BOLETIM DO INSTITUTO DE PESCA
Short Communication

Functional assessment of citral dietary supplementation on growth performance, intestinal parameters, and specific activity of the digestive enzymes of *Sardinella brasiliensis* reared in recirculating aquaculture system

Bruna Tomazetti Michelotti[1], Marco Shizuo Owatari[2]*, Caio Magnotti[2], Ana Carolina Kohlrausch Klinger[1], Ulyssses da Silva Palma[2], Marina Cesconeto dos Santos[2], Sharine Nunes Descovi[1], Vinícius Ronzani Cerqueira[2], Sílvio Teixeira da Costa[1], Letícia Bogoni Bianchin[1], Bernardo Baldisserotto[1]

[1]Universidade Federal de Santa Maria – Santa Maria (RS), Brazil.
[2]Universidade Federal de Santa Catarina – Florianópolis (SC), Brazil.
*Corresponding author: owatarimarco@hotmail.com

ABSTRACT

This study assessed the impact of dietary citral supplementation on growth performance, intestinal parameters, and digestive enzyme activity in Brazilian sardine *Sardinella brasiliensis*. The experiment involved 240 juvenile *S. brasiliensis* distributed in a recirculating aquaculture system with 12 tanks. Fish were fed diets with varying levels of citral (0.5, 1, and 2 mL·kg feed^{-1}) for 20 days. Results showed improved survival with citral supplementation, increased lipase and amylase activity at 2 mL·kg feed^{-1}, and enhanced intestinal morphology. However, growth parameters were negatively affected at lower citral levels. Overall, the study recommends a citral inclusion level of around 1.25–1.3 mL·kg feed^{-1} for optimal results in *S. brasiliensis* aquaculture.

Keywords: Brazilian sardine; Feed supplementation; Marine fish farming; Sustainability.

Avaliação funcional da suplementação dietética de citral sobre o desempenho zootécnico, parâmetros intestinais e atividade específica das enzimas digestivas de *Sardinella brasiliensis* cultivada em sistema de recirculação para aquicultura

RESUMO

Este estudo avaliou o impacto da suplementação dietética de citral no desempenho do crescimento, parâmetros intestinais e atividade de enzimas digestivas na sardinha brasileira *Sardinella brasiliensis*. O experimento envolveu 240 juvenis de *S. brasiliensis* distribuídos em um sistema de aquicultura de recirculação com 12 tanques. Os peixes foram alimentados com dietas com níveis variados de citral (0,5, 1 e 2 mL·kg feed^{-1}) por 20 dias. Os resultados mostraram melhor sobrevivência com suplementação de citral, aumento da atividade de lipase e amilase em 2 mL·kg feed^{-1} e morfologia intestinal aprimorada. No entanto, os parâmetros de crescimento foram afetados negativamente em níveis mais baixos de citral. No geral, o estudo recomenda um nível de inclusão de citral de cerca de 1,25–1,3 mL·kg feed^{-1} para resultados ótimos no cultivo de *S. brasiliensis*.

Palavras-chave: Sardinha brasileira; Suplementação alimentar; Piscicultura marinha; Sustentabilidade.

Received: Aug 16, 2023 | **Approved:** Nov 25, 2024
Section editor: Fabiana Garcia

INTRODUCTION

The Brazilian sardine *Sardinella brasiliensis* is one of the most important species for the fishery in the Southwest Atlantic Ocean and Southern Brazil (SAP, 2021; Schroeder et al., 2022), has good characteristics for aquaculture, such as short life cycle, high fertility, fast growth, well-established consumer market, and is low-trophic level species (Cottrell et al., 2021; Perin & Vaz-dos-Santos, 2014).

In recent years, *S. brasiliensis* has gained prominence in Brazilian marine fish farming. Several studies have been carried out aiming at the development of technologies for *S. brasiliensis* farming (Baloi et al., 2016; Baloi et al., 2017b; Cerqueira et al., 2020), nutritional improvements (Sterzelecki et al., 2017, 2018, 2021a, 2021b), pathology investigations (Owatari et al., 2020), and physiological aspects under different cultivation situations (Angelo et al., 2021; Baloi et al., 2017a; Owatari et al., 2023), indicating that the species presents advantageous aspects when compared to other marine fish species, mainly accelerated growth even with low feeding rates (Baloi et al., 2016), and spontaneous spawning in captivity (Magnotti et al., 2020).

For the sustainable development of marine fish farming, it is necessary to implement new environmental-friendly strategies (Lieke et al., 2020). The use of feed additives or functional diets can be sustainable alternatives, as they can increase the nutritional status and prevent diseases, increasing fish resistance to parasites and bacteria, in addition to reducing the use of antibiotics and the spread of antimicrobial resistance in aquaculture environments (Brum et al., 2017; Jesus et al., 2021). In this way, nutritional strategies become valuable tools that can help condition fish for the sustainable growth of marine fish farming worldwide.

Studies have shown that dietary supplementation with essential oils improves fish growth and disease resistance (Souza et al., 2019; Sutili et al., 2019). Essential oils from lemon verbena *Aloysia citriodora* (syn. *A. triphylla*) and cochin grass *Cymbopogon flexuosus* improved growth and oxidative status (Zeppenfeld et al., 2016, 2017), protein deposition and carcass yield when added to the diet of silver catfish *Rhamdia quelen* (Rampelotto et al., 2018), and growth in Nile tilapia *Oreochromis niloticus* (Souza et al., 2020a, 2020b). Furthermore, it was also proven that citral (3,7-dimethyl-2,6-octadienal) is the major compound of these essential oils, whose compound demonstrates antimicrobial, antifungal and antiparasitic characteristics, making citral a natural food additive (Berk, 2016; Maarse, 1991; Saddiq & Khayyat, 2010; Zheng et al., 2015).

