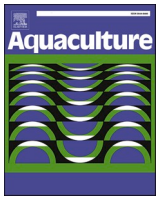
Aquaculture 590 (2024) 741084

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Effectiveness of lycopene dietary enrichment for growth performance, digestive enzymes, blood health, immunity, and antioxidant status of common carp (*Cyprinus carpio*) against chronic glyphosate toxicity

Rabia Tahir [a,c,1], Samra [b,1], Fozia Afzal [c,1], Abdul Ghaffar [c], Ji Liang [d], Abhimanyu Shrestha [a,e], Ume Habiba [f], Song Yang [a,*]

[a] College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan 611130, China
[b] School of Life Sciences, Lanzhou University, Lanzhou, Gansu 730000, China
[c] Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur, Punjab 63100, Pakistan
[d] School of Humanities, Universiti Sains Malaysia, Minden, Penang 11800, Malaysia
[e] Rainbow Trout Fishery, Research Station, Rasuwa 45000, Nepal
[f] Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab 63100, Pakistan

ARTICLE INFO

Keywords:
Aquaculture
Antioxidants
Food safety
Ecotoxicology
Exposure
Pesticides

ABSTRACT

For food safety challenges, sustainable aquaculture emerges as a significant source in recent years; however, despite its potential, the industry still facing challenges, notably the exposure of cultured animals to pesticidal pollution. This pollution originating from agricultural practices that can enter aquaculture system directly: to integrated-agriculture aquaculture practices, or indirectly via soil leakage. Current research based on glyphosate (GLY) toxicity and its amelioration by lycopene (LYC). Four fish groups used for six-weeks experiment in which four groups were used. Control group (CL) was fed with basal commercial diet only without any LYC and GLY exposure; 2) LYC group: exposed to LYC supplemented diet (15 mg/kg per fish diet); 3) GLY group: exposed to glyphosate only (1/5th of 96 h LC_{50}: 0.0892 mg/L) with basal commercial diet, and; 4) GLY + LYC group: exposed to both lycopene supplemented diet (15 mg/kg per fish diet) and glyphosate (1/5th of 96 h LC_{50}: 0.0892 mg/L). GLY observed to decrease growth parameters and feed utilization whereas, lycopene ameliorated growth rate (WG, SGR, HSI, CF) and feeding utilization (FCR) as compared to the control group. Also, GLY induced toxicity within hematobiochemical parameters with alleviation by LYC supplementation. GLY induced cytotoxicity was observed within RBCs as lobbing, notching, vacuolation, blebbing, micronuclei, and condensation. Increase in reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS) were observed by GLY exposure. Also, there is observed reduction in antioxidant enzyme activities (CAT, SOD, POD, TPC and GSH) upon GLY exposure. Lipid peroxidation (malondialdehyde: MDA), 8-OHdG (8-hydroxy-2′-deoxyguanosine) and DIY (dityrosine) observed to increase by GLY toxicity. There was improvement in immune responses; increased AChE (acetylcholinesterase) activity, lysozyme content, ACP (acid phosphatase), NBT (nitro blue tetrazolium), NO (nitric oxide) and IgM levels (immunoglobulin M) and digestive enzyme activities (protease, lipase and amylase) observed by LYC supplemented diet. Taken together, LYC supplementation observed to alleviate GLY induced oxidative stress and cytotoxicity with improved immunity, digestive actions and blood health within *C. carpio*. Therefore, dietary supplementation with lycopene can protect common carp from the harmful effects by glyphosate within agri-integrated aquaculture practices, so suggesting it as potential feed additive.

1. Introduction

Herbicides are extensively being used in agriculture for weeds control. However, upon entering water sources, they exhibit extensive ecotoxicological effects on aquatic organisms (Sharma et al., 2020; Ali et al., 2021). Herbicides, in this regard, constitute almost half of global

* Corresponding author.
E-mail address: ysys210@hotmail.com (S. Yang).
[1] To be considered as joint first authors.

https://doi.org/10.1016/j.aquaculture.2024.741084
Received 13 January 2024; Received in revised form 2 May 2024; Accepted 11 May 2024
Available online 11 May 2024

pesticide usage; among which, glyphosate N–(phosphonomethyl) is most widely used for weed control in numerous countries, including Pakistan, China and Iran (Maggi et al., 2020). Glyphosate (GLY), often utilized in its commercial form as Roundup®, acts as a broad-spectrum herbicide by inhibiting amino acid synthesis in plants (Leino et al., 2021; Emerenciano et al., 2022). Glyphosate holds the title of being the world's top-selling broad-spectrum agrochemical. Within the agricultural domain, the presence of various glyphosate-resistant crops allows for its frequent application (Hendlin et al., 2020; Hervé et al., 2024). This widespread use has led to heightened levels of glyphosate in the environment, including surface water and groundwater. Studies have detected peak concentrations of glyphosate in soy plants (ranging from 1.9 to 4.4 mg/kg), grains (0.1–1.8 mg/kg), soybeans (3.3 mg/kg), and lentils from Canada (10.5 mg/kg) (Ojelade et al., 2022). Exposure to glyphosate has been linked to adverse effects on freshwater species. Various research efforts have highlighted glyphosate, bring about alterations in water bodies surrounding cultivation areas, rainwater, and groundwater through processes such as volatilization, spray drift, surface runoff, chemical-biological degradation, leaching, and sorption (Annett et al., 2014; Brovini et al., 2021).

Glyphosate, classified as an organophosphorus chemical, has been linked to neurotoxic effects, although international agencies controversially classify it as non-neurotoxic even at high concentration (Tresnakova et al., 2021). The widespread use of glyphosate has led to its residues being found in various foods, food products, and water sources. Studies have detected glyphosate metabolites in humans' urine, blood, umbilical cord blood, and maternal blood (Agostini et al., 2020). While glyphosate inhibits a crucial plant enzyme, 5-enolpyruvylshikimate-3-phosphate synthase, it doesn't induce toxic effects in non-target organisms due to the absence of this enzyme in animals (Verster, 2018; Singh et al., 2020). Reports suggested that the frequent and extensive use of pesticides results in various side effects on physiology, behavior, and haemato-biochemistry, leading to oxidative stress and reduced organism efficiency (Khoma et al., 2020; Sharma et al., 2020; Ames et al., 2022). Yet, numerous studies have demonstrated the harmful effects of GLY on aquatic species, as reduced growth and survival rate, genotoxicity, oxidative stress, immune system suppression, liver damage, and reduced digestion ability (Tresnakova et al., 2021; Brovini et al., 2021; Uchenna et al., 2022; Ames et al., 2022). Additionally, environmentally significant concentration of GLY observed to elevate the risk of fish diseases (Le Du-Carrée et al., 2021), which further emphasized the substantial threat posed by agriculture-related pesticides to fish production.

To ensure the required growth in aquaculture production, effective strategies are essential to mitigate herbicides toxicity in fish (Naiel et al., 2020; Zhao et al., 2021). Over the past decade, there has been extensive research on the positive impacts of supplementing fish diets with medicinal plants, focusing on areas such as growth, immunity, and disease resistance (Kumar et al., 2022; Ahmadifar et al., 2021; Li et al., 2022). More recent studies indicate that plant-based supplementation can also enhance fish resistance and reduce oxidative stress induced by various stressors like hypoxia (Abdel-Tawwab et al., 2018; Hoseini et al., 2021; Montaser et al., 2021), crowding stress (Yousefi et al., 2019) or exposure to disinfectants (Khalili et al., 2020). As of now, the potential of plant-derived compounds in safeguarding fish against herbicides-related toxicity has been largely unexplored. Limited recent studies on the topic indicate that supplementing fish diet with extract from curcumin, resveratrol, geranium or oregano alleviated immune-depressive effects linked to herbicides exposure (Rafieepour et al., 2019; Rahman et al., 2020; Zhao et al., 2021; Rohmah et al., 2022). These findings underscore the potential of diets enriched with plant components in reducing herbicides toxicity in fish.

Natural antioxidants, such as carotenoids, a family of fat-soluble pigments prevalent in numerous fruits and vegetables, (including beta-carotene, lycopene, and alpha-tocopherol), play crucial role in animal health by neutralizing harmful free radicals generated during normal cellular activity and under various stressors (Jideani et al., 2021). Therefore, they have been extensively focused for their potential to mitigate oxidative stress. Antioxidant potential of these micronutrients is vital for maintaining the functional and structural integrity of essential immune cells, thereby enhancing immunity (Alagawany et al., 2021). Moreover, while exact mechanism responsible for the antioxidant properties of carotenoids remain unknown, its distinctive feature as a potent antioxidant and free radical scavenger positioned it as a protective agent against induced cardiotoxicity and hepatotoxicity in various studies (Van Doan et al., 2016; Abdel-Daim et al., 2019; Dawood et al., 2020). Lycopene (LYC), naturally occurring in tomatoes, has gained significant attention owing to its highly efficient antioxidant activity against singlet oxygen and free radicals as compared to alpha-tocopherol and beta-carotene (Yonar, 2012; Madia et al., 2021). Common carp, *Cyprinus carpio*, holds the status of being the fourth most significant cultured fish globally and is particularly valued in numerous Asian countries like Pakistan, China and Iran (Rahman et al., 2020). It is abundantly found within freshwater sources; most of which are located near agricultural land; thereby, it found exposed to herbicides pollution from agricultural water (Rohmah et al., 2022). Therefore, current study was aimed to explore the potential of LYC supplementation for amelioration of GLY induced toxicity over blood health, growth, antioxidant status and immune responses of common carp.

2. Materials and methods

2.1. Chemicals

Glyphosate, (GLY), {$(HO)_2P(O)CH_2NHCH_2CO_2H$), N-(Phosphonomethyl) glycine, 96% purity, molecular weight: 169.07, (CAS 1071-83-6)} was obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA). Lycopene, (LYC), {Redivivo TM, 10% FS, ~10% in corn oil, ≥ 95.0% (sum of isomers), molecular weight: 536.87, (empirical formula: $C_{40}H_{56}$; CAS: 502–65-8)} was obtained from Sigma-Aldrich Chemical (St. Louis, MO, USA).

2.2. Fish maintenance

Apparently healthy common carp (*C. carpio*) fingerlings were obtained from Fish Hatchery, Karachi Mor, Government of the Punjab, Bahawalpur, Pakistan to Aquaculture, Genetic Toxicity and Molecular Biology laboratory in Department of Zoology, The Islamia University of Bahawalpur, Pakistan. All the experimental work was carried out according to protocol and guidelines approved by Ethical Committee of Animal Care and Use in Research, The Islamia University of Bahawalpur, Pakistan. Common carp with average body weight of 44 ± 0.07 g were acclimatized for two weeks before starting experiment. Water quality parameters were recorded and kept under optimum range during experimental work as: water temperature: 25 ± 1.01 °C, pH: 6.8 ± 0.2, dissolved oxygen: 6.7 ± 0.35 mg/L, ammonia: 0.031 ± 0.02 mg/L, photoperiod: 12 h light/12 h dark. Aquaria with size of 100 cm × 40 cm × 40 cm having 160 L water capacity was used to keep the fish. Oxygen levels were maintained in aquaria by using air stones via air pumps. One third of water volume was changed after every two days using siphon tubes, whereas water was spiked for maintaining the GLY level within GLY exposed groups. Fish behavior and clinical signs were observed during experimental period.

The LC_{50} for 96 h of GLY for common carp fingerlings were determined as 0.446 mg/L by probit regression analysis as proposed by Raymond (1985). Therefore, safe concentration of glyphosate used in experiment as the 1/5th of 96 h LC_{50} (0.0892 mg/L).

