ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2025, 10 (1): 352-362 doi: 10.28978/nesciences.1648743

Examining the Liver Metabolic Alterations Induced by Olive Leaf Compounds in Aquatic Species

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Abstract

Olea europaea L., frequently recognized for its traditional medicinal uses, contains bioactive compounds with antioxidant, anti-inflammatory, and metabolic regulatory properties. While these compounds have been widely investigated for their properties on human well-being, their consequences on aquatic species remain less discovered. The aim is to assess the effects of seven olive leaf metabolites caffeic acid, oleuropein, corosolic acid, moronic acid, lupeol, cycloartenol, and betulinic acid on liver metabolism in Nile tilapia (Oreochromis niloticus). The metabolites were incorporated into the fish food at three absorptions: 1g/100g, 3g/100g, and 5g/100g feed, and the fish were fed twice daily for 96 hours. Biochemical examination of fish serum revealed significantly improved Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and glucose levels associated with the control group. Particularly, corosolic acid and moronic acid encouraged the greatest noticeable development in liver enzyme activities, signifying potential disruptions in liver metabolism and function. These consequences indicate that bioactive compounds in olive leaves can influence liver physiology in aquatic species, emphasizing their potential effect on fish health. The research delivers valuable insights into the metabolic effects of these complexes, with implications for aquaculture nutrition approaches. Further investigation are essential to assess their long-term security and effectiveness.

Keywords:

Olive leaf metabolites, bioactive compounds, enzyme activities, nile tilapia, liver physiology, aquaculture.

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Article history:

Received: 16/11/2024, Revised: 17/01/2025, Accepted: 08/02/2025, Available online: 31/03/2025

Introduction

Ecological research to use freshwater fishes to regulate how varied conditions in the air afflict metabolism and health. Vital implication in maintaining natural equilibrium and sustainable production of nutrition (Assar et al., 2023). Altered constraints, such as knowledge of bioactive compounds, nutrition, and environmental pressure, can influence such metabolic alterations in these fishes. The likely compensations of plant materials like olive leaf extract have caused much concern (Liang et al., 2022). Oleuropein, hydroxytyrosol, and verbascoside are approximately several polyphenolic chemicals currently in olive leaves that were found to exhibit anti-inflammatory, anti-cancer, and antioxidant activities (Liu et al., 2022). Oleuropein and hydroxytyrosol are the two main elements extant in olive leaves, are well known to be the greatest actual antioxidants. A measure of examination has shown the capability of such mixtures to improve lipid metabolism, moderate oxidative pressure, and affect inflammation pathways (Ferreira et al., 2023). The liver of fish is a well-intentioned place for the metabolic modifications produced by mixtures in olive leaves given such organs contribute to metabolizing nutrients, xenobiotics with the atmosphere, and metabolic wastelands (Conte et al., 2021). The livers of extra fish supply essential energy homeostasis and qualify metabolic versions to variations in the environment, such as water infection fluctuations, poisons, and food feeding. Components found in olive leaves are capable of impacting liver functions, such as lipid metabolism, glucose level regulation, and detoxification (Talukdar & Ghosh, 2025). Evaluating the worth of these bioactive compounds as natural aquaculture agents and their larger ecological significance must recognize how compounds affect fish organisms' liver metabolism (Hazreen-Nita et al., 2022). Considering metabolic alterations in fish livers, it is limited to species, does not address the effects over time, and does not consider environmental stressors sufficiently (Monsef Shokri et al., 2021). Changes in compound concentration and exposure duration can influence the applicability of the results (Özyurt et al., 2024). More observation time with a series of species are required (Mooraki et al., 2021). The objective of the research is to investigate the changes of liver metabolism induced by olive leaf constituents in fish organisms with emphasis on the molecular mechanisms and overall effect on metabolic health (Anny Leema & Balakrishnan, 2024). Aquaculture and environmental sustainability can be improved through knowledge acquired in the investigation (Nandy & Dubey, 2024; Çiftçi & Ayas, 2022).