This study evaluated the effectiveness of citral as a dietary supplement for Brazilian sardine *S. brasiliensis*, examining its impact on growth performance, intestinal histomorphometry, and specific activity of digestive enzymes.

MATERIAL AND METHODS

Fish and rearing conditions

Second generation (F2) of juvenile *S. brasiliensis* (total length 9.7 ± 0.04 cm and weight 8.6 ± 0.04 g) were obtained by spontaneous spawning of a broodstock from Marine Fish Culture Laboratory of the Universidade Federal de Santa Catarina. The broodstock was kept in an outdoor 8,000-L black circular tank with constant aeration and water renewal of 350% per day (19–20 L·min^{-1}). The water was continuously supplied by direct pumping from the ocean, collected at Mozambique beach, Florianopolis (SC), Brazil (27°34′02″S, 48°25′44″W), as indicated by Magnotti et al. (2020). Larviculture and juvenile growth were performed as described by Cerqueira et al. (2020).

Two hundred and forty juvenile *S. brasiliensis* were randomly distributed in a recirculating aquaculture system (RAS) composed of 12 circular tanks of 150 L (n = 20 per tank). The RAS was filled with ocean water from Mozambique beach. Mechanical (50 µm) and biological filters, an ultraviolet sterilizer (60 W) and a foam fractionator were used for water purification. The photoperiod was mimicked using 20 W fluorescent lamps and controlled by an automatic timer, making a cycle of 12-h light/12-h dark (light start at 8 a.m.). During the 20-day experimental period, the water quality variables remained at a salinity of 33.15 ± 0.25 ppt, temperature of 27.11 ± 0.11°C, dissolved oxygen at 6.13 ± 0.05 mg·L^{-1}, pH at 8.24 ± 0.01, total ammonia 0.2 ± 0.01 mg·L^{-1}, nitrite 0.27 ± 0.03 mg·L^{-1}, and alkalinity ≥ 150 mg $CaCO_3$·L^{-1}.

The sardines received a basal diet without any feed additives during four days before the beginning of the experimental period. The remains of food and feces were removed by siphoning, and the loss of water by evaporation was replaced daily. Temperature, dissolved oxygen, and pH were checked daily, and ammonia and nitrite were measured every week, as described by Michelotti et al. (2018).

Citral and experimental diets

Citral (α-citral = 60.15%, β-citral = 39.85%) was purchased from Sigma-Aldrich (St. Louis, United States of America). The quantification of the isomers was executed in an Agilent 6890A gas chromatography coupled with a 5,973-mass selective detector using a HPCHIRAL capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) and electron ionization mode at 70eV. Helium was used as carrier gas in a flow rate of 1 mL·min^{-1}, injector temperature was set at 250°C and detector at 280°C. Oven

temperature was kept at 40°C for 4 min and raised to 240°C at a rate of 4°C·min^{-1}. Sample solutions of 1 µL (2:1,000 in hexane, v/v) were injected in a splitless mode. Kovats retention indices were calculated using a homologous series of C8-C40 n-alkanes injected under the same conditions of the samples. The isomers were identified by mass spectra and Kovats retention index comparison with data from the National Institute of Standards and Technology Mass Spectral Library. Compounds relative percent was estimated by under peak area integration obtained from the chromatogram according to Michelotti et al. (2020).

The basal experimental diet was produced according to the formulation described by Sterzelecki et al. (2017). Afterwards, citral was added to the diet together with fish oil (before cold drying at 16°C and 2-mm pelletizing) at different inclusion levels, making four treatments (0-control, 0.5, 1, and 2 mL kg feed^{-1}) with three replications each (Table 1). The sardines were fed four times a day (9 a.m., 1 p.m., 3 p.m., and 6 p.m.) up to apparent satiation for 20 days. The fish fasted 24 hours before the final sampling.

Table 1. The basal experimental diet formulation and analyzed proximate average composition*.

Ingredients	g kg^{-1}
Dextrin	309.3
Casein	206.3
Fish meal	200
Cellulose	110.3
Fish oil	66.1
Gelatin	40
Vitamins and minerals (premix)	40
Dibasic calcium phosphate	20
Sunflower oil	8
Composition	(%)
Dry matter content	88.14
Protein	34.69
Lipids	8.79
Mineral matter	5.51
Carbohydrate	30
CHO: L	0.34
Energy (J·kg^{-1})	1491

*Vitamin and mineral mixture (security levels per kilogram of product) — folic acid: 250 mg, pantothenic acid: 5,000 mg, antioxidant: 0.60 g, biotin: 125 mg, cobalt: 25 mg, copper: 2,000 mg, iron: 820 mg, iodine: 100 mg, manganese: 3750 mg, niacin: 5,000 mg, selenium: 75 mg, vitamin A: 1,000,000 UI, vitamin B1: 1,250 mg, vitamin B12: 3,750 mcg, vitamin B2: 2,500 mg, vitamin B6: 2,485 mg, vitamin C: 28,000 mg, vitamin D3: 500,000 UI, vitamin E: 20,000 UI, vitamin K: 500 mg, zinc: 17,500 mg.

