil.
[5]Laboratório de Nanotecnologia, Universidade Franciscana (UFN), Santa Maria, RS, Brasil.

ABSTRACT: This study examined the intestinal morphology and the oxidative status of different tissues of silver catfish (*Rhamdia quelen*) naturally infected or not by *Aeromonas hydrophila* and fed diets supplemented with *Cymbopogon flexuosus* essential oil (EOCF) nanoemulsion (0 - control, 0.25, 0.5, and 1.0 mL of EOCF per kg of diet) for 25 days. Dietary EOCF supplementation did not alter thiobarbituric acid reactive substances levels in the liver; however, in muscle, reduced superoxide anion production. Regarding muscle, the infected group fed with 0.25 mL of EOCF per kg of diet showed higher activity of the total antioxidant capacity compared to the uninfected group. Fish fed 0.25 mL per kg of diet had a survival rate of 72.05%, compared to 17.64% for the infected control. The intestinal fold height was significantly lower in the 0.5 mL EO group compared to the uninfected control group. Fish fed 0.5 and 1 mL per kg of diet presented a lower number of goblet cells than uninfected controls. The addition of 0.25 mL of EO per kg of diet is recommended for use as a feed additive since it increases the antioxidant responses, survival, and the resistance of silver catfish to *A. hydrophila* infection.
Key words: antioxidant, bacterial infection, histopathology, immunostimulant, prophylactic.

Nanoemulsão de óleo essencial de *Cymbopogon flexuosus* adicionada à dieta de jundiá naturalmente infectado por *Aeromonas hydrophila*: sobrevivência, biomarcadores de estresse oxidativo e morfometria intestinal

RESUMO: Este estudo examinou a morfologia intestinal e o estado oxidativo de diferentes tecidos de jundiá (*Rhamdia quelen*) naturalmente infectados ou não por *Aeromonas hydrophila* e alimentados com dietas suplementadas com nanoemulsão de óleo essencial de *Cymbopogon flexuosus* (EOCF) (0 - controle, 0,25, 0,5 e 1,0 mL de OECF por kg de dieta) por 25 dias. A suplementação dietética de nanoemulsão do OECF não alterou os níveis de substâncias reativas ao ácido tiobarbitúrico no fígado; entretanto, no músculo, reduziu a produção de ânions superóxido. Em relação ao músculo, o grupo infectado e alimentado com 0,25 mL de OECF por kg de dieta apresentou maior atividade da capacidade antioxidante total em comparação com o grupo não infectado. Os peixes alimentados com 0,25 mL por kg de dieta tiveram uma taxa de sobrevivência de 72,05%, em comparação com 17,64% para o controle infectado. A altura da dobra intestinal foi significativamente menor no grupo OE de 0,5 mL em comparação com o grupo controle não infectado. Os peixes alimentados com 0,5 e 1,0 mL por kg de dieta apresentaram menor número de células caliciformes do que os controles não infectados. A adição de 0,25 mL de OE por kg de dieta é recomendada para uso como aditivo alimentar, pois aumenta as respostas antioxidantes, a sobrevivência e a resistência do jundiá à infecção por *A. hydrophila*.
Palavras-chave: antioxidante, histopatologia, infecção bacteriana, imunoestimulante, profilática.

INTRODUCTION

The gastrointestinal tract (GIT) in fish is a multifunctional organ, with a leading role in several physiological functions (GROSELL et al., 2010). In addition to the well-known and described functions in diet digestion and nutrient absorption, it has a decisive role in endocrine regulation, metabolism, and in the defense of the organism against the entry of pathogens (SALINAS & PARRA, 2015). In recent years, the term “gut health” has been coined to describe an ideal state of nutrition, metabolism, immunity, pathogen

Received 05.20.25 Approved 09.10.25 Returned by the author 01.29.26
CR-2025-0271.R1
Editor: Rudi Weiblen

control, zootechnical performance, behavior, and animal welfare (FIRMINO et al., 2021a). Despite the ambiguity of the term, elements such as diet, functional mucosal barrier structure, effective digestion and absorption of nutrients, appropriate and stable microbiota, and effective immunity are considered to be key elements in defining good gastrointestinal health (CELI et al., 2017).

The plasticity and compartmentalized ecology of the GIT is particularly attractive to the fish farming industry, since the gut is the main source of pathogen entry, allows the entry of drugs and supplements incorporated in the feed, modulates the colonization of the microbial community from the gastrointestinal immune system, contributing to the welfare of the host by cooperating in key elements such as digestion and pathogen defense (SALINAS & PARRA, 2015). Understanding this as a multifactorial eco-physiological system will allow a better understanding and manipulation of it in aquaculture. In this sense, functional additives have been postulated as a tool to manipulate these factors, given their benefits in regulating metabolism, improving nutrient absorption, activating immunity by stimulating the innate immune system, promoting colonization of specific microorganisms (probiotics), inhibiting pathogens in the GIT (HOSEINIFAR et al., 2019), or by their antioxidant properties that act as free radicals scavengers (LEE et al., 2004). This antioxidant property has been associated with functional additives such as essential oils (EOs), recognized to be biocompatible, biodegradable, and safe for the ecosystem, animals, and human health, and categorized as eco-friendly products (VASEEHARAN & THAYA, 2013). These phytochemicals have shown positive results in improving survival rates against bacterial infections by improving the antioxidant responses of channel catfish (*Ictalurus punctatus*) (ZHENG et al., 2009) and juvenile Jian carp (*Cyprinus carpio* var. Jian) (ZHAO et al., 2013). There are also multiple reports in which supplementation with these bioactives generates improvements in the antioxidant responses in several tissues of different species of fish (DOS SANTOS et al., 2017; SOUZA et al., 2018a).

Although, many of the EOs meet the characteristics to be considered functional additives, physicochemical characteristics of these bioactives, such as their high volatility, high hydrophobicity, highly unsaturated nature, and high content of double bonds, make them very sensitive to environmental stressors such as light, humidity, and temperature (AMEUR et al., 2022; ZHOU et al., 2018). In addition to the above, degradation by gastric juices, resistance to bile salts, or continuous changes in pH and enzyme types are other challenges that EOs must face in their transit through the GIT (YANG et al., 2016). Considering the above, nanoemulsions have been used in recent years as a coating and protection system against adverse conditions. This transport system helps to stabilize and deliver lipophilic nutraceuticals (ZHOU et al., 2018), potentiate the biological and functional characteristics of the oils and enable the bioavailability of the supplement throughout the GIT (HWANG et al., 2016).

In this regard, this study chose the EO of *Cymbopogon flexuosus* (EOCF) in a nanocarrier system as a functional additive. The EOCF has been previously described for its antimicrobial, antioxidant, insecticidal, analgesic, and immunostimulant properties (AVOSEH et al., 2015). Studies on EOCF nanoemulsions reported how this phytocomponent can reduce the adhesion of pathogenic microorganisms to surfaces by inhibiting biofilm formation (GÜNDEL et al., 2018). Furthermore, incorporating EOCF in a nanocarrier system has been shown to enhance its antifungal activity compared to the EO in its free state form (GÜNDEL et al., 2020). An example is the inhibition of growth and biofilm formation of the antibiotic-resistant (SEMWAL et al., 2023) opportunistic pathogen *A. hydrophila* by EOs from plants of the genus *Cymbopogon* (FELIX E SILVA et al., 2022). This bacterium causes epidemic outbreaks of motile *Aeromonas* septicemia (MAS) in species such as carp (*Cyprinus carpio*), bream (*Megalobrama amblycephala*), and channel catfish (*Ictalurus punctatus*) (HOSSAIN et al., 2014; KHAN et al., 2023). The damage caused by this pathogen has been linked to the bacteria's capacity to compromise the antioxidant status by inducing protein oxidation and inhibiting antioxidant enzymes (TKACHENKO et al., 2014). In this context, the present study aimed to evaluate the effects of the inclusion of EOCF nanoemulsion as a functional ingredient in the diet of silver catfish (*Rhamdia quelen*) naturally infected by *A. hydrophila*. The study assessed its impact on oxidative stress biomarkers, intestinal histomorphology, and performance indicators.

MATERIALS AND METHODS

Fish

The silver catfish juveniles (7.60 ± 3.29 g; 10.01 ± 1.40 cm) were obtained from a fish farming in Cruz Alta, Rio Grande do Sul State, Brazil. The experiments were conducted on a recirculation

aquaculture system in the Fish Physiology Laboratory at the UFSM. The animals were randomly distributed in twenty polypropylene boxes (70 L), 17 fish per tank, and acclimated to the laboratory conditions for one week. Water temperature, pH, dissolved oxygen, and mortality were checked daily, while ammonia concentrations were measured weekly.

