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Original Article

Influence of dietary garlic powder (*Allium sativum*) on growth performance, hemato-biochemical parameters, antioxidant status, and hepatic enzyme activity in *Catla catla* fingerlings

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Abstract
This study evaluated the impact of dietary garlic powder (*Allium sativum*) on growth performance, hematology, lipid profile, hepatic enzyme activity, and antioxidant activity in *Catla catla* fingerlings over a 90-day feeding trial. A total of 120 fish (initial weight: 5.86±0.14 g) were divided into four dietary groups: 0% (Group-1), 2% (Group-2), 4% (Group-3), and 6% (Group-4) garlic powder. Results indicated that final weight (41.17±7.04 g), weight gain (35.5±7.11 g), feed intake (52.2±0.96 g), and specific growth rate (3.59±0.08%) were higher in Group 4 ($p < 0.001$). Feed conversion ratio improved significantly (1.22±0.015; $p < 0.001$). Hematological values, including red blood cell counts (2.63±0.014 ×10^6 mm^{-3}), hemoglobin (13.58 ± 0.106 g 100 mL^{-1}), and packed cell volume (40.9±0.5%) increased significantly ($p < 0.001$) in Group-4. In contrast, white blood cell counts (WBCs) were significantly reduced ($p = 0.008$). Biochemical parameters such as triglycerides (102.3±4.72 mg dL^{-1}), cholesterol (134.2±2.26 mg dL^{-1}), low-density lipoprotein (45.56±2.19 mg dL^{-1}), and very low-density lipoprotein (20.24±0.94 mg dL^{-1}) decreased significantly, while high-density lipoprotein improved. Hepatic enzymes AST (53.8±0.37 U L^{-1}), ALT (18.2±1.14 U L^{-1}), ALP (172.6±0.4 U L^{-1}), and LDH (170±1.04 U L^{-1}) were reduced ($p < 0.001$). Antioxidant enzymes catalase (26.2±0.23 KU L^{-1}), superoxide dismutase (16±0.31 U mL^{-1}), and glutathione peroxidase (65.8±0.58 U L^{-1}) increased ($p < 0.001$), while MDA levels were lowered (2.38±0.058 mmol L^{-1}), indicating reduced oxidative stress. In conclusion, garlic powder supplementation, particularly at 6%, significantly enhanced growth, hematological status, lipid regulation, liver function, and antioxidant defense in *C. catla*, supporting its potential use as a natural feed additive in freshwater aquaculture.

Keywords: dietary supplement; glutathione peroxidase; malondialdehyde; specific growth rate

1 | INTRODUCTION

The rapid expansion of aquaculture has transformed global food systems by providing a reliable source of high-quality protein. Yet, the intensification of aquaculture practices has introduced new challenges, including physiological stress, metabolic disorders, and immunosuppression in cultured fish species (Aich *et al*. 2018; Chary *et al*. 2024). These complications can adversely impact growth, feed efficiency, and overall survival, necessitating the exploration of sustainable nutritional interventions to improve fish health and performance (Henriksson *et al*. 2021; Hossain *et al*. 2024). Among various strategies, the use of functional feed additives, especially phytogenic compounds derived from medicinal plants, has gained increasing attention for their roles in promoting growth, enhancing immunity, and maintaining physiological homeostasis (Ceccotti *et al*. 2019; Adetunji *et al*. 2025).

Garlic (*Allium sativum*), an extensively studied medicinal herb, contains a diverse array of bioactive compounds, including allicin, alliin, and various organosulfur molecules. These components exhibit broad-spectrum biological properties, including antimicrobial, antioxidant, hypolipidemic, and immunomodulatory effects (Sharma *et al*. 2019; El-Saadony *et al*. 2024). In aquaculture, garlic has emerged as a promising natural supplement capable of supporting both growth performance and systemic health across different fish species (Abdel-Latif *et al*. 2024). Its inclusion in fish diets has been linked to enhanced feed utilization, improved antioxidant capacity, and modulation of lipid and hematological profiles (Yu *et al*. 2018; Ahmed 2024; Edrees *et al*. 2025).

