Aquaculture Science and Management

RESEARCH Open Access

Effects of dietary supplementation with *Lippia sidoides* essential oil on the growth, histopathological and hematological parameters of koi carp (*Cyprinus carpio* linnaeus, 1758)

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Abstract

The aim of this research was to evaluate the effects of dietary supplementation with *Lippia sidoides* essential oil on the hematological, histopathological and growth parameters of koi carp (*Cyprinus carpio*). A total of 300 fish with an initial average weight of 3.56 ± 0.68 g were randomly distributed in 15 net cages and fed twice daily with diets containing 0.0, 0.25, 0.5, 0.75, and 1.0% essential oil, all in triplicate. Histological and hematological analyses were conducted after 30 and 60 days of feeding, along with growth performance assessment at the conclusion of the 60-day period. No mortalities were observed during the period. Fish in the 0.75% treatment exhibited significantly lower final weight and specific growth rate ($p = 0.012$ and $p = 0.011$) compared to fish in the 0.25% treatment and the control group. Histological analysis of renal tissue revealed a significant increase ($p < 0.05$) in capillary dilation in fish that were fed diets containing 1.0% *L. sidoides* essential oil compared with those fed diets containing 0.25%, 0.50%, and 0.75% essential oil. Hematological parameters were not altered at any of the essential oil concentrations. Supplementing the diet with *L. sidoides* essential oil was found to be safe and did not result in any mortality over a 60-day period. Among the treated groups, the 0.25% dose resulted in improved growth. Nevertheless, it is advisable to exercise caution when using higher dosages approaching 1.0%.

Keywords Aquaculture, Hematology, Ornamental fish, Phytobiotics

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Introduction

The market for ornamental fish has been steadily increasing, driven by the demand for exotic species and the growing popularity of aquariums as leisure and business activities [7, 19, 32, 34]. According to the FAO [14], the global trade in ornamental fish generates over US$350 million annually, with Asia responsible for more than 50% of exports, particularly Singapore, Japan, and Thailand.

In Brazil, ornamental koi carp (*Cyprinus carpio*) has become a popular commercial species [39] because of its vibrant colors, ability to thrive in various culture systems (aquariums and ponds), and acceptance of different commercial diets [26, 36]. Koi carp are highly valued ornamental fish known for their intricate patterns, graceful body shapes, and elegant swimming behavior. They are in high demand in the global fish market because of their popularity among aquarium enthusiasts worldwide [5]. Thus, research on enhancing production efficiency and enhancing care practices is essential for the sustainable management of koi carp farming.

In addition to being ornamental, the common carp (*C. carpio*) is one of the most important species in aquaculture worldwide [15]. As production systems become more intensive, there is a growing interest in finding strategies to increase fish health and performance, with a focus on natural products [9]. In this situation, the advantages of plant-derived dietary compounds are being thoroughly explored as effective alternatives for sanitation in aquaculture [2–4]. For example, Cornelian cherry (*Cornus mas* L.) fruit extract enhances growth performance, disease resistance, and serum immune- and antioxidant-related gene expression in common carp [3], whereas dietary supplementation with *Plantago ovata* at 0.25% of the diet for 60 days boosts growth performance and enhances the health of common carp by mitigating oxidative stress induced by ammonia [4].

Likewise, the immunomodulatory, antimicrobial, antioxidant, and antiparasitic properties of essential oils, which are volatile compounds derived from aromatic plants, have been extensively researched [2, 20, 21]. Species of the *Lippia* genus, which is native to Brazil, have high essential oil yields and a broad spectrum of biological activities. In particular, *Lippia sidoides* (pepper rosemary) oil is rich in thymol, a compound known for its antimicrobial and anti-inflammatory effects [41].

Despite their significant potential, the use of essential oils requires specific precautions to ensure safety, efficacy, and sustainability [2, 9, 22, 23]. Factors such as the chemical composition and standardization of extracts are considered essential for reproducibility and the safety of effects [22]. Additionally, subtherapeutic doses may be ineffective, whereas high doses may lead to toxicity in fish [11, 22], depending on the species and developmental stage [22]. Therefore, further research on essential oils, such as *L. sidoides*, in aquacultured fish diets is crucial.

While numerous studies have demonstrated the beneficial impacts of essential oils on economically significant fish species such as tilapia (*Oreochromis niloticus*) [22, 35], there remains a lack of research on the effects of these phytobiotics on ornamental fish species, particularly concerning hematological and histopathological parameters following dietary supplementation [23, 27]. Assessing morphological and blood parameters is crucial for monitoring fish health and identifying physiological changes and tissue damage associated with the use of natural additives [10, 44], thereby indicating any signs of physiological intolerance to the phytobiotic or the administered doses.

Therefore, this study aimed to investigate the impact of incorporating *L. sidoides* essential oil into the diet of ornamental koi carp (*C. carpio*) on growth, hematological and histopathological parameters, with the goal of promoting the safe and effective utilization of this phytobiotic in ornamental aquaculture.

Materials and methods

The fish used in the study were provided by Vale dos Bettas fish farm in Biguaçu, Santa Catarina, Brazil, where the fish feed supplementation trial was conducted. All animal procedures were approved by the Animal Use Ethics Committee (CEUA/UFSC 1440100217).