Water parameters

The temperature (23.2 ± 2.24 ºC) and dissolved oxygen levels (4.6 ± 1.31 mg L^{-1}) were measured with a YSI oxygen meter (Model Y5512; YSI Inc., Yellow Springs, OH, USA). The pH (7.2 ± 0.4) was monitored with a DMPH-2 pH meter (Digimed, São Paulo, Brazil). Nesslerization was used to verify the total ammonia nitrogen levels (0.025 ± 0.04 mg L^{-1}) according to the method of EATON & FRANSON (2005).

Reagents

The EOCF was obtained from the FERQUIMA Indústria e Comércio Ltda (Brazil). Polysorbate 80 was acquired from Synth (Brazil), sorbitan monooleate was obtained from Sigma Aldrich (Brazil), and Crodamol GTCC (triglycerides of capric and caprylic acid) from Alpha Chemistry (Brazil). The physicochemical description of EOCF was made by (GÜNDEL et al., 2018).

Preparation of Nanoemulsions

The nanoemulsions (n = 2) were prepared employing the method of homogenization under high agitation according to (GÜNDEL et al., 2018) with some modifications. The oil phase was composed of 5% EOCF and 2% sorbitan monooleate (Span 80), and the aqueous phase was formed of 2% polysorbate 80 (Tween 80) and ultrapure water. It was designed as a control blank replacing the EO with a saturated medium-chain triglyceride (CrodamolTM) without antimicrobial activity. To ensure reproducibility and confirm the stability of the nanoemulsion, key physicochemical parameters were assessed. The average droplet size was 78.46 ± 0.51 d.nm, with a polydispersity index of 0.25 ± 0.002, indicating a homogeneous distribution. The zeta potential was -10.2 ± 0.34 mV, and the pH of the formulation was 4.02 ± 0.03. These measurements were obtained using a Nano-ZS Zetasizer® (model ZEN 3600, Malvern, UK) and a DM-22 pH meter (Digimed®, São Paulo, Brazil).

Diets and experimental design

Four diets were produced based on the formulation of ZEPPENFELD et al. (2016). The diet contained 30% soybean meal, 35% meat meal, 12% rice bran, 15% corn bran, 3% canola oil, 1 % salt, 3 % vitamins and minerals (Premix), and 1 % phosphate dicalcium. Three different doses of EOCF nanoemulsion (0.25, 0.5, and 1.0 mL per kg of diet) were added to the mixture together with the canola oil (Table 1). The control diet was composed by the same formulation with 2 mL of crodamolTM nanoemulsion per kg of diet. Water was added to the diets, which were then pelletized. A drying process was performed in a forced air circulation oven for 24 h (40 ºC), and finally stored at -18 ºC. The animals received experimental feeds until apparent satiation twice a day (9 am and 7 pm) for 25 days.

The experimental design resulted in four groups, each with four replicates. However, during the experiment, fish suffered natural infection by *A. hydrophila*, which led to mortality of all fish from the control treatment. Faced with the need to have a control to compare the supplemented treatments, a new control was implemented. This new control complied with all experimental conditions (25 days, 4 replicates, diet, etc.) with the exception that it did not present symptoms of bacterial pathologies during and at the end of the experiment (Figure 1). These last fish were used in all the posterior analysis, except for the mortality. Mortality in groups with bacteria was used to calculate the Relative Percentage Survival (RPS) following the equation described in (BABA et al., 2016).

RPS = 100 - [(Mortality (%) in treated group) / (Mortality (%) in control group)] ×100

After 25 days of the trial, ten fish by treatment were anesthetized with 50 µL L^{-1} of eugenol for two minutes (CUNHA et al., 2010) and euthanized by sectioning the spinal cord. Muscle and liver were removed, frozen, and stored at -80 ºC for further analysis. The intestine was removed and placed in 10% neutral formalin for histological tests.

Microbiology analysis

During the experiment, deceased fish were subjected to necropsy. Samples from the kidney, gill, and superficial lesions were collected for microbiological analysis. An oxidase-positive, Gram-negative bacterium was isolated from all samples and identified as *Aeromonas hydrophila* through biochemical assays (MARKEY et al., 2013).

Oxidative stress biomarkers

For the oxidative stress biomarkers, tissues (muscle and liver) were homogenized according to DA ROSA et al. (2019). The following measures were

Table 1 - Formulation of the experimental diets.

Ingredients (g/kg)	----------Diets (mL of EOCF nanoemulsion per kg of diet)----------			
	Control	0.25	0.5	1
Soybean meal	300	300	300	300
Meat flour	350	350	350	350
Rice bran	120	120	120	120
Corn bran	150	150	150	150
Canola oil	30	30	30	30
Salt	10	10	10	10
Mineral and vitamin (premix)	30	30	30	30
EOCF nanoemulsion	0	0.25	0.5	1
Dicalcium phosphate	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	

1Vitamin 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: 1000 000 UI, vitamin B2: 2500 mg, vitamin C: 28000 mg, vitamin K: 500 mg, vitamin D3: 500000 UI, vitamin E: 20000 UI, , zinc: 17500 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.

performed in the muscle: superoxide anion ($O_2^{\cdot-}$) (WANG et al., 1998), total superoxide dismutase (SOD) (MISRA & FRIDOVICH, 1972), catalase (CAT) (AEBI, 1984), glutathione S-transferase (GST) (HABIG et al., 1974) and total antioxidant capacity (TAC) (CAMPOS & LISSI, 1997). In the liver, the lipid peroxidation was estimated using thiobarbituric acid reactive substances (TBARS) technique (HERMES-LIMA et al., 1995) and total glutathione levels were determined as (ELLMAN, 1959).

Histological examination

The intestine fixed with 10% neutral formalin was sliced and dehydrated with alcohol, embedded in paraffin, and cut into 6 µm slices with a microtome (Easy path®). The slides were stained with hematoxylin and eosin (HE), and the PAS and Alcian Blue staining were used to identify the goblet cells (ZHENG et al., 2021). All the samples were viewed and photographed under the light microscope Axio Scope.A1 adapted with a photographer system Axiocam 105 color (ZEISS®, Germany). Gastrointestinal morphometry was analyzed by measuring the thickness of the muscle layer, villus height, number of goblet cells, number of villi, total intestinal area, total area of the muscle layer, area of villi, and light area of the intestine (Figure 2). The light area of the intestine allowed for determining the

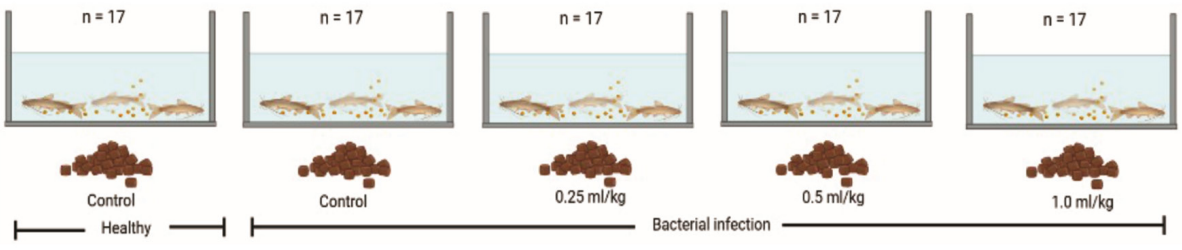


Figure 1 - Final experimental design. An uninfected (healthy) control group was added to the experimental design due to total mortality of the *A. hydrophila*-naturally infected group (four replicates by treatment).

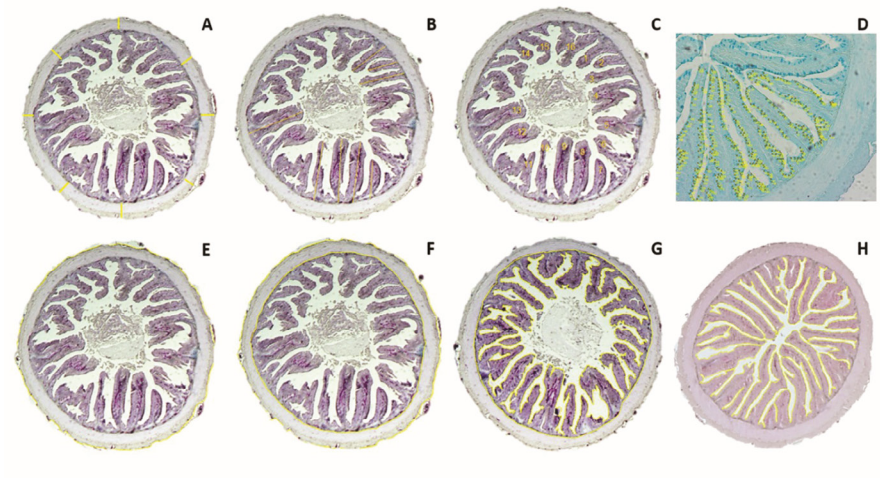


Figure 2 - Morphophysiological measures of the posterior intestine. All of them are indicated in yellow. A. The thickness of the muscle layer, B. Villus height, C. Number of villi, D. Number of Goblet cells, E. Total intestinal area, F. Total area of the muscle layer, G. Area of villi, H. Light area of the intestine. 20x magnification.

absorption surface. Picture measurements were made with the software ZEN and ImageJ v.1.49.

