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ANIMAL SCIENCE

Addition of *Lippia sidoides* Essential Oil to Tilapia Diet: Performance Analysis, Blood Parameters, and Challenges of Air Exposure and Experimental Infection

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Abstract: This study evaluated the effects of *Lippia sidoides* essential oil (LSEO) as a growth and health promoter for juvenile tilapia subjected to stress induced by air exposure and experimental infection with *Aeromonas hydrophila*. Juvenile were allocated to 15 tanks (in triplicate) and fed diets containing 0.0% (Control), 0.05%, 0.1%, 0.2%, or 0.4% LSEO for 33 days. For the air exposure challenge, five fish from each tank were exposed to air for five minutes after the feeding period. Blood samples were collected for hematological analysis. For the infection challenge, six fish from each tank were intraperitoneally inoculated with 100 µL of *A. hydrophila* and monitored for ten days. Hematological and histomorphometric parameters were assessed. The results indicated that the 0.4% LSEO supplementation reduced feed conversion ratios ($P < 0.05$). Before the challenges, LSEO concentrations influenced total counts of neutrophils, basophils, and monocytes ($P < 0.05$). After the air exposure and infection challenges, no significant differences were observed among treatments for these parameters ($P > 0.05$). Regarding hematimetric indices, all parameters were affected by LSEO concentrations prior to the challenges ($P < 0.05$). Although LSEO supplementation improved feed conversion, it did not mitigate the hematological alterations caused by the stress challenges.

Key words: Aquaculture, Haematology, Immunity, *Oreochromis niloticus*, Phytotherapy.

INTRODUCTION

The aquaculture sector has been growing worldwide over the years, compensating for the volume derived from fishing, which remains constant but requires greater effort to maintain the same quantities as always (FAO 2024). In Brazil, a country with vast territorial expanses and significant potential for activity expansion, fish farming has seen continuous growth, reaching approximately 887,000 tons of farmed fish in 2023 (PEIXEBR 2024). Within this context, tilapia emerges as the most significant species in Brazilian aquaculture, with a production of approximately 579,000 tons in 2023, solidifying Brazil's position as the world's fourth-largest producer (PEIXEBR 2024).

The intensification of production systems often leads to higher stocking densities, which can compromise water quality, such as reducing dissolved oxygen availability, increasing concentrations of ammonia, nitrite, and nitrate, among other parameters (Boyd 2015, Jatobá & Silva 2015). This, in turn, causes stress, making fish more susceptible to bacterial infections

and impairing their zootechnical performance (Torrecillas et al. 2007).

Stress is commonly observed in fish following routine aquaculture management practices. It is highly detrimental to their health, triggering physiological and adaptive responses that can influence blood parameters (either reducing or increasing them) and, in chronic cases, negatively impact zootechnical and reproductive performance (Gorissen & Flik 2016). Therefore, it is essential to adopt strategies aimed at reducing stress and/or preventing or treating infections, such as water treatments during handling procedures (e.g., transportation) (Mirghaed & Ghelichpour 2019, Yousefi et al. 2022) or dietary supplementation (da Silva et al. 2022, de Moraes et al. 2022).

Many plants and their derivatives or by-produtcs, particularly essential oils, have been associated with stress mitigation, growth promotion, antimicrobial effects, and enhanced immune response (Hoseini et al. 2019, da Silva et al. 2021, Alnahass et al. 2023, Wickramanayake et al. 2023, Pereira et al. 2024). An additional advantage of essential oils is their versatility, as they can be applied via water (da Silva et al. 2020) or incorporated into feed (Pereira et al. 2024) or even combined with other additives such as probiotics (Jatobá et al. 2024).

Lippia sidoides, commonly known in Brazil as "alecrim-pimenta", it is a medicinal plant characterized by its various bioactive compounds, predominantly the monoterpenes thymol and carvacrol found in its leaves (Almeida et al. 2010, Soares & Tavares-Dias 2013). The essential oil of *L. sidoides* (LSEO) has demonstrated biological potential for antimicrobial activity and stress mitigation during transport (Soares & Tavares-Dias 2013, Majolo et al. 2017, Brandão et al. 2022). Cardoso et al. (2024) observed improved health outcomes in *Danio rerio* fed a diet supplemented with 0.25% *L. sidoides* and subsequently challenged with Ehrlich ascitic carcinoma. Additionally, it reduced stress during transport in *Pterophyllum scalare* (de Oliveira et al. 2022).