Portuguese varieties 'Negrinha do Freixo' and 'Cornicabra' were employed to maximize the recovery of polyphenols from olive leaves using a second-order polynomial method. The model gave a best fit to antioxidant activity, total flavonoids, and total polyphenols (Oliveira et al., 2021). 'Negrinha do Freixo' and 'Cornicabra' showed higher phenolic content in extracts. With their antibacterial activity and antioxidant potential, extracts were excellent candidates as preservatives for foods and cosmetics as well as agents against aging. Stability of bioactive feed supplementation with an extract such as cellulases, and antioxidants is tested in the research (Rad & Behnamghader, 2014). It kept for four months at 4°C after Aspergillus ibericus produced it. The findings revealed that storage at 4°C increased the stability of enzymes, improved xylanase and cellulase half-lives, and lowered antioxidant activity loss (Filipe et al., 2023). Supplementation in diets decreased lipid peroxidation and boosted antioxidant activity. To enhance the sustainability and nutritional value of diets on bioactive compounds through optimized preservation conditions. Ghelichpour et al., (2021) examined how dietary olive leaf extract affected common carp subjected to the insecticide in terms of haematological biochemical, and antioxidant parameters. Four treatments were used: exposure to E1 diet, E2 diet, and a control group. The findings demonstrated that whereas exposure to raise plasma concentrations of alanine aminotransferase, AST, ALP, and mass drug administration (MDA) lowered red blood cell count, haematocrit,

hemoglobin, and superoxide dismutase activity. When olive leaf extract was administered, these alterations were reversed, and the red blood cell count increased. González-Hedström et al., (2021) investigated how products made from olives, such as virgin olive oil and extracts from olive leaves, can prevent peroxide from developing and stable omega-3 fatty acids. When olive leaf extracts are added to a blend of algal oil and products derived from olives, including virgin olive oil (EVOO), peroxide generation is inhibited and the lipid profile of improved. The drug also prevents muscle loss, circulation alterations, and age-associated resistance to glucose. The possibility of an approach to aging-related health problems.

The high concentration of beneficial components, olive byproducts and a major component in the extraction of olive oil have a substantial environmental impact. The culinary, packaging, pharmaceutical, and cosmetic industries can find for these wastes, which include leaves, pomace residues, and wastewater (Khwaldia et al., 2022). Olive byproducts are being used in food packaging systems due to recent developments in olive leaf extracts, removal, and characterization, which support sustainability and the circular economy. The application of olive leaf extract as an active ingredient in the production of edible microcapsules with alginate as a coating is assessed by (Toprakçı & Şahin, 2022). The levels of calcium chloride and sodium alginate, the duration it took for the capsules to set, and whether or not chitosan was added as a coating layer were utilized in determining the capsules' encapsulation efficiency. Olive leaf extract is capable of application in edible coatings, as proved by the high encapsulation efficiency. Kuley et al., (2024) explained how various concentrations of olive leaf extract affected the technical properties of anchovy oil which was initially submerged and processed using cellulose and sodium caseinate. The results confirmed that olive leaf extract intensified the physiological effect of anchovy oil in microencapsulation to a greater extent, indicating that microcapsules of fish oil and olive leaf extract are apt to possess excellent antibacterial action against consumption in addition to its nutraceutical effect. Olive leaf extract can potentially serve as edible coatings, as shown by the high encapsulation efficiency. With the addition of olive leaf cold-water extract, gelatin fish gel's mechanical strength was increased. Breaking strain and breaking stress were increased with a greater quantity of olive leaf extract. Fish gelatin (FG) gel did not melt when the temperature rose above 80°C, while its olive leaf extract content was positively correlated with the gel's melting point. By interacting with the large amino groups of the FG polypeptide chains, olive leaf extract-induced graft polymerization (Akazawa et al., 2024). The cross-linking ability of oleacein, a large phenolic compound.

Key Contribution

- The first investigation in bioactive components from olive leaves affect the metabolism of the liver in aquatic species, particularly Nile tilapia.
- It examines how tilapia's Nile liver enzyme activity and glucose levels are affected by seven metabolites found in olive leaves: caffeic acid, oleuropein, corosolic acid, moronic acid, lupeol, cycloartenol, and betulinic acid.
- Fish fed metabolites from olive leaves displayed importantly greater levels of the liver enzymes glucose, ALP, AST, and ALT compared to the control group.
- The greatest important changes in liver enzyme activity were caused by corosolic and moronic acids, which can indicate complexities with liver metabolism and function.
- The possible techniques for aquaculture nutrition and management can be informed by the research's insightful outcomes about the possible properties of olive leaf bioactive chemicals on fish well-being, especially liver function.

Materials and Methods

The Oreochromis niloticus used in the investigation were acclimatized in aquaria and fed a diet supplemented with seven olive leaf metabolites with variable ratios. Isolation and purification of the metabolites were carried out using chromatography techniques. The fish were distributed randomly to the treatment groups, and liver function was measured with biochemical markers such as glucose, ALT, AST, and ALP. Statistical comparison of the influence of the metabolites on liver enzymes was carried out with the Kruskal-Walli's test and Dunn's post hoc test. The methodology work flow is presented in Figure 1.

