

**Research Article**

Evaluation of Citronella Essential Oil as a Functional Feed Additive for Improving Growth Performance, Nutrient Utilization, Tissue Health, and Antioxidant Defense in Common Carp (*Cyprinus carpio*)

Saima Unbreen¹, Dilawar Hussain^{1,*}, Manahil Ibrahim¹, Muhammad Mudassar Shahzad²¹Department of Zoology, Faculty of Science & Technology, University of Central Punjab, Lahore-54000 Pakistan.²Department of Zoology, Division of Science & Technology, University of Education, Lahore.*Corresponding author: Dilawar Hussain (dilawar.hussain@ucp.edu.pk)
Tel.: +923007790314**Abstract**

The current study evaluated the impacts of supplementation of Citronella Essential Oil (CEO) on feed utilization, macro nutritional profile, liver histology, and detoxifying enzyme activity in common carp (*Cyprinus carpio*). Eighty healthy fingerlings were randomly assigned to four treatment groups and fed diets supplemented with different levels of CEO: 0% (G₁ Control) 0.5% (G₂), 1.0% (G₃), and 1.5% (G₄) for a period of 10 weeks. The parameters of growth performance, feed utilization, free amino acids, total lipid levels, liver histological observations, and the level of detoxifying enzymes were measured at the end of the trial. Fish fed with 1% CEO diet (G₃) not only had the best feed conversion ratio (4.77 ± 0.18), but they also exhibited the greatest weight gain (34.33 ± 1.24 g), specific growth rate ($0.716 \pm 0.021\%$ day⁻¹), feed intake (163.67 ± 1.53 g), and efficiency of feed conversion ratio (0.210 ± 0.013) compared to the non-CEO supplemented diets. In addition to the results mentioned above, fish in the CEO-supplemented groups showed significantly elevated amounts of free amino acids and total lipids relative to those in the P diet group. Histological examination revealed mild hepatic alterations in CEO-treated fish, although overall liver architecture remained preserved. In addition, there were significant increases in detoxifying enzyme activities of glutathione-S-transferase and total esterases in both supplemented groups. Based on the results, it is concluded that citronella essential oil can be used as a natural feed additive to improve growth rate, nutrient utilization, physiological health, and metabolic performance of common carp; G₃ fish with 1% CEO had the best results.

Keywords: *Cyprinus carpio*, Citronella essential oil, Feed utilization, Growth performance, Free amino acids

1. Introduction

Aquaculture has changed the world's food supply in recent years. Farmed fish and other seafood now make up a significant and growing share of the global animal protein supply and provide jobs for millions of people while playing a direct role in food and nutrition security [1]. Regular fish consumption is associated with several positive health outcomes, such as improved cardiovascular function and lower rates of malnutrition. Therefore, the provision of food from fisheries and aquaculture contributes to both 2 improved food security and enhanced population health, especially in developing nations [2]. Aquaculture and fishery sectors are crucial for providing food and jobs to people worldwide, but they also face a variety of obstacles that make them less sustainable and productive. Capture fisheries are increasingly pressured by the overexploitation of natural fish stocks, habitat degradation, climate change, and pollution [3]. Despite its importance, Aquaculture also faces many challenges to environmental sustainability and aquaculture, each

representing a major hindrance to the fisheries industry. Farmers have a limited ability to deal with pollution resulting from industrial runoff, wastewater discharge from homes, and agricultural runoff, which leads to the deterioration of their aquatic habitats, thus harming fish health and survival [4].

Pakistan's fisheries sector is benefiting from aquaculture and aquaculture has a great opportunity to enhance food security, generate jobs, and stimulate economic growth through its ability to produce more fish [5]. Inland aquaculture dominates fish production in Pakistan and is primarily practiced in freshwater systems. Carp polyculture systems are widely adopted due to their compatibility, efficient resource utilization, and relatively low production costs. These systems contribute substantially to domestic fish supply and rural livelihoods, particularly in Punjab and Sindh provinces [6].

Farmers typically employ outdated techniques and lack knowledge of sustainable feeding practices, health monitoring, and biosecurity precautions. Fish health, the environment, and

public health are all at risk from the overuse of pesticides and antibiotics, which are frequently applied unregulated. Alternative approaches, such as creating natural feed additives and putting improved fish health management techniques into practice, are desperately needed [7]. The common carp (*Cyprinus carpio*) belongs to the family Cyprinidae and is the most frequently grown freshwater fish species because of its rapid development, adaptability, and economic significance [8].

Common carp is one of the top contributors to aquaculture production worldwide, providing almost 7.7% of total freshwater aquaculture production from around the globe. The common carp's capability to inhabit a variety of environments due to its different subspecies and wide geographical range means that the species can be advantageous when studying the impact of different environmental stress factors. The difference in the differences associated with various types of carps will assist in learning about the genetic as well as phenotypic differences between the various subspecies of carp that would subsequently guide the endeavor in studying environmental stress [9]. Functional feed additives are bioactive substances added to aquafeed to improve the nutrition, well-being, and output of cultured animals. By boosting immunity, these compounds increase disease resistance and decrease the need for antibiotics, supporting ecologically friendly practices. They also help sustainable aquaculture development, enhance overall productivity, and serve environmental and global food security objectives [10].

Essential oils (EOs) are naturally occurring bioactive substances with antibacterial, antioxidant, anti-inflammatory, and immunostimulant qualities that are extracted from medicinal plants and herbs. They have drawn a lot of interest as natural feed additives that can enhance fish growth, health, and overall aquaculture productivity because of these advantageous qualities [11].