For tilapia, *L. sidoides* has been evaluated as an antiparasitic agent (de Oliveira Hashimoto et al. 2016); however, its effects when administered through dietary supplementation remain unexplored. Based on the positive results observed in fish using essential oils containing *L. sidoides*, this study aimed to evaluate the effects of different doses of LSEO as a growth and health promoter (based on blood parameters) in juvenile tilapia, both before and after exposure to stressors, such as air exposure and experimental infection with a sub-lethal dose of *Aeromonas hydrophila*.

MATERIALS AND METHODS

Experimental Design

The study was conducted at the Laboratório de Aquicultura do Instituto Federal de Educação, Ciência e Tecnologia Catarinense (IFC) with approval from the Animal Use Ethics Committee under protocol number 287/2025. The experiment was carried out in a recirculating system (RAS) with constant aeration and natural photoperiod (approximately 12:12, spring), where Nile tilapia juveniles were randomly distributed across 15 polyethylene tanks (800 L – 30 fish per tank) and divided into five treatments in triplicate.

The diets were prepared using commercial feed (GUABI® - Pirá Crescimento 4-6 mm: 32% crude protein, 6% ether extract, 16% mineral matter, 7% fiber). The addition of essential oil to the diet was based on Dairiki et al. (2013), using grain alcohol as a diluent. For this, 100 mL of grain alcohol was sprayed per kg of feed containing the desired concentrations (0.05%, 0.1%, 0.2%, and 0.4%), and in the control diet, only alcohol was sprayed. The diets were dried

for 24 h at room temperature and then stored at -18°C until feeding.

The fish were fed three times daily (8:00 am, 12:00 pm, and 5:00 pm) for 33 days with an offer of 3% of the total biomass. Water quality parameters were monitored continuously during the experiment, with the following recorded: dissolved oxygen levels of 6.58 ± 0.78 mg L^{-1} and temperature of 27.39 ± 1.72 °C (measured using a YSI PRO20 Oximeter); ammonia levels of 0.05 ± 0.05 NH_3 mg L^{-1}; nitrite levels of 1.76 ± 2.68 mg L^{-1}; nitrate levels of 1.71 ± 2.35 mg L^{-1}, pH of 6.95 ± 0.07, and alkalinity of 97.84 ± 14.31 mg $CaC0_2$ L^{-1}.

Essential Oil Extraction

L. sidoides specimens were cultivated in the medicinal plants and vegetables sector of Embrapa Western Amazon (Manaus, Amazonas, Brazil). After harvest, the leaves were air-dried and stored. The essential oil was extracted through hydrodistillation using a Clevenger apparatus for two hours. The extracted LSEO was stored in amber vials at -4°C. The oil's chemical composition was determined by gas chromatography and mass spectrometry at Embrapa Food Agroindustry (Rio de Janeiro, RJ, Brazil), as described by Potzernheim et al. (2012). The collected plant material was identified and deposited in the herbarium of the Federal Institute of Amazonas under registration number 13887.

Stress challenges (Air Exposure Stress and Experimental Infection with sub-lethal dose of *Aeromonas hydrophila*)

After the feeding period, the fish were fasted for 24 h, and five fish per tank were removed from the water using a dip net and subjected to air exposure for five minutes. Blood was then collected, and blood samples were used for hematological analysis as previously described.

For the pathogenic challenge conducted at the end of the experimental period, *A. hydrophila* was cultured in brain-heart infusion (BHI, Kasvi) at 30°C for 24 hours, followed by centrifugation at 1000 x g for 15 minutes. The bacterial suspension was diluted in sterile saline solution (0.85%) to achieve a concentration of 0.5 McFarland (1.5 × 106 CFU mL^{-1}).

Six fish from each unit were transferred to aquariums (60 L), all equipped with biological filters and maintained at a constant temperature (27°C). Each fish was intraperitoneally inoculated with 100 µL of *A. hydrophila* and then returned to the tanks for a 10-day mortality observation period. After this period, a new hematological collection was performed on three fish per tank.

Blood Collection and Analysis

Blood samples were collected (n = 5 fish per tank) at the end of the 33-day feeding period and after the air exposure challenge. Blood was drawn by puncture of blood vessels in the caudal region using syringes rinsed internally with ethylenediaminetetraacetic acid (EDTA) 10% as an anticoagulant. Erythrocyte counts were performed using a Neubauer chamber, with Dacie's solution as the stain and diluent. Hemoglobin concentration (Hb) was determined by the cyanmethemoglobin method using a commercial kit (Labtest, Brazil). Hematocrit (Ht) was measured by the microhematocrit method, following Goldenfarb et al. (1971). The following corpuscular constants were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH), according to Ranzani-Paiva et al. (2013). Total leukocyte counts were determined indirectly (Ishikawa et al. 2008) using blood smears stained with May-Grünwald/Giemsa/Wright, and differential leukocytes were calculated according to Jatobá et al. (2011).