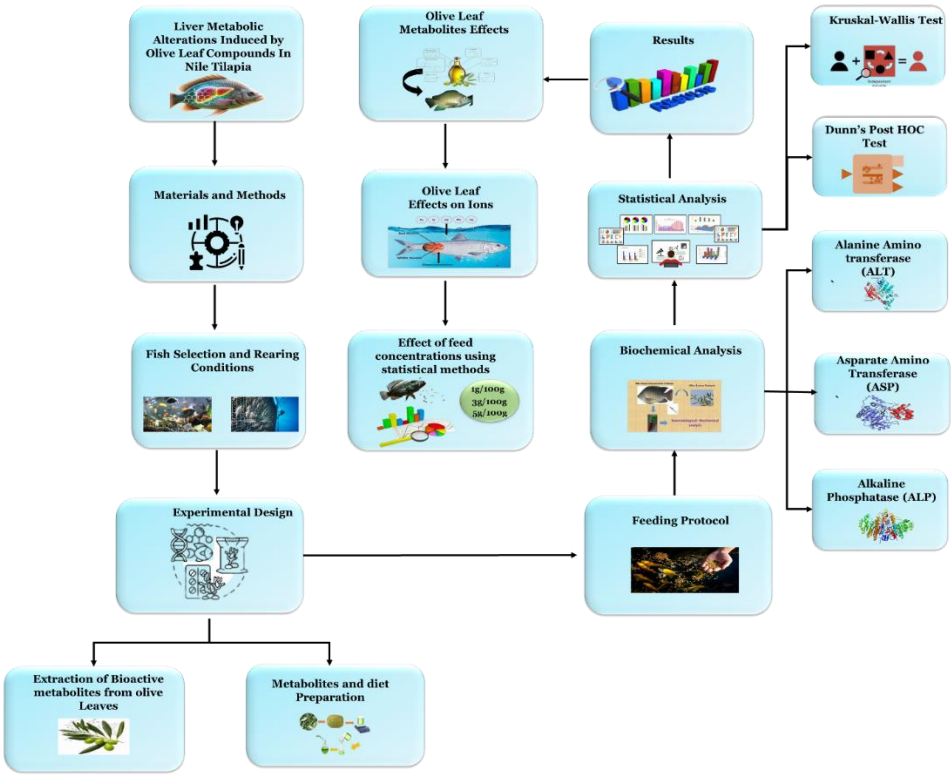

Figure 1. Work flow for changes in liver metabolism brought on by chemicals found in olive leaves in aquatic species

Fish Selection and Rearing Conditions

Oreochromis niloticus, the greatest common freshwater species applied in aquaculture examination, was preferred for the experimentation. The fish were accepted from a commercial hatchery and assumed two weeks of accommodation to regulate to lab conditions preceding to difficult. The fish were preserved in 100-liter glass aquariums with air, and water superiority was observed thoroughly in accommodation. The tank circumstances were preserved among 26–28°C, 6.0–7.5 mg/L liquefied oxygen, and 7.0–7.5 pH. Ammonia and nitrite levels were below detectable limits and 30% of the water was renewed daily to maintain optimal quality throughout the experiment.

Experimental Design

The purpose of the experiment was to assess how seven metabolites from olive leaves affected the liver function of Nile tilapia.

➢ ***Extraction of Bioactive Metabolites from Olive Leaves***

The extraction of caffeic acid, oleuropein, corosolic acid, moronic acid, lupeol, cycloartenol, and betulinic acid from Olea europaea leaves involves solvent extraction, chromatography, and purification techniques shown in Table 1. Fresh or dried leaves are cleaned, air-dried at ~25°C for 7–10 days, and ground into a fine powder. Caffeic acid and oleuropein are extracted using a methanol/water (80:20) mixture, followed by filtration, solvent evaporation, and purification via liquid-liquid partitioning and column chromatography. Triterpenoids are extracted using Soxhlet extraction (hexane/ethanol) for 8–12 hours, then purified using silica gel chromatography and preparative TLC/HPLC. Identification and quantification are performed using TLC, HPLC, and GC-MS/LC-MS. The purified metabolites are stored at -20°C in amber vials to prevent oxidation and can be lyophilized for feed preparation.