Obtaining and chemical composition of essential oils

The essential oil was extracted from leaves of plants grown and processed at the Medicinal Plants and Phytochemistry Laboratory of the Brazilian Agricultural Research Corporation – EMBRAPA Western Amazonia, Brazil, situated in Manaus, Amazonas. The leaves were collected in the morning, dried in a continuous air circulation oven at 45 °C for 48 h, and then subjected to hydrodistillation in a Clevenger-type apparatus to obtain the essential oils.

The chemical composition of *L. sidoides* oil was analysed using gas chromatography with an Agilent 6890 instrument and an Agilent 5973 N mass-selective detector. The separation of components was performed on an HP5-MS capillary column (30 m × 0.25 mm × 0.25 µm) with a temperature gradient from 60 °C to 240 °C at a rate of 3 °C/min. A 1.0% essential oil solution was injected into the flow divider at a 1:100 ratio and maintained at 250 °C. The relative quantification of the essential oil components was conducted on an Agilent 6890 N gas chromatograph with a flame ionization detector at 280 °C, using an HP5 capillary column (30 m × 0.32 mm × 0.25 µm) and hydrogen as the carrier gas at a flow rate of 1.5 mL/min. The essential oil components were identified by comparing mass spectra from a Wiley 6th edition

spectral library and by comparing the calculated retention index of each component with literature data. The retention index calculation was carried out by injecting a series of n-alkanes under the same analytical conditions as those used for essential oils, following the method described by Adams and Sparkman [1].

Experimental design

The dietary supplementation experiment was conducted at the Vale dos Bettas fish farm. *L. sidoides* essential oil was incorporated into a commercial diet GuabiTech® Impulse 45 (45% crude protein, 9% ether extract, 10% moisture, 16% mineral matter, 3% fiber, 1.5% calcium, 1.4% phosphorus, 2,000 mg/kg vitamin C, and 400 IU/kg vitamin E). The essential oil concentrations tested were 0.0% (control group), 0.25%, 0.5%, 0.75%, and 1.0% according to Cardoso et al. [11].

The essential oil was initially weighed with the aid of a digital scale and later diluted with 100 g of alcohol per kilo of feed. The essential oils at their respective concentrations were added to a hand sprayer and sprayed on the diet, which was subsequently dried for 24 h at room temperature and then stored in a freezer (−20 °C) until use according to Dairiki et al. [13].

Three hundred carp with an average initial weight of 3.56 ± 0.68 g from the same spawning were randomly distributed into 15 net cages (1.0 × 1.0 × 1.30 m) with 5.0 mm mesh, which were installed in an earthen pond with a total area of approximately 1.0 ha. After acclimatization, the fish were subjected to five dietary treatments: 0.0, 0.25, 0.50, 0.75, or 1.0% *L. sidoides* essential oil in the diet, all in triplicate. The fish were fed ad libitum twice daily (9:00 am and 4:00 pm) for a period of 60 days, after which their final weight (g), specific growth rate $(\text{SGR}\ \%\ \text{day}^{-1}) = 100 \times [(\ln\text{finalweight}) - (\ln\text{initialweight}) / \text{cultivation days}]$, and survival rate $(\%) = [(\text{final number of fish} / \text{initial number of fish}) \times 100]$ were assessed to measure growth performance.

Additionally, on the 30th and 60th day, samples of five fish were collected from each experimental unit for hematological and histological analysis. The water quality parameters of dissolved oxygen, temperature, pH, electrical conductivity, and total solids were monitored daily with a Hanna instrument (HI 9828) multiparameter. Total ammonia and nitrite were measured twice a week with a commercial kit (Alcon pet®). Transparency was assessed once a week with a Secchi disk. Throughout the experiment, the water quality parameters remained stable, with an average of 6.08 ± 1.55 mg/L of dissolved oxygen, 20.50 ± 0.88 °C temperature, 5.40 ± 0.82 pH, 40.73 ± 6.52 μS/cm^3 electrical conductivity, 19.25 ± 4.20 mg/L total solids, 0.58 ± 0.27 mg/L total ammonia, 0.0 mg/L nitrite, and 24.17 ± 1.83 cm transparency.

Blood collection and analysis

Five fish from each net cages were collected at 30 and 60 days of the experiment. The fish were anaesthetized with eugenol (75 mg/L), and then, blood was collected via puncture of the caudal vessel using 1.0 mL syringes containing EDTA (10%). The collected blood was used for counting the total erythrocytes in a Neubauer chamber after dilution (1:200) in Dacie's fluid. Additionally, total and differential counting of leukocytes was performed after the confection of blood extensions previously stained with May-Grünwald-Giemsa-Wright (MGGW) according to Tavares-Dias and Moraes [38]. The plasma glucose concentration was determined with an Accu-Chek® Advantage portable glucometer (Roche Diagnóstica, Brazil).