Growth performance remains the fundamental metric for assessing aquaculture productivity, directly influencing economic viability (Bostock *et al*. 2016; Garlock *et al*. 2024). Numerous studies have demonstrated that appropriate garlic supplementation can enhance key growth indicators, including weight gain, feed conversion efficiency, and specific growth rate across various commercially important fish species (Lugert *et al*. 2016). These improvements appear to result from garlic's ability to optimize protein metabolism, enhance nutrient utilization efficiency, and promote gastrointestinal health (Suleria *et al*. 2015). Simultaneously, biochemical parameters such as glucose, total cholesterol, triglycerides, and liver enzyme activities provide critical insights into the metabolic and hepatic status of fish. Garlic has demonstrated the potential to stabilize these biomarkers by supporting liver function and modulating glucose and lipid metabolism (Adineh *et al*. 2024).

In addition to metabolic health, the regulation of oxidative stress is essential for maintaining cellular integrity, especially under aquaculture conditions that often expose fish to environmental and nutritional stressors (Li *et al*. 2025). The antioxidant defense system comprising enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) neutralizes reactive oxygen species (ROS) and limits oxidative damage (Emami *et al*. 2016). Studies have indicated that garlic supplementation can significantly enhance these enzymatic activities while reducing malondialdehyde (MDA), a marker of lipid peroxidation (Chen *et al*. 2019).

Moreover, lipid profile parameters, including high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), are vital indicators of cardiovascular and metabolic health (Arsenault *et al*. 2009). Garlic has shown hypolipidemic effects in fish by decreasing serum cholesterol and triglyceride levels and improving the HDL/LDL ratio, thereby contributing to a healthier lipid profile (Guo *et al*. 2025).

Haematological analysis is a critical diagnostic tool in aquaculture, providing insights into the overall physiological condition of fish (Seibel *et al*. 2021; Anwar *et al*. 2025). These parameters reflect essential biological functions, including oxygen transport efficiency, immune competence, and stress adaptation (Koch *et al*. 2017). Among the most significant haematological markers are haemoglobin concentration, haematocrit percentage, and differential leukocyte counts, collectively providing a comprehensive assessment of fish health (Docan *et al*. 2018). The dietary interventions can substantially alter these haematological indices. Specifically, garlic (*Allium sativum*) supplementation has notably affected erythrocyte parameters (Pourmand *et al*. 2025). The significant increases in red blood cell counts and haemoglobin levels following garlic administration were reported in various fish species (Kairalla *et al*. 2022; Kusi *et al*. 2025). These improvements likely enhance oxygen-carrying capacity, potentially leading to better growth performance and stress tolerance in cultured fish (Shen *et al*. 2023). The immunomodulatory properties of garlic also manifest in leukocyte profiles (Antache *et al*. 2025). Several investigations have documented increased lymphocyte proliferation and enhanced phagocytic activity in fish receiving garlic-supplemented diets (Khang *et al*. 2022). These changes suggest improved immune readiness, which may translate to greater disease resistance in aquaculture settings (Khanzadeh *et al*. 2025).

Although garlic has shown promising benefits in various aquaculture species, limited information is available on its dose-dependent effects in *Catla catla*, an economically important freshwater species in South Asia. This study investigates the impact of graded inclusion of garlic powder (0%, 2%, 4%, and 6%) on growth performance, hematological health, lipid profile, antioxidant enzyme activity, and serum biochemistry in *C. catla* fingerlings. The outcomes aim to advance functional feed strategies

for enhancing fish health and aquaculture productivity.

2 | METHODOLOGY

2.1 Experimental feeds

Fresh garlic bulbs (*Allium sativum*) were sourced from a local agricultural market in Muzaffargarh, Punjab, Pakistan, ensuring regional consistency and uniform quality. The bulbs were manually separated into cloves, peeled, and thoroughly rinsed with potable water to remove surface impurities. To retain the bioactive integrity of organosulfur compounds, the cloves were shade-dried for 10–12 days in a well-ventilated area, minimizing direct sunlight exposure, which can degrade sensitive phytochemicals (Delgado *et al*. 2023). Once dried, the cloves were coarsely ground using a mechanical grinder. The resulting powder was sieved to remove fibrous debris and subsequently processed in a fine-grinding apparatus to achieve uniform particle size, ensuring even distribution in feed formulation (Gao *et al*. 2020; Abdulrahman and Al Sulivany 2025). To further reduce residual moisture and prevent microbial contamination, the garlic powder was air-dried for an additional 48 hours on sterile trays. The final product was stored in airtight, light-resistant containers under climate-controlled conditions (18±2°C, RH < 40%) to prevent oxidative degradation and preserve bioactive components (Razavi Amri *et al*. 2024). The powder was incorporated into experimental diets at inclusion levels of 0% (control), 2%, 4%, and 6% by weight. All feed batches were prepared under identical hygienic conditions using sanitized equipment to avoid cross-contamination and ensure consistency across treatments (Hor *et al*. 2017). This preparation protocol was essential for maintaining the functional integrity of garlic powder as a dietary additive in aquaculture nutrition trials