Growth performance

All fish were sampled at the beginning and the end of the experiment, anesthetized (50 mg L^{-1} benzocaine), measured, and weighed. Survival (S), weight gain (WG), specific growth rate, and feed conversion rate (FC) were calculated as described by Michelotti et al. (2018). The condition factor (K) was calculated with Eq. 1:

$$K = W_f/L_f^3 \times 100 \quad (1)$$

Where: W_f: the final weight; L_f: the final length.

Feed intake (FI) was measured by weighing the feed at the beginning and end of the day after feeding the sardines, and the difference between the two values divided by the number of animals in the tank was considered the feed intake per animal per day.

Digestive enzymes

At the end of the experiment, nine fish per treatment (three from each tank) were anesthetized (50 mg·L^{-1} of benzocaine for 3 min) and euthanized by sectioning the spinal cord. Stomach and midgut portions were collected and frozen in liquid nitrogen and then stored at -20°C for digestive enzyme analysis.

Preparation of extracts from the stomach and intestine was performed as described by Michelotti et al. (2020). The experimental protocol for analysis of amylase activity in the intestine followed Benfold (1955), and starch hydrolysis was determined according to Park and Johnson (1949). One unit of the enzyme was defined as 1 mmol of glycosyl-glucose released from starch per minute per milligram of protein. The activity of intestinal lipase was determined as stated in Gawlicka et al. (2000), and pepsin activity in the stomach according to Hidalgo et al. (1999). The activity of the enzymes was calculated as stated in Almeida et al. (2018).

Intestinal histology and histomorphometry

For histological analysis, at the end of the experiment, nine fish per treatment (three from each tank) were anesthetized (50 mg·L^{-1} of benzocaine for 3 min) and euthanized by sectioning the spinal cord. Midgut portions were collected, and the samples were fixed in 10% formaldehyde and cleaved, dehydrated in increasing alcohol (70–100%), diaphanized in xylol (xylene), and embedded in paraffin. The blocks were cut into 6-µm cross sections (PAT-MR10 microtome) that were stained with Harris Haematoxylin-Eosin (HHE) to posterior mounting in Entellan medium to be analyzed in a Axio Imager A.2. The number of villi was counted, and the intestine diameter, villus height, crypt

depth, and muscularis layer width (estimated in 10 villi) were measured according to Le Roux et al. (2016). Morphometry measurements were performed using ImageJ software.

Statistical analysis

Data are expressed as the mean ± standard error of the mean. The homogeneity of variances was evaluated with Levene's test. The relationships between the studied parameters and dietary citral supplementation were analyzed with the software SigmaPlot 11.0 (SYSTAT Software, Inc.). In the situations in which there was no significant relationship, as variances were homoscedastic, comparisons between treatments were performed by one-way analysis of variance followed by Tukey's test with Statistica Software 7.0 (Stat Soft, Tulsa, OK, United States of America). Differences were considered significant at $p < 0.05$.

RESULTS

There was a significant relationship between dietary citral levels and all growth performance parameters analyzed. According to the equation, the highest survival value corresponded to 1.25–1.3 mL citral·kg feed^{-1}. The citral treatments had a survival around 80%, while in the control it was 40%. The lowest values of weight gain, specific growth rate, and condition factor, as well as the worst feed conversion rate, corresponded to 0.96–1.2 mL citral·kg feed^{-1} (Fig. 1), according to regression analyses.

The activity of the digestive enzymes amylase and lipase increased in 2 mL of citral·kg feed^{-1}, while pepsin was increased in 2 and 0.5 mL of citral·kg feed^{-1} (Fig. 2).

There were no changes in the height of villi and muscularis layer width in the intestine of the fish fed different dietary citral