Histological analysis

The same fish used for blood collection were euthanized by sectioning the spine to collect fragments of the intestine, kidney, spleen, heart and liver. These fragments were fixed in 10% buffered formalin at pH 7.4 to observe possible changes in tissues caused by the addition of different concentrations of *L. sidoides* oil to the diet. All organ fragments were subsequently dehydrated in a progressively graduated alcohol series and then in xylol and embedded in paraffin at 60 °C. Thin sections of 5.0 μm from each organ were made, stained with hematoxylin and eosin, and the slides were assembled and analysed via differential interference contrast microscopy (DIC) (ZEISS, Axio Imager A.2, Gottingen, Germany). Histological changes in the kidney, spleen, liver, heart and intestine were evaluated semiquantitatively, assigning values according to the degree of severity of lesions: 0 (no lesion), 1 (mild, < 25% of tissue area), 2 (moderate, 25% to 50% of the tissue area) and 3 (severe, >50% of the tissue area) according to the modified method of Schwaiger et al. [33]. The morphometric characteristics of the intestinal tissue, such as the quantity, length and width of the villi, number of goblet cells, perimeter and area of the villi, were determined via differential interference contrast microscopy (DIC) (ZEISS, Axio Imager A.2, Gottingen, Germany).

Statistical analysis

The results of the hematological and histopathological parameters were initially subjected to the Shapiro–Wilk normality test and the Levene homoscedasticity test. The data were subjected to analysis of variance (ANOVA) via Statistica 13.0 (StatSoft Inc., Tulsa, USA). Tukey's test was used to compare means, with a significance level of 5%. The nonparametric Kruskal–Wallis test followed by the Dunn test was used for histological analysis.

Results

Chemical composition of lippia Sidoides essential oil

The results of the chemical composition analysis of *L. sidoides* are shown in Supplementary file 1. All the chemical components were quantified, and 99.4% of them were identified, with the highest percentages being the compounds thymol (72.2%), p-cymene (8.15%) and (E)-caryophyllene (4, 9%).

Growth performance

Supplementing the diet with *L. sidoides* essential oils had a significant impact on the growth of koi carp (*C. carpio*). The final weight ($p = 0.012$) and specific growth rate ($p = 0.011$) were significantly lower in the 0.75% treatment group compared to the 0.25% treatment group and the control group. However, there were no significant differences in final weight and specific growth rate between the 0.50%, 0.75%, and 1.0% treatment groups (Table 1).

Histological analysis

In kidney tissue, lesions such as cell vacuolization, hyaline degeneration, or tubular degeneration were not observed, either on the 30th or 60th day of analysis. A significant increase in capillary dilation in renal tissue was observed in fish fed diets containing 1.0% *L. sidoides* essential oil for 60 days (Table 2 and Fig. 1AB).

In heart tissue, eosinophilic infiltration was not observed on the 30th day of analysis. Compared with those in the 30-day group, all the experimental groups presented an increase in the intensity of eosinophilic infiltration in cardiac tissue at the end of 60 days of supplementation (Table 2 and Fig. 1CD).

On the 30th day, in liver tissue eosinophilic infiltration was significantly reduced ($p < 0.05$) in the 1.0% treatment group, whereas on the 60th day, it was significantly reduced ($p < 0.05$) in the control group and in the 1.0% treatment group. The loss of hepatocyte nuclei was significantly reduced ($p < 0.05$) in the 0.5% and 0.75% groups on the 30th day, whereas on the 60th day, it was significantly reduced ($p < 0.05$) in the 1.0% treatment and control groups (Table 3).

In the spleen tissue, fibrous capsule integrity significantly increased ($p < 0.05$) in the 0.25% treatment group, whereas on day 60, it significantly decreased ($p < 0.05$) in the 0.50% treatment group on the 30th day (Table 3).

No significant differences ($p > 0.05$) were detected in the intestinal histomorphometry of koi carp at either 30 or 60 day (Table 4).

Hematological parameters

The hematological parameters of koi carps fed diets containing *L. sidoides* essential oil did not significantly change at the end of days 30 and 60 (Table 5).

Discussion

Plant-based compounds are becoming increasingly important in contemporary fish farming because of their positive impact on fish. This is attributed to a variety of compounds that offer nutritional, functional, and environmentally friendly advantages. Compounds like flavonoids, tannins, alkaloids, and saponins possess antioxidant, antimicrobial, and anti-inflammatory properties, which enhance the fish's immune system and protect against diseases [2–4].

In the present study, chromatographic analysis of *L. sidoides* essential oil confirmed that thymol was the major compound, followed by p-cymene and (E)-caryophyllene. This chemical composition has been consistently observed in different studies [18, 41] and is associated with the antimicrobial, antioxidant, and anti-inflammatory properties of the oil [20]. The high concentration of thymol (> 70%) reinforces its therapeutic potential, especially in functional diets for ornamental fish.

Natural polyphenols found in essential oils have the potential to enhance fish growth performance. Nevertheless, the ideal dosages and potential negative impacts on growth performance may differ significantly based on the fish species and the origin of the essential oil [2]. For instance, a 60-day dietary supplementation with *P. ovata* seed extract at concentrations of 0.0%, 0.25%, 0.50%, and 1.0% was found to be beneficial for common carp (*C. carpio*) exposed to ammonia toxicity. However, the fish that received diets with 0.25% extract showed a significant enhancement in growth [4]. Likewise, in the current investigation, the varying levels of *L. sidoides* essential oil resulted in both advantageous and detrimental impacts on the evaluated organ tissues. Nevertheless, the 0.25% inclusion led to improvements in growth performance compared to higher doses like 0.75%, suggesting a negative impact on growth.