Data analysis

The data are registered as the mean ± standard deviation (SD). The normality and homoscedasticity were tested by the tests of Shapiro-Wilk, Pearson, Anderson-Darling, and Kolmogorov-Smirnov. A Kruskal-Wallis non-parametric test was performed for non-parametric variables. The variables that met the assumption of normality were analyzed using a one-way ANOVA test, followed by a multiple comparisons post hoc test (Tukey test) to find the significant differences of each treatment.

The survival of fish in each treatment group was estimated using Kaplan-Meier analysis, and differences between groups were assessed with the log-rank (Mantel-Cox) test for pairwise comparisons (YILMAZ & ERGÜN, 2014). Differences were considered significant at $P < 0.05$. All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, California, USA, <www.graphpad.com>).

RESULTS

Survival

All treatment diets were widely consumed during the experiment. During the first five days of the experiment, animals in the infected control and 0.5 mL of EOCF per kg of diet treatments exhibited dermal lesions. From days 5 to 10, similar symptoms appeared in the 0.25 and 1.0 of EOCF mL per kg of diet treatments. Subsequently, sporadic mortality occurred, and the animals experienced loss of barbels and fins until the trial's conclusion. At the end of the 25-day experimental essay, the treatment fed with 0.25 mL nanoemulsion of EOCF per kg of diet exhibited significantly lower mortality than the control-infected group ($P < 0.05$) (Figure 3).

Antioxidants analyses

Fish supplemented with the nanoemulsion of EOCF exhibited lower $O_2^{\bullet-}$ levels in the muscle compared with the uninfected control ($P < 0.05$). Inversely, the activity of SOD in the muscle increased significantly in the group fed in 0.25 mL nanoemulsion of EOCF per kg of diet ($P < 0.05$). In the same way, the activity of CAT in the muscle was significantly higher in the groups receiving diets with 0.5 and 1.0 mL nanoemulsion of EOCF per kg of feed than the uninfected control group ($P < 0.05$) and 0.25 mL nanoemulsion of EOCF per kg of diet ($P < 0.05$). GST activity in the muscle was higher in the fish receiving 0.25 and 0.5 mL nanoemulsion of EOCF per kg of diet than in the uninfected control group ($P < 0.05$) and 1.0 mL nanoemulsion of EOCF per kg of diet ($P < 0.05$). TAC in the muscle was the highest in the group fed 1.0 mL nanoemulsion of EOCF per kg of diet and fish fed with 0.25 mL nanoemulsion of EOCF per kg of diet had higher TAC compared to the uninfected control group ($P < 0.05$) and 0.5

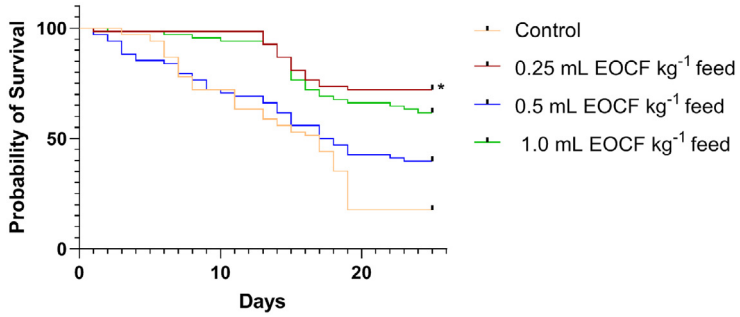


Figure 3 - Kaplan-Meier survivorship curves (cumulative survival [%] over time [days]) for *Rhamdia quelen* with a natural infection of *A. hydrophila*. Survival was analyzed using the log-rank (Mantel-Cox) test. Asterisk indicates significant difference from the control group ($P < 0.05$).

mL nanoemulsion of EOCF per kg of diet ($P < 0.05$). By contrast, the total glutathione levels in the liver were lower in fish from all treatments fed with EOCF nanoemulsion than the uninfected control group ($P < 0.05$), whilst TBARS did not differ significantly between groups ($P > 0.05$) (Table 2).

Histology

The supplementation with different doses of nanoemulsion of EOCF did not affect the thickness of the muscle layer, number of villi, total intestinal area, area of the muscle layer, area of the villus, light area of the intestine, and absorption surface. However, the fish fed 0.5 and 1.0 mL nanoemulsion of EOCF per kg of diet had a decrease in the height of villi and the number of goblet cells in the intestine compared with the uninfected control ($P < 0.05$) (Table 3).

DISCUSSION

Considering the close relationship between diet and "good GIT health" and the impacts this has on elements such as oxidative stress, zootechnical development, and animal health, it is important to determine the relationships between dietary ingredients and GIT. This is especially relevant when evaluating functional foods that promote animal health and nutrition by affecting the physiological functions of the gut (MAHMOUD et al., 2017).

It should be noted that the supplemented animals with the EOCF nanoemulsion were affected by a natural infection of *A hydrophila.* This infection is characterized by symptoms such as internal and external hemorrhages, ulcers, changes in swimming behavior, septicemia, and red-sore disease. All these

Table 2 - Oxidative stress biomarkers of *Rhamdia quelen* fed with feed containing different doses of the nanoemulsion of *Cymbopogon flexuosus* essential oil (EOCF) (mL per kg of feed).

	Healthy control	0.25 mL EOCF kg^{-1}	0.5 mL EOCF kg^{-1}	1.0 mL EOCF kg^{-1}
----------Muscle----------				
O_2^- (µmol min^{-1} g $tissue^{-1}$)	48.04 ± 2.31^a	22.12 ± 7.75^b	26.19 ± 5.36^b	26.30 ± 7.96^b
SOD (USOD g $tissue^{-1}$)	1.23 ± 017^a	1.65 ± 0.16^b	1.47 ± 0.09^a	1.27 ± 0.16^a
CAT (ρmol g $tissue^{-1}$)	0.28 ± 0.05^a	0.41 ± 0.08^a	0.63 ± 0.07^b	0.57 ± 0.08^b
GST (ρmol min^{-1} g $tissue^{-1}$)	1.68 ± 0.40^a	3.78 ± 0.35^b	3.61 ± 0.42^b	2.49 ± 0.38^a
TAC (µmol g $tissue^{-1}$)	11.22 ± 2.02^a	18.16 ± 2.70^b	15.12 ± 1.68^{ab}	23.50 ± 2.90^c
----------Liver----------				
Total glutathione (nmol g $tissue^{-1}$)	29.60 ± 5.33^a	15.26 ± 3.46^b	12.28 ± 6.31^b	16.79 ± 1.75^b
TBARS (nmol g $tissue^{-1}$)	1.30 ± 0.35	5.52 ± 4.76	5.52 ± 4.25	4.07 ± 2.25

Values are expressed as mean ± standard deviation (n = 10). Within a row, means with differing letters are significantly different ($P < 0.05$). Data were analyzed using one-way ANOVA with Tukey's post hoc test, except for TBARS, which was evaluated using the Kruskal-Wallis test due to non-normal distribution.

Table 3 - Histological parameters of the intestine of *Rhamdia quelen* fed with diets containing different doses of nanoemulsion of *Cymbopogon flexuosus* essential oil (EOCF) (mL per kg of feed).

