



OPEN ACCESS

EDITED BY
Gladstone Sagada,
Victory Farms, Kenya

REVIEWED BY
Peng Hu,
Neijiang Normal University, China
Nitesh Kumar Yadav,
Maharana Pratap University of Agriculture
and Technology, India

*CORRESPONDENCE
Abdallah Tageldein Mansour
✉ amansour@kfu.edu.sa
Yasmina M. Abd-Elhakim
✉ yasmina.forensic@gmail.com

RECEIVED 17 April 2026
REVISED 27 May 2026
ACCEPTED 28 May 2026
PUBLISHED 16 June 2026

CITATION
El-Houseiny W, Mansour AT, Behairy A,
Alsaqufi AS, Alkhamis YA, Abd-
Elhakim YM, Ashour M, Mathew RT,
Abbas AO, Hassan BA, Mohamed EMM
and Mahmoud YK (2026) Dietary
Crataegus sinaica leaves enhance
growth performance, antioxidant status,
intestinal integrity, and regulate
inflammation-related genes in Nile
tilapia under melamine exposure.
Front. Mar. Sci. 13:1858761.
doi: 10.3389/fmars.2026.1858761

COPYRIGHT
© 2026 El-Houseiny, Mansour, Behairy,
Alsaqufi, Alkhamis, Abd-Elhakim, Ashour,
Mathew, Abbas, Hassan, Mohamed and
Mahmoud. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication
in this journal is cited, in accordance
with accepted academic practice. No
use, distribution or reproduction is
permitted which does not comply with
these terms.

Dietary *Crataegus sinaica* leaves enhance growth performance, antioxidant status, intestinal integrity, and regulate inflammation-related genes in Nile tilapia under melamine exposure

Walaa El-Houseiny¹, Abdallah Tageldein Mansour^{2*},
Amany Behairy³, Ahmed Saud Alsaqufi²,
Yousef Ahmed Alkhamis², Yasmina M. Abd-Elhakim^{4*},
Mohamed Ashour⁵, Roshmon Thomas Mathew⁵,
Ahmed O. Abbas², Bayan A. Hassan⁶, Engy M. M. Mohamed⁷
and Yasmina K. Mahmoud⁸

¹Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, ²Animal and Fish Production Department, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia, ³Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia, Egypt, ⁴Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, ⁵Fish Resources Research Center, King Faisal University, Al-Ahsa, Saudi Arabia, ⁶Pharmacology Department, Faculty of Pharmacy, Future University, Cairo, Egypt, ⁷Fish Diseases and Management, Veterinary Clinic, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt, ⁸Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

Introduction: Dietary exposure to melamine (MELA) adversely affects growth performance and intestinal health in Nile tilapia. This study evaluated the protective effects of *Crataegus sinaica* leaf extract (CSL) against MELA-induced toxicity.

Methods: In this study, a total of 240 fish (26.39 ± 0.22) were randomly assigned to four groups (60 fish/group; four replicates of 15 fish each). The control group received a basal diet, the CSL group received 1% CSL, the MELA group received 10 g/kg MELA, and the CSL+MELA group received both CSL (1%) and MELA (10 g/kg) for 60 days.

Results: MELA exposure significantly reduced growth performance, including final body weight (−26.78%), weight gain (−50.59%), specific growth rate (−41.41%), and feed intake (−20.50%) compared to the control, and increased feed conversion ratio (FCR). Conversely, CSL supplementation enhanced growth parameters, with increases in weight gain (33.88%), specific growth rate (21.88%), and FCR reduced by (20.57%). Co-administration of CSL with MELA partially restored growth, with final body weight, weight gain, and FCR approaching control values. MELA also suppressed digestive enzyme activities (lipase −21.38%, α -amylase −34.72%, protease −32.42%) and impaired intestinal antioxidant defenses (SOD −43.73%, CAT −37.88%, GPx −38.01%, GST −50%; MDA increased by 128.58%). CSL supplementation increased antioxidant enzyme

activities (SOD + 17.20%, CAT + 57.58%, GPx +13.43%, GST + 28.95%) and restored digestive enzyme activity (lipase: +10.11%, α - amylase: +11.50%, and protease: +8.82%). At the molecular level, MELA significantly upregulated inflammatory genes (*tnf- α* : 5.22 ± 0.35 , *tlr-4*: 3.01 ± 0.34 , *il-6*: 2.66 ± 0.11 , *il-8*: 2.65 ± 0.15) while CSL co-administration downregulated these genes and normalized *il-10* expression (2.67 ± 0.15). Histopathological analysis confirmed that CSL mitigated MELA-induced intestinal damage, including epithelial denudation, inflammatory infiltration, hemorrhage, and goblet cell hyperplasia.

Conclusion: These findings showed that dietary CSL markedly promotes growth, enhances antioxidant defenses, modulates inflammatory responses, and protects intestinal integrity, demonstrating its potential as a natural dietary strategy to counteract MELA toxicity in *Oreochromis niloticus*.

KEYWORDS

body composition, *Crataegus sinaica*, growth, inflammation, intestine, melamine, Nile tilapia, oxidative stress

1 Introduction

Melamine (MELA) contamination in food presents a significant public health risk (Sadek et al., 2026), with reports of its presence in food products of animal origin, including fish and seafood (Andersen et al., 2008; Zhu and Kannan, 2019). As a nitrogen-rich compound, MELA is widely produced and distributed across various sectors of human activity. It is persistently released into the water cycle from numerous sources (Warner and Licha, 2025). Owing to its high solubility in water and low lipophilicity, MELA and its derivatives preferentially accumulate in aquatic environments (Liu et al., 2025). Studies have confirmed the widespread detection of MELA in diverse water sources, such as rivers, lakes, seawater, tap water, bottled water, rainwater, wastewater, and even swimming pool water, with detection rates exceeding 94% in most cases (Zhu and Kannan, 2020).

Melamine has been shown to have significant toxic effects on fish, particularly when included in their diet, either alone or in combination with compounds like cyanuric acid. Fish species such as red tilapia, Nile tilapia, and Asian seabass exposed to MELA-contaminated diets exhibit poor growth performance and decreased feed utilization (Phromkunthong et al., 2013, 2015; Abd El-Hakim et al., 2020). Moreover, MELA exposure has been linked to reduced body crude lipid content in Nile tilapia, indicating an adverse impact on their nutritional status (Abd El-Hakim et al., 2020). The toxicity of MELA primarily affects multiple organ systems through mechanisms involving oxidative stress, histopathological damage, and inflammation. Oxidative stress induced by MELA manifests through augmented release of reactive oxygen species (ROS) and disturbance of antioxidant defenses. This is evidenced by reduced enzyme activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), together with high concentrations of malondialdehyde (MDA), a marker of lipid peroxidation (Iheanacho et al., 2021). Additionally, MELA's interaction with albumin, a protein that binds arachidonic acid, may displace arachidonic acid, leading to the production of pro-inflammatory mediators (Rajpoot et al., 2017). In Nile tilapia, MELA exposure alters the expression of immune-related genes,

suppressing anti-inflammatory responses while promoting pro-inflammatory cytokine production (Abd El-Hakim et al., 2020). Furthermore, MELA-induced elevated intracellular calcium and ROS production activate apoptotic pathways, leading to tissue damage and inflammation (Yiu et al., 2017). Overall, MELA contamination in fish feed poses serious risks, including reduced growth, immune suppression, and increased susceptibility to infections.

In the field of aquaculture, the inclusion of herbal plants with antioxidant qualities in fish diets has gained popularity as a means to enhance overall fish growth, immune function, and resistance to diseases (El-Houseiny et al., 2024; Dahran et al., 2025; El-Houseiny et al., 2025). Plant leaf powders have been widely used as dietary supplements in aquaculture due to their beneficial effects on growth performance, immunity, and antioxidant status in fish (Abdel Rahman et al., 2018; Kapinga et al., 2018; Abdel-Razek et al., 2019). This approach allows evaluation of the combined effects of all leaf constituents, including fibers and secondary metabolites, rather than focusing solely on compounds recovered by solvent extraction. *Crataegus sinaica* Boiss (Sinai Hawthorn), a hybrid between *C. monogyna* and *C. azarolus*, is notably cultivated in the mountainous regions of the St. Catherine Protectorate in Egypt (El-Shahaby et al., 2020). Known for its bioactive compounds, including quercetin, hyperoside, and procyanidins, *C. sinaica* has demonstrated notable antioxidative and anti-inflammatory activities (Refaat et al., 2010; Alsharif et al., 2022). These properties suggest its potential to improve fish health and resilience, particularly in the context of environmental toxins. While other plant-based additives, such as adlay and traditional Chinese medicine mixtures, have shown benefits in aquaculture, such as enhanced antioxidant capacity, disease resistance, and immune response (Abarike et al., 2019; Jiang et al., 2025), *C. sinaica* stands out for its efficacy in mitigating the effects of specific environmental toxins, like acrylamide, and enhancing survival under such stressors (El-Houseiny et al., 2023). Dietary supplementation with *C. sinaica* (1%) significantly improved the health, growth, and survival rates of African catfish (*Clarias gariepinus*) reared in stressed environments caused by waterborne acrylamide exposure. It counteracted the negative

effects of acrylamide, such as reduced growth, anemia, and oxidative stress, and restored antioxidant levels (e.g., GPx and SOD) and immune components (e.g., lysozyme, complement 3), while reducing tissue damage in the liver, kidney, and spleen. However, to date, the use of *C. sinaica* leaves (CSL) in aquafeeds to mitigate the impacts of MELA on fish growth, intestinal architecture, and related mechanisms has not yet been investigated. This study objective to explore the usefulness of CSL supplementation in fish feed as a strategy to reduce the harmful effects of MELA exposure in Nile tilapia (*Oreochromis niloticus*).

2 Materials and methods

2.1 Tested compounds

MELA ($C_3H_6N_6$, 99.5% purity) was purchased from Alpha Chemica, Mumbai, India. CSL were harvested from the Saint Catherine mountainous region (South Sinai, Egypt). Plant authentication was conducted by a specialist from the Department of Plant Sciences, Faculty of Agriculture, Zagazig University, Egypt, and a voucher specimen was preserved. The collected *C. sinaica* leaves were carefully washed and air-dried in the shade at $25 \pm 2^\circ\text{C}$ with a relative humidity of approximately 50% until constant weight was achieved to ensure complete moisture removal while preserving bioactive compounds. The dried leaves were then finely ground using an electric grinder and stored in airtight containers at 4°C until further use.

For proximate analysis, the leaves were dried until constant weight was achieved to ensure complete moisture loss, then ground, and the proximate composition of the resulting dried CSL powder was determined in triplicate following standard procedures outlined by the Association of Official Analytical Chemists (A.O.A.C., 2005). On a dry matter basis, CSL contained $9.49 \pm 0.65\%$ ash, $10.06 \pm 0.70\%$ protein, $8.06 \pm 0.43\%$ ether extract, $26.48 \pm 1.19\%$ crude fiber, $90.51 \pm 0.65\%$ organic matter, and $45.91 \pm 1.44\%$ nitrogen-free extract.

The phytochemical profile of CSL powder was further characterized using gas chromatography-mass spectrometry (GC-MS) (Trace GC-TSQ, Thermo Scientific, USA) equipped with a capillary column (TG-5MS, $0.25\text{ mm} \times 0.25\text{ }\mu\text{m} \times 30\text{ m}$). The program of oven temperature commenced at 50°C , maintained for 2 minutes, then held for 2 minutes at $5^\circ\text{C}/\text{min}$ to 250°C , followed by a rapid ramp of $30^\circ\text{C}/\text{min}$ to 300°C , and continued for an additional 2 minutes. The injector temperature was set at 270°C , and the transfer line temperature at 260°C . Helium served as the carrier gas with a flow rate of $1\text{ mL}/\text{min}$. In split mode, a volume of $1\text{ }\mu\text{L}$ of diluted sample was injected by an AS1300 autosampler, with a 4 min solvent interval. Mass spectra were developed in electron ionization mode at 70 eV , covering a 50–650 mass-to-charge (m/z), with the ion source kept at 200°C . The identification of the constituents was performed by matching the obtained mass spectra against the WILEY 09 and NIST 14 spectral libraries.

