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Effects of Dietary Green Microalgae *(Chlorella vulgaris)* and Iron Nanoparticles on Biochemical, Enzymatic, and Tissue Health in *Cyprinus carpio*

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ABSTRACT

This study investigates the effects of *Chlorella vulgaris* and iron nanoparticles (Fe-NPs) as dietary supplements on the health of *Cyprinus carpio*. Eighty juvenile carp (156.8 ± 2.14 g) were divided into four groups: control (T0), 10% *C. vulgaris* (T1), 85 mg/kg Fe-NPs (T2), and a combination of both (T3), fed for 60 days. Biochemical, enzymatic, and histological parameters were analysed. The results showed that *C. vulgaris* supplementation (T1) provided the most significant benefits by reducing glucose levels. The combination group (T3) also showed improved glucose regulation. Serum ALT and AST were lowest in T3, while T1 showed muscle enzyme reduction (ALT: 50.53 ± 2.49 U/l; AST: 596.1 ± 88.15 U/l). Histologically, T1 enhanced muscle fibre density and spleen immune activity, whereas T3 mitigated Fe-NPs-induced iron accumulation. In conclusion, while all treatments improved fish health, *C. vulgaris* alone offered the most consistent metabolic, enzymatic, and tissue benefits, highlighting its potential as a sustainable aquaculture feed additive.

Keywords: biochemistry, *Chlorella vulgaris*, *Cyprinus carpio*, enzyme activity, iron nanoparticle.

INTRODUCTION

Aquaculture is a relatively recent addition to the global food production system, and due to its rapid production growth, it has surpassed fisheries as the primary source of seafood for human consumption (Garlock *et al.*, 2024; Asad *et al.*, 2024). In recent years, aquaculture has gained greater importance due to concerns that livestock farming, agriculture, and fisheries are no longer sufficient to meet the growing demand for food necessary to support the population. Therefore, aquaculture provides an option that includes food security, reduces food shortages, alleviates poverty, and reduces exploitation of the global fishing market (Contreras *et al.*, 2024).

Global fisheries and aquaculture production reached a significant milestone in 2020, reaching a production capacity of 214 million tons. This included 178 million tons of aquatic animals and 36 million tons of algae, most of which originated in Asia (Abdullah, 2022). In the same year, the European aquaculture sector reported a sales volume of 1.2 million tons and a turnover value of €3.9 billion, directly employing around 57,000 people working for approximately 14,000 enterprises (Alsaleh, 2024).

The common carp (*Cyprinus carpio*) is the third most important fish species in global aquaculture production (Ahmed, 2023, 2024), accounting for 8.3% of global aquaculture fish production (Karnai and Szűcs, 2018). It is important to enhance diets in aquaculture. Fish has maximum protein value, and its edibility surpasses 90% (Selamoglu and Naeem, 2023). It requires a balanced diet that not only enables the provision of energy required for various

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functions in the body and supports optimal growth and reproduction, but also enhances the immune function of the fish (Al-Obaidi, 2025).

Microalgae, a diverse group of oxygen-evolving, photosynthetic organisms, are characterized by widespread distribution, rapid growth and reproduction, and strong tolerance to harsh environments (Gao *et al.*, 2024). Among them, *C. vulgaris* is known for being a rich source of nutrients, including proteins, vitamins, minerals, and various bioactive compounds, such as carotenoids, chlorophyll, and polysaccharides, all of which contribute to its numerous therapeutic effects (Panahi *et al.*, 2016). These compounds have been linked to antioxidant, immunomodulatory, detoxifying properties, and anti-inflammatory properties (Mendes *et al.*, 2024). Microalgae are one of the promising alternative energy sources due to their high oil yield per land area (Chisti, 2007).

Nanoparticles (NPs) offer a promising alternative to conventional antibiotics for inhibiting antibiotic-resistant strains that threaten human and animal health, offering efficient and sustainable solutions (Abdelkarim *et al.*, 2025). In recent years, NPs have been increasingly used in aquaculture, with applications aimed at improving feed quality, nutrient absorption, and mineral utilization (Mahmud and Haque, 2025). Iron is one of the most important trace elements in fish and is present in all tissues and organs. It plays a crucial role in cellular components, cellular respiration, lipid peroxidation, immune system modulation, and body defence against infections. However, excess iron levels can be toxic, leading to reduced growth, increased mortality, diarrhea, and histological damage to liver cells (Akbary *et al.*, 2019; Omar and Al Sulivany, 2025).

This study assesses the response of *C. carpio* to iron nanoparticles, *C. vulgaris*, and a combination of both. The main objectives are to evaluate the biochemical indicators, enzyme activities, and histological alterations in muscle and spleen tissues.

MATERIALS AND METHODS

Eighty juvenile common carp of both sexes, weighing 156.8 ± 2.14 g, with a total length (TL) of 19.75 ± 1.83 cm, fork length (FL) of 16.5 ± 0.62 cm, and standard length (SL) of 15.7 ± 0.4 cm, were obtained from a local farm and acclimatized to the laboratory conditions at the Fish Laboratory, Department of Biology, College of Science, University of Zakho for two weeks to adapt to healthy laboratory conditions. The fish were kept in a 1.2 m^3 plastic tank with a capacity of 400 liters and fed a control diet every 24 hours. The feed composition was obtained from Amedi Animal Feed Company. To control pathogens, *C. carpio* was immersed in a 2% saline solution (sodium chloride; NaCl) obtained from Sigma-Aldrich for 1-2 minutes (Das *et al.*, 2025; Abdulrahman and Al Sulivany, 2025). Continuous aeration was provided for each tank using small air pumps (Luckiness 828, 5 W power, 3.5 L/min air flow) and Chinese air compressors (Hailea ACO-318, 45 W power, 70 L/min air flow; Hailea ACO-328, 55 W power, 82 L/min air flow; Resun ACO-010, 200 W power, 0.135 m^3/min air flow). Culture conditions were monitored daily and adjusted to optimal conditions, with pH, dissolved oxygen, temperature, and salinity measured as follows: 8.28 ± 0.23, 7.4 ± 0.4, 16 ± 0.3, and 0.06 g/L (Owais *et al.*, 2024).

