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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*)

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ABSTRACT

This study evaluated the effects of dietary *Chlorella vulgaris* (*C. vulgaris*) and iron nanoparticles (Fe-NPs) on growth, blood parameters, and physiological status in common carp (*Cyprinus carpio*). The study was done at the Fish Lab, Biology Department, University of Zakho. The trial was conducted on 80 juvenile common carp divided into four groups: T0 (control, basal diet), T1 (10% *C. vulgaris*), T2 (85mg/ kg Fe-NPs), and T3 (both *C. vulgaris* and Fe-NPs) during 60 days of experiment. The results indicated an enhancement in growth performance, as the T3 group recorded the highest final total length (24.29±0.35cm, $P<0.05$), final body weight (167.7 ± 5.67g, $P<0.05$), and relative growth rate (RGR_{length}: 22.35±6.1, $P<0.05$). The hematological parameters demonstrated a remarkable improvement, with T3 recording the highest red blood cell (RBC) count (15.4±0.25 $\times 10^6 mm^{-3}$) and preserving hemoglobin (Hb) levels (10.97±0.37g/ 100ml). The white blood cell (WBC) counts, on the other hand, were preserved equally in all of the experimental groups. PCV was significantly decreased in T1 ($P<0.05$), but it remained stable in T2 and T3. Condition factors, such as Fulton's condition factor (F. *K*), were at the highest in T3 (1.242 ± 0.05). Furthermore, the modified condition factor (F. *Kb*) and relative condition factor (F. *Kn*) were also significantly improved in T1 and T3 ($P<0.05$). The hepatosomatic index (HSI: 2.58 ± 0.54%, $P<0.05$) and gill somatic index (GSI: 2.63 ± 0.33%, $P < 0.05$) exhibited their highest values in T3. It can be concluded that *C. vulgaris* and Fe-NPs profoundly improved the growth, hematological parameters, and some physiological parameters of common carp, indicating their potential as green dietary supplements in aquaculture.

INTRODUCTION

Aquaculture is the fastest-growing global food-producing sector, expanding and intensifying across nearly all regions **(Ceccotti *et al.*, 2019)**. With the global population rising, the demand for aquatic food products continues to increase **(Subasinghe *et al.*, 2009)**. Aquaculture is farming aquatic organisms such as fish, crustaceans, mollusks, and aquatic plants **(Aly *et al.*, 2024)**. Aquaculture production growth in recent decades has been remarkable, with global production increasing from 2.6 million metric tons in 1970 to 87.5 million in 2020, with total global aquaculture production exceeding global capture

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fisheries by more than 18.32 million tonnes **(Afewerki *et al*., 2023)**. Fish is especially beneficial for a healthy diet due to its low carbohydrate content and high levels of unsaturated fats, including omega-3 fatty acids **(Calder, 2010)**. The common carp (*Cyprinus carpio*) is one of the most extensively farmed aquatic fish species worldwide, including regions like the Middle East and Iraq. *C. carpio* meat is highly valued since it has unique taste and it is easy to be digested. This fish is omnivorous, highly adaptable, and can thrive in diverse environmental conditions **(Rahman *et al*., 2015; Ahmed, 2023)**. Fish meal is a high-quality protein source in aquafeeds due to its well-balanced nutrient profile, minimal antinutritional components, and high palatability **(Zhou *et al*., 2005)**. Nonetheless, its application in aquafeeds is significantly constrained by its elevated cost and limited availability **(Li *et al*., 2021)**. Therefore, exploiting new alternative protein sources for fish meal is pressing. Numerous protein sources, including plant protein sources, have been studied to replace fish meal **(Xi *et al*., 2022)**.

In recent years, microalgae have been recognized as a single-cell protein source in aquafeeds, which contain balanced amino acid composition, high polyunsaturated fatty acid, and good palatability. *Chlorella vulgaris* (*C. vulagris*) is the most cultivated eukaryotic green microalga since it is widely used as a health food and feed supplement in the pharmaceutical and cosmetics industries **(Long *et al*., 2024)**. It contains proteins, carotenoids, lipids, immunostimulator compounds, polysaccharides, vitamins, antioxidants, and minerals **(Sharma *et al*., 2012)**. *C. vulgaris* is one of the promising alternative energy sources due to its high oil yield per land area **(Chisti, 2007)**, while carbon dioxide (CO_2) is reduced for its cultivation **(Glaser, 2009)**. *C. vulagris* are commonly used in regulatory tests and are a key constituent in aquatic systems (**Romero *et al*., 2020**). Microalgae biomass is used in aquaculture as feed, growth enhancers, and immunostimulants. *C. vulgaris* is an important species with a good biomolecular composition. Commercially, it is one of the most commonly used microalgae in aquaculture **(Ahmed *et al*., 2020)**.

In aquaculture farming, iron deficiency in fish causes anemia and improper growth. Biologically synthesized nanoparticles are widely used in aquaculture for micro-nutrient delivery as they are eco-friendly and non-toxic to the environment. Fe-NPs can be an alternative supplement to overcome iron deficiency **(Thangapandiyan *et al*., 2020)**. Due to their enormous area-to-volume ratio, these metallic nanoparticles have been shown to have exceptional antibacterial properties by increasing the formation of reactive oxygen (ROS) species, such as hydrogen peroxide (H_2O_2) **(Rakhi *et al*., 2022)**. Iron (Fe) is one of fish's most important trace elements, found in all tissues and organs; it plays a crucial role in the cellular components, cellular respiration, lipid peroxidation, immune system modulation, and body defense against infections **(Akbary & Jahanbakhshi, 2019)**. Fe-NPs are essential for body functions such as oxygen transport and fat oxidation, and their presence forms a vital line of defense to protect fish from infectious diseases **(El-Shenawy *et al*., 2019)**. Iron deficiency causes microcytic anemia in rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), and *C. carpio*. In addition, excess iron levels can also be toxic and include reduced growth, increased mortality, diarrhea, and histological damage to liver cells. Therefore, determining the optimal concentration of Fe-NPs in feed for growth and organ function in fish is essential **(Akter *et al.*, 2018)**. This study aimed to evaluate the effects of dietary *C. vulgaris* and iron nanoparticles on growth performance, and hematological parameters in common carp. By examining these factors, the research aimed to provide a comprehensive understanding of how these additives influence fish health and development, potentially leading to more sustainable and effective aquaculture practices.

