

Dietary *Quercus infectoria* Mitigates Lead Nitrate Toxicity in Common Carp (*Cyprinus carpio*): Impacts on Growth Performance, Condition Factors, Weight Length Relationship, Hematological Responses, and Detoxification Potential During 60-Day Exposure

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ABSTRACT

This study investigated the effects of dietary *Quercus infectoria* seeds (QIS) supplementation on lead nitrate $Pb(NO_3)_2$ toxicity in common carp (*Cyprinus carpio*) during a 60-day experiment. Four experimental groups were established: T0 (control, basal diet), T1 (basal diet + 5 mg/L $Pb(NO_3)_2$), T2 (basal diet + 10g/ kg QIS), and T3 (basal diet + 10g/ kg QIS + 5mg/ L $Pb(NO_3)_2$). Growth performance, hematological parameters, and somatic indices were evaluated. Results showed that T2 exhibited a significantly highest final weight ($257.4 \pm 12.7g$, $P < 0.001$) and daily weight gain ($1.8 \pm 0.28g/ day$, $P < 0.05$), while T1 had the lowest final weight ($171.1 \pm 4.7g$, $P < 0.05$) and daily weight gain ($0.33 \pm 0.26g/ day$, $P < 0.05$). Hematological analysis revealed that T1 had elevated white blood cell counts ($12.8 \pm 1.15 \times 10^3/mm^3$, $P < 0.05$) and reduced red blood cell counts ($9.6 \pm 1.20 \times 10^6/mm^3$, $P < 0.05$) compared to T0. QIS supplementation in T3 partially mitigated Pb-induced toxicity, as evidenced by intermediate growth and hematological values. These findings suggest that QIS enhances growth performance and alleviates Pb toxicity in common carp, highlighting its potential as a natural detoxifying agent in aquaculture.

INTRODUCTION

Aquaculture is the fastest-expanding sector in global agriculture, driven by advancements in fish genetics, nutrition, feed technology, culture systems, and management practices (Ceccotti *et al.*, 2019). Fish is considered a staple food in many cultures and has long been known to be rich in essential nutrients such as protein, omega3 fatty acids (Hosomi *et al.*, 2012; Abera & Adimas, 2024), and sources of minerals (iodine and selenium), vitamin-D, and amino acids (taurine, carnitine, melatonin, tryptophan, and polyamines) (Mendivil, 2021). All these make the fish an important and delicious part of a healthy diet. Due to such reasons, the production and consumption of fish has been increasing across the world. Consuming fish, meat, and oil offers significant nutritional and health benefits, such as reducing the risk of diabetes, heart disease, cancer, and other chronic conditions. As a result, the global demand for fish meat and aquatic-based foods has grown substantially. Fish are regarded as the most vulnerable aquatic

organisms to toxic substances found in water (**Hemmadi, 2017**). Fish serves as a dependable bioindicator for evaluating heavy metal pollution levels in aquatic ecosystems (**Authman, 2015**). Additionally, public awareness of the health advantages of fish consumption has steadily risen in recent years (**Carlos., 2009; Lichtenstein, 2021; Tsoupras et al., 2022**). Due to the presence of environmental contaminants and other potential hazards, consumption of fish poses potential human health risks. Methyl mercury, polychlorinated biphenyls (PCBs), dioxins, and pesticides, and also currently, plastic wastes are common contaminants (**Bhuyan, 2022; Montano, 2022; Alberghini, 2023; Asad et al., 2024**). In addition, arsenic, cadmium, lead, selenium, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbon pesticides are some other contaminants in fish (**Soerensen et al., 2022**). Generally, the sources of fish contaminants can be natural or anthropogenic. Natural sources include metals like mercury and arsenic from the Earth's crust, while anthropogenic sources include pollutants from human activities like industrial chemicals and pesticides. Industrial chemicals and pesticides enter the environment through manufacturing, waste disposal, agricultural practices, and PCBs, which were once widely used in industrial applications (**Jaishankar et al., 2014**).

Lead (Pb) is a naturally occurring element in the Earth's crust and is typically present in small quantities in soil, plants, and water (**Sharma & Dubey, 2005; Cheng & Hu, 2010**). Pb is among the most toxic heavy metals that contribute to pollution and pose health risks in both occupational settings and the natural environment (**Mansour & Sidky, 2002; Rose & Lakshmanan, 2024**). **Ashour et al. (2007)** highlighted multiple organs that are particularly vulnerable to the harmful effects of Pb exposure, such as the nervous, blood, and kidney systems. This heavy metal infiltrates the tissues of aquatic organisms via the gastrointestinal tract, diffusion, and inhalation. It induces various forms of toxicity in fish, such as oxidative stress, reproductive dysfunction, and alterations in biochemical parameters (**Łuszczek-Trojnar et al., 2013**). Pb toxicity leads to severe damage to fish organs, including the liver, kidneys, and muscles and alters hematological parameters (**Önen et al., 2012; Al-Balawi et al., 2013**). The common carp (*Cyprinus carpio* L.) is classified under the Kingdom Animalia (**McCrimmon, 1968**). *Cyprinus carpio* (*C. carpio*) is one of the most extensively farmed aquatic fish species worldwide, such as the Middle East and Iraq (**Ahmed, 2023**). Known for its unique flavor and ease of digestion, common carp meat is highly valued. This species is characterized by its omnivorous diet, hardiness, and remarkable ability to thrive in diverse environmental conditions (**Murai, 1992; Hoseini & Al Sulivany 2024**). Molecular genetic studies have identified two distinct subspecies of common carp: *C. carpio*, native to Europe and Central Asia, and *C. haematopterus*, found in East Asia (**Kohlmann et al., 2003, 2005**). Multiple studies have assessed the levels of lead (Pb) in different fish species, revealing that its presence can cause various physiological and metabolic disorders in fish (**Lee et al., 2019; Li et al., 2021**). According to **Omidi et al. (2022)**, scientific observations revealed that increased Pb levels in aquatic environments are associated with diminished

mucosal protein quantities in carp. Furthermore, **Kour et al. (2023)** noted that Pb toxicity intensifies under rising thermal conditions. Pb exposure in fish is linked to behavioral and morphological anomalies, disrupted blood chemistry, and immune system degradation, with cascading neurophysiological impairments (**Li et al., 2019; Kour et al., 2023**).