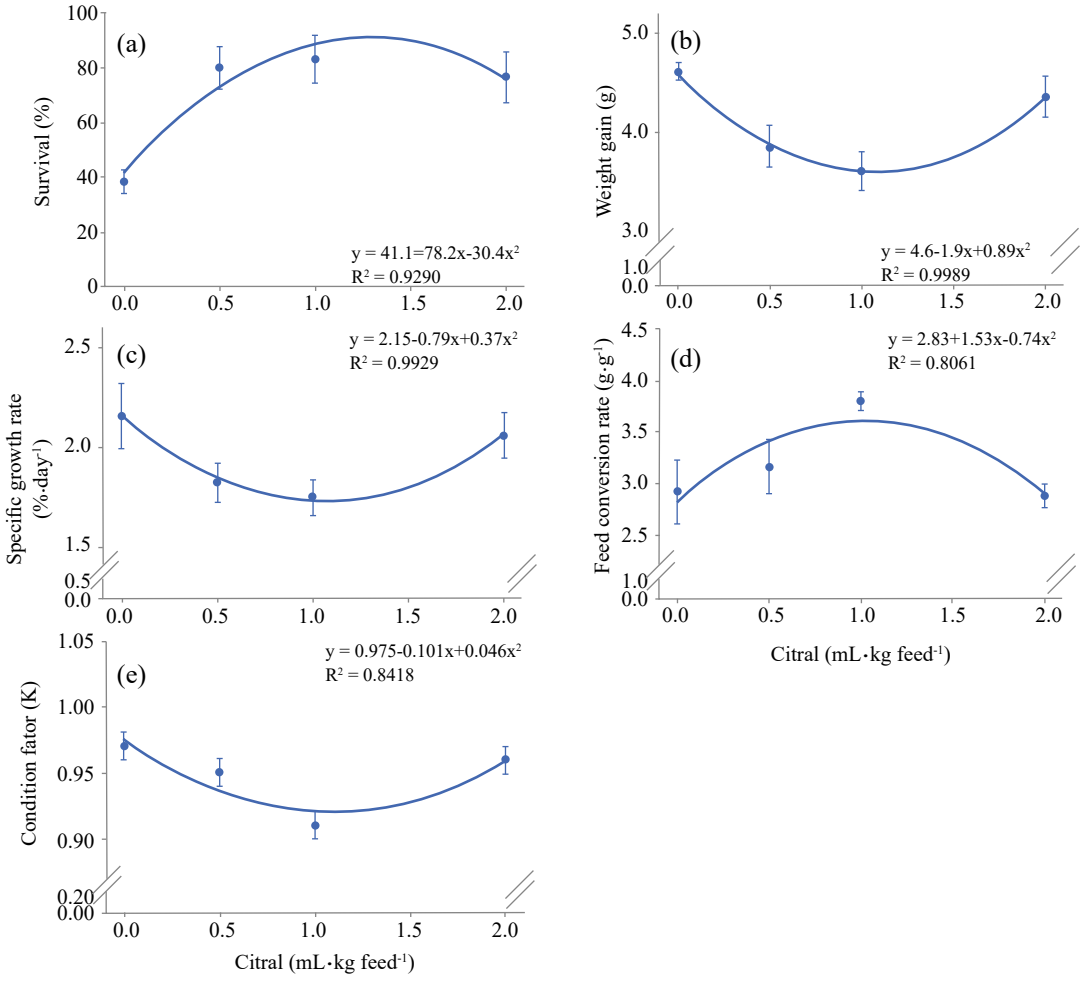

Figure 1. Growth performance indexes of the Brazilian sardine *Sardinella brasiliensis* after 20 days of feeding with citral dietary supplementation in concentrations at 0.5, 1, and 2 mL·kg feed^{-1}, or not supplemented (control). (a) Survival, (b) weight gain, (c) specific growth rate, (d) feed conversion ratio, (e) condition factor.

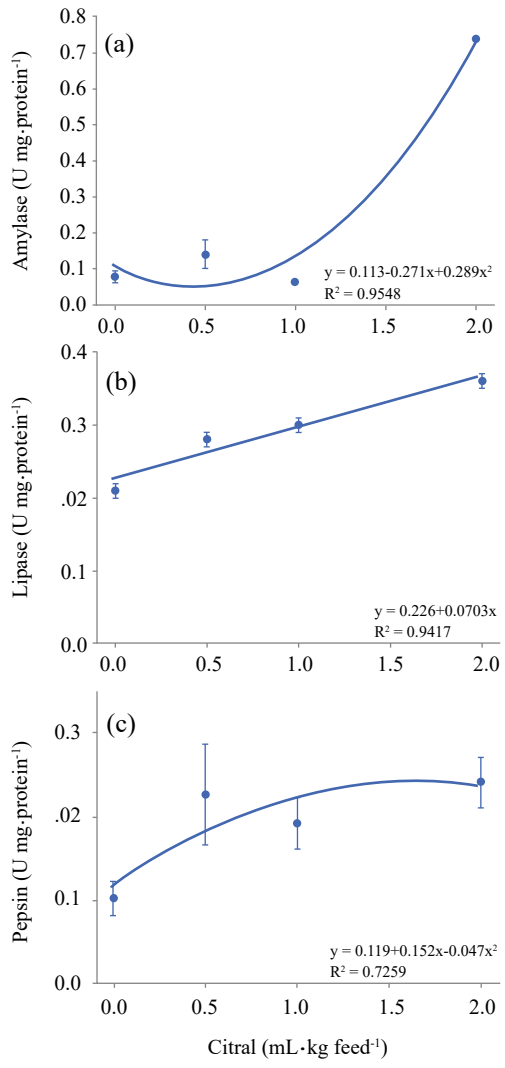

Figure 2. Specific activity of the digestive enzymes in the Brazilian sardine *Sardinella brasiliensis* after 20 days of feeding with citral dietary supplementation in concentrations at 0.5, 1, and 2 mL·kg feed^{-1}, or not supplemented (control). (a) Amylase, (b) lipase, (c) pepsin.

levels ($p > 0.05$). However, the intestinal diameter was larger with 0.5 mL of citral·kg^{-1} feed compared to the fish from control group. The number of villi and crypt depth increased with increasing dietary citral levels (Table 2).

DISCUSSION

The current research examined the effects of adding citral to the diet of Brazilian sardine *S. brasiliensis*. Results that showed supplementing the diet with citral led to increased survival rates, with the highest rates observed in the citral-treated groups. One possible reason for this finding could be that citral helped to reduce the stress induced by the experimental conditions in the fish. Thus, stress worsened in the control group, causing more deaths. This would be an unprecedented case of altered behavior in these experimental units, given that on other occasions in the same experimental units and with other marine species such as common snook *Centropomus undecimalis* (Michelotti et al., 2020) and Lebranche mullet *Mugil liza* (Silva et al., 2020) this phenomenon did not occur. Another factor to be considered is that citral has sedative and anesthetic effects (Santos et al., 2022), and this may have contributed to keeping the fish in the treated groups under less stressful conditions.

Sardines, even juveniles, form a dense school and react very quickly to any potential threat. Reports of abnormal behavior, deformity, and mortality, when they are kept in small tanks and high density, have already been made (Cerqueira et al., 2020). As we did not measure stress parameters, it was not possible to prove whether stress was reduced in citral treatments, which would explain the higher survival in these groups.

