*Ann. Anim. Sci., Vol. 24, No. 1 (2024) 139–149* *DOI: 10.2478/aoas-2023-0057*

sciendo

# THE AMELIORATIVE EFFECTS OF DIETARY ROSEMARY (*ROSMARINUS OFFICINALIS*) AGAINST GROWTH RETARDATION, OXIDATIVE STRESS, AND IMMUNOSUPPRESSION INDUCED BY WATERBORNE LEAD TOXICITY IN NILE TILAPIA FINGERLINGS

Mohsen Abdel-Tawwab[1♦], Heba S. Hamed[2], Mohamed N. Monier[1], Rehab M. Amen[3]

[1]Department of Fish Biology and Ecology, Central Laboratory for Aquaculture Research, Agricultural Research Center, Abbassa, Abo-Hammad, Sharqia 44662, Egypt
[2]Department of Zoology, Faculty of Women for Arts, Science & Education, Ain Shams University, Cairo 11757, Egypt
[3]Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt
♦Corresponding author: mohsentawwab@arc.sci.eg, mohsentawwab@gmail.com

**Abstract**

**The dietary effects of rosemary (*Rosmarinus officinalis*) leaves powder (RLP) were tested on the performance and welfare of Nile tilapia (*Oreochromis niloticus*) fingerlings subjected to lead (Pb) toxicity. Two hundred fish (31.9±0.28 g) were stocked into 20 85-L glass aquaria (10 fish/aquarium) to represent four treatments with five replicates each. Fish were fed on 0.0% (control; T1) and 1.0% RLP (T2) along with continuous exposure to either 0.0 (T3) or 7.94 mg Pb/L (T4) for 60 days. Compared with the control diet, feeding Nile tilapia on RLP alone improved the growth performance and hemato-biochemical, antioxidant, and immunological indices. Conversely, the Pb toxicity negatively affected hematocrit, hemoglobin, white and red blood cell counts, and growth indices. Furthermore, Pb exposure raised blood cortisol, glucose, total cholesterol, serum transaminases (ALT and AST levels), alkaline phosphatase, and lactate dehydrogenase accompanied with significant reductions in serum total lipids, protein, albumin, and globulin values. Furthermore, Pb exposure decreased respiratory burst, lysozyme, total immunoglobulins, malondialdehyde, superoxide dismutase, catalase, and total antioxidant capacity. On the other hand, dietary RLP significantly reduced the negative impacts of Pb toxicity on the above-mentioned indices. Additionally, the dietary RLP reduced the Pb accumulation in Pb-intoxicated fish leading to significant enhancements in Nile tilapia's growth and welfare. These findings suggest that dietary 1% RLP significantly enhanced the fish performance and welfare status and could alleviate the Pb toxicity effects on Nile tilapia performance and welfare.**

**Key words: *Rosmarinus officinalis*, lead toxicity, hemato-biochemical indices, oxidative/antioxidative status, innate immunity, Nile tilapia**

Lead (Pb) is a non-essential and toxic metal, which may reach water systems naturally from the rocks and soils as well as from anthropogenic activities from batteries and electrical, pigments and paints, refining of ores, pesticides, fertilizers, fuel, domestic effluents, among others (Abd El-Kader et al., 2022). The Pb pollution is due to its direct discharge into aquatic ecosystems or through indirect routes such as dry and wet deposition and land run-off and it was 7 µg/L in Manzala Lake, Egypt (Abd El-Kader et al., 2022) and 5.0 µg/L in High-Dam Lake, Egypt (Rashed, 2001). Sometimes, fish cannot migrate away from the polluted ecosystem as fish-ponds and lakes; so, fish are better experimental animals suitable for detecting the effects of heavy metals pollution that may occur on aquatic ecosystems.

Nile tilapia (*Oreochromis niloticus*) is one of the major freshwater fish species cultivated in Egypt and worldwide (El-Sayed, 2019). Due to the serious development of the aquaculture industry in Egypt and other countries, a drastic shortage in freshwater resources is much more expected. This leads to the possible use of wastewater in charging tilapia farms. In this case, Pb may reach the tilapia farms causing oxidative stress that retards their growth performance and causes immune suppression in fish. Previous studies found that Pb exposure caused several physio-pathological problems in aquatic animals characterized by inflammation, apoptosis, oxidative stress damage, and innate immune impairment in several fish species (Giri et al., 2018; Lee et al., 2019; Giri et al., 2021; Hamed et al., 2022).

To mitigate the negative effects of Pb on a range of fish species, some researchers have used medicinal herbs as feed additives (Abdel Rahman et al., 2019; El-Houseiny et al., 2019; Dawood et al., 2020; Hamed and Abdel-Tawwab, 2021; Hamed et al., 2022). Rosemary (*Rosmarinus officinalis* L.) is an evergreen, perennial, branched shrub that reaches one-meter high with fragrant needle-shaped dark green leaves (Lešnik et al., 2021). The rosemary plant is native to the Mediterranean region, and its leaves are used extensively in foods as spices and flavorings because of its therapeutic role in the prevention and treatment of colds, rheumatoid arthritis as well as muscle and joint pain (Ali et al., 2019; Lešnik et al., 2021). Mena et al. (2016) stated that the biological properties of rosemary have been attributed to its phytochemical composition rich in (poly)phenolic compounds, mainly diterpenoids such as carnosic acid and carnosol where the rosemary extract contained 24 flavonoids (mainly flavones), 5 phenolic acids, 24 diterpenoids (carnosic acid, carnosol, and rosmanol derivatives), 1 triterpenoid (betulinic acid), and 3 lignans (medioresinol derivatives). For this reason, rosemary exhibited beneficial health effects and has been used in traditional and complementary alternative medicine for its digestive, tonic, astringent, diuretic, and diaphoretic properties (Borges et al., 2019; Lešnik et al., 2021).

Rosemary leaves powder (RLP) was previously used as a natural feed additive in fish diets and exhibited significant enhancements in fish performance and welfare status (Yilmaz et al., 2012; Hernández et al., 2015; Ayoub et al., 2019; Kubiriza et al., 2019; Naiel et al., 2019; Yousefi et al., 2019; Naiel et al., 2020). This promising effect could be attributed to its considerable content of bioactive compounds, which have free radical scavenging capability and exhibit antioxidants and biological properties (Mena et al., 2016; Nieto et al., 2018; Borges et al., 2019; Karatas et al., 2020; Lešnik et al., 2021). Previous studies showed that dietary RLP played a significant role in reducing the aflatoxin B1 toxicity in Nile tilapia (Naiel et al., 2019) and overpopulation stress in common carp (*Cyprinus carpio*) fingerlings (Yousefi et al., 2019). Thus, this research was done to evaluate the effects of dietary RLP and/or Pb toxicity on growth performance, hemato-biochemical, oxidative stress, innate immunity indicators, and Pb residues in Pb-intoxicated Nile tilapia fingerlings.

## Material and methods

### Diets formulation and fish husbandry

Rosemary (*R. officinalis*) leaf powder (RLP) was purchased from a nearby store in Cairo, Egypt, and was added to the control basal diet (30% crude protein) at levels of 0% (control) and 1.0% (Table 1) as recommended by Naiel et al. (2020). Feed components were mixed with RLP levels and 200 mL of water was added to each kilogram of diet during the mixing process. The dough was combined in a grinding machine and dried for 24 hours before being crushed into 1–2 mm pellets; after that, the experimental diets were kept at –4°C until further use.

Nile tilapia fingerlings were obtained from the nursery ponds at the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. The fish were acclimated to laboratory conditions in a 1-$m^3$ plastic tank for 14 days. Fluorescent lamps provided a 12-hour photoperiod. Lead nitrate ($Pb(NO_3)_2$) was purchased from El-Gomhouria Company for Trading Chemicals and Medical Appliances (Cairo, Egypt). The sublethal dose ($LC_{50}$) of Pb was previously calculated in our previous study (Hamed et al., 2022), and the current investigation used 7.94 mg Pb/L as 1/10 of the $LC_{50}$ to induce sub-lethal toxicity effects.

