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Essential oil of *Lippia alba* in the diet of *Macrobrachium rosenbergii*: Effects on antioxidant enzymes and growth parameters

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Abstract

The juveniles of *Macrobrachium rosenbergii* (0.060 ± 0.004 g and 2.018 ± 0.071 cm) were fed during 60 days with diets containing different doses of *Lippia alba* essential oil (EOLA) (0.0—control, 1.0, or 2.0 ml EOLA/kg diet; in triplicate, with 20 prawns/replicate). After the experimental period, were verified the survival, growth parameters and the antioxidant enzymes, lipid peroxidation and Na/K-ATPase activities in hepatopancreas and gills, respectively, of the animals. There were no significant differences on survival, growth parameters and gill Na/K-ATPase activity. However, glutathione peroxidase and glutathione-*S*-transferase activities were lower in hepatopancreas of prawns fed with 1.0 ml EOLA/kg diet compared to the control. Moreover, the activity of the enzyme superoxide dismutase was higher in hepatopancreas of prawns that received 2.0 ml EOLA/kg diet compared to those fed with 1.0 ml EOLA/kg diet. Although the addition of both EOLA doses has not improved the survival and growth parameters of *M. rosenbergii*, these doses contributed to decrease lipid peroxidation. Additionally, the dose of 2.0 ml EOLA/kg diet contributed for increasing the antioxidant enzymes activities in the hepatopancreas, improving antioxidant status, and therefore, it can be recommended as diet supplementation for *M. rosenbergii*.

KEYWORDS

antioxidant enzymes, biochemical analysis, feed additives, freshwater prawn, growth metrics

1 | INTRODUCTION

The crustacean's production has shown a constant increase in the last years (from 2010 to 2016) reaching 7,862 million ton (FAO, 2018), of which 3% (about of 234,000 ton) are related to the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879). Thus, *M. rosenbergii* is considered a commercially important human food source, and moreover, several studies showed that this species can be adapted to farming or laboratory conditions (Ballester et al., 2017; Ballester, Maurente, Heldt, & Dutra, 2018; Cohen, Ra'anan, & Brody, 1981; Cohen, Ra'anan, Rappaport, & Arieli, 1983; Negrini et al., 2017; Valenti, de Mello, & Castagnolli, 1993). Therefore, *M. rosenbergii* is an adequate biological model for investigation and development of new methodologies that reduce the stress due to aquaculture procedures, such as biometry, transport and handling.

In the last years, several studies regarding the use of biological additives, such as essential oils (EOs) obtained from plants, in aquatic organisms diet have been reported (Asadi, Gharaei, Harijani, & Arshadi, 2018; Saccol et al., 2013; Souza et al., 2015; Zeppenfeld et al., 2016). This approach is related to the increase of human population and the necessity for a higher and natural aquaculture production in the shortest possible time, without causing adverse effects in the aquatic organisms (Asadi et al., 2018). Phenolic compounds and terpenoids from plant additives have antioxidant activity, acting as radical scavengers, modifying the intestinal flora, increasing digestibility and nutrient absorption, and, therefore, improving the growth performance and immune response (Giannenas et al., 2012; Harikrishnan, Balasundaram, & Heo, 2011; Niciforovic et al., 2010; Tayag, Lin, Li, Liou, & Chen, 2010). However, the addition of new dietary components can result in some stress, such as nutritional imbalance, which affects health and animal performance and lead to oxidative stress.