Citronella (*Cymbopogon nardus*) is a medicinal plant whose essential oil is primarily composed of citronellal, citronellol, and geraniol, compounds known for their antiseptic, bactericidal, and insect-repellent properties [12].

Since CEO has been shown to improve growth performance (including average daily gain), feed utilization, and disease resistance, aquaculture has begun getting attention for using CEO as a natural feed additive [13].

In addition to providing nutritional benefits, CEO can also stimulate physiological responses, such as an increase in muscle relaxation, modulation of swimming behaviour, and a decrease in cortisol levels, suggesting that CEO have some potential for reducing stress [14]. Most importantly, the CEO has a non-toxic mode of action, and in vitro toxicity assessments suggest (including Microtox testing) limited risk to aquatic life, as well as the surrounding ecosystem. This makes the CEO a viable, environmentally safe alternative to antibiotics in sustainable aquaculture while supporting cultured fish health, production level and welfare [15]. Citronellal, the main compound in citronella (*Cymbopogon nardus*) essential oils, has many bioactive properties beneficial for aquaculture. It has powerful antioxidant properties that help reduce oxidative stress in fish and promote cellular health [16]. Additionally, citronellal supports enhanced immune response in fish, increased feed efficiency and growth, and improved disease resistance in cultivated fish. All of these benefits demonstrate that citronella is a natural feed additive that is safe and environmentally friendly and serves as a sustainable substitute for antibiotics and other artificial growth promoters in aquaculture [6].

2. Materials and Methods

2.1. Experimental fish and study site

Healthy common carp (*Cyprinus carpio*) were sourced from the Manawan Fish Hatchery in Lahore, Pakistan (Figure 1). The fish were transferred in polyethylene bags filled with oxygen to ensure minimum stress during the transportation process. The fish that arrived were checked visually to select healthy and disease-free fish for use in the study. Before the feeding trial, fish were acclimatized to the laboratory conditions in aerated aquaria. During the acclimatization period, fish were fed a basal diet twice a day, and 20% of the aquaria water was changed daily.

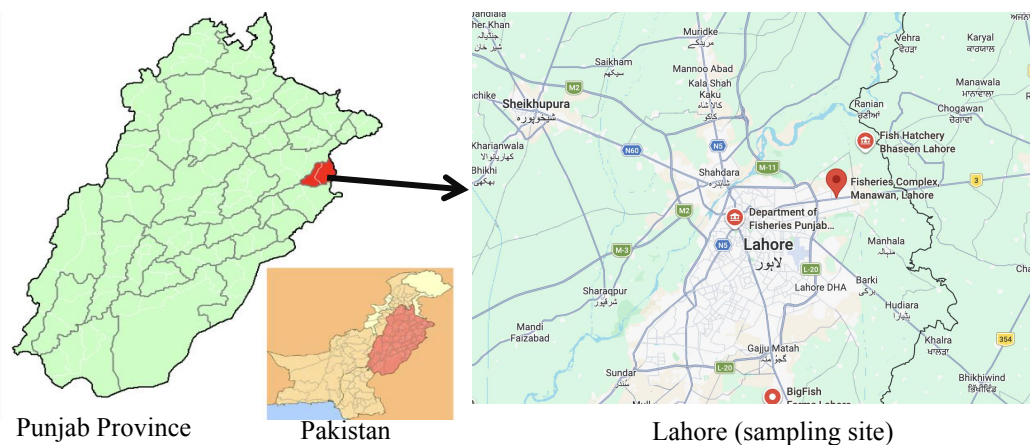


Figure 1. Fish sampling site map.

2.2. Experimental design

In the current study, a completely randomized design (CRD) was utilized. The experimental procedure involved 80 healthy common carp that were similar in size and distributed randomly into four treatment groups with 20 fish per group. The control group (G1) received the basal diet containing no citronella essential oil. The treatment groups G2, G3, and G4 received diets containing 0.5, 1.0, and 1.5 mL citronella essential oil/kg feed, respectively, in addition to the basal diet. The feeding trial lasted for 60 days in a controlled laboratory environment with continuous aeration.

2.3. Diet formulation and preparation

Experimental diets were developed with the inclusion of menhaden meal, soybean meal, dextrinized starch, vitamin premix, mineral premix, carboxymethyl cellulose (CMC), soybean oil, dibasic calcium phosphate (CaPO_4), glycine, and DL-methionine. The control diet (G1) had no citronella essential oil (CEO), while diets G2, G3, and G4 had 0.5, 1.0, and 1.5 mL CEO/kg feed in the diets. The dry ingredients were precisely measured and blended well. Soybean oil was carefully added, and in case of supplemented diets, CEO was mixed previously with soybean oil before incorporation in the mixes. Distilled water was added based on need to achieve dough-like consistency, and this was converted into pellets by using a pelleting machine.

2.4. Feeding and husbandry management

For 60 days, fish were fed the experimental diets twice a day (at 9:00 and 16:00) at a rate of 3% of their body weight. Throughout the experiment, standard husbandry procedures were followed, including as constant aeration, daily removal of feces and uneaten feed, and replacement of about 20% of the aquarium water with fresh dechlorinated water.