2.3. Feed preparation and feeding regimes

Fish commercial diet having 30% crude protein, purchased from Hi-Tech Feeds Private limited company Sahiwal, was used to feed the fish

consisting of: wheat gruel (20%), rice broken (10%), maize gruel (17%), maize glutelin 1% (60%), maize glutelin 7% (30%), cotton seed meal (decorticated) (7%), ghar meal (3.0%), fish meal (6.0%), soybean meal (6.0%), rice polishing (10%), dicalcium phosphate (DCP) (1.5%), limestone (7.5%), vitamin/mineral (premix) (0.5%) and molasses (3.5%). Lycopene (15 mg/kg per fish diet) was evenly sprayed over the basal commercial diet, then dried at room temperature and stored at 4 °C as described by Yonar (2013). Fish were fed twice a day at 9:00 am and 4:00 pm, at 2% and 5% feeding rate, with adjustment according to biweekly body weight estimation. Proper siphoning of leftover feed and feces were done daily to maintain the proper water quality.

2.4. Experimental design

After acclimation period, three hundred sixty common carp (*C. carpio*) were divided into four groups with three replicates (30 fish per replicate). Four groups used in experiment were as follows: 1) control group (CL group) was fed with basal commercial diet only without any LYC and GLY exposure; 2) LYC group: group was exposed to LYC (15 mg/kg per fish diet) supplemented diet without any GLY exposure; 3) GLY group: exposed to glyphosate only (1/5th of 96 h LC_{50}: 0.0892 mg/L) without LYC supplemented diet but fed with basal commercial diet, and; 4) GLY + LYC group: exposed to both lycopene supplemented diet (15 mg/kg per fish diet) and glyphosate (1/5th of 96 h LC_{50}: 0.0892 mg/L). Experiment was carried out for 6-weeks.

2.5. Growth parameters and feed utilization

Initial body weight (IBW) and final body weight (FBW) was measured during the trial period. Hence, weight gain (WG, g) was calculated using difference between initial and final body weight. Specific growth rate was calculated using formula: (SGR, %/day) = 100 (ln final weight of fish −ln Initial weight of fish)/No. of days. Feed conversion ratio (FCR) calculated as; quantity of consumed feed (g)/ weight gain (g). Whereas, survival rate (SR, %) was calculated as; 100 (final number of fish/ initial number of fish); hepatosomatic index (HSI, %) measured as: 100 × [(liver weight (g)/whole body weight (g)]; and condition factor (CF, %) measured as: 100 (body weight/total length^3).

2.6. Hematobiochemical parameters and cytotoxicity analysis

After 3- and 6-weeks, five random blood samples of reared fish from each group were collected from the caudal vein for blood health assessment. Before sample collection, all fish were starved for 24 h. Fish were anaesthetized using 4.5 mg/L clove oil for avoiding any stress during fish handling as given by Ghaffar et al. (2021). Blood was sampled using sterile syringe containing EDTA (1.0 mg/mL); whereas non-heparinized syringes used for serum collection. Serum and plasma were further stored at −20 °C for post-analysis. Plasma parameters were assessed using hematology analyzer (Sysmax, KX21) whereas biochemical parameters measured using biochemistry analyzer (Microlab 300, Merck). Observed plasma parameters were as: hemoglobin (Hb), hematocrit (HCT), red blood cells (RBCs), white blood cells (WBCs), platelets (PLTs), whereas biochemical parameters were as: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), urea (UR), creatinine (CRT) and triglycerides (TR). For cytotoxicity observation within RBCs, thin blood smears of freshly collected blood of experimental fish was prepared on glass slides. All the blood smears were air-dried and fixed with absolute ethanol and stained using Giemsa stain. All of these analyses were carried out as given in our previous research (Ghaffar et al., 2016, 2021).

2.7. Oxidant/ antioxidant status

Liver, kidney, gills, muscles and intestinal tissues of five fish from each group were excised and processed after 3- and 6-weeks of experimental peroids, for measuring the oxidant/antioxidant status. Tissues were kept within chilled saline water after dissection. Homogenates of excised tissue were prepared and antioxidant markers were then assessed as: catalase (CAT, units/min), superoxide dismutase (SOD, units/mg protein), peroxidase (POD, units/min), thiobarbituric acid reactive substance (TBARS, nmol/TBARS formed/mg protein/min), reduced glutathione (GSH, μmol/g tissue), Total proteins (TPC, μg/mg tissue) and reactive oxygen species (ROS, optical density) by following the method given by Ghaffar et al. (2021). Similarly, ELISA kit (Catalog No: MBS764814, MyBiosource, Southern California, USA) was used to detect 8-OHdG (organic 8- hydroxy 2-deoxyguanosine, ng/mL) levels according to the manufacture's protocol with an absorbance at 450 nm. Whereas, DIY (dityrosine, ng/mL) measured using ELISA kit (Catalog No: NWK-DIY01, Northwest Life Science Specialties, LLC., US) according to the manufacture's protocol with an absorbance at 450 nm.

2.8. Digestive enzyme activity

Gastrointestinal tract was separated, at the end of experimental period, from the five reared fish samples and washed with PBS (pH 7.4; 1 g/10 ml), which was then homogenized and centrifuged for 5 min at 8000 rpm. Supernatant of samples were kept at 4 °C for the assessment of digestive enzyme activities. Activity of intestinal protease was calculated through non-specific protease activity assay by using casein as a substrate, as described by Cupp-Enyard (2008). Whereas, amylase and lipase activities were measured spectrophotometrically at A_{540} and A_{714} with some modification according to method described by Wang et al. (2019). All the digestive enzyme activities for *C. carpio* were assessed after 3 and 6 weeks of exposure to experimental conditions.

2.9. Immunological parameters

Immunological parameters for *C. carpio* were assessed after 3 and 6 weeks of exposure to experimental conditions. Quantitative Sandwich ELISA kit (Catalog No. MBS042385) was used for measuring immunoglobulin M (IgM) in the serum samples of fish as the method described by Overkamp et al. (1988). Lysozyme activity was measured spectrophotometrically by using turbidity assay method as given by Parry Jr et al. (1965) and the nitric oxide (NO) level was measured according to method described by Montgomery and Dymock (1962). Whereas, AChE activity within gills was spectrophotometrically measured using a colorimetric method as given by Ellman et al. (1961). Nitro blue tetrazolium (NBT) assay was performed for spectrophotometric assessment of whole blood respiratory burst activities at 630 nm as described by Secombes (1990). Alternative complement pathway (ACP) activities of serum was analyzed by method described by Yanno (1992).

2.10. Statistical analysis

Data was statistically evaluated by using SPSS (windows version 17, SPSS Inc., IL, and USA). Homogeneity and normality of variance for data was checked by Shapiro-Wilk and Levene tests. Means± SEM values of these parameters were compared using one-way ANOVA, followed by Duncan's multiple range *post-hoc* test. Differences were statistically significant at $P < 0.05$.

3. Results

3.1. Clinical signs and behavior of Cyprinus carpio

Constant exposure of sublethal concentration of GLY for 6 weeks caused stress condition within *C. carpio*. Clinical signs observed during trial period were as: equilibrium loss, irregular swimming, jerky movement, hyperactivity, darkened skin, surface breathing, over-excitability, restlessness and faintness. Whereas, normal feeding behavior and morphological features were observed in CL and LYC

group. No mortality was observed during trial period (Table 1).

3.2. Growth performance and feed utilization

C. carpio fingerlings fed with lycopene supplemented diet for 6 weeks exhibited significant ($P < 0.05$) final mean weight (FW), weight gain (WG) and specific growth rate (SGR) as compared to control group (Table 2). Feed conversion ratio (FCR) also shown significantly lower value within LYC group as compared to control group. Fish fed with lycopene supplemented diet shown significantly lower hepatosomatic indices (HSI). Whereas, no mortality observed during the experiment so survival rate was 100% in all groups (Table 2).

3.3. Haemato-biochemical parameters

Haemato-biochemical parameters of *C. carpio* after six-week exposure to glyphosate and lycopene treatment are given in Table 3. Significant increase found in Hb, HCT, RBCs; whereas, significant decrease observed for WBCs and PLTs by lycopene supplemented diet as compared to control group. In context of biochemical parameters, significant decrease observed for ALT, AST ALP, UR, CRT, while significant increase observed in TRG by lycopene supplemented diet as compared to control group.

3.4. Cytotoxicity within erythrocytes

Cytotoxic changes within erythrocytes of *C. carpio* after six-week exposure to glyphosate and lycopene treatment were observed and given in Fig. 1. No morphological and nuclear alterations found within control and LYC group i.e., all cells were oval-shaped. Whereas GLY and GLY + LYC groups have shown cytotoxic alteration as vacuolated nuclei, notching, blebbing, condensation, spherocytes, micronuclei, spindle shaped nuclei and pear-shaped nuclei as given in Fig. 1. Erythrocytic alteration observed by 1500 cells per sample, after 3- and 6- weeks are given in Table S1.

3.5. Oxidant/antioxidant status

Oxidative stress biomarkers significantly increase ($P < 0.05$) whereas antioxidant enzymes decrease ($P < 0.05$) upon exposure to glyphosate as compared to control group. ROS: reactive oxygen species (optical density) and TBARS: thiobarbituric acid reactive species (nmol/TBARS formed/mg protein/min) and malondialdehyde (MDA, nmol/g protein) increase was observed ($P < 0.05$) in liver, gills and kidney of *C. carpio* by GLY exposure for 6-weeks. Whereas, CAT: catalase (units/min), SOD: superoxide dismutase (units/mg protein), peroxidase POD (units/min) and reduced glutathione: (GSH, μmol/g tissue) has shown decrease ($P < 0.05$) in gills, liver and kidney of *C. carpio* by GLY exposure for 6-weeks as compared to control group, as shown in Fig. 2. Likewise, total proteins (TPC, μg/mg tissue) decrease, organic 8- hydroxy 2-deoxyguanosine (8-OHdG, ng/mL) and DIY (dityrosine, ng/mL) level increase by glyphosate exposure ($P < 0.05$) as compared to control group, as shown in Fig. 3a. Whereas, amelioration by lycopene supplemented diet was observed within LYC and GLY + LYC group ($P < 0.05$).

Table 1
Behavioral alteration and clinical signs observed within common carp (*C. carpio*) exposed to glyphosate and lycopene for 6 weeks.

Observation	Groups			
	CL	LYC	GLY	GLY + LYC
Equilibrium loss	−	+	+++	+++
Irregular swimming	−	++	++	++
Jerky movement	−	+	++	++
Hyperactivity	−	+	+++	+++
Darkened skin	−	++	+++	+++
Surface breathing	−	+	+++	++
Over-excitability	−	+	+++	+++
Restlessness	−	+	+++	++
Faintness	−	+	+++	++

Signs for observation indicating follows: Absent (−); rare (+); frequent (++); abundant (+++).

Table 2
Growth performance and feed utilization of *C. carpio* after 6-week exposure to glyphosate and lycopene supplemented diet.

Growth Parameters	Groups			
	CL	LYC	GLY	GLY + LYC
IW (g)	15.13 ± 0.72	15.57 ± 0.67	15.04 ± 0.56	15.40 ± 0.44
FW (g)	41.25 ± 0.35^{b}	43.08 ± 0.63^{a}	29.13 ± 0.32^{d}	31.79 ± 0.02^{c}
WG (g)	25.95 ± 0.42^{b}	27.51 ± 0.05^{a}	14.09 ± 0.76^{c}	16.39 ± 0.51^{d}
SGR (%/day)	2.05 ± 0.02^{b}	2.09 ± 0.01^{a}	1.76 ± 0.02^{c}	2.01 ± 0.01^{ab}
FCR (g/g)	1.51 ± 1.09^{b}	1.49 ± 1.10^{a}	1.67 ± 0.87^{d}	1.57 ± 1.55^{c}
HSI (%)	3.63 ± 0.09^{c}	3.71 ± 0.11^{d}	4.41 ± 0.07^{a}	4.01 ± 0.05^{b}
CF (%)	1.79 ± 0.12^{a}	1.81 ± 0.12^{a}	1.56 ± 0.03^{c}	1.66 ± 0.05^{b}
SR (%)	100	100	100	100

Values with different superscript letters within each row are significantly different ($P < 0.05$). IW: initial mean weight, FW: final mean weight, WG: weight gain, SGR: specific growth rate, FCR: feed conversion ratio, HSI: hepatosomatic index, CF: condition factor, SR: survival rate.