This oxidative stress results from the imbalance between the production of reactive species of oxygen (ROS) and the antioxidant system, which is essential to maintain the organism homeostasis (Betteridge, 2000). The antioxidant defence system includes the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST), and also non-enzymatic antioxidants, such as reduced glutathione, carotenoids, ascorbic acid, retinol and α-tocopherol (Azambuja et al., 2011; Lushchak, 2011; Yang et al., 2011). Stressful situations can interfere in the intermediary metabolism of crustaceans, through specific actions on important enzymes related to carbohydrate, lipid, and amino acid degradation processes (Jiang et al., 2009) or even osmoregulatory proteins (Jahn, Cavagni, Kaiser, & Kucharski, 2006). Moreover, antioxidant compounds are synthesized by plants as secondary products acting as defence mechanisms against ROS production and avoiding oxidative damage (Niciforovic et al., 2010). Several studies have reported the use of medicinal herbs to improve growth, antioxidant status, immune response and survival in crustacean species (Asadi et al., 2018; Immanuel, Vincybai, Sivaram, Palavesam, & Marian, 2004; Liu et al., 2010; Vaseeharan, Sai Prasad, Ramasamy, & Brennan, 2011; Wang et al., 2017; Yudiati, Isnansetyo, & Handayani, 2016). However, the responses to the addition of essential oil of *Lippia alba* (EOLA) in the diet of *M. rosenbergii* were not investigated to the moment.

Lippia alba (Mill.) N.E. Brown (Verbenaceae) is an aromatic shrub worldwide distributed (Hennebelle, Sahpaz, Joseph, & Bailleul, 2008). The EOLA has showed anaesthetic effect, improved water quality during fish transportation, besides physiological and biochemical parameters and antioxidant responses in some aquatic organisms (Azambuja et al., 2011; Becker et al., 2012, 2016; Cunha et al., 2010, 2011; Hohlenwerger et al., 2016, 2017; Parodi et al., 2012; Salbego et al., 2017; Simões et al., 2017; Souza, Baldissera, et al., 2018; Toni et al., 2014). Positive effects of dietary EOLA addition were found in silver catfish, *Rhamdia quelen*, in which there was a decrease of the lipid peroxidation and an increase in the lactate and glycogen reserves, and the tissue antioxidant response (Saccol et al., 2013), and, also, an improving feed conversion and immunological activity in Nile tilapia (*Oreochromis niloticus*, Souza, Souza, et al., 2019). Thus, the analysis of the oxidative stress seems relevant to the comprehension of physiology and growth performance of prawn fed with dietary EOLA supplementation. The present study aimed to evaluate the survival, growth performance and antioxidant enzymes in *M. rosenbergii* fed with diets supplemented with different EOLA doses. Moreover, this is the first description of the effects of dietary addition of an EO on gill Na^+K^+-ATPase activity in crustaceans.

2 | MATERIALS AND METHODS

2.1 | Material

All solutions were prepared using Millipore Milli-Q ultrapure, apyrogenic water and all reagents were of the highest purity commercially available. Tris, ATP ditris salt, pyruvate kinase (PK), phosphoenolpyruvate (PEP), NADH, N-(2-hydroxyethyl) piperazine-*N′*-ethanesulfonic acid (HEPES), lactate dehydrogenase (LDH), agarose, sodium borate, alamethicin, EDTA and ouabain were purchased from Sigma-Aldrich Chemical Co. (USA).

2.2 | Plant material and essential oil extraction

The essential oil of *Lippia alba* (EOLA) was obtained by steam distillation of fresh leaves for 3 hr in a Clevenger apparatus (European Pharmacopoeia, 2007) and stored at −4°C until utilization. The EO was analysed on an Agilent 7890A GC coupled to a 5975C mass spectrometer with a non-polar HP5-MS fused silica capillary column (5% phenyl—95% methylsiloxane, 30 m × 0.25 mm i.d. × 0.25 mm film thickness) and EI-MS of 70 eV. Analysis parameters are as follows—carrier gas: He (1 ml/min); split inlet: 1:100; injector and detector temperatures: 250°C; temperature programme: 40°C for 4 min and 40–320°C at 4°C/min. The EO components were identified by comparison of the mass spectra and Kovats retention index with the literature data (NIST/EPA/NIH, 2010). Quantitative analysis was carried out on an Agilent 7890A gas chromatograph equipped with a flame ionization detector, using a column with the same features and programme above described, except the injection in splitless mode and both injector and detector temperatures at 300°C. The EO sample was injected in triplicate, and the components relative per cent were estimated by under peak area integration obtained from FID chromatograms (Table 1).