MATERIALS AND METHODS

Eighty juvenile common carp of both sexes, weighing 156.8 ± 2.14g, with a total length (TL) of 19.75 ± 1.83cm, fork length (FL) of 16.5 ± 0.62cm, and standard length (SL) of 15.7 ± 0.4cm, were obtained from a local farm and acclimatized to the laboratory conditions (Fish lab of Biology Department, College of Science, University of Zakho) for two weeks to adapt to healthy laboratory settings. The fish were kept in a 1.2m^3 tank and fed the control diet over 24 hours acquired from Amedi Animal Feed Company. To eliminate pathogens, *C. carpio* was immersed in a saline solution (sodium chloride; NaCl) obtained from Sigma-Aldrich for 1-2 minutes **(Das *et al*., 2025; Hassan *et al*., 2025)**.

After acclimation, fish with no external abnormalities were randomly distributed into eight polyethylene circular tanks (100×71.4cm) with a capacity of about 400 liters, with 10 fish per tank. All the tanks were equipped with an air stone for aeration, and 30% of the water was replaced daily with clean water. Continuous aeration was provided to each tank using small aquarium air pumps (Luckiness 828, power: 5 W, airflow: 3.5 L/min) and Chinese air compressors (Hailea ACO-318, power: 45W, airflow: 70L/ min; Hailea ACO-328, power: 55W, airflow: 82L/ min; Resun ACO-010, power: 200W, airflow: 0.135m^3/ min). Physicochemical parameters were monitored daily with portable multi-parameter HANNA HI 9828 pH, dissolved oxygen, temperature, and salinity were measured as 8.28±0.23, 7.4 ± 0.4mg/ L, 16±0.3°C, and 0.06g/ L **(Owais *et al*., 2024a)**.

Diet collection and experimental design

C. vulgaris powder of the highest quality was obtained from Natura Vitalis, Netherlands. The nutritional composition of *C. vulgaris* per 100g includes an energy value of 1450kJ (343 kcal), 2.3g of fat (of which 6g are saturated fat), 14g of carbohydrates (with less than 0.1g of sugar), 12g of fiber, and 61g of protein. It also contains 0.15g of salt. Additionally, it provides 59mg of Vitamin B3, 50µg of vitamin B12, and 1000µg of iodine. Fe-NPs with code 746835 were purchased from Sigma-Aldrich, China. The size of Fe-NPs is 25nm, composed of 99.5% trace material basis.

During this experiment, a control diet and three other different diets were prepared. All the ingredients were grounded and thoroughly mixed in a blender to prepare the diets with *C. vulgaris* and Fe-NPs. Then, small quality water was incorporated to make a smooth dough, which was extruded using an electric-extruder homemade pasta machine with a mesh plate of 2mm in size. Extruded pellets were dried overnight at 50°C and stored at −18°C until further utilization **(Hoseini *et al.*, 2025)**. The diet composition of each diet is shown in Table (1).

The experiment commenced on October 15, 2024, with fish allocated into four treatment groups, each having duplicate replicates. These groups were designated as T0, T1, T2, and T3. T0 served as the control group, receiving only the basal diet pellets (BDP). T1 was fed the BDP enriched with 10% *C. vulgaris*. T2 received the BDP supplemented with 85mg/ kg of Fe-NPs, while T3 was provided with the BDP containing both *C. vulgaris* and Fe-NPs. The fish were fed daily at 10:00 AM for 60 days, with a feeding rate of 3% of body weight **(Al Sulivany *et al.*, 2024a)**. Feces and uneaten food were removed using a vacuum system. No mortalities were observed throughout the study period.

Table 1. Feed ingredient and proximate composition analysis of fish-fed basal pellets with *C. vulgaris* and Fe-NPs and a combination of both during 60-day intervals

Ingredient	**T0**	**T1**	**T2**	**T3**
Fish Meal	16	16	16	16
Corn	14	14	14	14
Soybean Meal	28	28	28	28
Barley	17	17	17	17
Wheat	22	22	22	22
Premix	2	2	2	2
Ascorbic acid	1	1	1	1
Proximate composition of fish 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
Ether Extract (%)	4.80	4.80	4.80	4.80
Ash (%)	7.22	7.22	7.22	7.22
Moisture (%)	7.10	7.10	7.10	7.10
Organic Matter (%)	75.68	75.68	75.68	75.68

Length parameter and relative growth rate of length (RGR_{Length})

At the end of the experiment, the fish were placed on a ruler with their snout aligned at the zero cm mark to determine its various length measurements. These included TL, measured from the tip of the snout to the end of the caudal fin; FL, measured from the midpoint of the tail's concave edge to the snout; and SL, measured from the snout to the base of the tail **(Asad *et al.*, 2025)**. The relative growth rate of the length (RGR_{length}) in fish was calculated as the percentage increase in this parameter over a specific period relative to their initial length **(Myszkowski, 1997)**.

$$RGR_{Length} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}} \times 100$$

Growth morphometry and survival rate

The fish's final body weight (FBW) was measured, and the daily weight gain (DWG) was computed to assess weight increment over 24 hours **(Al Sulivany *et al*., 2024b)**. Total weight gain (TWG) was calculated to evaluate the cumulative increase in body mass over the experimental duration. The relative growth rate of the weight (RGR_{weight}) was also calculated to measure proportional growth over time **(Lieke *et al*., 2021)**. Moreover, the metabolic growth rate (MGR) was employed to analyze the relationship between growth and metabolic processes **(White *et al*., 2022)**. The specific growth rate (SGR) was computed to express the percentage increase in body weight over a specified time interval **(Ahmed, 2023; Owais *et al*., 2024b; Abdulrahman & Al Sulivany, 2025)**. The fish's survival value was measured at the end of the experimental rearing days by counting the number of dead and surviving fish within the group's treatment **(Aminikhoei *et al*., 2015)**.