Table 3
Haemato-biochemical parameters of *C. carpio* after 6-week exposure to glyphosate and lycopene supplemented diet.

Parameters	Groups			
	CL	LYC	GLY	GLY + LYC
Hb (g/dL)	7.95 ± 0.01^{b}	8.19 ± 0.02^{a}	6.53 ± 0.21^{d}	7.16 ± 0.11^{c}
HCT (%)	25.61 ± 0.12^{b}	29.43 ± 0.11^{a}	19.98 ± 0.04^{d}	20.66 ± 0.27^{c}
RBCs (10^6/mm^3)	2.15 ± 0.12^{b}	2.42 ± 0.02^{a}	1.83 ± 0.04^{d}	1.99 ± 0.01^{c}
WBCs (10^3/mm^3)	54.47 ± 8.48^{c}	57.66 ± 6.48^{d}	89.33 ± 5.89^{a}	61.00 ± 4.09^{b}
PLT (x10^3/L)	14.66 ± 4.05^{b}	16.33 ± 2.02^{d}	36.0 ± 1.05^{a}	27.33 ± 6.01^{b}
ALT (IU/L)	20.66 ± 1.16^{c}	19.33 ± 1.51^{c}	24.0 ± 2.78^{a}	16.0 ± 5.03^{b}
AST (IU/L)	33.66 ± 2.03^{c}	32.0 ± 3.01^{c}	46.33 ± 2.33^{a}	39.33 ± 4.17^{b}
ALP (mg/dl)	18.33 ± 3.31^{c}	17.11 ± 2.08^{c}	36.33 ± 2.13^{a}	25.31 ± 1.05^{b}
UR (mg/dl)	11.18 ± 0.04^{c}	12.04 ± 0.12^{c}	18.94 ± 0.76^{a}	19.70 ± 1.01^{b}
CRT (mg/dl)	0.14 ± 0.02^{c}	0.13 ± 0.03^{c}	0.44 ± 0.01^{a}	0.19 ± 0.02^{b}
TR (mg/dL)	111.01 ± 4.02^{b}	110.62 ± 3.05^{a}	82.33 ± 5.09^{d}	94.0 ± 4.05^{c}

Values with different superscript letters within each row are significantly different (analysis of variance, $P < 0.05$).
Hb: hemoglobin, HCT: hematocrit, RBCs: red blood cells, WBCs: white blood cells, PLT: platelets, ALT: alanine aminotransferase, ALP: alkaline phosphatase, AST: aspartate aminotransferase, UR: urea, CRT: creatinine and TR: triglycerides.

3.6. Digestive enzyme activities

Activity of intestinal protease (U/mg), amylase (U/mg) and lipase (U/mg) significantly decreased by the glyphosate exposure as compared to control group ($P < 0.05$), whereas lycopene supplemented diet observed to ameliorate their levels ($P < 0.05$) as given in Fig. 3b.

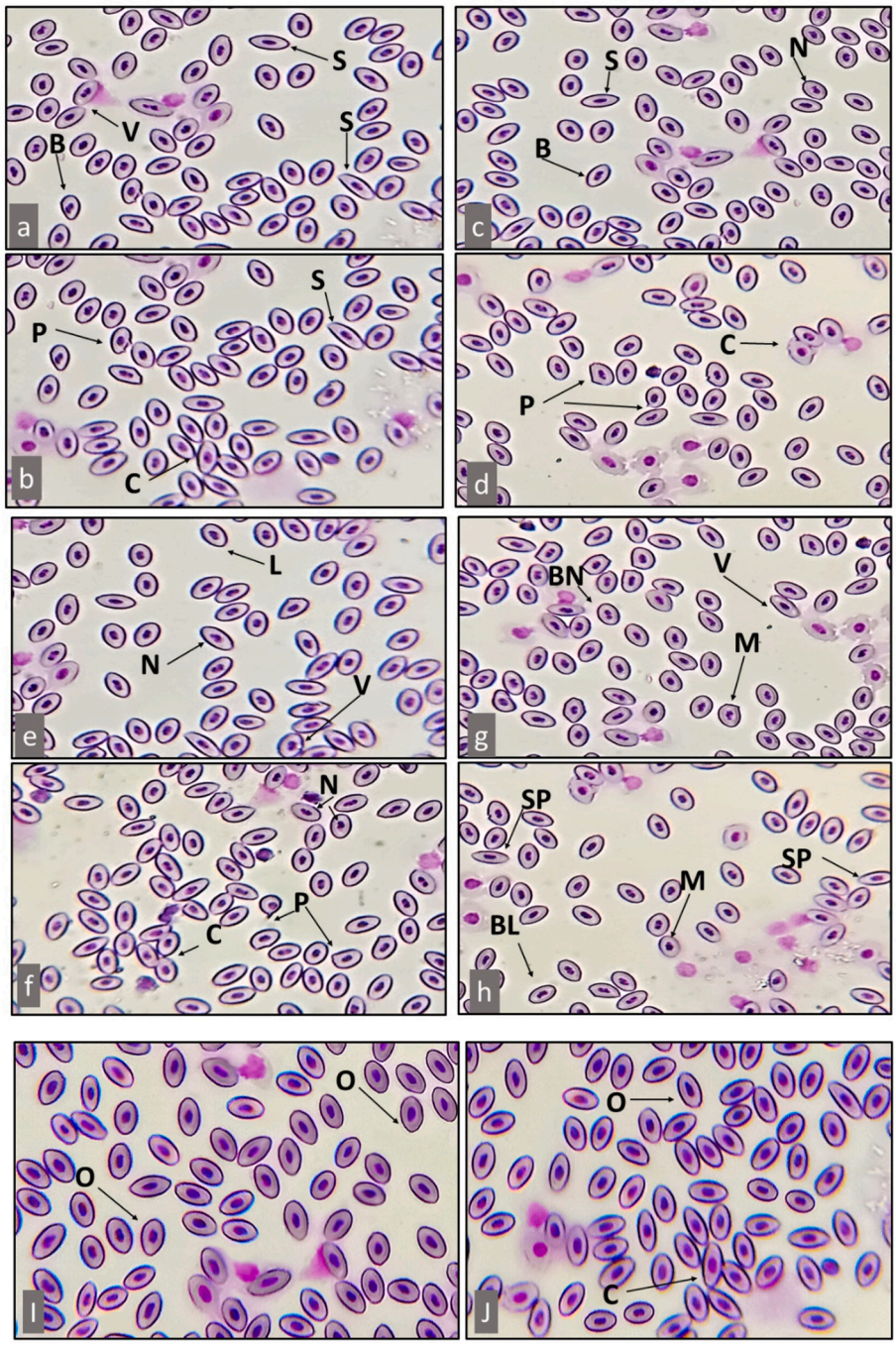


Fig. 1. Morphological and nuclear alterations found in erythrocytes of *C. carpio* exposed to glyphosate and lycopene for 6 weeks. a-d: showing blood cells from GLY, e-h: showing blood cells GLY + LYC group, while i-j: CL and LYC group. S: spherocytes, P: pear shaped cells, C: condensation, O: oval cells, B: blebbed nuclei, BL: bilobed nuclei, BN: binucleated cells, V: vacuolation, N: notched nuclei, MN: micronuclei, SP: spindle shaped nuclei, L: lobed nuclei. CL: control group, LYC: lycopene supplemented group (15 mg/kg per fish diet), GLY: glyphosate group (0.0892 mg/L), GLY + LYC: group exposed to both glyphosate (0.0892 mg/L) and lycopene (15 mg/kg per fish diet). Giemsa stain: ×1000.

3.7. Immunological parameters

Immunoglobulin M (IgM, μg/mL) in the serum samples of fish, lysozyme activity (LZM, μg/mL) and nitric oxide (NO, μmol/mL) level significantly decreased by the glyphosate exposure as compared to control group ($P < 0.05$) as given in Fig. 4. In similar manner, AChE activity (U/mL), nitro blue tetrazolium (NBT, %) of whole blood respiratory burst activities and alternative complement pathway (ACP, U/ml) activities of serum significantly decreased ($P < 0.05$) by glyphosate exposure as compared to control group ($P < 0.05$). whereas, all these immune responses ameliorated by lycopene supplemented diets ($P < 0.05$).

4. Discussion

The widespread issues of herbicides-contaminated water possess a significant challenge in land-based aquaculture globally (Yousefi et al., 2021). Fish in integrated agriculture-aquaculture systems may be directly exposed to herbicides through application, while indirect exposure through soil leakage is another common pathway. This