2.2 Experimental fish

Apparently healthy Nile tilapia (average weight $26.39 \pm 0.22\text{ g}$) were obtained from the aquaculture unit of the Faculty of Veterinary Medicine, Zagazig University, Egypt. Fish were adapted for two weeks under laboratory conditions and fed a basal diet before the experiment. The fish were reared in glass aquaria (75-L capacity, $30 \times 40 \times 80\text{ cm}$) containing dechlorinated water, with continuous aeration supplied via air stones connected to a central compressor. A controlled 12 h light and 12 h dark photoperiod was continued. Each aquarium contained 15 fish. To maintain water quality, approximately 25–30% of the water was replaced daily with fresh dechlorinated water, and tank bottoms were siphoned regularly to remove uneaten feed and waste. Water quality parameters, including pH, dissolved oxygen, temperature, ammonia, and nitrite, were monitored throughout the experiment to ensure optimal rearing conditions. Water quality parameters, including pH, dissolved oxygen, temperature, ammonia, and nitrite, were monitored throughout the experiment to ensure optimal rearing conditions. Water quality indices remained within recommended ranges for tilapia culture, with mean values of pH (7.67 ± 0.38), dissolved oxygen ($6.85 \pm 0.31\text{ mg/L}$), temperature ($26.50 \pm 0.37^\circ\text{C}$), and ammonia ($0.11 \pm 0.02\text{ mg/L}$), consistent with Boyd and Tucker (2012).

2.3 Diet formulation and experimental design

The feeding trial was conducted from March to May 2025 under controlled laboratory conditions. The basal diet was designed to fulfill the dietary needs of tilapia, following the guidelines provided by the National Research Council (2011) (Table 1). Nutrient profile of the experimental diets was determined according to AOAC (2006) methods, including crude protein (macro-Kjeldahl method), crude fat (ether extraction), ash (muffle furnace), moisture (oven drying), and crude fiber. A total of 240 fish were arbitrarily assigned into four experimental groups (60 fish per group), each subdivided into four replicates (each replicate containing 15 fish). The four experimental groups included a control group fed the non-supplemented basal diet, CSL group fed a basal diet containing 1% CSL, MELA group fed a diet containing MELA (10 g/kg diet) (Abd El-Hakim et al., 2020), and the CSL+MELA group fed a diet containing both 1% CSL and MELA (10 g/kg). The chosen inclusion level of CSL (1%) was determined based on a preliminary trial, where different dietary levels (0, 0.5, 1, 1.5, and 2%) of CSL were tested for their impact on the physiological parameters of Nile tilapia. The findings indicated that a 1% CSL inclusion yielded the most favorable effects on fish health and performance. Additionally, in our earlier study, the addition of 1% of CSL to another freshwater fish, *C. gariepinus*, significantly enhanced the fish's overall health, disease resistance, and growth (El-Houseiny et al., 2023).

Experimental diets were prepared by carefully mixing the components with CSL powder, followed by the addition of 100 mL of water per kg diet to form a homogenous paste. The mixture

TABLE 1 Ingredients and proximate chemical analysis of the experimental diet.

Ingredients	Experimental diets			
	Control	CSL	MELA	CSL+MELA
Fish meal, 65%	11	11	11	11
Ground corn	24	23	23	22
Soybean meal 48%	42	42	42	42
Corn oil	4	4	4	4
Wheat bran	10	10	10	10
Cod liver oil	2	2	2	2
Starch	4	4	4	4
<i>Crataegus Sinaica</i> (CS)	0	1	0	1
Melamine	0	0	1	1
Vitamin premix ¹	1	1	1	1
Mineral premix ²	2	2	2	2
Total	100	100	100	100
Chemical analysis				
Crude protein (N×6.25)	30.85	30.86	34.93	34.94
Crude lipids	8.57	8.63	8.55	8.61
Crude fiber	4.69	4.93	4.67	4.91
Ash	7.20	7.27	7.32	7.39
Nitrogen-free extract ³	48.69	48.31	44.53	44.15

¹Vitamin premix (per kg of premix): vitamin A, 800,000 IU; vitamin E, 7000 mg; vitamin D3, 2,000,000 IU; vitamin K3, 1500 mg; biotin, 50 mg; folic acid, 700 mg; nicotinic, 20,000 mg; pantothenic acid, 7000 mg; vitamin B1, 700 mg; vitamin B2, 3500 mg; vitamin B6, 1000 mg; vitamin B12, 7 mg.

²Mineral premix (per kg of premix): zinc sulfate, 4.0 g; iron sulfate, 20 g; manganese sulfate, 5.3 g; copper sulfate, 2.7 g; calcium iodine, 0.34 g; sodium selenite, 70 mg; cobalt sulfate, 70 mg, and CaHPO₄·2H₂O up to 1 kg.

³Calculated by difference (100 –protein% + lipids% + ash% + crude fiber %).

was pelleted using a laboratory pelletizer, dried in air for 24 h with periodic turning, and kept at 4 °C until usage. Fish were fed daily at 3% of their body weight, distributed into three equal meals (09:00, 12:00, and 16:00), for a period of 60 days.

2.4 Assessment of growth performance and mortality

The fish were initially weighed to define their initial body weight (IBW, g) and re-weighed at the end of the study to measure the final body weight (FBW, g). The growth performance and feed efficiency were then calculated by these equations:

Weight gain (WG, g) = FBW – IBW (Pitcher and Hart, 1982).

Weight gain percentage(WG, %) = [(FBW – IBW)/IBW] × 100 (Ogunji, 2008).

Daily weight gain (DWG, g/day) = WG/duration of the experiment(days) (Pitcher and Hart, 1982).

Specific growth rate (SGR) = [(ln FBW – ln IBW)/duration of the experiment (days)] × 100 (Jobling, 1995).

Condition factor (K) = [BW(g)/Length³ (cm³)] × 100 (Datta et al., 2013)

Feed intake(FI) = Total feed consumed(g)/no. of surviving fish (Abdel-Hakim et al., 2008)

Feed conversion ratio(FCR) = Dry FI(g)/wet WG(g) (Ogunji et al., 2020).

Survival rate(%) = (no. of fish that survived/total no. of fish at the start) × 100 (El-Saidy et al., 2009).

Mortality rate(%) = (no. of fish that died/total no. of fish at the start) × 100.

2.5 Whole-body chemical composition assessment

After the completion of the feeding experiment, eight fish from each treatment group were randomly chosen, retained in plastic containers, and frozen at –20 °C for proximate analysis. To estimate the moisture amount, the samples were dried at 105 °C until they reached a constant weight. Crude protein was analyzed using the Kjeldahl distillation unit (UDK 129, VELP Scientifica, Usmate Velate, Italy), while total lipid content was quantified using Soxhlet extraction with petroleum ether for a duration of 16 hours. The ash content was determined through burning the samples at 550 °C in a muffle furnace (Barnstead/Thermolyne

Benchtop 47900, Thermo Scientific, Massachusetts, United States) for 6 hours, and the percentage of ash was calculated based on the weight loss, following the procedures outlined by AOAC (2005).

2.6 Sample collection

At the accomplishment of the 60-day experiment, eight fish per group were humanely euthanized using benzocaine solution (250 mg L⁻¹) as described by Neiffer and Stamper (2009). The small intestines were dissected and divided into three portions for subsequent analyses. One set of samples was preserved in 10% neutral buffered formalin for histopathological examination. Another portion was immediately placed in TRIzol reagent (Thermo Scientific) and kept at -80 °C for RNA extraction and assessment of inflammation-related gene expression. The remaining intestinal samples were collected on ice, rinsed with sterile saline, blotted dry, and stored at -20 °C for the determination of digestive enzyme activities and oxidative stress markers.

2.7 Biochemical examination of intestinal homogenate

2.7.1 Measurement of intestinal enzyme activities

Immediately following dissection, the intestine was carefully removed, cleaned with ice-cold saline solution to eliminate any remaining contents, and weighed. The tissues were then homogenized in ice-cold phosphate buffer at a 1:9 ratio. The homogenates were then centrifuged for 15 minutes at 8000 × g 4 °C by a Hettich EBA 20 centrifuge (Hettich, Germany), and the supernatants were collected for subsequent enzyme assays. The total protein content in the supernatant was estimated by the Bradford technique (Kruger, 2009).

Lipase activity was assessed by monitoring the hydrolysis of p-nitrophenyl palmitate (p-NPP), leading to the release of p-nitrophenol, which appears yellow in color (Winkler and Stuckmann, 1979). The reaction was performed at 37 °C, and the absorbance of the liberated p-nitrophenol was recorded at a wavelength of 405 nm. A unit of lipase activity was quantified as the enzyme amount needed to produce 1 μmol of p-nitrophenol per minute. Amylase activity was measured using soluble starch as the substrate, following the method established by Bernfeld (1955). Once the reaction was completed, it was terminated with dinitrosalicylic acid (DNS) reagent, and the mixture was heated in a water bath to produce a color change. The absorbance was then read at 540 nm, with enzyme activity defined as the amount of maltose released per minute per milligram of protein. For protease activity, casein was used as the substrate, and the procedure outlined by Walter (1984) was followed. After the incubation at 37 °C, the reaction was halted by the addition of trichloroacetic acid, and the absorbance of the released tyrosine-containing peptides was determined at 280 nm. Protease activity was recorded as the amount of enzyme needed to generate 1 μg of tyrosine each minute, corresponding to one unit of activity.

2.7.2 Evaluation of oxidative stress and lipid peroxidation markers

The activities of intestinal glutathione S-transferases (GST), CAT, GPx, and SOD were quantified using colorimetric assay kits from Biodiagnostic Co. (Cairo, Egypt), with the following catalog numbers: GT 2519, CA 25 17, GP 25 24, and SD 25 21, respectively, in accordance with the manufacturer's recommendations. The intestinal MDA levels were determined using the Biodiagnostic MDA colorimetric kit (Catalog No: MD 25 29).

2.8 Histopathological examination

Intestinal samples were placed in 10% formalin for 48 hours, dehydrated through a graded ethanol series, cleared in xylene, and inserted in paraffin to prepare tissue blocks. Five μm-thick sections were cut using a microtome and subsequently stained with hematoxylin and eosin for histological evaluation using a light microscope (Olympus CX23, Tokyo, Japan) (Suvarna et al., 2018).

For each fish, five randomly selected, non-overlapping microscopic fields per organ were examined blindly by a pathologist under a 10× objective, and intestinal parameters were evaluated based on the observed histological features. Histopathological intestinal alterations, including denuded lamina epithelia, goblet cell hyperplasia, inflammatory cell infiltrates, and hemorrhages were quantitatively assessed using a semi-quantitative scoring system described by Bernet et al. (1999). Scores ranged from 0 (no observable alteration) to 4 (widespread lesion), indicating the severity of the changes. The results were expressed as mean ± SE.