2.3 | Prawns, experimental design and manufacture of experimental diet

Macrobrachium rosenbergii juveniles (*n* = 180) were obtained from the a Commercial Laboratory LACQUA, Palotina, Brazil, transported to the Prawn Farming Laboratory of the Federal University of Paraná (UFPR), Palotina Sector, and maintained during the acclimation period (15-days) in circular tanks (200-L, density of 50 prawns/m^2), with mechanical and biological filtration.

The experimental design consisted of nine tanks (300-L, at a density of 40 prawns/m^2, equivalent to 20 prawns/tank), with a recirculating water system, proper aeration and temperature control under a natural photoperiod of 12 hr daylight. The prawns used in the experiments showed an initial weight and length of 0.060 ± 0.004 g and 2.018 ± 0.071 cm respectively (mean ± *SEM*). Moreover, the water quality parameters were monitored as reported by Ballester et al. (2018), and the values were the following (mean ± *SEM*): temperature (27.28 ± 2.17°C), pH (8.18 ± 0.46), dissolved oxygen (7.97 ± 0.92 mg/L), total ammonia nitrogen (0.001 ± 0.0002 mg N/L), nitrite (0.004 ± 0.0007 mg/L), nitrate (3.46 ± 1.15 mg/L), alkalinity (95.0 ± 9.0 mg $CaCO_3$/L) and hardness (53.0 ± 10.0 mg $CaCO_3$/L).

The nine tanks were, randomly, divided in three experimental groups with the following diets: 0.0 (control), 1.0 or 2.0 ml of EOLA per kg diet (36% crude protein; Mitra, Chattopadhyay, & Mukhopadhyay, 2005; Reed & D'Abramo, 1989; Table 2). For the preparation of experimental diets, all solid ingredients were grounded (0.5 mm) in a hammer mill, and subsequently, all ingredients were mixed according to the formulation (Table 2) for processing. The pellets were made in an experimental pelletizer by pre-moistening the mixture with water at a temperature of 35°C. After pelleting, the pellets (1 mm) were dried in a forced air oven for 24 hr at 40°C and stored refrigerated for later use. Prawns were fed, during 60 days, three times a day (8:30, 13:30 and 18:30 hr) with initial feeding rate equivalent to 15% of the

TABLE 1 Chemical composition of the essential oil of *Lippia alba*

RI experimental	RI literature[a]	Chemical compound	Per cent composition
971	973	Sabinene	0.68
992	985	β-Pinene	6.07
1,003	1,010	Limonene	1.57
1,029	1,031	1,8-Cineole	6.58
1,048	1,050	β-*E*-ocimene	3.67
1,111	1,100	Linalool	59.93
1,129	1,130	1,3,8-p-Menthatriene	3.47
1,200	1,200	Carveol	1.07
1,205	1,202	Myrtenol	0.77
1,267	1,269	α-Citral	1.81
1,415	1,415	β-Caryophyllene	3.45
1,476	1,481	β-Cubebene	1.30
1552	1,550	Selina-3,7(11)-diene	0.30
Identified compounds			90.74

Abbreviation: RI, Retention Index.
[a]NIST/EPA/NIH (2010).