The addition of citral (0.3 mL·kg feed^{-1}) to the diet of Atlantic salmon (*Salmo salar*) did not affect growth after 30 days (Jensen et al., 2015), whereas 0.5 mL·kg feed^{-1} impaired growth performance parameter of common snook (Michelotti et al., 2020),

Table 2. Histomorphometric changes in the midgut of Brazilian sardine *Sardinella brasiliensis* after 20 days of dietary supplementation with citral*.

	Citral inclusion (mL·kg feed^{-1})			
Intestinal parameter	**0.0 (control)**	**0.5**	**1.0**	**2.0**
Villi height (µm)	228.29 ± 11.04^{a}	237.72 ± 21.81^{a}	209.90 ± 10.05^{a}	255.13 ± 26.81^{a}
Number of villi**	49.33 ± 4.80	50.66 ± 2.90	54.00 ± 5.03	62.00 ± 1.15
Intestine diameter (µm)	1,355.54 ± 36^{b}	1,623.03 ± 54^{a}	1,438.33 ± 69^{ab}	1,540.36 ± 72^{ab}
Crypt depth (µm)**	147.14 ± 8.31	153.34 ± 8.63	168.31 ± 8.24	184.81 ± 10.88
Muscularis layer width (µm)	27.72 ± 0.88^{a}	28.18 ± 1.69^{a}	30.18 ± 1.08^{a}	27.49 ± 0.89^{a}

*Data expressed as mean ± standard error. Different letters indicate significant difference between the treatments using one-way analysis of variance and Tukey's test ($p < 0.05$); **significant relationships with dietary citral supplementation levels. The relationships are given by the equations y = 48.3 + 6.5x ($r^2 = 0.9693$) and y = 146.3 + 19.5x ($r^2 = 0.9828$), where y: number of villi and crypt depth (µm), respectively, and x: citral supplementation (mL·kg of feed^{-1}).

as observed here in Brazilian sardine fed the same dietary levels of citral. Essential oils and/or their major compounds can act as prooxidant inducers and affect cell structures such as mitochondria, causing cytotoxic effects depending on the dosage. Thus, it is important to understand that compounds such as citral are not just feed additives, but complex substances in their chemical composition that can cause diverse and adverse effects to animals (Bakkali et al., 2008).

Citral also caused oxidative damage in the liver and gills of common snook (Mori et al., 2019). However, dietary supplementation with essential oils obtained from ginger *Zingiber officinale* and containing citral as the main compound did not affect (at 5 or 10 mL·kg feed^{-1}) or reduced (15 mL·kg feed^{-1}) the weight of the Nile tilapia *O. niloticus* compared to control fish (Brum et al., 2017). The addition of *A. citriodora* essential oil (2 mL·kg feed^{-1}) to the diet did not interfere with the growth of zebrafish *Danio rerio* (Zago et al., 2018), while the same level of inclusion of essential oil from *C. flexuosus* improved the growth of catfish (Zeppenfeld et al., 2016) and Nile tilapia (Souza et al., 2020a). These results indicate that the effects of dietary supplementation with citral or essential oils containing citral as the major compound may adversely influence fish growth, depending on the species and the synergy of other compounds present in the essential oils. In addition, citral may have its chemical instability increased in acidic environments (Chat et al., 2019), making the stomach a favorable environment for its degradation and absorption.

The digestion process in fish is widely investigated as it is less known than in mammals. Elevated activity of digestive enzymes may indicate some sign of inflammation or disease in the pancreas, and when the concentration is low it may indicate pancreatic insufficiency or serious liver disease. However, they have been also proposed as indicators of the nutritional status and functioning of the digestive organs in fish (Hidalgo et al., 1999; Sugita et al., 1997).

A possible mechanism of action of essential oils to improve feed digestion could be the stimulation of digestive enzyme activities (Mitsch et al., 2004). According to Hidalgo et al. (1999), omnivorous species showed greater amylase activity than carnivorous ones, and the activity of digestive enzymes may decrease with a decrease in incubation temperature, depending on the fish species and tissue analyzed.

Depending on the dose, dietary citral supplementation proportionally increased the activity of the enzymes' amylase, lipase, and protease, as well as the number of villi and crypt depth in Brazilian sardines, which would indicate an increase in intestinal area for nutrient absorption. Similarly, dietary supplementation with the essential oil of *A. citriodora* (*A. triphylla*) ncreased the length of intestinal folds in silver catfish (Zeppenfeld et al., 2016), corroborating the histological results of the current study with Brazilian sardines.

CONCLUSION

The results of the experiment suggested that the addition of citral as an additive in the diet of *S. brasiliensis* improved the survival of fish reared in RAS. Based on the findings of the current study, it is advised to supplement the diet with citral at inclusion levels around 1.25–1.3 mL citral·kg feed^{-1}.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The data related to this research are available upon request.

AUTHORS' CONTRIBUTIONS

Conceptualization: Michelotti, B.T., Baldisserotto, B.; **Methodology:** Michelotti, B.T.; **Formal analysis:** Michelotti, B.T., Owatari, M.S., Magnotti, C., Klinger, A.C.K., Santos, M.C., Descovi, S.H., Costa, S.T., Letícia B. Bianchin; **Investigation:** Michelotti, B.T., Magnotti, C., Klinger, A.C.K., Santos, M.C., Descovi, S.H.; **Writing – original draft:** Owatari, M.S., Magnotti, C.; **Writing – review & editing:** Owatari, M.S., Magnotti, C.; **Supervision:** Cerqueira, V.R., Baldisserotto, B.; **Resources:** Baldisserotto, B.; **Project administration:** Baldisserotto, B.; **Funding acquisition:** Baldisserotto, B; **Final approval:** Owatari,M.S.

