BOLETIM DO INSTITUTO DE PESCA
Scientific Article

Effect of *Aloysia triphylla* essential oil on the productive performance and digestive tract histology of juvenile *Apistogramma cacatuoides*

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ABSTRACT

The objective of this study was to evaluate different concentrations of *Aloysia triphylla* essential oil (ATEO) on the productive performance of juvenile *Apistogramma cacatuoides*, as well as on liver morphology and intestinal villi morphometry. The experiment followed a completely randomized design with four treatments (0, 3, 6, or 9 g of ATEO per kg of feed), each with four replicates. A total of 80 juvenile *A. cacatuoides* (average weight of 28.4 ± 14.5 mg) was fed twice daily over a period of 45 days. At the end of the experimental period, final survival rates were determined, and the fish were measured to assess zootechnical performance. Additionally, eight juveniles per treatment were processed histologically to evaluate liver and intestinal tissues. The results showed that the use of ATEO in the feed did not result in statistically significant differences in growth or survival. However, juveniles that received feed containing 6 and 9 $g \cdot kg^{-1}$ of ATEO exhibited higher averages in the intestinal morphometry parameters evaluated, with no liver damage. These findings highlight the potential of ATEO in the production of this species. Further studies with extended periods of administration and elevated oil concentrations are recommended, as well as research to evaluate its immunostimulant effects on fish.

Keywords: Amazonian cichlid; Feed additive; Ornamental fish farming.

Efeito do óleo essencial de *Aloysia triphylla* sobre o desempenho produtivo e a histologia do trato digestivo de juvenis *Apistogramma cacatuoides*

RESUMO

O objetivo deste estudo foi avaliar diferentes concentrações de óleo essencial de *Aloysia triphylla* (OEAT) no desempenho produtivo de juvenis de *Apistogramma cacatuoides*, bem como na morfologia hepática e morfometria das vilosidades intestinais. O experimento seguiu um delineamento inteiramente casualizado com quatro tratamentos (0, 3, 6 ou 9 g de OEAT por kg de ração), cada um com quatro repetições. O total de 80 juvenis de *A. cacatuoides* (peso médio de 28,4 ± 14,5 mg) foi alimentado duas vezes ao dia, por 45 dias. No fim do período experimental, a taxa de sobrevivência foi determinada, e os peixes foram medidos para avaliação do desempenho zootécnico. Além disso, seis juvenis de cada tratamento foram processados histologicamente para avaliar os tecidos hepático e intestinal. Os resultados mostraram que o uso de OEAT na ração não resultou em diferenças estatisticamente significativas no crescimento ou na sobrevivência, entretanto juvenis que receberam ração contendo 6 e 9 $g \cdot kg^{-1}$ de OEAT apresentaram médias maiores nos parâmetros de morfometria intestinal avaliados, sem danos hepáticos. Esses achados destacam o potencial do OEAT na produção dessa espécie. Estudos posteriores com períodos de administração prolongados ou maiores concentrações de óleo são recomendados, juntamente com pesquisas para avaliar seus efeitos imunoestimulantes nos peixes.

Palavras-chave: Ciclídeo amazônico; Aditivo alimentar; Piscicultura ornamental.

Received: October 3, 2024 | **Approved:** March 12, 2025
Section editor: Claudia Maris Ferreira

INTRODUCTION

Apistogramma cacatuoides, a dwarf cichlid native to the Amazon basin (Alves et al., 2009), is highly valued in the ornamental fish market due to its morphological and behavioral traits, as well as its vibrant colors (Quérouil et al., 2015). In its natural environment, fish of the genus *Apistogramma* feed on zooplankton, insect larvae, periphyton and detritus. However, in captivity, they accept both dry and moist feeds (Ferreira et al., 2018). In the Brazilian ornamental fish market, individuals of this species can sell for up to R$ 35 (US$ 6.36), while in the international market a specimen measuring 3–4 cm can fetch up to USD 49 (Aquanimal, 2023), with prices varying based on color patterns and country/region where its sale takes place.