$$\text{DWG (gr/day)} = \frac{FW - IW}{t}$$

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

$$\text{RGRweight} = \frac{\text{FW} - \text{IW}}{\text{IW}} \times 100$$

$$\text{MGR (gkg}^{0.8}\text{ day}^{-1}) = \text{(TWG)} / [\{(\text{IW}/1000)^{0.8} + (\text{IW}/1000)^{0.8}\}]/2$$

$$SGR = \frac{\ln_{(FW)} - \ln_{(IW)}}{t} \times 100$$

$$\text{Survival rate (\%)} = \frac{\textit{Fish}\text{ numbers at the end of the trails}}{\textit{Fish}\text{ number were stocked at the beginig of the trails}} \times 100$$

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

Measurements of condition factors

Fulton's condition factor (*K*) is a morphometric indicator to evaluate fish's general health and physiological state by analyzing their weight and length **(Hvas *et al*., 2022)**. An adjusted version of this factor, the modified condition factor (*Kb*), enhances sensitivity by integrating the cube root of the fish's weight **(Mrdak *et al*., 2023)**. Additionally, the relative condition factor (*Kn*) further refined Fulton's condition factor, incorporating the anticipated weight-length correlation observed in a healthy fish population **(Dietz *et al.*, 2019)**.

$$K = 100 \times \frac{W}{L^3} \qquad Kb = 100 \times \frac{W}{L^b} \qquad Kn = \frac{W}{^\wedge W}$$

Where, W is the weight of fish in g; L is the length of fish in cm; a is the rate of change in weight with length (intercept); b is the weight at unit length (slope), and ^W denotes the anticipated weight

Hematological parameters

The hematological parameters were done after the experimental trials. Five were anaesthetized using an MS-222 (tricaine methane sulfonate, 0.1 g. L^{-1}, Sigma-Aldrich, USA) to reduce stress and avoid harm. 3 mL syringes were used to draw blood samples from the caudal vein. The blood samples were moved to an Ethylenediaminetetraacetic acid (EDTA) tube to prevent coagulation. The routine hematologic parameters of fish include the determination of total RBCs, total WBCs, PCV, and Hb.

The methodology measured the RBCs and WBCs using a hemocytometer and the Neubauer Counting Chamber method **(Blaxhall & Daisley, 1973)**. The concentration of Hb was determined using **Wedemeyer's (1977)** methodology. The following formulas were used to determine blood indices such as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) **(Campbell, 2004)**.

Biological somatic indices

After collecting the blood, the fish was killed with a sharp blow to the head. Individual fish were carefully dissected. The weight of select organs, including the liver, spleen, gills, and heart, was meticulously measured and recorded according to the protocol outlined by **Lagler (1956).** This protocol provided a standardized approach to data collection and enabled comparisons with other studies.

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

Statistical analysis

The data obtained from the experiment were statistically analyzed by GraphPad Prism 9, Analysis of Variance (ANOVA). Duncan's Multiple Range Test mean comparisons were used to make group differences. A $P < 0.05$ was used as the indicator of statistical significance. Means and standard errors were presented in each result.

RESULTS

The results of the 60-day feeding trial demonstrated significant variations in the growth parameters of *C. carpio* across different dietary treatments (Table 2 & Fig. 1A, B, C, and D). Fish in the T0 group fed basal diet pellets (BDP) exhibited an ITL of 19.57 ± 0.36cm and an FTL of 22.79 ± 0.3cm. In contrast, the group supplemented with 10% *C.*

vulgaris (T1) showed a slight increase in ITL (19.71 ± 0.54cm) and a significant improvement in FTL (23.57 ± 0.2cm, $P< 0.05$). The group receiving 85mg/ kg Fe-NPs (T2) displayed an ITL of 20 ± 0.54cm and an FTL of 22.79 ± 0.4cm. At the same time, the combination of *C. vulgaris* and Fe-NPs (T3) resulted in the highest FTL of 24.29 ± 0.35cm ($P< 0.05$), indicating a synergistic effect of the combined supplementation. Similarly, the FFL and FSL were significantly higher in T3 (21.86 ± 0.23cm and 18.43 ± 0.27cm, respectively) compared to T0 (19.5 ± 0.21cm and 17.14 ± 0.2cm, respectively, $P < 0.05$). The RGR_{length} was also significantly enhanced in T1 (19.62 ± 1.4) and T3 (22.35 ± 6.1) compared to T0 (14.31 ± 0.8, $P < 0.05$), suggesting that both *C. vulgaris* and Fe-NPs, either alone or in combination, positively influenced the growth performance of *C. carpio*.

Table 2. The length parameters and metabolic growth weight of the length in fish fed basal diet pellets supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials

	T0 Control	**T1 10% *C. vulgaris***	**T2 85 mg/kg Fe-NPs**	**T3 *C. vulgaris* + Fe-NPs**
ITL (cm)	19.57±0.36	19.71±0.54	20±0.54	20.07±0.76
FTL (cm)	22.79a±0.3	23.57ab±0.2	22.79a±0.4	24.29b±0.35
IFL (cm)	17.43±0.2	17.14±0.26	17.71±0.28	16.71±0.8
FFL (cm)	19.5a±0.21	20.43ab±0.29	19.64a±0.23	21.86b±0.23
ISL (cm)	15.43±0.23	15.29±0.28	15.64±0.17	15.43±0.2
FSL (cm)	17.14a±0.2	17.86ab±0.26	17.36a±0.23	18.43b±0.27
RGR_{Length}	14.31a±0.8	19.62b±1.4	14.05a±2.5	22.35b±6.1

ITL: initial Total length. FTL: Final total length. IFL: Initial fork length. FFL: Final fork length. ISL: Initial standard length. FSL: Final standard length. RGR_{Length}: Relative growth rate of the length.