2.2 Experimental design and fish acclimatization

A total of 120 healthy *C. catla* fingerlings, with an average initial weight of 5.86±0.14 g, were obtained from a certified hatchery in Multan, Punjab, Pakistan. The fish were transported in oxygenated polythene bags to the Aquatic Research Laboratory at the Saline Water Aquaculture Research Centre (SWARC), Fisheries Department, Muzaffargarh. Upon arrival, they underwent a 15-day acclimatization period under controlled laboratory conditions. To minimize pathogen contamination, the fingerlings were treated with a 2.5 g L^{-1} sodium chloride (NaCl) solution for 1–2 minutes, following established disinfection protocols (Burrell *et al*. 2016; Omar and Al Sulivany 2025).

2.3 Dietary treatments and tank setup

After acclimatization, the fish were randomly distributed among 12 glass aquaria (100×40×40 cm^3, 120 L water volume per tank) in a completely randomized design. Four experimental diets were formulated with varying levels of garlic powder supplementation: 0% (control, Group-1), 2% (Group-2), 4% (Group-3), and 6% (Group-4) (Irkin *et al*. 2014). Each treatment was replicated three times, with 10 fish per aquarium. To maintain optimal water quality, each tank was equipped with an air pump and air stones to ensure proper aeration. Water temperature was regulated using submersible heaters and monitored daily, while dissolved oxygen and pH levels were measured weekly. Throughout the trial, the water parameters remained stable, with a temperature of 25.2±0.6°C, pH of 7.3±0.2, and dissolved oxygen of 7.6±0.3 mg L^{-1} (Lucey *et al*. 2020; Zulfiqar *et al*. 2025).

2.4 Feeding management and diet preparation

The fish were hand-fed their respective diets twice daily at 3% of their body weight, with feed rations adjusted biweekly based on weight gain (Karim *et al*. 2023; Owais *et al*. 2025). Uneaten feed and waste were removed daily to maintain water quality. The experimental diets were prepared using locally sourced ingredients, including fish meal, soybean meal, wheat bran, corn flour, and a vitamin-mineral premix. The ingredients were finely ground, mixed, and pelletized using a manual pelletizer before being dried and stored at −18°C. Before the feeding trial, all diets were analyzed for proximate composition to ensure consistency in nutritional content (Table 1). This experimental setup provided a controlled environment to evaluate the effects of garlic powder supplementation on the growth performance of *C. catla* fingerlings.

2.5 Growth performance

Throughout the 90-day feeding trial, *C. catla* fingerlings were maintained under uniform environmental conditions to evaluate the effects of dietary garlic powder supplementation on growth performance. To minimize digestive variation and ensure accurate weight assessments, all fish underwent an overnight fast once each week. This protocol, including a fasting session on day sixteen, helped stabilize metabolic rates before measurements were taken. Throughout the fasting intervals, continuous access to clean, aerated water was maintained to avoid dehydration or unnecessary stress. On the morning following each fasting period, individual fish from each treatment group were weighed using a precision digital balance (Model: A&D HT-120, Adventure Series) to record their body weights consistently.

Key growth metrics, including weight gain (WG), specific growth rate (SGR), feed intake (FI), and feed conversion ratio (FCR), were monitored weekly for each group. Data collection followed the methodology outlined by Al Sulivany *et al*. (2024), ensuring standardized and replicable observations throughout the experiment. Calculations of growth performance were conducted using widely accepted formulas (Fore *et al*. 2016; Hassan *et al*. 2025), which allowed for reliable comparisons across all garlic supplementation levels.

WG (g) = FW - IW
FCR = FG / WG
GR = (WG / IW) × 100
FI= FCR × WG
SGR= ln FW - ln IW / t × 100

In these equations, WG denotes the gain in weight (g), FW and IW represent final and initial body weights (g), respectively, and refers to the number of days in the trial. FCR indicates the efficiency of feed conversion, GR reflects relative weight increase, FI is the total feed consumed, and SGR expresses the daily growth rate as a percentage.