2.9 Evaluation of inflammation-related gene expression

For transcriptomic analysis, approximately 50 mg of intestinal tissue from eight fish per group was used to extract total RNA by QIAzol reagent (Qiagen, Germany) at a volume of 1 mL per sample. The purity concentration of RNA was evaluated by a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), with an acceptable A260/A280 ratio ranging from 1.8 to 2.0. Complementary DNA (cDNA) was synthesized by the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following Khamis et al. (2021). The expression of intestinal inflammation-related genes, including interleukin-10 (*il-10*), interleukin-1β (*il-1β*), interleukin-8 (*il-8*), interleukin-6 (*il-6*), tumor necrosis factor (*tnf-α*), and toll-like receptor (*tlr-4*), was quantified through real-time quantitative PCR (RT-qPCR) by gene-specific primers itemized in Table 2. RT-qPCR was carried out by first denaturing the samples for 10 minutes at 95 °C. Then, 40 amplification cycles consisting of 10 seconds at 95 °C, 15 seconds at 60 °C, and 15 seconds at 72 °C were applied. A melt-curve analysis was performed at the end of the cycles. The relative expression of genes was determined by the 2^{-ΔΔCt} method, with elongation factor 1 alpha (*ef-1α*) serving as the reference gene, as defined by Schmittgen and Livak (2008).

TABLE 2 Primers sequences of *Oreochromis niloticus* target genes used in the real-time quantitative PCR study.

Primer		Primer sequences (F: forward and R: reverse)	Efficiency	Accession no.	Size
<i>ef-1α</i>	F	ACGTCAAGAACGTCTCCGTC	99.64	NM_001279647.1	141
	R	CATAGCCTGCAGCGATCTGA			
<i>il-10</i>	F	ATGAGCAGAAGGCCTGTCAC	100.28	XM_013269188.3	102
	R	ACCCAGTGAGGTGATGGGTA			
<i>il-1b</i>	F	CTCATGTCTGTCCGTACCC	96.5	KF747686.1	103
	R	TGAAGCTTCTGTAGCGTGGG			
<i>il6</i>	F	GGCATCTCCTCAGGCAAAGT	100.45	MN870642.1	93
	R	AGCTGACCGTCAAGGAACTG			
<i>il-8</i>	F	CAAGATCATGTCCAGCAGATCC	98.52	NM_001279704.1	119
	R	TCGTGAAAGGAACACGGTGA			
<i>tnf-α</i>	F	CTGCTCCCTTCCACTCCTTG	95.81	XM_013266975.3	74
	R	CCGCTATCTGTGAGAGGCTG			
<i>tlr-4</i>	F	TTCGCAGCAGATGACCACTT	94.63	XM_005453840.4	82
	R	CAATGCGCTCTGATGCTGTC			

ef-1α, Elongation Factor 1 Alpha; *il-10*, Interleukin 10; *il-1β*, Interleukin 1 Beta; *il-6*, Interleukin 6; *il-8*, Interleukin 8; *tnf-α*, Tumor Necrosis Factor Alpha; *tlr-4*, Toll-Like Receptor 4.

2.10 Statistical analysis

The normality of the data and the homogeneity of variances were evaluated by the Kolmogorov-Smirnov test (Berger and Zhou, 2014) and Levene's test (Nordstokke and Zumbo, 2010), respectively. For data meeting parametric assumptions, statistical analyses were accomplished with IBM SPSS Statistics version 21 (IBM Corp., Armonk, NY, USA) (Spss, 2011). Group differences were assessed using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test for pairwise comparisons (Benjamini and Braun, 2002). The histopathological scores as non-parametric data were analyzed using the Kruskal-Wallis test and Dunn's multiple comparisons test for *post hoc* analysis. Data are displayed as mean ± standard error (SE), with significance determined at $P < 0.05$. Graphical presentation was done by GraphPad Prism (Prism, 2019).

3 Results

3.1 GC-MS profile of CSL

The GC-MS analysis of CSL revealed a total of 13 identified compounds, as summarized in Table 3, along with their retention times, molecular characteristics, and relative peak areas (Figure 1). The identified constituents mainly belonged to flavonoids, fatty acids and their derivatives, terpenes, and aromatic compounds. The predominant compound was 4H-1-benzopyran-4-one derivative (2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-), representing 16.56% of the total peak area, followed by n-hexadecanoic acid (palmitic acid) (13.65%) and hexadecanoic acid, 2,3-dihydroxypropyl ester (13.11%). Other major constituents included 9-octadecenoic acid (oleic acid) (10.40%) and phytol (7.65%). Moderately abundant compounds comprised phthalic acid

derivatives (6.81%) and fumaric acid ester (6.75%), while lower percentages were recorded for palmitic acid methyl ester (4.45%), terpene ketone (2.23%), flavonoid glycoside (2.16%), polyunsaturated fatty acid methyl ester (1.90%), isochiapin B (1.77%), and fatty alcohol ester (1.64%).

3.2 Growth performance and nutrient utilization indices

As shown in Table 4, the groups receiving CSL supplementation exhibited a significant ($P < 0.001$) rise in FBW (17.92%), WG (33.88%), and WG% (34.78%), DWG (33.33%), SGR (21.88%), K (16.45%), and FI (6.87%) relative to the CON group. Compared to the CON group, the CSL-supplemented group showed a considerable ($P < 0.001$) reduction in FCR (20.57%). A significant ($P < 0.001$) reduction was noted in the FBW (26.78%), WG (50.59%), WG % (51.01%), DWG (50.98%), SGR (41.41%), K (23.68%), and FI (20.50%) when the group of fish subjected to MELA for 60 days was compared to the CON group ($P < 0.001$). Moreover, this group showed the highest FCR compared to all other fish groups. In contrast, the CSL + MELA group showed notably ($P < 0.001$) superior values compared to the MELA group regarding FBW, WG, WG %, DWG, SGR, K, and FI ($P < 0.001$), along with a significant reduction in FCR which very close to CON values by 7.81%, 13.68%, 12.76%, 13.73%, 8.59%, 13.16%, 7.40%, and 7.09% respectively. Survival % exhibited a notable decline in the group exposed to MELA relative to the CON group (18.19%), whereas no significant differences in survival % were observed among the other experimental groups ($P > 0.05$).

3.3 Effects on whole-body composition

Table 4 shows that incorporating CSL into the diet of *O. niloticus* led to a considerable rise ($P < 0.001$) in crude protein levels (6.82%)

TABLE 3 Retention time (RT) and peak area (%) of the different compounds identified in *Crataegus sinaica* leaves powder analyzed by GC-MS.

Peak no.	Compound	RT (min)	Peak area %	Molecular formula	MW	Classification
1.	4H-1-Benzopyran-4-One, 2-(3,4-Dimethoxyphenyl)-3,5-Dihydroxy-7-Methoxy-	41.40	16.56	C ₁₈ H ₁₆ O ₇	344	Flavonoid derivative
2.	n-Hexadecanoic acid, palmitic acid	27.06	13.65	C ₁₆ H ₃₂ O ₂	256	Saturated fatty acid
3.	Hexadecanoic Acid, 2,3-Dihydroxypropyl Ester	30.33	13.11	C ₁₉ H ₃₈ O ₄	330	Saturated fatty acid derivatives
4.	9-Octadecenoic Acid, Oleic acid	39.27	10.40	C ₁₈ H ₃₄ O ₂	282	Unsaturated fatty acid
5.	Phytol	29.69	7.65	C ₂₀ H ₄₀ O	296	Terpene alcohol
6.	1,2-Benzenedicarboxylic Acid, Phthalic Acid	36.67	6.81	C ₂₄ H ₃₈ O ₄	390	Aromatic carboxylic acid
7.	Fumaric acid, 3-methylbut-3-enyl undecyl ester	6.57	6.75	C ₂₀ H ₃₄ O ₄	338	Ester of unsaturated fatty acid
8.	Palmitic Acid methyl ester	26.23	4.45	C ₁₇ H ₃₄ O ₂	270	Saturated fatty acid derivatives
9.	2-Pentadecanone, 6,10,14-trimethyl	24.53	2.23	C ₁₈ H ₃₆ O	268	Terpene ketone
10.	4H-1-Benzopyran-4-One, 2-(3,4-Dihydroxyphenyl)-6,8-Di- α -D-Glucopyranosyl-5,7-Dihydroxy	45.23	2.16	C ₂₇ H ₃₀ O ₁₆	610	Flavonoid glycoside
11.	7,10-Octadecadienoic acid, methyl ester	29.36	1.9	C ₁₉ H ₃₄ O ₂	294	Polyunsaturated fatty acid methyl ester
12.	Isochiapin B	39.34	1.77	C ₁₉ H ₂₂ O ₆	346	Flavonoid
13.	10-Methyl-8-tetradecen-1-ol acetate	17.55	1.64	C ₁₇ H ₃₂ O ₂	268	Fatty alcohol ester

when compared to the CON group. *O. niloticus* subjected to MELA displayed a significant ($P < 0.001$) decrease in crude protein levels (9.80%), paired with an increase in crude lipid levels (19.29%) compared to the CON group. Significantly, in contrast to the MELA group, the CSL+MELA group showed a substantial enhancement in crude protein and reductions in lipid levels ($P < 0.001$), and very near to the CON group by 1.08% and 5.31%, respectively. No notable differences were observed in moisture and ash levels across the various experimental groups ($P > 0.05$).

3.4 Effects on intestinal enzymes

As indicated in Figure 2, the intestinal enzyme activities, including lipase, α -amylase, and protease, were notably ($P < 0.001$) increased in the CSL group by 13.98%, 23.70%, and

25.55%, respectively, in comparison to the CON group. On the contrary, the activities of these intestinal enzymes were notably ($P < 0.001$) reduced in the MELA group by 21.38%, 34.72%, and 32.42%, respectively, relative to the CON group. A notable ($P < 0.001$) rise in intestinal enzyme activities was recorded in the CSL+MELA group relative to the MELA group, with levels nearly matching those in the CON group by 10.11%, 11.50%, and 8.82%, respectively.

3.5 Effect on lipid peroxidation and oxidative stress indices in intestinal tissue

As revealed in Figure 3, the intestinal tissues from fish consuming CSL-containing diets had a significant ($P < 0.001$) rise in

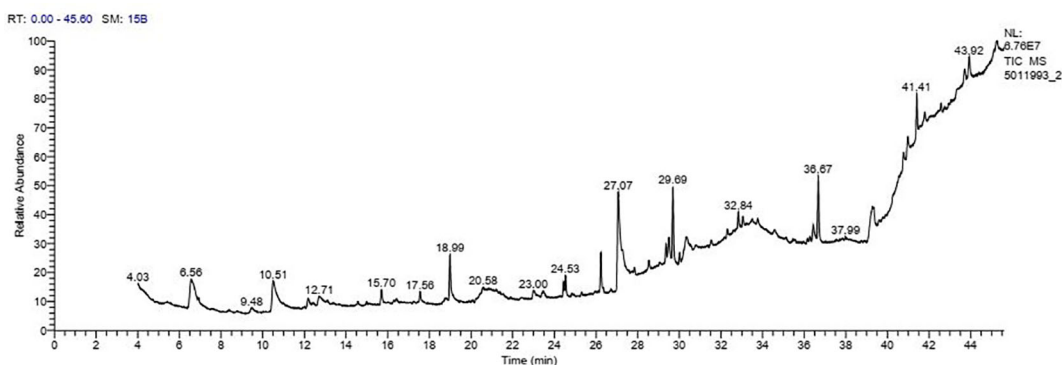


FIGURE 1
GC-MS chromatogram of *Crataegus Sinaica* leaves powder.

TABLE 4 Effect of *Crataegus Sinaica* (CSL) supplementation on growth performance and whole body composition (% dry matter basis) of *Oreochromis niloticus* fed melamine (MELA) containing diet for 60 days.