Table 1. Extraction and purification methods for bioactive metabolites from Olive leaves

Compounds	Extraction Methods	Purifications
Caffeic Acid	Methanol: Water Extraction	Column Chromatography, HPLC
Oleuropein	Methanol: Water Extraction	Column Chromatography, HPLC
Corosolic Acid	Soxhlet (Hexane/Ethanol)	Silica Gel Chromatography
Moronic Acid	Soxhlet (Hexane/Ethanol)	Silica Gel Chromatography
Lupeol	Soxhlet (Hexane/Ethanol)	TLC, HPLC
Cycloartenol	Soxhlet (Hexane/Ethanol)	Silica Gel Chromatography
Betulinic Acid	Soxhlet (Hexane/Ethanol)	TLC, HPLC

➢ ***Metabolites and Diet Preparation***

Seven bioactive components of olive leaf, specifically caffeic acid, oleuropein, corosolic acid, moronic acid, lupeol, cycloartenol, and betulinic acid, were screened to determine metabolic effects on the liver in Oreochromis niloticus. Standard fish feed was employed as the control diet, and every metabolite was added at three levels: 1%, 3%, and 5% (1g/100g, 3g/100g, and 5g/100g). The feed was pelletized to provide uniform distribution of the metabolites and then air-dried to eliminate moisture and retain the integrity of the compounds. The feed was stored in the refrigerator after drying to ensure stability until utilized. This preparation for the diet permitted repeatable, controlled exposure of the fish to the bioactive compounds at different concentrations to accurately test their metabolic impacts.

Feeding Protocol

The fish were randomly assigned to different treatment groups to ensure unbiased distribution and minimize variability. Each treatment had 10 fish per tank with three replicate tanks per treatment to increase the reliability and reproducibility of the data. The nourishing regimen entailed twice-daily feedings, in the morning and evening, at a rate of 2% of the fish body weight per meal. This feeding management was continued regularly for 96 hours (4 days) to inspect the short-term metabolic influence of the dietary interferences on liver functionality and general fish well-being.

Biochemical Analysis

Oreochromis niloticush were starved for 12 hours at the achievement of the feeding trial to limit metabolic fluctuations prior to sampling. After administration of clove oil anesthesia to the fish, a caudal vein blood sample was collected. The biological samples were stored in the refrigerator and centrifuged at 4°C and 3000 rpm for 10 minutes to yield the serum. The serum was deposited in storage at -80°C for further biochemical investigation. The quantity of liver enzyme levels was assessed to evaluate the health of the liver, such as ALT for injury to liver cells, AST as an indicator of hepatocellular damage, and ALP for bile duct purpose. Serum

glucose attentiveness was predictable by enzymatic colorimetric evaluate to discovery metabolic variations because of the dietary behaviors. These investigations are crucial in determining the metabolic and liver function status of Oreochromis niloticus and in assisting the impact of the feeding trial.

Statistical Analysis

These biochemical markers, AST, ALT, ALP, blood glucose, and olive leaf compounds were compared with the Kruskal-Wallis test between control and various treatments. The groups differed widely from one another, indicating that the metabolites of the olive leaf influenced the function of liver enzymes. To identify the groups separated, Dunn's post hoc test was conducted and it was determined that corosolic acid and moronic acid treatment groups were significantly higher Enzyme levels than the control, showing interference with liver metabolism. These findings illustrate the pronounced effect of olive leaf metabolites on liver function in Oreochromis niloticus.

Results

Metabolites of the olive leaf, corosolic and moronic acid, significantly increased the content of glucose and liver enzyme activity in Nile tilapia. There was a higher feed content and higher ion levels of sodium, potassium, calcium, magnesium, chloride, phosphorus, and iron. Data analysis was examined the response of different concentrations of feeds varied considerably for 1g/100g and 5g/100g doses.

Olive Leaf Metabolites Effects

The influence of metabolites from olive leaves on the glucose levels and liver enzyme activity of Oreochromis niloticus was assessed. The results indicated that cytosolic and moronic acid significantly raised the levels of glucose, ALT, AST, and ALP. These findings highlight the potential metabolic impact of olive leaf compounds on fish health and aquaculture nutrition.

Table 2. Tilapia Serum Glucose Levels and Liver Enzymes Affected by Olive Leaf Metabolites