TABLE 1 Ingredients and proximate composition of experimental diets for *Catla catla.*

Ingredients (g/kg)	Groups			
	1	2	3	4
Fish meal	180	180	180	180
Wheat bran	300	295	290	285
Corn flour	180	180	180	180
Soybean meal	140	135	130	125
Rice polish	100	100	100	100
Garlic powder	0	20	40	60
Soybean oil	18	18	18	18
Vitamin-mineral premix	12	12	12	12
Fish meal	180	180	180	180
Wheat bran	300	295	290	285
Corn flour	180	180	180	180
Proximate composition (% dry matter)				
Crude protein (%)	30.0	30.1	30.1	30.2
Crude lipids (%)	4.0	4.1	4.1	4.2
Crude fiber (%)	5.8	5.9	6.0	6.1
Ash (%)	9.3	9.5	9.6	9.7
Other carbohydrates* (%)	50.9	50.4	50.2	49.8
Total (Dry Matter) (%)	100	100	100	100

Notes: (1) To meet the essential micronutrient requirements of *Catla catla*, a commercial vitamin premix was incorporated into all experimental diets. Each kilogram of diet contained the following vitamins: Vitamin A: 7500 IU, Vitamin D_3: 1500 IU, Vitamin E: 200 IU, Vitamin C: 100 mg, Vitamin B_1: 2 mg, Vitamin B_2: 5 mg, Vitamin B_6: 3 mg, Vitamin B_{12}: 0.01 mg, Niacin: 40 mg, and Folic Acid: 1 mg. The vitamin premix was procured from AquaVita Industries (Karachi) and NutriTech Aquafeeds (Lahore); (2) To support enzymatic functions, osmoregulation, and skeletal development, a balanced mineral premix was added to all diets. Each kilogram of feed included: Calcium (Ca): 1.2 g, Phosphorus (P): 0.9 g, Zinc (Zn): 40 mg, Iron (Fe): 50 mg, Manganese (Mn): 20 mg, Copper (Cu): 5 mg, and Magnesium (Mg): 600 mg. These minerals were sourced from PureMinerals (Islamabad) and AquaTrace Additives (Faisalabad) to ensure high quality and bioavailability.

2.6 Evaluation of hemato-immunological profiles

At the end of the 90-day feeding trial, hematological and immunological parameters were analyzed to assess the physiological response of *C. catla* to dietary inclusion of garlic powder. From each replicate tank, four fish were randomly selected and anesthetized using tricaine methanesulfonate (MS-222) at a concentration of 0.1 g L^{-1} to reduce handling stress. Blood samples were collected aseptically from the caudal vein using sterile 3 mL syringes. For hematological analysis, blood was immediately transferred into ethylenediaminetetraacetic acid (EDTA)-coated tubes to prevent coagulation. Red blood cell (RBC; 10^6 mm^{-3}) and white blood cell (WBC; 10^3 mm^{-3}) counts were determined using a Neubauer hemocytometer under a light microscope, following the protocol of Blaxhall and Daisley (1973). Hemoglobin (Hb; g 100 mL^{-1}) concentration was measured using the cyanmethemoglobin method, as outlined by Wedemeyer and Yasutake (1977), while packed cell volume (PCV; %) was estimated using the standard microhematocrit centrifugation technique. To evaluate immune cell distribution, thin blood smears were prepared from fresh, non-anticoagulated blood samples. After fixation and staining, the slides were examined under the microscope to determine the percentages of lymphocytes and neutrophils (Klontz 1994). A total of 100 leukocytes were counted per slide, and the relative abundance of each cell type was expressed as a percentage of the total leukocyte count.

2.7 Serum biochemical profiling and hepatic enzyme diagnostics

The enzymatic markers analyzed included alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), and lactate dehydrogenase (LDH), all of which are commonly associated with liver and tissue health. In addition to enzymatic profiles, the biochemical parameters assessed comprised glucose and lipid profiles, including total cholesterol, triglycerides, and high-density lipoprotein (HDL). These biomarkers were selected to provide insight into energy metabolism, lipid utilization, and potential hepatic responses to dietary garlic powder.

All serum assays were performed using the FUJIFILM DRI-CHEM NX500 analyzer (Czech Republic), following the manufacturer's guidelines using slide reagent kits. For parameters not directly measured, specific formulas were applied to estimate additional lipid fractions:

VLDL = Triglycerides ÷ 5
LDL = Total Cholesterol − (Triglycerides ÷ 5)

These calculations were based on the methodology described by Ahmed (2023), allowing for a comprehensive assessment of lipid transport dynamics and garlic's influence on circulatory lipid fractions.