Items	Experimental groups				P-value
	CON	CSL	MELA	CSL+MELA	
Initial body weight (g)	26.43 ± 0.41	26.27 ± 0.54	26.67 ± 0.55	26.17 ± 0.43	0.893
Final body weight (g)	57.13 ± 1.06 ^b	67.37 ± 1.14 ^a	41.83 ± 0.78 ^d	52.67 ± 0.66 ^c	0.000
Weight gain (g)	30.70 ± 0.66 ^b	41.10 ± 0.61 ^a	15.17 ± 0.24 ^d	26.50 ± 0.23 ^c	0.000
Weight gain (%)	116.12 ± 0.82 ^b	156.51 ± 0.97 ^a	56.89 ± 0.52 ^d	101.30 ± 0.80 ^c	0.000
Daily Weight gain (g)	0.51 ± 0.01 ^b	0.68 ± 0.01 ^a	0.25 ± 0.004 ^d	0.44 ± 0.004 ^c	0.000
Specific growth rate (%)	1.28 ± 0.01 ^b	1.56 ± 0.01 ^a	0.75 ± 0.01 ^d	1.17 ± 0.01 ^c	0.000
Condition factor (K)	1.52 ± 0.02 ^b	1.77 ± 0.01 ^a	1.16 ± 0.004 ^d	1.32 ± 0.01 ^c	0.000
Feed intake (g)	43.23 ± 0.43 ^b	46.20 ± 0.61 ^a	34.37 ± 0.41 ^d	40.03 ± 0.52 ^c	0.000
Feed conversion ratio	1.41 ± 0.02 ^c	1.12 ± 0.01 ^d	2.27 ± 0.01 ^a	1.51 ± 0.01 ^b	0.000
Survival %	97.78 ± 2.22 ^a	100.00 ± 0.00 ^a	79.997 ± 3.85 ^b	93.33 ± 3.85 ^a	0.006
Whole body composition (% dry matter basis)					
Moisture	74.70 ± 0.21	74.45 ± 0.10	75.23 ± 0.35	74.97 ± 0.14	0.148
Crude lipid	5.08 ± 0.06 ^{bc}	4.58 ± 0.15 ^c	6.06 ± 0.02 ^a	5.35 ± 0.21 ^b	0.000
Crude protein	14.80 ± 0.06 ^b	15.81 ± 0.17 ^a	13.35 ± 0.10 ^c	14.64 ± 0.14 ^b	0.000
Ash	5.42 ± 0.13	5.16 ± 0.09	5.35 ± 0.30	5.04 ± 0.21	0.562

^{a, b, c, d}Means with different superscripts are statistically different. Values are presented as means ± SE, and *n* = 4 aquarium/treatment, 15 fish/aquarium.

SOD (17.20%), CAT (57.58%), GPX (13.43%), and GST (28.95%) activities compared to the CON group, and showed a trend toward a reduction, although not significant, in MDA levels compared to fish given a basal diet without supplements. Conversely, the intestinal tissues of fish exposed to MELA exhibited markedly (*P* < 0.001) reduced SOD (43.73%), CAT (37.88%), GPX (38.01%), and GST

(50%) activities, alongside markedly (*P* < 0.001) increased MDA levels by 128.58% relative to the CON group. The CSL + MELA group demonstrated significantly (*P* < 0.001) elevated activities of SOD (23.91%), GPX (9.01%), and GST (13.16%), along with reduced MDA levels in their intestinal tissues when compared to the MELA group and very close to CON fish by 22.78%.

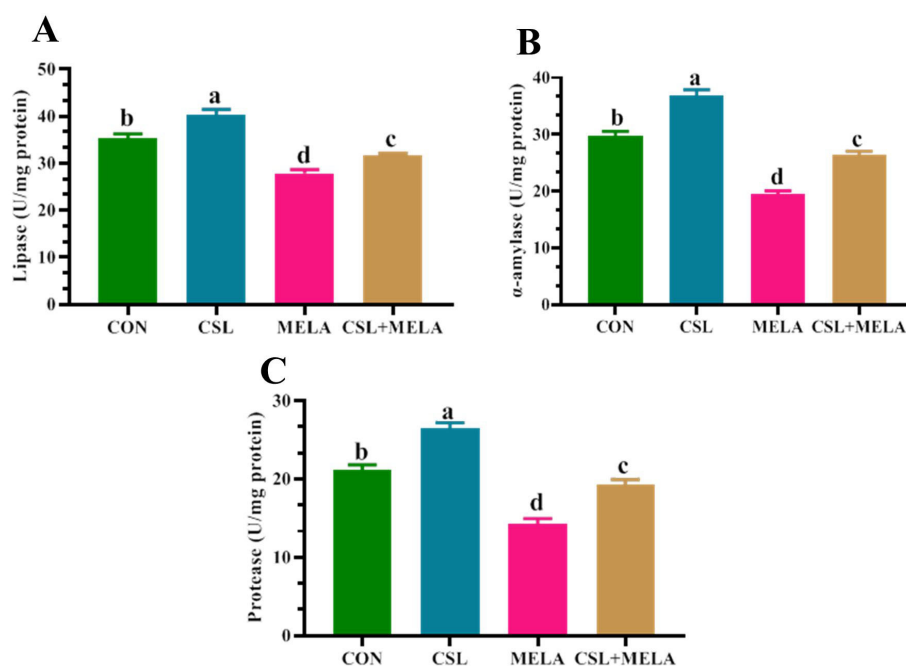


FIGURE 2

Activities of digestive enzymes in intestinal tissues of *Oreochromis niloticus* fed melamine (MELA) and/or *Crataegus sinaica* leaves powder (CSL) diets for 60 days. (A) Lipase, (B) α-Amylase, (C) Protease. Values are mean ± SE. *n* = 8 for each group. Bars with different letters (a, b, c, and d) are significantly different at *P* < 0.001.

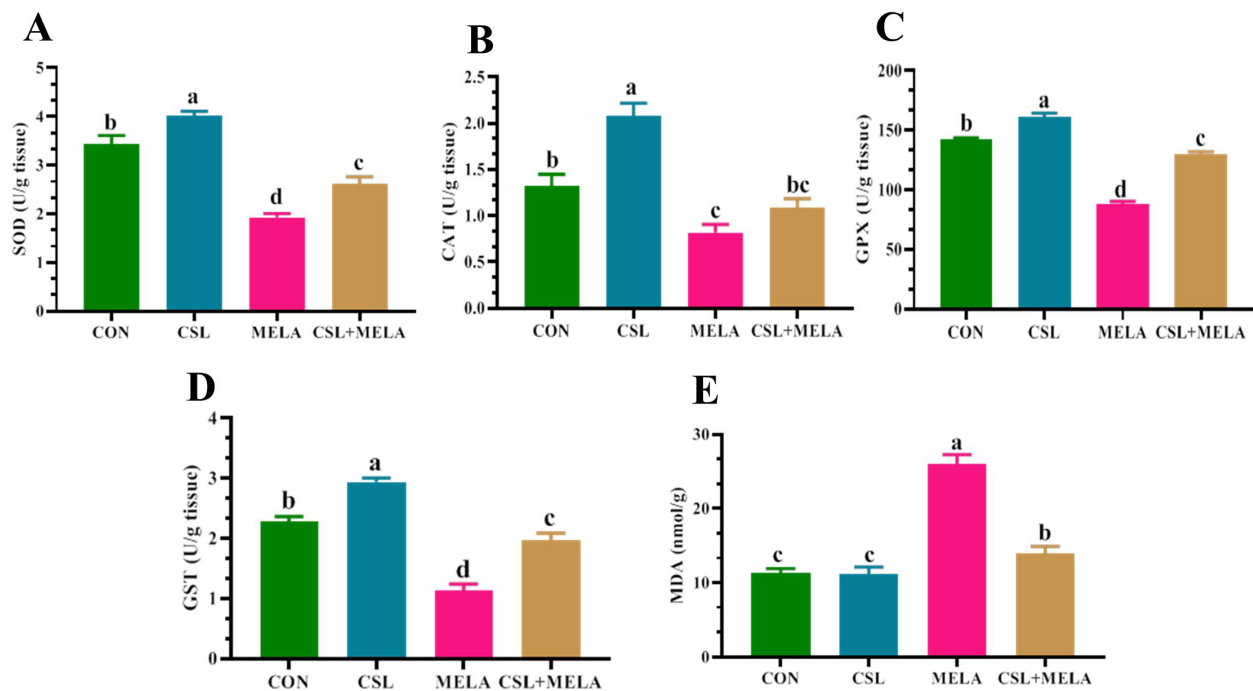


FIGURE 3

Activities of antioxidant enzymes and lipid peroxidation in intestinal tissues of *Oreochromis niloticus* fed melamine (MELA) and/or *Crataegus sinaica* leaves powder (CSL) diets for 60 days. (A) Superoxide dismutase (SOD), (B) Catalase (CAT), (C) Glutathione peroxidase (GPX), (D) Glutathione-S-transferases (GST), (E) Malondialdehyde (MDA). Values are mean ± SE. n=8 for each group. Bars with different letters (a, b, c, and d) are significantly different at $P < 0.001$.

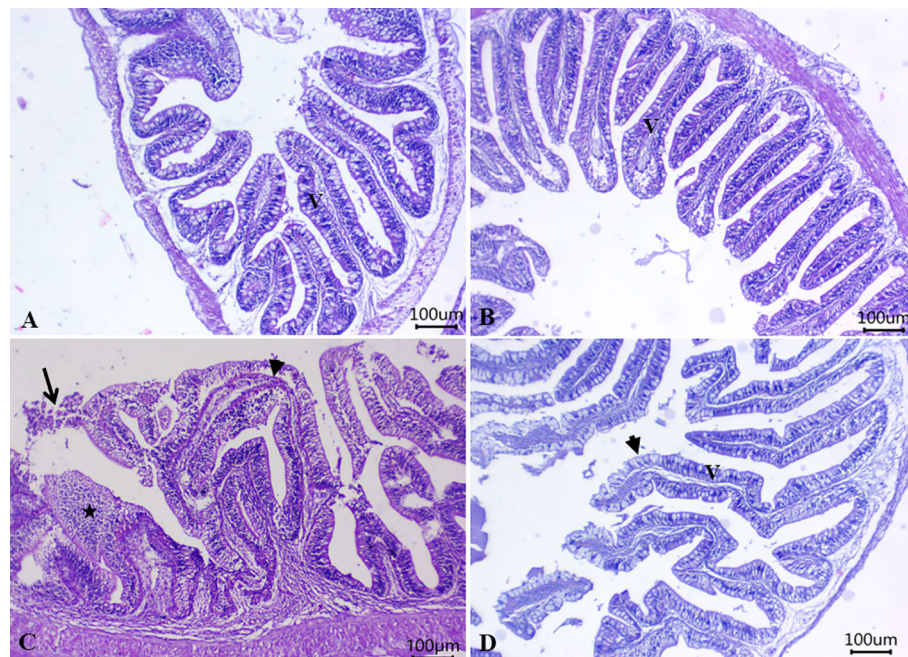


FIGURE 4

Photomicrograph of H&E-stained intestinal sections. (A) Control group showing normal histology with intact columnar epithelial lining, mucosa, submucosa, and muscular layer. (B) *Crataegus sinaica* leaves powder-fed group (CSL) showing normal histological architecture comparable to control. (C) Melamine (MELA) group showing denuded sheets of lamina epithelial cells (arrow) with inflammatory cell infiltrates, few extravasated erythrocytes within the lamina propria (arrowhead), and submucosal hemorrhage (star). (D) MELA + CSL group showing goblet cell hyperplasia (arrowhead) within the mucosal epithelium with maintained morphology of most mucosal villi (V), submucosa, and muscular layer. H&E; bar = 100 μm.