Zootechnical Parameters

The zootechnical indices of Nile tilapia fed diets supplemented with LSEO were evaluated. At the beginning and end of the experiment, the fish were weighed using a precision balance, and the length was measured using calipers. The following zootechnical parameters were calculated: weight gain (g) = final weight – initial weight, final length, specific growth rate, feed conversion ratio (FCR = feed offered/(Final biomass - Initial biomass), and productivity (kg/m³), as described by Jatobá et al. (2024).

Histological and Histomorphometric Analysis

The anterior intestine portion of four fish per experimental unit was collected and fixed in 10% buffered formalin. After 24 hours, samples were dehydrated in ethyl alcohol and processed using routine histological techniques, with paraffin inclusion at 60°C. Four µm thick sections were cut using a PAT-MR10 microtome and stained with Harris hematoxylin and eosin (H&E). The slides were prepared in Entellan® medium and analyzed using a Zeiss Axio Imager A.2 phase contrast microscope (DIC) (Zeiss, Göttingen, Germany). Intestinal morphology, including the length, width, and area of intestinal folds, was measured using Zen Pro software.

Statistical Analysis

The data were subjected to the Kolmogorov-Smirnov test to assess normality and Levene's test to verify homoscedasticity. Data meeting the prerequisites of normality and homoscedasticity were analyzed using ANOVA or two-way ANOVA, followed by the SNK test for mean comparisons among treatments (Zar 2010). Pearson correlation and linear regression analyses were performed for normally distributed data (BioEstat®). A significance level of 5% was adopted for all evaluations.

RESULTS

Chemical Composition of the Essential Oils

The main components of LSEO were thymol (75.4%), p-cymene (7.3%), (E)-caryophyllene (4.3%), and γ-terpinene (3.0%) among the 37 identified compounds (Table I).

Growth Performance Parameters and Histological and Histomorphometric Analysis

The addition of 0.4% LSEO significantly reduced ($P < 0.05$) the feed conversion ratio of tilapia (Table II). Although no statistical differences were observed in the other growth performance parameters among treatments ($P > 0.05$), the final average weight and productivity (Kg/m³) showed a very strong direct correlation ($r = 0.99$ for both). A linear regression was observed between these parameters and the LSEO concentration in the diet [$y = 76.4478 + (1.8615\times)$; $R^2 = 0.97$; and $y = 2.8655 + (0.0710\times)$; $R^2 = 0.98$], respectively (Table II). The villi and crypts did not show any alterations among the treatments ($P > 0.05$).

Hematological Parameters

Regarding defense cells (Table III), the LSEO concentration influenced the total counts of neutrophils, basophils, and monocytes before the challenges ($P < 0.05$). After air exposure and infection challenges, no significant variation was observed among treatments ($P > 0.05$). However, when comparing values before and after the challenges, thrombocytes and total leukocytes decreased after the challenges; lymphocytes decreased after the infection in all groups but only after air exposure in the control group; neutrophils increased in the LSEO-treated groups after the infection; and monocytes showed the highest values after air exposure ($P < 0.05$). Regarding hematimetric indices (Table IV), LSEO concentrations influenced all parameters before the challenges, the mean corpuscular

Table I. Chemical composition (%) of essential oil of *Lippia sidoides*.

Components	RI	Quantity (%)
α-Thujene	925	0.8
α-Pinene	931	0.3
β-Pinene	975	0.1
Myrcene	989	1.5
α-Phellandrene	1004	0.1
δ-3-Carene	1009	0.1
α-Terpinene	1015	1
n.i.	1016	0.1
p-Cymene	1023	7.3
Limonene	1026	0.5
1,8-Cineole	1029	0.3
(Z)-β-Ocimene	1034	0.1
(E)-β-Ocimene	1045	0.1
γ-Terpinene	1056	3
Terpinolene	1087	n.d.
p-Cymenene	1088	n.d.
Linalool	1099	0.1
n.i.	1101	0.4
Ipdienol	1144	0.3
Terpinen-4-ol	1175	0.6
n.i.	1176	0.2
Thymol methyl ether	1233	0.6
Thymol	1298	75.4
Carvacrol	1303	0.3
α-Copaene	1373	0.3
(E)-Caryophyllene	1417	4.3
trans-α-Bergamotene	1433	n.d.
Aromadendrene	1436	0.4
α-Humulene	1450	0.2
Allo-Aromadendrene	1457	n.d.
n.i.	1486	n.d.
Bicyclogermacrene	1492	n.d.
α-Muurolene	1497	0.4
β-Bisabolene	1506	0.1
δ-Cadinene	1521	0.2
Spathulenol	1575	0.1
Caryophyllene oxide	1580	0.6
Total identified		100