Table 1. Ingredients and proximate analysis (%; on dry matter basis) of diets containing different levels of rosemary (*R. officinalis*) leaves powder (RLP)

| Ingredients | RLP levels (%) | |
|---|---|---|
| | 0.0 (Control) | 1.0 |
| Fish meal (72% crude protein) | 8.5 | 8.5 |
| Soybean meal (45% crude protein) | 46.5 | 46.5 |
| Wheat bran | 18.3 | 18.3 |
| Ground corn | 10.0 | 9.0 |
| Corn oil | 2.0 | 2.0 |
| Cod liver oil | 2.0 | 2.0 |
| Mineral mixture[a] | 3.0 | 3.0 |
| Vitamin mixture[b] | 3.0 | 3.0 |
| Starch | 6.7 | 6.7 |
| Rosemary leaves powder | 0.0 | 1.0 |
| Total | 100 | 100 |
| Chemical composition (%) | | |
| dry matter | 89.94 | 89.07 |
| crude protein | 29.68 | 29.59 |
| total lipids | 7.42 | 7.38 |
| crude fiber | 4.33 | 4.31 |
| total ash | 6.35 | 6.34 |
| NFE | 52.22 | 52.38 |
| GE (MJ/kg)[c] | 17.68 | 17.51 |

[a]Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; paraaminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[b]Mineral premix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7{\cdot}3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2{\cdot}4H_2O$, 2.5; $Cu(OAc)_2{\cdot}H_2O$, 0.785; $CoCl_3{\cdot}6H_2O$, 0.477; $CaIO_3{\cdot}6H_2O$, 0.295; $CrCl_3{\cdot}6H_2O$, 0.128; $AlCl_3{\cdot}6H_2O$, 0.54; $Na_2SeO_3$, 0.03.

[c]GE (Gross energy) was from NRC (2011) as 16.7, 37.4, and 16.7 kJ/g for protein, lipid, and carbohydrates, respectively.

Two hundred fish (31.9±0.28 g) were stocked into twenty 85-L glass aquaria (10 fish/aquarium), which were aerated by air pumps via air stones to represent four treatments with five replicates each. Fish were fed on 0.0% (control; T1) and 1.0% RLP (T2) to apparent satiety thrice a day (9:00, 13:00, and 17:00 h) with continuous exposure to either 0.0 (T3) or 7.94 mg Pb/L (T4) for 60 days. Aquaria's water was regularly changed every two days and substituted with new aerated water containing the same Pb levels. Water quality parameters in each aquarium were regularly monitored where 1-L samples were collected 20 cm underneath surface water. Dissolved oxygen (DO) level and water temperature were measured daily in sites by an oxygen-meter (Jenway, London, UK). The pH values (Digital Mini-pH Meter, USA) were weekly monitored in sites and levels of total alkalinity and total hardness were determined every week as described in Boyd (1984). DO level was 6.7±0.7 mg/L, pH value was 7.7±0.5, water temperature was

27.6±1.6°C, total alkalinity was 125 mg/L as $CaCO_3$, and total hardness was 150 mg/L as $CaCO_3$. The Pb levels in aquaria without and with Pb were 0.17–0.19 and 8.01–8.07 g/L, respectively.

After the feeding experiment, fish in every aquarium were harvested, counted, and group-weighed to determine the survival rate and growth indices as follows:

Weight gain (g) = final weight (g) – initial weight (g);

Specific growth rate (SGR; %/day) = 100 (Ln final weight – Ln initial weight) / 60;

Feed consumption (g feed/fish) = total feed consumed/number of fish in the aquarium;

Feed conversion ratio (FCR) = feed consumption / weight gain;

The survival rate (%) = 100 (fish number at the end / fish number at the start).

**The assessment of blood biochemistry**

Fish were anesthetized with 0.02% benzocaine and were fasted for 24 h before sampling. Blood samples were taken from caudal veins of three fish from each aquarium (15 fish per treatment). Hematological analysis was performed on a subset of the blood samples stored in heparin-filled Eppendorf tubes. After letting the second portion be clotted at room temperature for 30 min, it was transferred to Eppendorf tubes and centrifuged at 1500 × g for 15 minutes to separate the serum. The counts of red blood cells (RBC) and white blood cells (WBC) were measured as previously reported (Brown, 1980). The cyanmethemoglobin method of Van Kampen and Zijlstra (1961) was used to determine the Hb levels. Hematocrit levels were measured via centrifuging fresh blood in capillary glass tubes for 10 min in a micro-hematocrit centrifuge and then packed cell volume was measured. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were computed by Dacie and Lewis (1984).

Diagnostic reagent kits (Biodiagnostic Co., Cairo, Egypt) were used to assess biochemical, oxidative stress, and immunological indices according to the manufacturer's guidelines. Methods developed by Trinder (1969) and Foster and Dunn (1974) were used to measure blood cortisol and glucose, respectively. Serum total protein (TP) and albumin (ALB) concentrations were determined using the methods of Henry (1964). The serum globulin (GLO) concentration was calculated by subtracting ALB values from TP values. Colorimetric commercial kits (Diamond Diagnostic Co. for Modern Laboratory Chemicals, Egypt) were used to measure serum aspartic aminotransferase (AST) and alanine aminotransferase (ALT) activities as well as serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities as described by Reitman and Frankel (1957) as well as Bergmeyer (1974) and Belfield and Goldberg (1971), respectively. Spectrophotometric analysis was used to determine total cholesterol (T-CHO) concentrations according to Allain et al. (1974).

**Liver DNA fragmentation**

After blood sampling, fish were dissected and liver tissues were taken and crushed within hypotonic lysis buffer (1 g : 9 mL). After centrifugation (13,000 × g for 15 min), the supernatant containing DNA fragments was used for the colorimetric determination by diphenylamine (DPA) assay. The DNA fragmentation percentage was assessed using a reagent blank and spectrophotometer according to Perandones et al. (1993). The percentage of DNA fragmentation was expressed by the following formula:

*Fragmented DNA% = 100 [fragmented DNA / (fragmented + intact DNA)].*

**Oxidative stress and immunological assays**

After drawing blood samples from the fish, liver tissues were removed and washed with distilled water; then 1.0 g of tissue was homogenized in 9 mL of cold phosphate buffer saline (0.1 M pH 7.4) utilizing a Potter-Elvehjem glass/Teflon homogenizer. The samples were centrifuged at 1600 × g for 10 min at 4°C and the supernatant was collected and frozen until further analysis. Catalase (CAT) and sodium dismutase (SOD) activities were determined as described by Aebi (1984) and McCord and Fridovich (1969), respectively. Alternatively, thiobarbituric acid was used to assess malondialdehyde (MDA) concentrations (Ohkawa et al., 1979). The total antioxidant capacity (TAC) value was evaluated utilizing the ferric-reducing antioxidant power technique (Benzie and Strain, 1996).

Turbidimetric analysis (Ellis, 1990) was used to quantify lysozyme (LYZ) activity in blood using *Micrococcus luteus* as a substrate in a phosphate buffer of pH 6.2. Whole blood samples were analyzed for respiratory burst activity (RBA) using nitroblue tetrazolium dye, as described by Secombes (1990). Total immunoglobulin (total Ig) was determined using the method described by Siwicki and Anderson (1993).

**Statistical analysis**

Statistical significance between experimental groups was determined using SPSS version 26 (SPSS, Richmond, VA, USA) according to Dytham (2011). However, data obtained were subjected to a two-way analysis of variance followed by Duncan's multiple-range test, which was used to determine whether there were statistically significant changes between means at the $P<0.05$ level.

## Results

The two-way ANOVA revealed that dietary RLP, Pb exposure, and their interaction significantly ($P<0.001$) influenced growth indices of Nile tilapia fingerlings but not FCR or fish survivability (Table 2). The inclusion of RLP in fish diets (T2) resulted in substantial ($P<0.001$) improvements in FBW, WG%, SGR, and feed consumption compared with the control group (T1). On the other hand, Pb exposure alone (T3) caused substantial (P<0.001) delays in FBW, WG%, and SGR and reduced the amount of feed consumption. Feeding Nile tilapia with a RLP-enriched diet along with Pb exposure (T4) minimized the adverse impacts of Pb toxicity (T3) on growth indices. Dietary RLP resulted in a marked increase in feed intake; however, exposing fish to Pb toxicity led to a substantial (P<0.001) reduction in feed consumption (Table 2). Feed consumption was pointedly improved in Pb-treated fish that were fed with RLP (T3) compared to Pb-exposed fish alone (T4). The fish survival rate varied from 94% to 100%, and it was not statistically affected by RLP supplementation (P = 0.301) or Pb exposure (P = 0.153), or their interaction (P = 1.00).

