



Antioxidant status and performance of *Macrobrachium rosenbergii* juveniles fed diets containing non-nutritive *Aloysia triphylla* essential oil

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Abstract

Incorporating essential oils as dietary supplements in aquaculture is increasing in popularity. In prawn farming, their use is associated with reduced disease outbreaks, enhanced immunity, and improved animal growth and welfare. Herein, we explored the use of lemon beebrush essential oil (*Aloysia triphylla*; EOAT) in the diet of juvenile giant freshwater prawns (*Macrobrachium rosenbergii*) and its potential impact on growth performance and oxidative status of the hepatopancreas. Four hundred juvenile *M. rosenbergii* (0.40 ± 0.19 g, similar weights between sexes) were fed diets containing concentrations of EOAT at 0.0% (control), 0.1%, 0.2%, or 0.3% for 50 days. Each experimental group had five replicates, with 20 prawns per experimental unit. Prawn survival remained above 84% for all experimental groups. Prawn weight gain, specific growth rate, feed conversion ratio, and body condition were similar among all experimental groups. Dietary EOAT increased activities of the enzymatic antioxidants, catalase and superoxide dismutase, and varied glutathione S transferase activity in the hepatopancreas of prawns when compared to those fed the control diet. Dietary EOAT did not affect malondialdehyde (lipid peroxidation) levels. While EOAT had no impact on survival and growth of *M. rosenbergii*, it may modulate the detoxification capacity of the hepatopancreas (i.e., metabolic resistance). Future work should prospect EOAT as part of a nutraceutical blend, especially when combined with known growth enhancers and immune stimulants. Based on our results, dietary inclusion of 0.2% EOAT is the most suitable for juvenile *M. rosenbergii* farming.

Keywords Antioxidant enzymes · Feed supplement · Lemon verbena · Crustacean welfare · Innate immunity

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Introduction

The giant river prawn, *Macrobrachium rosenbergii*, is a freshwater species found in tropical and subtropical environments. It is a commercially important prawn species in tropical producer countries, with a global yield of ~ 294,000 tonnes in 2020 (FAO 2022). During the last two decades, commercial interest in prawn farming has resurged, particularly in the Americas and Asia. Although still not at a level to compete with marine penaeid farming, freshwater prawn farming is a sustainable way to diversify the aquatic food industry (New et al. 2000). Aimed at improving productivity and economic gains in prawn farming, research efforts have focused on the potential of growth enhancers and dietary immune stimulants (Jahanbakhshi et al. 2022; Naksing et al. 2022), including prebiotics, probiotics, organic acids, and herbs (Chen et al. 2017; Wee et al. 2018; Amiruddin et al. 2021). Interest in natural compounds has intensified in recent years, especially the use of essential oils.

Essential oils (EO) are natural mixtures of compounds distilled from plant materials. EOs are volatile, odoriferous, lipophilic, and rich in monoterpenes and sesquiterpenes with low molecular weight(s) and broad biological activity, e.g., antibacterial, antifungal, antioxidant, and anti-inflammatory properties (Noriega 2020; Badr et al. 2021; Silva et al. 2021). In culture settings, EOs are applied as growth promoters and immune stimulants (Dawood et al. 2022; Liu et al. 2022; Hoa et al. 2023), natural anaesthetics (Hoseini et al. 2019; de Souza Valente 2022; de Souza Valente et al. 2023), phytotherapeutics (Dawood et al. 2021), and food preservatives (Rout et al. 2022).

The aromatic plant lemon beebrush (*Aloysia triphylla*), Verbenaceae family, is originally from Latin America and Africa and is currently naturalized in North America and the Mediterranean. Lemon beebrush is a medicinal ornamental sub-shrub, with its active medicinal component derived from the leaves, which contain 0.9% to 1.5% of EO (Golparyan et al. 2018). The EO of *A. triphylla* (EOAT) is associated with different benefits for aquaculture. Immersion of silver catfish (*Rhamdia quelen*) in water with EOAT reduces gross oxidative stress during transportation (Zeppenfeld et al. 2014). Therapeutic baths with *A. triphylla* have antiparasitic activity on tambaqui (*Colossoma macropomum*; dos Santos et al. 2023). It is an effective anesthetic for fish (*R. quelen*, Gressler et al. 2014; *Oreochromis niloticus*, Teixeira et al. 2017; *Lophiosilurus alexandri*, Becker et al. 2017) and shrimp (*Penaeus vannamei*, Parodi et al. 2014). As a food additive, EOAT promotes the growth of silver catfish (*R. quelen*) (Zeppenfeld et al. 2016) and Nile tilapia (*O. niloticus*) (de Souza et al. 2020) and improves zebrafish (*Danio rerio*) welfare due to an anxiolytic effect (Zago et al. 2018).