RI = retention index; n.d. = not detected.

volume (MCV) after both challenges, and the mean corpuscular hemoglobin (MCH) only after the infection. Except for erythrocytes, all other parameters were affected by air exposure or infection. Total proteins were not influenced by the LSEO concentration ($P > 0.05$), but they significantly increased ($P < 0.05$) after the stress and infection challenges (Figure 1).

Stress challenges (Air Exposure Stress and Experimental Infection with sub-letal dose of *Aeromonas hydrophila*)

No mortality was observed at any of the oil concentrations for both stress challenges.

DISCUSSION

The analysis of LSEO revealed its major compounds as thymol (75.4%) and p-cymene (7.3%). Our results are consistent with findings from Brandão et al. (2021), dos Santos et al. (2015), and Monteiro et al. (2021), who also reported thymol as the main compound in LSEO. In our experiment, the thymol content was very similar to the amounts reported by Brandão et al. (2021) and Monteiro et al. (2021) (72.2% and 76.6%, respectively). On the other hand, Copatti et al. (2022) reported a different result, identifying carvacrol (44.5%) as the major compound. Although essential oils are effective alternatives to synthetic compounds, their efficacy and effects depend on their chemical composition, concentration, and chemotype. Therefore, characterizing their chemical composition is of paramount importance (Souza et al. 2019).

Similar to our findings, where LSEO dietary supplementation improved zootechnical parameters, Copatti et al. (2022) observed quadratic effects on growth variables—final weight, weight gain, feed conversion ratio, and specific growth rate—when testing 0.00, 0.25, 0.50, 1.00, and 1.50 mL/kg of LSEO in the diet of

Table II. Growth performance parameters and histological and histomorphometric analysis of tilapia fed with different concentrations of *Lippia sidoides* essential oil.

Variables	*Lippia sidoides* Concentrations				
	0.0%	0.05%	0.1%	0.2%	0.4%
Initial Weight (g)	14.72 ± 1.34	16.26 ± 1.41	16.01 ± 1.28	16.43 ± 2.02	14.22 ± 1.28
Final Weight (g)*	76.64 ± 6.41	76.73 ± 9.93	78.35 ± 6.90	80.86 ± 4.54	83.62 ± 4.81
Weight Gain (g)*	61.92 ± 3.38	60.47 ± 5.17	62.34 ± 3.59	64.43 ± 3.28	69.40 ± 3.05
FCR	1.03 ± 0.01[a]	1.01 ± 0.06[a]	1.08 ± 0.13[a]	1.01 ± 0.01[a]	0.90 ± 0.04[b]
Length (cm)	15.59 ± 0.54	16.07 ± 0.42	15.77 ± 0.10	15.74 ± 0.38	15.75 ± 0.17
Productivity (Kg/m³)*	2.87 ± 0.24	2.88 ± 0.37	2.94 ± 0.26	3.03 ± 0.17	3.14 ± 0.18
Villus (µm)	299.29 ± 25.89	299.91 ± 53.48	266.31 ± 47.3	296.23 ± 33.46	271.33 ± 43.12
Cripta (µm)	34.52 ± 7.8	35.42 ± 1.19	34.02 ± 3.72	38.97 ± 5.06	35.09 ± 11.61

FCR = Feed Conversion Ratio. * Indicates significant linear regression (P < 0.05). Different letters indicate significant differences (P < 0.05).

Colossoma macropomum. They suggested that 0.50 mL/kg yielded the best performance. In our experiment, the initial weight was statistically similar among treatments, reinforcing that the observed higher final weight resulted from the treatment and not from the initial weight. However, Monteiro et al. (2021) did not observe significant differences in *Colossoma macropomum* fed diets supplemented with 0.625 and 1.250 g/kg of LSEO. It is worth noting that the major LSEO compound differed between these studies, being carvacrol (44.5%) and thymol (76.6%), respectively. A similar observation was made in Nile tilapia fed turmeric hydrolate at various doses; however, the authors reported a significant reduction in the hepatosomatic index, indicating potential alterations in the fish's metabolism (Pereira et al. 2020).