Dietary RLP, Pb exposure, and their interactions exhibited significant (P<0.05) effects on all hemato-biochemical variables except MCH, which showed no response (Table 3). Pb toxicity (T3) caused substantial reductions in WBCs counts (P<0.001), RBCs counts (P = 0.001), Hb levels (P<0.001), and Ht % (P<0.001), but feeding RLP to Pb-exposed fish (T4) markedly reduced their worth performance. Regarding the control treatment (T1), fish fed exclusively on RLP-supplemented diets (T2) showed highest levels of the above-mentioned parameters. Fish from treatments T1 and T2 displayed no substantial (P>0.05) changes in Hb, MCV, MCH, or MCHC, while fish exposed to Pb (T3) showed higher MCV and MCH levels, which were reduced by dietary supplementation with RLP (T4; Table 3). The Pb exposure (T3) significantly reduced the MCHC value (P<0.001), whereas dietary RLP (T2) increased it to the highest level (P = 0.010).

Table 2. Growth performance and feed utilization of Nile tilapia (*O. niloticus*) fed with rosemary (*R. officinalis*) leaves powder (RLP) along with exposure to sublethal levels of lead (Pb) toxicity for 60 days

| RLP levels (%) | Pb levels (mg/L) | | Initial weight (g) | Final weight (g) | Weight gain % | SGR (%/day) | Feed consumption (g feed/fish) | Food conversion ratio | Fish survival (%) |
|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0 | T1 | 31.9±0.28 | 64.±0.56 b | 101.9±2.71 b | 1.171±0.023 b | 47.7±1.91 b | 1.47±0.068 | 98.0±2.00 |
| 1.0 | 0 | T2 | 31.6±0.27 | 71.6±1.25 a | 126.6±2.91 a | 1.363±0.021 a | 59.3±2.06 a | 1.48±0.084 | 100.0±0.00 |
| 0.0 | 7.94 | T3 | 31.9±0.18 | 43.0±0.47 d | 34.8±1.59 d | 0.498±0.019 d | 16.5±0.51 d | 1.49±0.034 | 96.0±2.44 |
| 1.0 | 7.94 | T4 | 31.6±0.28 | 54.4±0.92 c | 72.2±3.98 c | 0.905±0.038 c | 33.9±0.89 c | 1.49±0.046 | 98.0±2.00 |
| Two-way ANOVA | | | P value | | | | | | |
| RLP levels | | | 0.203 | <0.001 | <0.001 | <0.001 | <0.001 | 0.950 | 0.301 |
| Pb exposure | | | 0.878 | <0.001 | <0.001 | <0.001 | <0.001 | 0.771 | 0.153 |
| RLP × Pb exposure | | | 0.939 | 0.026 | 0.042 | 0.001 | 0.017 | 0.833 | 1.00 |

Mean values followed by different letters in the same column are significantly different at P<0.05 (n = 5).

Table 3. Hematological parameters of Nile tilapia (*O. niloticus*) fed with rosemary (*R. officinalis*) leaves powder (RLP) along with exposure to sublethal levels of lead (Pb) toxicity for 60 days

| RLP levels (%) | Pb levels (mg/L) | | WBCs ($\times 10^3$ µL) | RBCs ($\times 10^6$ µL) | Hemoglobin (g/dL) | Hematocrit (%) | MCV | MCH | MCHC |
|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0 | T1 | 9.4±0.326 b | 3.76±0.157 a | 9.64±0.098 b | 34.90±0.428 b | 92.8±2.78 b | 25.6±1.01 | 27.6±0.42 b |
| 1.0 | 0 | T2 | 12.2±0.374 a | 4.02±0.177 a | 11.90±0.365 a | 37.94±1.025 a | 94.4±3.80 b | 29.6±1.89 | 31.4±0.76 a |
| 0.0 | 7.94 | T3 | 6.4±0.430 c | 1.68±0.037 c | 4.96±0.323 d | 22.06±0.451 d | 131.3±4.09 a | 29.5±2.30 | 22.5±1.51 c |
| 1.0 | 7.94 | T4 | 8.6±0.292 b | 2.78±0.132 b | 7.10±0.187 c | 28.76±0.493 c | 104.5±5.81 b | 25.5±0.93 | 24.7±1.05 bc |
| Two-way ANOVA | | | P value | | | | | | |
| RLP levels | | | <0.001 | <0.001 | <0.001 | <0.001 | 0.009 | 0.967 | 0.010 |
| Pb exposure | | | <0.001 | 0.001 | <0.001 | <0.001 | <0.001 | 0.909 | <0.001 |
| RLP × Pb exposure | | | 0.014 | 0.007 | 0.024 | 0.012 | 0.004 | 0.162 | 0.047 |

Mean values followed by different letters in the same column are significantly different at P<0.05 (n = 5).

Table 4. Changes in the levels of blood cortisol, glucose, total lipids, total cholesterol (T-CHO), total protein (TP), albumin (ALB), and globulin (GLO) of Nile tilapia (*O. niloticus*) fed with rosemary (*R. officinalis*) leaves powder (RLP) along with exposure to sublethal levels of lead (Pb) toxicity for 60 days

| RLP levels (%) | Pb levels (mg/L) | | Cortisol (ng/mL) | Glucose (mg/dL) | T-CHO (mg/L) | Total lipids (mg/L) | TP (mg/L) | ALB (mg/L) | GLO (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | T1 | 5.83±0.106 c | 48.0±0.51 c | 146.1±0.45 c | 11.11±0.197 b | 6.4±0.24 b | 3.4±0.05 b | 3.0±0.25 a |
| 1.0 | 0 | T2 | 5.83±0.109 c | 48.3±0.69 c | 146.4±0.38 c | 12.58±0.514 a | 7.4±0.27 a | 4.2±0.09 a | 3.2±0.30 a |
| 0 | 7.94 | T3 | 12.84±0.224 a | 77.2±0.60 a | 181.8±0.52 a | 6.10±0.139 d | 3.3±0.04 d | 2.7±0.08 c | 0.6±0.04 c |
| 1.0 | 7.94 | T4 | 9.07±0.275 b | 59.6±1.26 b | 154.6±1.13 b | 9.75±0.241 c | 4.7±0.15 c | 3.2±0.04 b | 1.5±0.15 b |
| Two-way ANOVA | | | P value | | | | | | |
| RLP levels | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.001 | 0.012 |
| Pb exposure | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| RLP × Pb exposure | | | <0.001 | <0.001 | 0.001 | 0.003 | 0.032 | 0.013 | 0.018 |

Mean values followed by different letters are significantly different at P<0.05 (n = 5).

Table 5. Changes in the levels of serum lactate dehydrogenase (LDH), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and DNA fragmentation percentage of Nile tilapia (*O. niloticus*) fed with rosemary (*R. officinalis*) leaves powder (RLP) along with exposure to sublethal levels of lead (Pb) toxicity for 60 days

| RLP levels (%) | Pb levels (mg/L) | | LDH (IU/L) | ALP (IU/L) | ALT (IU/L) | AST (IU/L) |
|---|---|---|---|---|---|---|
| 0 | 0 | T1 | 26.4±0.33 c | 11.6±0.20 a | 45.0±0.16 c | 50.5±0.51 c |
| 1.0 | 0 | T2 | 26.7±0.41 c | 11.7±0.38 a | 44.3±1.02 c | 50.8±0.64 c |
| 0 | 7.94 | T3 | 41.2±0.10 a | 6.7±0.09 c | 59.2±0.31 a | 68.6±1.58 a |
| 1.0 | 7.94 | T4 | 32.2±0.94 b | 9.5±0.34 b | 49.8±1.08 b | 57.7±0.53 b |
| Two-way ANOVA | | | P value | | | |
| RLP levels | | | <0.001 | <0.001 | <0.001 | <0.001 |
| Pb exposure | | | <0.001 | <0.001 | <0.001 | <0.001 |
| RLP × Pb exposure | | | <0.001 | <0.001 | <0.001 | <0.001 |

Mean values followed by different letters are significantly different at P<0.05 (n = 5).