Although highly valued by aquarists, there are few studies related to the rearing of this species in captivity. Some research has focused on its reproduction (Alves, 2007; Alves et al., 2009; Engelking et al., 2010; Römer et al., 2014; Romer & Beisenherz, 2005) or feeding protocols for larviculture (Zanfurlin-Lima et al., 2024). However, nutritional studies and research on immunonutrition for *A. cacatuoides* are nonexistent and are essential for the consolidation of native species in the ornamental aquaculture sector.

In this context, the use of essential oils in aquaculture has demonstrated excellent potential for these compounds when used as feed additives (Sutili et al., 2018). They act as growth promoters (Giannenas et al., 2012) and immunostimulants (Mahboub & Tartor, 2020), improve feed conversion rates (Chung et al., 2021; Hassaan & Soltan, 2016), promote antioxidant activity (Diler et al., 2017; Zheng et al., 2009), exhibit antibacterial properties (Bandeira-Junior et al., 2022; Sutili et al., 2015), and have anxiolytic effects (Zago et al., 2018).

Among these, the *Aloysia triphylla* essential oil (ATEO) is a product that has been already tested in fish and exhibited antioxidant, anti-inflammatory, anesthetic, and immunostimulant effects, as well as bactericidal, fungicidal, and parasiticidal properties (Brandão et al., 2021; Gressler et al., 2014; Souza et al., 2019). Furthermore, the use of ATEO as an additive in diets for juvenile Nile tilapia promoted greater growth and immunostimulant action (Souza et al., 2020). In *Rhamdia quelen*, ATEO increased antioxidant activity (Zeppenfeld et al., 2017), leading to greater growth and positive changes in intestinal morphology (Zeppenfeld et al., 2016).

However, ATEO has been used to enhance the production of juvenile forms, especially those of ornamental species, and may be a viable alternative to improve the productive performance of *A. cacatuoides* during this phase. Thus, the present study aimed to evaluate the effect of using the ATEO on the productive performance and survival of *A. cacatuoides* when used as a feed additive at different concentrations in the diet and its effect on liver morphology and the morphometry of intestinal villi.

MATERIALS AND METHODS

Fish and experimental conditions

The present study was approved by the Ethics Committee on Animal Use at Universidade Nilton Lins, under protocol No. 005/2022. The experiment was conducted in the Laboratory of Reproduction and Molecular Biology of Aquatic Organisms (LRBMOA) at Universidade Nilton Lins, Manaus, Amazonas, Brazil.

For this purpose, 80 juveniles obtained from the natural reproduction of *A. cacatuoides* breeders held in the LRBMOA were used. The larvae were separated from their parents five days after hatching and placed in an aquarium with 40 L of water coupled to a water recirculation system with a mechanical and biological filter, heating and constant aeration. The larvae were fed according to the protocol defined by Zanfurlin-Lima et al. (2024). They were initially fed brine shrimp nauplii and later fed to apparent satiety twice a day (at 8 a.m. and 5 p.m.) with commercial feed for tropical ornamental fish (manufacturer's guaranteed levels: crude protein 35.5%; ether extract 4.0%; crude fiber 3.0%; mineral matter 8.0%; moisture 12.0%; calcium 1.6%; phosphorus 1.0%). The feed was subjected to a process of maceration, after which it was presented in a powdered form.

Acquisition and characterization of the oil

The *A. triphylla* plants utilized for oil extraction were cultivated at Embrapa Amazônia Ocidental, Manaus, Amazonas, Brazil. The essential oil extraction process involved harvesting and drying the leaves and flowers of the plants until they reached a constant weight. The essential oil was then extracted by hydrodistillation in a Clevenger apparatus for a period of two hours at the institution's Medicinal Plants and Phytochemistry Laboratory (Potzernheim et al., 2012). After extraction, an aliquot of each essential oil was utilized for the purpose of determining its primary chemical composition by means of gas chromatography and mass spectrometry.