Table 1 Growth performance (mean ± standard deviations) Koi carp (*Cyprinus carpio*) supplemented with different levels of *Lippia Sidoides* essential oils (0.0, 0.25, 0.50, 0.75, or 1.0%) in their diet for 60 days. SGR = specific growth rate. Significant differences among treatments were assessed using the Tukey test ($p < 0.05$) and are indicated by different letters in the lines

Parameters	Treatments					
	Control	0.25%	0.50%	0.75%	1.0%	*p*-value
Final weight (g)	7.35 ± 0.21^{a}	7.45 ± 0.49^{a}	6.23 ± 0.79^{ab}	5.94 ± 0.83^{b}	6.79 ± 1.24^{ab}	0.012
SGR (% day^{-1})	1.20 ± 0.38^{a}	1.23 ± 0.26^{a}	0.93 ± 0.59^{ab}	0.85 ± 0.17^{b}	1.08 ± 0.10^{ab}	0.011
Survival (%)	100	100	100	100	100	–

Table 2 Histological evaluation (mean ± standard deviations) of kidney and heart tissues in Koi carp (*Cyprinus carpio*) supplemented with different levels of *Lippia Sidoides* essential oils (0.0, 0.25, 0.50, 0.75, or 1.0%) in their diet for 30 and 60 days

kidney parameters	**Treatments**				
30 days	Control	0.25%	0.50%	0.75%	1.00%
Capillary dilation	1.50 ± 0.87	1.33 ± 0.29	1.33 ± 0.58	1.33 ± 0.58	1.67 ± 0.58
Bowman space reduction	1.50 ± 0.87	1.00 ± 0.00	1.00 ± 1.00	2.33 ± 0.58	0.83 ± 0.29
Presence of red blood cells	2.00 ± 0.00	1.67 ± 0.58	1.33 ± 0.58	1.33 ± 0.58	1.17 ± 0.76
Blood leakage	1.33 ± 0.58	1.33 ± 0.29	1.00 ± 0.00	1.00 ± 0.00	1.17 ± 0.76
Melamacrophagus	0.67 ± 0.58	0.33 ± 0.58	0.67 ± 0.58	1.00 ± 0.00	1.50 ± 0.87
Lymphocyte infiltrate	1.33 ± 1.53	1.00 ± 0.00	0.67 ± 1.15	2.00 ± 1.00	1.17 ± 0.76
Hyaline balls	0.50 ± 0.50	0.33 ± 0.58	1.67 ± 1.53	0.67 ± 0.58	0.50 ± 0.50
Eosinophilic infiltrate	1.00 ± 1.00	1.00 ± 1.73	0.00 ± 0.00	1.33 ± 1.15	1.50 ± 0.50
60 days	Control	0.25%	0.50%	0.75%	1.00%
Capillary dilation	1.83 ± 0.29^{ab}	1.33 ± 0.58^{b}	1.33 ± 0.58^{b}	1.00 ± 0.00^{b}	3.00 ± 0.00^{a}
Bowman space reduction	2.17 ± 0.76	2.17 ± 0.76	1.50 ± 0.50	1.67 ± 1.04	2.00 ± 1.75
Presence of red blood cells	1.00 ± 0.00	1.33 ± 0.58	1.17 ± 0.29	1.17 ± 0.29	1.00 ± 0.00
Blood leakage	0.83 ± 0.29	1.00 ± 1.00	1.17 ± 0.29	1.00 ± 0.00	0.67 ± 0.58
Melamacrophagus	1.00 ± 1.00	1.33 ± 0.58	1.50 ± 0.50	1.67 ± 0.15	1.00 ± 0.00
Lymphocyte infiltrate	2.00 ± 0.00	2.50 ± 0.50	2.00 ± 0.00	1.67 ± 0.76	2.67 ± 0.58
Hyaline Balls	1.50 ± 1.50	1.33 ± 0.58	1.33 ± 1.53	1.33 ± 1.15	1.00 ± 0.00
Eosinophilic infiltrate	1.67 ± 0.76	2.67 ± 0.58	1.17 ± 0.29	1.83 ± 1.04	2.00 ± 0.00
Heart parameters	**Treatments**				
30 days	Control	0.25%	0.50%	0.75%	1.00%
Cardiac steatosis	1.33 ± 0.58	1.50 ± 0.87	1.17 ± 0.29	1.67 ± 0.58	1.67 ± 1.15
Congestion	1.33 ± 1.53	1.83 ± 0.29	0.50 ± 0.50	1.67 ± 1.53	0.50 ± 0.50
Necrosis	0.83 ± 0.50	0.83 ± 1.04	0.33 ± 0.50	1.00 ± 0.87	0.67 ± 1.15
Lymphocyte infiltrate	0.67 ± 0.58	0.83 ± 0.29	1.00 ± 0.00	0.67 ± 0.76	1.33 ± 0.58
60 days	Control	0.25%	0.50%	0.75%	1.00%
Cardiac steatosis	1.67 ± 1.15	1.67 ± 0.29	0.67 ± 0.58	1.33 ± 0.58	1.67 ± 1.15
Congestion	2.17 ± 1.04	1.33 ± 0.29	1.00 ± 1.00	1.50 ± 0.50	1.17 ± 0.76
Necrosis	0.33 ± 0.58	1.00 ± 0.87	0.33 ± 0.58	0.83 ± 1.04	0.50 ± 0.50
Lymphocyte infiltrate	1.83 ± 0.29	2.00 ± 0.50	1.00 ± 0.00	1.50 ± 1.32	1.50 ± 0.50
Eosinophilic infiltrate	0.67 ± 0.29	0.67 ± 0.28	0.67 ± 0.58	0.71 ± 0.28	0.83 ± 0.29