3.6 Histopathological findings

Normal intestinal histological architecture, including intact columnar epithelial lining of the mucosa, as well as well-organized submucosal and muscular layers, was observed in both the CON group (Figure 4A) and the CSL group (Figure 4B). Notably, the CSL group exhibited slightly elongated intestinal villi compared to the control. On the contrary, the MELA group (Figure 4C) showed marked histopathological alterations, including denudation of the lamina epithelium, accompanied by inflammatory cell infiltration and the presence of extravasated erythrocytes within the lamina propria and submucosal layer. Interestingly, the CSL+MELA group (Figure 4D) demonstrated evident histological improvement, as indicated by goblet cell hyperplasia within the mucosal epithelium and preservation of the overall morphology of most mucosal villi, as well as the submucosal and muscular layers.

Histopathological lesion scoring further supported these observations (Figure 5). Neither the CON nor the CSL groups exhibited any pathological alterations. In contrast, the MELA-exposed group showed significant ($P < 0.001$) intestinal damage, characterized by increased scores for denuded lamina epithelium (2.50 ± 0.22), goblet cell hyperplasia (2.70 ± 0.21), inflammatory cell infiltration (3.20 ± 0.25), and hemorrhage (1.90 ± 0.28), compared to the control group. Conversely, co-treatment with CSL (CSL+MELA group) significantly ameliorated ($P < 0.001$) these histopathological lesions, as evidenced by markedly reduced scores for denuded lamina epithelium (0.50 ± 0.17), goblet cell hyperplasia (0.90 ± 0.23), inflammatory cell infiltration (0.40 ± 0.16), and hemorrhage (0.30 ± 0.15), relative to the MELA group.

3.7 Changes in inflammatory gene expression in the intestinal tissue

As shown in Figure 6, in contrast to the CON group, tilapia on CSL-enriched diets showed no significant difference ($P > 0.05$) in inflammation-related (*il-1 β* , *il-10*, *tlr-4*, *il-6*, *tnf- α* , and *il-8*) gene expression in their intestines. Conversely, fish subjected to MELA exhibited a considerable ($P < 0.001$) upregulation of inflammatory (*tnf- α* (5.22 ± 0.35), *tlr-4* (3.01 ± 0.34), *il-6* (2.66 ± 0.11), and *il-8* (2.65 ± 0.15)) genes relative to the CON group. In the CSL+MELA group, the intestinal levels of *tnf- α* (2.35 ± 0.11) and *tlr-4* (1.76 ± 0.06) were considerably ($P < 0.001$) reduced, whereas the intestinal levels of *il-10* were significantly ($P < 0.001$) increased (2.67 ± 0.15) in comparison to the MELA-exposed group.

4 Discussion

In this research, fish that consumed diets with MELA for 60 days exhibited significantly slowed growth, evident from reduced FBW, WG%, DWG, SGR, K, and FI compared to the control group. Similar growth depression has been documented before and in other fish species, and crustaceans fed MELA in their diet (Nuntapong et al., 2019; Abd El-Hakim et al., 2020). This growth inhibition may result from MELA-induced oxidative stress, impaired digestive enzyme activity, and altered nutrient absorption, which together limit energy availability and protein utilization for growth (Nuntapong et al., 2019). In contrast, a notable

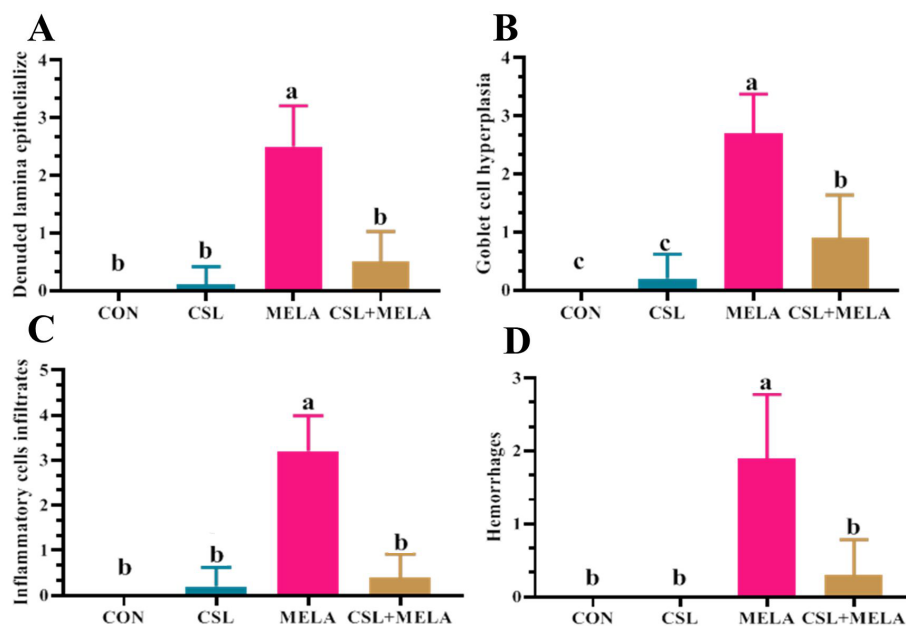


FIGURE 5

Changes in intestinal histological lesions frequency in *Oreochromis niloticus* fed melamine (MELA) and/or *Crataegus Sinaica* (CSL) containing diets for 60 days. (A) denuded lamina (B) goblet cell hyperplasia, (C) inflammatory cells infiltrates, and (D) hemorrhages. Values are mean $\pm$ SE. $n=8$ for each group. Bars with different letters (a, b, and c) are significantly different at $P < 0.001$.

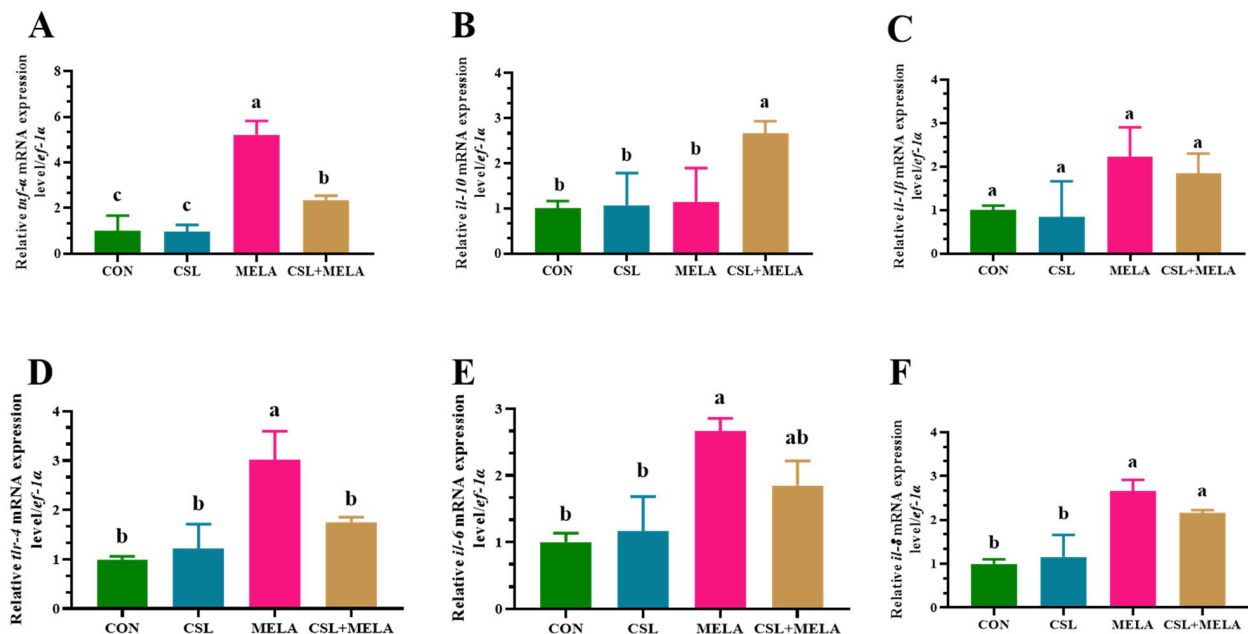


FIGURE 6

Relative expression of inflammatory markers in intestinal tissues of *Oreochromis niloticus* fed melamine (MELA) and/or *Crataegus sinaica* leaves powder (CSL) diets for 60 days. (A) tumor necrosis factor α (*tnf-α*), (B) interleukin10 (*il-10*), (C) interleukin1 beta (*il-1β*), (D) Toll-like receptor 4 (*tlr-4*), (E) interleukin6 (*il-6*) and (F) interleukin8 (*il-8*). *ef-1α*, elongation factor 1 alpha. Values are mean $\pm$ SE. $n=8$ for each group. Bars with different letters (a, b, and c) are significantly different at $P < 0.001$.

enhancement in growth metrics was noted in fish subjected to MELA and provided with diets enriched with CSL, aligning with findings from El-Houseiny et al. (2023). Similarly, the addition of 0.50 g of *C. monogyna* extract/kg to the diets of juvenile golden pompano (*Trachinotus ovatus*) over eight weeks notably enhanced the growth of the fish (Sarian et al., 2017). The observed growth improvement with CSL may be mechanistically attributed to the dietary inclusion of whole leaf powder, which provides a complex mixture of bioactive compounds, fibers, and secondary metabolites which enhance antioxidant defenses, reduce intestinal inflammation, and improve nutrient utilization. In this regard, the GC-MS profile of CSL indicated a predominance of flavonoid derivatives (e.g., benzopyran compounds and isochiapin B), fatty acids (oleic and palmitic acids), and their esters, along with terpenoid constituents such as phytol. These bioactives can scavenge ROS, protect intestinal epithelial cells, modulate gut microbiota, and suppress pro-inflammatory signaling, collectively supporting enhanced growth and feed efficiency. For example, Flavonoids are known for their strong antioxidant effects, while also exhibiting anti-inflammatory and antimicrobial activities (Barros et al., 2011; Al-Khayri et al., 2022; Chagas et al., 2022). Besides, fatty acids and their derivatives are known to exert antibacterial activity through membrane disruption and contribute to antioxidant defense mechanisms (Obukhova and Murzina, 2024). Terpenoid compounds such as phytol further enhance these effects owing to their reported antioxidant and antimicrobial activities (Pejin et al., 2014; Sarian et al., 2017). The synergistic action of these compounds likely underlies the biological efficacy of CSL in mitigating MELA-induced toxicity. Similar benefits have been reported in other fish species with herbal leaf powders (Abd El-Gawad et al., 2020; El-Kassas et al., 2022; Ujan et al., 2025).

The activity of digestive enzymes, which is influenced by diet composition, reflects feeding behaviors, digestive processes, and nutrient utilization, and is closely linked to growth (Duan et al., 2017). The present research revealed that tilapia fed MELA-containing diets showed notably reduced enzyme activities, suggesting impaired digestion and nutrient absorption. In contrast, CSL dietary supplementation for MELA-exposed fish significantly restored digestive enzyme activities, likely through its bioactive compounds promoting gut integrity and possibly acting as prebiotics to support beneficial microbiota (Sammari et al., 2021). Likewise, Ahmadipour et al. (2020) suggested that the beneficial impacts of dietary hawthorn on digestive enzyme function, together with intestinal structure and microbiome, might account for their growth-promoting effects.