2.8 Assessment of antioxidant enzyme activity

To investigate the antioxidant defense mechanisms in *C. catla* following dietary garlic supplementation, serum

antioxidant enzyme activities were analyzed at the end of the 90-day feeding period. The enzymes assessed included catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA), which serves as an indicator of lipid peroxidation.

Catalase (CAT) activity was evaluated based on the method described by Goth (1991). In this assay, 0.2 mL of serum was added to 1 mL of hydrogen peroxide substrate (65 µmol mL^{-1} in 60 mmol L^{-1} sodium-potassium phosphate buffer, pH 7.4) and incubated at 37°C for one minute. Following incubation, 1 mL of ammonium molybdate (32.4 mM) was added to terminate the reaction. This formed a yellow complex between molybdate and residual hydrogen peroxide. The absorbance of the resulting suspension was measured at 405 nm using a Jasco V-550 UV-visible spectrophotometer.

Superoxide dismutase (SOD) activity was determined following the method of Marklund and Marklund (1974), which relies on the enzyme's ability to inhibit pyrogallol autoxidation. The reaction mixture was prepared by zeroing the spectrophotometer with a Tris-EDTA buffer solution, after which the absorbance of both the sample and control was recorded at 420 nm immediately and again one minute after the addition of pyrogallol. Enzymatic activity was expressed in units per milliliter (U mL^{-1}), where one unit corresponds to the amount of enzyme that inhibits 50% of pyrogallol oxidation.

Glutathione peroxidase (GPx) activity was analyzed using the protocol described by Hafeman *et al*. (1974). The assay involved mixing 0.1 mL of serum with 0.1 mL of 5 mM reduced glutathione (GSH), 0.1 mL of 1.25 mM hydrogen peroxide (H_2O_2), 0.1 mL of 25 mM sodium azide (NaN_3), and 0.05 mM phosphate buffer (pH 7.0), forming a total reaction volume of 2.5 mL. GPx activity was determined by the decrease in GSH concentration after reaction with hydrogen peroxide in the presence of NaN_3.

Lipid peroxidation, indicated by malondialdehyde (MDA) levels, was assessed through the thiobarbituric acid reactive substances (TBARS) assay, following the method of El-Demerdash (2012) and Habbib and Al-Sulaivany (2013). In brief, the serum was mixed with 1 mL of 20% trichloroacetic acid (TCA) and 2 mL of 0.67% thiobarbituric acid (TBA), then heated at 100°C for 60 minutes. After cooling, the mixture was centrifuged to remove the precipitate. The absorbance of the clear supernatant was measured at 535 nm using a spectrophotometer. TBARS concentrations were calculated using the extinction coefficient for MDA (1.56×10^5 M^{-1} cm^{-1}), with MDA considered the primary marker of lipid peroxidation.

2.9 Data analysis

Statistical analysis was performed using SPSS version 23.0, and data were analyzed using one-way analysis of variance (ANOVA) to identify significant differences among groups. Post hoc analysis was conducted using Duncan's multiple range test to compare mean values.

3 | RESULTS

The growth performance parameters of *C. catla* are presented in Table 2 and Figure 1. The IW were comparable across all groups (4.67 ± 0.98 to 6.00 ± 0.44 g; $p = 0.5014$). By day 90, the FW, WG and FI increased significantly with GP level ($p = 0.043$ and $p < 0.001$, respectively), with Group 4 recording the highest values (41.17±7.04 g, 35.50±7.11 g, and 52.20±0.96 g) compared to Group 1 (18.50±3.73 g, 13.83±2.79 g, and 24.80±0.58 g). Conversely, FCR declined from 1.49±0.03 in Group 1 to 1.22±0.015 in Group 4 ($p < 0.001$), while SGR rose from 2.15±0.17% day^{-1} to 3.59±0.08% day^{-1} ($p < 0.001$).