	Healthy control	0.25 mL EOCF kg^{-1}	0.5 mL EOCF kg^{-1}	1.0 mL EOCF kg^{-1}
Thickness of the muscle layer (µm)	178.74 ± 74.76	133.23 ± 61.01	89.78 ± 25.32	104.02 ± 12.88
Villus height (µm)	606.04 ± 155.84^{a}	481.74 ± 166.39^{ab}	355.06 ± 78.39^{b}	395.49 ± 85.90^{ab}
Number of villi (n)	21.63 ± 4.85	20.04 ± 4.94	19.88 ± 6.10	19.31 ± 4.92
Goblet cells (n)	72.63 ± 21.44^{a}	57.59 ± 18.44^{ab}	41.42 ± 8.63^{b}	45.81 ± 9.07^{b}
The total intestinal area (mm^2)	3.70 ± 2.00	3.50 ± 2.59	1.58 ± 0.43	2.19 ± 1.06
Area of the muscle layer (mm^2)	1.19 ± 0.70	0.93 ± 0.78	0.41 ± 0.16	0.54 ± 0.18
Area villus (mm^2)	1.21 ± 0.24	1.57 ± 1.06	0.73 ± 0.19	1.03 ± 0.45
Light area of the intestine (mm^2)	0.41 ± 0.04	0.99 ± 0.76	0.47 ± 0.17	0.61 ± 0.43
Absorption surface (mm)	2.28 ± 0.13	3.33 ± 1.30	2.40 ± 0.43	2.64 ± 0.96

Values are expressed as mean ± standard deviation (n=10). Within a row, means with differing letters are significantly different ($P < 0.05$; one-way ANOVA followed by Tukey's test).

symptoms can lead to negative impacts on growth performance and heavy mortalities (SEMWAL et al., 2023). The fact that infected fish supplemented with 0.25 mL nanoemulsion of EOCF per kg of diet had a lower mortality rate than infected fish without supplementation is an indicator that some doses of this phytobiotic may be a protective factor against bacteriosis. The findings reported in juveniles of *Colossoma macropomum* confirm that supplementation with 0.25 mL of *Cymbopogon citratus* EO (a phytocompound from a plant of the same genus as *C. flexuosus*, with citral as its main component) per kg of diet resulted in a higher survival rate following a challenge with *A. hydrophila*, compared to treatments with a higher concentration of this EO (COPATTI et al., 2022). Although, the antimicrobial potential of nanoemulsion of EOCF has been widely described (GÜNDEL et al., 2018), this could be discarded as the primary reason why EO contributes to the protection against Aeromonasis since the lowest minimal inhibitory and bactericidal concentration (MIC and MBC) of EO reported for bacteria (195.31 µg mL^{-1}) for *Aeromonas* spp. (SOUZA, et al., 2018b), 0.351 mg mL^{-1} for *Mycobacterium fortuitum, M. massiliense, M. abscessus* (ROSSI et al., 2017), 0.288-0.567 mg mL^{-1} for *Candida albicans* (TYAGI & MALIK, 2010) cannot be obtained either in the external or internal environment with dietary supplementation alone. In addition, LEE et al. (2004) rule out the accumulation of EO in tissues due to its rapid metabolic conversion and excretion.

Although, it is not known whether EOCF nanoemulsion can reach MBC and MIC in the GIT, there are records that citral (0.25 g EO per kg of feed), loaded in various nanosystems, significantly decreased the bacterial population in the intestine of silver catfish (SUTILI et al., 2019). Such changes could potentially influence gut homeostasis and contribute to dysbiosis. For instance, SUN et al. (2022) described how grass carp (*Ctenopharyngodon idella*) was more susceptible to *A. hydrophila* infection after undergoing intestinal dysbiosis. While we did not directly assess gut microbiota or pathogen loan in this study, it is plausible that doses higher than 0.25 mL of the EOCF nanoemulsion per kg of diet may have triggered unidentified adverse effects possibly related to intestinal alterations, microbial balance, or other physiological mechanisms that could have compromised the fish's ability to cope with the infection. This might explain why these treatments showed no significant differences in mortality compared to unsupplemented infected fish.

Oxidative stress biomarkers of the fish were also evaluated in this study, since it is affected by dietary supplementation (GAO et al., 2012; ZHAO et al., 2013). A notable example is the study by SACCOL et al. (2013), which found that dietary supplementation with *Lippia alba* EO (rich in linalool, β-citral, and α-citral) improved the oxidative status of silver catfish. It was correlated with a greater survival rate against *A. hydrophila* infections in channel catfish (*Ictalurus punctatus*) (ZHENG et al., 2009), and in juvenile Jian carp (*Cyprinus carpio var. Jian*) (ZHAO et al., 2013). In turn, it has been shown that during infections of this bacterium in silver catfish, the production of reactive oxygen species (ROS) is excessive compared to a control treatment (BALDISSERA et al., 2017). These changes have been widely described in infections of the genus *Aeromonas* (BANDEIRA JUNIOR & BALDISSEROTTO, 2021).

In this sense, when evaluating oxidative stress through lipid peroxidation (LPO) by TBARS, we found no significant differences between the uninfected control treatment and the fish fed all three diets supplemented with the EOCF nanoemulsion that were naturally infected by *A. hydrophila*. Numerous studies on *Aeromonas* infections in fish have reported elevated TBARS levels as a marker of oxidative damage induced by the pathogen (BANDEIRA JUNIOR & BALDISSEROTTO, 2021). In this context, the findings suggested that supplementation with this phytobiotic may exert a protective effect on hepatic oxidative status in infected fish.

Another biomarker of oxidative stress is respiratory burst activity (RBA), indicated directly from the production of $O_2^{\bullet-}$ (SUTILI et al., 2014). *A. hydrophila* infections affect this biomarker by increasing $O_2^{\bullet-}$ radical production in goldfish *Carassius auratus* (HARIKRISHNAN et al., 2009) and silver catfish (DA ROSA et al., 2019), likely as an innate immune response (ELUMALAI et al., 2021). The significant reduction in muscle $O_2^{\bullet-}$ production in animals treated with EOCF nanoemulsion suggests a protective effect against oxidation. However, studies reviewed by BANDEIRA JUNIOR & BALDISSEROTTO (2021) show varying effects of *Aeromonas* infection on RBA.

To better elucidate the mechanisms by which EOCF nanoemulsion alters the oxidative status in silver catfish, several enzymes of the antioxidant defense system, such as SOD, CAT, and GST activity, were evaluated. These enzymes play a key role in cellular defense and protection against the damaging action of ROS. The importance of SOD (present in mitochondria and cytosol) is due to its ability to catalyze the reaction of dismutation of the highly toxic $O_2^{\bullet-}$ to oxygen and hydrogen peroxide (H_2O_2) (BANDEIRA JUNIOR & BALDISSEROTTO, 2021; LOPES et al., 2019; ZHENG et al., 2009). The latter by itself can lead to the generation of lipid peroxidation by the production of the hydroxyl radical, a potent oxidant that can cross cell membranes and affect lipids, proteins, and DNA (HALLIWELL & GUTTERIDGE, 2015), which is why the CAT enzyme (found mainly in peroxisomes) takes on special importance in the antioxidant defense system, by catalyzing H_2O_2 into oxygen and water (BALDISSERA et al., 2017; KAUSHAL et al., 2018). Finally, the GST family of enzymes detoxifies metabolites such as xenobiotics or LPO products, utilizing reduced glutathione (GSH) as a cofactor in phase II metabolism, conjugating GSH to the electrophilic center of a broad spectrum of hydrophilic molecules (BACKOS et al., 2012). This GSH-xenobiotic conjugate is more water soluble than the parent compound and transforms it into an excretable molecule (LUSHCHAK, 2011).

In the present study, EOCF nanoemulsion significantly increased SOD (0.25 mL nanoemulsion of EOCF per kg of diet), GST (0.25 and 0.5 mL nanoemulsion of EOCF per kg of diet), and CAT (0.5 and 1.0 mL nanoemulsion of EOCF per kg of diet) activity in muscle. Other studies of antioxidant supplementation in silver catfish have evidenced that SOD and GST activities are susceptible to changes in muscle depending on the diet (PÊS et al., 2016). Regarding EOs, the results are contrasting. Consistent with to findings from the present trial, in silver catfish supplementation with *A. triphylla* EO, a significant increase in SOD (DOS SANTOS et al., 2017) and with *L. alba* EO increased GST activity in muscle (SACCOL et al., 2013). In contrast, studies in silver catfish fed with microencapsulated EOCF showed no differences in the enzymatic activity of SOD, GST, and CAT at the gonad level (SOUZA et al., 2018a), or as reported by LOPES et al. (2019) where the addition of *Citrus aurantium* EO in the diet of silver catfish resulted in a decrease of GST activity, an increase of SOD, and no alteration of CAT in the liver.

Other research approaches have ventured into the use of these functional foods to prevent and treat redox imbalance in different fish species affected by *Aeromonas*. This imbalance has been described in many fish species, and in most studies, it has been recorded that after *Aeromonas* infection, SOD activity is stimulated, GST is depleted, and CAT and TAC do not show an apparent pattern (BANDEIRA JUNIOR & BALDISSEROTTO, 2021). Total glutathione, which encompasses both its reduced (GSH) and oxidized (GSSG) forms, is a key biomarker of oxidative stress in cells and tissues. The liver, as the central organ for detoxification, is particularly vulnerable to oxidative damage. Under conditions of elevated oxidative stress – such as during bacterial infections – hepatic GSH levels can significantly decline due to increased consumption and impaired regeneration (ZHAO et al., 2011). This has been demonstrated in infections caused by *Helicobacter pylori* and *Pseudomonas aeruginosa*, where a marked reduction in hepatic GSH was observed following infection (AHMAD et al., 2011; SHIRIN et al., 2001). This observation is supported by the present findings, in which animals naturally infected with *A. hydrophila* exhibited a significant reduction in total glutathione levels compared to the uninfected control group, indicating a disruption in redox homeostasis likely driven by infection-induced oxidative stress.