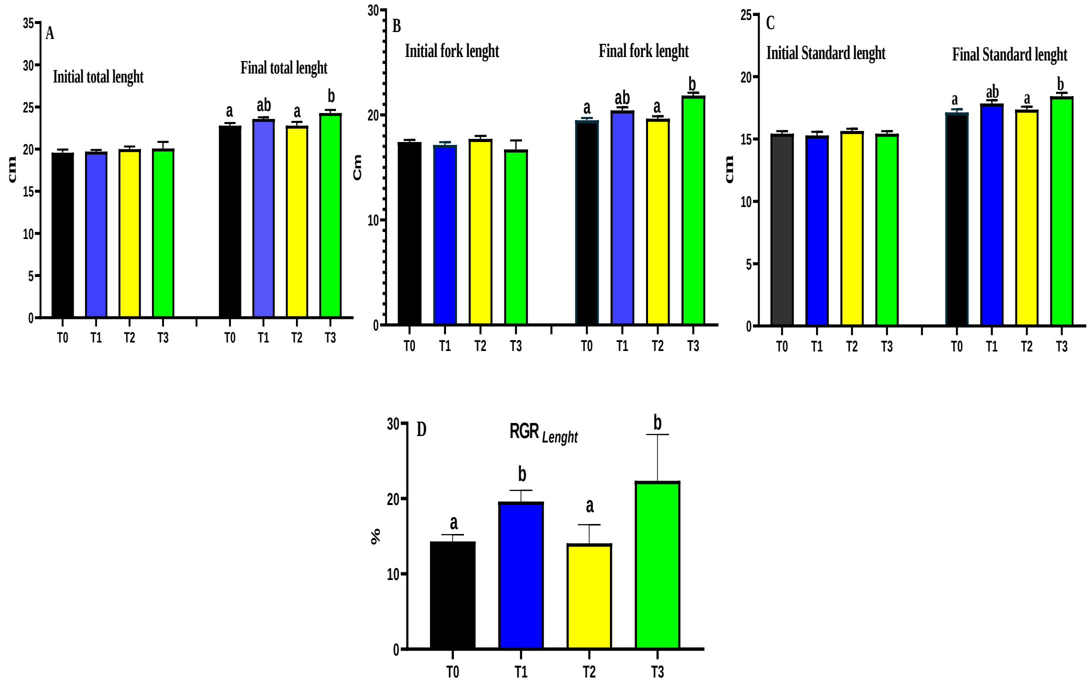

Fig. 1. The length and relative growth rate of the length parameters in fish feds BDP supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials. Significant differences ($P < 0.05$) are indicated by distinct superscripts (a, b, c, and d).

The growth morphometry of *C. carpio* across the dietary treatments is shown in Table (3) and Fig. (2). Fish in the T0 group fed BDP had an IBW of 107.2 ± 8.056g (Fig. 2A) and an FBW of 125.8 ± 7.45g (Fig. 2B), with a DWG of 0.31 ± 0.012g/ day (Fig. 2C) and a TWG of 18.67 ± 3.5g (Fig. 2D). In contrast, the group supplemented with 10% *C. vulgaris* (T1) showed a higher FBW of 156.8 ± 9.43g ($P < 0.05$), a DWG of 0.9 ± 0.04g/ day ($P < 0.05$), and a TWG of 53.3 ± 5.6g ($P < 0.05$), indicating a significant enhancement in growth compared to the control. The group receiving 85mg/ kg Fe-NPs (T2) exhibited an FBW of 141.3 ± 4.35g, a DWG of 0.6 ± 0.06g/day, and a TWG of 36.7 ± 4.6g. Although the results in T2 are lower than T1, they still represented an improvement over T0. The combination of *C. vulgaris* and Fe-NPs (T3) resulted in the highest growth performance, with an FBW of 167.7 ± 5.67g ($P < 0.05$), a DWG of 0.98 ± 0.04g/day ($P < 0.05$), and a TWG of 59.17 ± 4.7g ($P < 0.05$). Additionally, the WGR and MGR were significantly higher in T1 (64.76 ± 12.43% and 285.8 ± 82.3gkg$^{0.8}$ day^{-1}, respectively) and T3 (56.69 ± 6.3% and 294 ± 23.39 gkg$^{0.8}$ day^{-1}, respectively) compared to T0 (18.38 ± 2.63% and 107.6 ± 12.3 gkg$^{0.8}$ day^{-1}, respectively, $P < 0.05$) (Fig. 2E, F). The RGR$_{weight}$ varied significantly across treatments. The T0 had an RGR of 2.86 ± 0.13%, while the *C. vulgaris* T1 and Fe-NPs T2 groups showed higher RGR$_{weight}$ of 3.76

± 0.43% and 3.52 ± 1.9%, respectively. The combination of *C. vulgaris* and Fe-NPs (T3) achieved the highest RGR_{weight} of 4.01 ± 0.11% ($P < 0.05$) (Fig. 2G).

Table 3. The growth parameters and metabolic growth weight in fish fed basal diet pellets supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials

Parameter	T0 Control	T1 10% *C. vulgaris*	T2 85 mg/kg Fe-NPs	T3 *C.vulgaris*+Fe-NPs
IBW	107.2±8.056	103.5±13.8	104.7±6.1	108.5±7.94
FBW	125.8c±7.45	156.8ab±9.43	141.3cb±4.35	167.7a±5.67
DWG	0.31c±0.012	0.9ab±0.04	0.6ac±0.06	0.98b±0.04
TWG	18.67a±3.5	53.3b±5.6	36.7a±4.6	59.17b±4.7
WGR	18.38c±2.63	64.76ab±12.43	36.76cb±5.7	56.69a±6.3
MGR	107.6a±12.3	285.8b±82.3	200a±28.22	294b±23.39
RGR*weight*	2.86a±0.13	3.76cb±0.43	3.52ab±1.9	4.01c±0.11
SR (%)	100	100	100	100

IW (g): Initial body weight. FBW (gr): Final body weight. DWG (g/day): Daily weight gain. TWG (g): Total weight gain. WGR (%): Weight growth rate. MGR ($gkg^{0.8}$ day^{-1}): Metabolic growth rate. RGR_{weight} (%): Relative growth rate of weight. SR (%): Survival rate.