2.5. Growth performance and feed utilization

Growth performance was evaluated by recording the body weight of fish at the beginning and end of the 60-day feeding trial. Before weighing, fish were fasted for 24 h to ensure complete evacuation of the gastrointestinal tract. Body weight was measured using a digital electronic balance after gently blotting excess water from the fish. Growth performance parameters, including weight gain (WG) and specific growth rate (SGR), were calculated using Equations (1) and (2), respectively. Feed utilization was assessed by determining feed intake (FI), feed conversion ratio (FCR), and feed efficiency ratio (FER) using Equations (3), (4), and (5), respectively.

$$\text{WG (g)} = \text{Final Weight} - \text{Initial Weight} \quad (1)$$

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\ln(W_f) - \ln(W_i)}{T} \times 100 \quad (2)$$

Where W_f = final weight, W_i = initial weight, and T = experimental duration (days).

$$\text{FI (g)} = \text{Total Feed Offered} - \text{Uneaten Feed} \quad (3)$$

$$\text{FCR} = \text{Feed Intake} / \text{Weight Gain} \quad (4)$$

$$\text{FER} = \text{Weight gain (g)} / \text{Feed intake (g)} \quad (5)$$

2.6. Sample collection

Fish from each treatment group were chosen at random for biochemical and histological examinations at the conclusion of the feeding trial. While muscle samples were taken to measure the amounts of free amino acids and total lipids, liver tissues were carefully removed for histological analysis and tissue homogenates. Before undergoing additional laboratory analysis, all gathered materials were properly labeled and stored.

2.7. Determination of free amino acids

The ninhydrin method was used to evaluate the free amino acid (FAA) content in muscle tissue spectrophotometrically. The normal procedure was followed to create the reaction mixture and homogenize the muscle samples. Following incubation, absorbance at 570 nm was measured with a UV-visible spectrophotometer, and the concentration of FAA was determined using a standard curve made with recognized amino acid standards. The results were given in mg g^{-1} tissue.

2.8. Determination of total lipids

The total lipid content of muscle tissue was measured using the Folch extraction method with a chloroform:methanol (2:1, v/v) solvent mixture. From homogenized muscle tissue, lipids were isolated, separated from non-lipid components, and measured gravimetrically. The proportion of tissue weight was used to express the total lipid content.

2.9. Histological examination of liver

Liver tissues were treated using conventional histological methods after being promptly fixed in 10% neutral buffered formalin. After fixation, tissues were cleaned in xylene, embedded in paraffin wax, and dehydrated using a graded ethanol series. A rotary microtome was used to create sections of the proper thickness, which were then mounted on glass slides and stained with hematoxylin and eosin (H&E). Under a light microscope, histological observations were made, and representative photomicrographs were taken so that treatment groups could be compared.

2.10. Preparation of liver homogenates

Using a tissue homogenizer the liver tissues were homogenized in buffer under cooled conditions. After that the homogenates were centrifuged to yield clear supernatants, which were then used in the study of detoxifying enzymes. The homogenates were prepared under cold conditions to secure the enzyme activity.

2.11. Determination of Glutathione-S-Transferase (GST) activity

Glutathione-S-transferase (GST) activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate and the usual spectrophotometric technique. Reduced glutathione, CDNB, buffer solution, and tissue homogenate were all included in the reaction mixture. Spectrophotometric monitoring of absorbance changes was done, and the normal

approach was followed to calculate GST activity.

2.12. Determination of total esterase activity

The conventional colorimetric approach was used to evaluate total esterase activity. Enzyme activity was measured spectrophotometrically using changes in absorbance after liver homogenates were incubated under controlled circumstances with the proper substrate. The suggested procedure was followed in calculating the enzyme activity.

2.13. Statistical analysis

The mean $\pm$ standard deviation (SD) was used to express all experimental results. SPSS software was used to conduct

statistical analysis. One-way analysis of variance (ANOVA) was used to assess treatment group differences, and Tukey's multiple comparison test was used to find significant mean differences. At $P < 0.05$, differences were deemed statistically significant.

3. Results

The effects of dietary citronella essential oil (CEO) supplementation on growth performance, feed utilization, muscle biochemical composition, detoxifying enzyme activities, and liver histology in common carp (*Cyprinus carpio*) were evaluated after the feeding trial (Table 1)

Table 1. Growth performance of common carp treated with CEO for 10 weeks.

Parameters	G1 (Control)	G2 (0.5% CEO)	G3 (1% CEO)	G4 (1.5% CEO)
Weight gain (g)	27.04 $\pm$ 0.98 ^b	28.11 $\pm$ 1.02 ^b	34.33 $\pm$ 1.24 ^a	31.82 $\pm$ 1.52 ^a
Feed intake (g)	146.11 $\pm$ 2.14 ^c	153.70 $\pm$ 1.98 ^b	163.67 $\pm$ 1.53 ^a	155.33 $\pm$ 3.09 ^b
FCR	5.41 $\pm$ 0.31 ^{ab}	5.47 $\pm$ 0.22 ^a	4.77 $\pm$ 0.18 ^c	4.91 $\pm$ 0.09 ^{bc}
FER	0.183 $\pm$ 0.009 ^b	0.185 $\pm$ 0.005 ^b	0.210 $\pm$ 0.013 ^a	0.205 $\pm$ 0.011 ^a
SGR	0.34 $\pm$ 0.011 ^c	0.576 $\pm$ 0.019 ^b	0.716 $\pm$ 0.021 ^a	0.683 $\pm$ 0.031 ^a

Note: Values are expressed as mean $\pm$ SD. Different superscript letters within the same row indicate statistically significant differences among treatments ($p < 0.05$).