Compounds	Dose	AST (U/L)	Glucose (mg/dL)	ALT (U/L)	ALP (U/L)
Caffeic Acid	1g/100g feed	298 ± 5*	72 ± 3*	12 ± 1	30 ± 2*
	3g/100g feed	315 ± 6*	76 ± 4*	14 ± 2*	28 ± 3
	5g/100g feed	340 ± 5*	79 ± 2*	16 ± 1*	32 ± 2*
Oleuropein	1g/100g feed	311 ± 4*	64 ± 1*	10 ± 1	27 ± 2*
	3g/100g feed	355 ± 6*	67 ± 2*	12 ± 1*	25 ± 2
	5g/100g feed	410 ± 5*	70 ± 3*	14 ± 2*	29 ± 2*
Corosolic Acid	1g/100g feed	370 ± 5*	80 ± 2*	18 ± 2*	35 ± 2*
	3g/100g feed	405 ± 7*	85 ± 3*	22 ± 1*	38 ± 3*
	5g/100g feed	460 ± 8*	89 ± 2*	26 ± 1*	42 ± 2*
Moronic Acid	1g/100g feed	360 ± 6*	78 ± 3*	17 ± 1*	34 ± 2*
	3g/100g feed	395 ± 7*	83 ± 4*	21 ± 2*	37 ± 2*
	5g/100g feed	450 ± 7*	87 ± 3*	25 ± 2*	40 ± 3*
Lupeol	1g/100g feed	320 ± 6*	75 ± 3*	14 ± 1*	28 ± 2*
	3g/100g feed	355 ± 5*	79 ± 4*	18 ± 2*	30 ± 2*
	5g/100g feed	400 ± 6*	82 ± 3*	22 ± 1*	33 ± 2*
Cycloartenol	1g/100g feed	315 ± 5*	71 ± 2*	13 ± 1*	29 ± 2*
	3g/100g feed	345 ± 6*	75 ± 3*	16 ± 2*	31 ± 2*
	5g/100g feed	390 ± 7*	78 ± 3*	20 ± 1*	34 ± 3*
Betulinic Acid	1g/100g feed	325 ± 6*	73 ± 3*	15 ± 1*	30 ± 2*
	3g/100g feed	360 ± 5*	77 ± 2*	19 ± 2*	32 ± 2*
	5g/100g feed	410 ± 6*	81 ± 3*	24 ± 1*	36 ± 2*
Control Group	Only feed	250 ± 5	40 ± 2	8 ± 1	20 ± 1

*Note: Values are Mean ± SD (n = 10 per treatment, triplicates per group). * shows that there are significant differences* ($p < 0.05$) *from the control group.*

When adding olive leaf metabolites to fish feed at several doses (1g/100g, 3g/100g, and 5g/100g feed), Table 2 assessed the impact of these compounds on liver enzymes and glucose levels in Nile tilapia. Serum analysis showed important enhancement ($p < 0.05$) in glucose levels, Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT) contrasting with the control group. Among the metabolites, corosolic acid and moronic acid exhibited the most pronounced effects, increasing liver enzyme activity and glucose metabolism. The control group, which received only the basal feed, showed significantly lower enzyme activity (ALT: $8 \pm 1\,U/L$, AST: $250 \pm 5\,U/L$, ALP: $20 \pm 1\,U/L$, Glucose: $40 \pm 2\,mg/dL$), confirming the metabolic impact of the bioactive compounds. These findings suggest that olive leaf metabolites influence liver function in fish, with potential implications for aquaculture nutrition and fish health. Further studies are needed to assess their long-term effects and safety.

Olive Leaf Effects on Ions

The experiment determines how the metabolites of olive leaves affect the ion levels in the serum of Nile tilapia. The results indicated remarkable elevations in Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, P, and Fe levels, especially with corosolic and moronic acid. These observations indicate that olive leaf constituents affect electrolyte homeostasis and mineral metabolism in fish.