The maintenance of normal intestinal function relies on the integrity of the intestinal morphological structure (Gao et al., 2013). The documented histological intestinal changes in fish exposed to MELA supported these results and were enhanced by CSL dietary supplementation. The intestine showed marked denuded lamina epithelialization, goblet cell hyperplasia, infiltration of inflammatory cells, and hemorrhaging relative to the control group. Similarly, Andersen et al. (2008) observed that fish administered MELA (400 mg/kg) daily for 3 days developed crystals in the mucosa and submucosa of the stomach, pyloric caeca, and intestines, with some fish also showing crystals accompanied by a minor lymphocyte infiltrate in the muscularis of these organs. In contrast, CSL supplementation preserved the intestinal villi structure, reduced inflammatory cell infiltration, and supported goblet cell function, indicating improved mucosal barrier integrity and enhanced nutrient absorption. The observed improvement in antioxidant status and the concomitant reduction in intestinal inflammatory markers

in the MELA+CSL group likely contributed to these beneficial effects. Such morphological preservation is consistent with reports of hawthorn extracts enhancing intestinal structure and microbiota (Tan et al., 2020).

In the current experiment, MELA exposure also altered body composition, resulting in decreased crude protein and increased crude lipid content. This may be linked to oxidative stress induced by MELA, as evidenced by reduced activities of antioxidant enzymes and elevated lipid peroxidation, which can impair normal metabolic processes, including protein and lipid metabolism (Phromkunthong et al., 2015). Additionally, MELA has been reported to cause renal and hepatic damage, including nephropathy and crystal formation in renal tubules, which could disrupt nitrogen excretion and protein metabolism due to the critical role of the kidneys in these processes (Mahardika et al., 2017). Previous studies in fish have documented MELA-induced histopathological changes in the liver and kidneys, which may indirectly affect metabolic functions such as protein and fat metabolism (Phromkunthong et al., 2015; Abd El-Hakim et al., 2020). On the contrary, CSL supplementation restored protein levels and reduced lipid accumulation, likely through enhanced digestive efficiency and modulation of metabolic pathways via its bioactive compounds. Additionally, CSL is rich in bioactive compounds known for their hepatorenal protective effects, largely attributed to their antioxidant properties, which could positively influence protein metabolism (Refaat et al., 2010; Hoseini et al., 2021). Similarly, hawthorn extract added to the diet boosted the overall crude protein and crude lipid levels in fish bodies, yet decreased the lipid content in muscles, indicating that hawthorn extract may influence fat distribution and enhance abdominal subcutaneous fat in golden pompano (Tan et al., 2020).

Herein, fish fed MELA-containing diets exhibited significantly reduced antioxidant enzyme activities and increased MDA levels in the intestinal tissue of fish, indicating enhanced oxidative stress. This response can be mechanistically explained by the ability of MELA to generate ROS, which overwhelms the endogenous antioxidant defense system (Iheanacho et al., 2021). The activation of NADPH oxidase (NOX) enzymes, including NOX1, NOX2, and NOX4, has been implicated in MELA-induced ROS production, amplifying oxidative damage and promoting lipid peroxidation (Kuo et al., 2013). Consequently, the suppressed activities of key antioxidant enzymes such as SOD, CAT, and GPX reduce the fish's capacity to neutralize ROS, further exacerbating cellular injury. These mechanistic insights align with previous observations of oxidative stress and tissue damage following MELA exposure (Lee et al., 2016; Nuntapong et al., 2019; Abd El-Hakim et al., 2020). Conversely, intestinal tissues from fish fed CSL-supplemented diets exhibited a marked increase in the activities of SOD, CAT, GPx, and GST, accompanied by a trend toward reduced MDA levels compared to control fish. Additionally, in the CSL+MELA group, antioxidant enzyme activities were notably restored, and lipid peroxidation was reduced relative to the MELA-only group. While the initial rise in antioxidant enzymes could reflect a compensatory response to oxidative stress, the concurrent decrease in MDA indicates that CSL supplementation effectively mitigates oxidative damage by strengthening the organism's intrinsic antioxidant defenses. This dual effect highlights CSL's role in both

lowering the pro-oxidant burden and enhancing endogenous antioxidant capacity. These protective effects are likely linked to the bioactive compounds present in CSL. The extract contains flavonoid derivatives, such as benzopyran compounds and isochiapin B, which are recognized for their potent antioxidant and free radical scavenging properties (Gaspar et al., 2015; Salama et al., 2024). These molecules function primarily by donating hydrogen atoms and stabilizing reactive oxygen species, thereby safeguarding cellular components from oxidative damage (Sarian et al., 2017). In addition, phenolic constituents within CSL further augment antioxidant defenses through free radical trapping and inhibition of lipid peroxidation (Zhang et al., 2001; Martinelli et al., 2021). The current findings are consistent with El-Houseiny et al. (2023), who reported a significant recovery of antioxidant enzyme activities and reduced lipid peroxidation in acrylamide-exposed fish receiving CSL-supplemented diets. Similarly, Zhang et al. (2014) demonstrated that an 80% hawthorn ethanol extract, rich in compounds such as procyanidin B2, isoquercitrin, chlorogenic acid, and epicatechin, significantly augmented SOD and CAT activities while lowering MDA levels.

In this study, MELA exposure significantly upregulated *tlr-4*, *tnf- α* , *il-6*, and *il-8* gene expression in the fish intestine, indicating that MELA alters immune-related organs and triggers inflammatory responses. *tnf- α* and *il-1 β* are key proinflammatory cytokines secreted by activated macrophages that modulate innate immune responses and drive inflammation (Dinarello, 2018). *il-10* serves as an anti-inflammatory cytokine, whereas *il-8* promotes inflammation (Luo et al., 2025). Mechanistically, excessive ROS production induced by MELA can activate the *nf- κ b* signaling pathway (Alsharif et al., 2023). Under basal conditions, *nf- κ b* remains inactive in the cytoplasm; upon activation, it translocates to the nucleus, regulating target genes involved in inflammatory responses (Habotta et al., 2023). These observations are consistent with Abd El-Hakim et al. (2020), who reported elevated splenic *il-1 β* and *tnf- α* expression following MELA exposure. Similarly, Habotta et al. (2023) observed significant upregulation of *tlr-4*, along with activation of high-mobility group box-1 (*hmgb-1*) and receptor for advanced glycation end products (*rage*), in cortical tissues of MELA-exposed rats. *hmgb-1* interaction with *rage* and *tlr-4* initiates inflammation by triggering multiple intracellular pathways, leading to the release of cytokines such as *il-6*, *il-8*, *tnf- α* and activation of *nf- κ b* (Abdeen et al., 2021). Importantly, fish exposed to MELA and fed a diet supplemented with CSL demonstrated a notable increase in *il-10* expression, accompanied by reductions in *tnf- α* and *tlr-4*. This anti-inflammatory effect may be mediated through modulation of *ampk/sirt1/nf- κ b* signaling pathways, as suggested by studies on hawthorn polyphenol extracts (Liu et al., 2021). Similarly, Luo et al. (2025) reported that hawthorn polysaccharides enhanced *il-10* while reducing *il-8* in crucian carp under stress conditions. The bioactive compounds in CSL, including polyphenols and flavonoids, are recognized for their ability to prevent and alleviate inflammation (Li et al., 2022). For instance, hawthorn-derived flavonoids reduced *tnf- α* -induced intestinal epithelial barrier damage in Caco-2 cells, improving epithelial integrity (Liu et al., 2020). Oligomeric proanthocyanidins from hawthorn have also been shown to suppress proinflammatory cytokine expression, mitigating

inflammatory responses in rats (Elango and Devaraj, 2010). Moreover, antioxidant phytochemicals can modulate the *hmgb-1/rage/tlr-4* pathway to attenuate inflammation in various models (Badawi et al., 2021). Habotta et al. (2023) further demonstrated that sesquiterpene nootkatone inhibits the combined activity of *hmgb-1*, *rage*, *tlr-4*, and *nf-kb*, highlighting the potential of natural compounds, such as those in CSL, to reduce MELA-induced inflammation and tissue injury.

5 Conclusion

From the results shown, it can be inferred that the incorporation of MELA in *O. niloticus* diets adversely affects their growth, intestinal digestive enzymes, overall body composition, oxidative stress indicators, intestinal histological structure, inflammatory response, and associated gene expression. Nonetheless, the research showed that these negative effects can be largely reduced by adding CSL to the diet. CSL supplementation effectively returns most parameters to levels similar to those of the controls and may be proposed as an effective feed additive to mitigate the previous adverse effects. Additional studies are required to clarify other potential underlying mechanisms of CSL and assess its effectiveness on different fish species.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

All experimental procedures were performed at the Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Zagazig University, Egypt, following approval by the Institutional Animal Care and Use Committee (ZU-IACUC/2/F/342/2026) and in line with NIH recommendations for the care and use of laboratory animals. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

WE-H: Conceptualization, Data curation, Investigation, Methodology, Software, Writing – original draft. AM: Conceptualization, Resources, Software, Writing – review & editing. AB: Conceptualization, Data curation, Methodology, Software, Writing – original draft. ASA: Conceptualization, Resources, Software, Writing – review & editing. YA: Conceptualization, Resources, Software, Writing – review & editing. YA-E: Conceptualization, Software, Visualization, Writing – original