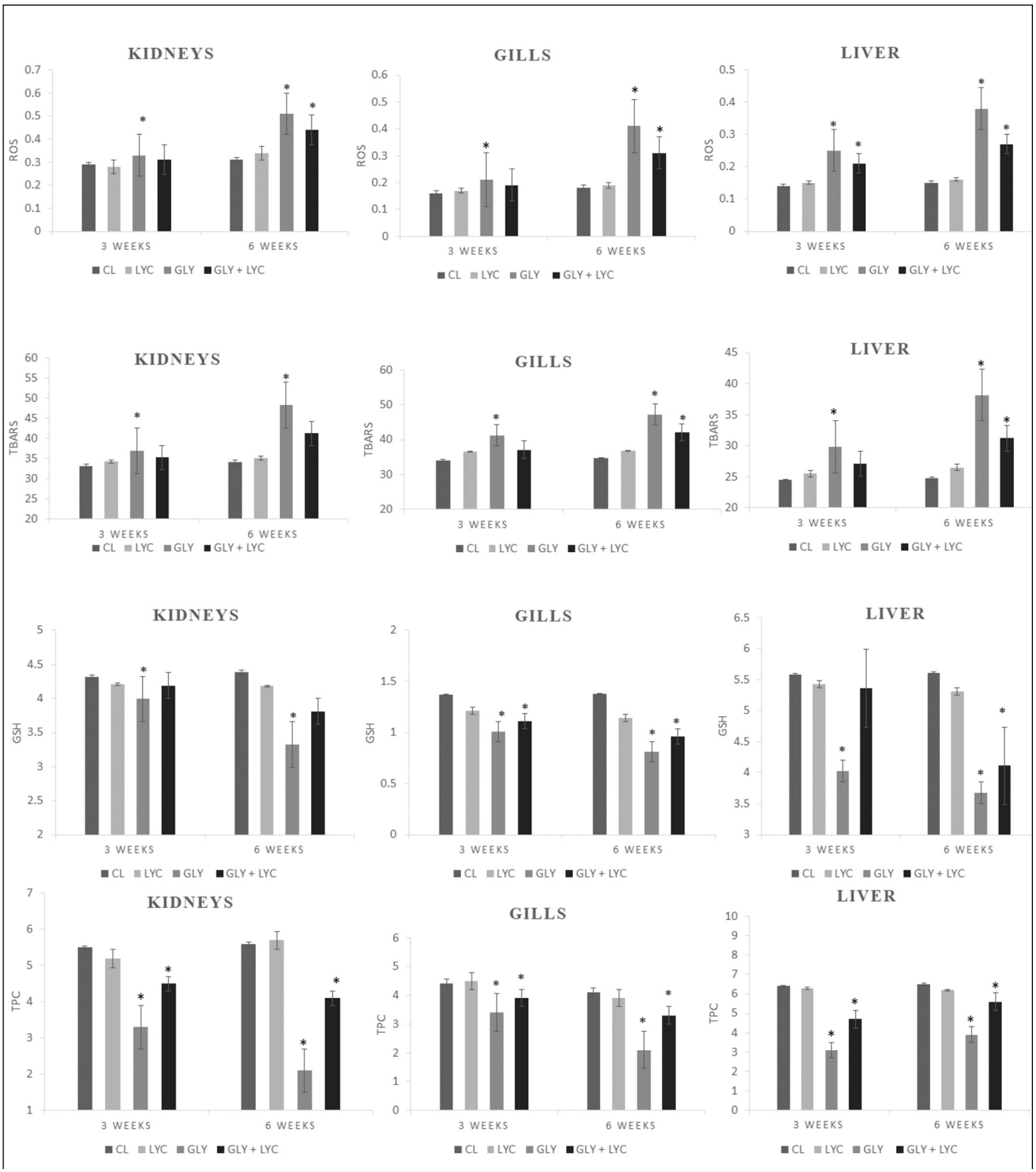


Fig. 2. a. Impact of glyphosate stress and lycopene supplementation over oxidant/antioxidant status of *C. carpio* during 6 weeks exposure period. ROS: reactive oxygen species (optical density) and TBARS: thiobarbituric acid reactive species (nmol/TBARS formed/mg protein/min), GSH: reduced glutathione (μmol/g tissue) and TPC: total proteins (μg/mg tissue). CL: control group, LYC: lycopene supplemented group (15 mg/kg per fish diet), GLY: glyphosate group (0.0892 mg/L), GLY + LYC: group exposed to both glyphosate (0.0892 mg/L) and lycopene (15 mg/kg per fish diet). * is showing significant differences at the level of 0.05. b. Impact of glyphosate stress and lycopene supplementation over oxidant/antioxidant status of *C. carpio* during 6 weeks exposure period. CAT: catalase (units/min), SOD: superoxide dismutase (units/mg protein), peroxidase POD (units/min) and MDA: malondialdehyde (nmol/g protein). CL: control group, LYC: lycopene supplemented group (15 mg/kg per fish diet), GLY: glyphosate group (0.0892 mg/L), GLY + LYC: group exposed to both glyphosate (0.0892 mg/L) and lycopene (15 mg/kg per fish diet). * is showing significant differences at the level of 0.05.

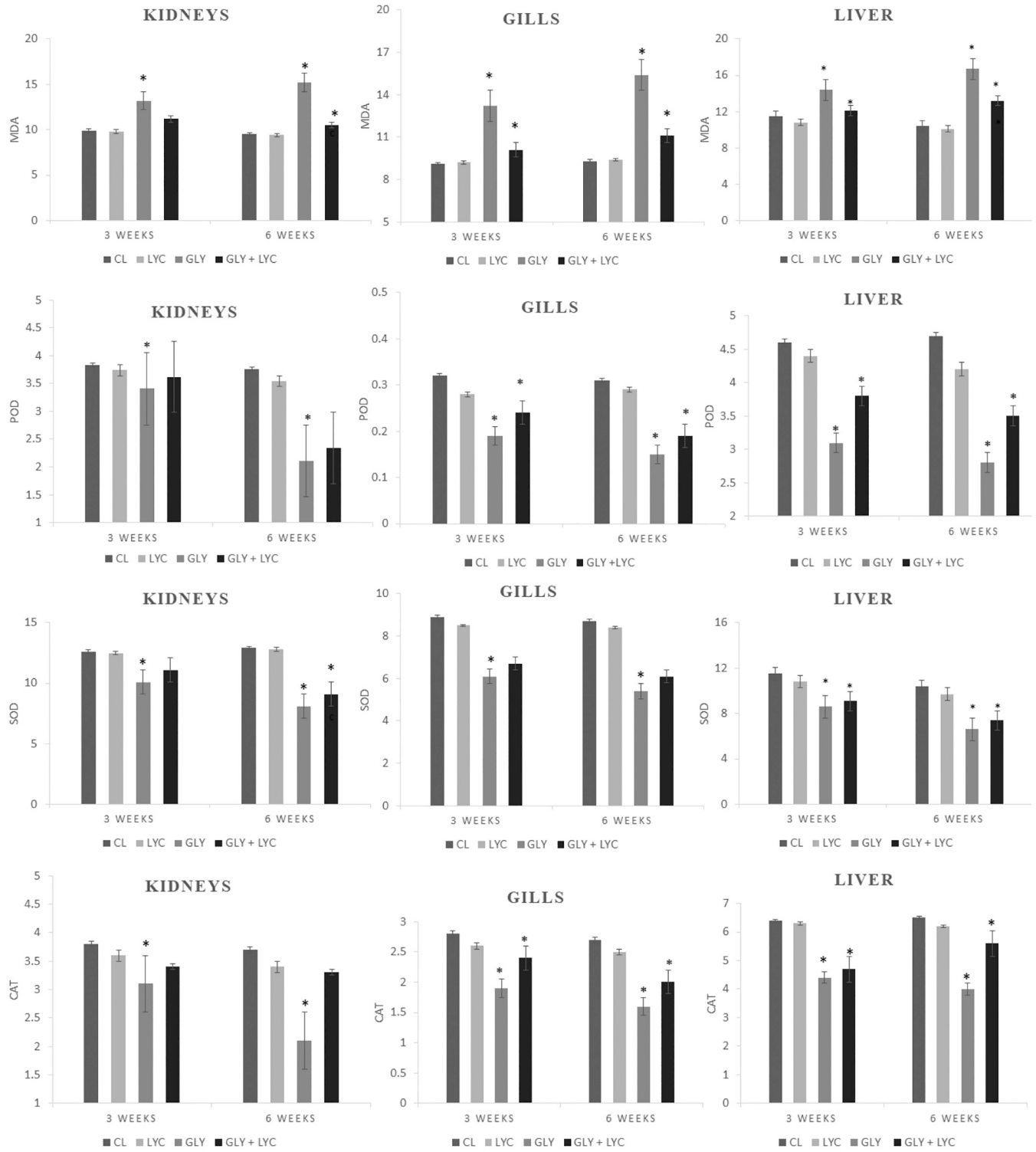


Fig. 2. (*continued*).

exposure can lead to fish encountering sub-lethal concentrations of pesticides, thereby posing serious implications for their health (Latif et al., 2023). Whereas, plant-based supplementation can enhance fish growth and reduce oxidative stress induced by various stressors (Hoseini et al., 2021; Montaser et al., 2021). Therefore, in current research, potential of lycopene diet supplementation to mitigate or reduce the toxic effects of the glyphosate herbicide in common carp fingerlings was investigated. Fish receiving lycopene-supplemented diets exhibited better resilience to glyphosate exposure compared to the control group.

4.1. Growth performance and feed utilization

In the current study, GLY stress was observed to induce alterations in behavior of *C. carpio* whereas similar results were observed by Yonar (2019) and El Basuini et al. (2020) AChE activity reduction and oxidative stress may cause alteration in behavior and induced clinical signs as

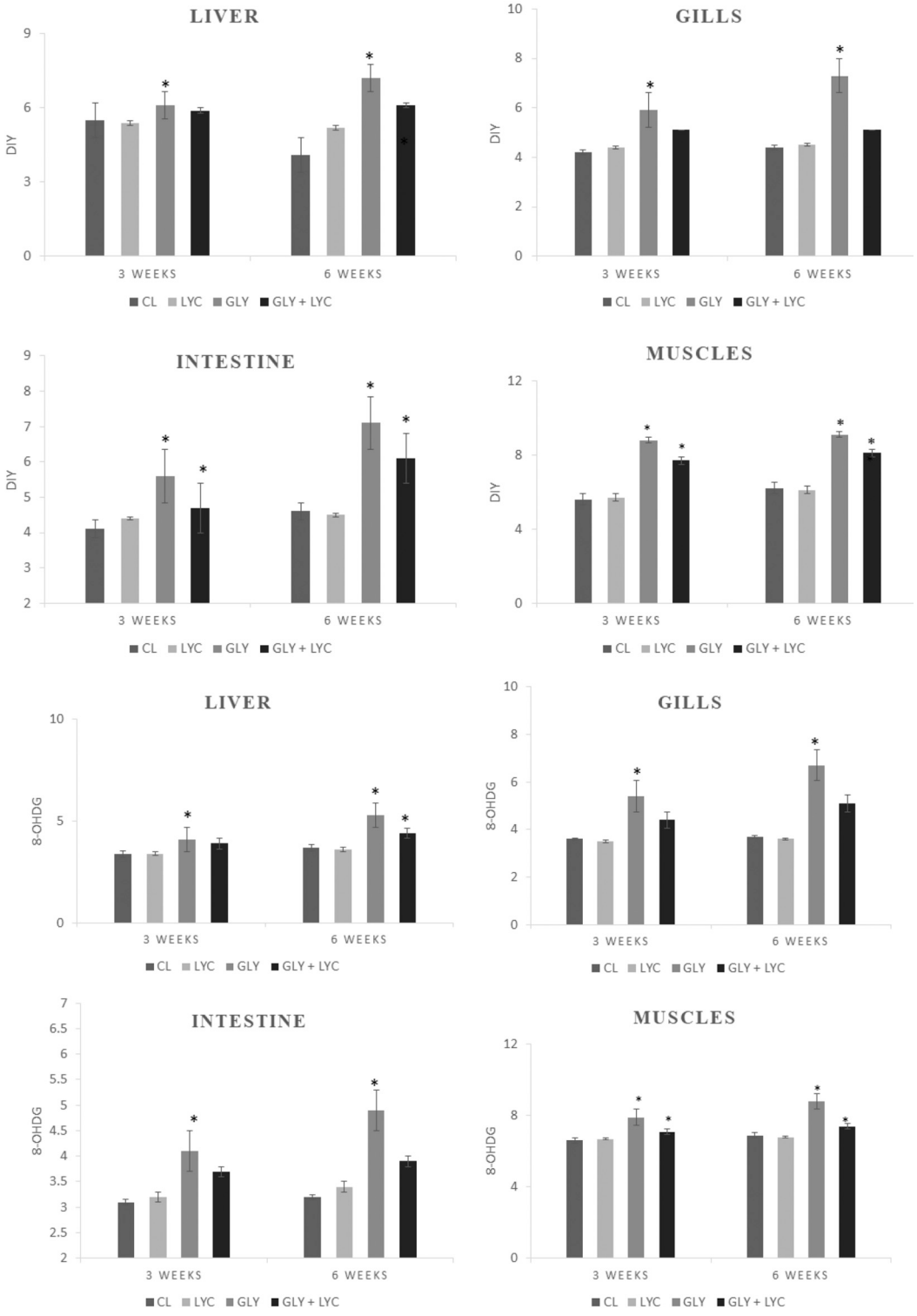


Fig. 3. a. Impact of glyphosate stress and lycopene supplementation over DIY (dityrosine, ng/mL) and organic 8- hydroxy 2-deoxyguanosine (8-OHdG, ng/mL) level within *C. carpio* during 6 weeks exposure period. CL: control group, LYC: lycopene supplemented group (15 mg/kg per fish diet), GLY: glyphosate group (0.0892 mg/L), GLY + LYC: group exposed to both glyphosate (0.0892 mg/L) and lycopene (15 mg/kg per fish diet). * is showing significant differences at the level of 0.05. b. Impact of glyphosate stress and lycopene supplementation over digestive enzymes activities within *C. carpio* during 6 weeks exposure period. CL: control group, LYC: lycopene supplemented group (15 mg/kg per fish diet), GLY: glyphosate group (0.0892 mg/L), GLY + LYC: group exposed to both glyphosate (0.0892 mg/L) and lycopene (15 mg/kg per fish diet). * is showing significant differences at the level of 0.05.