TABLE 2 Ingredients and proximate composition of the experimental diets (wet basis)

	Diet		
	Control	EOLA 1.0	EOLA 2.0
Ingredients (%)			
Ground corn	41.98	41.98	41.98
Fish meal	25.00	25.00	25.00
Soybean meal	25.00	25.00	25.00
Soybean oil	5.00	4.90	4.80
Starch	2.00	2.00	2.00
Vitamin and mineral supplement[a]	1.00	1.00	1.00
Butylated hydroxytoluene (BHT)	0.02	0.02	0.02
EOLA[b]	0.00	0.10	0.20
Total	100.0	100.0	100.0
Proximate composition			
Dry matter (%)	91.28	91.84	91.57
Moisture (%)	8.72	8.16	8.43
Gross energy (kJ/kg)	18.57	18.54	18.60
Crude protein (%)	36.00	36.00	36.00
Ether extract (%)	10.49	10.67	10.79
Ash (%)	11.11	10.92	11.07
Crude fibre (%)	6.28	6.25	7.97
NFE (%)	27.40	28.00	25.74

Abbreviation: NFE, nitrogen-free extract.
[a]Vitamin and mineral supplement—vitamin (guarantee levels per kg): 16,875 IU vitamin A, 3,375 IU vitamin D3, 200 IU vitamin E, 6.7 mg vitamin K3, 120 mg vitamin B, 36 mg vitamin B2, 25.5 mg vitamin B6, 45 mg vitamin B12, 1,200 mg vitamin C, 11.2 mg folic acid, 67.5 mg pantothenic acid, 170 mg nicotinic acid, 1.68 mg biotin, 265 mg inositol, and, mineral (guarantee levels per kg): 65 mg iron, 13.8 mg copper, 150 mg zinc, 85 mg manganese, 0.35 mg cobalt, 1.3 mg iodine and 0.4 mg selenium.
[b]EOLA (essential oil of *Lippia alba*: the percentage corresponds to 0.00, 1.00 and 2.00 ml/kg EOLA.

biomass. The amount of feed was adjusted every two weeks based on weight and the number of shrimp in each tank, but maintaining the percentage reported above. The tanks were syphoned to remove the uneaten food and faeces 30 min before feeding.

2.4 | Growth performance detection

After the experimental period, the survival and growth parameters of animals were evaluated. Prawns were measured using a 402 digital caliper (King Tools®) and weighed (Analytical Scale AY 220; Marte®) on days 0 and 60 of experiment for evaluation of final weight, weight gain (WG), feed conversion rate (FCR), specific growth rate (SGR) and total (measured by linear distance from the rostrum extremity to the telson tip) and standard (linear distance from the posterior margin of the ocular orbit to the base of the telson) lengths respectively. These parameters were calculated as follows:

$$\text{survival}\,(\%) = (\text{final number of prawns}/\text{initial number of prawns}) \times 100;$$

$$\text{weight gain}\,(\%) = ((\text{final weight} - \text{initial weight})\,/\text{initial weight}) \times 100;$$

$$\text{specific growth rate}\ (\text{SGR\% per day}) = \left[(\ln \text{final weight} - \ln \text{initial weight})\,/\text{days of the experiment}\right] \times 100;$$

$$\text{condition factor} = [\text{weight (g)}/[\text{standard length (cm)}^3]] \times 100;$$

$$\text{feed conversion ratio (FCR)} = (\text{feed consumed, g})\,/\,(\text{weight gain, g}).$$

2.5 | Sample collection for biochemical analysis

After 60 experimental days, the juvenile prawns (*n* = 15 per experimental group) were cryoanesthetized (covered with ground ice) for about 1 min, until fully immobile. The hepatopancreas and gills were removed and immediately stored at −80°C for determination of antioxidant enzyme activities and lipid peroxidation and Na^+/K^+-ATPase, respectively, following the protocols described by Souza et al. (2018) and Lucena, McNamara, and Leone (2017).

The assay of the total SOD activity (expressed as SOD units per mg protein) was based on pyrogallol oxidation, a process highly dependent on superoxide (Marklund, 1985). GPx activity was estimated through the NADPH oxidation at 340 nm (Wendel, 1981), and the results were expressed as units per milligram of protein. GST activity (expressed as units per mg protein) was determined according to Mannervik and Guthenberg (1981) with slight modifications, estimating the rate of formation of dinitrophenyl-S-glutathione at 340 nm. The lipid peroxidation (LPO) levels were estimated according to the thiobarbituric acid reactive substances (TBARS) assay (Buege & Aust, 1978), and the results were expressed as nmol MDA/mg protein.