FUNDING

Conselho Nacional de Desenvolvimento Científico e Tecnológico

Grant No.: CNPq 309101/2017-4

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

Finance code: 001

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

Almeida, A. P. G., Zardo, E. L., Toni, C., Behr, E. R., Picolli da Silva, L., Vieira, J. P., & Baldisserotto, B. (2018). Composition of gastrointestinal content, protease and lipase activities in summer and winter of four freshwater Siluriforms (Teleostei: Actinopterygii) with two different feeding habits. *Zoologia*, *35*, 1-8. https://doi.org/10.3897/zoologia.35.e13286

Angelo, M., Lisboa, M. K., Magnotti, C., Pilotto, M. R., Mattos, J. J., & Cerqueira, V. R. (2021). Temperature influence on the initial development of *Sardinella brasiliensis* larvae. *Aquaculture Research*, *52*(12), 6497-6503. https://doi.org/10.1111/are.15517

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils — a review. *Food and Chemical Toxicology*, *46*(2), 446-475. https://doi.org/10.1016/j.fct.2007.09.106

Baloi, M. F., de Carvalho, C. V., Sterzelecki, F. C., Passini, G., & Cerqueira, V. R. (2016). Effects of feeding frequency on growth, feed efficiency and body composition of juveniles Brazilian sardine, *Sardinella brasiliensis* (Steindacher 1879). *Aquaculture Research*, *47*(2), 554-560. https://doi.org/10.1111/are.12514

Baloi, M. F., Magnotti, C. F., Carneiro Sterzelecki, F., Vieira Rodrigues, R., & Ronzani Cerqueira, V. (2017a). Acute toxicity of ammonia in juvenile Brazilian sardine *Sardinella brasiliensis*. *Boletim do Instituto de Pesca*, *43*(1), 135-139. https://doi.org/10.20950/1678-2305.2017v43n1p135

Baloi, M. F., Sterzelecki, F. C., Sugai, J. K., Passini, G., Carvalho, C. V. A., & Cerqueira, V. R. (2017b). Growth performance, body composition and metabolic response to feeding rates in juvenile Brazilian sardine *Sardinella brasiliensis*. *Aquaculture Nutrition*, *23*(6), 1458-1466. https://doi.org/10.1111/anu.12521

Benfold, D. (1955). Amylase β and α. In S. P. Colowick & N. O. Kaplan (Eds.), *Method in Enzymology* (pp. 149-154). Academic Press.

Berk, Z. (2016). History, production, trade, and utilization. In Z. Berk (Ed.), *Citrus Fruit Processing* (pp. 1-8). Academic Press. https://doi.org/10.1016/B978-0-12-803133-9.00001-1

Brum, A., Pereira, S. A., Owatari, M. S., Chagas, E. C., Chaves, F. C. M., Mouriño, J. L. P., & Martins, M. L. (2017). Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture*, *468*(1), 235–243. https://doi.org/10.1016/j.aquaculture.2016.10.020

Cerqueira, V. R., Sterzelecki, F. C., Baloi, M. F., Magnotti, C., Cipriano, F. S., & Manzoni, G. C. (2020). Cultivo da sardinha verdadeira. In B. Baldisserotto (Ed.), *Espécies nativas para piscicultura no Brasil* (pp. 57-72). Santa Maria: Editora da UFSM.

Chat, O. A., Bhat, P. A., Nazir, N., & Dar, A. A. (2019). Self-assembled systems based on surfactants and polymers as stabilizers for citral in beverages. In *Value-Added Ingredients and Enrichments of Beverages* (pp. 487-521). Academic Press. https://doi.org/10.1016/B978-0-12-816687-1.00015-1

Cottrell, R. S., Metian, M., Froehlich, H. E., Blanchard, J. L., Sand Jacobsen, N., McIntyre, P. B., Nash, K. L., Williams, D. R., Bouwman, L., Gephart, J. A., Kuempel, C. D., Moran, D. D., Troell, M., & Halpern, B. S. (2021). Time to rethink trophic levels in aquaculture policy. *Reviews in Aquaculture*, *13*(3), 1583-1593. https://doi.org/10.1111/raq.12535

Gawlicka, A., Parent, B., Horn, M. H., Ross, N., Opstad, I., & Torrissen, O. J. (2000). Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): indication of readiness for first feeding. *Aquaculture*, *184*(3-4), 303-314. https://doi.org/10.1016/S0044-8486(99)00322-1

Hidalgo, M. C., Urea, E., & Sanz, A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, *170*(3-4), 267-283. https://doi.org/10.1016/S0044-8486(98)00413-X

Jensen, L. B., Provan, F., Larssen, E., Bron, J. E., & Obach, A. (2015). Reducing sea lice (*Lepeophtheirus salmonis)* infestation of farmed Atlantic salmon (*Salmo salar L.*) through functional feeds. *Aquaculture Nutrition*, *21*(6), 983-993. https://doi.org/10.1111/anu.12222

Jesus, G. F. A., Owatari, M. S., Pereira, S. A., Silva, B. C., Syracuse, N. M., Lopes, G. R., Addam, K., Cardoso, L., Mouriño, J. L. P., & Martins, M. L. (2021). Effects of sodium butyrate and *Lippia origanoides* essential oil blend on growth, intestinal microbiota, histology, and haemato-immunological response of Nile tilapia. *Fish & Shellfish Immunology*, *117*, 62-69. https://doi.org/10.1016/j.fsi.2021.07.008