According to the two-way ANOVA, levels of cortisol, glucose, and T-CHO in Nile tilapia fingerlings were markedly (P<0.05) affected by RLP feeding, Pb exposure, and their interaction (Table 4). However, their levels were not substantially altered among T1 and T2 treatments. When Pb-exposed fish were fed with a RLP-supplemented diet (T4), cortisol, glucose, and T-CHO levels were dramatically attenuated as compared with the T3 fish group. Compared to the control group (T1), dietary RLP (T2) markedly raised the blood levels of total lipids (P<0.001), TP (P<0.001), ALB (P = 0.001), and GLO (P = 0.012; Table 4). Conversely, the levels of the above-mentioned parameters were remarkably decreased when the fish were exposed to Pb toxicity alone (T3) as compared with the control (T1). The two-way ANOVA also showed that fish exposed to Pb toxicity (T3) exhibited higher activities of LDH (P<0.001), ALT (P<0.001), AST (P<0.001), and ALP (P<0.001) compared with the T1 fish group (Table 5). On the other hand, feeding Nile tilapia on a RLP-containing diet only (T2) showed approximately the same levels of previous enzymes as the T1 fish group. The addition of RLP to diets of Pb-intoxicated fish (T4) substantially reduced the activities of the previously mentioned enzymes as compared with the T3 treatment (P>0.001).

MDA levels and DNA fragmentation % were substantially (P<0.001) elevated in the hepatic tissues of Pb-intoxicated *O. niloticus* (T3) over the control group (T1). The dietary RLP only (T2) showed no significant (P>0.05) differences compared with the T1 fish group (Figure 1). Feeding Pb-intoxicated fish on RLP diets (T4) partially minimized MDA levels and DNA fragmentation % but did not reach levels in T1 and T2 groups. Table 6 shows that the RLP-fed fish (T2) had considerably higher levels of hepatic SOD (P<0.001), CAT (P<0.001), and TAC (P<0.001), in addition to higher levels of LYZ (P<0.001), RBA (P<0.001), and total Ig (P<0.001), than those of the control fish group (T1). Conversely, the Pb-intoxicated fish (T3) had lowest levels (P<0.001) of the above-mentioned biomarkers compared with the T1 treatment (Table 6). When Pb-subjected fish were fed with RLP-enriched diets (T4), the antioxidant and immunological parameters showed partial enhancements as compared with those of the control group (T1 vs. T4; Table 6).

According to the two-way ANOVA, levels of Pb residue in the whole body of Nile tilapia were markedly (P<0.001) affected by RLP feeding, Pb exposure, and their interaction. When Nile tilapia were exposed to Pb toxicity (T3), a large amount of Pb was accumulated throughout the fish body (23.3 mg/g wet weight), as compared to 0.48 mg/g wet weight in the control treatment (Figure 2). Fish fed with RLP (T2) did not induce substantial variances (P>0.05) in Pb residue in the body of the control fish (T1). In Pb-exposed fish, dietary RLP effectively reduced the Pb accumulation in the fish body compared with fish exposed to Pb only (T3 vs. T4). Fish fed with 0.0 (T1) or 1.0% RLP-supplemented diet alone (T2) exhibited significantly (P<0.001) lower accumulation in Pb concentrations than fish subjected to Pb only (T3), or fish given RLP-supplemented diets combined with Pb exposure (T4).

Table 6. Changes in the levels of hepatic superoxide dismutase (SOD), catalase (CAT), and total antioxidant activity (TAC) as well as blood lysozyme (LYZ), respiratory burst activity (RBA), and total immunoglobulin (total Ig) of Nile tilapia (*O. niloticus*) fed with rosemary (*R. officinalis*) leaves powder (RLP) along with exposure to sublethal levels of lead (Pb) toxicity for 60 days

| RLP levels (%) | Pb levels (mg/L) | | LYZ (µg/mL) | RBA (mg/mL) | Total Ig (mg/mL) | SOD (U/mg protein) | CAT (U/mg protein) | TAC (U/mg protein) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | T1 | 5.58±0.054 b | 1.91±0.032 b | 14.7±0.09 b | 35.8±0.71 b | 26.3±0.37 b | 42.1±0.55 b |
| 1.0 | 0 | T2 | 7.36±0.211 a | 2.08±0.069 a | 16.0±0.21 a | 41.5±1.22 a | 29.5±1.13 a | 46.2±0.68 a |
| 0 | 7.94 | T3 | 3.62±0.059 c | 1.20±0.016 d | 8.2±0.16 d | 26.0±0.47 d | 16.7±0.35 d | 29.4±0.30 d |
| 1.0 | 7.94 | T4 | 5.19±0.179 b | 1.59±0.025 c | 12.7±0.10 c | 32.4±0.49 c | 22.0±0.42 c | 37.1±0.27 c |
| Two-way ANOVA | | | P value | | | | | |
| RLP levels | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Pb exposure | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| RLP × Pb exposure | | | 0.019 | 0.016 | <0.001 | 0.028 | 0.012 | 0.002 |

Mean values followed by different letters in the same column are significantly different at P<0.05 (n = 5).

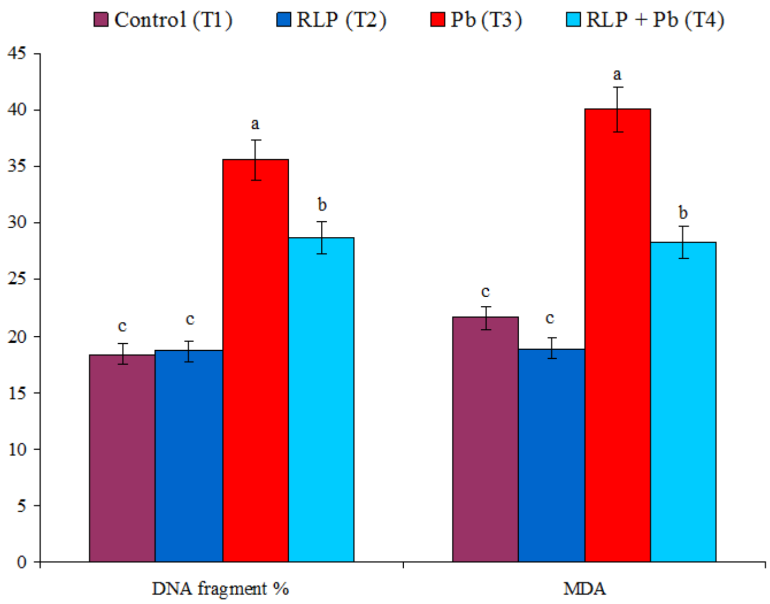


Figure 1. Changes in hepatic DNA fragmentation (%) and malondialdehyde levels (MDA; nmol/mg protein) in Nile tilapia (*O. niloticus*) fed with rosemary (*R. officinalis*) leaves powder (RLP) along with exposure to sublethal levels of lead (Pb) toxicity for 60 days. Bars with different letters for each variable are significantly different at P<0.05 (n = 5). The control group (T1): fish fed on the control diet with no RLP or Pb exposure; RLP (T2): fish fed on the RLP-enriched diet only with no Pb exposure; Pb (T3): fish fed on the control diet with Pb exposure only; RLP + Pb (T4): fish fed on the RLP-enriched diet accompanied with Pb exposure