Total antioxidant capacity (TAC) reflects the cumulative action of all antioxidants present in a sample, including both enzymatic and non-enzymatic components (APAK et al., 2016). While glutathione levels decreased in the liver following *A. hydrophila* infection, TAC values did not show a corresponding decline in muscle tissue, suggesting the activation of compensatory antioxidant mechanisms or the involvement of alternative antioxidant systems. This interpretation is supported by SAHREEN et al. (2021), who reported increased antioxidant enzyme activity in muscle and liver tissues of fish under parasitic infection, highlighting tissue-specific redox adaptations in response to oxidative stress. In this sense, DA ROSA et al. (2019) evidenced a decrease in TAC in silver catfish muscle supplemented with a flavonoid, after being challenged with *A. hydrophila*. This contrasts with our results, where the 0.25 and 1.0 mL nanoemulsions of EOCF per kg of diet showed the highest TAC compared to uninfected, un-supplemented fish.

It should be noted that; although, previous studies on oxidative stress have been described in silver catfish with EO with active components similar to EOCF, it is not possible to generate deterministic conclusions given that tissues and organs have different rates of oxygen consumption, metabolic activity and antioxidant concentrations, so differences or similarities described may be due to different rates of free radical generation, differences in susceptibility to oxidative damage or antioxidant capacity of the tissue (SACCOL et al., 2013).

To better understand the effects of EOCF nanoemulsion at the tissue level, a description of the changes in the intestinal morphology was made, considering that it is closely linked to nutrient assimilation and the health status of the animal (BRUM et al., 2018; PIRARAT et al., 2011). The intestinal histomorphology showed that the number of goblet cells and height of the villi were reduced in animals that received a dose of EOCF nanoemulsion higher than 0.5 mL of EOCF nanoemulsion per kg of diet. A reduction in absorptive surface area is directly associated with impaired nutrient and electrolyte assimilation (ADEOYE et al., 2016). In parallel, secretory goblet cells play a critical role by producing the mucus layer that serves as the first physicochemical barrier between the host and pathogens, being of vital importance in the animal's innate immunity (CORNICK et al., 2015) and in the lubrication of the food bolus through the GIT.

Although 0.25 mL/kg was identified as the optimal dose, higher concentrations of the EOCF nanoemulsion resulted in reduced survival and compromised intestinal morphology. This may suggest that high doses of EOCF nanoemulsion negatively affect intestinal morphology. Similar trends have been observed in other studies, such as the inverse relationship between *Zingiber officinale* EO concentration and villus thickness in juvenile Nile tilapia (CHUNG et al., 2021), and the histological damage caused by high concentrations of clove basil (*Ocimum gratissimum* L.) and ginger EO in Nile tilapia tissues (BRUM et al., 2018). A plausible explanation for these effects lies in the bioactivity of citral, the main component of EOCF, which has been shown to depolarize the membrane of Caco-2 intestinal epithelial cells, likely through interactions with receptor channels that alter ionic conductance (CARNESECCHI et al., 2002). This depolarization may compromise tight junction integrity, increasing epithelial permeability ROCHA-DE-SOUZA et al. (2003) demonstrated that the adhesion of *Aeromonas caviae* to Caco-2 cells is influenced by the polarization state of the monolayer, with depolarized membranes exposing basolateral receptors that facilitate bacterial adhesion. This was further confirmed in depolarized HEp-2, Caco-2, and T-84 cell lines exposed to over 50 *Aeromonas* strains (COUTO et al., 2007; DOS SANTOS et al., 2015), suggesting that high doses of EOCF may disrupt mucosal integrity and promote pathogen colonization, ultimately compromising host survival.

However, prior studies using similar doses of EOCF in silver catfish did not report histopathological lesions in other tissues. For instance, histological evaluations of the gonads (SOUZA et al., 2018a), liver, skin, and gill (VALÉRIO-JÚNIOR et al., 2025) showed no tissue damage associated with this EO. Moreover, studies in the same biological model, with another EO with similar components (β-citral, and α-citral) and doses, reported positive effects on the increase in the number of intestinal folds (ZEPPENFELD et al., 2016). These findings suggest that the adverse effects observed at higher doses may be tissue-specific or dose-dependent and highlight the importance of evaluating mucosal responses in detail.

Considering the above reports, our results appear consistent when considering the effects of *A. hydrophila* infection. It is important to note that the observed histological alterations might also be influenced by the *A. hydrophila* infection itself, rather than being solely attributable to EOCF dosage. Previous research has shown that this pathogen can reduce villus length during gastrointestinal colonization in blunt snout bream (*Megalobrama amblycephala*) (NGAMKALA et al., 2020), and Nile

tilapia (ZHANG et al., 2022). Therefore, the reduction in the adsorption surface area and in the mean number of goblet cells in the 0.5 and 1.0 mL nanoemulsion of EOCF per kg of diet may be an explanation for the recorded mortality and confirms the importance of these cells in the resistance to *A. hydrophila* infection. In addition, studies in other biological models have demonstrated dose-dependent EO deposition (BOTSOGLOU et al., 2002), which would explain the positive results associated with the lower concentration of the EOCF nanoemulsion (0.25 mL per kg of diet). Nevertheless, the lack of an infected, un-supplemented control group in the histological analysis constrains the ability to distinguish the specific effects of the EOCF nanoemulsion from those induced by *A. hydrophila* infection.

It is important to highlight that the comparison of the effects caused by phytobiotics used as functional foods must be careful, taking into account that the composition of these plant extracts are mixtures of different biomolecules that may or may not have been characterized within the different studies, that are administered with different dosages, exposure times (FIRMINO et al., 2021b), in biological models with different feeding habits (detritivores, herbivores, carnivores, or omnivores, different forms of presentation (nanoemulsion, microemulsion, powder forms, hydroethanolic extracts or dissolved solutions) and in many cases without a follow-up on the biodegradation potential in feed storage or during traffic through the GIT (FIRMINO et al., 2021b).

CONCLUSION

This study indicated that low concentrations of EOCF nanoemulsion (0.25 ml of EO per kg of diet) enhance the survival of silver catfish when infected by *A. hydrophila*. This increased resistance is attributed to the positive effects of the treatment on the antioxidant enzymatic defense system (GST and SOD) and the overall increase in total antioxidant activity within tissues. However, the lack of a concurrent infected control group limits the ability to draw strong causal conclusions about the protective effects of EOCF nanoemulsion. Therefore, while the findings are promising, they should be interpreted with caution, and further studies including appropriate control groups are needed to confirm these effects.

ACKNOWLEDGMENTS

Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq - Brasil) for providing research fellowship to B. Baldisserotto (proc. 301816/2022-0). And was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil - Finance code 001.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The experimental protocol was approved by the Committee on Animal Experimentation of Universidade Federal de Santa Maria (UFSM) under the registration code nº 6899161121.

DATA AVAILABILITY STATEMENT

Data supporting the results and conclusions of this study are available and may be requested from the author upon reasonable request.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

No AI tools were used in the preparation of this manuscript.

REFERENCES

ADEOYE, A. A. et al. Supplementation of formulated diets for tilapia (*Oreochromis niloticus*) with selected exogenous enzymes: overall performance and effects on intestinal histology and microbiota. **Animal Feed Science and Technology**, v.215, p.133-143, 2016. Available from: <https://doi.org/10.1016/j.anifeedsci.2016.03.002>. Accessed: Apr. 20, 2024. doi: 10.1016/j.anifeedsci.2016.03.002.

AEBI, H. Catalase *in Vitro*. **Methods in Enzymology**, v.105, p.121-126, 1984. Available from: <https://doi.org/10.1016/S0076-6879(84)05016-3>. Accessed: Jul. 14, 2024. doi: 10.1016/s0076-6879(84)05016-3.

AHMAD, I. M. et al. Oxidation of thiols and modification of redox-sensitive signaling in human lung epithelial cells exposed to *Pseudomonas pyocyanin*. **Journal of Toxicology and Environmental Health - Part A: Current Issues**, v.74, n.1, p.43-51, 2011. Available from: <https://doi.org/10.1080/15287394.2010.514233>. Accessed: Oct. 16, 2024. doi: 10.1080/15287394.2010.514233.