Histopathology is a sensitive tool for detecting the subclinical effects of food additives. In this study, capillary dilation was observed in the renal tissue of carp fed 1.0% essential oil for 60 days, suggesting that this concentration may exceed the safe limit for prolonged exposure. Although previous studies have not reported this specific lesion, similar renal changes have been documented in fish exposed to deficient or contaminated diets [21, 25]. Such damage may compromise the excretory function and ionic homeostasis of fish. Low water pH, such as the levels observed in the present study, could also harm fish kidneys by causing metabolic acidosis, impairing kidney function, and increasing injury risk [37].

The eosinophilic infiltrate observed in cardiac tissue after 60 days, although mild in intensity, indicates a possible delayed inflammatory response. This change has been associated with immune responses induced by dietary or parasitic stimuli [6, 16]. However, as the intensity was classified as nonpathological (< 25% of the tissue area), it does not constitute a limiting factor for the use of the oil at the concentrations tested up to 0.75%.

The liver is a crucial organ that performs essential functions in the body. It is responsible for various biological processes, including nutrient metabolism and detoxification. Moreover, the liver contributes to the immune system by producing defense proteins such as ferritin and other inflammatory response proteins. Additionally, the liver aids in removing microorganisms and foreign particles from the blood, which helps safeguard the body against infections [28]. The present study revealed no serious liver or pancreas damage after 30 or 60 days of dietary supplementation with *L. sidoides*. These findings suggest that the treatment did not cause significant acute or chronic toxicity in these organs. In contrast, Cardoso et al. [11] reported significant histopathological changes in the liver tissues of *Danio rerio* fed diets containing 1.0% *L. sidoides* essential oil.

Eosinophilic infiltration indicates an inflammatory response [28], suggesting an activated inflammatory or immune response. The decrease in infiltration over time, particularly in the treated groups (1.0%), may suggest that *L. sidoides* had an anti-inflammatory or restorative effect.