Despite such reported benefits of EOAT for fish and some shellfish, there has been no effort to assess the effect of dietary EOAT on the growth and antioxidant responses of *M. rosenbergii*. Therefore, this study aimed to evaluate the growth parameters of juvenile *M. rosenbergii* when reared on diets supplemented with increasing amounts of EOAT. In addition, we recorded the activities of enzymatic antioxidants and lipid peroxidation levels of the hepatopancreas in *M. rosenbergii*.

Material and methods

Plant origin and essential oil extraction

Specimens of *Aloysia triphylla* were raised and sampled in Frederico Westphalen, Brazil. A certified specimen was deposited in the Biology Department herbarium, UFSM, Brazil, Reg. No. SMDB 11169.

EOAT extracts were obtained from leaves via steam distillation using a Clevenger-type apparatus for 3 h (European Pharmacopoeia 2007) and subsequently stored at -20°C in amber glass bottles. The EOAT was analyzed using an Agilent 7890A gas chromatograph (Agilent Technology®, USA) coupled to a 5975C Mass Spectrometer with HP-5-ms non-polar fused silica capillary column (5% phenyl—95% methylsiloxane phase; $30\text{ m} \times 0.25\text{ mm}$ id $\times 0.25\text{ }\mu\text{m}$ film thickness) and EI-MS of 70 eV. Analysis parameters were carrier gas: He (1 mL min^{-1}); split input: 1:100; inlet and detector temperature: 250°C ; temperature program: 40°C for 4 min and 40°C to 320°C at a rate of $4^{\circ}\text{C min}^{-1}$ (Bandeira Jr et al. 2017). EOAT components were identified by comparing the mass spectra and Kovats retention indices with published data (NIST 2010). Quantitative analysis was performed using an Agilent 7890A gas chromatograph equipped with a flame ionization detector, using a column with the same features and program as described above, except for split-less injection mode and inlet and detector temperatures at 300°C . Samples of EOAT were injected in triplicate and the relative percentage of components was estimated by integrating the peak area obtained from the flame ionization detector (FID) chromatograms (Silva-Flores et al. 2019). The main identified phytochemicals of the EOAT leaves were β -citral (207.8 g kg^{-1}) and α -citral (294.1 g kg^{-1}).

Experimental diets

A total of four experimental diets were investigated: 0.0% (control), 0.1%, 0.2%, and 0.3% (v/w) of EOAT (Table 1). EOAT was added to diets by replacing the respective amount of soybean oil. The crude protein inclusion level (30%) used in this study followed the optimum protein requirement published for juvenile *M. rosenbergii* (30–35%) (Antiporda 1986; D'Abramo and New 2000). Similarly, the lipid inclusion level (5%) falls within the 3–7% optimum dietary lipid level recommended for all *M. rosenbergii* developmental stages (Mitra et al. 2005). For feed preparation, solid ingredients were pulverized in a hammer mill, graded to 0.8-mm particle size, and mixed for processing. Feed pellets were manufactured by pre-wetting the mixture with water at 35°C . The diet, extruded into 1-mm-diameter pellets, was dried in a forced air oven (24 h, 45°C) and stored at 4°C .

Proximate composition was carried out on diets, in triplicates, using AOAC (2016) methods, including gross energy (bomb calorimetry, IKA® model C 5000), ether extract (Soxhlet extraction method), moisture (heating at 105°C for 24 h), dry matter (dry at 40°C until constant weight), crude protein (Kjeldahl method), ash (incineration at 550°C for 16 h), crude fiber (Weende method), and nitrogen-free extract (sum of ether extract, moisture, crude protein, ash, and crude fiber subtracted from 100).