Stress challenges are routinely used to evaluate the stress-inducing or stress-mitigating potential of substances or environments. Following exposure, animals primarily release catecholamines and cortisol, which trigger secondary adaptive changes observable in the blood. This makes hematological analyses valuable tools for assessing fish health in the face of sanitary, physiological (e.g., stress), or nutritional challenges (Saravanan et al. 2011, Gorissen & Flik 2016, Yousefi et al. 2022, da Silva et al. 2023).

In this experiment, air exposure did not affect total leukocyte counts, while infection reduced them. According to Barton & Iwama (1991), stress hormones inhibit lymphocyte production, and air exposure is a known stressor for fish (Louison et al. 2017). The delayed mobilization of defense cells is considered a mechanism enabling the immune system to respond effectively after the primary threat (Frank et al. 2013). Infections significantly influence leukocyte production and distribution and may yield antagonistic responses in total leukocyte counts under stress (Seibel et al. 2021).

Monocyte proliferation is regulated in the bone marrow via β-adrenergic signaling, a typical stress response (Korytář et al. 2016). In our study, monocytes increased significantly following air exposure. Similarly, Korytář et al. (2016) observed increased monocyte levels in *Coregonus maraena* subjected to density stress,

Table III. Total and differential leukocyte count of tilapias fed with *Lippia sidoides*.

Parameters	*Lippia sidoides* Concentrations				
	0.00%	**0.05%**	**0.10%**	**0.20%**	**0.40%**
Before challenges					
Thrombocytes (10^3 µL^{-1})	57.55 ± 14.94^{A}	53.74 ± 9.86^{A}	50.39 ± 10.69^{A}	62.66 ± 10.20^{A}	43.82 ±12.05^{A}
Total leukocytes (10^3 µL^{-1})	163.36 ± 60.01^{A}	118.83 ± 11.84^{A}	103.27 ± 11.31^{A}	108.25 ± 6.70^{A}	122.34 ± 16.02^{A}
Lymphocytes (10^3 µL^{-1})	158.90 ± 60.25^{A}	115.01 ± 11.9	99.08 ± 11.20	104.54 ± 6.16	119.66 ±16.71
Neutrophils (10^3 µL^{-1})	3.11 ± 0.14^{Aa}	1.85 ± 0.30^{Bb}	1.55 ± 0.56^{bc}	0.74 ± 0.47^{Cc}	1.54 ± 0.46^{Bc}
Basophils (10^3 µL^{-1})	0.46 ± 0.23^{a}	0.61 ± 0.19^{a}	0.87 ± 0.28^{ab}	1.36 ± 0.41^{b}	0.75 ± 0.22^{ab}