AMEUR, A. et al. Application of oil-in-water nanoemulsions based on grape and cinnamon essential oils for shelf-life extension of chilled flathead mullet fillets. **Journal of the Science of Food and Agriculture**, v.102, n.1, p.105-112, 2022. Available from:

<https://doi.org/10.1002/jsfa.11336>. Accessed: Aug. 10, 2024. doi: 10.1002/jsfa.11336.

AVOSEH, O. et al. *Cymbopogon* species; ethnopharmacology, phytochemistry and the pharmacological importance. **Molecules**, v.20, n.5, p.7438-7453, 2015. Available from: <https://doi.org/10.3390/molecules20057438>. Accessed: Sept. 24, 2024. doi: 10.3390/molecules20057438.

BABA, E. et al. Evaluation of *Citrus limon* peels essential oil on growth performance, immune response of Mozambique tilapia *Oreochromis mossambicus* challenged with *Edwardsiella tarda*. **Aquaculture**, v.465, p.13-18, 2016. Available from: <https://doi.org/10.1016/j.aquaculture.2016.08.023>. Accessed: Oct. 10, 2024. doi: 10.1016/j.aquaculture.2016.08.023.

BACKOS, D. S. et al. The role of glutathione in brain tumor drug resistance. **Biochemical Pharmacology**, v.83, n.8, p.1005-1012, 2012. Available from: <https://doi.org/10.1016/j.bcp.2011.11.016>. Accessed: Feb. 25, 2024. doi: 10.1016/j.bcp.2011.11.016.

BALDISSERA, M. D. et al. *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**, v.61, n.2, p.1-8, 2017. Available from: <https://doi.org/10.1016/j.fsi.2016.12.016>. Accessed: Jun. 14, 2024. doi: 10.1016/j.fsi.2016.12.016.

BANDEIRA JUNIOR, G.; BALDISSEROTTO, B. Fish infections associated with the genus *Aeromonas*: a review of the effects on oxidative status. **Journal of Applied Microbiology**, v.131, n.3, p.1083-1101, 2021. Available from: <https://doi.org/10.1111/jam.14986>. Accessed: Apr. 01, 2024. doi: 10.1111/jam.14986.

BOTSOGLOU, N. A. et al. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. **British Poultry Science**, v.43, n.2, p.223-230, 2002. Available from: <https://doi.org/10.1080/00071660120121436>. Accessed: Jun. 03, 2024. doi: 10.1080/00071660120121436.

BRUM, A. et al. Histological changes in Nile tilapia fed essential oils of clove basil and ginger after challenge with *Streptococcus agalactiae*. **Aquaculture**, v.490, p.98-107, 2018. Available from: <https://doi.org/10.1016/j.aquaculture.2018.02.040>. Accessed: Nov. 20, 2024. doi: 10.1016/j.aquaculture.2018.02.040.

CAMPOS, A. M.; LISSI, E. A. Kinetics of the reaction between 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) derived radical cations and phenols. **International Journal of Chemical Kinetics**, v.29, n.3, p.219-224, 1997. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/(SICI)1097-4601(1997)29:3%3C219::AID-KIN9%3E3.0.CO;2-X>. Accessed: Jul. 09, 2024. doi: 10.1002/(SICI)1097-4601(1997)29:3<219::AID-KIN9>3.0.CO;2-X.

CARNESECCHI, S. et al. Perturbation by geraniol of cell membrane permeability and signal transduction pathways in human colon cancer cells. **The Journal of Pharmacology and Experimental Therapeutics**, v.303, n.2, p.711-715, 2002. Available from: <https://jpet.aspetjournals.org/article/S0022-3565(24)35992-0/abstract>. Accessed: Jan. 24, 2025. doi: 10.1124/jpet.102.039263.

CELI, P. et al. Gastrointestinal functionality in animal nutrition and health: new opportunities for sustainable animal production. **Animal Feed Science and Technology**, v.234, n.May, p.88-100, 2017. Available from: <https://doi.org/10.1016/j.anifeedsci.2017.09.012>. Accessed: Aug. 05, 2024. doi: 10.1016/j.anifeedsci.2017.09.012.

CHUNG, S. et al. Essential oil from ginger influences the growth, haematological and biochemical variables and histomorphometry of intestine and liver of Nile tilapia juveniles. **Aquaculture**, v.534, 2021. Available from: <https://doi.org/10.1016/j.aquaculture.2020.736325>. Accessed: Feb. 25, 2024. doi: 10.1016/j.aquaculture.2020.736325.

COPATTI, C. E. et al. Addition of essential oil from lemongrass to the tambaqui (*Colossoma macropomum*) diet: effects on growth, intestinal enzymes, haematological and metabolic variables, and antimicrobial challenge. **Aquaculture Research**, v.53, n.16, p.5656-5666, 2022. Available from: <https://doi.org/10.1111/are.16048>. Accessed: Apr. 02, 2024. doi: 10.1111/are.16048.

CORNICK, S. et al. Roles and regulation of the mucus barrier in the gut. **Tissue Barriers**, v.3, n.1, 2015. Available from: <https://doi.org/10.4161/21688370.2014.982426>. Accessed: Nov. 21, 2024. doi: 10.4161/21688370.2014.982426.

COUTO, C. R. A. et al. Interactions of clinical and environmental *Aeromonas isolates* with Caco-2 and HT29 intestinal epithelial cells. **Letters in Applied Microbiology**, v.45, n.4, p.405-410, 2007. Available from: <https://doi.org/10.1111/j.1472-765X.2007.02199.x>. Accessed: Feb. 18, 2025. doi: 10.1111/j.1472-765X.2007.02199.x.

CUNHA, M. A. et al. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet. **Ciência Rural**, v.40, n.10, p.2107-2114, 2010. Available from: <https://doi.org/10.1590/S0103-84782010005000154>. Accessed: Oct. 04, 2024. doi: 10.1590/S0103-84782010005000154.

DAROSA, V. M. et al. Dietary addition of rutin impairs inflammatory response and protects muscle of silver catfish (*Rhamdia quelen*) from apoptosis and oxidative stress in *Aeromonas hydrophila* induced infection. **Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology**, v.226, p.108611, 2019. Available from: <https://doi.org/10.1016/j.cbpc.2019.108611>. Accessed: Jan. 15, 2025. doi: 10.1016/j.cbpc.2019.108611.

DOS SANTOS, P. A. et al. Adhesion, invasion, intracellular survival and cytotoxic activity of strains of *Aeromonas spp*. in HEp-2, Caco-2 and T-84 cell lines. **Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology**, v.107, n.5, p.1225-1236, 2015. Available from: <http://link.springer.com/article/10.1007/s10482-015-0416-4>. Accessed: Oct. 10, 2024. doi: 10.1007/s10482-015-0416-4.

DOS SANTOS, A. C. et al. *Aloysia triphylla* essential oil as additive in silver catfish diet: blood response and resistance against *Aeromonas hydrophila* infection. **Fish and Shellfish Immunology**, v.62, p.213-216, 2017. Available from: <https://doi.org/10.1016/j.fsi.2017.01.032>. Accessed: Feb. 10, 2025. doi: 10.1016/j.fsi.2017.01.032.

EATON, A. D.; FRANSON, M. A. H. **Standard methods for the examination of water and wastewater**. 1 Edition., American Public Health Association, Washington, DC, 2005. v. 21.

ELLMAN, G. L. Tissue sulfhydryl groups. **Archives of biochemistry and biophysics**, v.82, p.70-77, 1959. Available from:

<https://doi.org/10.1016/0003-9861(59)90090-6>. Accessed: Dec. 10, 2024. doi: 10.1016/0003-9861(59)90090-6.

ELUMALAI, P. et al. Herbal immunomodulators in Aquaculture. **Reviews in Fisheries Science & Aquaculture**, v.29, n.1, p.33-57, 2021. Available from: <https://doi.org/10.1080/23308249.2020.1779651>. Accessed: Mar. 23, 2025. doi: 10.1080/23308249.2020.1779651.

FELIX E SILVA, A. et al. Antibacterial and antibiofilm activities and synergism with florfenicol from the essential oils of *Lippia sidoides* and *Cymbopogon citratus* against *Aeromonas hydrophila*. **Journal of Applied Microbiology**, v.132, n.3, p.1802-1812, 2022. Available from: <https://doi.org/10.1111/jam.15336>. Accessed: Aug. 05, 2024. doi: 10.1111/jam.15336.

FIRMINO, J. P. et al. Diet, immunity, and microbiota interactions: an integrative analysis of the intestine transcriptional response and microbiota modulation in gilthead seabream (*Sparus aurata*) fed an essential oils-based functional diet. **Frontiers in Immunology**, v.12, p.1-19, 2021a. Available from: <https://doi.org/10.3389/fimmu.2021.625297>. Accessed: Nov. 15, 2024. doi: 10.3389/fimmu.2021.625297.