Total (Na^+, K^+)-ATPase activity was performed at 25°C in a PK/LDH coupling system in which the ATP hydrolysis was coupled to NADH oxidation according to Leone et al. (2012). In this system, the phosphate released during ATP hydrolysis by the (Na^+, K^+)-ATPase is converted to ATP while phosphoenolpyruvate is converted to pyruvate by pyruvate kinase. Pyruvate is converted to lactate concomitant with NADH oxidation. The rate of ATP hydrolysis by the (Na^+, K^+)-ATPase was estimated by monitoring NADH titres, and the oxidation of NADH was monitored at 340 nm ($\varepsilon_{340\ nm,\ pH\ 7.5}$ = 6,200 mol L^{-1} cm^{-1}) in a Shimadzu UV 1800 spectrophotometer equipped with thermostatted cell holders.

2.6 | Ethics statement

Studies with invertebrates do not require any authorization from the Ethical and Animal Welfare Committee of the Universidade Federal do Paraná.

2.7 | Statistical analysis

The results were expressed as the mean ± standard error of the mean (*SEM*). Levene's test was used to evaluate the homogeneity of data variances. All data showed homogeneous variances and were compared using a one-way analysis of variance (ANOVA) followed by Tukey's test. All analyses were performed with Statistica Software 7.0 (Stat Soft), and the differences were considered significant at $p < .05$.

3 | RESULTS

3.1 | Growth performance

The prawns survival had no significant difference ($p > .05$) between treatments and ranged between 76% and 83% (Table 3). Moreover, there were no significant differences in the weight gain and SGR and other zootechnical parameters ($p > .05$) of the prawns fed with diets containing EOLA compared to the control group (Table 3).

3.2 | Biochemical analyses

The SOD activity in hepatopancreas was 39% higher ($p < .05$) in prawns fed with 2.0 ml EOLA per kg of diet than those fed with 1.0 ml EOLA per kg of diet (Figure 1a). Activities of GPx and GST were lower (about of 20% and 42.5% respectively; $p < .05$) in the hepatopancreas of *M. rosenbergii* fed with 1.0 ml EOLA per kg of diet compared to control and 2.0 ml EOLA per kg of diet groups (Figure 1b,c). The TBARS levels were lower ($p < .05$) in the hepatopancreas of prawns fed 1.0 (55%) and 2.0 (50%) mL EOLA per kg of

TABLE 3 Growth performance and survival of the prawn *Macrobrachium rosenbergii* fed with diets containing different doses of *Lippia alba* essential oil

	Diet (ml EOLA per kg diet)		
	0.0	1.0	2.0
Final weight (g)	0.29 ± 0.02	0.30 ± 0.01	0.28 ± 0.02
Final total length (cm)	3.25 ± 0.11	3.21 ± 0.08	3.22 ± 0.14
Final standard length (cm)	2.77 ± 0.09	2.79 ± 0.07	2.73 ± 0.12
Survival (%)	83.33 ± 8.33	76.67 ± 1.67	81.67 ± 11.67
Weight gain (%)	333.33 ± 20.76	319.05 ± 20.77	318.05 ± 31.22
SGR (% per day)	2.44 ± 0.08	2.38 ± 0.08	2.37 ± 0.13
Condition factor (g/cm^3)	1.39 ± 0.08	1.40 ± 0.07	1.41 ± 0.09
FCR (g/g)	3.75 ± 0.22	4.23 ± 0.35	4.34 ± 1.16

Abbreviations: FCR, feed conversion ratio; SGR, specific growth rate.

Values are expressed as the mean ± *SEM* ($n = 3$, $p < .05$). The initial total lengths and weights were the following, respectively: 2.018 ± 0.071 cm and 0.060 ± 0.004 g.

diet, compared with control group (Figure 1d). The diets containing EOLA did not lead to any significant effect ($p > .05$) on the branchial (Na^+, K^+)-ATPase activity (Figure 2).