Experimental design and setup

In total, 400 mixed sex juveniles (*Macrobrachium rosenbergii*; similar starting weights for females and males) were obtained from the Shrimp Culture Laboratory, UFPR, Brazil. Prawns were acclimated for 7 days in circular tanks (200 L capacity, density 50 prawns m^{-2}), with mechanical and biological filtration, and fed the control diet (Table 1 absent EOAT). After acclimation, prawns were randomly allocated into 20 tanks (100 L; $n = 5$ replicates per experimental group), with 20 prawns in each tank, in a recirculating aquaculture system with constant aeration. Throughout the feeding trial of 50 days, prawns were fed three times daily (9h30, 14h30, 20h30) with an initial feeding rate equivalent to 5% of biomass. The amount of feed offered was adjusted daily based on consumption.

Table 1 Feed formulation and proximate composition of the experimental diets for *Macrobrachium rosenbergii*, with different concentrations of *Aloysia triphylla* essential oil

Ingredients (%)	Experimental diets			
	Control	0.1%	0.2%	0.3%
Ground corn	41.98	41.98	41.98	41.98
Fish meal	25.00	25.00	25.00	25.00
Soybean meal	25.00	25.00	25.00	25.00
Soybean oil	5.00	4.90	4.80	4.70
Corn starch	2.00	2.00	2.00	2.00
Vitamin and mineral supplement ¹	1.00	1.00	1.00	1.00
Butylated hydroxytoluene	0.02	0.02	0.02	0.02
EOAT ²	0.00	0.10	0.20	0.30
Total	100.00	100.00	100.00	100.00
Proximate composition				
Gross energy (kcal g ⁻¹)	4.49	4.50	4.45	4.43
Ether extract (%)	4.85	4.77	5.10	5.18
Moisture (%)	4.68	4.52	4.79	4.54
Dry matter (%)	95.32	95.48	95.21	95.46
Crude protein (%)	30.81	30.51	30.46	30.60
Ash (%)	9.23	8.21	10.24	10.05
Crude fiber (%)	4.19	4.35	3.98	4.19
Nitrogen-free extract (%)	51.73	52.67	50.68	50.58

¹ Vitamin guarantee levels kg⁻¹: 36 mg vitamin B2, 25.5 mg vitamin B6, 45 mg vitamin B12, 1200 mg vitamin C, 11.2 mg folic acid, 67.5 mg pantothenic acid, 170 mg nicotinic acid, 1.68 mg biotin, 265 mg inositol. Mineral guarantee levels kg⁻¹: 65 mg iron, 13.8 mg copper, 150 mg zinc, 85 mg manganese, 0.35 mg cobalt, 1.3 mg iodine, 0.4 mg selenium

² Essential oil of *Aloysia triphylla*: percentage corresponds to 0.00, 1.00, 2.00, and 3.00 mL of EOAT kg of feed⁻¹ (v/w)

Tanks were siphoned to remove uneaten food and feces 30 min before feeding. The photoperiod was 12 h light:12 h dark cycle.

Water quality parameters were monitored daily for the entire trial. Observed values were (mean ± SD): temperature (29.12 ± 1.46 °C; digital thermometer-CE®), pH (7.97 ± 0.27; pH meter AT-315-Alfakit®), dissolved oxygen (6.47 ± 0.54 mg L⁻¹; oximeter AT-170-Alfakit®), total ammonia nitrogen (0.047 ± 0.030 mg N L⁻¹; according to Mackerth 1978), nitrite (0.035 ± 0.044 mg L⁻¹; according to Mackerth 1978), nitrate (4.453 ± 4.377 mg L⁻¹; according to Mackerth 1978), alkalinity (83.0 ± 7.51 mg CaCO₃ L⁻¹; according to Walker 1978), and hardness (84.0 ± 22.0 mg CaCO₃ L⁻¹; according to Walker 1978).

Growth parameters

A total of 20 prawns ($n = 5$ per experimental group), randomly selected on days 0 and 50, were measured with a digital caliper 402 (King Tools®, Canada) and weighed (Analytical Scale AY 220; Shimadzu®, Japan). Two lengths were considered:

Prawn length (cm) =	linear distance between the tip of the rostrum (anterior) to the tip of the telson (posterior, uropods)
Standard prawn length (cm) =	linear distance from the posterior margin of the ocular orbit to the base of the telson

Survival was determined at the end of the feeding trial. Survival and growth parameters were calculated as follows:

- Survival (%) = (final number of prawn/initial number of prawn) $\times$ 100
- Final weight gain (%) = [(final prawn weight – initial prawn weight)/initial prawn weight] $\times$ 100
- Daily weight gain (g day^{-1}) = final weight gain/days of feeding trial
- Weekly weight gain (g week^{-1}) = final weight gain/weeks of feeding trial
- Specific growth rate (g day^{-1}) = [(ln final prawn weight – ln initial prawn weight)/days of feeding trial] $\times$ 100
- Feed conversion ratio (g g^{-1}) = weight of total feed provided/prawn weight gain
- Condition factor (g cm^{-3}) = [final prawn weight/(standard length $^{-3}$)] $\times$ 100

Enzyme activities in the hepatopancreas

After the feeding trial, 12 prawns were randomly collected ($n = 3$ per experimental group), euthanized by thermal shock (iced water, 5 min), surface sterilized with ethanol (70%, v/v), and dissected for the hepatopancreas. Tissues were immediately stored in sterile 2-mL microtubes at $-20\text{ }^{\circ}\text{C}$. The hepatopancreas was subsequently sent to the Laboratory of Aquatic Animal Reproduction Technology (Unioeste, Brazil), homogenized in cold 0.8% sterile saline solution with the use of an electric homogenizer (T10 basic Ultra-Turrax®, China), and centrifuged at $12,000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$ (Sigma 3-16KL, Germany). Supernatants were used to evaluate the activity of oxidative stress biomarkers, including catalase (CAT), total superoxide dismutase (SOD) and glutathione S-transferase (GST), and the levels of lipid peroxidation. CAT activity was determined by measuring H_2O_2 catalysis into O_2 and H_2O (de Souza et al. 2014), and expressed as U mg^{-1} , where one unit refers to the amount of CAT necessary to degrade 1 mM of $\text{H}_2\text{O}_2\text{ min}^{-1}$. SOD activity was determined using nitro blue tetrazolium (NBT), following Campa-Córdova et al. (2009), and expressed as U mg^{-1} , where one unit refers to the amount of SOD necessary to inhibit 50% of O_2^- reaction with NBT. GST activity was determined by monitoring the conjugation of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB; de Souza et al. 2014; Tu et al. 2008) and expressed as U mg^{-1} , where one unit refers to the amount of GST necessary to conjugate 1 mM of CDNB min^{-1} . Lipid peroxidation was determined by the thiobarbituric acid reactive species (TBARS) assay (Oakes and Van Der Kraak 2003; Mensah et al. 2012) and expressed as pmol of malondialdehyde (MDA) levels mg^{-1} of protein.

Statistical analyses

Data are expressed as mean $\pm$ standard error (SEM). Homoscedasticity and normality between experimental groups were evaluated using Levene's and Shapiro-Wilk's tests, respectively. Data were analyzed using orthogonal polynomial contrasts analysis (Zar 2014; Motulsky 2018). This analysis was applied to verify which regression model best describes

the relationships between experimental groups (predictors) and the variables (responses). Linear, quadratic, and cubic models were tested (Carvalho et al. 2023). Model selection took into consideration both the lowest P -value and the highest coefficient of determination (r^2), also considering the absence of residual trends (Motulsky 2018). Analyses were performed using Minitab® 17 (USA). Differences were considered significant when $P < 0.05$.

Results

Growth performance

Upon completion of the feeding trial, there were no significant differences in prawn survival or growth levels among the treatment groups (0.1%, 0.2%, and 0.3% EOAT) and the control (0.0%; Table 2). Notably, all the experimental groups displayed high survival rates, $> 84\%$ (Table 2).

Detoxification enzymes

Catalase activity levels were 1.3- to 1.8-fold higher ($0.078 \text{ U mg protein}^{-1}$) in the prawns fed 0.3% EOAT compared to those fed all other experimental diets ($0.044\text{--}0.060 \text{ U mg protein}^{-1}$; $P = 0.0034$; Fig. 1A). Three- to six-fold higher SOD activity was observed for prawns fed EOAT, with all dietary treatments significantly different from the control group ($P = 4.61 \times 10^{-5}$; Fig. 1B). GST activity levels fluctuated, being reduced in prawns fed 0.1% EOAT in comparison to those fed 0.2% EOAT and the control group ($P = 0.0094$), though like prawns fed 0.3% EOAT (Fig. 1C). Levels of malondialdehyde (lipid peroxidation) were consistent across all treatments, and much lower than the control group, $0.6\text{--}0.7 \text{ pmol MDA per mg protein}$ ($P < 0.0001$; Fig. 1D).