FIRMINO, J. P. et al. Phytogenic bioactive compounds shape fish mucosal immunity. **Frontiers in Immunology**, v.12, 2021b. Available from: <https://doi.org/10.3389/fimmu.2021.695973>. Accessed: Jan. 23, 2025. doi: 10.3389/fimmu.2021.695973.

GAO, J. et al. Effects of dietary oxidized fish oil with vitamin E supplementation on growth performance and reduction of lipid peroxidation in tissues and blood of red sea bream *Pagrus major*. **Aquaculture**, v.356-357, p.73-79, 2012. Available from: <https://doi.org/10.1016/j.aquaculture.2012.05.034>. Accessed: May, 19, 2024. doi: 10.1016/j.aquaculture.2012.05.034.

GROSELL, M. et al. **Fish physiology**: the multifunctional gut of fish, 2010. 30v. Available from: <https://books.google.ca/books?id=eddWw%5C_nyRIYC>. Accessed: Aug. 14, 2024.

GÜNDEL, S. S. et al. *In vivo* antifungal activity of nanoemulsions containing eucalyptus or lemongrass essential oils in murine model of vulvovaginal candidiasis. **Journal of Drug Delivery Science and Technology**, v.57, p.101762, 2020. Available from: <https://doi.org/10.1016/j.jddst.2020.101762>. Accessed: Oct. 12, 2024. doi: 10.1016/j.jddst.2020.101762.

GÜNDEL, S. S. et al. Nanoemulsions containing *Cymbopogon flexuosus* essential oil: development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. **Microbial Pathogenesis**, v.118, n.4, p.268-276, 2018. Available from: <https://doi.org/10.1016/j.micpath.2018.03.043>. Accessed: Feb. 14, 2025. doi: 10.1016/j.micpath.2018.03.043.

HABIG, W. H. et al. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. **The Journal of Biological Chemistry**, v.249, n.22, p.7130-7139, 1974. Available from: <https://doi.org/10.1016/S0021-9258(19)42083-8>. Accessed: Apr. 26, 2024. doi: 10.1016/S0021-9258(19)42083-8.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free radicals in biology and medicine**. New York, NY: Oxford University Press, 2015. 4th Edition.

HARIKRISHNAN, R. et al. Effect of chemotherapy, vaccines and immunostimulants on innate immunity of goldfish infected with *Aeromonas hydrophila*. **Diseases of Aquatic Organisms**, v.88, n.1, p.45-54, 2009. Available from: <https://www.int-res.com/articles/dao2010/88/d088p045.pdf>. Accessed: Jun. 23, 2024. doi: 10.3354/dao02143.

HERMES-LIMA, M. et al. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. **Free Radical Biology and Medicine**, v.19, n.3, p.271-280, 1995. Available from: <https://doi.org/10.1016/0891-5849(95)00020-X>. Accessed: Jul. 05, 2024. doi: 10.1016/0891-5849(95)00020-x.

HOSEINIFAR, S. H. et al. Feed additives, gut microbiota, and health in finfish aquaculture. **Feed Additives**, p.121-142, 2019. Available from: <https://ouci.dntb.gov.ua/en/works/7Wej3zOl/>. Accessed: Jan. 20, 2025. doi: 10.1007/978-3-030-16190-3_6.

HOSSAIN, M. J. et al. An asian origin of virulent *Aeromonas hydrophila* responsible for disease epidemics in united states-farmed catfish. **mBio**, v.5, n.3, p.1-7, 2014. Available from: <https://doi.org/10.1128/mbio.00848-14>. Accessed: Sept. 07, 2024. doi: 10.1128/mBio.00848-14.

HWANG, J. et al. Physicochemical property and oxidative stability of whey protein concentrate multiple nanoemulsion containing fish oil. **Journal of Food Science**, v.82, n.2, p.437-444, 2016. Available from: <https://doi.org/10.1111/1750-3841.13591>. Accessed: Mar. 16, 2025. doi: 10.1111/1750-3841.13591.

KAUSHAL, J. et al. Catalase enzyme: application in bioremediation and food industry. **Biocatalysis and Agricultural Biotechnology**, v.16, p.192-199, 2018. Available from: <https://doi.org/10.1016/j.bcab.2018.07.035>. Accessed: May, 22, 2024. doi: 10.1016/j.bcab.2018.07.035.

KHAN, C. D. et al. A global survey of hypervirulent *Aeromonas hydrophila* (vAh) identified vAh strains in the lower Mekong river basin and diverse opportunistic pathogens from farmed fish and other environmental sources. **Microbiology Spectrum**, v.11, n.2, p.1-15, 2023. Available from: <https://doi.org/10.1128/spectrum.03705-22>. Accessed: Jul. 12, 2024. doi: 10.1128/spectrum.03705-22.

LEE, K. W. et al. Essential oils in broiler nutrition. **International Journal of Poultry Science**, v.3, n.12, p.738-752, 2004. Available from: <https://scialert.net/abstract/?doi=ijps.2004.738.752>. Accessed: Dec. 15, 2024. doi: 10.3923/ijps.2004.738.752.

LOPES, J. M. et al. *Citrus* x *aurantium* essential oil as feed additive improved growth performance, survival, metabolic, and oxidative parameters of silver catfish (*Rhamdia quelen*). **Aquaculture Nutrition**, v.25, n.2, p.310-318, 2019. Available from: <https://doi.org/10.1111/anu.12854>. Accessed: Feb. 19, 2025. doi: 10.1111/anu.12854.

LUSHCHAK, V. I. Environmentally induced oxidative stress in aquatic animals. **Aquatic Toxicology**, v.101, n.1, p.13-30, 2011. Available from: <https://doi.org/10.1016/j.aquatox.2010.10.006>. Accessed: Nov. 21, 2024. doi: 10.1016/j.aquatox.2010.10.006.

MAHMOUD, D. et al. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. **Reviews in Aquaculture**, v.10, n.4, p.1-25, 2017. Available from: <https://doi.org/10.1111/raq.12209>. Accessed: Mar. 23, 2025. doi: 10.1111/raq.12209.

MARKEY, B. K. et al. **Clinical Veterinary Microbiology**, 2013. 2nd Edition. Available from: <https://books.google.com/books?id=hJ8GswEACAAJ>. Accessed: Mar. 23, 2025.

MISRA, H. P.; FRIDOVICH, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. **Journal of Biological Chemistry**, v.247, n.10, p.3170-3175, 1972. Available from: <https://doi.org/10.1016/S0021-9258(19)45228-9>. Accessed: Jan. 25, 2025. doi: 10.1016/S0021-9258(19)45228-9.

NGAMKALA, S. et al. Histopathological study and intestinal mucous cell responses against *Aeromonas hydrophila* in Nile tilapia administered with *Lactobacillus rhamnosus* GG. **Veterinary World**, v.13, n.5, p.967-974, 2020. Available from: <https://doi.org/10.14202/vetworld.2020.967-974>. Accessed: Dec. 10, 2024. doi: 10.14202/vetworld.2020.967-974.

PÊS, T. S. et al. Quercetin in the diet of silver catfish: Effects on antioxidant status, blood parameters and pituitary hormone expression. **Aquaculture**, v.458, p.100-106, 2016. Available from: <https://doi.org/10.1016/j.aquaculture.2016.02.020>. Accessed: Feb. 16, 2025. doi: 10.1016/j.aquaculture.2016.02.020.

PIRARAT, N. et al. Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. **Research in Veterinary Science**, v.91, n.3, p.e92-e97, 2011. Available from: <https://doi.org/10.1016/j.rvsc.2011.02.014>. Accessed: Feb. 10, 2025. doi: 10.1016/j.rvsc.2011.02.014.

ROCHA-DE-SOUZA, C. M. et al. Influence of polarisation and differentiation on interaction of 43-kDa outer-membrane protein of *Aeromonas caviae* with human enterocyte-like Caco-2 cell line. **International Journal of Molecular Medicine**, v.11, p.661-667, 2003. Available from: <https://doi.org/10.3892/ijmm.11.5.661>. Accessed: Feb. 13, 2025. doi: 10.3892/ijmm.11.5.661.

ROSSI, G. G. et al. Antibiofilm activity of nanoemulsions of *Cymbopogon flexuosus* against rapidly growing mycobacteria. **Microbial Pathogenesis**, v.113, p.335-341, 2017. Available from: <https://doi.org/10.1016/j.micpath.2017.11.00>. Accessed: Jan. 21, 2025. doi: 10.1016/j.micpath.2017.11.002.