Quercus infectoria (*Q. infectoria*), commonly referred to as Mazuphal, belongs to the family Fagaceae, genus *Quercus*, and species *infectoria* (**Hashim, 2013**). This small shrub, usually growing up to 2 meters tall, is characterized by its grayish bark and galls, which are gathered for their medicinal properties (**Hashim, 2013; Anwer et al., 2024**). The oak is recognized as a medicinal plant and has been traditionally utilized in folk medicine across various cultures (**Dar et al., 1976**). The seeds of *Q. infectoria* (QIS) have been pharmacologically proven to exhibit astringent, antibacterial, antifungal, larvicidal, antidiabetic, local anesthetic, antiviral, and anti-inflammatory properties (**Khare, 2007**). The QIS, a protective secretion produced by the small shrub *Q. infectoria*, has gained growing recognition for its medicinal uses in countries like Greece, Syria, Iraq, and Iran. The oak galls are known for their diverse biological properties, including antibacterial, antiviral, antifungal, antioxidant, astringent, antidiabetic, antiparkinsonian, antitumor, local anesthetic, antipyretic, and anti-inflammatory effects (**Dar & Ikram, 1979; Wan Nor Amilah et al., 2014**). This microalga has potent antibacterial, antioxidant, and anti-inflammatory properties, along with antitumor, antifungal, antiviral, antiprotozoal, antiamebic, antiulcer, larvicidal, tooth and gum tonic, antipyretic, analgesic/local anesthetic, antidiabetic, cardioprotective, hepatoprotective, antiparkinsonian, antitumor, and wound-healing effects (**Purbowati et al., 2023**). Because of the astringent properties of hydrolyzable tannins, they can lead to adverse effects, such as gastric mucosa irritation, nausea, and vomiting (**Sariozlu & Kivanc, 2011**). This research aimed to evaluate how lead contamination, dietary inclusion of *Quercus infectoria* seeds, and their synergistic interaction influence physiological responses in common carp (*Cyprinus carpio*). Parameters assessed encompass growth dynamics, morphometric ratios, somatic indices, and blood-related biomarkers.

MATERIALS AND METHODS

Ethical approval and consent

The authors provided verbal informed consent for their involvement in the study. The research design and methodology were evaluated and approved by the Animal Ethics Committee at the College of Science, University of Zakho, ensuring adherence to ethical guidelines (AEC-058; 2024).

Study areas and fish collection

Juvenile common carp (*Cyprinus carpio*) of both sexes (total number 80 fish) with mean weight (151.4 ± 6.4 g), total length (19.71 ± 2.33 cm), fork length (17.69 ± 2.55 cm), and standard length (15.6 ± 2.4 cm) were collected from a specialized

hatchery in Khanke township located in the Summil district of Duhok Governorate in the Kurdistan Region of Iraq. Khanke lies near the Mosul Dam (Chambarakat Dam), located approximately 50km north of Mosul and about 70-80km south of Duhok. It sits on the Tigris River, the region's key water and energy resource. This strategic location makes Khanke a vital region for water resources and fisheries (Al Sulivany, 2024a). The fish were then transported and acclimatized in laboratory conditions (Fish lab of Biology Department, College of Science, University of Zakho) for fifteen days, allowing them to adapt to normal laboratory settings. During acclimatization, the fish were kept in circular polyethylene tanks (100×71.4cm) with a capacity of about 400 liters. To kill the pathogens, juvenile *C. carpio* was submerged in a saline solution (Sodium chloride; NaCl) purchased from Sigma-Aldrich for 1-2 minutes (Das *et al.*, 2025; Hassan *et al.*, 2025). During acclimatization, fish were fed a basal diet twice over 24 hours acquired from Amedi Animal Feed Company, as shown in Table (1). Continuous aeration was provided to each tank using small aquarium air pumps (Luckiness 828, power: 5 W, airflow: 3.5L/ min) and Chinese air compressors (Hailea ACO-318, power: 45 W, airflow: 70L/ min; Hailea ACO-328, power: 55 W, airflow: 82 L/min; Resun ACO-010, power: 200 W, airflow: 0.135m³/ min). Culture conditions were monitored daily and were adjusted to optimum conditions where pH, dissolved oxygen, temperature, and salinity were measured as 8.28± 0.23, 7.4 ± 0.4, 16± 0.3, and 0.06g/ L (Owais *et al.*, 2024a).

Diet preparation and experimental design

Quercus infectoria seeds (QIS) were collected from a mountain near the Batifa district, Zakho, Duhok, KRG, Iraq. All the ingredients were ground and thoroughly mixed in a blender to prepare the diets mixed with QIS. Then, small quality water was incorporated to make a smooth dough, which was extruded using an electric-extruder homemade pasta machine with a mesh plate of 2mm in size. Extruded pellets were dried overnight at 50°C and stored at -18°C until further utilization. The diet composition of each diet is shown in Table (1). Lead nitrate Pb (NO₃)₂ was sourced from Sigma-Aldrich, Turkey.

On the 15th of October 2024, the fish were divided into four treated groups, each with duplicate replicates (10 fish per tank). The groups were labelled as T0, T1, T2, and T3. T0 was the control group, where fish were fed only the basal diet pellets. T1 consisted of a fish-fed basal diet and exposure to lead nitrate at a 5mg/ L concentration. T2 included fish fed the basal diet pellets mixed with 10g/ kg of QIS. T3 comprised fish fed the basal diet pellets mixed with 10g/ kg of QIS and exposed to lead nitrate at 5mg/ L. The carp were fed every morning at 9 am for 60 days at a ratio of 3mg/ kg body weight. The contents of the faeces and remaining food were extracted using a vacuum. There were no recorded deaths during the examination.

Table 1. Ingredient and proximate composition analysis of fish-fed diets with different concentrations of *Q. infectoria* during 60-day intervals

Ingredient	T0	T1	T2	T3
Fish Meal	16	16	16	16
Corn	14	14	14	14
Soybean Meal	28	28	28	28
Barley	17	17	17	17
Wheat	22	22	22	22
Premix	2	2	2	2
Ascorbic acid	1	1	1	1
Proximate composition of fish feds				
Dry Matter (%)	92.90	92.90	92.90	92.90
Crude Protein (%)	30.0	30.0	30.0	30.0
Crude Lipid (%)	8.3	8.3	8.3	8.3
Crude Fiber (%)	3.30	3.30	3.30	3.30
Ether Extract (%)	4.80	4.80	4.80	4.80
Ash (%)	7.22	7.22	7.22	7.22
Moisture (%)	7.10	7.10	7.10	7.10
Organic Matter (%)	75.68	75.68	75.68	75.68