The hematological analysis of fish fed different levels of GP supplementation showed clear differences among the groups. The RBC counts increased progressively with higher GP inclusion, from $1.92\pm0.006\times10^6$ mm^{-3} in Group 1 (basal diet) to $2.63\pm0.014\times10^6$ mm^{-3} in Group 4 (6% GP), with all differences being highly significant ($p < 0.001$). The Hb levels followed a similar trend, rising from 7.52±0.13 g $100ml^{-1}$ in Group 1 to 13.58±0.106 g $100ml^{-1}$ in Group 4 ($p < 0.001$), indicating improved oxygen-carrying capacity. On the other hand, the PCV also increased significantly, from 29.36±0.39% in Group 1 to 40.9±0.5% in Group 4 ($p < 0.001$), further supporting enhanced blood health. Furthermore, WBC counts were reduced with GP supplementation, decreased from $7.46\pm0.29\times10^3$ mm^{-3} in Group 1 to $5.96\pm0.32\times10^3$ mm^{-3} in Group 4 ($p = 0.008$). The percentage of lymphocytes also decreased from 54.8±0.55% in Group 1 to 39.8±0.73% in Group 4 ($p < 0.001$). Similarly, neutrophil percentages also diminished, from 49.2±0.58% to 33.4±0.5% ($p < 0.001$) (Table 3 and Figure 2).

The analysis of serum lipid profile revealed significant improvements with increasing dietary GP supplementation over the feeding trial. *Catla catla* receiving the 6% GP diet (Group 4) showed the most favorable lipid profile, with triglycerides decreasing significantly from 124±0.7 mg dl^{-1} in the control group to 102.3±4.72 mg dl^{-1} ($p < 0.001$). A similar pattern was observed for VLDL levels, which decreased from 24.6±0.14 mg dl^{-1} in Group 1 to 20.24±0.94 mg dl^{-1} in Group 4 ($p < 0.001$). Cholesterol levels exhibited an interesting pattern, remaining unchanged between Groups 1 (144.4±3.3 mg dl^{-1}) and 2 (144.6±3.48 mg dl^{-1}), but decreasing significantly in Groups 3 (134±1.9 mg dl^{-1}) and 4 (134.2±2.26 mg dl^{-1}) ($p = 0.028$). Clear effects were observed in the serum HDL and LDL. The serum HDL increased markedly from 52.6±2.58 mg dl^{-1} in Group 1 to 67.4±2.33 mg dl^{-1} in Group 4 ($p < 0.001$). Conversely, LDL was nearly halved in fish fed higher GP levels, decreasing from 66.2±3.01 mg dl^{-1} in Group 1 to 45.56±2.19 mg dl^{-1} in Group 4 ($p < 0.001$) (Table 4 and Figure 3).

Serum enzyme activities revealed striking improve-

ments in liver health markers with GP supplementation. Across all measured enzymes, we observed a clear dose-dependent decrease in activity levels as GA concentrations in the diet increased. Starting with serum AST levels decreased progressively from 77±0.7 U L^{-1} in the control group to 53.8±0.37 U L^{-1} in the 6% GP group ($p < 0.001$). The pattern was even more pronounced for ALT, where values decreased from 41.8±0.96 U L^{-1} to 18.2±1.14 U L^{-1} across the same groups ($p < 0.001$).

TABLE 2 Effects of Dietary Garlic Powder Supplementation on Growth Performance Parameters of *Catla catla* Over a 90-Day Feeding Trial.

Growth parameter	Group 1	Group 2	Group 3	Group 4	*p*-value
Initial weight (g)	4.67±0.98	5.16±0.7	6±0.44	5.66±0.21	0.501
Final weight (g)	18.5±3.73	25.17±4.68	34.67±6.19	41.17±7.04	0.043
Weight gain (g)	13.83±2.79^{a}	20±4.06a^{b}	28.67±5.8a^{c}	35.5±7.11^{d}	<0.001
Feed intake	24.8±0.58^{a}	33.2±1.2^{b}	46±1.48^{c}	52.2±0.96^{d}	<0.001
FCR (%)	1.49±0.03^{a}	1.38±0.004^{b}	1.33±0.008^{b}	1.22±0.015^{c}	<0.001
SGR	2.15±0.17^{a}	2.73±0.1^{b}	3.09±0.08^{b}	3.59±0.08^{c}	<0.001

Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

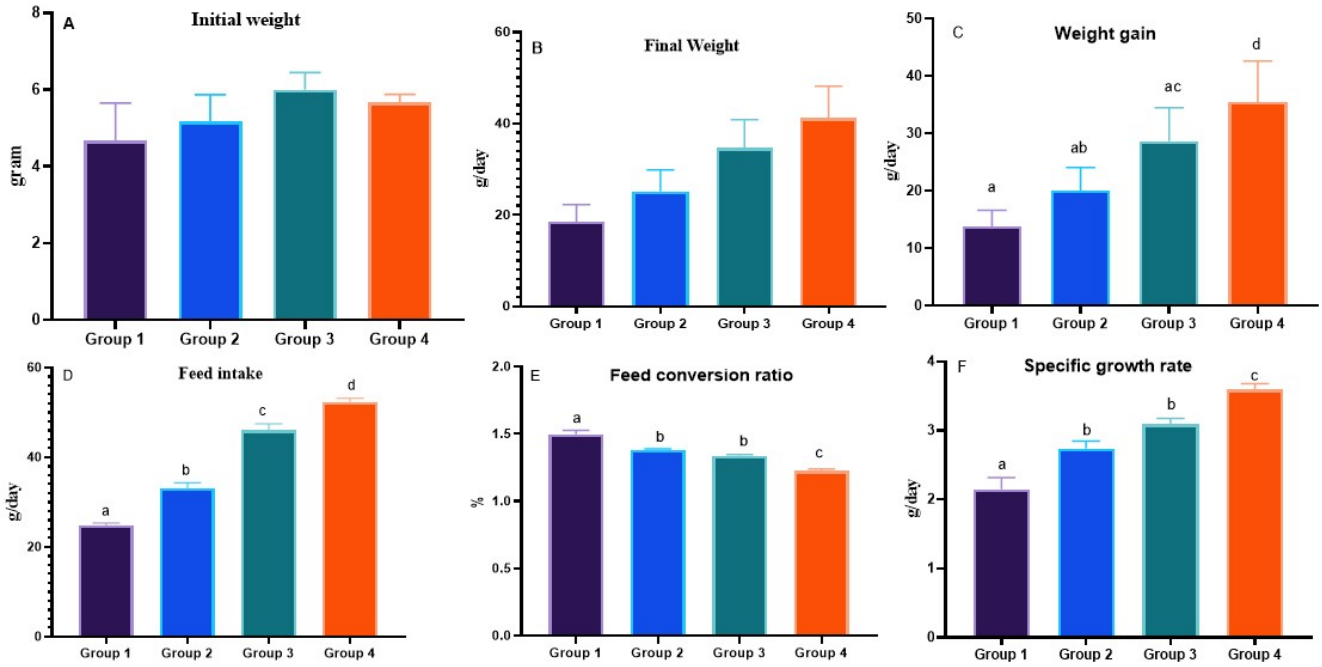

FIGURE 1 Effects of dietary garlic powder supplementation on growth performance parameters of *Catla catla* over a 90-day feeding trial. Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

TABLE 3 Hematological responses of *Catla catla* fed diets containing varying levels of garlic powder over a 90-day feeding period.

Parameters	Group 1	Group 2	Group 3	Group 4	*p*-value
RBC ($10^{6}mm^{-3}$)	1.92±0.006^{a}	2.22±0.037^{b}	2.53±0.011^{c}	2.63±0.014^{d}	<0.001
WBC ($10^{3}mm^{-3}$)	7.46±0.29^{a}	6.76±0.14^{c}	6.13±0.13^{b}	5.96±0.32^{b}	0.008
Hb (g/100ml)	7.52±0.13^{a}	9.42±0.037^{b}	11.3±0.1^{c}	13.58±0.106^{d}	<0.001
PCV (%)	29.36±0.39a	31.86±0.23^{b}	36.58±0.11^{c}	40.9±0.5^{d}	<0.001
Lymphocyte (%)	54.8±0.55^{a}	50.8±0.58^{b}	46±0.7^{c}	39.8±0.73^{d}	<0.001
Neutrophil (%)	49.2±0.58^{a}	44±0.31^{b}	39.6±0.92^{c}	33.4±0.5^{d}	<0.001

Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

TABLE 4 Effects of dietary garlic powder supplementation on serum lipid profile of *Catla catla* after a 90-day feeding trial.