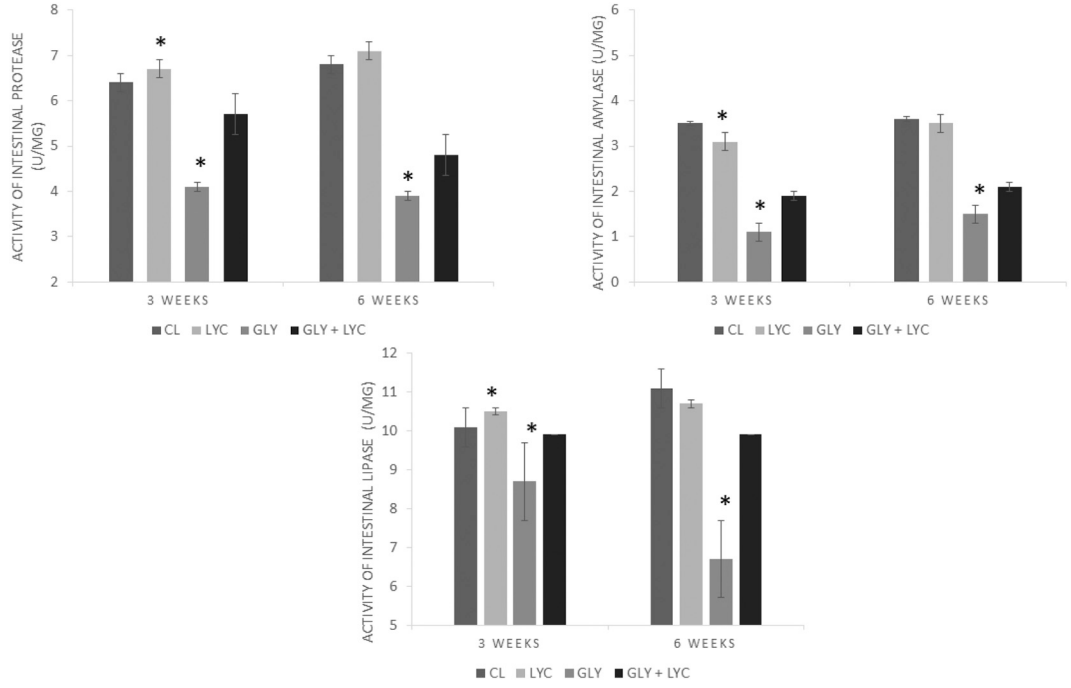


Fig. 3. (*continued*).

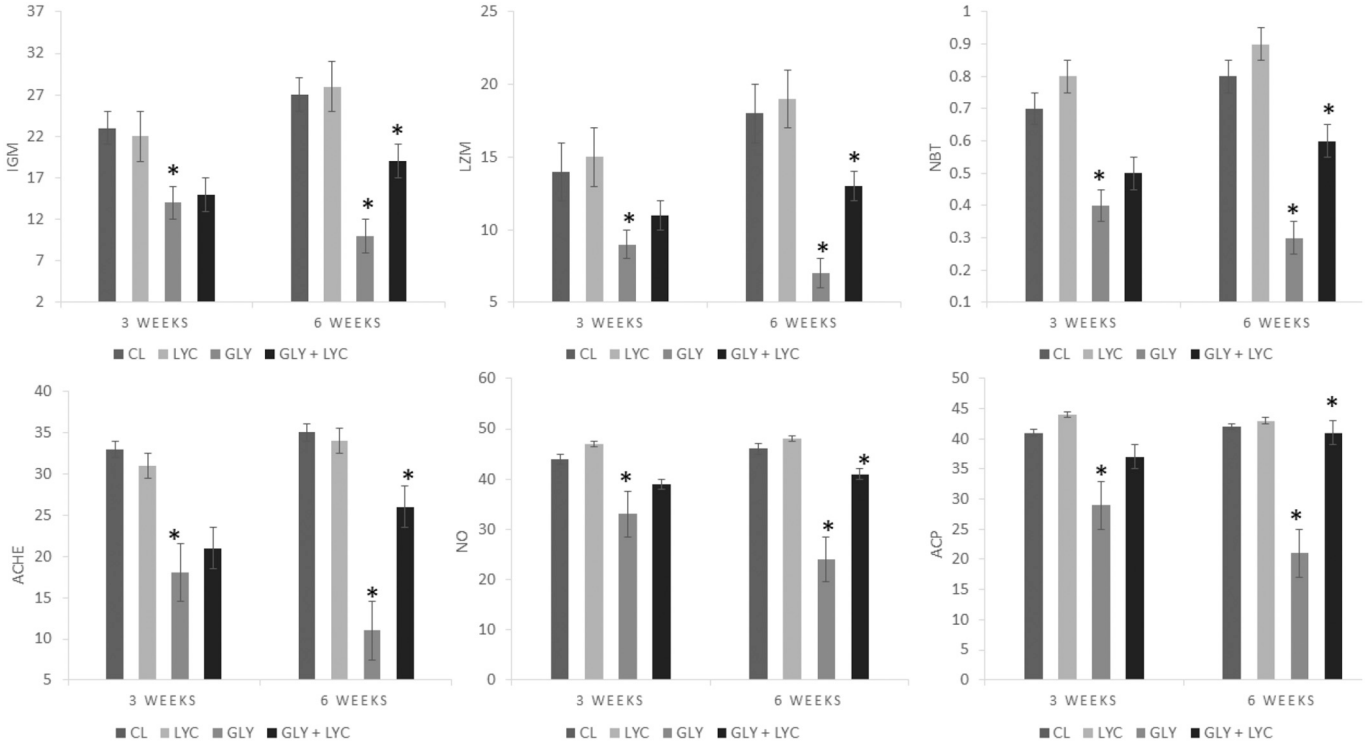


Fig. 4. Impact of glyphosate stress and lycopene supplementation over immunological parameters within *C. carpio* during 6 weeks exposure period. Immunoglobulin M (IgM, μg/mL), lysozyme activity (LZM, μg/mL), nitric oxide (NO, μmol/mL), AChE activity (U/mL), nitro blue tetrazolium (NBT, %) and alternative complement pathway (ACP, U/ml). CL: control group, LYC: lycopene supplemented group (15 mg/kg per fish diet), GLY: glyphosate group (0.0892 mg/L), GLY + LYC: group exposed to both glyphosate (0.0892 mg/L) and lycopene (15 mg/kg per fish diet). * is showing significant differences at the level of 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

GLY irreversible binding to enzymatic site playing role for hydrolysis of acetylcholine (AChE) to choline and acetate resulting in over stimulated cholinergic activity (Cacabelos, 2020). Disturbance in sodium channels within neurons of common carp by GLY stress may also responsible for these behavioral alterations (Sabra and Mehana, 2015). These results in current study also supported by chlorpyrifos and cypermethrin toxicity within *Heteropneustes fossilis*, which observed prominent reduction AChE activity within brain, gills and muscle (Tiwari et al., 2019). There is

positive relationship between AChE activity and locomotion behavior within fish (Kavitha and Rao, 2008). Supplementation of lycopene has ameliorated the cypermethrin (CYP) toxicity as it is rich in antioxidants and vitamins, which has AChE- restoring features (Hoseini et al., 2021; Montaser et al., 2021; Mundkar et al., 2022).

The improvement in growth and feed utilization by dietary lycopene may be attributed to the indirect influence on metabolic pathways regard (El Basuini et al., 2020). Similarly, previous studies have reported improved feeding efficiency, growth performance and weight gain with plant-based supplementation in different animal models as observed in current study (Johnson et al., 2015; Morales et al., 2016; Martinez et al., 2021). While GLY exposure reduced the growth and feed utilization rate of *C. carpio* as observed in previously (Yousefi et al., 2019; El Basuini et al., 2020). Further investigations are required to understand the accumulation of herbicides, particularly glyphosate, in fish muscle, as this could potentially pose additional human health concerns (Yousefi et al., 2019).

4.2. Haemato-biochemical parameters

Exposure of animals to toxic substances, including herbicides, has been associated with alterations and dysfunction in fish metabolism and biochemical processes (Bojarski and Witeska, 2020). Therefore, changes in certain haemato-biochemical parameters, such as metabolic enzymes or biomarkers related to various blood parameters and metabolic pathways (e.g., lipid, carbohydrate, protein), are considered diagnostic tools in toxicology (Roux et al., 2011). Aminotransferases (ALT, AST) play a role in amino acids metabolism, while ALP is a polyfunctional enzyme involved in membrane transport activities (Cuykx et al., 2018). Consistent with prior research, our results demonstrate a significant increase in ALT, AST, and ALP in fish exposed to pesticides, including glyphosate (Yang et al., 2019). Elevated levels of these enzymes in the blood serum are likely associated with cytolysis and the leakage of enzymes into the bloodstream, indicating tissue damage in organs such as the liver and kidney (Zheng et al., 2022). Furthermore, our findings reveal that common carp fingerlings fed with diets enriched with lycopene displayed significantly lower levels of ALT, AST, and ALP after exposure to glyphosate, which highlighted the protective potential of lycopene supplementation on the metabolism of common carp fingerlings, potentially linked to significantly lower stress levels (Yang et al., 2019). Additionally, glyphosate exposure was observed to impact fish lipid and protein metabolism, leading to significant increase in creatinine, urea and reduced triglycerides. Similar effects were observed in tilapia (*Oreochromis niloticus*) exposed to CYP for 80 days (Abdel-Tawwab and Hamed, 2020) and bisphenol-A toxicity (Hamed and Abdel-Tawwab, 2017). Moreover, decrease in hematological values signifies anemia in fish exposed to GLY, potentially attributed to erythropoietic, haemosynthetic, and osmoregulatory dysfunction or an elevated rate of erythrocyte destruction in hematopoietic organs (Burgos-Aceves et al., 2019). Lycopene has been shown to ameliorate these values against GLY toxicity as previously observed in other studies (Yonar, 2012, 2013; Yousefi et al., 2019). Acute pesticide exposure can induce a severe anemic state by causing hemolysis of red blood cell membranes (Farag and Alagawany, 2018). Nevertheless, dietary supplementation with lycopene and/or vitamin E has demonstrated improvements in hematological parameters compared to control and other groups (Yousefi et al., 2019).

Additionally, assessing morphological variations within erythrocytes has been established as an effective indicator of oxidative stress (De Faria et al., 2018). Ghaffar et al. (2015) documented similar cytotoxic damage in male Japanese quail, observing micronuclei and lobbing in erythrocytes. Furthermore, Ghaffar et al. (2018) and Ghaffar et al. (2019) reported fipronil-induced nuclear and cellular abnormalities in *C. carpio* and *Labeo rohita*, respectively. Increased proportion of RBCs displaying micronuclei and lobed nuclei by GLY in our investigation may be attributed to an excess of caspase-activated DNase, an enzyme responsible for cleaving aneuploid proteins, cytoskeletal proteins, and nuclear proteins, inducing oxidative damage to the mitochondrion (Hussain et al., 2014). Various studies have observed diverse morphological and nuclear alterations in erythrocytes of fish (*C. carpio* and L. *rohita*) and avian species in response to oxidative stress (Shahjahan et al., 2020; Ashaf-Ud-Doulah et al., 2019). Furthermore, an increase in lipid peroxidation products is linked to heightened permeability and reduced symmetry of the red blood cell membrane, leading to increased osmotic fragility and abnormalities on the surface of erythrocytes (Łuczaj et al., 2016; Esmaeilnejad et al., 2018).