Procedures used in the experiment and water quality

The experiment was conducted using a completely randomized design with four treatments defined according to the following concentrations of ATEO: 0, 3, 6, and 9 $g \cdot kg^{-1}$ of feed, each with four replicates. The experimental units consisted of polyethylene containers in a semi-static system supplied with 5 L of water and constant aeration, at the density of 1 fish·L^{-1}. Partial water changes (50%) were performed daily two hours after the last feeding, and the experiment lasted for 45 days.

During the experiment, the fish were fed to apparent satiety twice a day (at 8 a.m. and 5 p.m.) with commercial feed for tropical ornamental fish (manufacturer's guaranteed levels described above) and with the addition of ATEO in the previously determined proportions. To incorporate the EO into the feed, cereal alcohol (diluent) was used at the ratio of 1.5 mL of oil for every 100 g of alcohol to facilitate even dispersion throughout the feed, in accordance with Dairiki et al. (2013).

The physical-chemical variables of the water, such as pH, temperature, electrical conductivity, total dissolved solids, and dissolved oxygen, were monitored daily using two multiparameter probes (Combo 5 and AK88V2, Akso). Total ammonia and nitrite levels were measured every five days, using a photocolorimeter (ACQUA, Alfakit). The photoperiod was maintained at 12-h light and 12-h darkness.

Analysis of production performance

At the end of the experimental period, survival was evaluated, and all the juveniles were anesthetized in benzocaine hydrochloride (100 mg·L^{-1}) to biometrics assessment. From this, the following were calculated (Eqs. 1 and 2):

$$\text{Weight gain (Wg)=Final weight (Wf)-Initial weight (Wi)} \quad (1)$$

$$\text{Length gain (Lg)=(Final length Lf-Initial length)} \quad (2)$$

Specific growth rate was calculated according to Lugert et al. (2016) (Eq. 3):

$$\text{Specific growth rate} = \left(\frac{\mathrm{l(Wf)} - \mathrm{l(W_i)}}{\mathrm{t}}\right) \times 100 \quad (3)$$

Where: t: number of days of the experiment.

Batch uniformity in weight (Uw) or length (Ul) was calculated according to Furuya et al. (1998) (Eq. 4):

$$\text{Uw or Ul} = \frac{\mathrm{X}}{\mathrm{X1}} \times 100 \quad (4)$$

Where: Uw: batch uniformity in weight; Ul: batch uniformity in length; X: total number of fish in each experimental unit; X1: final number of fish with final weight (FP) or final length (FL) within 20% of the mean of each experimental unit.

Relative condition factor (Kn) was calculated according to Le Cren (1951) (Eq. 5):

$$\mathrm{K}n = \frac{\mathrm{W}}{\mathrm{aL}^{n}} \quad (5)$$

Where: W: weight; L: length; a: a constant; n: an exponent usually lying between 2.5 and 4.0.

Survival (S) was calculated by Eq. 6:

$$\mathrm{S} = \left(\frac{\mathrm{N_f}}{\mathrm{N_i}}\right) \times 100 \quad (6)$$

Where: Nf: final number of fish; Ni: initial number of fish.