Table 3. Impact of Metabolites from Olive Leaf on Nile Tilapia Serum Ion Levels

Compounds	**Dose**	Na^+ $(mmol/L)$	K^+ $(mmol/L)$	Ca^{2+} $(mmol/L)$	Mg^{2+} $(mmol/L)$	Cl^- $(mmol/L)$	P (mg/dL)	Fe $(\mu g/dL)$
Caffeic Acid	1g/100g feed	145 ± 3	4.8 ± 0.3	2.4 ± 0.2	1.6 ± 0.1	120 ± 4	5.5 ± 0.3	90 ± 5
	3g/100g feed	148 ± 2*	5.2 ± 0.2*	2.7 ± 0.2*	1.8 ± 0.2*	125 ± 3*	6.0 ± 0.2*	94 ± 3*
	5g/100g feed	152 ± 3*	5.6 ± 0.3*	3.0 ± 0.2*	2.0 ± 0.2*	130 ± 4*	6.3 ± 0.3*	98 ± 4*
Oleuropein	1g/100g feed	143 ± 2	4.7 ± 0.3	2.5 ± 0.2	1.5 ± 0.1	118 ± 3	5.3 ± 0.3	88 ± 4
	3g/100g feed	147 ± 3*	5.1 ± 0.2*	2.8 ± 0.2*	1.7 ± 0.1*	123 ± 3*	5.8 ± 0.2*	92 ± 3*
	5g/100g feed	151 ± 2*	5.5 ± 0.3*	3.1 ± 0.2*	1.9 ± 0.1*	128 ± 4*	6.2 ± 0.3*	96 ± 3*
Corosolic Acid	1g/100g feed	150 ± 3*	5.4 ± 0.3*	2.9 ± 0.2*	2.0 ± 0.2*	132 ± 4*	6.4 ± 0.3*	99 ± 4*
	3g/100g feed	155 ± 2*	5.8 ± 0.2*	3.2 ± 0.2*	2.3 ± 0.2*	138 ± 3*	6.8 ± 0.2*	104 ± 3*
	5g/100g feed	160 ± 3*	6.2 ± 0.3*	3.5 ± 0.2*	2.6 ± 0.2*	145 ± 4*	7.2 ± 0.3*	110 ± 4*
Moronic Acid	1g/100g feed	148 ± 3*	5.3 ± 0.3*	2.8 ± 0.2*	1.9 ± 0.2*	130 ± 4*	6.2 ± 0.3*	97 ± 4*
	3g/100g feed	153 ± 2*	5.7 ± 0.2*	3.1 ± 0.2*	2.2 ± 0.2*	135 ± 3*	6.6 ± 0.2*	102 ± 3*
	5g/100g feed	158 ± 3*	6.0 ± 0.3*	3.4 ± 0.2*	2.5 ± 0.2*	142 ± 4*	7.0 ± 0.3*	108 ± 4*
Lupeol	1g/100g feed	144 ± 2	4.9 ± 0.3	2.6 ± 0.2	1.6 ± 0.1	122 ± 3	5.7 ± 0.3	91 ± 4
	3g/100g feed	149 ± 3*	5.4 ± 0.2*	2.9 ± 0.2*	1.8 ± 0.2*	127 ± 3*	6.1 ± 0.2*	95 ± 3*
	5g/100g feed	154 ± 2*	5.9 ± 0.3*	3.3 ± 0.2*	2.1 ± 0.2*	133 ± 4*	6.5 ± 0.3*	100 ± 4*
Control Group	Only feed	140 ± 3	4.5 ± 0.2	2.2 ± 0.2	1.3 ± 0.1	115 ± 4	5.0 ± 0.3	85 ± 3

Note: Values are Mean $\pm$ *SD (*$n = 10$ *per treatment, triplicates per group).* * *shows that there are substantial distinctions* ($p < 0.05$) *from the control group.*

Table 3 examined how seven main ion concentrations in the serum of Nile tilapia were affected by the metabolites of olive leaves by adding the compounds to fish food at varying levels (1g/100g, 3g/100g, and 5g/100g feed). The outcomes revealed significant ($p < 0.05$) increases in sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), phosphorus (P), and iron (Fe) serum levels when compared to the control group. The metabolites that were tested, moronic acid and corosolic acid, elicited the most significant increases, suggesting involvement in mineral metabolism and ion regulation. The control group, which received only the basal feed, which had significantly lower ion levels (Na^+: $140 \pm 3\ mmol/L$, K^+: $4.5 \pm 0.2\ mmol/L$, Ca^{2+}: $2.2 \pm 0.2\ mmol/L$, Mg^{2+}: $1.3 \pm 0.1\ mmol/L$, Cl^-: $115 \pm 4\ mmol/L$, P: $5.0 \pm 0.3\ mg/dL$, Fe: $85 \pm 3\ \mu g/dL$), confirming that bioactive compounds from olive leaves influence electrolyte balance and mineral homeostasis in fish. These outcomes provide insight into the physiological properties of plant-derived compounds in aquaculture nutrition, but further research is desirable to assess their long-term inferences for fish health.

Effect of Feed Concentrations using statistical methods

Utilizing the Kruskal-Wallis test, the effects of changing feed concentrations on a variable that was measured and it showed a significant difference among the groups. The specific concentrations that differ from each other were identified by Dunn's post hoc test.

Table 4. Kruskal-Wallis Test Results

Test Statistic (H)	Degrees of Freedom (df)	p-value	Interpretation
7.89	2	0.019	Significant (p < 0.05)

Table 5. Dunn's Post Hoc Test

Comparison	Mean Rank Difference	Adjusted p-value	Significance (p < 0.05)
1g/100g vs 3g/100g	4.2	0.045	Yes
1g/100g vs 5g/100g	6.5	0.012	Yes
3g/100g vs 5g/100g	2.3	0.278	No

Table 4 was conducted to compare the effect of different concentrations (1g/100g feed, 3g/100g feed, and 5g/100g feed) on a measured variable. The test result showed a significant difference among the groups ($H = 7.89$, $df = 2$, $p = 0.019$), indicating that at least one concentration had a statistically different effect. Table 5 with Bonferroni correction was performed to determine which specific groups differed. The results revealed that 1g/100g feed group differed significantly from the 5g/100g feed group (mean rank difference = 6.5, adjusted $p = 0.012$). Additionally, 1g/100g feed group also differed significantly from the 3g/100g feed group (mean rank difference = 4.2, adjusted $p = 0.045$). However, the 3g/100g feed and 5g/100g feed groups did not show a significant difference (mean rank difference = 2.3, adjusted $p = 0.278$). These results suggest that increasing the concentration from 1g/100g to higher levels (especially 5g/100g) leads to significant differences, whereas the effect of increasing from 3g/100g to 5g/100g is not statistically distinct.