Growth morphometry

After 60 days of exposure, the fish individual was placed on the ruler with its snout at the 0cm mark to determine its length. Juvenile carp were measured for three different lengths: total length (TL), which is measured from the nose to the caudal fin; standard length (SL), which is measured from the snout to the end of the tail; and fork length (FL), which is measured from the snout to the middle of a concave tail (Asad *et al.*, 2024). Additionally, the fish's weight gained in a single day was measured by their daily weight gain (DWG); total weight gain (TWG) is the sum of the weight that fish have gained over a specific period; the fish's weight growth rate (WGR) determines the percentage increase in weight over a given period about its starting weight, condition factor (K). The relative growth rate (RGR) is a measurement of the proportionate growth in fish size over time, while the metabolic growth rate (MGR) is the correlation between the growth and metabolic rate of a single fish. The specific growth rate (SGR) was used to define the percentage increase in a fish's body weight over a given period. Those measurements were calculated, according to the following equations (Ahmed, 2023):

$$DWG \text{ (gr/day)} = FW - IW/t \quad (\text{Al Sulivany et al., 2024b})$$

$$TWG \text{ (gr)} = FW - IW$$

$$RGR(\%) = FW - IW/IW \times 100 \quad (\text{Lieke et al., 2021})$$

$$MGR \text{ (gkg}^{0.8} \text{ day}^{-1}) = (TWG) / [\{ (IW/1000)^{0.8} + (FW/1,000)^{0.8} \}] / 2 \quad (\text{White et al., 2022})$$

$$SGR = \frac{\ln FW - \ln IW}{t} \times 100 \quad (\text{Owais et al., 2024b})$$

$$K = 100 \times W/TL^3 \quad (\text{Ahmed, 2023})$$

Where, (W) stands for wet weight; (FW) is final weight; (IW) is initial weight; (TL) is the total length; and (t) is the duration of the experiment.

Hematological parameter

At the end of the experimental trials, the hematological parameters were determined. Fish (5 per tank) were anesthetized using an MS-222 (tricaine methane sulfonate, 0.1g L⁻¹, Sigma-Aldrich, USA) to minimize stress and prevent injury during the procedure. Blood samples were collected from the caudal vein using 3mL syringes. The blood samples were transferred to an Ethylenediaminetetraacetic acid (EDTA) tube to prevent coagulation. Hematocrit levels were determined through the micro-hematocrit technique (Gallaugher & Farrell, 1998). Red blood cells (RBCs) and white blood cells (WBCs) were quantified using a hemocytometer and the Neubauer Counting Chamber method, following the protocol of Stolen *et al* (1990). Haemoglobin (Hb) concentration was measured according to the method of Wedemeyer and Yasutake (1977). Blood indices, including mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC), were calculated using the following formulas:

$$MCV = \frac{10 \times PCV}{RBCs}, \quad MCH = \frac{10 \times Hb}{RBCs}, \quad MCHC = \frac{100 \times Hb}{PCV} \quad (\text{Arsalan et al., 2016}).$$

Biological somatic indices

At the end of the experiment, the biological somatic indices were determined. Fish were caught in a dip net, killed by a sharp blow to the head, and dissected with scissors. The weight of the whole organs, including the liver, spleen, gills, heart, kidneys, brain, intestines, and intestinal length, was recorded according to the method of the U.S. (Rehman, 2013).

$$\text{Organsomatic index (\%)} = \frac{\text{organ weight (gr)}}{\text{fish weight (gr)}} \times 100$$

$$\text{Intestinal length index (\%)} = \frac{\text{Intestinal length (cm)}}{\text{fish length (cm)}} \times 100$$

Statistical analysis

The experimental data were analyzed statistically using GraphPad Prism 9, employing Analysis of Variance (ANOVA). Duncan's Multiple Range Test was conducted for mean comparisons to identify significant differences among groups. A significance level of $P < 0.05$ was considered statistically meaningful. Results are presented as means with standard errors. Additionally, the length-weight relationship was determined using the linear correlation coefficient.

RESULTS

Growth morphometry and survivability

Following the administration of different dietary treatments specifically, the basal diet pellets (T0), the basal diet with exposure to 5mg/ l lead nitrate (T1), diet pellets supplemented with 10g/ kg QIS (T2), and the QIS-supplemented diet combined with lead nitrate exposure (T3), the results are presented in Table (2) and Fig. (1A, B, C, D, E, F, G, H, and J). The IW was statistically non-significant ($P > 0.05$) between all the groups (T0, T1, T2, and T3), with means ranging from 149 ± 16.1 g (T0) to 152.6 ± 16.7 g (T2).

The FW showed significant variation: Fish feds basal diet pellets (T0) had a mean FW of 235.4 ± 11.6 g, T1 (lead nitrate exposing) had the lowest FW at 171.1 ± 4.7 g; ($P < 0.05$), T2 had the highest FW at 257.4 ± 12.7 g; ($P < 0.001$), whereas the fish feds diet with QIS and exposing to lead nitrate (T3) showed an intermediate final weight of 223 ± 12.4 g ($P < 0.05$).

The DWG also varied significantly, with T2 exhibiting the DWG gain (1.8 ± 0.28 g/ day), followed by T0 (1.44 ± 0.26 g/ day), T3 (1.2 ± 0.4 g/ day), and T1 (0.33 ± 0.26 g/ day). After 60 days of mixing fish-feeding basal diet pellets with QIS, the SGR reveals significant elevation compared to the control. On the other hand, the SGR reveals a decrease after exposing the fish to (5g/ kg) lead nitrate. Whereas, when fish fed a diet supplied with QIS and exposed to lead nitrate, the SGR showed significant ($P < 0.01$) elevation compared to the fish exposed to lead nitrate alone. The RGR_{weight} was highest in T2 ($79.2 \pm 13.5\%$; $P < 0.001$) and lowest in T1 ($26.36 \pm 12.4\%$). Metabolic growth rates followed a similar pattern of RGR_{weight} , with QIS (T2) groups showing the highest rate (193.8 ± 33.5 g/kg $^{0.8}$ /day) and lead nitrate exposing fish (T1) the lowest (52.1 ± 22.8 g/kg $^{0.8}$ /day). The survival rate was 100% for T0, T2, and T3 but decreased to 85% in T1.

Table 2. Impact of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combined effects on growth morphometry and survivability

	T0 (Basal diet)	T1 Pb(NO ₃) ₂	T2 (QIS)	T3 Pb(NO ₃) ₂ +QIS
IW (gr)	149±16.1	151.4±15.4	152.6±16.7	150.7±21.6
FW (gr)	235.4 ^a ±11.6	171.1 ^b ±4.7	257.4 ^a ±12.7	223 ^{ab} ±12.4
DWG (gr)	1.44 ^a ±0.26	0.33 ^b ±0.26	1.8 ^c ±0.28	1.2 ^{ad} ±0.4
TWG (g/day)	86.4 ^a ±15.8	19.7 ^b ± 3.7	104.8 ^a ±16.6	72.3 ^{±a} ±21.3
SGR (%)	0.82 ^a ±0.12	0.27 ^b ±0.23	0.9 ^c ±0.24	0.75 ^{ad} ±0.21
WGR (%)	71.6a±16.6	26.3 ^b ±12.4	79.2c±13.5	66.9ad±15.6
MGR (gk.g ^{0.8} day ⁻¹)	169.3 ^a ±37.1	52.1 ^b ±22.8	193.8 ^c ±33.5	151.2 ^{cd} ±46.7
RGR _{weight} (%)	1.19 ^a ±0.23	0.43 ^b ±0.07	1.32 ^c ±0.4	1.12 ^{ad} ±0.21
Survival rate (%)	100	85	100	100