Eosinophils (10^3 µL^{-1})	0.59 ± 0.47	0.36 ± 0.52	1.27 ± 0.64	1.21 ± 0.68	0.32 ± 0.21
Monocytes (10^3 µL^{-1})	0.31 ± 0.41^{Bab}	0.62 ± 0.21^{Bb}	0.50 ± 0.27^{Bb}	0.40 ± 0.10^{Bb}	0.07 ± 0.13^{Ca}
Giant cells (10^3 µL^{-1})	0.00 ± 0.00	0.38 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
After exposure to air					
Thrombocytes (10^3 µL^{-1})	7.34 ± 8.41^{B}	4.57 ± 2.37^{B}	3.97 ± 3.38^{B}	12.51 ± 5.71^{B}	5.3 ± 2.83^{B}
Total leukocytes (10^3 µL^{-1})	123.51 ± 7.25^{A}	123.13 ± 40.13^{A}	126.89 ± 33.69^{A}	145.25 ± 41.75^{A}	118.17 ± 40.97^{A}
Lymphocytes (10^3 µL^{-1})	115.22 ± 1.39^{B}	111.54 ± 35.95^{A}	116.24 ± 33.73	133.77 ± 38.17	105.73 ± 35.06
Neutrophils (10^3 µL^{-1})	1.03 ± 0.61^{B}	1.42 ± 0.89^{B}	1.88 ± 1.95	1.91 ± 1.03^{B}	2.07 ± 0.29^{A}
Basophils (10^3 µL^{-1})	0.39 ± 0.40	0.11 ± 0.17	0.31 ± 0.21	0.09 ± 0.15	0.08 ± 0.14
Eosinophils (10^3 µL^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.07
Monocytes (10^3 µL^{-1})	6.08 ± 5.46^{A}	9.25 ± 8.57^{A}	7.16 ± 4.16^{A}	8.66 ± 3.63^{A}	8.41 ± 8.58^{A}
Giant cells (10^3 µL^{-1})	0.59 ± 0.58	0.82 ± 1.25	1.31 ± 0.97	0.41 ± 0.36	1.84 ± 1.91
After experimental infection with *Aeromonas hydrophila*					
Thrombocytes (10^3 µL^{-1})	14.84 ± 10.57^{B}	9.81 ± 4.02^{B}	8.75 ± 3.46^{B}	8.3 ± 2.71^{B}	7.97 ± 4.29^{B}
Total leukocytes (10^3 µL^{-1})	64.31 ± 29.54^{B}	51.67 ± 13.77^{B}	59.77 ± 14.66^{B}	58.52 ± 19.8^{B}	42.91 ± 4.42^{B}
Lymphocytes (10^3 µL^{-1})	58.01 ± 26.79^{C}	44.46 ± 15.43^{B}	54.86 ± 12.56^{B}	50.68 ± 16.94^{B}	39.61 ± 8.47^{B}
Neutrophils (10^3 µL^{-1})	4.04 ± 1.4^{A}	4.19 ± 1.83^{A}	2.51 ± 0.84	4.79 ± 1.58^{A}	1.98 ± 0.37^{A}
Basophils (10^3 µL^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.07
Eosinophils (10^3 µL^{-1})	0.00 ± 0.00	0.09 ± 0.12	0.07 ± 0.11	0.04 ± 0.04	0.09 ± 0.08
Monocytes (10^3 µL^{-1})	2.26 ± 1.64^{AB}	2.17 ± 1.26^{B}	1.78 ± 0.27^{B}	3.01 ± 2.34^{AB}	1.19 ± 0.43^{AB}
Giant cells (10^3 µL^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Different uppercase letters indicate significant (P < 0.05) differences before and after stress, lowercase letters indicate significant (P < 0.05) differences among doses in bifactorial (two-way) ANOVA and the SNK tests.