draft. MA: Conceptualization, Resources, Software, Writing – review & editing. RM: Conceptualization, Software, Visualization, Writing – review & editing. AOA: Conceptualization, Software, Visualization, Writing – review & editing. BH: Conceptualization, Investigation, Methodology, Writing – review & editing. EM: Conceptualization, Investigation, Methodology, Writing – review & editing. YM: Conceptualization, Investigation, Methodology, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU261548]. Also, the authors thank all staff of the Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, for their support during the work. The authors also thank Dr. Mohamed S. Sobh, Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt, for his assistance in the histopathological evaluation of this study.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author YA-E declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Abarike, E. D., Jian, J., Tang, J., Cai, J., Yu, H., and Chen, L. (2019). Traditional Chinese medicine enhances growth, immune response, and resistance to *Streptococcus agalactiae* in Nile tilapia. *J. Aquat. Anim. Health* 31, 46–55. doi: 10.1002/aah.10049
- Abdeen, A., Samir, A., Elkomy, A. A., Aboubaker, M., Habotta, O. A., Gaber, A., et al. (2021). The potential antioxidant bioactivity of date palm fruit against gentamicin-mediated hepato-renal injury in male albino rats. *Biomed. Pharmacother.* 143, 112154. doi: 10.1016/j.biopha.2021.112154
- Abd El-Gawad, E. A., El Asely, A. M., Soror, E. I., Abbass, A. A., and Austin, B. (2020). Effect of dietary Moringa oleifera leaf on the immune response and control of *Aeromonas hydrophila* infection in Nile tilapia (*Oreochromis niloticus*) fry. *Aquac. Int.* 28, 389–402. doi: 10.1016/j.aquac.2019.46730
- Abd El-Hakim, Y. M., El-Houseiny, W., El-Murr, A. E., Ebraheim, L. L. M., Moustafa, A. A., and Rahman Mohamed, A. A. (2020). Melamine and curcumin enriched diets modulate the haemato-immune response, growth performance, oxidative stress, disease resistance, and cytokine production in *Oreochromis niloticus*. *Aquat. Toxicol.* 220, 105406. doi: 10.1016/j.aquatox.2020.105406
- Abdel-Hakim, N., Lashin, M., Al-Azab, A., and Nazmi, H. (2008). "Effect of replacing soybean meal protein by other plant protein sources on growth performance and economic efficiency of mono sex Nile tilapia (*Oreochromis niloticus*) cultured in tanks", In: Elghobashy, H., Fitzsimmons, K., and Diab, A. S. (Eds.), Proceedings of the 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, 12–14 October 2008, pp. 739–755.
- Abdel Rahman, A. N., Elhady, M., and Hassanin, M. E. (2018). Effect of Indian lotus (*Nelumbo nucifera* Gaertn.) leaf powder on immune status and disease resistance of Nile tilapia. *Aquac. Res.* 49, 3392–3399. doi: 10.1111/are.13803
- Abdel-Razek, N., Awad, S. M., and Abdel-Tawwab, M. (2019). Effect of dietary purslane (*Portulaca oleracea* L.) leaves powder on growth, immunostimulation, and protection of Nile tilapia, *Oreochromis niloticus* against *Aeromonas hydrophila* infection. *Fish Physiol. Biochem.* 45, 1907–1917. doi: 10.1007/s10695-019-00685-8
- Ahmadipour, B., Kalantar, M., Schreurs, N. M., Raza, S. H. A., Khan, R., Khan, S., et al. (2020). Flavonoid bioactive compounds of hawthorn extract can promote growth, regulate electrocardiogram waves, and improve cardiac parameters of pulmonary hypertensive chickens. *Poult. Sci.* 99, 974–980. doi: 10.1016/j.psj.2019.10.022
- Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., and Al-Mssalleem, M. Q. (2022). Flavonoids as potential anti-inflammatory molecules: A review. *Molecules* 27, 2901. doi: 10.3390/molecules27092901
- Alsharif, I. A., Abd-Elsalam, R. M., Amer, M. S., El-Desoky, A. H., and Abdel-Rahman, R. F. (2022). *Crataegus sinaica* defatted methanolic extract ameliorated monosodium iodoacetate-induced oxidative stress and inhibited inflammation in a rat model of osteoarthritis. *Res. Pharm. Sci.* 17, 493–507. doi: 10.4103/1735-5362.355209
- Alsharif, K. F., Albrakati, A., Al Omairi, N. E., Almalki, A. S., Alsanie, W., Abd Elmageed, Z. Y., et al. (2023). Neuroprotective efficacy of the bacterial metabolite, prodigiosin, against aluminium chloride-induced neurochemical alterations associated with Alzheimer's disease murine model: Involvement of Nrf2/HO-1/NF- κ B signaling. *Environ. Toxicol.* 38, 266–277. doi: 10.1002/tox.23718
- Andersen, W. C., Turnipseed, S. B., Karbiwnyk, C. M., Clark, S. B., Madson, M. R., Gieseke, C. M., et al. (2008). Determination and confirmation of melamine residues in catfish, trout, tilapia, salmon, and shrimp by liquid chromatography with tandem mass spectrometry. *J. Agric. Food. Chem.* 56, 4340–4347. doi: 10.1021/jf800295z
- Aoac (2005). *Official Methods of Analysis of the Association of Analytical Chemists International*. 18th Ed (Gaithersburg, Md. USA: Association of Official Analytical Chemists), 18.
- A.O.A.C. (2005). *Official Methods of Analysis of the Assoc. of Official Agricultural Chemists*. 12th ed (USA, Washington, DC: AOAC International, Gaithersburg, MD), 12.
- Aoac (2006). *Official methods of analysis*. 18th edn (USA, Washington,DC), 18.
- Badawi, G. A., Shokor, M. M., Zaki, H. F., and Mohamed, A. F. (2021). Pentoxifylline prevents epileptic seizure by modulating HMGB1/RAGE/TLR4 signalling pathway and improves memory in pentylenetetrazol kindling rats. *Clin. Exp. Pharmacol. Physiol.* 48, 1111–1124. doi: 10.1111/1440-1681.13508
- Barros, L., Carvalho, A. M., and Ferreira, I. C. (2011). Comparing the composition and bioactivity of *Crataegus monogyna* flowers and fruits used in folk medicine. *Phytochem. Anal.* 22, 181–188. doi: 10.1002/pca.1267
- Benjamini, Y., and Braun, H. (2002). John W. Tukey's contributions to multiple comparisons. *Ann. Stat.* 30, 1576–1594. doi: 10.1002/j.2333-8504.2002.tb01891.x
- Berger, V. W., and Zhou, Y. (2014). Kolmogorov-smirnov test: overview. In Wiley StatsRef: Statistics Reference Online. John Wiley & Sons, Ltd. doi: 10.1002/9781118445112.stat06558
- Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., and Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *J. Fish Dis.* 22, 25–34. doi: 10.1046/j.1365-2761.1999.00134.x
- Bernfeld, P. (1955). α -amylase. *Methods Enzymol.* 1, 149–151. doi: 10.1016/0076-6879(55)01021-5
- Boyd, C. E., and Tucker, C. S. (2012). *Pond aquaculture water quality management* (New York, USA: Springer Science & Business Media).
- Chagas, M. D. S. S., Behrens, M. D., Moragas-Tellis, C. J., Penedo, G. X., Silva, A. R., and Gonçalves-De-Albuquerque, C. F. (2022). Flavonols and flavones as potential anti-inflammatory, antioxidant, and antibacterial compounds. *Oxid. Med. Cell. Longev.* 2022, 9966750. doi: 10.1155/2022/9966750
- Dahran, N., Alotaibi, B. S., Abd-Elhakim, Y. M., Mohamed, A.-R., Ibrahim, R. E., Metwally, M. M., et al. (2025). Dietary purslane (*Portulaca oleracea* L.) leaf powder maintains growth and intestinal health in *Oreochromis niloticus* under chronic water-borne cadmium exposure by strengthening the gut barriers, modulating the intestinal nutrient transporters, and relieving oxidative stress. *Fish Physiol. Biochem.* 51, 8. doi: 10.1007/s10695-024-01415-5
- Datta, S. N., Kaur, V. I., Dhawan, A., and Jassal, G. (2013). Estimation of length-weight relationship and condition factor of spotted snakehead *Channa punctata* (Bloch) under different feeding regimes. *Springerplus* 2, 436. doi: 10.1186/2193-1801-2-436
- Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol. Rev.* 281, 8–27. doi: 10.1111/immr.12621
- Duan, Y., Zhang, Y., Dong, H., Zheng, X., Wang, Y., Li, H., et al. (2017). Effect of dietary poly- β -hydroxybutyrate (PHB) on growth performance, intestinal health status and body composition of Pacific white shrimp *Litopenaeus vannamei* (Boone 1931). *Fish Shellfish Immunol.* 60, 520–528. doi: 10.1016/j.fsi.2016.11.020
- Elango, C., and Devaraj, S. N. (2010). Immunomodulatory effect of Hawthorn extract in an experimental stroke model. *J. Neuroinflamm.* 7, 97. doi: 10.1186/1742-2094-7-97
- El-Houseiny, W., Abd-Allah, N. A., Abd-Elhakim, Y. M., Abdel-Warith, A.-W., Younis, E. M., Davies, S. J., et al. (2024). Dietary garden cress (*Lepidium sativum*) seeds mitigate the effect of aflatoxin B1 contamination on growth, antioxidant status, AFB1 residues, immune response, and tissue architecture of *Oreochromis niloticus*. *Aquac. Rep.* 36, 102040. doi: 10.1016/j.aqrep.2024.102040
- El-Houseiny, W., Abdelmageed, M., Abd-Elhakim, Y. M., Abdel-Warith, A.-W., Younis, E. M., Abd-Allah, N. A., et al. (2023). The effect of dietary *Crataegus sinaica* on the growth performance, immune responses, hemato-biochemical and oxidative stress indices, tissues architecture, and resistance to *Aeromonas sobria* infection of acrylamide-exposed *Clarias gariepinus*. *Aquac. Rep.* 30, 101576. doi: 10.1080/10408398.2014.914889
- El-Houseiny, W., Basher, A. W., Mahmoud, Y. K., Bayoumi, Y., Abdel-Warith, A.-W., Younis, E. M., et al. (2025). Mitigation of sodium fluoride-induced growth inhibition, immunosuppression, hepatorenal damage, and dysregulation of oxidative stress, apoptosis, and inflammation-related genes by dietary artichoke (*Cynara scolymus*) leaf extract in *Oreochromis niloticus*. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* 277, 111068. doi: 10.1016/j.cbpb.2024.111068
- El-Kassas, S., Aljahdali, N., Abdo, S. E., Alaryani, F. S., Moustafa, E. M., Mohamed, R., et al. (2022). Moringa Oleifera leaf powder dietary inclusion differentially modulates the antioxidant, inflammatory, and histopathological responses of normal and *Aeromonas hydrophila*-infected mono-sex Nile tilapia (*Oreochromis niloticus*). *Front. Vet. Sci.* 9, 918933. doi: 10.3389/fvets.2022.918933
- El-Saidy, D., Mahmoud, S., and Tonsy, H. (2009). Nutrition evaluation of sesame seed meal, *Sesamum indicum* (L.) as alternative protein source in diets of juvenile mono-sex Nile tilapia (*Oreochromis niloticus*). *Egypt. J. Aquat. Biol. Fish.* 13, 93–106. doi: 10.21608/efab.2009.2026
- El-Shahaby, O., Reicha, F., Aboushadi, M. M. N., and El-Zayat, M. (2020). Green synthesis and biological assessments of silver nanoparticles using the plant extract of *Crataegus sinaica* Boiss. *Fruits. Prog. Chem. Biochem. Res.* 3, 105–113. doi: 10.33945/sami/pcbr.2020.2.3
- Gao, Y., Han, F., Huang, X., Rong, Y., Yi, H., and Wang, Y. (2013). Changes in gut microbial populations, intestinal morphology, expression of tight junction proteins, and cytokine production between two pig breeds after challenge with *Escherichia coli* K88: a comparative study. *J. Anim. Sci.* 91, 5614–5625. doi: 10.2527/jas.2013-6528
- Gaspar, A., Milhazes, N., Santana, L., Uriarte, E., Borges, F., and Matos, M. J. (2015). Oxidative stress and neurodegenerative diseases: looking for a therapeutic solution inspired on benzopyran chemistry. *Curr. Top. Med. Chem.* 15, 432–445. doi: 10.2174/1568026614666141229124141
- Habotta, O. A., Abdeen, A., El-Hanafy, A. A., Yassin, N., Elgameel, D., Ibrahim, S. F., et al. (2023). Sesquiterpene nootkatone counteracted the melamine-induced neurotoxicity by repressing of oxidative stress, inflammatory, and apoptotic trajectories. *Biomed. Pharmacother.* 165, 115133. doi: 10.1016/j.biopha.2023.115133
- Hoseini, S. M., Gharavi, B., Taheri Mirghaed, A., Hoseinifar, S. H., and Van Doan, H. (2021). Effects of dietary phytol supplementation on growth performance, immunological parameters, antioxidant and stress responses to ammonia exposure in common carp, *Cyprinus carpio* (Linnaeus 1758). *Aquaculture* 545, 737151. doi: 10.1016/j.aquaculture.2021.737151
- Iheanacho, S. C., Odo, G. E., and Ezewudo, B. I. (2021). Adulteration of aquafeed with melamine and melamine-formaldehyde chemicals; Ex situ study of impact on haematology and antioxidant systems in *Clarias gariepinus*. *Aquac. Res.* 52, 2078–2084. doi: 10.1111/are.15059