4.3. Oxidant/antioxidant status

Numerous studies have demonstrated that glyphosate exposure in aquatic animals leads to oxidative stress, which upon long term exposure, can result in cellular damage (Nwani et al., 2013; Hong et al., 2018). Pesticide-induced oxidative stress may arise from the production of reactive oxygen species (ROS) or direct interaction with lipid membranes. The primary mechanism of organophosphate pesticide toxicity involves their interaction with the cytoplasmic membrane (Zhu et al., 2018). In this study, malondialdehyde (MDA), a byproduct of oxidation known for its toxic effects, was utilized as an indicator of lipid peroxidation (Gill et al., 2018; Zheng et al., 2022). Our findings indicate a significant increase in MDA levels in fish exposed to glyphosate, consistent with previous observations in the Chinese mitten crab (Yang et al., 2019). However, fish fed with plant-based diets exhibited significantly lower MDA levels (Zheng et al., 2022).

Antioxidant enzymes such as catalase (CAT), reduced glutathione (GSH), peroxidase (POD) and superoxide dismutase (SOD) play protective role by preventing the uncontrolled generation of ROS, representing an important adaptation to stress induced by pollutants (Hong et al., 2017). Earlier research has indicated that herbicides, including glyphosate, hinder the antioxidant defenses in fish (Sarker et al., 2021), as observed in the current study. In this study, the level of superoxide dismutase (SOD) and reduced glutathione peroxidase (GSH) in fish significantly reduced after glyphosate exposure. Conversely, fish fed on diets supplemented with lycopene exhibited significantly higher values, suggesting that the inclusion of lycopene might have stimulated certain antioxidant enzymes (SOD, POD, and GSH). This activation could be linked to the observed lower levels of oxidative stress, as indicated by reduced MDA, ROS and TBARS in the treated fish (Zheng et al., 2022). Carotenoids are known for their highly effective scavenging of singlet oxygen ($^1O_2$) and other excited oxygen species. When singlet oxygen is quenched, energy is transferred from it to the lycopene molecule, converting lycopene to the energy-rich triplet state (Ateşşahin et al., 2006). However, trapping other reactive oxygen species (ROS) like hydroxyl radicals (OH), nitrogen dioxide (NO^2), or peroxy-nitrite can lead to the oxidative breakdown of lycopene. This process suggests that lycopene may protect against the oxidation of lipids, proteins, and DNA in vivo (Yonar, 2013).

On the contrary, elevated levels of total protein (TPC) were detected in fish receiving lycopene, suggesting potential improvement in fish health or a response to post-injury or infection adaptation (El Basuini et al., 2020). The 8-OHdG and DIY increased by GLY exposure and its amelioration by LYC is supported by earlier study by Wang et al., 2021and Zhang et al. (2022). However, 8-OHdG is crucial biomarker for assessing oxidative stress and cancer risk assessment associated with exposure to different carcinogenic substances and environmental pollutants (Omari Shekaftik and Nasirzadeh, 2021). Moreover, 8-hydroxydeoxyguanosine (8OHDG) and dityrosine (DIY) serve as specific biomarkers indicative of DNA and protein damage resulting from oxidative stress. Given that their origins are guanosine (a nucleoside comprising guanine and ribose) and tyrosine (an amino acid), respectively, these biomarkers find utility in assessing drug toxicity in aquaculture (Oğuz et al., 2018; Chowdhury and Saikia, 2020). LYC demonstrates significant antioxidant efficacy, with its singlet oxygen

quenching ability being 47 times and 100 times greater than that of vitamin E and β-carotene, respectively. Thus, the reinforcement of the antioxidant defense system underscores the beneficial antagonistic effect of LYC against GLY (Abass et al., 2016).

4.4. Digestive enzyme activities and immunity

As a remarkable edible antioxidant, LYC has shown no reported toxic side effects to date (Gutiérrez Galán et al., 2021). Within the gastrointestinal tract, LYC undergoes isomerization into the CIS configuration and is emulsified by the scavenger receptor class B type 1 protein. Ultimately, it is dissolved and absorbed into the intestinal tract (Liang et al., 2019). Hence, the incorporation of lycopene into diets not only led to enhanced growth and feeding efficiency in *C. carpio* fingerlings but also improved digestive enzyme activities as observed in current study. Given that herbicides are recognized to disrupt and diminish digestive enzymes, the inclusion of lycopene in the diet may offer protective effects in this regard (El Basuini et al., 2020). However, it is essential to note that this aspect was not specifically examined in our study and warrants further investigation.

Immune-depressant impacts of glyphosate over aquatic species, including Chinese mitten crab, tilapia, and silver catfish, has been documented in previous observations (Hong et al., 2017; Yang et al., 2019; Zheng et al., 2021), similar to the current study. Phagocytic cells play a crucial role as the primary cellular components of the innate immune system in fish. Phagocytic activity is considered a fundamental defense mechanism and a key part of the nonspecific immune system. The current study demonstrated that administering lycopene alone notably boosts phagocytic activity (Yonar, 2013; Zheng et al., 2021). Lysozyme, a proteolytic enzyme, plays a crucial role in the innate immune system by eliminating pathogenic bacteria and triggering other immune responses, such as the complement system and phagocytic cells (Nigam et al., 2012). While, immunoglobulins contribute to both innate and adaptive immunity by generating specific antibodies against various antigens (Abós et al., 2022). The reduction in serum NO and lysozyme values observed in fish exposed to chlorpyrifos and cypermethrin toxicity might be linked to a decline in macrophage numbers due to water pollution (Zahran et al., 2018; Zhao et al., 2020). AChE activity observed to decrease in current study on exposure to GLY within common carp. Inhibition by GLY for binding of AChE to its active site and inhibition of sodium channels within neuron are reason behind this decrease. Whereas, antioxidants found within lycopene help to ameliorate this situation as previously observed (Singh et al., 2016; Arora and Arora, 2021). Phagocytosis represents a crucial defense mechanism in fish, involving lysozyme activity, bactericidal activity (BA), respiratory burst activity (Nitroblue Tetrazolium, NBT), and alternative complement pathway activities (ACP) (El Basuini et al., 2020). The NBT activity serves as a crucial indicator of the innate immune defense mechanism in fish (Kumar et al., 2018). The observed enhancement in immunological parameters with lycopene supplements can be attributed to the maintenance of a healthy energy level, stress reduction, improved mitochondrial respiration, and protection of cell membranes (Kumar et al., 2018; Yonar, 2019). Consequently, a reduction in these immune responses, can significantly impact fish health by compromising their disease resistance. Indeed, an increased risk of fish diseases and associated mortality has been observed in fish (*Galaxias anomalus*) exposed to glyphosate (Van Bruggen et al., 2018). Whereas, the use of plant-derived supplement, including lycopene as used in current study, is recognized for its ability to enhance fish immune responses as consistent with previous literature observed (Yonar, 2013; Harikrishnan et al., 2022; Sarker et al., 2021).

5. Conclusion

Lycopene partially ameliorated the glyphosate-induced toxicity by enhancing growth, liver functioning, serum proteins, blood health, and antioxidant levels, while reducing lipid peroxidation, within common carp (*C. carpio*). It also improved feeding utilization, immune responses and digestive enzyme activities of *C. carpio*. Thereby, we suggest incorporating lycopene into fish diets as a natural remedy to mitigate the toxicity caused by glyphosate. These findings can be further extended to be tested for other pesticides and herbicides for widening the knowledge and implementation at practical level.

CRediT authorship contribution statement

Rabia Tahir: Writing – original draft, Data curation, Conceptualization. **Samra:** Writing – review & editing, Validation, Supervision. **Fozia Afzal:** Writing – review & editing, Funding acquisition, Conceptualization. **Abdul Ghaffar:** Supervision, Project administration, Investigation. **Ji Liang:** Writing – review & editing, Formal analysis. **Abhimanyu Shrestha:** Writing – review & editing, Formal analysis. **Ume Habiba:** Investigation, Data curation. **Song Yang:** Writing – review & editing, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Data availability

No data was used for the research described in the article.

Acknowledgements

Rabia Tahir wants to pay thanks to three most important mentors in her life; Dr. Yang Song, Dr. Muhammad Ali Raza and Dr. Abdul Ghaffar, the former one who always inspires her to succeed, while the latter both to make sure she do succeed. RT is indebted to them for her learnings, courage and strength.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741084.

References

Abass, M.A., Elkhateeb, S.A., Abd EL-Baset, S.A., Kattaia, A.A., Mohamed, E.M., Atteia, H.H., 2016. Lycopene ameliorates atrazine-induced oxidative damage in adrenal cortex of male rats by activation of the Nrf2/HO-1 pathway. Environ. Sci. Pollut. Res. 23, 15262–15274.

Abdel-Daim, M.M., Eissa, I.A., Abdeen, A., Abdel-Latif, H.M., Ismail, M., Dawood, M.A., et al., 2019. Lycopene and resveratrol ameliorate zinc oxide nanoparticles-induced oxidative stress in Nile tilapia, *Oreochromis niloticus*. Environ. Toxicol. Pharmacol. 69, 44–50.

Abdel-Tawwab, M., Hamed, H.S., 2020. Antagonistic effects of dietary guava (*Psidium guajava*) leaves extract on growth, hemato-biochemical, and immunity response of cypermethrin-intoxicated Nile tilapia, *Oreochromis niloticus*, fingerlings. Aquaculture 529, 735668.

Abdel-Tawwab, M., Samir, F., Abd El-Naby, A.S., Monier, M.N., 2018. Antioxidative and immunostimulatory effect of dietary cinnamon nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus* (L.) and its susceptibility to hypoxia stress and *Aeromonas hydrophila* infection. Fish Shellfish Immunol. 74, 19–25.

Abós, B., Bailey, C., Tafalla, C., 2022. Adaptive immunity. In: Principles of Fish Immunology: From Cells and Molecules to Host Protection. Springer International Publishing, Cham, pp. 105–140.

Agostini, L.P., Dettogni, R.S., Dos Reis, R.S., Stur, E., Dos Santos, E.V., Ventorim, D.P., et al., 2020. Effects of glyphosate exposure on human health: insights from epidemiological and in vitro studies. S. Total Environ. 705, 135808.

Ahmadifar, E., Pourmohammadi Fallah, H., Yousefi, M., Dawood, M.A.O., Hoseinifar, S. H., Adineh, H., et al., 2021. The gene regulatory roles of herbal extracts on the growth, immune system, and reproduction of fish. Animals 11 (8), 2167.