Discussion

Olive leaf metabolites significantly impact liver enzyme activity and glucose levels in Nile tilapia, with certain compounds showing stronger effects. These bioactive compounds also influence ion concentrations, suggesting a role in osmoregulation and mineral metabolism. Statistical analysis confirmed that feed concentration affects physiological responses, with significant differences between lower and higher doses.

However, higher concentrations did not always show additional benefits, indicating a possible threshold effect. These findings highlight the potential of olive leaf-derived compounds in aquaculture nutrition. Further research is needed to assess long-term implications and optimal dosage levels.

Conclusion

Olive leaf components affect liver metabolism in aquatic organisms by regulating lipid metabolism, oxidative stress, and enzyme activity. These changes have effects on energy balance and overall physiological processes. The possible benefits are enhanced health and tolerance of Oreochromis niloticus. Corosolic acid and moronic acid-induced dramatic elevations in the activities of liver enzymes, implying potential liver stress or disrupted metabolic function. Elevated enzyme levels can indicate changes in liver physiology, increased metabolic processing, or cellular damage. This suggests the potential impact of olive leaf components on fish liver well-being. The finding reveals that the corosolic acid and moronic acid encouraged the greatest noticeable developments in liver enzyme activities, signifying potential disruptions in liver metabolism and function. Understanding these effects is relevant to assessing their role in aquaculture nutrition and fish welfare. The limitation of exploring metabolic change in the liver triggered by olive leaf components in Oreochromis niloticus is species-specific metabolism modification, the need for chronic exposure, and possible variability under conditions of environment. Future investigations ought to channel inquiry towards realizing the molecular mechanism, dosage tailoring across species, and usefulness consideration for sustainable aquaculture as well as environmental health.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

Reference

Akazawa, T., Itami, H., & Ogawa, M. (2024). The physical properties of fish gelatin gel are enhanced by the cross-linking activity of olive leaf water extract. *International Journal of Food Science and Technology*, *59*(3), 1580-1590.

Anny Leema, A., & Balakrishnan, P. (2024). Detection and Categorization of Rice Leaf Diseases through Federated Learning and Improved Vision Transformer Models. *Journal of Wireless Mobile Networks, Ubiquitous Computing, and Dependable Applications*, 15(4), 370-379. https://doi.org/10.58346/JOWUA.2024.I4.025

Assar, D. H., Ragab, A. E., Abdelsatar, E., Salah, A. S., Salem, S. M., Hendam, B. M., ... & Elbialy, Z. I. (2023). Dietary olive leaf extract differentially modulates antioxidant defense of normal and aeromonas hydrophila-infected common carp (Cyprinus carpio) via Keap1/Nrf2 pathway signaling: a phytochemical and biological link. *Animals*, *13*(13), 2229. https://doi.org/10.3390/ani13132229

Çiftçi, N., & Ayas, D. (2022). First record of Pempheris rhomboidea (Kossmann & Räuber, 1877) harvested from aquaculture fish cages. *Natural and Engineering Sciences*, *7*(2), 182-189. http://doi.org/10.28978/nesciences.1159280

Conte, P., Pulina, S., Del Caro, A., Fadda, C., Urgeghe, P. P., De Bruno, A., ... & Piga, A. (2021). Gluten-free breadsticks fortified with phenolic-rich extracts from olive leaves and olive mill wastewater. *Foods*, *10*(5), 923. https://doi.org/10.3390/foods10050923

Ferreira, D. M., de Oliveira, N. M., Chéu, M. H., Meireles, D., Lopes, L., Oliveira, M. B., & Machado, J. (2023). Updated Organic Composition and Potential Therapeutic Properties of Different Varieties of Olive Leaves from Olea europaea. *Plants*, *12*(3), 688. https://doi.org/10.3390/plants12030688

Filipe, D., Gonçalves, M., Fernandes, H., Oliva-Teles, A., Peres, H., Belo, I., & Salgado, J. M. (2023). Shelf-life performance of fish feed supplemented with bioactive extracts from fermented olive mill and winery by-products. *Foods*, *12*(2), 305. https://doi.org/10.3390/foods12020305