which aligning with our findings and suggesting that these cells may indicate unfavorable conditions for tilapia.

Another notable finding was the reduction in thrombocyte levels following air exposure and infection. Supporting our results, Korytář et al. (2016) also reported a decrease in thrombocytes in *Coregonus maraena* subjected to high-density stress. Thrombocytes play a role analogous to mammalian platelets, assisting in blood coagulation (Köllner et al. 2004). Similarly, Brum et al. (2017) observed reduced thrombocyte levels in *O. niloticus* fed 1.0% *Zingiber officinale* after an infection challenge, attributing this

Table IV. Hematological parameters of Nile tilapia fed with *Lippia sidoides*.

Parameters	*Lippia sidoides* Concentrations				
	0.00%	**0.05%**	**0.10%**	**0.20%**	**0.40%**
Before challenges					
HT%	27.97 ± 1.16^{Aab}	28.07 ± 0.96^{Bab}	25.63 ± 2.70^{Ba}	30.67 ± 1.58^{Ab}	26.00 ± 1.68^{Ba}
HB (g/dL)	9.87 ± 1.11^{Ab}	11.53 ± 0.63^{Aab}	10.43 ± 1.50^{Aab}	12.37 ± 0.43^{Aa}	11.99 ± 2.31^{Aab}
Ery (10^6 cells/µL)	1.53 ± 0.06^{a}	1.41 ± 0.07^{ab}	1.27 ± 0.11^{b}	1.30 ± 0.12^{b}	1.26 ± 0.09^{b}
MCV (fL)	187.95 ± 11.74^{Aa}	199.7 ± 12.43^{Aa}	214.81 ± 22.65^{Aab}	239.06 ± 8.38^{b}	211.48 ± 20.51^{Aab}
MCH (pg)	66.51 ± 4.45^{Aa}	82.40 ± 2.02^{Ab}	96.46 ± 1.11^{Ac}	96.99 ± 5.47^{Ac}	102.18 ± 9.67^{Ac}
MCHC (pg)	35.72 ± 3.62^{Aa}	41.52 ± 3.10^{Ab}	43.48 ± 5.72^{Ab}	41.09 ± 1.31^{Ab}	51.00 ± 7.47^{Ab}
After exposure to air					
HT%	33.61 ± 2.94^{A}	32.89 ± 2.80^{A}	34.56 ± 2.86^{A}	33.39 ± 1.23^{A}	31.84 ± 1.65^{A}
HB (g/dL)	8.51 ± 0.13^{A}	8.67 ± 0.67^{B}	8.75 ± 0.86^{A}	8.51 ± 0.12^{B}	8.54 ± 0.46^{B}
Ery (10^6 cells/µL)	1.79 ± 0.37	1.98 ± 0.98^{A}	1.75 ± 0.86	2.00 ± 0.50	1.94 ± 0.79
MCV (fL)	193.92 ± 34.68^{Aab}	169.5 ± 34.65^{Ab}	223.12 ± 16.86^{Aa}	176.16 ± 40.66^{ab}	172.76 ± 9.75^{Ba}
MCH (pg)	48.79 ± 5.35^{B}	44.67 ± 5.61^{C}	56.09 ± 6.86^{B}	43.21 ± 8.61^{B}	46.02 ± 9.73^{B}
MCHC (pg)	25.51 ± 2.64^{B}	26.46 ± 2.41^{C}	25.39 ± 8.86^{B}	24.54 ± 0.88^{C}	26.92 ± 1.5^{B}
After experimental infection with *Aeromonas hydrophila*					
HT%	25.25 ± 6.29^{AB}	20.78 ± 2.47^{C}	23.47 ± 0.71^{B}	22.67 ± 2.84^{B}	23.14 ± 2.31^{B}
HB (g/dL)	6.29 ± 1.02^{B}	5.98 ± 0.95^{C}	6.19 ± 0.44^{B}	5.94 ± 0.62^{C}	5.69 ± 0.73^{C}
Ery (10^6 cells/µL)	1.58 ± 0.46	1.09 ± 0.13	1.36 ± 0.22	1.32 ± 1.36	1.42 ± 1.15
MCV (fL)	151.71 ± 6.58^{Bb}	200.43 ± 23.37^{Aa}	168.54 ± 24.85^{Bb}	195.62 ± 1.41^{a}	154.01 ± 1.48^{Cb}
MCH (pg)	40.59 ± 4.7^{Bb}	60.9 ± 12.28^{Ba}	41.44 ± 1.92^{Cb}	49.13 ± 4.55^{Bb}	41.5 ± 4.03^{Bb}
MCHC (pg)	27.08 ± 4.53^{B}	31.43 ± 3.23^{B}	26.31 ± 2.27^{B}	29.03 ± 2.92^{B}	27.64 ± 2.24^{B}

Different uppercase letters indicate significant (P < 0.05) differences before and after stress; lowercase letters indicate significant (P < 0.05) differences among doses in bifactorial (two-way) ANOVA and the SNK tests. HT = Hematocrit; HB = Hemoglobin concentration; Ery = Erythrocytes count; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration.

reduction to thrombocyte migration to lesion sites caused by the infection. In contrast, Monteiro et al. (2021) found that the inclusion of LSEO did not influence thrombocyte counts in *Colossoma macropomum* infected with *A. hydrophila*.

In our study, hematological parameters remained within reference values for *O. niloticus* (Azevedo et al. 2016). Hemoglobin reduction following stress, as observed in our study, is consistent with findings by Saravanan et al. (2011), who reported similar reductions in *Cyprinus carpio* exposed to toxic stress. Hemoglobin is a reliable secondary stress response indicator in fish (Seibel et al. 2021), and its reduction may result from disturbances in hematopoiesis (Kori-Siakpere & Ubogu 2008). We also noted decreased MCH and MCHC levels after stress and infection, as reported by Köprücü et al. (2006) and Saravanan et al. (2011) in *Silurus glanis* and *Cyprinus carpio*, respectively, following toxic stress. According to Saravanan et al. (2011), these reductions may stem from high percentages of immature red blood cells in circulation and