Discussion

For crustacean aquaculture, animal growth performance is paramount as it greatly influences the final product yield, quality, and profit. The addition of any feed supplement must ensure adequate growth and never impair animal performance (i.e., detrimental non-target effects). Regardless of the EOAT level used (up to 0.3% v/w), this dietary supplement does not impair prawn zootechnical performance. Our results corroborate other studies with EOs used as feed supplements. Dietary supplementation with EO of bushy lippia (*Lippia alba*) administered by feeding to *M. rosenbergii* did not affect prawn growth (de Almeida et al. 2022; Cagol et al. 2020). Similarly, EOAT did not diminish zebrafish growth (Zago et al. 2018). A blend of organic acids (citric and sorbic acids) and EO components (thymol and vanillin) added to *P. vannamei* diet does not affect shrimp performance (He et al. 2017). Nonetheless, EOAT used as a feed supplement for Nile tilapia had a positive effect on fish final weight, weight gain, and SGR, although without a gross effect on fish survival (de Souza et al. 2020). The inclusion of *Melaleuca alternifolia* EO in feed can increase prawn zootechnical performance (Liu et al. 2022).

In addition to routine zootechnical parameters, we analyzed the potential effect of EOAT as a modulator of the oxidative status of the hepatopancreas. Animal nutritionists have considered carefully feed compositions that promote animal health and growth. Nevertheless,

Table 2 Growth performance of juvenile *Macrobrachium rosenbergii* reared on diets supplemented with *Aloysia triphylla* essential oil

Growth parameters	Experimental groups (mL EOAT kg diet ⁻¹)					Trend	F	P-value	r ²
	Control	0.10%	0.20%	0.30%					
Survival (%)	87.14 ± 4.16	85.71 ± 4.52	84.29 ± 4.16	87.14 ± 4.74		Quadratic	0.1282	0.8804	0.015
Initial weight (g)	0.40 ± 0.19	0.40 ± 0.19	0.40 ± 0.19	0.40 ± 0.19		N/A			
Final weight (g)	1.56 ± 0.17	1.57 ± 0.04	1.54 ± 0.05	1.66 ± 0.05		Linear	1.0264	0.3244	0.054
Final weight gain (g)	1.17 ± 0.08	1.18 ± 0.04	1.15 ± 0.05	1.26 ± 0.05		Linear	1.0264	0.3243	0.054
Daily weight gain (g day ⁻¹)	0.023 ± 0.002	0.024 ± 0.001	0.023 ± 0.001	0.025 ± 0.001		Linear	1.0264	0.3244	0.054
Weekly weight gain (g week ⁻¹)	0.163 ± 0.011	0.165 ± 0.005	0.160 ± 0.007	0.177 ± 0.007		1.0264	0.3243	0.5013	0.054
Initial standard length (cm)	2.43 ± 0.50	2.43 ± 0.50	2.43 ± 0.50	2.43 ± 0.50		N/A			
Final standard length (cm)	3.84 ± 0.07	3.77 ± 0.07	3.71 ± 0.04	3.85 ± 0.02		Quadratic	2.2112	0.1401	0.206
Initial total length (cm)	3.06 ± 0.61	3.06 ± 0.61	3.06 ± 0.61	3.06 ± 0.61		N/A			
Final total length (cm)	5.07 ± 0.12	4.94 ± 0.09	4.94 ± 0.05	5.03 ± 0.04		Quadratic	1.7698	0.2003	0.172
SGR (g week ⁻¹)	2.74 ± 0.09	2.75 ± 0.05	2.71 ± 0.07	2.86 ± 0.06		Linear	1.1097	0.3061	0.058
FCR (g g ⁻¹)	2.36 ± 0.11	2.44 ± 0.12	2.52 ± 0.08	2.23 ± 0.10		Quadratic	1.8673	0.1849	0.180
Condition factor (g cm ⁻³)	2.75 ± 0.07	2.93 ± 0.11	3.02 ± 0.07	2.90 ± 0.09		Quadratic	2.6792	0.0974	0.240

SGR specific growth rate, FCR feed conversion ratio. Data presented as mean ± SEM, n = 5. Trend, F statistics, P-values, and coefficients of determination (r²) values from orthogonal polynomial contrast analysis