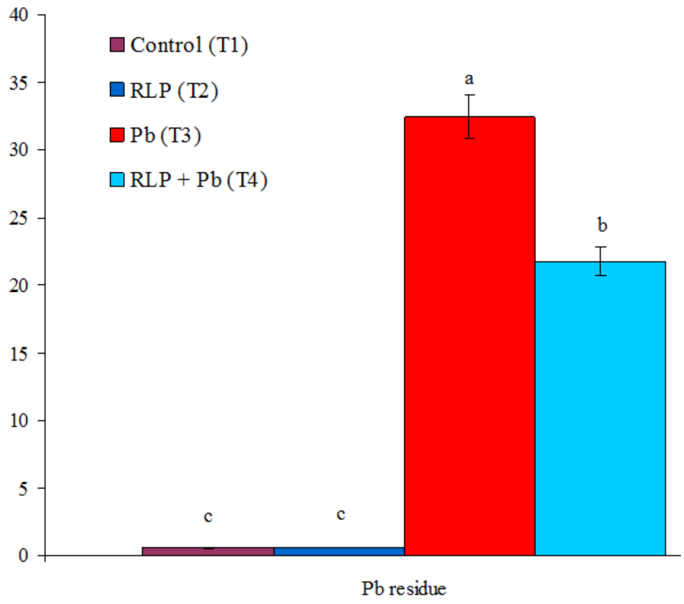


Figure 2. Pb residues in the entire body (mg/g fresh weight) of Nile tilapia (*O. niloticus*) fed with rosemary (*R. officinalis*) leaves powder (RLP) along with exposure to sublethal levels of lead (Pb) toxicity for 60 days. Bars with different letters are significantly different at P<0.05 (n = 5). The control group (T1): fish fed on the control diet with no RLP or Pb exposure; RLP (T2): fish fed on the RLP-enriched diet only with no Pb exposure; Pb (T3): fish fed on the control diet with Pb exposure only; RLP + Pb (T4): fish fed on the RLP-enriched diet accompanied with Pb exposure

## Discussion

The current investigation showed that feeding Nile tilapia fingerlings with dietary RLP alone (T2) markedly enhanced their growth performance compared to the control group (T1). These findings are consistent with those obtained by the previous investigations on common carp (Yousefi et al., 2019), European seabass, *Dicentrarchus labrax* (Yilmaz et al., 2012), Nile tilapia (Ayoub et al., 2019; Naiel et al., 2019, 2020), Arctic charr, *Salvelinus alpinus* (Kubiriza et al., 2019), and rainbow trout, *Oncorhynchus mykiss* (Karatas et al., 2020). The growth enhancement may be associated with the roles of functional bioactive ingredients present in RLP such as polyphenols, especially 1,8-cineole, carnosol, carnosic acid, rosmarinic acid, β- and α-pinene, camphor, and camphene among others (Mena et al., 2016; Nieto et al., 2018; Borges et al., 2019; Lešnik et al., 2021). These bioactive substances may be responsible for the enhanced feed palatability, stimulated appetite, and consequently higher feed intake, which is responsible for the growth enhancement (Ahmadifar et al., 2021). In addition, dietary RLP exerts a positive effect on nutrient absorption via the gastrointestinal system (Koga et al., 2006) by improving the health status of the intestinal mucosa (Bhattacharyya et al., 2014; Mohamed et al., 2016), which consequently promotes nutrient digestion and absorption.

On the other hand, exposing Nile tilapia to Pb toxicity for 60 days retarded their growth as indicated by marked reductions in FW, WG%, and SGR. This decline may be related to the negative impact of Pb toxicity on the various metabolic processes. According to Bengani et al. (2015), Pb is a toxic element that impairs biological functions. Ikeogu et al. (2016) hypothesized that fish exposed to Pb toxicity experienced slow growth and high mortality rates. Moreover, Pb-exposed Nile tilapia grew more slowly than the control one, according to Kiran Kumar et al. (2021) and Hamed et al. (2022).

Furthermore, feeding Nile tilapia fingerlings with RLP reduced the negative impacts of Pb toxicity regarding their growth performance. The protective effects of dietary RLP against Pb toxicity could be related to its effectiveness as a chelating feed additive. This hypothesis is supported by the considerable reduction of Pb residue in the body of the T4 fish group versus the T3 fish group. These results may be attributed to the bioactive compounds that are present in RLP, which have free radical scavenging capability and exhibited antioxidants and biological properties (Mena et al., 2016; Nieto et al., 2018; Borges et al., 2019; Adimcilar et al., 2019; Karatas et al., 2020; Ahmadifar et al., 2021; Lešnik et al., 2021). Similar investigations have confirmed the possible impact of supplemental curcumin in lowering cadmium accumulation in African catfish, *Clarias gariepinus,* and common carp, *C. carpio,* tissues (El-Houseiny et al., 2019; Giri et al., 2021). According to Hamed et al. (2022), cinnamon (*Cinnamomum zeylanicum*) could reduce the Pb residues in the body of Pb-exposed Nile tilapia. Giri et al. (2021) confirmed that dietary curcumin considerably decreased the toxicity caused by Pb in common carp. In the present study, the Pb-intoxicated Nile tilapia group (T3) showed higher Pb residues. This result was similar to that observed by Giri et al. (2021), who found a substantial amount of Pb residue in the hepatic and renal tissues in Pb-intoxicated common carp. Zhai et al. (2017) and Giri et al. (2018) declared comparable results in Pb-exposed hepatic, renal, and intestine tissues of *C. carpio* and *O. niloticus*, respectively. Moreover, *O. niloticus* juveniles subjected to 4.02 mg/L of Pb $(NO_3)_2$ in water for one month showed considerably higher Pb levels in their hepatic tissues than the control fish (Kiran Kumar et al., 2021).

Fish health could be evaluated by blood and serum biochemical testing. Pb-intoxicated fish (T3) had considerably lower WBCs, RBCs, Hb, Ht, TP, ALB, and GLO levels. These decreases may be related to the impacts of Pb on the iron-binding locations of Hb, which could ultimately result in impaired Hb production (Pyszel et al., 2005). On the other hand, dietary RLP normalized abnormal hematological parameters. These outcomes are similar to earlier studies, which reported that dietary RLP improved the hematological parameters of fish (Naiel et al., 2019; Yousefi et al., 2019; Naiel et al., 2020). The findings herein are concomitant with those of Sherif et al. (2020) and Hamed et al. (2022), who reported that Pb-subjected Nile tilapia exhibited acute anemia. A decrease in erythropoiesis and an increase in RBCs breakdown may also be attributed to the decrease in erythrocytes, PCV, Hb, and WBC count (Sherif et al., 2020). In a comparable study, Dutta et al. (2015) demonstrated that the erythrocyte levels, WBCs count, and Hb levels were reduced in *Channa punctatus* after exposure to Pb $(NO_3)_2$. Kiran Kumar et al. (2021) found also lower WBC and RBC counts, Hb, and Ht levels in Pb-subjected *O. niloticus*, but feeding this fish on diets containing *Moringa oleifera* flower induced a significant attenuation of the values mentioned above.

The blood cortisol, glucose, and T-CHO concentrations of *O. niloticus* were pointedly increased due to their exposure to subacute Pb toxicity. Cortisol and glucose are essential components of the stress response in fish and their interactions with toxicants may be the key to the fish physiology functions, which must not be ignored (Mommsen et al., 1999; Sadoul and Geffroy, 2019). Similarly, Sayed et al. (2017), Kiran Kumar et al. (2021), and Hamed et al. (2022) found elevated cortisol and glucose concentrations in Pb-subjected *C. gariepinus* and *O. niloticus*, respectively. The present study presented that Pb toxicity lowered total lipids, TP, ALB, and GLO values in *O. niloticus*. Pb can suppress oxidative metabolism as it inhibits oxygen production as well as energy production (Karatas et al., 2019). However, the concentrations of the biomarkers mentioned above were increased in the RLP-treated fish, which indicated better utilization of protein and lipids. Serum TP represents the most serum immunoglobulins, complement, and LYZ (Giri et al., 2021). These findings were consistent with those of Naiel et al. (2019), Yousefi et al. (2019), and Naiel et al. (2020), who mentioned that the inclusion of RLP in fish diets positively improved TP, ALB, and GLO levels.