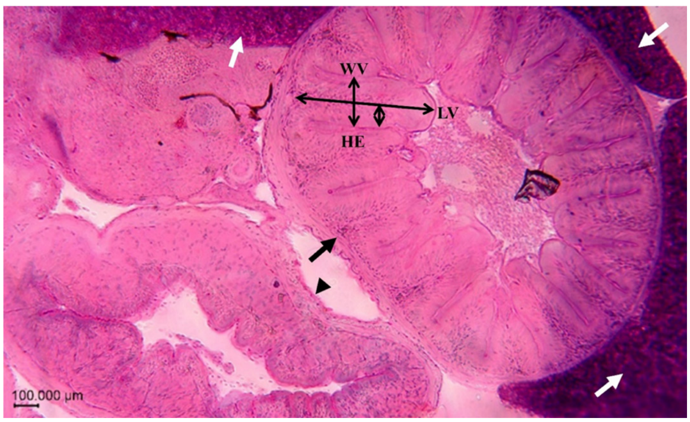
Histological analyses

A total of eight animals was collected per treatment (two from each experimental unit) for histological analyses of the liver and intestine after the end of the experimental period. The specimens were euthanized in benzocaine hydrochloride (300 mg·L^{-1}). Due to the relatively diminutive proportions of the animals, the intestinal tract and hepatic organ were not subject to removal to avoid any potential damage to these structures. Consequently, it was taken to proceed with excising the regions of the cephalic, dorsal and caudal, with a view to facilitating optimal preservation and fixation of the organs within the visceral cavity.

The samples were then fixed for 24 hours in a 4% paraformaldehyde solution in 0.2 M Sorensen phosphate buffer (pH 7.2). After fixation, the samples were washed three times in 0.2 M Sorensen phosphate buffer (pH 7.2) with intervals of at least one hour between washes and stored in 70% alcohol.

The inclusion in histological resin (methyl methacrylate) was carried out according to the manufacturer's recommendations (Leica Biosystems). The samples preserved in 70% alcohol were progressively dehydrated in alcoholic solutions (80% alcohol for one hour and 95% alcohol for three hours). Following this, they were embedded in a solution of 95% alcohol and resin (1/1 ratio) for four hours and pure resin (Resin 1) for overnight. The next day, they were embedded in pure resin (Resin 2) for four hours and then transferred to a new resin with the hardener. After hardening, the samples were sectioned to 4 μm using a microtome and high-profile metal blades. For the final assembly of the slides, these were stained with hematoxylin-floxin and subsequently visualized for image capture using an optical microscope (Bel Photonics) coupled to a digital camera (Micro Scientific 12 MP) and image capture software (Capture 2.1).

Histological alterations in the liver were classified according to the degree of morphological damage, following the methodology of Cengiz and Unlu (2006). For this, five sections of liver tissue were analyzed on each slide (n = 8). The classification of liver tissue degradation was assessed based on the integrity of liver nuclei, cytoplasm and hepatocytes, categorizing them as either healthy, intermediate, or degraded, according to Stejskal et al. (2021). The morphometry of the intestinal villi (Fig. 1) was analyzed according to Cunha et al. (2016).



LV: length of villi; WV: width of villi; HE: height of epithelium.

Figure 1. Micrograph of the intestinal tissue of juvenile *Apistogramma cacatuoides* fed a diet supplemented with 9 g of essential oil of *Aloysia triphylla* per kg of feed for 45 days. Black arrow: intestine; white arrow: liver; arrowhead: stomach; staining: hematoxylin-floxin. Magnification 10x.

Statistical analysis

The data obtained were subjected to Shapiro-Wilk and Brown-Forsythe's tests to verify the normality of errors and homoscedasticity of variances, respectively. Subsequently, the mean values of zootechnical performance were statistically analyzed using parametric one-way analysis of variance (ANOVA), and, when statistical differences were found, the means were compared using the Tukey's test at a 5% probability level. Data regarding the morphometric analyses of the intestine were analyzed using the Kruskal-Wallis' test, and when statistical differences were observed, the means were compared using Dunn's test. Statistical analyses were performed using the R program (R Core Team, 2024).

RESULTS

Essential oil composition

The ATEO showed two components in higher proportions, the β-pinene (16.6%) and *trans*-pinocamphone (13.3%) (Table 1).

Table 1. Components of the essential oil of *Aloysia triphylla*.

Components	%	LRI exp	LRI lit
β -pinene	16.6	973	974
trans-pinocamphone	13.3	1,156	1,158
trans-pinocarvyl acetate	7.6	1,295	1,298
(*E*)-caryophyllene	6.5	1,410	1,417
Guaiol	6.2	1,589	1,600
cis-pinocamphone	5.5	1,169	1,172
caryophyllene oxide	4.1	1,572	1,582
Nopinone	3.6	1,134	1,135
germacrene B	2.9	1,546	1,559
Pinocarvone	2.7	1,157	1,160
Limonene	2.6	1,024	1,024
Myrtenal	2.6	1,192	1,195
germacrene D	2.5	1,472	1,480
Myrcene	2.4	987	988
Bulnesol	2.1	1,658	1,670
alpha-pinene	2.0	930	932
Others	16.8	-	-

Others: components with low concentrations; LRI exp: linear retention index from the experiment; LRI lit: linear retention index from the literature.