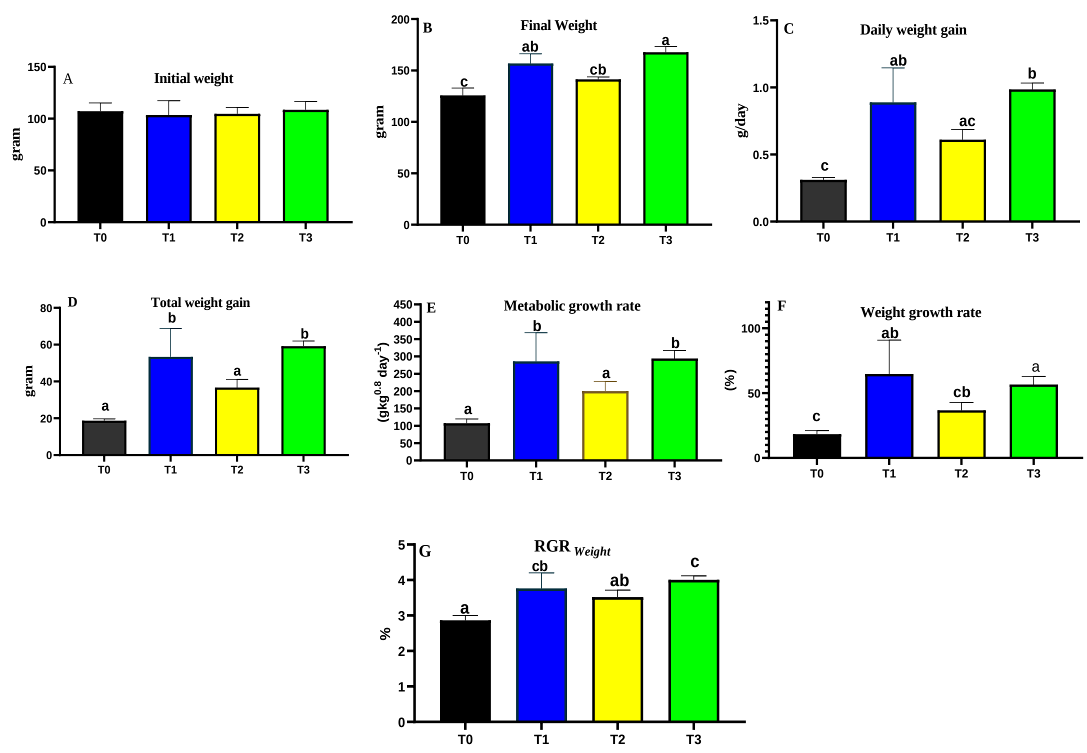

Fig. 2. The growth morphometry and metabolic rates parameters in fish fed BDP supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials. Significant differences ($P< 0.05$) are indicated by distinct superscripts (a, b, c, and d)

The effects of dietary supplements on the final condition factors of *C. carpio* over a 60-day feeding trial

The T0 group, BDP, had a final Fulton condition factor (F. *K*) of 1.052 ± 0.04. In contrast, the group supplemented with 10% *C. vulgaris* (T1) showed a significantly higher F. *K* of 1.19 ± 0.09 ($P< 0.05$). The group receiving 85mg/ kg Fe-NPs (T2) exhibited an F. *K* of 1.1 ± 0.2, while the combination of *C. vulgaris* and Fe-NPs (T3) achieved the highest F. *K* of 1.242 ± 0.05 ($P< 0.05$). Similarly, the final modified condition factor (F. *Kb)* was significantly improved in T1 (0.76 ± 0.04) and T3 (0.75 ± 0.06) compared to T0 (0.68 ± 0.02, $P < 0.05$). The final relative condition factor (F. *Kn*) also showed significant enhancements in T1 (1.265 ± 0.09) and T3 (1.266 ± 0.07) compared to T0 (1.151 ± 0.04, $P < 0.05$) (Table 4 & Fig. 3A, B, C, D, E, and F).

Table 4. The condition factor in fish fed basal diet pellets supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials

Parameter	T0 Control	T1 10% *C. vulgaris*	T2 85 mg/kg Fe-NPs	T3 *C. vulgaris* + Fe-NPs
I. *K*	1.315±0.07	1.335±0.1	1.27±0.09	1.289±0.12
F. *K*	1.052a±0.04	1.19b±0.09	1.1a±0.2	1.242b±0.05
I. *Kb*	0.879±0.05	0.8591±0,09	0.8737±0.07	0.8471±0,07
F. *Kb*	0.68a±0.02	0.76b±0.04	0.7a±0.04	0.75a±0.06
I. *Kn*	1.49±0.08	1.456±0.08	1.481±1.02	1.436±0.21
F. *Kn*	1.151a±0.04	1.265b±0.09	1.171a±0.17	1.266a±0.07

I. *K*: Initial Fulton condition factor. F. *K*: Final Fulton condition factor. I. *Kb*: Initial modified condition factor. F. *Kb*: Final modified condition factor. I. *Kn*: Initial relative condition factor. F. *Kn*: final relative condition factor.