IW; for initial weight. FW: for final weight. DWG for daily weight gain. TWG; for total weight gain. SGR is for a specific growth rate. WGR is for a weight growth rate. MGR; for metabolic growth rate. RGR_{weight}; for the relative growth rate of weight

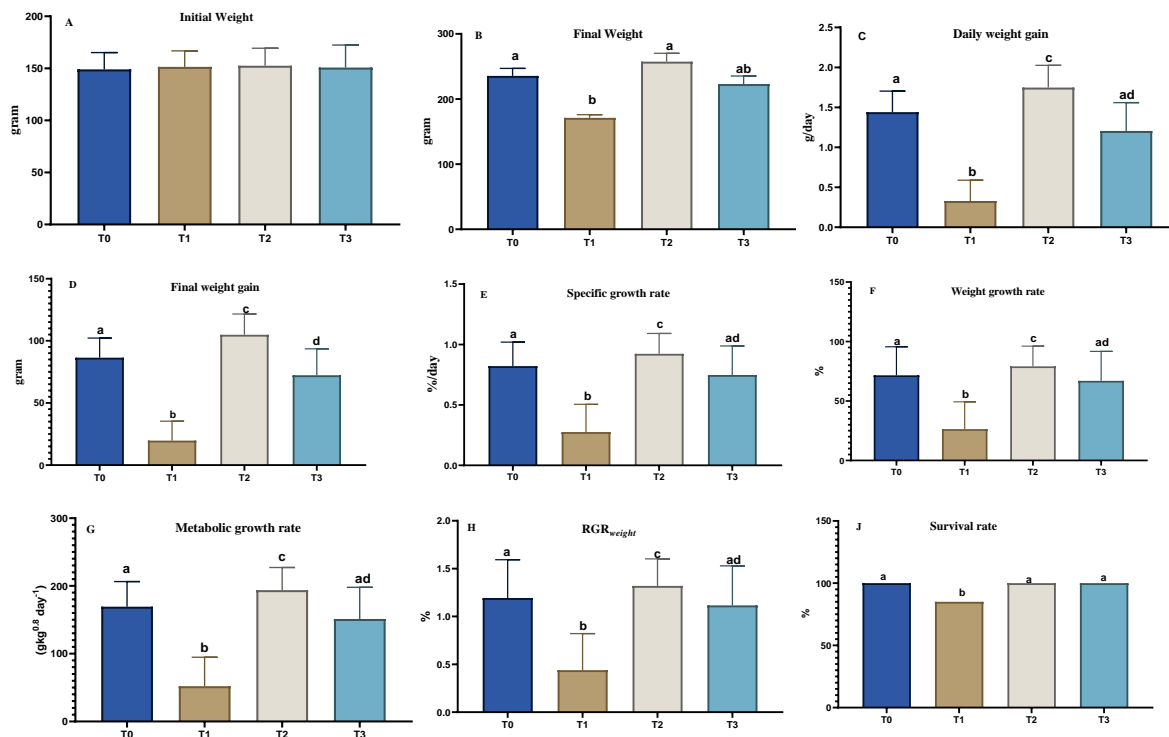


Fig. 1. Effects of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combination effects on growth parameter and survival rate after exposing the *C. carpio* for 60-day intervals. A: for initial weight. B: for final weight. C: for daily weight gain. D: for total weight gain. E: for specific growth rate, F: for weight growth rate. G: for metabolic growth rate. H for the relative growth rate of weight. J: for survival rate. Significant differences ($P < 0.05$) are indicated by distinct superscripts (a, b, c, and d).

Condition factors (Fulton; *K*. Modified; *Kb*, and Relative; *Kn*)

Fish's condition factors (Fulton, Modified, and Relative) across four treatment groups over a 60-day experimental period are shown in Table (3) and Fig. (2). The initial Fulton condition factor (Initial-*K*) for the control group (T0) was 1.72 ± 0.1 , which increased to 2.09 ± 0.2 by the end of the experiment (Fig. 3A). In contrast, the fish in the T1 group (basal diet + 5mg/ L lead nitrate) showed a significant decrease in the final Fulton condition factor (Final-*K*) to 1.64 ± 0.09 ($P < 0.05$), indicating a negative impact of lead nitrate on fish condition (Fig. 2B). The T2 group (basal diet + *Q. infectoria* at 10g/ kg) and T3 group (basal diet + *Q. infectoria* + 5mg/ L lead nitrate) exhibited Final-*K* values of 2.05 ± 0.17 and 2.0 ± 0.12 , respectively, which were not significantly different from the control ($P > 0.05$). Similarly, the modified condition factor (Final-*Kb*) showed a significant decline in T1 (1.04 ± 0.08) compared to T0 (1.35 ± 0.14 , $P < 0.05$), while T2 (1.34 ± 0.11) and T3 (1.27 ± 0.12) remained comparable to the control. The relative condition factor (Final-*Kn*) followed a similar trend, with T1 (1.76 ± 0.09) showing a

significant reduction compared to T0 (2.24 ± 0.2 , $P < 0.05$), while T2 (2.19 ± 0.18) and T3 (2.15 ± 0.17) were not significantly different from the control.

Table 3. Impact of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combined effects on condition factors

	T0 (Basal diet)	T1 Pb (NO ₃) ₂	T2 (QIS)	T3 Pb (NO ₃) ₂ +QIS
Initial- <i>K</i>	1.72±0.1	1.957±0.17	1.95±0.25	1.94±0.32
Final- <i>K</i>	2.09a±0.2	1.64b±0.09	2.05a±0.17	2.0a±0.12
Initial- <i>Kb</i>	1.1±0.12	1.26±0.11	1.25±0.16	1.25±0.21
Final- <i>Kb</i>	1.35a±0.14	1.04b±0.08	1.34a±0.11	1.27a±0.12
Initial- <i>Kn</i> ;	1.86±0.2	2.13±0.19	2.12±0.27	2.12±0.12
Final- <i>Kn</i>	2.24a±0.2	1.76b±0.09	2.19a±0.18	2.15a±0.17

Initial-*K*; for Initial Fulton condition factor. Final-*K*; for final Fulton condition factor. Initial-*Kb*; Initial modified condition factor. Final-*Kb*; for Final modified condition factor. Initial-*Kn*; Initial relative condition factor. Final-*Kn*; Final relative condition factor.