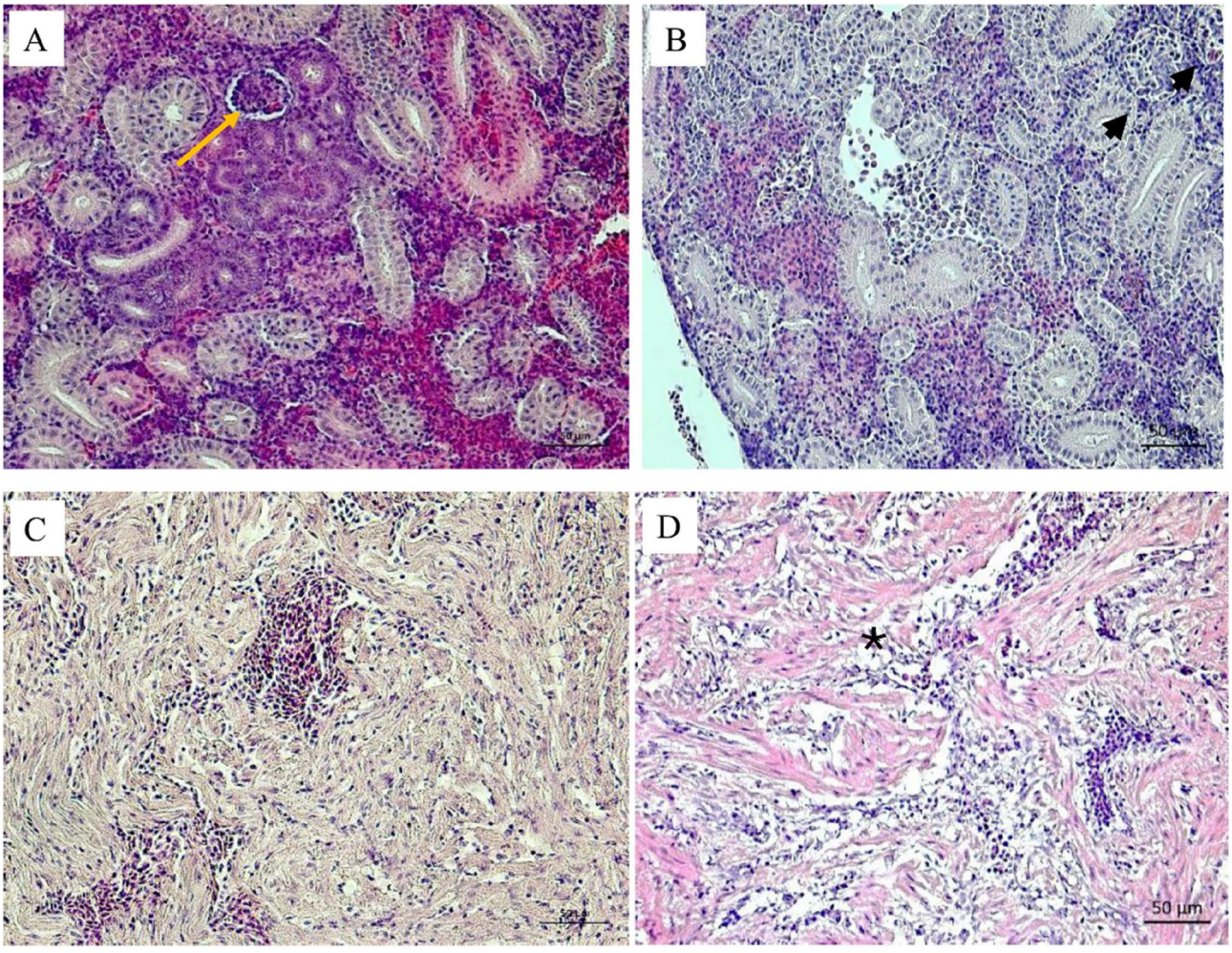

Fig. 1 Histological evaluation of kidney tissues in koi carp (*Cyprinus carpio*) supplemented with different levels of *Lippia sidoides* essential oils (0.0, 0.25, 0.50, 0.75, or 1.0%) in their diet for 30 and 60 days. **A** – Renal tissue without glomerular alterations at 30 days (arrow). **B** – Renal tissue with dilated capillaries in 1.0% oil at 60 days (arrow). **C** – Cardiac tissue without alterations at 30 days. **D** – Cardiac tissue with eosinophilic infiltration at 60 days of treatment (asterisk). Staining: HE, Bar: 50 µm

However, the control group also showed a reduction on day 60, indicating that the underlying condition (potentially initial inflammation) naturally decreased over time.

On the other hand, the loss of hepatocyte nuclei is a sign of cellular damage or degeneration [42]. The reduction in this loss in the 0.5% and 0.75% treatments on day 30 suggests that these treatments helped protect hepatocytes from cellular degeneration. On day 60, the 1.0% treatment was also effective, and the control group showed improvement. The improvement in the control group over time could indicate natural recovery or adaptation of the liver to some conditions related to the experimental environment itself.

Like the liver, the spleen is crucial for fish physiology. Serving as a lymphoid organ, the spleen is vital in the fish immune system, eliminating foreign cells and particles such as bacteria, viruses, and damaged blood cells [24, 28]. Owatari et al. [28] reported that the spleen is a severely affected organ in conditions of severe septicaemia caused by bacterial infection. On the other hand, Cardoso et al. [11] reported a significant reduction in splenic necrosis in *D. rerio* that received diets containing 0.25%, 0.50% and 1.00% *L. sidoides*. Similarly, in the present study, the absence of severe changes in the splenic tissue of koi carp suggests that, overall, the *L. sidoides* treatments did not cause severe structural damage to the spleen.

The absence of significant changes in intestinal tissue indicates that *L. sidoides* oil does not compromise structures fundamental to digestion, metabolism, or immunity in fish. Similar studies with basil (*Ocimum gratissimum*), mint (*Mentha piperita*), and thyme (*Thymus vulgaris*) oils corroborate these findings, demonstrating the safety of these compounds at moderate doses [8, 40, 43].

Hematological parameters serve as reliable indicators for monitoring changes in homeostasis and overall health

Table 3 Histological evaluation (mean ± standard deviations) of liver and spleen tissues in Koi carp (*Cyprinus carpio*) supplemented with different levels of *Lippia Sidoides* essential oils (0.0, 0.25, 0.50, 0.75, or 1.0%) in their diet for 30 and 60 days. Significant differences among treatments were assessed using the Tukey test ($p < 0.05$) and are indicated by different letters in the lines