4 | DISCUSSION

The effects of dietary supplementation with herbal components (flowers, leaves, seeds and roots) or EOs (obtained from leaves) for fish, shrimps and prawns on growth performance, antioxidant status, immune response and survival have been reported (Asadi et al., 2018; Francis, Makkar, & Becker, 2002; Immanuel et al., 2004; Ji et al., 2007; Liu et al., 2010; Saccol et al., 2013; Vaseeharan et al., 2011; Wang et al., 2017; Yudiati et al., 2016; Zeppenfeld et al., 2016; Zheng et al., 2009). Although some plant extracts or EOs have increased the growth performance, mainly due to the positive effects on digestion, absorption and assimilation of the several nutrients (Wang et al., 2017), the present study showed that the dietary addition of EOLA did not interfere significantly in the growth parameters and survival of *M. rosenbergii*. The dietary supplementation with EOLA (0.25–2.0 ml per kg diet) also did not affect the growth and survival of silver catfish (Saccol et al., 2013). However, the addition of 2.0 ml EOLA per kg diet improved feed conversion ratio and condition factor of Nile tilapia (Souza et al., 2019). Finally, the results found in our study could be related to a lower dietary supplementation of EOLA, mainly, when compared to the study performed by Talpur and Ikhwanuddin (2012) with Asian sea bass (*Lates calcarifer*) which were fed with higher garlic concentrations (5–20 g per kg feed) exhibiting a significant improvement of the growth, weight gain and feed conversion, or, yet, our results could be attributed to the animal species or farming/laboratory conditions (Wang et al., 2017).

The antioxidant defence system is a highly conserved biochemical mechanism in both invertebrates and vertebrates (Chainy, Paital, & Dandapat, 2016). Its main function is to protect the organisms from harmful effects of reactive oxygen species (ROS), which, in general, can oxidize biomolecules (lipids, carbohydrates, proteins and DNA) impairing the normal cellular functions (Chainy et al., 2016; Han et al., 2018). Thus, the interruption in the balance between oxidation and antioxidant systems by excess in ROS formation or depletion of antioxidants, characterize the oxidative stress. The ROS concentrations could interfere in two ways in the organisms: low concentrations of ROS regulate several physiological processes, while higher concentration could be toxic, impairing cellular functions (Chainy et al., 2016; Han et al., 2018).

The pro/antioxidant parameters analysed in hepatopancreas of *M. rosenbergii* (present study) indicated interesting and promising results, and in general, the antioxidant effect of EOLA agrees with the results obtained in other species. Moreover, it is important to consider that, in crustaceans, the hepatopancreas is the main metabolic centre for ROS production (Bianchini & Monserrat, 2007), and most EOs possess antioxidant potential due, mainly, to presence of radical scavengers, phenolic as well as non-phenolic compounds (Franz, Baser, & Windisch, 2010). As reported in the study of Saccol et al. (2013), the EOLA has constituents of terpenic origin, with linalool being the main component as the EOLA used in the present study.

In the present study, prawns fed with both diets supplemented with EOLA presented lower LPO (indicated by lower TBARS levels) in the hepatopancreas compared to the control. However, prawns fed with 2.0 ml EOLA per kg diet presented higher SOD, GPx and GST antioxidant enzymes activities comparatively those fed with 1.0 ml EOLA per kg diet. SOD is responsible for removing the superoxide radical ($O_2^{\cdot-}$) by increasing hydrogen peroxide (H_2O_2) production, which is, then, captured by CAT and/or GPx enzymes (Lushchak & Bagnyukova, 2006). In the sequence, the GPx catalyses the conversion of H_2O_2 and organic hydroperoxides to less reactive products, maintaining the free radical balance and reducing oxidative damage. Thus, the results found in our study indicate that the antioxidant system capacity of the hepatopancreas was efficient in reducing the oxidative damage: low LPO concomitantly with high SOD, GPx and GST levels.