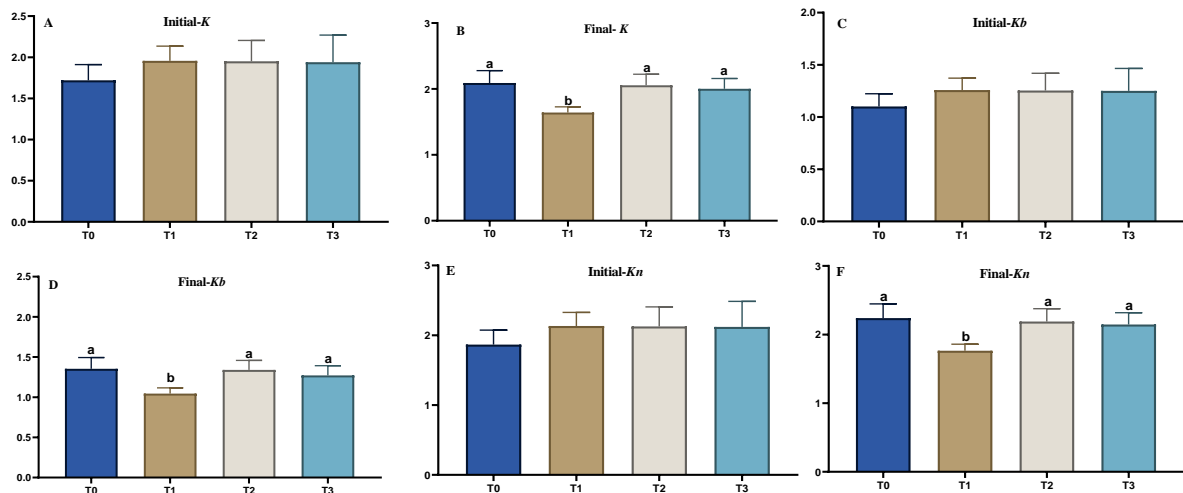


Fig. 2. Effects of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combination effects on condition factors after exposing the *C. carpio* for 60-day intervals. A; for Initial Fulton condition factor. B; for final Fulton condition factor. C: Initial modified condition factor. D; for Final modified condition factor. E: Initial relative condition factor. F; Final relative condition factor. Significant differences ($P < 0.05$) are indicated by distinct superscripts (a, b, c, and d)

Weight length relationship (WLR)

The results of the 60-day experiment revealed distinct relationships between weight and length across the groups (T0, T1, T2, T3), as indicated by the intercept, slope, and R^2 values (Fig. 3A, B, C, D, E, F, G, and H). The control group (T0), fed a basal diet pellet, had an intercept of $-0.0677 + 2.4605$, a slope of 1.75 ± 0.05 , and an R^2 value of

0.0011, reflecting a stable and consistent growth pattern. In contrast, the T1 group, exposed to 5mg/ kg of lead nitrate, showed an intercept of $0.1848 + 2.4749$, a reduced slope of 1.75 ± 0.05 , and a lower R^2 value of 0.05, indicating that lead exposure disrupted the WLR and impaired growth. The T2 group, co-supplemented with *Q. infectoria* seed at 10g/ kg, exhibited an intercept of $0.5844x + 1.608$, a slope of 1.56 ± 0.018 , and a higher R^2 value of 0.08. The T3 group, which received both *Q. infectoria* seed supplementation and lead nitrate exposure, had an intercept of $0.564 + 1.58$, a slope of 1.56 ± 0.018 , and an R^2 value of 0.055, demonstrating intermediate results that indicate partial protection against the toxic effects of lead.

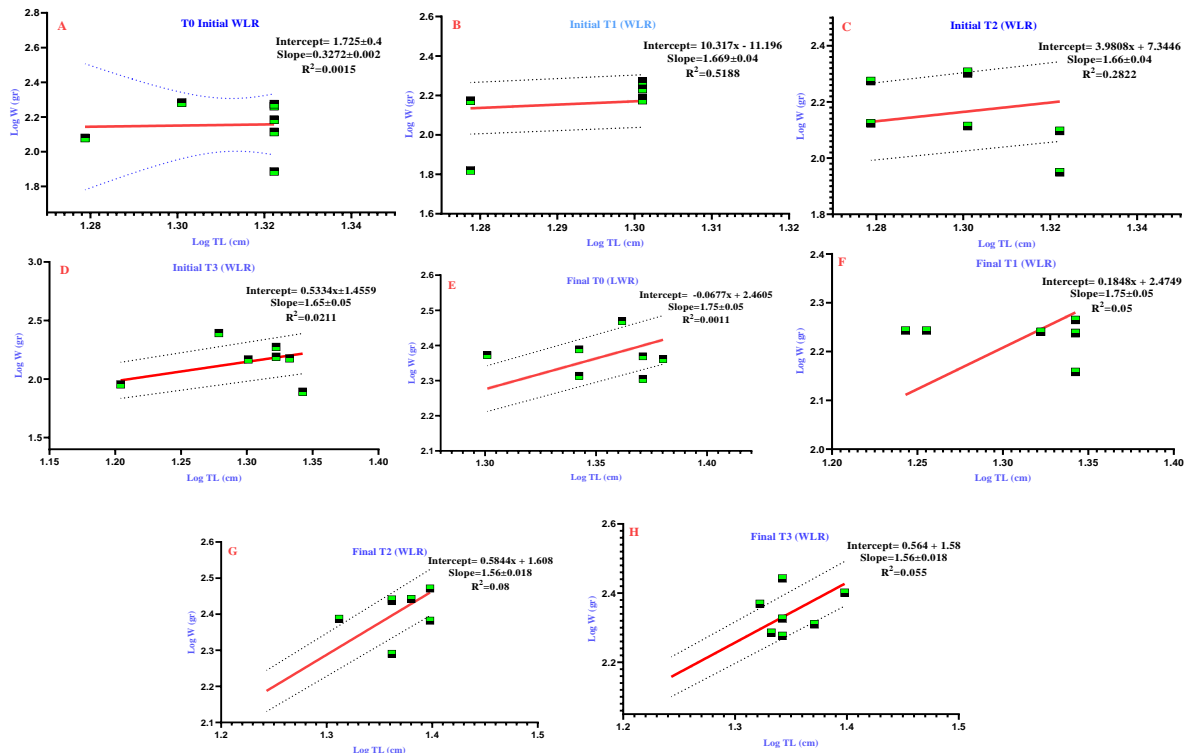


Fig. 3. Effects of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combination effects on weight length relationship (WLR) after exposing the *C. carpio* for 60-day intervals