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-071; 2024).

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Dietary Collection and Experimental Design

C. vulgaris powder was obtained from Natura Vitalis, the Netherlands. One hundred grams of *C. vulgaris* contains an energy value of 1450 kJ (343 kcal), 2.3 grams of fat (of which 6 grams are saturated), 14 grams of carbohydrates (with less than 0.1 grams of sugar), 12 grams of fiber, and 61 grams of protein. It also contains 0.15 grams of salt, 59 mg of vitamin B3, 50 µg of vitamin B12, and 1000 µg of iodine. Iron nanoparticles (Fe-NPs; code 746835) were purchased from Sigma-Aldrich, China. The Fe-NPs were 25 nm and comprised 99.5% trace elements.

After acclimatization, a control diet and three experimental diets were prepared. All ingredients were ground and mixed well in a blender to prepare diets containing *C. vulgaris* and iron nanoparticles. Water was added to form a smooth paste, which was then processed using an electric extruder (Jiaozuo Machine, China) equipped with a 2 mm diameter mesh plate. The extruded pellets were dried overnight at 50°C and stored at -18°C until use (Yousefi *et al.*, 2025). Table 1 shows the diet composition for each fish group.

Eighty fish were divided into four treatment groups, each containing duplicates. These groups were designated as T0, T1, T2, and T3. T0 was the control group, receiving only the basal feed pellet (BDP). T1 was fed BDP supplemented with 10% *C. vulgaris*. T2 received BDP supplemented with 85 mg/kg of iron nanoparticles, while T3 was provided with BDP containing both *C. vulgaris* and Fe-NPs. Fish were fed daily at 10:00 a.m. for 60 days, at a feeding rate of 3% of their body weight (Hoseini *et al.*, 2025). Feces and uneaten feed were removed using a vacuum system. No mortalities were observed throughout the study period.

Table 1. Feed and Proximate Composition of Fish Diet with *C. vulgaris* and Fe-NPs and a Combination of Both

Ingredient (g)	T0	T1	T2	T3
Wheat bran	16	12	15	11
Corn	14	14	14	14
Soybean meal	30	30	30	30
Barley	16	17	17	17
Wheat	22	22	22	22
Vitamin premix	2	2	2	2
C. vulgaris 10%	-	3	-	3
Iron nanoparticle	-	-	0.85	0.85
Proximate composition of fish feeds				
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
Ash (%)	10.01	10.01	10.01	10.01
Moisture (%)	12.10	12.10	12.10	12.10
Organic matter (%)	77.6	77.3	77.7	77.3

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Sample Collection and Analysis

After 60 days of feeding, the fish were anesthetized using MS-222 (tricaine methane sulfonate, 0.1 g/L, Sigma-Aldrich, USA) to minimize stress and prevent injury during the procedure. Blood samples were collected from the caudal vein using 3 ml syringes. The blood samples were inserted into gel tubes without anticoagulants and left at room temperature for five minutes to determine biochemical and enzyme activity parameters.

After blood collection, the fish were caught by a dip net and euthanized by a sharp blow to the head. Spleen and muscle tissues (2 g, 1×1 cm) were collected by a scalpel in the middle portion of the body to determine tissue enzyme activity and for histopathological examination of the spleen and muscle.

Determination of Biochemical and Enzyme Activity Parameters

After blood collection, the blood was centrifuged at 1500 rpm for 10 minutes to obtain serum for analysis. The serum biochemical (glucose, urea, creatinine, uric acid, cholesterol) and enzyme activity (alanine transaminase (ALT) and aspartate transaminase (AST)) parameters were assayed using a FUJIFILM (DRI_CHEM NX500-Czech Republic) analyzer following the manufacturer's instructions for the slide reagent kits.

Tissue Enzyme Activity Assays

Spleen and muscle tissues were homogenized. The samples were rinsed with a cold saline solution and dried before being weighed in 0.15 M Tris-HCl buffer with a pH of 7.4. A 10% (w/v) tissue homogenate was prepared in the same buffer to assess ALT and AST enzyme activities (Abdulla et al, 2024). The homogenates were centrifuged at 10,000 g for 10 minutes. Supernatant was used to determine the enzyme activities, which were analyzed using the COBAS C111 Automated Analyzer (Al Habbib and Al Sulivany, 2013).

Histopathological Examination

After dissecting the fish, the spleen and muscle were removed, cleaned with distilled water, and weighed. The tissues were fixed in 10% neutral buffered formalin, dehydrated through an ascending grade of ethyl alcohol, cleared in xylene, and fixed in paraffin wax. Sections were cut at 5 µm and stained with hematoxylin and eosin. The sections were examined under a compound microscope to assess the histological conditions of the tissues (Abd-Elhafeezd *et al.*, 2024; Haji *et al.*, 2024; Mustafa & Al Sulivany, 2025).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 9 from Finland. A one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons (Tukey's) was employed to identify significant differences between groups. A p-value of less than 0.05 was used as an indicator for statistical significance.