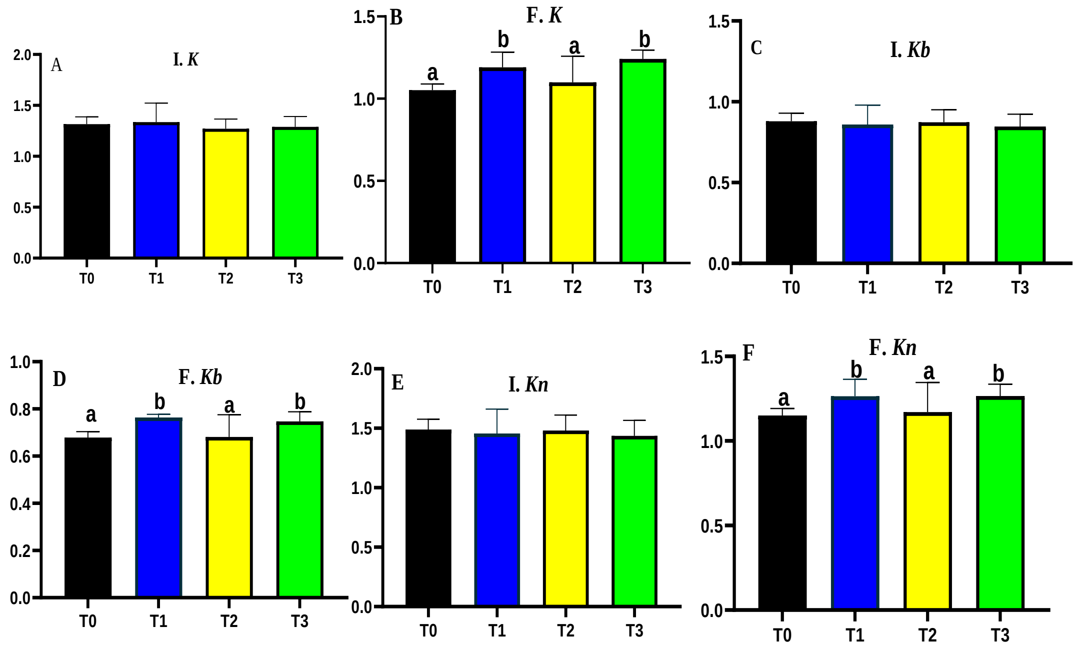

Fig. 3. The condition factor (Fulton, modified, ad relative) parameters in fish fed BDP supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials. Significant differences (P< 0.05) are indicated by distinct superscripts (a, b, c, and d).

The hematological parameters of fish were significantly also influenced. The carp in the T0 group fed normal pellets, had an RBC count of 12.17±0.4 $\times 10^6 mm^{-3}$, while the group supplemented with 10% *C. vulgaris* (T1) showed an increased RBC count of 13.23±0.2$\times$ $10^6 mm^{-3}$ (P< 0.05). The group receiving 85mg/ kg Fe-NPs (T2) exhibited a further rise in RBC count to 14.2±0.37 $\times 10^6 mm^{-3}$, and the combination of *C. vulgaris* and Fe-NPs (T3) resulted in the highest RBC count of 15.4±0.25$\times$ $10^6 mm^{-3}$ (P<0.05). The WBC count showed minimal variation across treatments, with T0 (7.5±0.34$\times$ $10^3 mm^{-3}$), T1 (7.61±0.41$\times$ $10^3 mm^{-3}$), T2 (7.52±0.3$\times$ $10^3 mm^{-3}$), and T3 (6.86±0.23 $\times$ $10^3 mm^{-3}$). Dietary supplementation with *C. vulgaris* and Fe-NPs did not significantly affect WBC counts in fish. The Hb levels were significantly lower in T1 (9.2±0.53 g/100ml) compared to T0 (11.6±0.17g/ 100ml, P < 0.05), but T2 (11.73±0.91g/ 100ml) and T3 (10.97±0.37g/ 100ml) maintained levels closer to the control. On the other hand, the percentage of PCV was significantly reduced in T1 (29.44±1.87%) compared to T0 (37.12±0.5%, P<0.05), while T2 (37.52±2.94%) and T3 (35.09±1.2%) showed values similar to the control. However, the MCV was highest in T0 (305.9±13.0 fL) and significantly lower in T1 (222.8±16.6 fL) and T3 (228.2±8.35 fL, P < 0.05). The MCH and MCHC did not show significant differences across groups (Table 5 & Fig. 4A, B, C, D, E, F, and G).

Table 5. The haematological parameters in fish feds basal diet pellets supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials

Parameter	T0 Control	T1 10% *C. vulgaris*	T2 85 mg/kg Fe-NPs	T3 *C. vulgaris* + Fe-NPs
RBCs	12.17a±0.4	13.23ab±0.2	14.2bc±0.37	15.4c±0.25
WBCs	7.5±0.34	7.61±0.41	7.52±03	6.86±0.23
Hb	11.6a±0.17	9.2b±0.53	11.73a±0.91	10.97ac±0.37
PCV	37.12b±0.5	29.44a±1.87	37.52b±2.94	35.09bc±1.2
MCV	305.9a±13.0	222.8bc±`16.6	245.2abc±16.8	228.2b±8.35
MCH	95.6a±5.1	69.63bc±5.3	76.62abc±6,1	71.25b±4.5
MCHC	31.25±1.2	32.25±2.2	32.92±1.9	31.92±1.7

RBCs ($10^6 mm^{-3}$): Red blood cells. WBCs ($10^3 mm^{-3}$): White blood cells. Hb (g/100ml): Haemoglobin. PCV (%): Packed cell volume. MCV (fL): Mean cell volume. MCH (pg): Mean cell Haemoglobin. MCHC (%): Mean cell hemoglobin concentration.