Biological somatic indices

The body mass indices in fish across four treatment groups over a 60-day experimental period are shown in Table (4) and Fig. (4). The hepatosomatic index (HSI) was significantly higher in the T1 group ($3.12 \pm 0.24\%$) compared to the control group (T0: $1.31 \pm 0.34\%$, $P < 0.05$). However, the T2 group ($1.48 \pm 0.51\%$) and T3 group ($2.08 \pm 0.27\%$) showed no significant difference from the control (Fig. 4A). The splenosomatic index (SSI) was also significantly higher in T1 ($0.29 \pm 0.039\%$) compared to T0 ($0.12 \pm 0.034\%$, $P < 0.05$), while T2 ($0.17 \pm 0.04\%$) and T3 ($0.24 \pm$

0.047%) showed intermediate values (Fig. 4B). Similarly, the renosomatic index (RSI) was significantly elevated in T1 ($0.95 \pm 0.02\%$) compared to T0 ($0.41 \pm 0.09\%$, $P < 0.05$), with T2 ($0.38 \pm 0.079\%$) and T3 ($0.54 \pm 0.13\%$) showing no significant difference from the control (Fig. 4C). The cardiosomatic index (CSI) followed a similar trend, with T1 ($0.22 \pm 0.016\%$) significantly higher than T0 ($0.11 \pm 0.025\%$, $P < 0.05$), while T2 ($0.13 \pm 0.021\%$) and T3 ($0.17 \pm 0.013\%$) were not significantly different from the control (Fig. 4D). The intestinal somatic index (ISI) was significantly higher in T1 ($2.22 \pm 0.42\%$) compared to T0 ($1.48 \pm 0.12\%$, $P < 0.05$), but T2 ($1.7 \pm 0.02\%$) and T3 ($1.76 \pm 0.36\%$) were not significantly different from the control (Fig. 4E). The gill somatic index (GSI) and brain somatic index (BSI) showed no significant differences among the groups ($P > 0.05$) (Fig. 4F, G).

Table 4. Impact of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combined effects on biological somatic indices

	T0 (Basal diet)	T1 Pb (NO ₃) ₂	T2 (QIS)	T3 Pb (NO ₃) ₂ +QIS
HIS (%)	1.312 ^a ±0.34	3.12 ^b ±0.24	1.48 ^a ±0.51	2.08 ^a ±0.27
SSI (%)	0.12 ^a ±0.034	0.29 ^b ±0.039	0.17 ^{ab} ±0.04	0.24 ^{ab} ±0.047
RSI (%)	0.41 ^a ±0.09	0.95 ^b ±0.02	0.38 ^{ab} ±0.079	0.54 ^{ab} ±0.13
CSI (%)	0.11 ^a ±0.025	0.22 ^b ±0.016	0.13 ^a ±0.021	0.17 ^{ab} ±0.013
ISI (%)	1.48 ^b ±0.12	2.22 ^a ±0.42	1.7 ^b ±0.02	1.76 ^b ±0.36
BSI (%)	0.14±0.01	0.15±0.02	0.16±0.02	0.16±0.01
GSI (%)	1.9±0.28	2.3±0.45	1.9±0.22	2.37±0.21
ILI (%)	12.81 ^c ±0.7	12.37 ^c ±1.8	24.08 ^a ±1.7	17.56 ^b ±1.08

HIS; hepatosomatic index, SSI; spleenosomatic index, RSI; renosomatic index, CSI; cardiosomatic index, ISI; intestinal somatic index, BSI; brain somatic index. GSI; gill somatic index, ILI; intestinal length index.

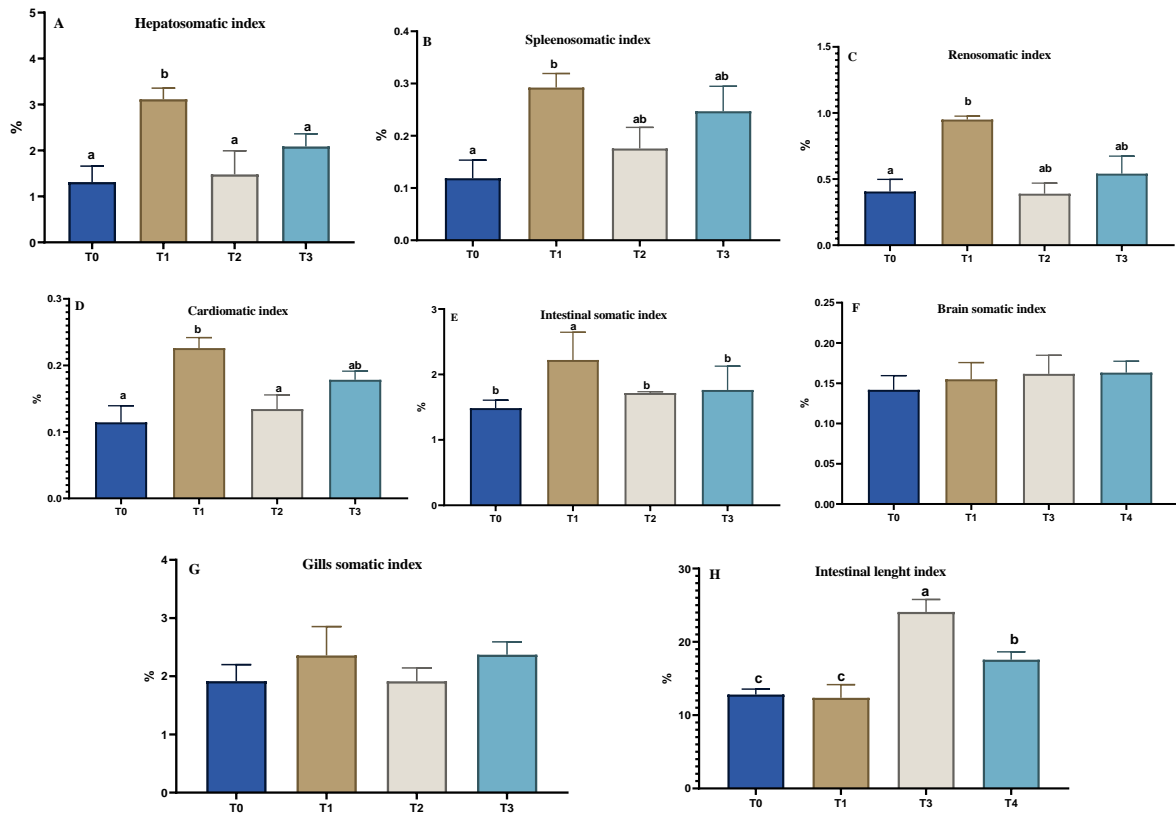


Fig. 4. Effects of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combination effects on biological somatic indices after exposing the *C. carpio* for 60-day intervals. A: hepatosomatic index, B: splenosomatic index, C: renosomatic index, D: cardiosomatic index, E: intestinal somatic index, F: brain somatic index. G: gill somatic index, H; intestinal length index. Significant differences ($P < 0.05$) are indicated by distinct superscripts (a, b, c, and d)