RESULTS AND DISCUSSION

The results showed significant variations in biochemical and enzyme activity parameters of *C. carpio* fed different diets over 60 days. Fish fed the control diet (T0) showed baseline glucose levels of 152.5 ± 2.219 mg/dl, which significantly decreased to 88.77 ± 24.21 mg/dl in the *C. vulgaris*-supplemented group (T1) ($p = 0.0283$). At the same time, the combined *C. vulgaris* and Fe-NPs diet (T3) also reduced glucose levels to 71.83 ± 6.585 mg/dl. The serum urea levels increased in the Fe-NPs group (T2) at 8.33 ± 0.88 mg/dl ($p = 0.0302$), whereas creatinine was lowest in T1 (0.036 ± 0.003 mg/dl) and highest in T2 (0.05 ± 0.016 mg/dl) ($p = 0.041$). Serum uric acid levels

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varied significantly, with T2 showing the highest (0.93 ± 0.38 mg/dl) and T1 the lowest (0.62 ± 0.1 mg/dl) (p = 0.033). Cholesterol was diminished in T1 (122.3 ± 1.18 mg/dl; p = 0.047), while serum enzyme activities (ALT and AST) were lowest in T3 (8.7 ± 2.2 U/l and 96.95 ± 32.65 U/l, respectively; p = 0.05 and 0.047).

Table 2. The Biochemical and Enzyme Activity Parameters in Fish Fed Basal Diet Pellets Supplemented with *C. vulgaris* and Fe-NPs and a Combination of Both

Biochemical parameters	T0	T1	T2	T3	p-value
Glucose (mg/dl)	152.5 ± 2.21^{a}	88.77 ± 24.2^{b}	150.5 ± 46.5^{a}	71.83 ± 6.585^{c}	0.0283
Urea (mg/dl)	5.76 ± 0.8^{a}	5.6 ± 1.15^{a}	8.33 ± 0.88^{b}	5.46 ± 0.4^{a}	0.0302
Creatinine (mg/dl)	0.048 ± 0.002^{a}	0.036 ± 0.003^{b}	0.05 ± 0.016^{c}	0.046 ± 0.006^{ac}	0.041
Uric acid (mg/dl)	0.83 ± 0.033^{a}	0.62 ± 0.1^{b}	0.93 ± 0.38^{c}	0.7 ± 0.4^{ab}	0.033
Cholesterol (mg/dl)	140 ± 28.69a	122.3 ± 1.18^{b}	135.9 ± 20.7^{a}	127 ± 20.45^{a}	0.047
ALT (U/l)	23.3 ± 1.99^{a}	10.9 ± 1.1^{b}	10.87 ± 5.38^{b}	8.7 ± 2.2^{c}	0.05
AST (U/l)	287.4 ± 13.95^{a}	196.5 ± 59.05^{b}	245.1 ± 91.01^{a}	96.95 ± 32.65^{c}	0.047