Alagawany, M., Elnesr, S.S., Farag, M.R., Tiwari, R., Yatoo, M.I., Karthik, K., et al., 2021. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. Vet. Q. 41 (1), 1–29.
Ali, S., Ullah, M.I., Sajjad, A., Shakeel, Q., Hussain, A., 2021. Environmental and health effects of pesticide residues. In: Sustainable Agriculture Reviews 48: Pesticide Occurrence, Analysis and Remediation Vol. 2 Analysis, pp. 311–336.
Ames, J., Miragem, A.A., Cordeiro, M.F., Cerezer, F.O., Loro, V.L., 2022. Effects of glyphosate on zebrafish: a systematic review and meta-analysis. Ecotoxicology 31 (8), 1189–1204.
Annett, R., Habibi, H.R., Hontela, A., 2014. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. J. Appl. Toxicol. 34 (5), 458–479.
Arora, S., Arora, S., 2021. Nutritional significance and therapeutic potential of *Moringa oleifera*: the wonder plant. J. Food Biochem. 45 (10), e13933.
Ashaf-Ud-Doulah, M., Shahjahan, M., Islam, S.M., Al-Emran, M., Rahman, M.S., Hossain, M.A.R., 2019. Thermal stress causes nuclear and cellular abnormalities of peripheral erythrocytes in Indian major carp, rohu *Labeo rohita*. J. Therm. Biol. 86, 102450.
Ateşşahin, A., Karahan, İ., Türk, G., Gür, S., Yılmaz, S., Çeribaşı, A.O., 2006. Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats. Reprod. Toxicol. 21 (1), 42–47.
Bojarski, B., Witeska, M., 2020. Blood biomarkers of herbicide, insecticide, and fungicide toxicity to fish—a review. Environ. Sci. Pollut. Res. 27, 19236–19250.
Brovini, E.M., Cardoso, S.J., Quadra, G.R., Vilas-Boas, J.A., Paranaíba, J.R., Pereira, R.D. O., Mendonça, R.F., 2021. Glyphosate concentrations in global freshwaters: are aquatic organisms at risk? Environ. Sci. Pollut. Res. 28 (43), 60635–60648.
Burgos-Aceves, M.A., Lionetti, L., Faggio, C., 2019. Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Sci. Total Environ. 670, 1170–1183.
Cacabelos, R., 2020. Pharmacogenetic considerations when prescribing cholinesterase inhibitors for the treatment of Alzheimer's disease. Expert Opin. Drug Metab. Toxicol. 16 (8), 673–701.
Chowdhury, S., Saikia, S.K., 2020. Oxidative stress in fish: a review. J. Sci. Res. 12 (1).
Cupp-Enyard, C., 2008. Sigma's non-specific protease activity assay-casein as a substrate. J. Vis. Exp. 19, e899.
Cuykx, M., Rodrigues, R.M., Laukens, K., Vanhaecke, T., Covaci, A., 2018. In vitro assessment of hepatotoxicity by metabolomics: a review. Arch. Toxicol. 92, 3007–3029.
Dawood, M.A., Abdel-Tawwab, M., Abdel-Latif, H.M., 2020. Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. Rev. Aquac. 12 (4), 2511–2526.
De Faria, D.B.G., Montalvão, M.F., de Souza, J.M., de Oliveira Mendes, B., Malafaia, G., Rodrigues, A.S.D.L., 2018. Analysis of various effects of abamectin on erythrocyte morphology in Japanese quails (*Coturnix japonica*). Environ. Sci. Pollut. Res. 25 (3), 2450–2456.
El Basuini, M.F., Teiba, I.I., Zaki, M.A., Alabssawy, A.N., El-Hais, A.M., Gabr, A.A., et al., 2020. Assessing the effectiveness of CoQ10 dietary supplementation on growth performance, digestive enzymes, blood health, immune response, and oxidative-related genes expression of Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol. 98, 420–428.
Ellman, G.L., Courtney, K.D., Andres Jr., V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7 (2), 88–95.
Emerenciano, M.G., Rombenso, A.N., Vieira, F.D.N., Martins, M.A., Coman, G.J., Truong, H.H., et al., 2022. Intensification of penaeid shrimp culture: an applied review of advances in production systems, nutrition and breeding. Animals 12 (3), 236.
Esmaeilnejad, B., Tavassoli, M., Samiei, A., Hajipour, N., Imani-Baran, A., Farhang-Pajuh, F., 2018. Evaluation of oxidative stress and antioxidant status, serum trace mineral levels and cholinesterases activity in cattle infected with *Anaplasma marginale*. Microb. Pathog. 123, 402–409.
Farag, M.R., Alagawany, M., 2018. Erythrocytes as a biological model for screening of xenobiotics toxicity. Chem. Biol. Interact. 279, 73–83.
Ghaffar, A., Hussain, R., Khan, A., Abbas, R.Z., Aslam, S., Mehreen, M., et al., 2015. Hemato-biochemical and testicular changes induced by sub-chronic doses of Triazophos in male Japanese quail. Pak. J. Agric. Sci. 52 (3), 801–807.
Ghaffar, A., Hussain, R., Aslam, M., Abbas, G., Khan, A., 2016. Arsenic and urea in combination alters the hematology, biochemistry and protoplasm in exposed Rahu fish (*Labeo rohita*) (Hamilton, 1822). Turk. J. Fish. Aquat. Sci. 16 (2), 289–296.
Ghaffar, A., Hussain, R., Abbas, G., Kalim, M., Khan, A., Ferrando, S., et al., 2018. Fipronil (Phenylpyrazole) induces hemato-biochemical, histological and genetic damage at low doses in common carp, *Cyprinus carpio* (Linnaeus, 1758). Ecotoxicology 27 (9), 1261–1271.
Ghaffar, A., Hussain, R., Abbas, G., Khan, R., Akram, K., Latif, H., et al., 2019. Assessment of genotoxic and pathologic potentials of fipronil insecticide in *Labeo rohita* (Hamilton, 1822). Toxin Rev. 1–12.
Ghaffar, A., Hussain, R., Ahmad, N., Ghafoor, R., Akram, M.W., Khan, I., et al., 2021. Evaluation of hemato-biochemical, antioxidant enzymes as biochemical biomarkers and genotoxic potential of glyphosate in freshwater fish (*Labeo rohita*). Chem. Ecol. 37 (7), 646–667.
Gill, J.P.K., Sethi, N., Mohan, A., Datta, S., Girdhar, M., 2018. Glyphosate toxicity for animals. Environ. Chem. Lett. 16, 401–426.
Gutiérrez Galán, D.K., Pacheco-Moisés, F.P., Silva Bezerra, F., Alves de Matos, N., González Rojas, N.A., Arratia-Quijada, J., et al., 2021. Hydrophilic lycopene-coated layered double hydroxide nanoparticles to enhance the antioxidant activity and the oxidative stress evaluation. Appl. Nanosci. 11, 2747–2758.
Hamed, H.S., Abdel-Tawwab, M., 2017. Ameliorative effect of propolis supplementation on alleviating bisphenol-a toxicity: growth performance, biochemical variables, and oxidative stress biomarkers of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Comp. Biochem. Physiol. C 202, 63–69.
Harikrishnan, R., Devi, G., Van Doan, H., Vijay, S., Balasundaram, C., Ringø, E., et al., 2022. Dietary plant pigment on blood-digestive physiology, antioxidant-immune response, and inflammatory gene transcriptional regulation in spotted snakehead (*Channa punctata*) infected with *Pseudomonas aeruginosa*. Fish Shellfish Immunol. 120, 716–736.
Hendlin, Y.H., Arcuri, A., Lepenies, R., Hüesker, F., 2020. Like oil and water: the politics of (not) assessing glyphosate concentrations in aquatic ecosystems. Eur. J. Risk Regul. 11 (3), 539–564.
Hervé, V., Sabatier, P., Lambourdière, J., Raymond, R., Foulquier, E., Le Berre, I., et al., 2024. Pesticide upsurge, cross-contamination and biodiversity: case studies from the Caribbean coast human-environment observatory. Compt. Rend. Géosci. 356 (S3), 1–22.
Hong, Y., Yang, X., Yan, G., Huang, Y., Zuo, F., Shen, Y., et al., 2017. Effects of glyphosate on immune responses and haemocyte DNA damage of Chinese mitten crab, *Eriocheir sinensis*. Fish Shellfish Immunol. 71, 19–27.
Hong, Y., Yang, X., Huang, Y., Yan, G., Cheng, Y., 2018. Assessment of the oxidative and genotoxic effects of the glyphosate-based herbicide roundup on the freshwater shrimp, *Macrobrachium nipponensis*. Chemosphere 210, 896–906.
Hoseini, S.M., Mirghaed, A.T., Iri, Y., Hoseinifar, S.H., Van Doan, H., Reverter, M., 2021. Effects of dietary Russian olive, Elaeagnus angustifolia, leaf extract on growth, hematological, immunological, and antioxidant parameters in common carp, *Cyprinus carpio*. Aquaculture 536, 736461.
Hussain, R., Khan, A., Mahmood, F., Rehan, S., Ali, F., 2014. Clinico-hematological and tissue changes induced by butachlor in male Japanese quail (*Coturnix japonica*). Pestic. Biochem. Physiol. 109, 58–63.
Jideani, A.I., Silungwe, H., Takalani, T., Omolola, A.O., Udeh, H.O., Anyasi, T.A., 2021. Antioxidant-rich natural fruit and vegetable products and human health. Int. J. Food Prop. 24 (1), 41–67.
Johnson, R.B., Kim, S.K., Watson, A.M., Barrows, F.T., Kroeger, E.L., Nicklason, P.M., et al., 2015. Effects of dietary taurine supplementation on growth, feed efficiency, and nutrient composition of juvenile sablefish (*Anoplopoma fimbria*) fed plantbased feeds. Aquaculture 445, 79–85.
Kavitha, P., Rao, J.V., 2008. Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, Gambusia affinis. Environ. Toxicol. Pharmacol. 26 (2), 192–198.
Khalili, M., Attar, M., Amirlatifi, R., Maleki, Z.N., Hoseini, S.M., 2020. Effects of dietary myrcene administration on antioxidant gene responses in common carp (*Cyprinus carpio*), exposed to copper sulphate. Aquac. Res. 51 (4), 1653–1659.
Khoma, V., Gnatyshyna, L., Martinyuk, V., Mackiv, T., Yunko, K., Formanchuk, R., et al., 2020. Combined exposures to low roundup concentration induce thiolome response in bivalve mollusk. Scien. Issue Ternopil. Volodymyr. Hnatiuk. Nat. Pedagogi. Univer. Ser.: Biol. 80 (3–4), 72–78.
Kumar, J., Priyadharshini, M., Madhavi, M., Begum, S.S., Ali, A.J., Musthafa, M.S., Faggio, C., 2022. Impact of *Hygrophila auriculata* supplementary diets on the growth, survival, biochemical and haematological parameters in fingerlings of freshwater fish *Cirrhinus mrigala* (Hamilton, 1822). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 263, 111097.
Kumar, P., Jain, K.K., Sardar, P., 2018. Effects of dietary synbiotic on innate immunity, antioxidant activity and disease resistance of *Cirrhinus mrigala* juveniles. Fish Shellfish Immunol. 80, 124–132.
Latif, F., Aziz, S., Iqbal, R., Iram, S., Nazir, M., Shakeel, M., 2023. Impact of pesticide application on aquatic environments and biodiversity. In: Xenobiotics in Aquatic Animals: Reproductive and Developmental Impacts. Springer Nature Singapore, Singapore, pp. 143–164.
Le Du-Carrée, J., Boukhari, R., Cachot, J., Cabon, J., Louboutin, L., Morin, T., et al., 2021. Generational effects of a chronic exposure to a low environmentally relevant concentration of glyphosate on rainbow trout, *Oncorhynchus mykiss*. Sci. Total Environ. 801, 149462.
Leino, L., Tall, T., Helander, M., Saloniemi, I., Saikkonen, K., Ruuskanen, S., et al., 2021. Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase) for assessing sensitivity of organisms to the herbicide. J. Hazard. Mater. 408, 124556.
Li, M., Wei, D., Huang, S., Huang, L., Xu, F., Yu, Q., et al., 2022. Medicinal herbs and phytochemicals to combat pathogens in aquaculture. Aquac. Int. 30 (3), 1239–1259.
Liang, X., Ma, C., Yan, X., Liu, X., Liu, F., 2019. Advances in research on bioactivity, metabolism, stability and delivery systems of lycopene. Trends Food Sci. 93, 185–196.
Łuczaj, W., Gindzienska-Sieskiewicz, E., Jarocka-Karpowicz, I., Andrisic, L., Sierakowski, S., Zarkovic, N., et al., 2016. The onset of lipid peroxidation in rheumatoid arthritis: consequences and monitoring. Free Radic. Res. 50 (3), 304–313.
Madia, V.N., De Vita, D., Ialongo, D., Tudino, V., De Leo, A., Scipione, L., et al., 2021. Recent advances in recovery of lycopene from tomato waste: a potent antioxidant with endless benefits. Molecules 26 (15), 4495.
Maggi, F., la Cecilia, D., Tang, F.H., McBratney, A., 2020. The global environmental hazard of glyphosate use. Sci. Total Environ. 717, 137167.
Martinez, T.M., Meyer, R.K., Duca, F.A., 2021. Therapeutic potential of various plant-based fibers to improve energy homeostasis via the gut microbiota. Nutrients 13 (10), 3470.
Montaser, M.M., El-Sharnouby, M.E., El-Noubi, G., El-Shaer, H.M., Khalil, A.A., Hassanin, M., et al., 2021. *Boswellia serrata* resin extract in diets of Nile tilapia,