Water quality parameters

The physical-chemical variables of the water did not differ statistically between treatments (Table 2). Concentrations of ammonia and nitrite remained at 0 ppm throughout the experimental period.

Productive performance

At the end of the experimental period, no statistical difference was observed for the parameters of zootechnical performance among the evaluated treatments (Table 3). Additionally, during the 45 days of feeding, no statistical difference was observed in the survival of the juvenile *A. cacatuoides*.

Histological analysis

In the morphometric analysis of the intestines of juveniles fed a diet containing 9 $g{\cdot}kg^{-1}$, higher mean values for length of intestinal villi (LV) and height of the intestinal epithelium (HE) were observed (Table 4), statistically differing from the means presented in the treatments with 0 and 3 $g{\cdot}kg^{-1}$. Regarding the mean width of the intestinal villi (WV), only juveniles fed with 3 $g{\cdot}kg^{-1}$ differed statistically from the other treatments ($p < 0.05$).

Table 2. Mean values ± standard deviation of water quality parameters for juvenile *Apistogramma cacatuoides* fed different concentrations of *Aloysia triphylla* essential oil in the diet over the 45 days of the experiment.

Treatment ($g \cdot kg^{-1}$)	pH	Temperature (°C)	Electrical conductivity ($\mu S \cdot cm^{-1}$)	Total dissolved solids (ppm)	Dissolved oxygen ($mg \cdot l^{-1}$)
0	5.3 ± 0.1	27.1 ± 0.5	64.7 ± 1.4	32.1 ± 2.4	6.3 ± 0.4
3	5.2 ± 0.4	26.6 ± 0.6	65.1 ± 3.5	33.4 ± 1.6	5.9 ± 0.4
6	5.1 ± 0.1	27.2 ± 1.0	58.8 ± 0.5	29.0 ± 1.4	6.6 ± 0.1
9	5.2 ± 0.2	26.9 ± 0.6	57.4 ± 3.7	29.3 ± 1.7	6.5 ± 0.2

Table 3. Mean values ± standard deviation of the zootechnical parameters of juvenile *Apistogramma cacatuoides* feed different concentrations of *Aloysia triphylla* essential oil per kg of feed over a period of 45 days.

Parameters	Concentrations of *Aloysia triphylla* essential oil in the feed ($g \cdot kg^{-1}$)				*p*-value
	0	3	6	9	
Wg (mg)	31.700 ± 22.200	35.100 ± 18.500	40.400 ± 22.600	45.400 ± 26.400	0.268
Lg (mm)	2.600 ± 1.900	2.800 ± 1.900	3.200 ± 1.600	3.600 ± 1.800	0.383
SGR (%)	1.500 ± 0.900	1.600 ± 0.700	1.900 ± 0.800	2.000 ± 0.800	0.301
S (%)	91.100 ± 10.200	88.200 ± 15.900	76.000 ± 8.300	95.800 ± 8.400	3.157
Ul (%)	100.000 ± 0.000	100.000 ± 0.000	100.000 ± 0.000	100.000 ± 0.000	0.425
Uw (%)	50.000 ± 24.20	58.700 ± 16.700	33.300 ± 20.400	47.400 ± 19.200	0.080
Kn	0.999 ± 0.004	1.011 ± 0.009	1.005 ± 0.029	1.000 ± 0.042	0.580

Wg: weight gain; Lg: length gain; SGR: specific growth rate; S: survival; Ul: batch uniformity in length; Uw: batch uniformity in weight; Kn: relative condition factor.