Additionally, GST also acts against LPO by conjugating xenobiotics and ROS to GSH, and favouring the detoxification (Yang

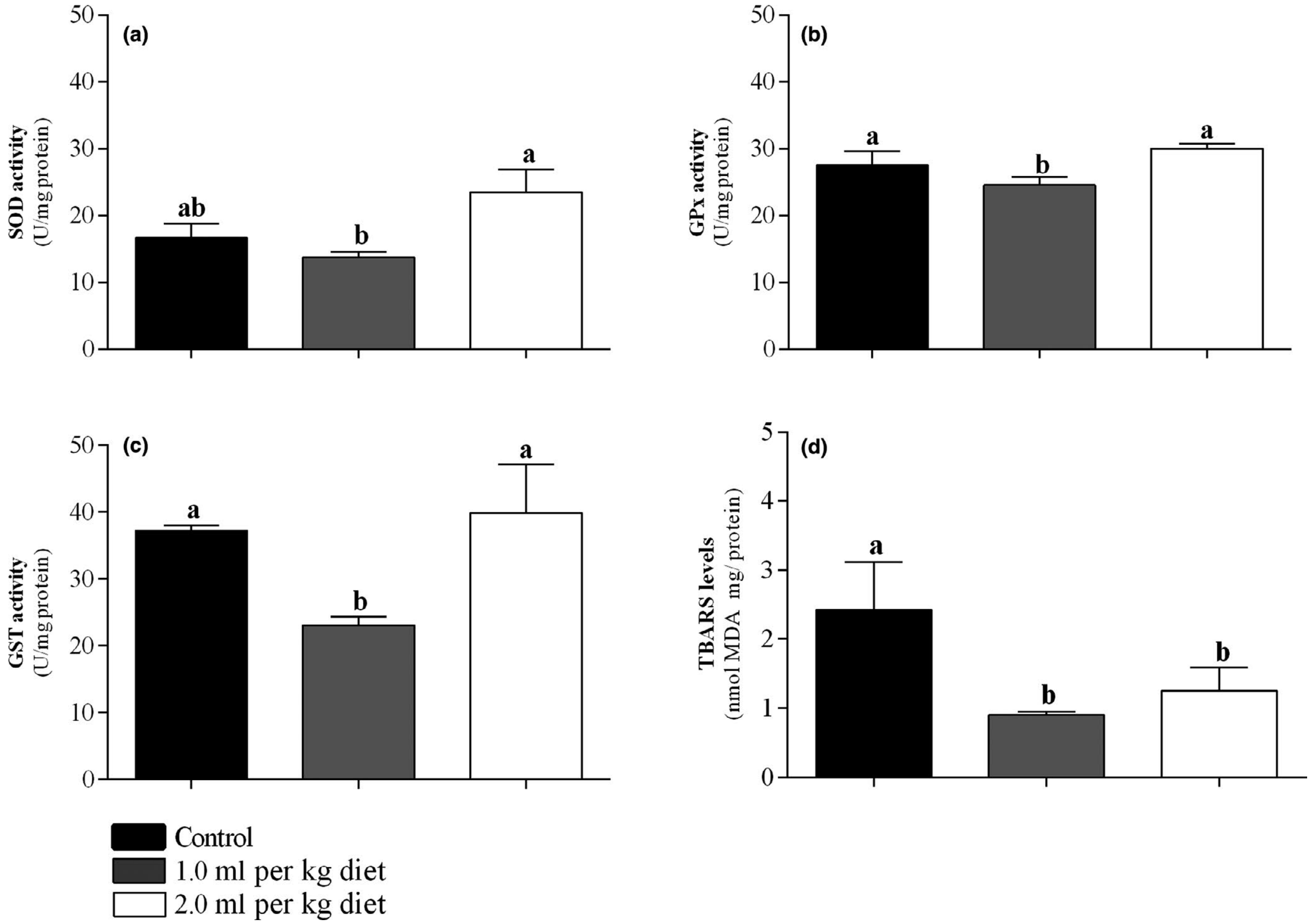

FIGURE 1 Superoxide dismutase (SOD) (a), glutathione peroxidase (GPx) (b), glutathione-s-transferase (GST) (c) activity and thiobarbituric acid reactive substances (TBARS) (d) levels in the hepatopancreas of *Macrobrachium rosenbergii* fed with diets supplemented with essential oil of *Lippia alba*. Values are expressed as the mean ± *SEM* (*n* = 3). Lowercase letters indicate significant differences between the diets (*p* < .05). Axis y represents the biochemical parameters analysed, and axis *x* represents the experimental groups (EOLA diets)

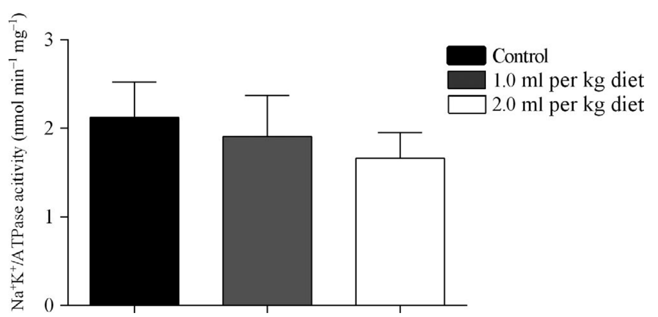

FIGURE 2 Total (Na^+, K^+)-ATPase activity in gills of *Macrobrachium rosenbergii* fed with diets supplemented with essential oil of *Lippia alba*. Values are expressed as the mean ± *SEM* (*n* = 3, *p* < .05). Axis y represents the biochemical parameter analysed, and axis x represents the experimental groups (EOLA diets)

et al., 2001). Interestingly, exposure of white shrimp *Litopenaeus vannamei* to 20–40 µl/L linalool (the main compound of EOLA chemotype used in the present study) for 8 hr increased LPO, CAT and GST activities in the gills and hepatopancreas (Becker et al., 2015). Dietary addition of EOLA (0.5–2.0 ml per kg diet) also reduced LPO levels in some tissues of silver catfish, but increased antioxidant enzymes activity (Saccol et al., 2013), possibly due to its sedative properties (Cunha et al., 2010), which could cause a reduction in metabolism and oxygen consumption. Consequently, the reduction of ROS justifies the lower levels of LPO found in the hepatopancreas of *M. rosenbergii* fed diets containing EOLA.