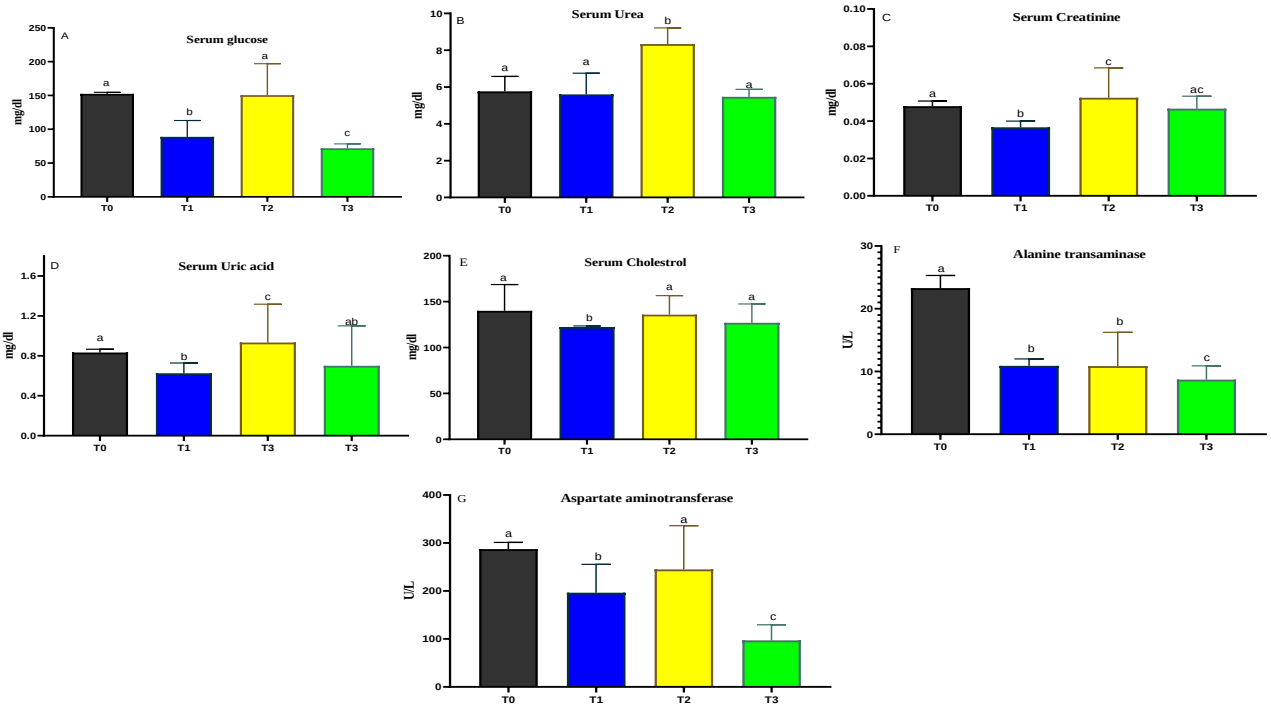

Figure 1. The biochemical and enzyme activity parameters in fish fed BDP supplemented with *C. vulgaris* and Fe-NPs, and a combination of both diets in *C. carpio* during 60-day experimental trials. Significant differences ($p < 0.05$) are indicated by distinct superscripts (a, b, c, and d).

This study also demonstrated significant changes in muscle and spleen enzyme activities in *C. carpio* fed different experimental diets for 60 days. In muscle tissue, the control group (T0) exhibited the highest ALT (85.33 ± 18.9 U/l) and AST (1140

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± 156.8 U/l) levels, while supplementation with *C. vulgaris* (T1) significantly reduced these values to 50.53 ± 2.49 U/l (P = 0.0052) and 596.1 ± 88.15 U/l (P = 0.0481), respectively. The Fe-NPs group (T2) showed intermediate enzyme levels (ALT: 67.47 ± 1.32 U/l; AST: 932.8 ± 51.78 U/l), whereas the combined *C. vulgaris* and Fe-NPs diet (T3) further lowered ALT (56.1 ± 6.9 U/l) and AST (680 ± 155.4 U/l). Similar trends were observed in spleen tissue, with T0 displaying the highest ALT (34.5 ± 2.5 U/l) and AST (105 ± 18 U/l), and T1 showing the lowest values (ALT: 19.5 ± 1.5 U/l, P = 0.0208; AST: 53.5 ± 5.5 U/l, P = 0.0336).

Table 3. Tissue Enzyme Activity Parameters in Fish Fed Basal Diet Pellets Supplemented with *C. vulgaris* and Fe-NPs and a Combination of Both

Biochemical parameters	T0	T1	T2	T3	p-value
Muscle					
ALT (U/l)	85.33 ± 18.9^{a}	50.53 ± 2.49^{b}	67.47 ± 1.32^{c}	56.1 ± 6.9^{bc}	0.0052
AST (U/l)	1140 ± 156.8^{a}	596.1 ± 88.15^{b}	932.8 ± 51.78^{c}	680 ± 155.4^{d}	0.0481
Spleen					
ALT (U/l)	34.5 ± 2.5^{a}	19.5 ± 1.5^{b}	26 ± 2^{c}	21 ± 2.4^{bc}	0.0208
AST (U/l)	105 ± 18^{a}	53.5 ± 5.5^{b}	74.5 ± 4.5^{c}	64 ± 3^{bc}	0.0336

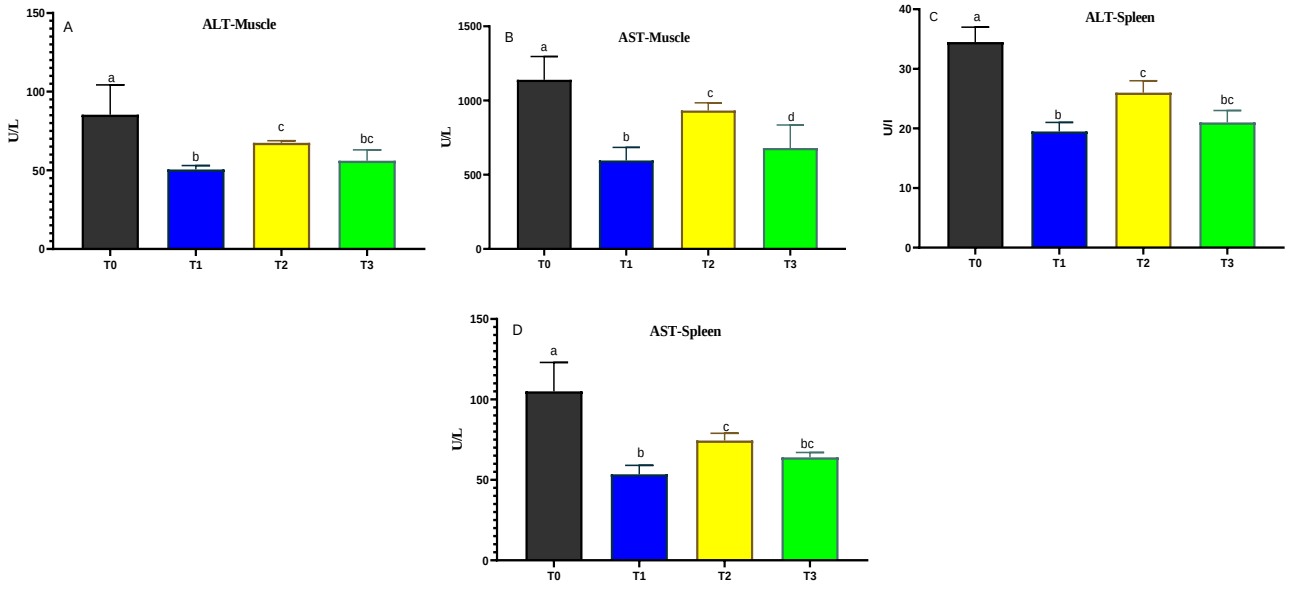

Figure 2. Serum homogenized muscle and spleen enzyme activity parameters (ALT and AST) in *C. carpio* during 60-day experimental trials. Significant differences ($p < 0.05$) are indicated by distinct superscripts (a, b, c, and d).