Table 2. Free amino acids (mg) and Total lipids (%) $\pm$ SD of CEO supplemented diets

Parameters	G1 (Control)	G2 (0.5% CEO)	G3 (1% CEO)	G4 (1.5% CEO)
Free Amino Acids (mg)	5.77 $\pm$ 0.18 ^d	6.37 $\pm$ 0.11 ^c	7.63 $\pm$ 0.25 ^a	6.742 $\pm$ 0.13 ^b
Total Lipids (%)	4.52 $\pm$ 0.16 ^c	5.15 $\pm$ 0.09 ^b	5.93 $\pm$ 0.21 ^a	5.83 $\pm$ 0.12 ^a

Note: Values are expressed as mean $\pm$ SD. Different superscript letters within the same row indicate statistically significant differences among treatments ($p < 0.05$).

Table 3. Effect of dietary citronella essential oil (CEO) supplementation on the GST and total Esterase activity of common carp.

Parameters	G1 (Control)	G2 (0.5% CEO)	G3 (1% CEO)	G4 (1.5% CEO)
GST ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$)	7.2 $\pm$ 0.5 ^d	10.1 $\pm$ 0.7 ^c	15.6 $\pm$ 1.0 ^a	13.8 $\pm$ 0.9 ^b
Total Esterase (U mg^{-1} Protein)	12.8 $\pm$ 0.8 ^d	15.7 $\pm$ 1.0 ^c	21.5 $\pm$ 1.4 ^a	18.9 $\pm$ 1.2 ^b

Note: Values are expressed as mean $\pm$ SD. Different superscript letters within the same row indicate statistically significant differences among treatments ($p < 0.05$).

Table 1 depicts the growth performance and feed consumption that differed between dietary interventions. G3 gained the most weight (1% CEO; 34.33 $\pm$ 1.24 g), followed by G4 (1.5% CEO; 31.82 $\pm$ 1.52 g), G2 (0.5% CEO; 28.11 $\pm$ 1.02 g), and G1 (control; 27.04 $\pm$ 0.98 g). There was no significant difference between G1 and G2 or between G3 and G4, although G3 and G4 gained considerably more weight than G1 and G2 ($p < 0.05$). The G3 group had the highest feed intake (163.67 $\pm$ 1.53 g), while the control group had the lowest (146.11 $\pm$ 2.14 g). While G2 (153.70 $\pm$ 1.98 g) and G4 (155.33 $\pm$ 3.09 g) did not substantially differ from one another, G3 differed considerably from the other treatments. G3 had the lowest feed conversion ratio (FCR) (4.77 $\pm$ 0.18), whereas G2 had the highest (5.47 $\pm$ 0.22). These findings indicate that

the 1% CEO treatment produced the highest growth and feed utilization values among the different groups

3.1. Free amino acids and total lipids

Table 2 depicts the effects of adding citronella essential oil to the meal on the biochemical makeup of the muscles of the common carp, as determined by the amount of free amino acids and total lipids. G3 (1% CEO; 7.63 $\pm$ 0.25 mg) had the highest free amino acid content, followed by G4 (1.5% CEO; 6.742 $\pm$ 0.13 mg) and G2 (0.5% CEO; 6.37 $\pm$ 0.11 mg). The control group (G1; 5.77 $\pm$ 0.18 mg) had the lowest value. G3 had the highest total lipid content (5.93 $\pm$ 0.21%), followed by G4 (5.83 $\pm$ 0.12%), G2 (5.15 $\pm$ 0.09%), and G1 (4.52 $\pm$

0.16%). Overall, the highest numerical values for both parameters were observed in fish receiving 1% CEO.

3.2. Detoxifying Enzymes

Glutathione-S-transferase and total esterase activities were measured to assess the impact of dietary citronella essential oil supplementation on the detoxifying enzyme activities of common carp. The responses of both enzymes to varying doses of dietary CEO supplementation are displayed in Table 3. GST activity was lowest in G1 ($7.2 \pm 0.5 \mu\text{mol min}^{-1} \text{mg}^{-1}$ protein) and increased to 10.1 ± 0.7 in G2, reaching the highest value in G3 (15.6 ± 1.0). G4 showed an intermediate value of 13.8 ± 0.9 . Total esterase activity showed a similar trend. The lowest value in this case was in G1 ($12.8 \pm 0.8 \text{ U mg}^{-1}$ protein) followed by G2 ($15.7 \pm 1.0 \text{ U mg}^{-1}$ protein). It was noted that both enzymes were at the highest concentration at 1% CEO treatment level and lower at 1.5% CEO level.

3.3. Liver Histology

The histological effects of dietary citronella essential oil (CEO) supplementation on the liver of common carp were

assessed by microscopic inspection after hematoxylin and eosin (H&E) staining. Table 4 summarizes the histological observations for each treatment group, and further figures display example photomicrographs.

Histological analysis showed that the experimental groups' liver architecture changed in a treatment-dependent manner. The hepatic architecture of the control group (G1) was normal, with intact sinusoids and portal tracts, minimal fatty infiltration, and no signs of necrosis or hepatocellular vacuolation. Chronic inflammatory cell infiltration, fatty degeneration, hepatic vacuolation, sinusoidal dilatation, vascular congestion, and localized necrosis were observed in fish given the meal supplemented with 0.5% CEO (G2). The liver in the 1% CEO group (G3) showed no necrosis but had intact lobular architecture with modest inflammatory infiltration, fatty degeneration, hepatocellular vacuolation, sinusoidal dilatation, and vascular congestion. Fish given 1.5% CEO (G4) showed hepatic vacuolation, sinusoidal dilatation, vascular congestion, mild-to-moderate inflammatory infiltration, fatty degeneration, and localized ceroid pigment deposition.