Table 4. Mean values ± standard deviation of the length (LV) and width (WV) of the intestinal villi, and the height of the intestinal epithelium (HE) of juvenile *Apistogramma cacatuoides* after 45 days of feeding different concentrations of the *Aloysia triphylla* essential oil per kg of feed*.

Variable	Concentrations of *Aloysia triphylla* essential oil in the feed ($g \cdot kg^{-1}$)				
	0	3	6	9	*p*-value
LV (µm)	177.8 ± 14.1 b	95.6 ± 7.9 c	187.2 ± 33.2 ab	208.7 ± 16.7 a	7.63e-09
WV (µm)	73.2 ± 19.3 a	42.5 ± 11.5 b	75.6 ± 26.4 a	88.9 ± 13.5 a	2.055e-06
HE (µm)	38.5 ± 12.8 b	21.2 ± 4.9 c	38.4 ± 13.0 ab	49.6 ± 7.4 a	6.538e-07

*Different letters differ statistically between treatment by the Kruskal-Wallis' test ($p < 0.05$).

In the histological evaluation of liver tissue, congestion of a mild degree was observed in all treatments. Grade I vacuolization was also observed in the control treatment, grade III in the 3 $g \cdot kg^{-1}$ treatment, and grade II in the other treatments (Table 5), as shown in Fig. 2.

Table 5. Histopathological alterations in the liver tissue of juvenile *Apistogramma cacatuoides* after 45 days of feeding different concentrations of *Aloysia triphylla* essential oil per kg of feed.

Alterations	Concentrations of *Aloysia triphylla* essential oil in the feed ($g \cdot kg^{-1}$)			
	0	3	6	9
Necrosis	-	-	-	-
Congestion	+	+	+	+
Hypertrophy hepatocellular	-	-	-	-
Vacuolization	+	+++	++	++

-: no clinical signs; +: mild; ++: moderate; +++: severe clinical signs.

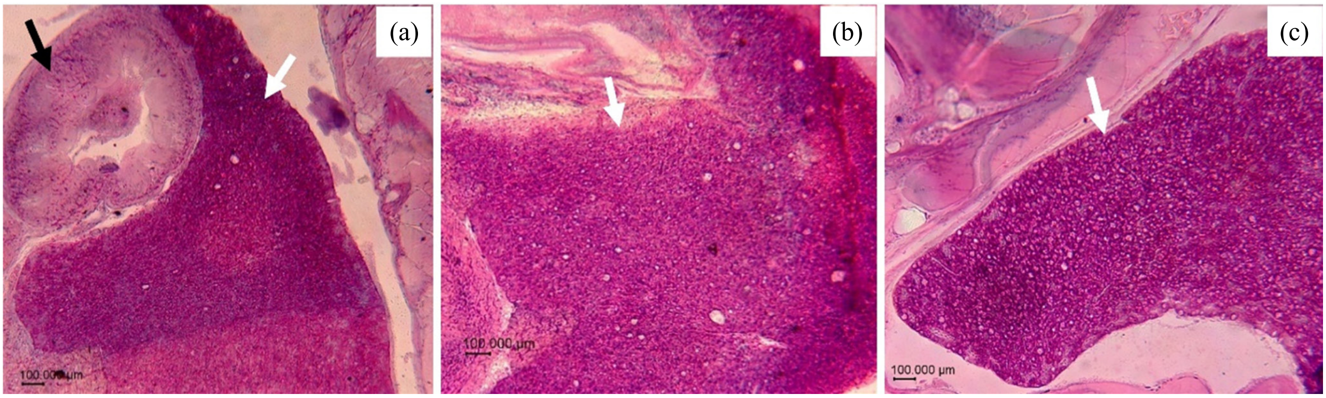


Figure 2. Micrograph of liver tissue of *Apistogramma cacatuoides* fed a diet supplemented with *Aloysia triphylla* essential oil (ATEO) for 45 days. (a) Vacuolization grade 1 in the liver of a juvenile fed with 0 mg·kg^{-1} of ATEO; (b) grade 2 in the liver of a juvenile fed with 9 g·kg^{-1} of ATEO; and (c) grade 3 in the liver of a juvenile fed with 3 g·kg^{-1} of ATEO. Black arrow: intestine; white arrow: liver; staining: hematoxylin-floxin. Magnification 10x.