Water pollutants may directly impact the fish's liver. Moreover, it is well known that fish liver plays a crucial role in detoxification and toxicity elimination. Consequently, serum biochemical and hepatic biomarkers will be noticeably changed due to fish exposure to toxins (Salamat and Zarie, 2012). The current research indicated that Nile tilapia exposed to Pb had higher values of serum ALT, AST, LDH, and ALP activities. These results may be due to that Pb toxicity caused oxidative stress damage to hepatocyte cell membranes, releasing enzymes into the circulation (Abdel Rahman et al., 2019). According to previous studies, blood levels of ALT, AST, and LDH of various fish species exposed to heavy metals including lead toxicity were markedly increased as compared with the control group (Giri et al., 2018; Ming et al., 2019; Giri et al., 2021; Kiran Kumar et al., 2021; Hamed et al., 2022). The reduced serum ALP values in Pb-exposed Nile tilapia could indicate apoptotic alterations and RBC membrane breakdown (Hamed et al., 2022). Additionally, exposing fish to Pb toxicity could induce liver dysfunction owing to lipid peroxidation, which generates substantial deformation and biological processes of hepatocyte cell membranes (El-Houseiny et al., 2019). According to prior studies, Nile tilapia exposed to Pb toxicity had elevated levels of AST, ALT, and ALP (Sherif et al., 2020; Kiran Kumar et al., 2021; Hamed et al., 2022). Nevertheless, dietary RLP substantially reduced AST and ALT activities, indicating the protective effects of RLP on liver functions that are linked to significant improvements in antioxidant activity. Earlier researches indicated the capacity of RLP to reduce AST and ALT activities in common carp, Nile tilapia, and gilthead sea bream, *Sparus aurata* (Hernández et al., 2015; Naiel et al., 2019; Yousefi et al., 2019; Naiel et al., 2020).

Furthermore, feeding Pb-subjected Nile tilapia fed on RLP-supplied diets reduced the Pb-induced changes in the serum levels of AST, ALT, and ALP. These findings indicate that feeding RLP might rescue Nile tilapia from Pb toxicity. Similar studies evoked the benefits of nutritional curcumin on liver biomarkers in common carp subjected to Pb (Giri et al., 2021), common carp provided a coffee-based complement and subjected to zinc (Abdel-Tawwab et al., 2018), and Nile tilapia subjected to Pb toxicity along with dietary *M. oleifera* flowers (Kiran Kumar et al., 2021).

The current research showed that Pb-intoxicated Nile tilapia had considerably lower SOD, CAT, and TAC levels, whereas their feeding on RLP-supplemented diets effectively reduced the Pb-induced oxidative stress. Previous studies have shown that Pb bioaccumulation increased the production of reactive oxygen species (ROS), which exert negative consequences on the metals-exposed fish (Abdel-Tawwab et al., 2017 a, b). According to Pokorny (1987), antioxidant enzymes serve as the first line of defense against oxidative stress damage, and the antioxidant enzyme defenses are activated to reduce ROS generation. However, as the activity of these enzymes decreased with time and ongoing stress, ROS causes cellular and DNA damage (genotoxicity) (Baş et al., 2015; Abdel-Wahhab et al., 2016; Hamed et al., 2022). In the current study, Pb exposure increased serum MDA levels, which are considered to be a sign of lipid peroxidation (LPO). ROS can cause cellular and molecular changes in the body due to oxidative damage and the modification of lipids, proteins, DNA, and antioxidants (Hamed and Osman, 2017; Karatas et al., 2021). LPO in fish caused by heavy metals has been documented in previous studies (Giri et al., 2021; Kiran Kumar et al., 2021; Hamed et al., 2022).

The DNA fragmentation % in the current investigation was higher in Pb-intoxicated fish confirming that Pb produces free radicals and increases LPO in hepatic tissues. These changes are related to the genotoxicity demonstrated by DNA adduct production and the disruption of $Ca^{2+}$ homeostasis induced by the dysfunction of the endoplasmic reticulum membrane (Jaishankar et al., 2014). Likewise, Sayed et al. (2017) observed that Pb toxicity resulted in hepatic dysfunction in African catfish, including significant decreases in SOD and CAT levels and increases in MDA levels and DNA fragmentation %. Moreover, serum SOD and CAT activities were lowered as the Pb residue in fish tissues increased (Sherif et al., 2020; Giri et al., 2021; Kiran Kumar et al., 2021; Hamed et al., 2022).

However, dietary RLP alone (T2) or in combination with Pb toxicity (T4) remarkably downregulated the MDA concentration and DNA fragmentation % compared with the Pb-intoxicated group alone (T3). Dietary RLP alone considerably increased hepatic SOD, CAT, and TAC concentrations in *O. niloticus* (T2). These findings suggest that dietary RLP stimulated the antioxidant activities in Nile tilapia and improved its defense capacity against Pb-induced oxidative stress. Therefore, the results obtained herein suggest that RLP supplementation to Nile tilapia markedly enhanced the antioxidant defense to transform ROS into safe molecules and shield biological components from oxidation. These findings may be due to the antioxidant properties of several bioactive compounds present in RLP (Mena et al., 2016; Nieto et al., 2018; Borges et al., 2019; Lešnik et al., 2021). These bioactive compounds show important roles in scavenging ROS with strong antioxidant activities (Adimcilar et al., 2019; Ahmadifar et al., 2021). In a comparable investigation, Giri et al. (2021) demonstrated that co-treating Pb-exposed fish with dietary curcumin decreased their MDA levels and increased their antioxidant activity. According to El-Houseiny et al. (2019), feeds enriched with curcumin and black pepper powder dramatically enhanced the antioxidant response of *C. gariepinus* exposed to cadmium. Thus, our findings imply that the inclusion of RLP in diets for Nile tilapia significantly enhanced the antioxidant defense. According to Yousefi et al. (2019), RLP-fed common carp exhibited considerably greater CAT and SOD activities than the control fish. Naiel et al. (2020) also found that providing *O. niloticus* with RLP-enriched diets significantly upregulated serum CAT levels and reduced glutathione levels, especially at the concentration of 10 g/kg diet.

The fish immune system is crucial for fish defense against pathogenic infections (Magnadóttir et al., 2006). Fish exposed to Pb (T3) showed lower levels of LYZ, RBA, and total Ig, which indicates Pb toxicity-related immune suppression. These findings align with previously published studies (Giri et al., 2018; El-Houseiny et al., 2019; Giri et al., 2021). In the current study, the decreased WBCs count observed in the T3 group could be explained by the decreased serum LYZ levels (Ghiasi et al., 2010). Additionally, Pb's affinity for disulfide groups when it attaches to certain proteins on the surface of lymphocytes may inhibit the lymphocytic activity or change the total amount of Ig (Albergoni and Viola, 1995). However, providing the control fish with RLP only (T2) or with Pb exposure (T4) dramatically enhanced the fish's innate immunity. The high content of polyphenols in dietary RLP, particularly carnosic and rosmarinic acids as well as carnosol among others (Mena et al., 2016; Nieto et al., 2018; Borges et al., 2019; Lešnik et al., 2021) are responsible for the high antioxidant and immunological capabilities (Adimcilar et al., 2019; Ahmadifar et al., 2021). In this regard, Yousefi et al. (2019) found that feeding common carp with RLP-supplemented diets significantly increased LYZ, total Ig, and ACH50 levels compared to those in the control group. Naiel et al. (2020) also found that feeding Nile tilapia with RLP-enriched diets led to significantly higher levels of immune biomarkers such as alternative complement, nitroblue tetrazolium, and LYZ, especially at the concentration of 10 g/kg diet.

**Conclusions**

Feeding Nile tilapia fingerlings on RLP-enriched diets supported their growth, innate immunity, and antioxidant capacity. Meanwhile, exposing fish to Pb toxicity deteriorated their growth and welfare status. However, feeding Pb-intoxicated Nile tilapia with 1% RLP significantly reduced the adverse effects of Pb toxicity concerning the growth performance, oxidant/antioxidant capacity, and immunological biomarkers of Nile tilapia. Hence, the current study recommends using 1.0% RLP in fish diets to enhance their performance and welfare and support their challenge against possible pollutants that may occur in aquatic ecosystems.

**Funding**

Authors self-funded this research.

**Data availability**

All data of this study are included in this article.

**Ethics approval and consent to participate**

The Committee Research Ethics Board of the Faculty of Women for Arts, Science & Education, Ain Shams University, Cairo, Egypt, reviewed and approved the present scientific work.

**Competing interests**

The authors declare no competing interests.