SACCOL, E. M. H. et al. Addition of *Lippia alba* (Mill) N. E. Brown essential oil to the diet of the silver catfish: an analysis of growth, metabolic and blood parameters and the antioxidant response. **Aquaculture**, v.416-417, p.244-254, 2013. Available from: <https://doi.org/10.1016/j.aquaculture.2013.09.036>. Accessed: Dec. 11, 2024. doi: 10.1016/j.aquaculture.2013.09.036.

SAHREEN, A. et al. Changes in the level of oxidative stress markers in Indian catfish (*Wallago attu*) infected with *Isoparorchis hypselobagri*. **Beni-Suef University Journal of Basic and Applied Sciences**, v.10, n.1, 2021. Available from: <https://doi.org/10.1186/s43088-021-00174-z>. Accessed: Nov. 21, 2024. doi: 10.1186/s43088-021-00174-z.

SALINAS, I.; PARRA, D. Fish mucosal immunity: intestine. **Mucosal Health in Aquaculture**, p.135-170, 2015. Available from: <https://doi.org/10.1016/B978-0-12-417186-2.00006-6>. Accessed: Apr. 24, 2025. doi: 10.1016/B978-0-12-417186-2.00006-6.

SEMWAL, A. et al. A review on pathogenicity of *Aeromonas hydrophila* and their mitigation through medicinal herbs in aquaculture. **Heliyon**, v.9, n.3, p.e14088, 2023. Available from: <https://pubmed.ncbi.nlm.nih.gov/36938468/>. Accessed: Jul. 13, 2024. doi: 10.1016/j.heliyon.2023.e14088.

SHIRIN, H. et al. Helicobacter pylori decreases gastric mucosal glutathione. **Cancer Letters**, v.164, n.2, p.127-133, 2001. Available from: <https://doi.org/10.1016/S0304-3835(01)00383-4>. Accessed: Jun. 19, 2024. doi: 10.1016/s0304-3835(01)00383-4.

SOUZA, E. M. et al. Chemical composition and evaluation of the antimicrobial activity of two essential oils. **Boletim do Instituto de Pesca**, v.44, n.2, p.44-47, 2018b. Available from: <https://doi.org/10.20950/1678-2305.2018.321>. Accessed: Jun. 22, 2024. doi: 10.20950/1678-2305.2018.321.

SOUZA. C. F. et al. Effects of dietary microencapsulated *Cymbopogon flexuosus* essential oil on reproductive related parameters in male *Rhamdia quelen*. **Fish Physiology and Biochemistry**, v.44, n.4, p.1253-1264, 2018a. Available from: <https://link.springer.com/article/10.1007/s10695-018-0518-9>. Accessed: Nov. 16, 2024. doi: 10.1007/s10695-018-0518-9.

SUN, B.-Y. et al. Dysbiosis, increased susceptibility to *Aeromonas hydrophila* infection in grass carp with antibiotic-induced intestinal. **Aquaculture**, v.552, 2022. Available from: <https://doi.org/10.1016/j.aquaculture.2022.737969>. Accessed: Jan. 21, 2025. doi: 10.1016/j.aquaculture.2022.737969.

SUTILI, F. J. et al. Effect of dietary supplementation with citral-loaded nanostructured systems on innate immune responses and gut microbiota of silver catfish (*Rhamdia quelen*). **Journal of Functional Foods**, v.60, p.103454, 2019. Available from: <https://doi.org/10.1016/j.jff.2019.103454>. Accessed: Jul. 13, 2024. doi: 10.1016/j.jff.2019.103454.

SUTILI, F. J. et al. The use of eugenol against *Aeromonas hydrophila* and its effect on hematological and immunological parameters in silver catfish (*Rhamdia quelen*), **Veterinary Immunology and Immunopathology**, v.157, n.3-4, p.142-148, 2014. Available from: <https://doi.org/10.1016/j.vetimm.2013.11.009>. Accessed: Nov. 14, 2024. doi: 10.1016/j.vetimm.2013.11.009.

TKACHENKO, H. et al. Oxidative stress biomarkers in liver of sea trout (*Salmo trutta* m. trutta L.) affected by ulcerative dermal necrosis syndrome. **Turkish Journal of Fisheries and Aquatic Sciences**, v.402, p.391-402, 2014. Available from: <https://www.trjfas.org/abstract.php?id=78>. Accessed: Sept. 26, 2024. doi: 10.4194/1303-2712-v14_2_09.

TYAGI, A. K.; MALIK, A. 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**, v.10, 2010. Available from: <https://doi.org/10.1186/1472-6882-10-65>. Accessed: Feb. 13, 2025. doi: 10.1186/1472-6882-10-65.

VALÉRIO-JÚNIOR, J. F. et al. 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**, v.54, e20240167, p.1-17, 2025. Available from: <https://doi.org/10.37496/rbz5420240167>. Accessed: May, 14, 2025. doi: 10.37496/rbz5420240167.

VASEEHARAN, B.; THAYA, R. Medicinal plant derivatives as immunostimulants: an alternative to chemotherapeutics and antibiotics in aquaculture, 2013. **Aquaculture International**, v.22, p.1079-1091, 2013. Available from: <http://link.springer.com/article/10.1007/s10499-013-9729-3>. Accessed: Jan. 22, 2025. doi: 10.1007/s10499-013-9729-3.

WANG, H. D. I. et al. Superoxide anion from the adventitia of the rat thoracic aorta inactivates nitric oxide. **Circulation**

Research, v.82, n.7, p.810-818, 1998. Available from: <https://doi.org/10.1161/01.RES.82.7.810>. Accessed: Dec. 10, 2024. doi: 10.1161/01.res.82.7.810.

YANG, Y. et al. Functional assessment of encapsulated citral for controlling necrotic enteritis in broiler chickens. **Poultry Science**, v.95, n.4, p.780-789, 2016. Available from: <https://doi.org/10.3382/ps/pev375>. Accessed: Aug. 05, 2024. doi: 10.3382/ps/pev375.

YILMAZ, S.; ERGÜN, S. Dietary supplementation with allspice pimenta dioica reduces the occurrence of streptococcal disease during first feeding of Mozambique tilapia fry. **Journal of Aquatic Animal Health**, v.26, n.3, p.144-148, 2014. Available from: <https://doi.org/10.1080/08997659.2014.893459>. Accessed: Oct. 12, 2024. doi: 10.1080/08997659.2014.893459.

ZEPPENFELD, C. C. et al. Essential oil of *Aloysia triphylla* as feed additive promotes growth of silver catfish (*Rhamdia quelen*). **Aquaculture Nutrition**, v.22, p.933-940, 2016. Available from: <https://doi.org/10.1111/anu.12311>. Accessed: Sept. 15, 2024. doi: 10.1111/anu.12311.

ZHANG, C. et al. The intestinal histopathology, innate immune response and antioxidant capacity of blunt snout bream (*Megalobrama amblycephala*) in response to *Aeromonas hydrophila*. **Fish and Shellfish Immunology**, v.124, p.525-533, 2022. Available from: <https://doi.org/10.1016/j.fsi.2022.04.037>. Accessed: Jan. 20, 2025. doi: 10.1016/j.fsi.2022.04.037.

ZHAO, J. et al. Immunology effects of dietary isoleucine on the immune response, antioxidant status and gene expression in the head kidney of juvenile Jian carp (*Cyprinus carpio* var. Jian). **Fish and Shellfish Immunology**, v.35, n.2, p.572-580, 2013. Available from: <https://doi.org/10.1016/j.fsi.2013.05.027>. Accessed: Nov. 23, 2024. doi: 10.1016/j.fsi.2013.05.027.

ZHAO, H. M. et al. Progress in the research of GSH in cells. **Chinese Science Bulletin**, v.56, n.28-29, p.3057-3063, 2011. Available from: <https://link.springer.com/article/10.1007/s11434-011-4689-3>. Accessed: Jul. 10, 2025. doi: 10.1007/s11434-011-4689-3.

ZHENG, Z. L. et al. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growthantioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). **Aquaculture**, v.292, n.3-4, p.214-218, 2009. Available from: <https://doi.org/10.1016/j.aquaculture.2009.04.025>. Accessed: Dec. 19, 2024. doi: 10.1016/j.aquaculture.2009.04.025.

ZHENG, T. et al. 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**, v.12, p.1-14, 2021. Available from: <https://doi.org/10.3389/fimmu.2021.740359>. Accessed: Feb. 11, 2025. doi: 10.3389/fimmu.2021.740359.

ZHOU, X. et al. Stability and in vitro digestibility of beta-carotene in nanoemulsions fabricated with different carrier oils. **Food Science Nutrition**, v.6, n.8, p.2537-2544, 2018. Available from: <https://doi.org/10.1002/fsn3.862>. Accessed: Jan. 14, 2025. doi: 10.1002/fsn3.862.