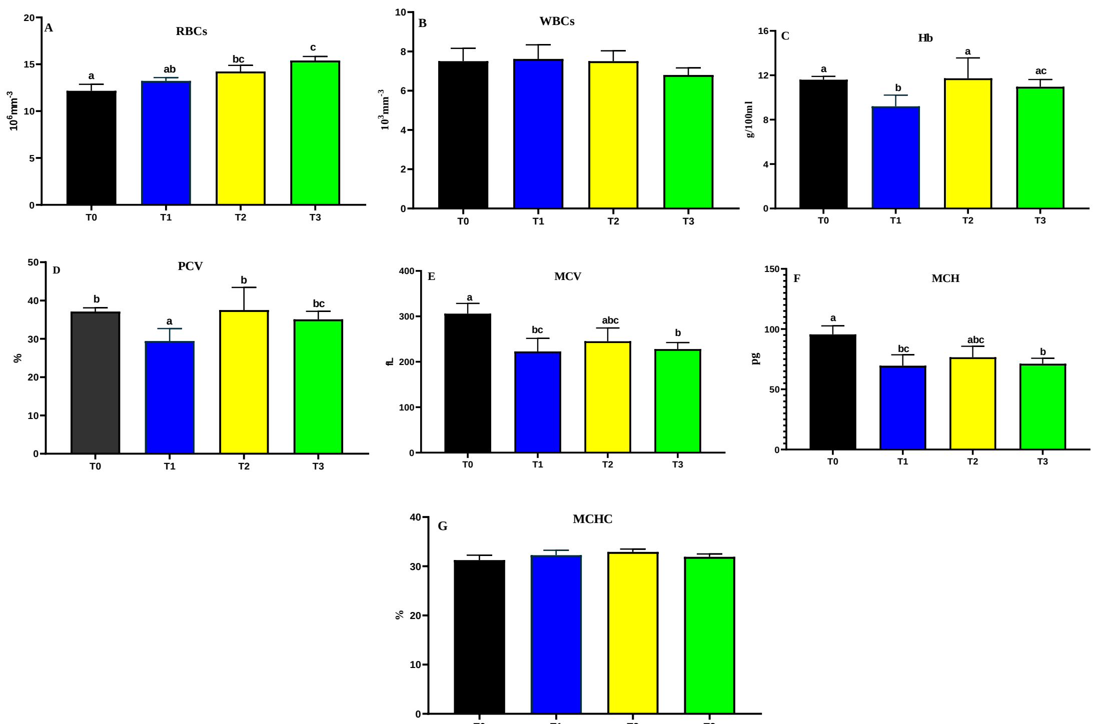

Fig. 4. The hematological parameters and RBCs indices in fish fed BDP were supplemented with *C. vulgaris* and Fe-NPs, and a combination of both diets was used in *C. carpio* during 60-day experimental trials. Significant differences ($P< 0.05$) are indicated by distinct superscripts (a, b, c, and d).

The biological somatic indices of the carp were significantly influenced by dietary supplementation over the experimental period (Table 6 & Fig. 5A, B, C, and D). The HSI in the T0 was 2.013±0.3%, while with 10% *C. vulgaris* T1 showed a significantly higher HSI of 2.854±0.61% ($P< 0.05$). The Fe-NPs group T2 and the combination of *C.*

vulgaris and Fe-NPs T3 exhibited intermediate HSI values of 2.5±0.44% and 2.58±0.54%, respectively. The SSI was significantly higher in T1 (0.36±0.082%) compared to T0 (0.36±0.05%, *P*< 0.05), while T2 (0.25±0.06%) showed a reduction. The CSI did not vary significantly across groups, with values of 0.3±0.041% in T0, 0.33±0.05% in T1, 0.31±0.019% in T2, and 0.36±0.018% in T3. The GSI was significantly higher in T2 (2.66±0.26%) and T3 (2.63±0.33%) compared to T0 (2.4±0.01%, *P*< 0.05), while T1 (2.18±0.2%) showed a slight decrease.

Table 6. The biological somatic index parameters in fish fed basal diet pellets supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials

Parameter	T0 Control	T1 10% *C. vulgaris*	T2 85 mg/kg Fe-NPs	T3 *C.vulgaris*+Fe-NPs
HIS	2.013a±0.3	2.854b±0.61	2.5ab±0.44	2.58ab±0.54
SSI	0.36a±0.05	0.36a±0.082	0.25b±0.06	0.32a±0.11
CSI	0.3±0.041	0.33±0.05	0.31±0.019	0.36±0.018
GSI	2.4ab±0.01	2.18a±0.2	2.66b±0.26	2.63b±0.33

HIS (%): Hepatosomatic index. SSI (%): splenosomatic index. CSI (%): Cardiosomatic index. GSI (%): Gill somatic index.

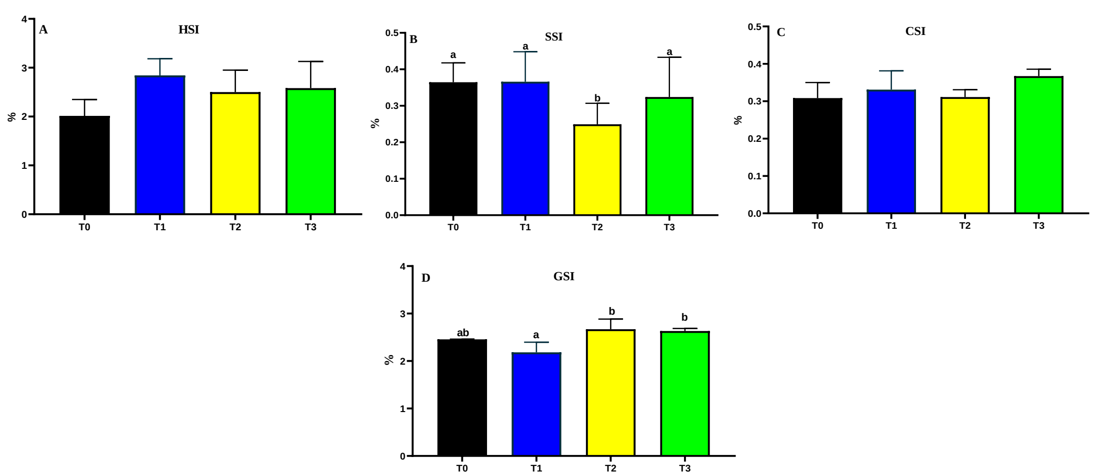

Fig. 5. The biological somatic index parameters in fish fed BDP supplemented with *C. vulgaris* and Fe-NPs and a combination of both diets in *C. carpio* during 60-day experimental trials. Significant differences (*P*< 0.05) are indicated by distinct superscripts (a, b, c, and d)

DISCUSSION

The significant improvements in the length, growth, condition factors, and somatic indices of *C. carpio* fed with *C. vulgaris*, Fe-NPs, and their combination can be attributed to several interconnected physiological and nutritional mechanisms. *C. vulgaris* is rich in proteins, essential amino acids, and PUFAs, which are critical for promoting fish growth, enhancing feed efficiency, and supporting cellular membrane integrity **(Shah *et al*., 2018; Eissa *et al*., 2024)**. These findings align with the work of **Safari *et al.* (2022)**, who reported that dietary supplementation with *C. vulgaris* significantly improved growth performance, feed utilization, and immune response in the juvenile narrow-clawed crayfish (*Pontastacus leptodactylus*). Similarly, the presence of bioactive compounds like carotenoids and antioxidants in *C. vulgaris* reduces oxidative stress and improves overall health, further contributing to enhanced growth performance and condition factors **(Alfaia *et al*., 2020)**. This is consistent with the findings of **Soliman *et al*. (2023)**, who demonstrated that *C. vulgaris* supplementation improved gut health and nutrient absorption in fish, leading to better growth and physiological conditions.