### References


Abd El-kader A.I., Bahnasawy M., Zaky M., El-Serafy M.A. (2022). Assessment of heavy metals concentration in water and the Nile tilapia of Lake Manzala, EL-Kapoty, Egypt. Egypt. J. Aquat. Biol. Fish., 26: 137–147.

Abdel Rahman A.N., ElHady M., Hassanin M.E., Mohamed A.A. (2019). Alleviative effects of dietary Indian lotus leaves on heavy metals-induced hepato-renal toxicity, oxidative stress, and histopathological alterations in Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture, 509: 198–208.

Abdel-Tawwab M., El-Sayed G.O., Monier M.N., Shady S.H. (2017 a). Dietary EDTA supplementation improved growth performance, biochemical variables, antioxidant response, and resistance of Nile tilapia, *Oreochromis niloticus* (L.) to environmental heavy metals exposure. Aquaculture, 473: 478–486.

Abdel-Tawwab M., El-Sayed G.O., Shady S.H. (2017 b). Effect of dietary active charcoal supplementation on growth performance, biochemical and antioxidant responses, and resistance of Nile tilapia, *Oreochromis niloticus* (L.) to environmental heavy metals exposure. Aquaculture, 479: 17–24.

Abdel-Tawwab M., Sharafeldin K.M., Ismaiel N.E.M. (2018). Interactive effects of coffee bean supplementation and waterborne zinc toxicity on growth performance, biochemical variables, antioxidant activity and zinc bioaccumulation in whole body of common carp, *Cyprinus carpio* L. Aquacult. Nutr., 24: 123–130.

Abdel-Wahhab M.A., Aljawish A., Kenawy A.M., El-Nekeety A.A., Hamed H.S., Abdel-Aziem S.H. (2016). Grafting of gallic acid onto chitosan nano particles enhances antioxidant activities *in vitro* and protects against ochratoxin A toxicity in catfish (*Clarias gariepinus*). Environ. Toxicol. Pharmacol., 41: 279–288.

Adimcilar V., Kalaycıoğlu Z., Aydoğdu N., Dirmenci T., Kahraman A., Erim F.B. (2019). Rosmarinic and carnosic acid contents and correlated antioxidant and antidiabetic activities of 14 *Salvia* species from Anatolia. J. Pharm. Biomed. Anal., 175: 112763.

Aebi H. (1984). Catalase *in vitro*. Methods Enzymol., 105: 121–126.

Ahmadifar E., Yousefi M., Karimi M., Raieni F.R., Dadar M., Yilmaz S., Dawood M.A.O., Abdel-Latif H.M.R. (2021). Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. Rev. Fish. Sci. Aquac., 29: 478–511.

Albergoni V., Viola A. (1995). Effects of cadmium on catfish, *Ictalurus melas*, humoral immune response. Fish Shellfish Immunol., 5: 89–95.

Ali A., Chua B.L., Chow Y.H. (2019). An insight into the extraction and fractionation technologies of the essential oils and bioactive compounds in *Rosmarinus officinalis* L.: past, present and future. Trac-Trends Anal. Chem., 118: 338–351.

Allain C.C., Poon L.S., Chan C.S.G., Richmond W., Fu P.C. (1974). Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470–475.

Ayoub H.F., El Tantawy M.M., Abdel-Latif H. (2019). Influence of moringa (*Moringa oleifera*) and rosemary (*Rosmarinus officinalis*), and turmeric (*Curcuma longa*) on immune parameters and challenge of Nile tilapia to *Aeromonas hydrophila*. Life Sci. J., 16: 8–15.

Baş H., Kalender Y., Pandir D., Kalender S. (2015). Effects of lead nitrate and sodium selenite on DNA damage and oxidative stress in diabetic and non-diabetic rat erythrocytes and leucocytes. Environ. Toxicol. Pharmacol., 39: 1019–1026.

Belfield A., Goldberg D. (1971). Colorimetric determination of alkaline phosphatase activity. Enzyme, 12: 561–566.

Bengani R., Gadhia M., Ansari E. (2015). Accumulation of lead in the muscle of brackish water fish (*Boleopthalmus dussumieri*). J. Appl. Nat. Sci., 7: 662–665.

Benzie I.F., Strain J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem., 239: 70–76.

Bergmeyer H.U. (1974). Methods of Enzymatic Analysis. Verlag Chemie, Weinheim, Germany.

Bhattacharyya A., Chattopadhyay R., Mitra S., Crowe S.E. (2014). Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev., 94: 329–354.

Borges R.S., Ortiz B.L.S., Pereira A.C.M., Keita H., Carvalho J.C.T. (2019). *Rosmarinus officinalis* essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J. Ethnopharmacol., 229: 29–45.

Boyd C.E. (1984). Water quality in warm water fishponds. Auburn University Agriculture Experimental Station, Auburn, Alabama, USA.

Brown B.A. (1980). Hematology: Principles and procedures. Lea and Febiger, Philadelphia, PA, USA.

Dacie J.V., Lewis S.M. (1984). Practical haematology, 6th ed. Churchill Livingstone, London, UK.

Dawood M.A., Abdel-Tawwab M., Abdel-Latif H.M.R. (2020). Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. Rev. Aquacult., 12: 2511–2526.

Dutta B., Sarma S.R., Deka P. (2015). Lead nitrate toxicity on haematological changes in a live fish species *Channa punctatus* (Bloch). Int. J. Fish. Aquat. Stud., 3: 196–198.

Dytham C. (2011). Choosing and using statistics: a biologist's guide. Blackwell Science Ltd., London, UK.

El-Houseiny W., Khalil A.A., Abd-Elhakim Y.M., Badr H.A. (2019). The potential role of turmeric and black pepper powder diet supplements in reversing cadmium-induced growth retardation, ATP depletion, hepatorenal damage, and testicular toxicity in *Clarias gariepinus*. Aquaculture, 510: 109–121.

Ellis A.E. (1990). Lysozyme assays. Tech. Fish Immunol., 1: 101–103.

El-Sayed A.-F.M. (2019). Tilapia culture, 2nd ed. Academic Press, Elsevier Science Publishing Co Inc., San Diego, USA.

Foster L., Dunn R. (1974). Single antibody technique for radioimmunoassay of cortisol in extracted serum or plasma. Clin. Chem., 20: 365.

Ghiasi F., Mirzargar S.S., Badakhshan H., Shamsi S. (2010). Effects of low concentration of cadmium on the level of lysozyme in serum, leukocyte count and phagocytic index in *Cyprinus carpio* under the wintering conditions. J. Fish. Aquat. Sci., 5: 113–119.

Giri S.S., Kim M.J., Kim S.G., Kim S.W., Kang J.W., Kwon J., Lee S.B., Jung W.J., Sukumaran V., Park S.C. (2021). Role of dietary curcumin against waterborne lead toxicity in common carp, *Cyprinus carpio*. Ecotoxicol. Environ. Saf., 219: 112318.

Giri S.S., Yun S., Jun J.W., Kim H.J., Kim S.G., Kang J.W., Kim S.W., Han S.J., Sukumaran V., Park S.C. (2018). Therapeutic effect of intestinal autochthonous *Lactobacillus reuteri* P16 against waterborne lead toxicity in *Cyprinus carpio*. Front. Immunol., 9: 1824.

Hamed H.S., Abdel-Tawwab M. (2021). Dietary pomegranate (*Punica granatum*) peel mitigated the adverse effects of silver nanoparticles on the performance, haemato-biochemical, antioxidant, and immune responses of Nile tilapia fingerlings. Aquaculture, 540: 736742.

Hamed H.S., Osman A.G.M. (2017). Modulatory effect of lycopene against carbofuran toxicity in African catfish, *Clarias gariepinus*. Fish Pysiol. Biochem., 43: 1721–1731.

Hamed H.S., Ismal S.M., Abdel-Tawwab M. (2022). Modulatory effects of dietary cinnamon (*Cinnamomum zeylanicum*) against waterborne lead toxicity in Nile tilapia fingerlings: Growth performance, haemato-biochemical, innate immunity, and hepatic antioxidant indices. Aquacult. Rep., 25: 101190.

Henry R. (1964). Colorimetric determination of total protein. Clinical Chemistry. Harper and Row Publ., New York, USA.