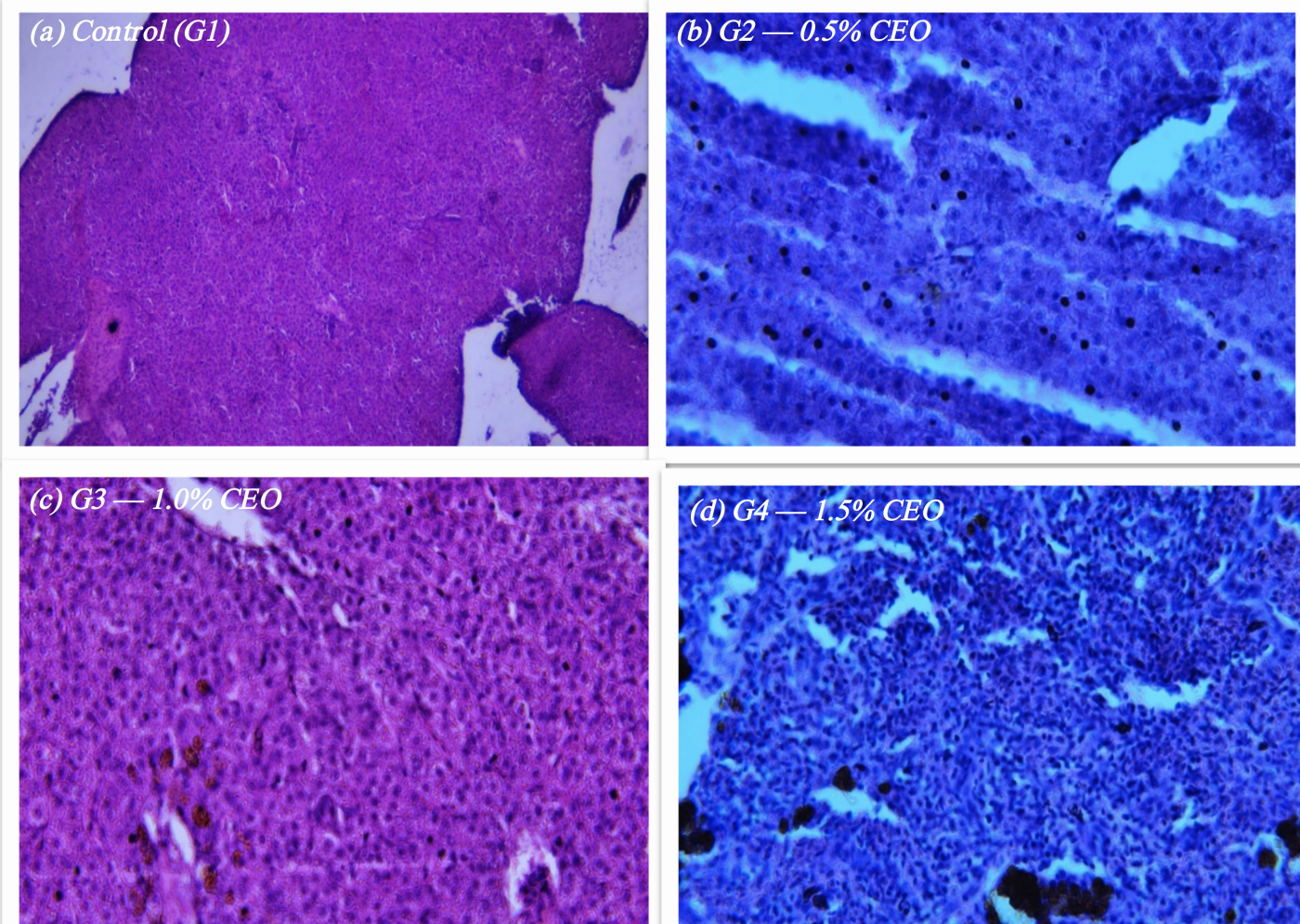


Figure 2. Liver histology of common carp (*Cyprinus carpio*) under different dietary citronella essential oil (CEO) treatments: (a) control (G1); (b) 0.5% CEO (G2); (c) 1.0% CEO (G3); and (d) 1.5% CEO (G4).

Table 4. Liver histological observations of common carp fed diets supplemented with different levels of citronella essential oil (CEO)

Treatment Groups	Main Histological Observations	Overall Interpretations	
G1 (Control)	Lobular architecture is preserved; portal tracts are intact; mild fatty infiltration is present; necrosis and vacuolation are absent; sinusoids are intact; and dysplastic cells are not present.	Normal liver histology	
G2 (0.5% CEO)	Maintained morphology of liver; chronic low-grade inflammatory infiltrate; fatty degeneration & vacuolation of hepatocytes; dilated sinusoids & vascular congestion; focal necrosis noted.	Mild inflammation	hepatic
G3 (1% CEO)	Intact lobular architecture with mild chronic inflammatory infiltration; fatty degeneration and vacuolation of hepatocytes; dilated sinusoids and vascular congestion; no necrosis detected.	Mild inflammation	hepatic
G4 (1.5% CEO)	Intact hepatic architecture; mild-to-moderate inflammatory infiltration; fatty degeneration and vacuolation of hepatocytes; sinusoidal dilation and vascular congestion; focal accumulation of ceroid pigment.	Moderate inflammation with ceroid pigment accumulation	hepatic

The histological appearance of liver tissues in common carp after dietary treatment with varied amounts of citronella essential oil (CEO). (A) Normal hepatic architecture in the control group; (B) inflammatory cell infiltration, hepatocellular vacuolation, vascular congestion, and focal necrosis in the 0.5% CEO group; (C) mild inflammatory infiltration in the 1.0% CEO group; and (D) hepatocellular vacuolation, sinusoidal dilatation, vascular congestion, $\times 100$ or $\times 400$ H&E stain) (Figure 2).