Le Roux, A. B., Granger, L. A., Wakamatsu, N., Kearney, M. T., & Gaschen, L. (2016). Ex vivo correlation of ultrasonographic small intestinal wall layering with histology in dogs. *Veterinary Radiology & Ultrasound*, *57*(5), 534-545. https://doi.org/10.1111/vru.12392

Lieke, T., Meinelt, T., Hoseinifar, S. H., Pan, B., Straus, D. L., & Steinberg, C. E. (2020). Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Reviews in Aquaculture*, *12*(2), 943-965. https://doi.org/10.1111/raq.12365

Maarse, H. (1991). Volatile compounds in foods and beverages. Routledge. https://doi.org/10.1201/9780203734285

Magnotti, C., Sterzelecki, F., Cipriano, F., Pedrotti, F., Rocha, V., Freitas, G. L., & Cerqueira, V. (2020). Spontaneous spawning of Brazilian sardine in captivity. *Boletim do Instituto de Pesca*, *46*(1), e539. https://doi.org/10.20950/1678-2305.2020.46.1.539

Michelotti, B. T., Mori, N. C., Magnotti, C. C. F., Heinzmann, B. M., Almeida, A. P. G., Cerqueira, V. R., & Baldisserotto, B. (2020). Citral as food additive for common snook zootechnical parameters and digestive enzymes. *Ciência Rural*, *50*(4), e20190577. https://doi.org/10.1590/0103-8478cr20190577

Michelotti, B. T., Passini, G., Carvalho, C., Salbego, J., Cossettin, N., Rodrigues, R. V., Baldisserotto, B., & Cerqueira, V. R. (2018). Growth and metabolic parameters of common snook juveniles raised in freshwater with different water hardness. *Aquaculture*, *482*, 31-35. https://doi.org/10.1016/j.aquaculture.2017.08.029

Mitsch, P., Zitterl-Eglseer, K., Köhler, B., Gabler, C., Losa, R., & Zimpernik, I. (2004). The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. *Poultry Science*, *83*(4), 669-675. https://doi.org/10.1093/ps/83.4.669

Mori, N. C., Michelotti, B. T., da Silva Pês, T., Bressan, C. A., Sutili, F., Kreutz, L. C., Garlet, Q., Baldisserotto, B., Pavanato, M. A., Cerqueira, V. R., Costa, S. T., & Cerqueira, V. R. (2019). Citral as a dietary additive for *Centropomus undecimalis* juveniles: Redox, immune innate profiles, liver enzymes and histopathology. *Aquaculture*, *501*, 14-21. https://doi.org/10.1016/j.aquaculture.2018.11.003

Owatari, M. S., Magnotti, C., Vargas, J. H., de Carvalho, C. V. A., Sterzelecki, F. C., & Cerqueira, V. R. (2023). Influence of salinity on growth and survival of juvenile *Sardinella brasiliensis*. *Boletim do Instituto de Pesca*, *49*. https://doi.org/10.20950/1678-2305/bip.2023.49.e808

Owatari, M. S., Sterzelecki, F. C., da Silva, C. S., Magnotti, C., Mouriño, J. L. P., & Cerqueira, V. R. (2020). Amyloodiniosis in farmed *Sardinella brasiliensis* (Steindachner, 1879): Case report of successful therapeutic control with copper sulfate. *Parasitology International*, *76*, 102091. https://doi.org/10.1016/j.parint.2020.102091

Park, J. T., & Johnson, M. J. (1949). A submicrodetermination of glucose. *Journal of Biological Chemistry*, *181*(1), 149-151.

Perin, S., & Vaz-dos-Santos, A. M. (2014). Morphometry and relative growth of the Brazilian sardine, *Sardinella brasiliensis* (Steindachner, 1879) in the southeastern Brazilian bight. *Arquivos de Zoologia*, *45*, 63-72. https://doi.org/10.11606/issn.2176-7793.v45iespp63-72

Rampelotto, C., de Lima, J., Pinheiro, C. G., Salbego, J., da Silva, L. P., & Emanuelli, T. (2018). Supplementation with microencapsulated lemongrass essential oil improves protein deposition and carcass yield in silver catfish (*Rhamdia quelen*). *Acta Scientiarum – Animal Sciences*, *40*, 1-8. https://www.scielo.br/j/asas/a/FKY5qGyks9p6RGBWyrFXSrM/?format=pdf&lang=en. Accessed on 7 Dec. 2024

Saddiq, A. A., & Khayyat, S. A. (2010). Chemical and antimicrobial studies of monoterpene: Citral. *Pesticide Biochemistry and Physiology*, *98*(1), 89-93. https://doi.org/10.1016/j.pestbp.2010.05.004

Santos, A. C., Bianchini, A. E., Bandeira Junior, G., Garlet, Q. I., Brasil, M. T. B., Heinzmann, B. M., Baldisserotto, B., Caron, B. O., Cunha, M. A. (2022). Essential oil of *Aloysia citriodora Paláu* and citral: sedative and anesthetic efficacy and safety in *Rhamdia quelen* and *Ctenopharyngodon idella*. *Veterinary Anaesthesia and Analgesia*, *49*(1), 104-112. https://doi.org/10.1016/j.vaa.2021.10.004

Schroeder, R., Correia, A. T., Medeiros, S. D., Pessatti, M. L., & Schwingel, R. (2022). Spatiotemporal variability of the catch composition and discards estimates of the different methods of onboard preservation for the Brazilian sardine fishery in the southwest Atlantic Ocean. *Thalassas: An International Journal of Marine Sciences*, *38*, 573-597. https://doi.org/10.1007/s41208-022-00398-5

Secretaria de Aquicultura e Pesca (SAP) (2021). *Balanço 2020*. *Ministério da Agricultura, Pecuária e Abastecimento*.