On the other hand, Fe-NPs enhance nutrient absorption and bioavailability due to their nano-sized structure, allowing for more efficient uptake in the gut compared to conventional iron sources **(El-Shenawy *et al*., 2019)**. This is supported by **Asad *et al*. (2025)**, who observed that Fe-NPs stimulated the activity of digestive enzymes such as proteases, lipases, and amylases, leading to better nutrient digestion and utilization. Furthermore, Fe-NPs upregulate the expression of growth-related genes, such as growth hormone (GH) and insulin-like growth factor (IGF), which are critical for growth and development, and improve the feed conversion ratio (FCR) by enabling fish to convert feed into body mass more efficiently **(Kumar *et al*., 2024; Nwanna & Ikusean, 2024)**. These findings are consistent with the research of **He *et al*. (2023)**, who found that Fe-NP supplementation in largemouth bass (*Micropterus salmoides*) diets improved growth rates and reduced oxidative stress.

The combination of *C. vulgaris* and Fe-NPs results in a synergistic effect, where *C. vulgaris* provides essential nutrients, and Fe-NPs enhance their bioavailability, leading to the FTL, RGR_{length}, FBW, DWG, and TWG observed in T3 **(Vargas *et al*., 2024)**. This synergistic interaction also improves the F. *K*, F. *Kb*, and F. *Kn*, indicating better overall health and nutritional status in the supplemented groups **(Zahran *et al*., 2024)**. These results agree with the findings of **Vargas *et al*. (2024)**, who demonstrated that the integration of microalgae and nanoparticles in aquaculture diets significantly improves growth performance and feed efficiency.

Additionally, the enhanced oxygen transport and energy metabolism facilitated by Fe-NPs contribute to improved respiratory efficiency, as indicated by the GSI in groups receiving Fe-NPs **(Gupta *et al*., 2016; Afshari *et al*., 2021; Abdel Rahman *et al*., 2023)**. Combining *C. vulgaris* and Fe-NPs resulted in the highest somatic indices, suggesting that integrating microalgae and nanoparticles in aquaculture diets significantly improves fish health, physiological function, and overall performance **(Mohammady *et al*., 2024)**. These findings are consistent with the studies that have demonstrated the beneficial effects of microalgae and nanoparticles on fish growth, health, and condition factors **(He *et al*., 2023; Soliman *et al*., 2023)**. For instance, **Zahran *et al.* (2024)** reported that combining microalgae with mineral nanoparticles in fish diets led to improved growth and feed utilization in the Nile tilapia, further supporting the synergistic effects observed in this study.

The hematological parameters of fish, including RBC, WBC, Hb, PCV, and blood index, are critical indicators of physiological and health status, and dietary interventions, environmental factors, and physiological stress can influence their variations **(Fazio, 2019; Esmaeili, 2021)**. The results indicate that RBC count exhibited a significant increase across the experimental groups, particularly in those supplemented with *C. vulgaris* and iron nanoparticles (Fe-NPs), individually or in combination. This elevation in RBC count can be attributed to the enhanced erythropoiesis stimulated by the nutritional components of *C. vulgaris*, which is rich in essential nutrients such as vitamins, minerals, and antioxidants that promote red blood cell production **(Galal *et al.*, 2018; Raji *et al.*, 2018; Safari *et al.*, 2022)**. Additionally, Fe-NPs are known to improve iron bioavailability, which is crucial for hemoglobin synthesis and erythrocyte maturation, thereby further boosting RBC counts **(Kumar *et al.*, 2024)**. The combination of *C. vulgaris* and Fe-NPs causes elevation in RBC count. Conversely, the WBC count remained relatively stable across all groups, indicating that the dietary supplements did not induce significant immune activation or stress responses that would typically elevate WBC levels. This stability suggests that the supplements were well-tolerated and did not provoke inflammatory or immune challenges in the fish **(Oliva-Teles, 2012)**.

The decline in Hb and PCV in the *C. vulgaris*-only group may be linked to the dilution effect caused by increased plasma volume or the potential interference of *C. vulgaris* with iron absorption or utilization **(Watanabe *et al.*, 1997; Lall & Kaushik, 2021)**. In contrast, the groups receiving Fe-NPs maintained Hb and PCV levels closer to the control, underscoring the role of iron in hemoglobin synthesis and erythrocyte production **(Eram *et al.*, 2022)**. On the other hand, the reduction in MCV suggests the production of smaller erythrocytes, potentially due to the influence of *C. vulgaris* on erythropoiesis or iron metabolism, leading to the formation of microcytic cells **(Jobling, 2012)**. The MCH and MCHC did not show significant variations across groups, indicating that the dietary supplements did not substantially alter the hemoglobin content **(Sherif *et al.*, 2024)**.

CONCLUSION

In conclusion, the supplementation of *C. vulgaris*, Fe-NPs, and their combination in the diet of *C. carpio* substantially improves growth performance, physiological condition, and hematological parameters. The synergistic nutrient profile of *C. vulgaris* and the bioavailability of Fe-NPs enhance feed utilization, nutrient absorption, and erythropoiesis with immune stability. These results indicate the promising value of integrating microalgae and nanoparticles in aquaculture to achieve fish health, growth, and productivity maximization and, hence, a sustainable strategy for advancing aquaculture nutrition.

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