4. Discussions

Plant-sourced feed additives are gaining popularity in aquaculture for their potential to enhance fish growth and health while minimizing dependence on artificial growth agents [17]. The present study demonstrated that dietary supplementation with citronella essential oil (CEO) significantly influenced growth performance, biochemical composition, and histological characteristics of carp. Researchers found, in general, that fish raised on a CEO diet outperformed control fish. The amount of CEO present in the diet did, however, influence the degree of difference between groups.

One of the most significant measures of dietary feed additive efficacy evaluation in aquaculture is growth performance. The study in question indicated that the use of citronella essential oil (CEO) in fish feed improved growth performance of common carp. Fish that were fed the diet containing 1% CEO (G3) showed the best results in terms of weight gain, SGR, feed intake, and FER as compared to the other test diets. In other research, it was noted that the incorporation of essential oils in dietary feed improves growth performance of fish due to increased nutrient utilization and digestive efficiency [18]. In contrast, another study provided evidence that plant-derived essential oils contribute to gut health improvement, thus enhancing nutrient absorption as well as efficiency of feed utilization [19].

Feed intake and feed conversion ratio (FCR) also significantly varied with the addition of CEO to the diet. Diets with 1% CEO gave the highest level of feed intake and the lowest FCR. This indicates better utilization of nutrients by

fish. The increased feed intake can be due to the presence of the aromatic compounds of citronella essential oil, which enhance palatability and promote feed intake [20]. Similarly, phytochemical additives have been reported to improve digestibility and feed conversion in fish. Fish given a diet containing 1.5% CEO had better performance in comparison to the control group, but no additional benefit was detected on any other parameter, hence indicating that moderate inclusion levels of CEO in the diet were more effective [18, 21].

The observed improvement in the growth performance of fish receiving the supplement can be confirmed by the biochemical findings. The levels of free amino acids, which are important for protein synthesis, tissue development, and metabolic regulation, increased significantly in fish fed fish oil, particularly in the group receiving 1% of the supplement. This increase in the availability of these important compounds may be due to better digestion, absorption of nutrients, and protein metabolism in those animals that consumed fish oil.

The biochemical composition of muscle is one of the key indicators of nutrient utilization and physiological status in cultured fish. In this study, the use of citronella essential oil (CEO) as a dietary supplement led to increased amounts of free amino acids and lipids in common carp. In particular, the largest values of these indices were observed in fish on diet G3, which included 1% of CEO. Numerous studies have shown that the addition of essential oils to fish feed results in better functionality of digestive enzymes and improved absorption of feed and nutrients. In another study, the improvement in digestive processes were reported to enhance leads absorption of amino acids and their availability in the body, which explains the results of diet G3 [22].

It has been observed that total lipid content in fish increases significantly with the use of CEO supplements, as the levels of lipids were found to be higher in CEO-treated fish than in the control group. Lipids are vital substrates in the body as they provide energy as well as perform many functions important for food growth, metabolism, and cellular processes. Therefore, it can also be deduced that the increased deposition of lipids in the body of fish in the CEO group proves better assimilation of nutrients and use of energy. The findings were consistent with previous research showing that feeding Nile

tilapia with essential oil can improve growth performance and stimulate metabolic processes. Through the findings of the current research, a good relationship between CEO supplementation, growth parameters, free amino acids and lipid content has been established and this advocates the positive impact of CEO supplementation on the performance of fish [23].

The dietary supplementation of citronella essential oil (CEO) positively influenced the detoxifying enzyme activities of glutathione-S-transferase (GST) and total esterase in common carp, with the peak enzyme activities attained in fish receiving 1% CEO supplementation (G3). However, although GST and total esterase activities at 1.5% CEO were greater than in the control group, they were lower than in G3, indicating that the use of 1% CEO was effective in stimulating hepatic detoxification. GST is essential in protecting cells from oxidative stress via glutathione conjugation, whereas total esterase contributes to the detoxification of both endogenous and exogenous compounds. The results of the present study were consistent with previous findings indicating that the incorporation of essential oils improves the antioxidant capacity of the fish in a dose-dependent fashion, and previous research has also reported the stimulation of the detoxifying enzyme activity and antioxidant protection in silver catfish via supplementation with *Lippia alba* essential oil. The increased enzymatic activities were also confirmed by the histological data, which showed that fish receiving 1% CEO experienced the least degree of alteration [18, 21].