Liver parameters	**Treatments**				
30 days	Control	0.25%	0.50%	0.75%	1.00%
Eosinophilic infiltrate	0.17 ± 0.29^{a}	0.17 ± 0.29^{a}	0.50 ± 0.50^{a}	0.17 ± 0.29^{a}	0.00 ± 0.00^{b}
Lymphocytic infiltrate	0.50 ± 0.50	1.33 ± 0.58	1.00 ± 0.00	0.51 ± 0.50	1.00 ± 0.00
Hepatocyte hypertrophy	1.50 ± 0.50	1.67 ± 0.29	1.33 ± 0.76	0.83 ± 0.29	0.50 ± 0.50
Macrosteatosis	1.67 ± 1.26	1.33 ± 1.04	1.17 ± 1.04	1.17 ± 1.04	0.83 ± 0.76
Microsteatosis	1.67 ± 1.26	2.33 ± 0.76	0.50 ± 0.50	0.67 ± 0.58	0.67 ± 0.29
Loss of hepatocytes nuclei	0.67 ± 0.58^{a}	1.00 ± 0.50^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.17 ± 0.29^{a}
60 days	Control	0.25%	0.50%	0.75%	1.00%
Eosinophilic infiltrate	0.00 ± 0.00^{b}	0.50 ± 0.50^{a}	0.67 ± 0.29^{a}	0.17 ± 0.29^{a}	0.00 ± 0.00^{b}
Lymphocytic infiltrate	1.17 ± 0.76	1.00 ± 0.50	0.83 ± 0.29	1.00 ± 0.00	0.67 ± 0.58
Hepatocyte hypertrophy	0.83 ± 0.29	1.50 ± 0.50	1.50 ± 0.87	1.00 ± 0.00	0.67 ± 0.58
Macrosteatosis	1.17 ± 1.04	1.17 ± 0.30	0.83 ± 0.76	0.50 ± 0.50	0.83 ± 0.76
Microsteatosis	0.50 ± 0.50	1.33 ± 0.76	1.33 ± 1.53	0.33 ± 0.58	1.33 ± 1.53
Loss of hepatocytes nuclei	0.00 ± 0.00^{b}	0.17 ± 0.29^{a}	0.17 ± 0.29^{a}	0.33 ± 0.58^{a}	0.00 ± 0.00^{b}
Spleen parameters	**Treatments**				
30 days	Control	0.25%	0.50%	0.75%	1.00%
Fibrous capsule integrity	0.00 ± 0.00^{b}	0.79 ± 0.69^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Melanomacrophage centers	1.50 ± 0.71	0.83 ± 0.47	0.50 ± 0.41	0.85 ± 0.85	0.83 ± 0.24
Melanomacrophages	0.83 ± 0.24	0.33 ± 0.47	1.00 ± 0.82	1.00 ± 0.82	1.00 ± 0.00
Eosinophilic infiltrate	0.17 ± 0.24	0.17 ± 0.14	0.33 ± 0.47	0.33 ± 0.47	0.33 ± 0.47
Lymphocyte infiltrate	0.17 ± 0.24	0.17 ± 0.14	0.17 ± 0.12	0.33 ± 0.47	0.17 ± 0.24
60 days	Control	0.25%	0.50%	0.75%	1.00%
Fibrous capsule integrity	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.70 ± 0.75^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Melanomacrophage centers	1.33 ± 0.47	0.83 ± 0.24	1.33 ± 1.25	1.17 ± 0.24	1.83 ± 0.85
Melanomacrophages	1.00 ± 0.00	0.83 ± 0.24	0.83 ± 0.85	0.67 ± 0.47	1.33 ± 0.47
Eosinophilic infiltrate	0.50 ± 0.50	0.55 ± 0.15	0.33 ± 0.47	0.17 ± 0.24	0.33 ± 0.47
Lymphocyte infiltrate	1.00 ± 0.82	0.17 ± 0.24	1.00 ± 0.12	0.17 ± 0.24	0.50 ± 0.41

Table 4 Histomorphometric evaluation (mean ± standard deviation) of intestinal tissues in Koi carp (*Cyprinus carpio*) supplemented with different levels of *Lippia Sidoides* essential oils (0.0, 0.25, 0.50, 0.75, or 1.0%) in their diet for 30 and 60 days. VC = villus length (μm), lv = villus width (μm), ncc = goblet cell. ATV = total villus area (μm)

Parameters			**Treatments**		
30 days	Control	0.25%	0.50%	0.75%	1.00%
Villi length (μm)	207.24 ± 69.90	254.59 ± 98.03	233.41 ± 57.48	233.00 ± 19.45	174.54 ± 61.83
Villi width (μm)	95.89 ± 23.63	98.00 ± 19.37	88.65 ± 12.28	99.00 ± 8.54	94.18 ± 4.98
Goblet cell	16.29 ± 8.37	19.3 ± 18.66	16.55 ± 3.47	13.56 ± 4.49	11.39 ± 6.17
Total villi area (μm)	1011213.39 ± 117507.62	748117.04 ± 283817.21	924244.80 ± 298963.96	1229649.41 ± 491044.07	991072.22 ± 459161.27
60 days	Control	0.25%	0.50%	0.75%	1.00%
Villi length (μm)	246.30 ± 30.19	220.79 ± 68.28	269.43 ± 52.31	208.87 ± 31.70	210.09 ± 40.07
Villi width (μm)	100.88 ± 17.43	94.04 ± 8.85	110.97 ± 23.87	95.51 ± 20.89	133.65 ± 16.47
Goblet cell	14.26 ± 1.71	10.87 ± 5.62	24.93 ± 16.39	14.40 ± 3.01	14.31 ± 4.76
Total villi area (μm)	1459004.79 ± 563249.74	1011638.39 ± 749840.25	1011638.39 ± 749840.25	886624.13 ± 358260.14	1395155.14 ± 728523.29

of fish, particularly in relation to the immune system [29]. Therefore, substances consumed through the diet, such as *L. sidoides* essential oil, have the potential to induce significant alterations [18]. However, the lack of significant changes in the hematological parameters of koi carp after 30 and 60 days in the present study suggests that the essential oil did not have toxic or disruptive effects on the hematological balance of the fish. These results indicate the absence of physiological stress, toxic effects, or systemic inflammatory processes, confirming the biocompatibility of the oil with carp physiology [30, 35].