Hematological parameters

The WBC count was significantly higher in the T1 group ($12.8 \pm 1.15 \times 10^3/\text{mm}^3$) compared to the control group (T0: $7 \pm 1.1 \times 10^3/\text{mm}^3$, $P < 0.05$), indicating an immune response to lead nitrate exposure. In contrast, the T2 group ($5.4 \pm 1.2 \times 10^3/\text{mm}^3$) showed a significant decrease in WBC count compared to T0 ($P < 0.05$), while the T3 group ($9.2 \pm 3.1 \times 10^3/\text{mm}^3$) was not significantly different from the control ($P > 0.05$) (Table 5 and Fig. 5A). The RBC count was significantly lower in T1 ($9.6 \pm 1.20 \times 10^6/\text{mm}^3$) compared to T0 ($13.33 \pm 2.4 \times 10^6/\text{mm}^3$, $P < 0.05$). However, the T2 ($15 \pm 1.15 \times 10^6/\text{mm}^3$) and T3 ($14.33 \pm 6.88 \times 10^6/\text{mm}^3$) groups showed no significant difference from the control (Fig. 5B). The Hb concentration did not differ significantly among the groups ($P > 0.05$), with values ranging from $10.33 \pm 0.3\text{g}/100\text{mL}$ in T1 to $11.83 \pm 0.6\text{g}/100\text{mL}$ in T3 (Fig. 5C). The PCV was significantly lower in T1 ($30 \pm 0.5\%$) compared to T0 ($33.33 \pm 0.88\%$, $P < 0.05$), while T2 ($35 \pm 0.7\%$) and T3 ($35.67 \pm 1.8\%$) showed no significant difference

from the control ($P > 0.05$) (Fig. 5D). The MCV was significantly higher in T1 (31.94 ± 3.67 fL) and T3 (36.93 ± 13.9 fL) compared to T0 (26.76 ± 4.98 fL, $P < 0.05$), while T2 (22.33 ± 0.87 fL) was significantly lower ($P < 0.05$) (Fig. 5E). The MCH was significantly higher in T1 (11.02 ± 1.34 pg) and T3 (12.26 ± 4.6 pg) compared to T0 (8.9 ± 1.71 pg, $P < 0.05$), while T2 (7.4 ± 0.29 pg) showed no significant difference ($P > 0.05$) (Fig. 5F). The MCHC did not differ significantly among the groups ($P > 0.05$) (Fig. 5G).

Table 5. Impact of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combined effects on hematological parameters

Parameters	T0 (Basal diet)	T1 Pb (NO ₃) ₂	T2 (QIS)	T3 Pb (NO ₃) ₂ +QIS
WBCs (10 ³ mm ⁻³)	7 ^{ab} ±1.1	12.8 ^b ±1.15	5.4 ^a ±1.2	9.2 ^{ab} ±3.1
RBCs (10 ⁶ mm ⁻³)	13.33 ^a ±2.4	9.6 ^b ±1.20	15 ^a ±1.15	14.33 ^a ±6.88
Hb (g/100ml)	10.8±0.2	10.33±0.3	11.07±0.5	11.83±0.6
PCV (%)	33.33 ^b ±0.88	30 ^a ±0.5	35 ^b ±0.7	35.67 ^b ±1,8
MCV (fL)	26.76 ^a ±4.98	31.94 ^b ±3.67	22.33 ^{ac} ±0.87	36.93 ^b ±13.9
MCH (Pg)	8.9 ^a ±1.71	11.02 ^{ab} ±1.34	7.4 ^a ±0.29	12.26 ^b ±4.6
MCHC (%)	33.39±0.17	34.44±1.11	33.21±0.6	33.18±0.03

WBCs; White blood cells. RBCs; Red blood cell. Hb; hemoglobin. PCV; Packet cell volume. MCV; Mean cell volume. MCH; Mean cell hemoglobin. MCHC; Mean cell hemoglobin concentration.

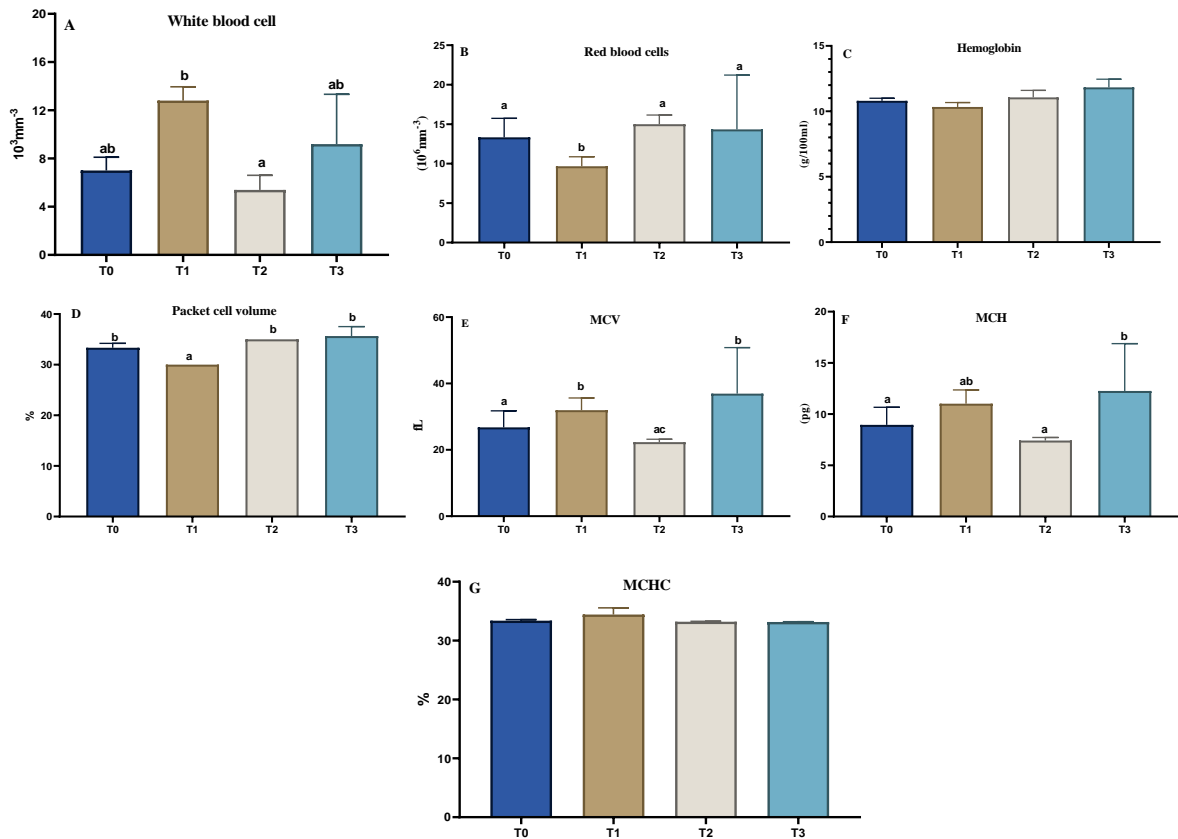


Fig. 5. Effects of dietary supplementation with *Q. infectoria*, lead nitrate exposure, and their combination effects on hematological parameters after exposing the *C. carpio* for 60-day intervals. WBCs: White blood cells. RBCs: Red blood cells. Hb; hemoglobin. PCV; Packet cell volume. MCV: Mean cell volume. MCH: Mean cell hemoglobin. MCHC: Mean cell hemoglobin concentration. Significant differences ($P < 0.05$) are indicated by distinct superscripts (a, b, c, and d).