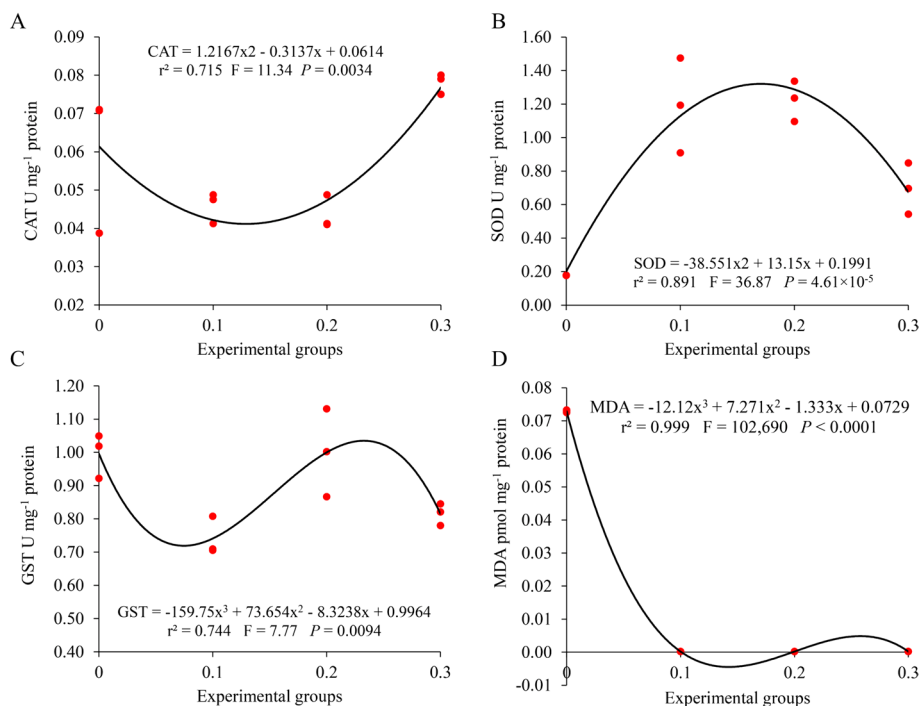


Fig. 1 Detoxification-associated activities in the hepatopancreas of *Macrobrachium rosenbergii*. Catalase activity—CAT (U mg⁻¹ protein) (A), superoxide dismutase activity—SOD (U mg⁻¹ protein) (B), glutathione S-transferase activity—GST (U mg⁻¹ protein) (C), and lipid peroxidation levels—MDA (pmol mg⁻¹ protein) (D) in prawns fed diets containing different concentrations of *Aloysia triphylla* essential oil as feed supplement. Data presented as mean $\pm$ SEM, $n = 3$. Models, coefficients of determination (r^2), F statistics, and P -values from orthogonal polynomial contrast analysis

non-nutritional strategies are gaining prominence, especially those that elicit immune stimulation. Immunostimulants are compounds that boost broad and/or specific (e.g., antibacterial) immune parameters, increasing animal resistance against stress factors and infectious diseases (Zhang and Mai 2014). The immune system of crustaceans is based on innate mechanisms that include physical, cellular, and humoral defenses that are linked inextricably to stress and welfare (Vazquez et al. 2009; Coates and Söderhäll 2021; Conneely and Coates 2024). The detoxification system, also known as metabolic resistance, includes enzymatic antioxidants that play a crucial role in disarming harmful oxygenic/nitrosative radicals generated during immunity (e.g., the intracellular killing of pathogens; Smith et al. 2014) and physiological activities (e.g., cuticle sclerotization). Antioxidant enzymes, such as CAT and SOD, and biotransformation enzymes like GST are crucial biological defenses forming part of the cellular stress response (Coates 2022). Those enzymes are often used as biomarkers of immune status, despite not being immune factors *sensu stricto* (Hui et al. 2013; van Rensburg et al. 2022).

Measurement of the antioxidant activity of a given EO is achieved by quantifying its free radicals scavenging ability or through the analysis of lipid peroxidation (Miguel 2010). Based on EOAT's ability to scavenge free radicals, our study found that *M. rosenbergii* fed with EOAT presented an increased antioxidant response in comparison to prawns without

dietary EOAT supplementation. That is, dietary supplementation with EOAT coincided with increased antioxidant levels in the hepatopancreas of juvenile *M. rosenbergii*. Dietary EOAT may act as a natural enhancer, promoting CAT SOD activities while reducing the risk of lipid peroxidation in the prawn hepatopancreas. Comparatively, the application of EOAT by water bath or dietary supplementation (0.2%) prevents lipid peroxidation by promoting CAT and GST activities and enhances finfish oxidative status (Gressler et al. 2014; Zeppenfeld et al. 2014, 2016). An anesthetic bath incorporating EOAT strongly improved antioxidative responses through increased CAT and GST activities in the hemolymph of Pacific white shrimp (*P. vannamei*, Parodi et al. 2012). Based on our findings and those in the literature, EOAT offered as a feed supplement is a putative natural antioxidant that acts as a non-nutritional health stimulant for juvenile *M. rosenbergii*.