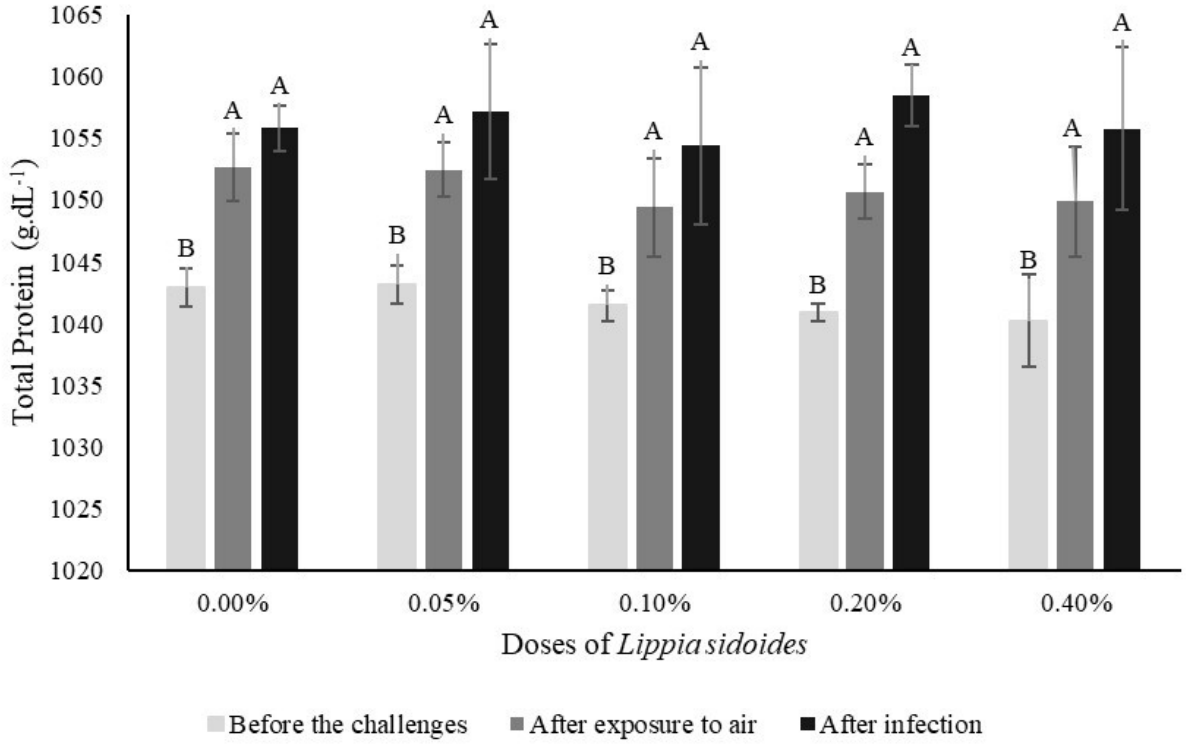

Figure 1. Total protein of Nile tilapia fed with different concentrations of *Lippia sidoides* essential oil. Different uppercase letters indicate significant (P < 0.05) differences before and after stress. Lowercase letters indicate significant (P < 0.05) differences among doses in bifactorial (two-way) ANOVA and the SNK tests.

decreased Hb synthesis. Despite these decreases, MCH and MCHC remained within recommended parameters for the species (Azevedo et al. 2016).

Unlike our study, Monteiro et al. (2021) found no effect of LSEO on total protein levels in *Colossoma macropomum* after infection. Increased protein synthesis is a secondary stress response, may help alleviate ionoregulatory issues associated with sodium loss in stressed fish (Mackett et al. 1992, Roberts et al. 2010). Additionally, during infections, the adaptive immune system produces immunoglobulins secreted by B lymphocytes, increasing protein levels (Mu et al. 2022), justifying the changes observed in our study

Overall, our experiment demonstrated that dietary LSEO inclusion in tilapia caused minimal alterations in blood parameters, indicating its safe use in feed formulations. These findings align with those of Monteiro et al. (2021), who reported no significant changes, and Copatti et al. (2022), who observed linear improvements in performance and hematology in *Colossoma macropomum*. However, specific knowledge is crucial for each scenario, as essential oils vary in active compound composition, and factors like species physiology, age, sex, nutritional status, management, and diet duration may influence outcomes (Azevedo et al. 2016, Monteiro et al. 2021).

Infection challenges with *A. hydrophila* are widely used to assess the effectiveness of natural additives in managing bacterial diseases in aquaculture (de Moraes et al. 2022, Jatobá et al. 2024). *A. hydrophila*, an opportunistic pathogen, causes septicemia, high mortality, and significant economic losses (Janda & Abbott 2010). In our experiment, we used a sub-lethal

dose of the pathogen to observe the changes caused by the stress of this infection and, therefore, did not observe mortality. However, we observed promising results that may suggest LSEO as a sustainable and effective alternative to antibiotics, aiding disease control and promoting animal welfare.

The supplementation of LSEO in Nile tilapia diets linearly increases final weight and productivity, while at a concentration of 0.4%, it reduces feed conversion ratio. Air exposure and infection directly affect blood parameters. Supplementation with LSEO did not impair blood indicators; however, it did not prevent their alteration following stress challenges. Further studies are needed to identify the effect of adding higher levels of LSEO.

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Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.