DISCUSSION

The observed changes in growth morphometry and survivability of *C. carpio* under different dietary treatments can be attributed to the distinct physiological and metabolic responses induced by QIS supplementation, Pb (NO₃)₂ exposure, and their combined effects. The significant enhancement in growth parameters, such as FW, DWG, and SGR, in the QIS-supplemented group suggests that QIS may possess bioactive compounds that promote growth and improve nutrient utilization. Previous studies have shown that plant-based supplements, particularly those rich in polyphenols and antioxidants, can enhance fish growth by improving feed efficiency, stimulating appetite, and modulating gut microbiota (Abdulrahman *et al.*, 2022; Fawzy *et al.*, 2022). In contrast, the marked decline in growth metrics in the Pb (NO₃)₂ -exposed group (T1) is

likely due to the toxic effects of Pb, which can disrupt metabolic processes, impair nutrient absorption, and induce oxidative stress, leading to reduced growth and SRs (Vinodhini & Narayanan, 2008; Javed & Usmani, 2016). The intermediate growth performance observed in the combination group (T3) indicates that QIS may partially mitigate the adverse effects of Pb (NO₃)₂, possibly through its antioxidant properties, which could counteract oxidative damage and support metabolic functions. This finding aligns with previous research demonstrating that dietary antioxidants can alleviate heavy metal toxicity in fish by reducing oxidative stress and enhancing detoxification pathways (Abdelkhalek *et al.*, 2017; Alagawany *et al.*, 2020).

The significant decline in Fulton, modified, and relative condition factors in the Pb-exposed group reflects Pb-induced oxidative stress and metabolic dysfunction, as heavy metals like Pb disrupt cellular homeostasis, impair enzyme activity, and damage lipid membranes, leading to reduced energy allocation for growth and maintenance (Javed & Usmani, 2016). QIS maintained condition factors, suggesting that QIS, at the tested concentration, does not inherently enhance growth but preserves physiological stability, potentially due to its polyphenolic constituents that support basal metabolic functions without overt antinutritional effects (Morales, 2021). The intermediate CFs in the combination group indicate partial mitigation of Pb toxicity by QIS, likely mediated through its antioxidant properties, such as scavenging reactive oxygen species (ROS) and chelating metal ions, thereby reducing oxidative damage and restoring metabolic efficiency. This aligns with studies demonstrating that plant-derived antioxidants, including tannins and flavonoids in QIS, counteract heavy metal toxicity by enhancing detoxification pathways and improving cellular resilience (Alagawany *et al.*, 2020). Similarly, the disrupted WLR in T1 underscores lead's interference with somatic growth dynamics, whereas the partial recovery in T3 highlights QIS's role in stabilizing nutrient partitioning and growth trajectories. These findings corroborate prior research on phytogetic supplements, which often exhibit dose-dependent protective effects against environmental pollutants, balancing growth modulation and toxicity alleviation (Vakili, *et al.*, 2023).

The marked elevation in HSI, SSI, and RSI indices in the Pb-exposed group aligns with lead's capacity to induce oxidative stress and organ hypertrophy, as heavy metals disrupt detoxification pathways, necessitating compensatory enlargement of metabolic organs to mitigate cellular damage (Özkan-Yilmaz *et al.*, 2014). QIS-supplemented group (T2) exhibited indices comparable to the control, suggesting that QIS at 10g/ kg does not impose additional metabolic strain, likely due to its balanced phytochemical profile, which may support baseline physiological functions without triggering antinutritional effects (Burlacu *et al.*, 2020). The partial attenuation of lead-induced stress is potentially mediated by QIS-derived antioxidants such as gallotannins and ellagic acid, which are known to scavenge ROS and enhance hepatic detoxification, thereby reducing organ-specific damage (Abd El-Rahman *et al.*, 2019). These findings

corroborate studies demonstrating that phenolic-rich plant extracts ameliorate heavy metal toxicity by improving antioxidant defenses and stabilizing cellular integrity in fish (Hamed *et al.*, 2022).

On the other hand, the alterations in hematological parameters observed under different treatments reflect the interplay between Pb (NO₃)₂, toxicity and QIS supplementation. The elevated WBC count in the Pb-exposed group aligns with lead-induced immune-stimulation or inflammation, as heavy metals like Pb trigger oxidative stress and cellular damage, prompting leukocyte proliferation to counteract tissue injury (Ahmed *et al.*, 2020). Conversely, the reduced WBC count in the QIS-supplemented group suggests potential anti-inflammatory properties of QIS, possibly mediated by its polyphenolic constituents, which modulate immune responses (Saxena & Flora, 2004). The anemia-like profile is T1-evidenced by RBC count, PCV, and elevated MCV and MCH, likely stems from lead's disruption of erythropoiesis and hemoglobin synthesis, consistent with lead's capacity to inhibit heme biosynthesis enzymes and induce oxidative hemolysis (Abdel-Warith *et al.*, 2020; Öz *et al.*, 2024). In contrast, the restored RBC and PCV levels in the T2 and T3 groups indicate QIS's protective role, potentially through antioxidant mechanisms that preserve erythrocyte membrane integrity and enhance iron utilization. The intermediate hematological values in T3 suggest partial mitigation of lead toxicity by QIS, likely via chelation of lead ions and scavenging of ROS, as observed with other phytogetic antioxidants like *Moringa oleifera* (Reda *et al.*, 2023). These findings corroborate studies demonstrating that plant-derived bioactive compounds ameliorate heavy metal-induced hematological dysregulation by enhancing antioxidant defenses and stabilizing cellular homeostasis (Bala *et al.*, 2020).

CONCLUSION

This study demonstrates that QIS supplementation mitigates Pb (NO₃)₂ nitrate toxicity in common carp, enhancing growth performance and restoring hematological and somatic indices. QIS alleviated Pb-induced oxidative stress, organ damage, and immune dysregulation, highlighting its potential as a natural detoxifying agent in aquaculture. These findings underscore QIS's dual role in promoting growth and reducing heavy metal toxicity, offering sustainable strategies for aquatic health management.

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