Hernández A., García García B., Caballero M.J., Hernández M.D. (2015). Preliminary insights into the incorporation of rosemary extract (*Rosmarinus offinalis* L.) in fish feed: influence on performance and physiology of gilthead seabream (*Sparus aurata*). Fish Physiol. Biochem., 41: 1065–1074.

Ikeogu C.F., Nsofor C.I., Igwilo I.O., Ngene A.A. (2016). Haematological and serological responses of *Clarias gariepinus* to sublethal concentrations of lead nitrate. J. Pharm. Sci. Biosci. Res., 6: 442–446.

Jaishankar M., Tseten T., Anbalagan N., Mathew B.B., Beeregowda K.N. (2014). Toxicity, mechanism and health effects of some heavy metals. Interdiscip. Toxicol., 7: 60–72.

Karatas T., Yildirim S., Arslan H., Aggul A.G. (2019). The effects on brown trout (*Salmo trutta fario*) of different concentrations of deltamethrin. Comp. Biochem. Physiol. (Part C), 226: 108606.

Karatas T., Korkmaz F., Karataş A., Yildirim S. (2020). Effects of rosemary (*Rosmarinus officinalis*) extract on growth, blood biochemistry, immunity, antioxidant, digestive enzymes and liver histopathology of rainbow trout, *Oncorhynchus mykiss*. Aquacult. Nutr., 26: 1533–1541.

Karatas T., Onalan S., Yildirim S. (2021). Effects of prolonged fasting on levels of metabolites, oxidative stress, immune-related gene expression, histopathology, and DNA damage in the liver and muscle tissues of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem., 47: 1119–1132.

Kiran Kumar E., Midhun S.J., Vysakh A., James T.J. (2021). Antagonistic effects of dietary *Moringa oleifera* on hematobiochemical and oxidative stress of lead nitrate intoxicated Nile tilapia, *Oreochromis niloticus*. Aquacult. Res., 52: 6164–6178.

Koga K., Shibata H., Yoshino K., Nomoto K. (2006). Effects of 50% ethanol extract from rosemary (*Rosmarinus officinalis*) on α-glucosidase inhibitory activity and the elevation of plasma glucose level in rats, and its active compound. J. Food Sci., 71: 507–512.

Kubiriza G.K., Árnarson J., Sigurgeirsson Ó., Hamaguchi P., Snorrason S., Tómasson T., Thorarensen H. (2019). Growth and hepatic antioxidant enzyme activity of juvenile Arctic charr (*Salvelinus alpinus*) fed on diets supplemented with ethoxyquin, rosemary (*Rosmarinus officinalis*), or bladder wrack (*Fucus vesiculosus*). Aquac. Int., 27: 287–301.

Lee J.-W., Choi H., Hwang U.-K., Kang J.-C., Kang Y.J., Kim K.I., Kim J.-H. (2019). Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: a review. Env. Toxicol. Pharmacol., 68: 101–108.

Lešnik S., Furlan V., Bren U. (2021). Rosemary (*Rosmarinus officinalis* L.): extraction techniques, analytical methods and health-promoting biological effects. Phytochem. Rev., 20: 1273–1328.

Magnadóttir B. (2006). Innate immunity of fish (overview). Fish Shellfish Immunol., 20: 137–151.

McCord J.M., Fridovich I. (1969). Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem., 244: 6049–6055.

Mena P., Cirlini M., Tassotti M., Herrlinger K.A., Dall'Asta C., Del Rio D. (2016). Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (*Rosmarinus officinalis* L.) extract. Molecules, 21: 1576.

Ming J.H., Ye J.Y., Zhang Y.X., Yang X., Shao X.P., Qiang J., Xu P. (2019). Dietary optimal reduced glutathione improves innate immunity, oxidative stress resistance and detoxifiation function of grass carp (*Ctenopharyngodon idella*) against microcystin-LR. Aquaculture, 498: 594–605.

Mohamed W.A.M., Abd-Elhakim Y.M., Farouk S.M. (2016). Protective effects of ethanolic extract of rosemary against lead-induced hepato-renal damage in rabbits. Exp. Toxicol. Pathol., 68: 451–461.

Mommsen T.P., Vijayan M.M., Moon T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish., 9: 211–268.

Naiel M.A., Ismael N.E., Negm S.S., Ayyat M.S., Al-Sagheer A.A. (2020). Rosemary leaf powder–supplemented diet enhances performance, antioxidant properties, immune status, and resistance against bacterial diseases in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 526: 735370.

Naiel M.A., Ismael N.E., Shehata S.A. (2019). Ameliorative effect of diets supplemented with rosemary (*Rosmarinus officinalis*) on aflatoxin B1 toxicity in terms of the performance, liver histopathology, immunity and antioxidant activity of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 511: 734264.

Nieto G., Ros G., Castillo J. (2018). Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis*, L.): a review. Medicines, 5: 98.

NRC (2011). Committee on the Nutrient Requirements of Fish and Shrimp. Nutrient Requirements of Fish and Shrimp. National Research Council. National Academies Press. https://doi.org/10.17226/13039.

Ohkawa H., Ohishi N., Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351–358.

Perandones C.E., Illera V.A., Peckham D., Stunz L.L., Ashman R.F. (1993). Regulation of apoptosis *in vitro* in mature murine spleen T cells. J. Immunol., 151: 3521–3529.

Pokorny J. (1987). Major factors affecting auto-oxidation. In: Autoxidation of unsaturated lipids, Chan H.W. (ed.). Academic Press, pp. 141–207.

Pyszel A., Wrobel T., Szuba A., Andrzejak R. (2005). Effect of metals, benzene, pesticides and ethylene oxide on the haematopoietic system. Med. Pr., 56: 249–255.

Rashed M.N. (2001). Cadmium and lead levels in fish (*Tilapia nilotica*) tissues as biological indicator for lake water pollution. Environ. Mon. Assess., 68: 75–89.

Reitman S., Frankel S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56–63.

Sadoul B., Geffroy B. (2019). Measuring cortisol, the major stress hormone in fishes. J. Fish Biol., 94: 540–555.

Salamat N., Zarie M. (2012). Using of fish pathological alterations to assess aquatic pollution: a review. WJFMS, 4: 223–231.

Sayed A.H., El-Sayed Y.S., El-Far A.H. (2017). Hepatoprotective efficacy of *Spirulina platensis* against lead-induced oxidative stress and genotoxicity in catfish; *Clarias gariepinus*. Ecotoxicol. Environ. Saf., 143: 344–350.

Secombes C.J. (1990). Isolation of salmonid macrophages and analysis of their killing activity. Techniques Fish Immunol., 1: 137–154.

Sherif A.H., Al-Sokary E.T., Rizk W.F., Mahfouz M.E. (2020). Immune status of *Oreochromis niloticus* subjected to long-term lead nitrate exposure and a *Arthrospira platensis* treatment trial. Environ. Toxicol. Pharmacol., 76: 103352.

Siwicki A., Anderson D. (1993). Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. In: Fish disease diagnosis, Siwicki A., Anderson D., Waluga J. (eds). Olsztyn, Poland, pp. 105–112.

Trinder P. (1969). Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol., 22: 246.

Van Kampen E.J., Zijlstra W.G. (1961). Recommendations for haemoglobinometry in human blood. Br. J. Haematol., 13: 71.

Yilmaz S., Ergün S., Şanver Çelik E. (2012). Effects of herbal supplements on growth performance of sea bass (*Dicentrarchus labrax*): change in body composition and some blood parameters. J. BioSci. Biotech., 1: 217–222.

Yousefi M., Hoseini S.M., Vatnikov Y.A., Kulikov E.V., Drukovsky S.G. (2019). Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (*Cyprinus carpio*) fingerlings. Aquaculture, 505: 473–480.

Zhai Q., Wang H., Tian F., Zhao J., Zhang H., Chen W. (2017). Dietary *Lactobacillus plantarum* supplementation decreases tissue lead accumulation and alleviates lead toxicity in Nile tilapia (*Oreochromis niloticus*). Aquacult. Res., 48: 5094–5103.

Received: 5 I 2023
Accepted: 27 IV 2023