The microscopic examination of muscle tissue showed apparent differences between the treatment groups. In the untreated fishes (T0), the muscle fibers appeared healthy, with even pink staining and distinct cross-striations, while the surrounding connective tissue remained undisturbed without signs of inflammation (Figure 3, A). When *C. vulgaris* was introduced (T1), the fibers became denser and more polygonal, with a noticeable increase in fiber count per field, hinting at possible muscle growth from the

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supplement (Figure 3, B). The group exposed to Fe-NPs (T2) displayed well-organized fibers with peripheral nuclei and intact striations, confirming that the nanoparticles did not disrupt standard muscle architectures (Figure 3, C). Meanwhile, the combination of *C. vulgaris* and Fe-NPs (T3) produced similar results, with fibers maintaining their typical shape and connective tissue integrity (Figure 3, D). Together, these observations suggest that both *C. vulgaris* and Fe-NPs support muscle tissue health, either alone or in combination.

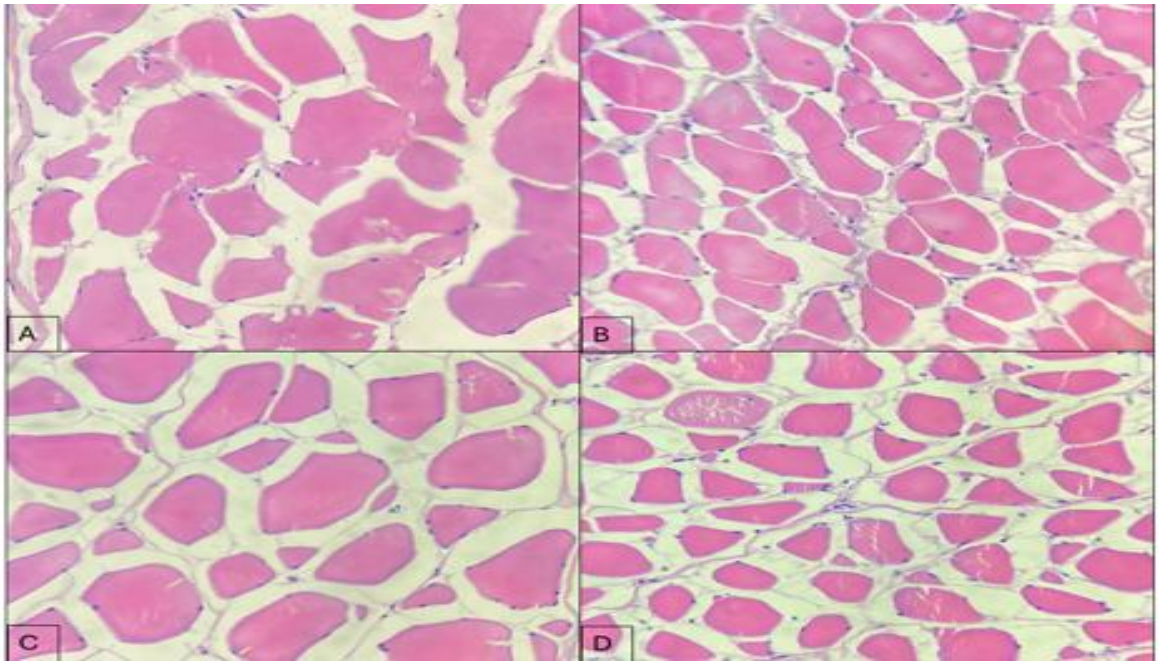

Figure 3. Histological observations of muscle [A] Control group. [B] *C. vulgaris* treated group [C]. The iron nano particles (Fe-NPs) treated group. [D] Co-treated with *C. vulgaris* and iron nano particles (Fe-NPs). (H&E) (A, B, C, D: 400x).

The histological examination of fish spleen tissues revealed distinct morphological changes across the treatment groups (Figure 4). In the control group (T0), the spleen exhibited normal lymphoid cell distribution and intact architecture, indicating healthy tissue (Figure 4, A). The *C. vulgaris*-treated group (T1) showed increased lymphoid cell density and mild white pulp hyperplasia, suggesting an immune response without tissue damage (Figure 4, B). In contrast, the Fe-NPs-treated group (T2) displayed disorganized splenic tissue, hemosiderin deposition, and mild red pulp congestion, indicative of iron accumulation and early structural disruption (Figure 4, C). Notably, the co-treatment group (T3) demonstrated improved splenic architecture, with reduced hemosiderin deposits and restored lymphoid organization, highlighting the potential protective role of *C. vulgaris* against Fe-NPs-induced toxicity (Figure 4, D). These findings underscore the varying impacts of the treatments on splenic histology, with the combined approach showing promise in mitigating adverse effects.