DISCUSSION

The physicochemical variables of the water samples remained in accordance with the parameters established in the study conducted by Zanfurlin-Lima et al. (2024). No statistically significant differences were observed between the treatment groups, indicating that all conditions were favorable.

The predominant components of the essential oil utilized in this study were β-pinene (16.6%) and trans-pinocamphone (13.3%). These components were different from those observed in other studies, in which the majority compounds were β-Citral e α-citral (De Camargo et al., 2022; Zago et al., 2018; Zeppenfeld et al., 2016; Zeppenfeld et al., 2017), In addition to α-Citral (39,91%), Souza et al. (2020) identified two other predominant components: carveol (25.36%) and limonene (21.52%). The chemical composition of essential oils is known to vary due to the influence of environmental factors. These factors include geographical origin, soil characteristics, climate, management techniques, extraction method, and the plant's growth phase (He et al., 2018; Moniodis et al., 2018; Zhang et al., 2023).

In this study, it was possible to observe a trend of increasing the means of the performance variables as the concentration of ATEO in the diet increased, although no statistical differences were found. According to Harikrishnan et al. (2011), the performance of animals can be impacted by the doses of essential oils that are administered. This may suggest that the concentrations used were either not ingested or absorbed sufficiently to promote significant differences in zootechnical performance, or that the duration of the supplemented diet was insufficient.

In studies using two concentrations (0.9 and 1.8 g) of ATEO·kg^{-1} in the diet of zebrafish (*Danio rerio*), no improvements in zootechnical performance indices were observed, even over a 90-day experimental period. However, a decrease in oxygen consumption and exploratory activity of the species was noted, indicating an anxiolytic effect of the oil (Zago et al., 2018). Another study, which used juvenile *Rhamdia quelen* (10.2 ± 4.3 g) fed a diet containing 1.8 g·kg^{-1} of ATEO for 32 days, also showed no significant effects of the supplementation on zootechnical parameters. However, the authors concluded that ATEO may be an alternative to prevent and/or reduce liver damage induced by mycotoxins, thus acting as a hepatoprotective agent (De Camargo et al., 2022).

On the other hand, studies with juvenile *Rhamdia quelen* (148.22 ± 16.94 g) fed a diet with ATEO for 60 days (Zeppenfeld et al., 2016; Zeppenfeld et al., 2017) and with juvenile Nile tilapia (*Oreochromis niloticus*) (10.79 ± 0.02 g) fed a diet with ATEO for 45 days (Souza et al., 2020) demonstrated that the inclusion of 2 mL·kg^{-1} of ATEO in the diet promoted greater growth. This inclusion increases the number of folds in the intestinal epithelium of the fish, as well as enhances the activity of gastrointestinal enzymes and benefits the hematological profile, thereby favoring the digestion and absorption of nutrients and electrolytes.

ATEO can be used in aquaculture to enhance production stability due to its immunostimulant action, as previously observed in *O. niloticus* (Souza et al., 2020), *R. quelen* (Dos Santos et al., 2017), and rainbow trout (*Oncorhynchus mykiss*) (Adeli et al., 2021). In this sense, the use of ATEO in the diet of *A. cacatuoides* also provided significant beneficial alterations to the intestinal morphology, but these were not sufficient to enhance productive performance during the evaluated period. The use of ATEO appears to show better results for growth performance when administered to medium and large species, which may be related to growth rate, duration of administration and the concentration of ATEO consumed and absorbed during the experimental period. Such factors may have influenced the results of this study, given that no statistical difference in growth was observed for *A. cacatuoides*, which is a small species that only doubled in size during the 45 days of feeding.