The (Na^+, K^+)-ATPase, a key enzyme involved in many physiological activities, is present in ionocytes of gills of aquatic organisms (McCormick, 1993; Stern, Borut, & Cohen, 1984). In the present study, dietary EOLA did not imply any effect on the gill (Na^+, K^+)-ATPase activity of *M. rosenbergii* prawn, but silver catfish fed with 0.25 ml EOLA per kg diet exhibited an increased activity of gill (Na^+, K^+)-ATPase, indicating that this supplementation had an effect on osmoregulatory capability (Souza et al., 2015), which was not observed for the prawns. Silver catfish anesthetized with EOLA (300 or 450 µl/L) showed increased gill (Na^+, K^+)-ATPase activity compared to the control group (Toni et al., 2014).

5 | CONCLUSIONS

Although not improving the growth performance and survival rate, the dietary supplementation with 2.0 ml EOLA per kg diet is recommended for *M. rosenbergii*, because it decreased LPO and increased antioxidant enzymes, improving the oxidative status of the prawn. However, further studies are needed to confirm the exact mechanism by which EOLA exerts its antioxidant properties as well as if the bioactivity observed from the results is due to the presence of major compounds or it is a consequence of a synergistic effect between the several molecules contained in EOLA.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data of this study can be made available by the authors upon request and reasonable arguments.

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