Results from histopathological examination of the liver confirmed additional evidence for the physiological effect of dietary supplementation with citronella essential oil (CEO). The control group showed normal liver architecture with normal hepatocytes, sinusoidal structure and no signs of necrosis or inflammation. Fish from the 0.5% CEO group showed mild inflammatory cell infiltration, fatty liver degeneration, hepatocellular vacuolation, sinusoidal dilation, vascular congestion and focal necrosis. The liver architecture was preserved. Fish from the 1% group showed mild to moderate inflammatory changes without any signs of necrosis with preserved lobular architecture, while showing good growth performance and biochemical response. The 1.5% CEO group showed more severity compared to 1% CEO group which indicates that going beyond 1% CEO does not give any further physiological benefit. This study is in agreement with other studies showing that the use of phytochemical additives at low doses can result in minor histological changes without having adverse effects in fish health.

5. Conclusion

The present study was conducted to evaluate the effects of citronella essential oil (CEO) as a dietary supplement to determine how this essential oil affects fish growth performance, biochemistry and liver site histology in fish. The results showed that the inclusion of essential oil in the diet had a positive impact on both growth and the use of feed as compared to the results of the control groups; however, the response depended upon the level of inclusion.

Among the experimental groups, fish fed the diet containing 1% CEO (G3) showed the best overall performance. This group recorded the highest weight gain, feed intake, feed efficiency ratio (FER), specific growth rate (SGR), free amino acid content, and total lipid content, along with the lowest feed conversion ratio (FCR). These results indicate that moderate CEO supplementation improved nutrient utilization and promoted growth.

The findings from biochemical analysis were in agreement with the growth results, namely that fish with CEO-supplied diets had a significantly higher concentration of free amino acids and lipids than the controls. These increases in nutrient consumption suggest that the fish have a greater capacity to absorb nutrients and the energy they provide. These findings suggest that CEO supplementation could be a useful feed additive in aquaculture. However, more research is needed to determine its safety and define the optimal inclusion level for practical application.

Author contributions

Saima Unbreen: Investigation, Data Curation, Formal Analysis, Writing – Original Draft. Dilawar Hussain: Supervision, Writing – Review & Editing. Manahil Ibrahim: Formal Analysis, Writing – Review & Editing. Muhammad Mudassar Shahzad: Formal Analysis, Writing – Review & Editing. All authors have read and approved the final manuscript for publication.

Ethical approval

The experimental procedures involving common carp (*Cyprinus carpio*) were conducted after obtaining approval from the Ethical Committee of the University of Central Punjab (UCP). All procedures were carried out in accordance with the relevant institutional guidelines for the care and handling of experimental fish.

Conflicts of Interest

The authors report no conflicts of interest.

Acknowledgment

The authors gratefully acknowledge Muhammad Shafqat and Laiba Sajid for their valuable support and technical assistance throughout the study.

Funding

Not applicable (N/A).

Data availability statement

The data will be made available upon reasonable request to the corresponding author.

REFERENCES

1. Boyd, Claude E., Aaron A. McNevin, and Robert P. Davis. The contribution of fisheries and aquaculture to the global protein supply: Aquaculture and global protein. Food security, 2022. 14(3), 805-827. <https://doi.org/10.1007/s12571-021-01246-9>