- Jiang, L., Tang, X., Lu, J., He, G., Xia, J., Li, Y., et al. (2025). Effect of adlay (Coix lachryma-jobi L.) on growth, antioxidant, immune modulation, and intestinal health of crucian carp (*Carassius auratus*). *Aquac. Int.* 33, 166. doi: 10.1007/s10499-025-01843-x
- Jobling, M. (1995). Fish bioenergetics. *Oceanogr. Lit. Rev.* 9, 785.
- Kapinga, I. B., Limbu, S. M., Madalla, N. A., Kimaro, W. H., and Tamatamah, R. A. (2018). *Aspilia mossambicensis* and *Azadirachta indica* medicinal leaf powders modulate physiological parameters of Nile tilapia (*Oreochromis niloticus*). *Int. J. Vet. Sci. Med.* 6, 31–38. doi: 10.1016/j.ijvsm.2018.03.003
- Khamis, T., Abdelalim, A. F., Saeed, A. A., Edress, N. M., Nafea, A., Ebian, H. F., et al. (2021). Breast milk MSCs upregulated β -cells PDX1, Ngn3, and PCNA expression via remodeling ER stress/inflammatory/apoptotic signaling pathways in type 1 diabetic rats. *Eur. J. Pharmacol.* 905, 174188. doi: 10.1016/j.ejphar.2021.174188
- Kruger, N. J. (2009). "The Bradford method for protein quantitation," in *The protein protocols handbook*, ed. J. M. Walker, 17–24. Totowa, NJ: Humana Press.
- Kuo, F. C., Tseng, Y. T., Wu, S. R., Wu, M. T., and Lo, Y. C. (2013). Melamine activates NF κ B/COX-2/PGE2 pathway and increases NADPH oxidase-dependent ROS production in macrophages and human embryonic kidney cells. *Toxicol. Vitro* 27, 1603–1611. doi: 10.1016/j.tiv.2013.04.011
- Lee, I. C., Ko, J. W., Park, S. H., Shin, I. S., Moon, C., Kim, S. H., et al. (2016). Melamine and cyanuric acid co-exposure causes renal dysfunction and structural damage via MAPKs and mitochondrial signaling. *Food Chem. Toxicol.* 96, 254–262. doi: 10.1016/j.fct.2016.08.013
- Li, T., Fu, S., Huang, X., Zhang, X., Cui, Y., Zhang, Z., et al. (2022). Biological properties and potential application of hawthorn and its major functional components: A review. *J. Funct. Foods* 90, 104988. doi: 10.1016/j.jff.2022.104988
- Liu, S., Fan, J., Wang, Y., Liu, Z., Yang, Z., and Chen, B. (2025). Occurrence and distribution of melamine and its derivatives in the diverse aquatic environments in China. *Sci. Rep.* 15, 22855. doi: 10.1038/s41598-025-05451-w
- Liu, S., Yu, J., Fu, M., Wang, X., and Chang, X. (2021). Regulatory effects of hawthorn polyphenols on hyperglycemic, inflammatory, insulin resistance responses, and alleviation of aortic injury in type 2 diabetic rats. *Food Res. Int.* 142, 110239. doi: 10.1016/j.foodres.2021.110239
- Liu, F., Zhang, X., and Ji, Y. (2020). Total flavonoid extract from hawthorn (*Crataegus pinnatifida*) improves inflammatory cytokines-evoked epithelial barrier deficit. *Med. Sci. Monit.* 26, e920170. doi: 10.12659/msm.920170
- Luo, L., Meng, X., Wang, S., Zhang, R., Guo, K., Wang, W., et al. (2025). Hawthorn (*Crataegus pinnatifida* Bunge) polysaccharide improves the adaptability of crucian carp (*Carassius auratus*) to high alkalinity by regulating immunity, intestinal microbiota, and intestinal metabolomics. *Int. J. Biol. Macromol.* 320, 146088. doi: 10.1016/j.jbiomac.2025.146088
- Mahardika, K., Mastuti, I., and Gondol, Z. Z. (2017). Histopathological study on nephropathy caused by oral administration with melamine and cyanuric acid in humpback grouper (*Cromileptes altivelis*). *Aquacult. Aquarium Conserv. Legis.* 10, 328–334.
- Martinelli, F., Perrone, A., Yousefi, S., Papini, A., Castiglione, S., Guarino, F., et al. (2021). Botanical, phytochemical, anti-microbial and pharmaceutical characteristics of hawthorn (*Crataegus monogyna* Jacq.), Rosaceae. *Molecules* 26, 7266. doi: 10.3390/molecules26237266
- National Research Council (2011). *Nutrient requirements of fish and shrimp* (Washington DC: National academies press).
- Neiffer, D. L., and Stamper, M. A. (2009). Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. *ILAR J.* 50, 343–360. doi: 10.1093/ilar.50.4.343
- Nordstokke, D. W., and Zumbo, B. D. (2010). A new nonparametric Levene test for equal variances. *Psicologica* 31, 401–430.
- Nuntapong, N., Phromkunthong, W., Wanlem, S., and Boonyaratpalin, M. (2019). Dietary exposure to melamine and cyanuric acid induced growth reduction, oxidative stress and pathological changes of hepatopancreas in Pacific white shrimp. *Int. Aquat. Res.* 11, 13–31. doi: 10.1007/s40071-019-0216-y
- Obukhova, E., and Murzina, S. (2024). Mechanisms of the antimicrobial action of fatty acids: a review. *Appl. Biochem. Microbiol.* 60, 1035–1043. doi: 10.1134/s0003683824605158
- Ogunji, J. (2008). Growth performance, nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (maggot) diets. *Turk. J. Fish. Aquat. Sci.* 8, 141–147.
- Ogunji, J. O., Iheanacho, S. C., Abe, G. A., and Ike, O. R. (2020). Assessing effects of substituting dietary fish meal with boiled donkey and cow blood meal on growth performance and digestive enzyme activities of *Clarias gariepinus* juvenile. *J. World Aquacult Soc* 51, 1066–1079. doi: 10.1111/jwas.12716
- Pejin, B., Savic, A., Sokovic, M., Glamoclija, J., Ciric, A., Nikolic, M., et al. (2014). Further *in vitro* evaluation of antiradical and antimicrobial activities of phytol. *Nat. Prod. Res.* 28, 372–376. doi: 10.1080/14786419.2013.869692
- Phromkunthong, W., Nuntapong, N., Boonyaratpalin, M., and Kiron, V. (2013). Toxicity of melamine, an adulterant in fish feeds: Experimental assessment of its effects on tilapia. *J. Fish Dis.* 36, 555–568. doi: 10.1111/jfd.12003
- Phromkunthong, W., Nuntapong, N., Wanlem, S., and Boonyaratpalin, M. (2015). A study on growth, histopathology and oxidative stress in Asian sea bass on diets with various loadings of melamine and cyanuric acid adulterants. *Aquaculture* 435, 336–346. doi: 10.1016/j.aquaculture.2014.10.009
- Pitcher, T., and Hart, P. (1982). *Fisheries ecology*, (American edition) Westport, CT: AVI Publishing Company, Inc.
- Prism, G. (2019). *Prism 8 for windows* (San Diego, CA, USA: GraphPad Software Inc).
- Rajpoot, M., Bhattacharyya, R., Banerjee, D., and Sharma, A. (2017). Melamine binding with arachidonic acid binding sites of albumin is a potential mechanism for melamine-induced inflammation. *Biotechnol. Appl. Biochem.* 64, 490–495. doi: 10.1002/bab.1512
- Refaat, A. T., Shahat, A. A., Ehsan, N. A., Yassin, N., Hammouda, F., Tabl, E. A., et al. (2010). Phytochemical and biological activities of *Crataegus sinaica* growing in Egypt. *Asian Pac. J. Trop. Med.* 3, 257–261. doi: 10.1016/s1995-7645(10)60062-4
- Sadek, M., Sarkis, N., and Jangi, G. (2026). Benchmark dose modeling of continuous renal biomarkers for melamine nephrotoxicity in rats to derive a health-protective tolerable daily intake. *J. Hazard. Mater. Adv.* 21, 101010. doi: 10.1016/j.hazadv.2026.101010
- Salama, A., El-Kassaby, M. I., Refaat, A., and Mohasib, R. M. (2024). GC–MS and molecular docking analyses of phytochemicals from *Calendula officinalis* L. hexane extract and evaluation of its antioxidant and wound healing properties in rats. *Egypt. J. Chem.* 67, 1037–1058. doi: 10.21608/ejchem.2024.328645.10642
- Sammari, H., Jedidi, S., Selmi, H., Ritbi, K., Jabri, M. A., Jridi, M., et al. (2021). Protective effects of *Crataegus azarolus* L. berries aqueous extract against castor oil-induced diarrhea, oxidative stress, and inflammation in rat. *Neurogastroenterol. Motil.* 33, e14065. doi: 10.14814/phy2.70393
- Sarian, M. N., Ahmed, Q. U., Mat So'ad, S. Z., Alhassan, A. M., Murugesu, S., Perumal, V., et al. (2017). Antioxidant and antidiabetic effects of flavonoids: a structure-activity relationship based study. *BioMed. Res. Int.* 2017, 8386065. doi: 10.1155/2017/8386065
- Schmittgen, T. D., and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* 3, 1101–1108. doi: 10.1038/nprot.2008.73
- Spss, I. (2011). *IBM SPSS statistics for Windows, version 20.0* (New York: IBM Corp).
- Suvarna, K. S., Layton, C., and Bancroft, J. D. (2018). *Bancroft's theory and practice of histological techniques E-Book* (Philadelphia, PA: Elsevier Health Sciences).
- Tan, X., Sun, Z., Zhou, M., Zou, C., Kou, H., Vijayaraman, S. B., et al. (2020). Effects of dietary hawthorn extracts supplementation on lipid metabolism, skin coloration and gut health of golden pompano (*Trachinotus ovatus*). *Aquaculture* 519, 734921. doi: 10.1016/j.aquaculture.2020.734921
- Ujan, J. A., Rind, K. H., Kesbiç, O. S., Masud, S., Habib, S. S., Al-Rejaie, S. S., et al. (2025). Green boost for Nile tilapia, *Oreochromis niloticus*: Unveiling the multifaceted effects of *Chenopodium album* leaves powder on growth, hematology, antioxidant defense, nonspecific immunity and tolerance against *Aeromonas hydrophila*. *Aquac. Res.* 2025, 8919050. doi: 10.1155/are/8919050
- Walter, H. E. (1984). "Proteinases: methods with hemoglobin casein and azocoll as substrates," in *Methods of enzymatic analysis*. Ed. H. J. Bergmeyer (Verlag Chemie, Weinheim, Germany), 270–277.
- Warner, W., and Licha, T. (2025). Melamine – a PMT/pVpM substance as a generic indicator for anthropogenic activity and urbanisation? An explorative study on melamine in the water cycle and soil. *Chemosphere* 370, 143918. doi: 10.1016/j.chemosphere.2024.143918
- Winkler, U. K., and Stuckmann, M. (1979). Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *J. Bacteriol.* 138, 663–670. doi: 10.1128/jb.138.3.663-670.1979
- Yiu, A. J., Ibeh, C. L., Roy, S. K., and Bandyopadhyay, B. C. (2017). Melamine induces Ca^{2+} -sensing receptor activation and elicits apoptosis in proximal tubular cells. *Am. J. Physiol. Cell Physiol.* 313, C27–C41. doi: 10.1152/ajpcell.00225.2016
- Zhang, Z., Chang, Q., Zhu, M., Huang, Y., Ho, W. K., and Chen, Z. (2001). Characterization of antioxidants present in hawthorn fruits. *J. Nutr. Biochem.* 12, 144–152. doi: 10.1016/s0955-2863(00)00137-6
- Zhang, Y., Shen, T., Liu, S.-W., Zhao, J., Chen, W., and Wang, H. (2014). Effect of hawthorn on *Drosophila melanogaster* antioxidant-related gene expression. *Trop. J. Pharm. Res.* 13, 353–357. doi: 10.1016/j.carbpol.2022.120121
- Zhu, H., and Kannan, K. (2019). Melamine and cyanuric acid in foodstuffs from the United States and their implications for human exposure. *Environ. Int.* 130, 104950. doi: 10.1016/j.envint.2019.104950
- Zhu, H., and Kannan, K. (2020). Occurrence and distribution of melamine and its derivatives in surface water, drinking water, precipitation, wastewater, and swimming pool water. *Environ. pollut.* 258, 113743. doi: 10.1016/j.envpol.2019.113743