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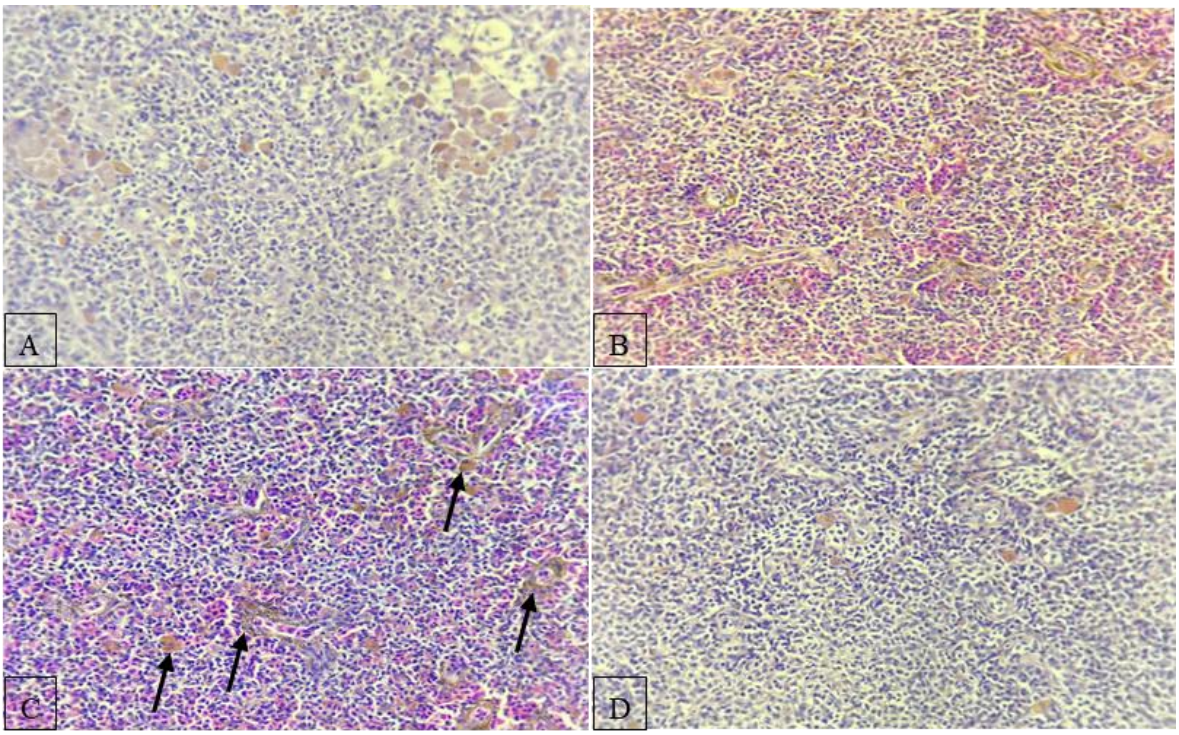

Figure 4. Histological observations of spleen tissues: [A] control group; [B] *C. vulgaris*-treated group; [C] iron nano particles (Fe-NPs)-treated group, arrows indicating iron accumulation; [D] co-treated with *C. vulgaris* and iron nano particles (Fe-NPs). (H&E) (A, B, C, D: 400x).

DISCUSSION

Glucose levels significantly decreased in the *C. vulgaris* (T1) and co-treatment (T3) groups. This reduction could be attributed to the improved metabolic efficiency of energy derived from *C. vulgaris*, potentially due to its fiber content or influence on carbohydrate metabolism (Panahi *et al.*, 2016). The increases in glucose, urea, creatinine, and uric acid in the Fe-NPs alone group (T2) compared to the control (T0) were observed. Elevated levels of these parameters can sometimes indicate metabolic stress or impaired organ function (Omar & Al Sulivany, 2025). The ability of *C. vulgaris* in T3 to counteract these increases suggests a beneficial role in maintaining metabolic homeostasis, even in the presence of supplementary iron. The serum ALT and AST are crucial indicators of fish health (Al Habbib & Sulaivany, 2013). The results showed that both *C. vulgaris* and the combined treatment reduced serum ALT and AST levels compared to the control. The reduction of these enzymes suggests a protective effect of *C. vulgaris* on hepatic tissue (Yarmohammadi *et al.*, 2021), possibly through its antioxidant and detoxifying properties (Panda *et al.*, 2023). The Fe-NPs group showed several increases in these enzymes compared to the control, although not always statistically significant, further underscoring the potential for iron overload to induce stress.

Similar trends were observed in the enzyme activities within the homogenized spleen and muscle tissues. Both ALT and AST activities in the spleen were reduced in the *C. vulgaris* and co-treatment groups compared to the control and Fe-NPs alone group. This reduction in splenic enzyme activity reinforces the protective and immunomodulatory effects of *C. vulgaris* on spleen health, suggesting reduced cellular stress (Gabr *et al.*, 2025). In muscle tissues, *C. vulgaris* and the combined

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treatment also reduced ALT and AST levels compared to the control and Fe-NPs groups. This indicates that *C. vulgaris* contributes to better muscle health and integrity, possibly by supporting cellular repair mechanisms (Zainul *et al.*, 2020).

The histological examination of the muscle tissues showed that *C. vulgaris* supplementation led to more compact and polygonal muscle fibers, with an apparent increase in fiber number, suggesting hypertrophy. *C. vulgaris* is recognized as a rich source of protein and other essential nutrients (Panahi *et al.*, 2016), which could directly contribute to muscle development and overall fish growth (Selamoglu and Naeem, 2023). The iron nanoparticle group (T2) and the co-treatment group (T3) showed normal muscle fiber architecture without signs of necrosis or inflammatory infiltration. This indicates that Fe-NPs did not negatively impact muscle integrity at the administered dosage, and the combination with *C. vulgaris* maintained healthy muscle morphology.