Another salient factor is that essential oils exert their effects based on the predominant and secondary phytocompounds that comprise their chemical composition (Souza Valente et al., 2024). In the ATEO utilized in this study, no significant phytocompound (> 20%) was identified, displaying substantial variation in its secondary components; it can be hypothesized that the relatively low concentration of the major phytocompounds may have had some influence on the animals' response.

Histological analyses are important for identifying possible responses and adverse reactions in juveniles exposed to ATEO. The liver, a crucial organ in lipid metabolism, is responsible for the synthesis and oxidation of fatty acids (Moustafa et al., 2020; Yilmaz & Genc, 2006). The hepatotoxic effects of essential oils from plants may be related to their toxic constituents, allergens or high doses. Therefore, it is necessary to conduct studies with longer exposure periods to better understand the long-term histopathological effects (Bandaranayake, 2006).

The vacuolization and congestion of the sinusoids observed in the juvenile *A. cacatuoides* are likely related to the storage substances in the hepatocytes, due to the nutrient intake received by the animals, rather than to toxic effects of ATEO at the concentrations used, as such observations were also identified in juveniles that were fed only on commercial feed. The hepatic vacuolization and congestion found are considered reversible alterations.

Moreover, constant handling during fish production can result in elevated stress levels, leading to a breakdown of homeostasis and negatively influencing the health of the animals. This can cause undesirable side effects, such as reduced growth, immunodepression, increased susceptibility to fungal and bacterial diseases and a higher mortality rate (Philippe et al., 2023; Sampaio & Freire, 2016; Souza et al., 2019). Therefore, it is essential to use immunostimulant agents to enhance the animals' resistance under stressful conditions, even if they do not promote a significant increase in fish growth.

CONCLUSION

The utilization of ATEO at concentrations of up to 9 $g \cdot kg^{-1}$ of feed had no deleterious effect on the productive performance of juvenile *A. cacatuoides*. It was also evident that concentrations of 6 and 9 $g \cdot kg^{-1}$ resulted in a significant increase in intestinal morphometry and no occurrence of hepatocyte degradation. In this regard, the results obtained demonstrated the potential of using ATEO in the production of *A. cacatuoides*, and studies with longer periods of administration of the oil or that use higher concentrations should be conducted to also examine its immunostimulant effects, given that the production of native ornamental fish requires management techniques that provide greater resilience to the animals.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data sets were generated or analyzed in the current study.

AUTHORS' CONTRIBUTION

Conceptualization: Gama, M.M., Freitas, T.M.; **Investigation:** Gama, M.M., Abe, H.A., Araújo dos Santos, A., Zanfurlin-Lima, E.; **Data curation:** Gama, M.M., Abe, H.A., Araújo dos Santos, A.; **Formal Analysis:** Gama, M.M.; **Writing – original draft:** Gama, M.M., Abe, H.A., Araújo dos Santos, A., Zanfurlin-Lima, E., Chaves, F.C.M., Chagas, E.C.; **Writing – review & editing:** Gama, M.M., Araújo dos Santos, A., Freitas, T.M.; **Supervision:** Abe, H.A., Araújo dos Santos, A., Freitas, T.M.; **Resources:** Chaves, F.C.M., Freitas, T.M.; **Methodology:** Chaves, F.C.M., Chagas, E.C.; **Project administration:** Freitas, T.M.; **Final approval:** Freitas, T.M.

FUNDING

Fundação de Amparo à Pesquisa do Estado do Amazonas

Grants No.: 001/2023 – PAIC/AM – FNL; 004/2024 - PEX-CT&I/FAPEAM.

ACKNOWLEDGMENTS

We would like to express our thanks to the Universidade Nilton Lins for providing the necessary space to carry out the experiment, to the team at the Laboratório de Reprodução de Peixes Amazônicos for their technical contributions and assistance throughout the experimental process, and to the Laboratório de Plantas Medicinais e Fitoquímica at Embrapa Amazonia Ocidental, for the process of characterizing and producing the essential oil used in this. Our thanks also to the scientific initiation coordinators, especially Professors Raquel Salgado Marques and Ilsa Maria Honorio de Valois Coelho.

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