However, the decrease in the absolute thrombocyte count may be interpreted as an adaptive response to a persistent stimulus, possibly the presence of the essential oil, influencing regulatory processes. Conversely, the increase in total leukocyte, lymphocyte, and neutrophil

Table 5 Hematological parameters (mean ± standard deviation) in Koi carp (*Cyprinus carpio*) supplemented with different levels of *Lippia Sidoides* essential oils (0.0, 0.25, 0.50, 0.75, or 1.0%) in their diet for 30 and 60 days

Parameters	Treatments					
30 days	Control	0.25%	0.50%	0.75%	1.00%	1.00%
Glucose (mg/dL)	21.08 ± 5.36	19.60 ± 3.19	16.12 ± 3.10	16.00 ± 1.28	16.82 ± 1.16	
Monocytes (10^3 cells/μL)	40.44 ± 2.66	31.10 ± 6.37	52.29 ± 15.15	35.35 ± 9.91	38.23 ± 6.18	
Thrombocytes (10^3 cells/μL)	1.68 ± 1.54	2.02 ± 0.79	1.28 ± 1.08	3.36 ± 3.02	2.06 ± 0.70	
Lymphocytes (10^3 cells/μL)	56.56 ± 13.53	61.23 ± 4.70	73.40 ± 20	71.27 ± 3.92	69.42 ± 22.43	
Neutrophils (10^3 cells/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Erythrocytes (10^6 cells/μL)	1.29 ± 0.15	1.32 ± 0.08	1.53 ± 0.35	1.41 ± 0.14	1.34 ± 0.19	
Total leukocytes (10^3 cells/μL)	48.57 ± 8.00	45.83 ± 4.04	62.77 ± 15.33	53.75 ± 3.49	53.82 ± 8.7	
60 days	Control	0.25%	0.50%	0.75%	1.00%	
Glucose (mg/dL)	16.00 ± 1.78	22.60 ± 2.59	15.79 ± 3.71	15.03 ± 2.91	16.85 ± 3.10	
Monocytes (10^3 cells/μL)	41.38 ± 22.25	42.38 ± 29.18	63.92 ± 10.01	50.25 ± 10.29	51.34 ± 26.47	
Thrombocytes (10^3 cells/μL)	0.54 ± 0.24	0.50 ± 0.61	1.42 ± 1.64	0.27 ± 0.27	0.26 ± 0.24	
Lymphocytes (10^3 cells/μL)	89.23 ± 60.18	96.67 ± 30.09	106.24 ± 10.90	91.78 ± 3.77	88.69 ± 17.9	
Neutrophils (10^3 cells/μL)	5.44 ± 5.48	3.22 ± 2.45	4.99 ± 2.81	2.25 ± 1.12	2.94 ± 2.76	
Erythrocytes (10^6 cells/μL)	1.30 ± 0.13	1.70 ± 0.45	2.02 ± 0.53	1.81 ± 0.24	1.57 ± 0.46	
Total leukocytes (10^3 cells/μL)	68.15 ± 43.17	74.34 ± 30.39	87.00 ± 10.12	71.42 ± 6.48	75.37 ± 26.91	

counts over time (from day 30 to day 60) indicates an immune response triggered by various factors, including the essential oil in the diet, which is beneficial for the organism's defense. This implies an immunomodulatory effect of *L. sidoides* essential oil, potentially enhancing the ability of the fish to combat infections or stressors. The maintenance of blood parameters within normal ranges may also be related to the antioxidant and immunomodulatory properties of thymol [17]. Additionally, studies with tilapia and tambaqui indicate that hematological changes are detected only after pathogen challenge, suggesting that the oils act as conditional immunomodulators [12, 30].

Conclusion

This study revealed that all concentrations of *L. sidoides* oil tested in this research can be safely incorporated into the diet of koi carp (*C. carpio*), as they did not result in any mortality over a 60-day period. Among the treated groups, the 0.25% dose resulted in improved growth. Nevertheless, it is advisable to exercise caution when using higher dosages approaching 1.0%. The use of this substance has been shown to be safe for koi carp at the recommended time and dose. Additional studies are suggested to verify the effectiveness of the essential oil against parasites and in dietary supplementation tests followed by challenge with pathogenic bacteria.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44365-025-00025-3.

Supplementary Material 1.

Acknowledgements
This study was funded in part by the Brazilian Higher Education Personnel Improvement Coordination (CAPES) - Financial Code 001. The authors thank CAPES and the National Council for Scientific and Technological Development (CNPq) M. L Martins Research Grant (CNPq 305869/2014-0, 306635/2018-6).

Authors' contributions
EMB: Experimental execution, Formal Analysis, Writing - Original Draft. MSO: Formal Analysis, Data curation, Writing - Original Draft, Manuscript review and final writing. DVVB: Investigation, Methodology. AB: Investigation, Methodology. LC: Investigation, Methodology. CFSF: Investigation, Methodology. FCMC: Investigation, Methodology. MQCS: Experimental execution, Investigation, Methodology. LSPF: Experimental execution, Investigation, Methodology. JLPM: Project Administration, Funding. MLM: Advisor, Review & Editing, Project Administration, Funding.

Funding
This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brazil (CAPES) — Finance Code 001, and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 303822/2022-8 and CNPq 301524/2017-3).

Data availability
The data related to this research are available upon prior request.

Declarations

Ethics approval and consent to participate
All animal procedures were approved by the Animal Use Ethics Committee (CEUA/UFSC 1440100217).

Competing interests
The authors declare no competing interests.

Received: 11 August 2025 / Accepted: 10 September 2025
Published online: 22 September 2025

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