The histological examination of the spleen revealed compelling evidence of immunomodulation. Fish fed with *C. vulgaris* (T1) exhibited a marked increase in lymphoid cells, suggesting an enhanced immune response without observable histopathological damage. This observation aligns with previous research highlighting the immunomodulatory properties of *C. vulgaris* due to its rich content of bioactive compounds such as polysaccharides and carotenoids (Mendes *et al.*, 2024; Panahi *et al.*, 2016). In contrast, the group supplemented with Fe-NPs alone (T2) showed hemosiderin deposition in the spleen, indicative of iron accumulation. While iron is an essential trace element for various physiological functions, including immune system modulation (Akbary *et al.*, 2019), excessive levels can lead to toxicity and tissue damage (Akbary *et al.*, 2019). The co-treatment group (T3) exhibited a normal splenic architecture with reduced hemosiderin deposition and well-organized lymphoid cells. This suggests a potential protective or ameliorative effect of *C. vulgaris* against iron-induced splenic alterations, possibly by aiding in iron detoxification or improving cellular health and antioxidant defense (Mendes *et al.*, 2024).

CONCLUSION

This study concluded that dietary supplementation with *C. vulgaris*, iron nanoparticles (Fe-NPs), and their combination improves the health and physiology of *C. carpio*. While all treatments showed beneficial effects, the *C. vulgaris* group exhibited the most significant enhancements in biochemical parameters, including reduced glucose and cholesterol levels, as well as lower ALT and AST activities, indicating improved metabolic and liver function. Histological examination further confirmed its superiority, with denser muscle fibers and enhanced spleen immune activity. The Fe-NPs group and the combined treatment also showed positive results, though *C. vulgaris* alone provided the most consistent benefits.

AUTHORS' CONTRIBUTIONS

M.O., B.S.A.A.S., and I.A.O. contributed to the concept and design of the work. A.I.Y., B.S.A.A.S., and I.A.O. were responsible for the acquisition, analysis, and interpretation of data. M.O., B.S.A.A.S., and I.A.O. drafted the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors declare no competing interests.

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REFERENCES

Abd-Elhafeezd, H. H., Al Sulivany, B. S., Salah, A. S., Ali, M., Mohamed, N. E., & Soliman, S. A. (2024). Microscopic Architecture of the Respiratory and Conducting System of the Lung of Nile monitor. Science Journal of University of Zakho, 12(4), 477–483.

Abdelkarim, E. A., Elsamahy, T., El Bayomi, R. M., Hussein, M. A., Darwish, I. A., El-tahlawy, A. S.& Sobhy, M. (2025). Nanoparticle-driven aquaculture: transforming disease management and boosting sustainable fish farming practices. Aquaculture International, 33(4), 1-49.

Abdullah, A. H. J. (2024). Evaluation of the Commercial Fish Catch at the Al-Fao Landing Site, Southern Iraq During January to December 2022. Egyptian Journal of Aquatic Biology and Fisheries, 28(3), 781-794.

Abdulrahman, P., Al Sulivany, B. (2025). 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. Egyptian Journal of Aquatic Biology and Fisheries, 29(2), 383-405.

Ahmed, B. (2023). Nutritional Effects of Dietary Spirulina (*Arthrospora platensis*) on Morphological Performance, Hematological Profile, Biochemical Parameters of Common Carp (*Cyprinus carpio* L.). *Egyptian Journal of Veterinary Sciences*, *54*(3), 515-524.

Ahmed, B. (2024). The Protective Effects of Blue-Green Algae (Spirulina) Against Arsenic-Induced Differences in Lipid Panel and Hematological Parameters in Female Rats (*Rattus norvegicus*). Egyptian Journal of Veterinary Sciences, 55(3), 785-793.

Akbary, P., & Jahanbakhshi, A. (2019). Nano and total iron oxide (Fe_2O_3) as nutritional additives: effects on growth, biochemistry, liver enzyme activity, pathological anatomy in the liver, and the version of genes associated with appetite in the golden fish (*Carassius auratus*). Aquaculture, 510, 191-197.

Al Habbib, O. A. M. & Al Sulivany, B. S. A. (2013). Effect Of Omega-3 And Multivitamins On Aluminum-Induced Changes in Serum and Tissue Enzyme Activities in Rats. Science Journal of University of Zakho, 1(1), 65-71.

Al-Obaidi, T. S. M. (2025). Effect of Adding Different Levels of Safflower Oil and Fish Oil Mixture in Common Carp (*Cyprinus carpio*) Diets. In IOP Conference Series: Earth and Environmental Science,1487(1), 012176.

Alsaleh, M. (2024). The impact of aquaculture economic expansion on marine water quality in the EU region. Regional Studies in Marine Science, 77, 103625.

Asad, F., Al Sulivany, B., Ali, S., Owais, M., Fazal, R. M. & Hussein, N. (2024). Origin, Physical Properties, biodegradation, and Potential Effects of Microplastics on Aquaculture. Aquatic Science and Fish Resources (ASFR), 5(1), 85–99.

Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology Advances, 25(3), 294-306.

Contreras, C. O., Pérez-Reynoso, F. D., Zamora-Antoniano, M. A., Alvarez-Alvarado, G. M., & Rodríguez-Resendez, G. (2024). An artificial intelligence-based aquaculture system for water quality improvement: a systematic analysis. Journal of Marine Science and Engineering, 12(1), 161.