Silva, E. C., Sterzelecki, F. C., Alves Musialak, L., Sugai, J. K., Castro, J. D. J. P., Pedrotti, F. S., Magnotti, C., Cipriano, F. S., & Cerqueira, V. R. (2020). Effect of feeding frequency on growth performance, blood metabolites, proximate composition and digestive enzymes of Lebranche mullet (*Mugil liza*) juveniles. *Aquaculture Research*, *51*(3), 1162-1169. https://doi.org/10.1111/are.14466

Souza, C. D., Baldissera, M. D., Baldisserotto, B., Heinzmann, B. M., Martos-Sitcha, J. A., 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., Souza, S. A., Souza, A. M., Copatti, C. E. (2020a). *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*(Suppl. 1), e20190140. https://doi.org/10.1590/0001-3765202020190140

Souza, R. C., Baldisserotto, B., Melo, J. F. B., Costa, M. M., Souza, E. M., Copatti, C. E. (2020b). Dietary *Aloysia triphylla* essential oil on growth performance and biochemical and haematological variables in Nile tilapia. *Aquaculture*, *519*, 734913. https://doi.org/10.1016/j.aquaculture.2019.734913

Sterzelecki, F. C., dos Santos Cipriano, F., Vasconcelos, V. R., Sugai, J. K., Mattos, J. J., Derner, R. B., Magnotti, C. F., Lopes, R. G., & Cerqueira, V. R. (2021a). Minimum rotifer density for best growth, survival and nutritional status of Brazilian sardine larvae, *Sardinella brasiliensis*. *Aquaculture*, *534*, 736264. https://doi.org/10.1016/j.aquaculture.2020.736264

Sterzelecki, F. C., Sugai, J. K., Baloi, M., Passini, G., de Carvalho, C. V. A., Fracalossi, D. M., & Cerqueira, V. R. (2017). Effect of dietary carbohydrate to lipid ratios on growth, digestive enzyme and blood metabolites of juvenile Brazilian sardines, *Sardinella brasiliensis* (Steindachner, 1879). *Aquaculture Research*, *48*(9), 5111-5121. https://doi.org/10.1111/are.13330

Sterzelecki, F. C., Sugai, J. K., Baloi, M., Passini, G., de Carvalho, C. V. A., Fracalossi, D. M., & Cerqueira, V. R. (2018). Effects of increasing protein level on the performance, enzyme activity and body composition of the Brazilian sardine, *Sardinella brasiliensis* (Steindachner, 1879). *Aquaculture Nutrition*, *24*(1), 366-374. https://doi.org/10.1111/anu.12567

Sterzelecki, F. C., Sugai, J. K., Mattos, J. J., Derner, R. B., Pedrotti, F. S., Magnotti, C. F., Lopes, R. G., & Cerqueira, V. R. (2021b). Live diet for first feeding of Brazilian sardine, *Sardinella brasiliensis* (Steindachner, 1879), larvae in captivity. *Aquaculture Research*, *52*(11), 5558-5565. https://doi.org/10.1111/are.15431

Sugita, H., Kawasaki, J., & Deguchi, Y. (1997). Production of amylase by the intestinal microflora in cultured freshwater fish. *Letters in Applied Microbiology*, *24*(2), 105-108. https://doi.org/10.1046/j.1472-765X.1997.00360.x

Sutili, F. J., Kreutz, L. C., Flores, F. C., da Silva, C. D. B., Kirsten, K. S., Voloski, A. P. D. S., & Baldisserotto, B. (2019). 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*, *60*, 103454. https://doi.org/10.1016/j.jff.2019.103454

Zago, D. C., Santos, A. C., Lanes, C. F. C., Almeida, D. V, Koakoski, G., de Abreu, M. S., & Baldisserotto, B. (2018). *Aloysia triphylla* in the zebrafish food: effects on physiology, behavior, and growth performance. *Fish Physiology and Biochemistry*, *44*(2), 465-474. https://doi.org/10.1007/s10695-017-0446-0

Zeppenfeld, C. C., Hernández, D. R., Santinón, J. J., Heinzmann, B. M., Cunha, M. A., Schmidt, D., & Baldisserotto, B. (2016). Essential oil of *Aloysia triphylla* as feed additive promotes growth of silver catfish (*Rhamdia quelen*). *Aquaculture Nutrition*, *22*(4), 933-940. https://doi.org/10.1111/anu.12311

Zeppenfeld, C. C., Saccol, E. M. H., Pês, T. S., Salbego, J., Koakoski, G., dos Santos, A. C., & Baldisserotto, B. (2017). *Aloysia triphylla* essential oil as food additive for *Rhamdia quelen* – Stress and antioxidant parameters. *Aquaculture Nutrition*, *23*(6), 1362-1367. https://doi.org/10.1111/anu.12511

Zheng, S., Jing, G., Wang, X., Ouyang, Q., Jia, L., & Tao, N. (2015). Citral exerts its antifungal activity against *Penicillium digitatum* by affecting the mitochondrial morphology and function. *Food Chemistry*, *178*, 76-81. https://doi.org/10.1016/j.foodchem.2015.01.077