2. Chen, L., Xu, J., Sun, X., & Xu, P. Research advances and future perspectives of genomics and genetic improvement in allotetraploid common carp. *Reviews in Aquaculture*, 2022. 14(2), 957-978. <https://doi.org/10.1111/raq.12636>
3. Gul, S., Shafiq, U., Mir, S. A., Iqbal, G., & Lone, H. Q. Enhancing global food security through sustainable fisheries and aquaculture: A comprehensive review. *Asian Journal of Agricultural Extension, Economics & Sociology*, 2024. 42(10), 60-70. <https://doi.org/10.9734/ajaees/2024/v42i102563>
4. Blanchard, J. L., Watson, R. A., Fulton, E. A., Cottrell, R. S., Nash, K. L., Bryndum-Buchholz, A., & Jennings, S. Linked sustainability challenges and trade-offs among fisheries, aquaculture and agriculture. *Nature ecology & evolution*, 2017. 1(9), 1240-1249. <https://doi.org/10.1038/s41559-017-0258-8>
5. Food and Agriculture Organization of the United Nations (FAO). Fisheries and aquaculture country profile: Pakistan. FAO, 2024.
6. Rokonzaman, M., Bhuia, M. S., Al-Qaaneh, A. M., El-Nashar, H. A., Islam, T., Chowdhury, R., & Torequl Islam, M. Biomedical perspectives of citronella: Biological activities, toxicological profile and molecular mechanisms. *Chemistry & Biodiversity*, 2025. 22(1), e202401973. <https://doi.org/10.1002/cbdv.202401973>
7. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. Antimicrobial resistance: A growing serious threat for global public health. In *Healthcare*, 2023. 11(13), 1946.
8. Yaqoob, S. A review of structure, origin, purpose & impact of common carp (*Cyprinus carpio*) in India. *Annals of the Romanian Society for Cell Biology*, 2021. 25(6), 34-47.
9. Wang, M.; Li, X.; Wang, C.; Zou, M.; Yang, J.; Li, X.D.; Guo, B. Asymmetric and parallel subgenome selection co-shape common carp domestication. *BMC Biology*, 2024. 22(1), 4. <https://doi.org/10.1186/s12915-023-01806-9>
10. Abeysinghe, K. C., Weeraratne, A. G. P. T., Colonne, D. K. C., & Bellanthudawa, B. K. A. Sustainable development goals (SDGs), climate change, and the development of aquaculture and fisheries industries. *Environmental Reviews*, 2025. 33, 1-24. <https://doi.org/10.1139/er-2024-0136>
11. Aydın, B., & Barbas, L.A.L. Sedative and anesthetic properties of essential oils and their active compounds in fish: A review. *Aquaculture* 2020, 520, 734999. <https://doi.org/10.1016/j.aquaculture.2020.734999>
12. Mahmud, F., Mahedi, M. R. A., Afrin, S., Haque, R., Hasan, M. S., Sum, F. A., & Kuri, O. C. Biological & insecticidal effect of citronella oil: A short review. *Clinical Medicine and Health Research Journal*, 2022. 2(6), 261-265. <https://doi.org/10.18535/cmhrj.v2i6.108>
13. Yilmaz, S., Ergün, S., Yiğit, M., & Yilmaz, E. An extensive review on the use of feed additives against fish diseases and improvement of health status of fish in Turkish aquaculture sector. *Aquaculture Studies*, 2022. 22(3). <https://doi.org/10.4194/AQUAST710>
14. Al-Sagheer, A. A., Mahmoud, H. K., Reda, F. M., Mahgoub, S. A., & Ayyat, M. S. Supplementation of diets for *Oreochromis niloticus* with essential oil extracts from lemongrass (*Cymbopogon citratus*) and geranium (*Pelargonium graveolens*) and effects on growth, intestinal microbiota, antioxidant and immune activities. *Aquaculture Nutrition*. 2018. 24(3), 1006-1014. <https://doi.org/10.1111/anu.12637>
15. Barbas, L.A.L.; Hamoy, M.; de Mello, V.J.; Barbosa, R.P.M.; de Lima, H.S.T.; Torres, M.F.; Nascimento, L.A.S.D.; da Silva, J.K.D.R.; Andrade, E.H.D.A.; Gomes, M.R.F. Essential oil of citronella modulates electrophysiological responses in tambaqui *Colossoma macropomum*: A new anaesthetic for use in fish. *Aquaculture* 2017, 479, 60-68. <https://doi.org/10.1016/j.aquaculture.2017.05.027>
16. Misriani, A., & Nur, I. Immunostimulatory potential of dietary citronella (*Cymbopogon nardus*) powder combined with probiotics in Nile tilapia infected with *Aeromonas hydrophila*. *Journal of Fish Health*, 2026. 6(1), 24-34. <https://doi.org/10.29303/jfh.v6i1.9444>
17. Boukid, F., Kumari, S., & Khan, Z. S. (2023). Plant Protein-Based Foods, Trend from a Business Perspective: Market, Consumers' Challenges, and Opportunities in Future. *Novel plant protein processing*, 267-282. <https://doi.org/10.1201/9781003369790-14>
18. Dawood, M. A., El Basuini, M. F., Yilmaz, S., Abdel-Latif, H. M., Alagawany, M., Kari, Z. A., & Van Doan, H. Exploring the roles of dietary herbal essential oils in aquaculture: A review. *Animals*, 2022. 12(7), 823. <https://doi.org/10.3390/ani12070823>
19. Öz, M., Üstüner, E., & Çifci, S. Medicinal and aromatic plant oils in aquafeeds: mechanistic perspectives on growth promotion, immunomodulation, and stress resilience. *Aquaculture Nutrition*, 2026. 2026(1), 8992384. <https://doi.org/10.1155/anu/8992384>
20. Sokol, M., Gulayev, I., Chirkina, M., Klimenko, M., Kamaeva, O., Yabbarov, N., & Nikolskaya, E. Fully green particles loaded with essential oils as phytobiotics: A review on preparation and application in animal feed. *Antibiotics*, 2025. 14(8), 803. <https://doi.org/10.3390/antibiotics14080803>
21. Inyang-Etoh, A. P., Ifon, H. T., Eteng, S. U., Ambo, A. A., Edem, B. E., & Ekanem, A. Comparative effects of phytogenic feed additives on growth and feed efficiency in Nile Tilapia. *Global journal of pure & applied sciences*, 2026. 32(3), 333-342. <https://doi.org/10.4314/gjpas.v32i3.2>
22. Faheem, M., Abbas, R. Z., Liaqat, I., Hoseinifar, S. H., Maneepitaksanti, W., & Van Doan, H. Bio-active components in medicinal plants: A mechanistic review of their effects on fish growth and physiological parameters: A review. *Annals of Animal Science*, 2022. 22(4), 1127-1149. <https://doi.org/10.2478/aoas-2022-0030>
23. Raslan, W. S., Shehab, A., Matter, A. F., Youssuf, H. A., Farid, O. A., Sabek, A., & Kadah, A. Impact of essential oil and probiotics supplementation on growth performance, serum biomarkers, antioxidants status, bioenergetics and histomorphometry of intestine of Nile tilapia fingerlings challenged with *Aeromonas veronii*. *BMC Veterinary research*, 2025. 21(1), 6. <https://doi.org/10.1186/s12917-024-04433-w>



Copyright © 2026 by the author(s).

Published by Science Research Publishers.

This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial-NoDerivatives (CC BY-NC-ND 4.0) License Visit <https://creativecommons.org/licenses/by-nc-nd/4.0/>

How to cite this article: Saima Unbreen, Hussain, D., Ibrahim, M., & Muhammad Mudassar Shahzad. (2026). Evaluation of Citronella Essential Oil as a Functional Feed Additive for Improving Growth Performance, Nutrient Utilization, Tissue Health, and Antioxidant Defense in Common Carp (*Cyprinus carpio*). *Journal of Zoology and Systematics*, 4(2), 12-19.