Das, P. S., Rouhani, F., Al-Sullivany, B. S. A., Nebir, S. S., Guthi, R. A., Sater, A., Hussain, M. S., & Ismail, S. S. (2025). Improving the growth performance and muscle composition of stinging catfish, Heteropneustes fossilis, Scientific Journal of the University of Zakho, 13(1), 33-39

Gabr, A., Mohamed, A. M., Abou Khalil, N. S., & Sayed, A. E. H. (2025). The protective effect of *Chlorella vulgaris* against diclofenac toxicity in *Clarias gariepinus*: haemato-immunological parameters and spleen histological features as outcome markers. Frontiers in immunology, 16, 1566496.

Gao, S., Chen, W., Cao, S., Sun, B., and Gao, X. (2024). Microalgae as alternatives to fishmeal in aquaculture: current status, existing problems, and possible solutions. Journal of Environmental Science and Pollution Research, 31(11), 16113-16130.

Garlock, T. M., Asche, F., Anderson, J. L. (2024). Environmental, economic, and social sustainability in aquaculture: the aquaculture performance indicators. Nat Commun 15, 5274.

Haji, A., Yousif, A., Mohammed, C., Bashir, T., & Mohammed, I. (2024). In Vivo Study of Physiological, and Histological Effects of Mercury Oxide on Liver and Kidney in Male Wistar Rats. Egyptian Journal of Veterinary Sciences, 1-10.

Hoseini, S. M., Al Sulivany, B. S. A., Afzali-Kordmahalleh, A., Abdollahpour, H., Rajabieste rabadi, H., & Yousefi, M. (2025). Effects of dietary citric acid, lactic acid, and potassium sorbate mixture on growth performance and intestinal immunological parameters in common carp (*Cyprinus carpio*) juveniles. Journal of the World Aquaculture Society, 56(1), e70004.

Karnai, L., & Szűcs, I. (2018). Outlooks and perspectives of the common carp production. Roczniki (Annals), 2018(1).

Mahmud, M. N., & Haque, M. M. (2025). Reassessing the Role of Nanoparticles in Core Fields of Aquaculture: A Comprehensive Review of

pISSN 2550-0910; eISSN 2579-4817
Available online at https://e-journal.unair.ac.id/JoAS

Applications and Challenges. Aquaculture Research, 2025(1), 6897333.

Mendes, A. R., Spínola, M. P., Lordelo, M., & Prates, J. A. (2024). Chemical Compounds, Bioactivities, and Applications of *Chlorella vulgaris* in Food, Feed and Medicine. Applied Sciences, 14(23), 10810.

Mustafa, A. F. E. Z., & Al Sulivany, B. S. A. (2025). Histological, Histochemical, And Immunohistochemical Characterization of the Efferent Ductules Of The Dove. Science Journal of University of Zakho, 13(1), 45–51.

Omar, I., & Al Sulivany, B. (2025). Sustainable Aquaculture Nutrition: Impact of *Chlorella vulgaris* and Iron Nanoparticles and their Combination on Growth, Hematology, and Immune Stability in the Common Carp (*Cyprinus carpio*). Egyptian Journal of Aquatic Biology and Fisheries, 29(2), 916-938.

Owais, M., Al Sulivany, B. S. A., Mohammed, P. & Fazal, R. M. (2024). The Pangas Catfish Pangasius pangasius; Growth Efficiency and Nutritional Composition Under Variety of Saltwater Challenges. Egyptian Journal of Aquatic Biology and Fisheries, 28(6), 1-13.

Panahi, Y., Darvishi, B., Jowzi, N., Beiraghdar, F., & Sahebkar, A. (2016). *Chlorella vulgaris*: A Multifunctional Dietary Supplement with Diverse Medicinal Properties. Curr Pharm Des, 22(2), 164-73.

Panda, C., Komarnytsky, S., Fleming, M. N., Marsh, C., Barron, K., Le Brun-Blashka, S., & Metzger, B. (2023). Guided Metabolic Detoxification Program Supports Phase II Detoxification Enzymes and Antioxidant Balance in Healthy Participants. Nutrients, 15*(*9), 2209.

Selamoglu, Z., & Naim, M. Y. Fish as an important source of nutrients. Journal of Public Health Nutrition, 2023(4), 1-14.

Yarmohammadi, S., Hosseini-Ghatar, R., Foshati, S., Moradi, M., Hemati, N., Moradi, S., Kermani, M. A. H., Farzaei, M. H., & Khan, H. (2021). Effect of *Chlorella vulgaris* on Liver Function Biomarkers: a Systematic Review and Meta-Analysis. Clinical nutrition research, 10(1), 83–94.

Yousefi, M., Adineh, H., Al Sulivany, B. S. A., Gholamalipour Alamdari, E., Yilmaz, S., Mahboub, H. H., & Hoseini, S. M. (2025). The Potential of the Inclusion of *Prosopis farcta* Extract in the Diet on the Growth Performance, Immunity, Digestive Enzyme Activity, and Oxidative Status of the Common Carp, *Cyprinus carpio*, in Response to Ammonia Stress. Animals, 15(6), 895.

Zainul Azlan, N., Mohd Yusof, Y. A., & Makpol, S. (2020). *Chlorella vulgaris* Ameliorates Oxidative Stress and Improves the Muscle Regenerative Capacity of Young and Old Sprague-Dawley Rats. Nutrients, 12(12), 3752.