EOs are composed of major and minor phytochemicals, which act in association, sometimes in an additive manner, potentiating or synergically, to confer the effects of EOs, including as antioxidants. The antioxidant activity of EOAT major phytochemicals was described by Gandhi et al. (2023) and may be a key reason for the observed bioactivity. The EOAT used herein was composed mainly of β -citral (neral or *cis*-citral) and α -citral (geranial or *trans*-citral). Citral is a non-phenolic, monoterpene aldehyde with a bimodal behavior. Its bioactivity is concentration dependent, i.e., oxidative protection increases with citral concentration, although excessive levels of citral can act as a pro-oxidant (Baschieri et al. 2017). Our results suggest the concentration of 0.2% EOAT promoted the best antioxidant protection, while the highest tested concentration (0.3% EOAT) led to the lowest antioxidant effect among the treatment groups, particularly on SOD activity. Citral, the major phytochemical of the studied EOAT, is likely responsible for the antioxidant effect observed in prawns fed EOAT. Beneficial effects of essential oils vary depending on the species of interest.

While our results showed no evident benefit on the zootechnical performance or survival of prawns fed EOAT, we postulate this non-nutritive dietary alternative may also promote defense responses, leading to improved outcomes post-stress. For instance, high survival has been observed in silver catfish (*Rhamdia quelen*) fed with EOAT (2.0 mg kg⁻¹) and challenged with *Aeromonas hydrophila* (dos Santos et al. 2017). Similarly, the essential oils of oregano (*Organum vulgare*, 2.5 and 5.0 mg kg⁻¹) and tea tree (*Melaleuca alternifolia*, 5.0 mg kg⁻¹) promote shrimp (*P. vannamei*) survival when challenged with *Vibrio campbellii* (Domínguez-Borbor et al. 2020). Future studies, for example, a challenge trial with prawns fed EOAT, could test this hypothesis. Likewise, studies on the blended use of EOAT with other EOs, as well as combined with growth enhancers and immune stimulants (e.g., prebiotics, probiotics, organic acids) could determine if combined compounds promote prawn growth performance and survival. Similarly, hemolymph biochemical parameters and fatty acid analysis would further expand the knowledge of the dietary use of EOAT on the health of *M. rosenbergii*.

In summary, we provide evidence that the essential oil of *A. triphylla* can be used as a non-nutritional feed supplement for juvenile *M. rosenbergii* as it is non-toxic lethal and promotes detoxification capacity of the hepatopancreas, particularly at a dose of 0.2% EOAT. Further studies evaluating EOAT effects during immune challenge would complement our findings and distinguish whether the apparent improved oxidative status is linked to reduced levels of collateral tissue damage and/or, an enhanced ability of prawns to recover post-infection.

Author contribution ELCB conceived and designed the research, provided funding, and supervised the study. BMH and BB chose the species for supplying the essential oil, and DS was responsible for the cultivation of plant material. RAB performed the extraction and chemical analysis of the essential oil of *Aloysia triphylla*. AGB and LC conducted the trial and performed the analyses. CJC and AMVS contributed to data handling, results interpretation, and intellectual content. LC, CSV and AMVS performed statistical analyses. LC and CSV wrote the first draft of the manuscript. All authors participated in the data interpretation, manuscript revisions, and approved the final version.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval In Brazil, the current animal protective legislation (law no. 11.794, 2008, Brazil) does not require the approval of studies with decapod crustaceans by an Animal Welfare Committee. Nevertheless, this study was performed in a research facility laboratory from a Brazilian federal university and followed the recommended commercial and academic standards for prawn farming, including adequate water quality and proper feed quality and ratio. Torpor with thermal shock was induced before euthanasia. This research does not contain any study with cephalopods, vertebrates, or humans.

Competing interests This study was part of Luana Cagol PhD thesis. The authors declare no additional conflict of interest.

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