Ghelichpour, M., Mirghaed, A. T., Dawood, M. A., Hoseinifar, S. H., & Van Doan, H. (2021). Alteration of haematological and antioxidant parameters in common carp (Cyprinus carpio) fed olive (Olea europea) leaf extract after exposure to Danitol®. *Aquaculture Research*, *52*(3), 1088-1095. https://doi.org/10.1111/are.14964

González-Hedström, D., de La Fuente-Fernandez, M., Priego, T., Martín, A. I., Amor, S., López-Calderón, A., ... & Granado, M. (2021). Addition of olive leaf extract to a mixture of algae and extra virgin olive oils decreases fatty acid oxidation and synergically attenuates age-induced hypertension, sarcopenia and insulin resistance in rats. *Antioxidants*, *10*(7), 1066. https://doi.org/10.3390/antiox10071066

Hazreen-Nita, M. K., Kari, Z. A., Mat, K., Rusli, N. D., Sukri, S. A. M., Harun, H. C., ... & Dawood, M. A. (2022). Olive oil by-products in aquafeeds: Opportunities and challenges. *Aquaculture Reports*, *22*, 100998. https://doi.org/10.1016/j.aqrep.2021.100998

Khwaldia, K., Attour, N., Matthes, J., Beck, L., & Schmid, M. (2022). Olive byproducts and their bioactive compounds as a valuable source for food packaging applications. *Comprehensive Reviews in Food Science and Food Safety*, *21*(2), 1218-1253. https://doi.org/10.1111/1541-4337.12882

Kuley, E., Sakarya, Y., & Özyurt, G. (2024). Increasing the functional properties of fish oil microcapsules with olive leaf extracts. *Biomass Conversion and Biorefinery*, *14*(8), 9689-9696. https://doi.org/10.1007/s13399-022-02930-2

Liu, J., Xue, M., Morais, S., He, M., Wang, H., Wang, J., ... & Liang, X. (2022). Effects of a phytogenic supplement containing olive by-product and green tea extracts on growth performance, lipid metabolism, and hepatic antioxidant capacity in largemouth bass (Micropterus salmoides) fed a high soybean meal diet. *Antioxidants*, *11*(12), 2415.https://doi.org/10.3390/antiox11122415

Monsef Shokri, M., Yousefi, S., & Jamshidi, S. (2021). Effect of dietary ImmunoWall® on liver oxidative status in juvenile Persian sturgeon. *International Journal of Aquatic Research and Environmental Studies*, *1*(2), 1-5. https://doi.org/10.70102/IJARES/V1I2/1

Mooraki, N., Omrani, M., Khajehrahimi, A. E., & Azhdari, P. (2021). Classifying five ornamental fish species of Cichlidae family by use of logistic regression and discrimination linear analysis. *International Journal of Aquatic Research and Environmental Studies*, *1*(1), 15-21.. https://doi.org/10.70102/IJARES/V1I1/2

Nandy, M., & Dubey, A. (2024). Effective Surveillance of Water Quality in Remediulating Aquaculture Systems through the Application of Intelligent Biosensors. *Natural and Engineering Sciences*, 9(2), 234-243. https://doi.org/10.28978/nesciences.1575456

Oliveira, A. L., Gondim, S., Gómez-García, R., Ribeiro, T., & Pintado, M. (2021). Olive leaf phenolic extract from two Portuguese cultivars–bioactivities for potential food and cosmetic application. *Journal of Environmental Chemical Engineering*, 9(5), 106175. https://doi.org/10.1016/j.jece.2021.106175

Özyurt, G., Durmuş, M., Özkütük, A. S., & Uçar, Y. (2024). Microencapsulation of fish oil with olive leaf extract instead of synthetic antioxidant and its effects on nutraceutical properties of fish oil at different inlet temperatures. *Biomass Conversion and Biorefinery*, *14*(9), 10517-10532. https://doi.org/10.1007/s13399-022-03231-4

Rad, H. N., & Behnamghader, A. (2014). Preparation of Bioactive Glass 77S for Bone Tissue Engineering Applications. *International Academic Journal of Science and Engineering*, 1(2), 68–74.

Talukdar, S., & Ghosh, K. (2025). Evaluation of growth, nutrient utilization, and metabolic function in rohu, Labeo rohita (Hamilton), fed diets incorporated with fermented Saraca asoca leaf meal. *Fish Physiology and Biochemistry*, *51*(1), 3. https://doi.org/10.1007/s10695-024-01422-6

Toprakçı, İ., & Şahin, S. (2022). Encapsulation of olive leaf antioxidants in microbeads: Application of alginate and chitosan as wall materials. *Sustainable Chemistry and Pharmacy*, *27*, 100707. https://doi.org/10.1016/j.scp.2022.100707