Oreochromis niloticus: effects on the growth, health, immune response, and disease resistance to *Staphylococcus aureus*. Animals 11 (2), 446.
Montgomery, H.A.C., Dymock, J.F., 1962. The rapid determination of nitrate in fresh and saline waters. Analyst 87 (1034), 374–378.
Morales, G.A., Denstadli, V., Collins, S.A., Mydland, L.T., Moyano, F.J., Øverland, M., 2016. Phytase and sodium diformate supplementation in a plant-based diet improves protein and mineral utilization in rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr. 22 (6), 1301–1311.
Mundkar, M., Bijalwan, A., Soni, D., Kumar, P., 2022. Neuroprotective potential of Moringa oleifera mediated by NF-kB/Nrf2/HO-1 signaling pathway: a review. J. Food Biochem. 46 (12), e14451.
Naiel, M.A., Shehata, A.M., Negm, S.S., Abd El-Hack, M.E., Amer, M.S., Khafaga, A.F., et al., 2020. The new aspects of using some safe feed additives on alleviated imidacloprid toxicity in farmed fish: a review. Rev. Aquac. 12 (4), 2250–2267.
Nigam, A.K., Kumari, U., Mittal, S., Mittal, A.K., 2012. Comparative analysis of innate immune parameters of the skin mucous secretions from certain freshwater teleosts, inhabiting different ecological niches. Fish Physiol. Biochem. 38, 1245–1256.
Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B., Lakra, W.S., 2013. DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. Environ. Toxicol. Pharmacol. 36 (2), 539–547.
Oğuz, E.K., Arihan, O., Oğuz, A.R., 2018. Oxidative and genotoxic effects of bisphenol a on primary gill cell culture of Lake Van fish (*Alburnus tarichi* Güldenstädt, 1814). Chem. Ecol. 34 (10), 914–924.
Ojelade, B.S., Durowoju, O.S., Adesoye, P.O., Gibb, S.W., Ekosse, G.I., 2022. Review of glyphosate-based herbicide and aminomethylphosphonic acid (AMPA): environmental and health impacts. Appl. Sci. 12 (17), 8789.
Omari Shekaftik, S., Nasirzadeh, N., 2021. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) as a biomarker of oxidative DNA damage induced by occupational exposure to nanomaterials: a systematic review. Nanotoxicology 15 (6), 850–864.
Overkamp, D., Mohammed-Ali, S., Cartledge, C., Landon, J., 1988. Production of polyclonal antibodies in ascitic fluid of mice: technique and applications. J. Immunoass. 9 (1), 51–68.
Parry Jr., R.M., Chandan, R.C., Shahani, K.M., 1965. A rapid and sensitive assay of muramidase. Proc. Soc. Exp. Biol. Med. 119 (2), 384–386.
Rafieepour, A., Hajirezaee, S., Rahimi, R., 2019. Dietary oregano extract (*Origanum vulgare* L.) enhances the antioxidant defence in rainbow trout, *Oncorhynchus mykiss* against toxicity induced by organophosphorus pesticide, diazinon. Toxin Rev. 39 (4), 397–407.
Rahman, A.N.A., Mohamed, A.A.R., Mohammed, H.H., Elseddawy, N.M., Salem, G.A., El-Ghareeb, W.R., 2020. The ameliorative role of geranium (*Pelargonium graveolens*) essential oil against hepato-renal toxicity, immunosuppression, and oxidative stress of profenofos in common carp, *Cyprinus carpio* (L.). Aquaculture 517, 734777.
Raymond, M., 1985. Log-probit analysis basic programme of microcomputer. Cah. Orstom. 117–121.
Rohmah, M.K., Salahdin, O.D., Gupta, R., Muzammil, K., Qasim, M.T., Al-Qaim, Z.H., et al., 2022. Modulatory role of dietary curcumin and resveratrol on growth performance, serum immunity responses, mucus enzymes activity, antioxidant capacity and serum and mucus biochemicals in the common carp, *Cyprinus carpio* exposed to abamectin. Fish Shellfish Immunol. 129, 221–230.
Roux, A., Lison, D., Junot, C., Heilier, J.F., 2011. Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: a review. Clin. Biochem. 44 (1), 119–135.
Sabra, F.S., Mehana, E.S.E.D., 2015. Pesticides toxicity in fish with particular reference to insecticides. Asian. J. Agric. Food. Sci. 3 (1).
Sarker, M.T., Wan, X., Yang, H., Wang, Z., 2021. Dietary lycopene supplementation could alleviate aflatoxin b1 induced intestinal damage through improving immune function and anti-oxidant capacity in broilers. Animals 11 (11), 3165.
Secombes, C.J., 1990. Isolation of salmonid macrophages and analysis of their killing activity. Tech. Fish Immunol. 1, 137–163.
Shahjahan, M., Khatun, M.S., Mun, M.M., Islam, S.M., Uddin, M.H., Badruzzaman, M., et al., 2020. Nuclear and cellular abnormalities of erythrocytes in response to thermal stress in common carp *Cyprinus carpio*. Front. Physiol. 11, 543.
Sharma, A., Shukla, A., Attri, K., Kumar, M., Kumar, P., Suttee, A., et al., 2020. Global trends in pesticides: a looming threat and viable alternatives. Ecotoxicol. Environ. Saf. 201, 110812.
Singh, S., Kumar, V., Datta, S., Wani, A.B., Dhanjal, D.S., Romero, R., et al., 2020. Glyphosate uptake, translocation, resistance emergence in crops, analytical monitoring, toxicity and degradation: a review. Environ. Chem. Lett. 18 (3), 663–702.
Singh, S.P., Sharma, J., Ahmad, T., Chakrabarti, R., 2016. Oxygen stress: impact on innate immune system, antioxidant defence system and expression of HIF-1α and ATPase 6 genes in *Catla catla*. Fish Physiol. Biochem. 42, 673–688.
Tiwari, R.K., Singh, S., Pandey, R.S., 2019. Assessment of the acute toxicity of chlorpyrifos and cypermethrin to *Heteropneustes fossilis* and their impact on acetylcholinesterase activity. Drug Chem. Toxicol. 42 (5), 463–470.
Tresnakova, N., Stara, A., Velisek, J., 2021. Effects of glyphosate and its metabolite AMPA on aquatic organisms. Appl. Sci. 11 (19), 9004.
Uchenna, U.B., Uka, A., Obiahu, O.H., 2022. The impact of sub-lethal concentrations of glyphosate on growth and haematology of African catfish under aquatic ecological micro-climate. Environ. Chem. Ecotoxicol. 4, 164–170.
Van Bruggen, A.H., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., et al., 2018. Environmental and health effects of the herbicide glyphosate. S. Total Environ. 616, 255–268.
Van Doan, H., Tapingkae, W., Moonmanee, T., Seepai, A., 2016. Effects of low molecular weight sodium alginate on growth performance, immunity, and disease resistance of tilapia, *Oreochromis niloticus*. Fish Shellfish Immunol. 55, 186–194.
Verster, E., 2018. The Influence of Manganese on Glyphosate Efficacy in Ryegrass (*Lolium Spp.*) (Doctoral dissertation). Stellenbosch University, Stellenbosch.
Wang, W., Ishikawa, M., Koshio, S., Yokoyama, S., Dawood, M.A., Hossain, M.S., et al., 2019. Effects of dietary astaxanthin and vitamin E and their interactions on the growth performance, pigmentation, digestive enzyme activity of kuruma shrimp (*Marsupenaeus japonicus*). Aquac. Res. 50 (4), 1186–1197.
Wang, Y., Zhao, H., Liu, Y., Li, J., Nie, X., Huang, P., et al., 2021. Environmentally relevant concentration of sulfamethoxazole-induced oxidative stress-cascaded damages in the intestine of grass carp and the therapeutic application of exogenous lycopene. Environ. Pollut. 274, 116597.
Yang, X., Song, Y., Zhang, C., Pang, Y., Song, X., Wu, M., et al., 2019. Effects of the glyphosate-based herbicide roundup on the survival, immune response, digestive activities and gut microbiota of the Chinese mitten crab, *Eriocheir sinensis*. Aquat. Toxicol. 214, 105243.
Yanno, T., 1992. 1992. Assays of haemolytic complement activity. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Kaattari, S.L., Rowley, A.F. (Eds.), Tech. Fish Immunol. SOS Publications, Fair Haven, NJ.
Yonar, M.E., 2012. The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (*Oncorhynchus mykiss*, W.). Fish Shellfish Immunol. 32 (6), 994–1001.
Yonar, M.E., 2013. Protective effect of lycopene on oxidative stress and antioxidant status in *Cyprinus carpio* during cypermethrin exposure. Environ. Toxicol. 28 (11), 609–616.
Yonar, S.M., 2019. Growth performance, haematological changes, immune response, antioxidant activity and disease resistance in rainbow trout (*Oncorhynchus mykiss*) fed diet supplemented with ellagic acid. Fish Shellfish Immunol. 95, 391–398.
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.
Yousefi, M., Adineh, H., Reverter, M., Hamidi, M.K., Vatnikov, Y.A., Kulikov, E.V., et al., 2021. Protective effects of black seed (Nigella sativa) diet supplementation in common carp (*Cyprinus carpio*) against immune depression, oxidative stress and metabolism dysfunction induced by glyphosate. Fish Shellfish Immunol. 109, 12–19.
Zahran, E., Risha, E., Awadin, W., Palić, D., 2018. Acute exposure to chlorpyrifos induces reversible changes in health parameters of Nile tilapia (*Oreochromis niloticus*). Aquat. Toxicol. 197, 47–59.
Zhang, J., Sundfør, E.B., Klokkerengen, R., Gonzalez, S.V., Mota, V.C., Lazado, C.C., et al., 2022. Determination of the oxidative stress biomarkers of 8-Hydroxydeoxyguanosine and Dityrosine in the gills, skin, dorsal fin, and liver tissue of Atlantic Salmon (*Salmo salar*) Parr. Toxics 10 (9), 509.
Zhao, H., Wang, Y., Guo, M., Mu, M., Yu, H., Xing, M., 2020. Grass carps co-exposed to environmentally relevant concentrations of cypermethrin and sulfamethoxazole bear immunodeficiency and are vulnerable to subsequent *Aeromonas hydrophila* infection. Environ. Pollut. 266, 115156.
Zhao, L., Tang, G., Xiong, C., Han, S., Yang, C., He, K., et al., 2021. Chronic chlorpyrifos exposure induces oxidative stress, apoptosis and immune dysfunction in largemouth bass (*Micropterus salmoides*). Environ. Pollut. 282, 117010.
Zheng, T., Jia, R., Cao, L., Du, J., Gu, Z., He, Q., et al., 2021. Effects of chronic glyphosate exposure on antioxdative status, metabolism and immune response in tilapia (GIFT, *Oreochromis niloticus*). Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 239, 108878.
Zheng, T., Jia, R., Cao, L., Du, J., Gu, Z., He, Q., et al., 2022. Alleviative effects of Ginkgo biloba extract on oxidative stress, inflammatory response and immune suppression induced by long-term glyphosate exposure in tilapia (*Oreochromis niloticus*). Aquaculture 546, 737325.
Zhu, J., Wang, J., Ding, Y., Liu, B., Xiao, W., 2018. A systems-level approach for investigating organophosphorus pesticide toxicity. Ecotoxicol. Environ. Saf. 149, 26–35.