Lipid profile	Group 1	Group 2	Group 3	Group 4	*p*-value
Triglyceride (mg/dl)	124±0.7[a]	120±1.64[a]	111.8±3.81[ab]	102.3±4.72[b]	<0.001
Cholesterol (mg/dl)	144.4±3.3[a]	144.6±3.48a	134±1.9[b]	134.2±2.26[b]	0.028
HDL (mg/dl)	52.6±2.58[a]	53.6±2.71[a]	65.4±1.28[b]	67.4±2.33[b]	<0.001
LDL (mg/dl)	66.2±3.01[a]	66.2±2.45[a]	45.44±1.9[b]	45.56±2.19[b]	<0.001
VLDL (mg/dl)	24.6±0.14[a]	23.8±0.3[a]	22.16±0.76[ab]	20.24±0.94[b]	<0.001

Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

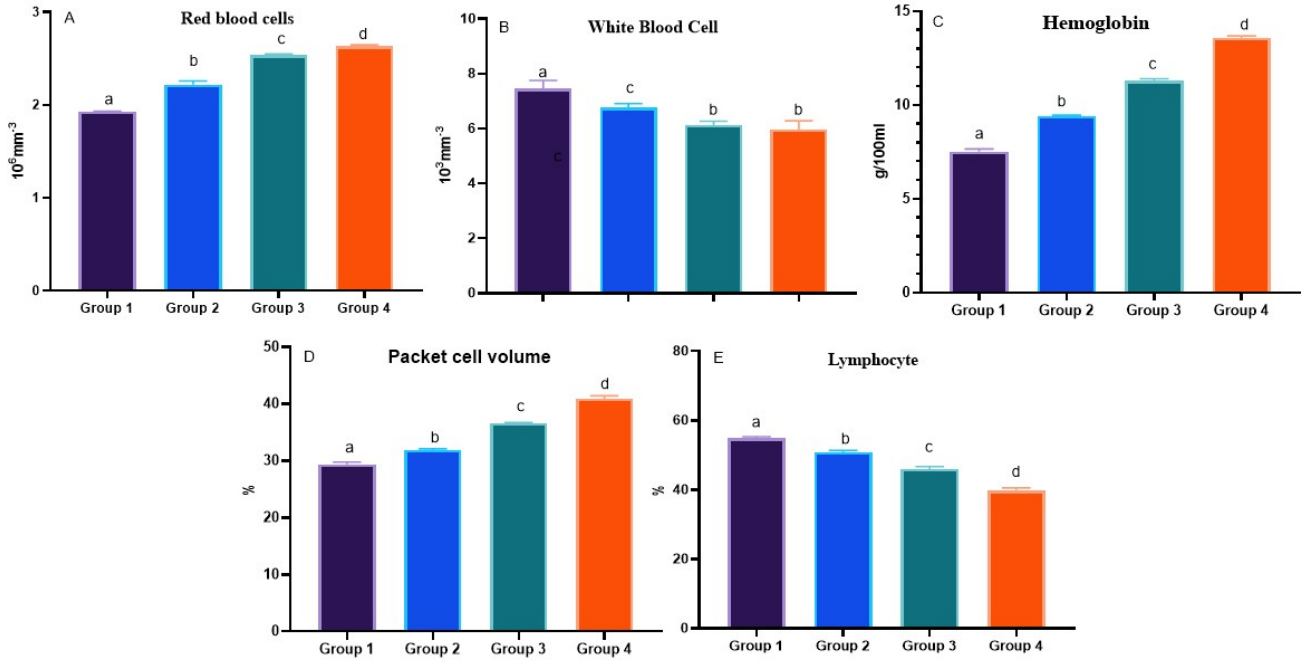

FIGURE 3 Effects of dietary garlic powder supplementation on serum lipid profile of *Catla catla* after a 90-day feeding trial. Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

The serum ALP activity consistently reduced from 210±0.89 U L^{-1} to 172.6±0.4 U L^{-1} ($p < 0.001$). The most remarkable change appeared in LDH levels, which were nearly halved, decreasing from 314±1.58 U L^{-1} to 170±1.04 U L^{-1} ($p < 0.001$). This substantial reduction in LDH (Table 5 and Figure 4).

Fish receiving GP-enriched diets showed a dose-dependent increase in antioxidant enzymes compared to the control group (Group 1). The activity of serum catalase increases from 20.4±0.67 U mg^{-1} in the basal diet group to 26.2±0.23 U mg^{-1} protein in the 6% GP group ($p < 0.001$). Similarly, the serum glutathione peroxidase levels increased progressively, starting at 52.8±0.37 U mg^{-1} in Group 1 and peaking at 65.8±0.58 U mg^{-1} in Group 4 ($p < 0.001$). However, Superoxide dismutase, nearly doubled in activity, from 9.4±0.24 U mg^{-1} protein in the control to 16±0.31 U mg^{-1} in the highest supplementation group ($p < 0.001$). Oxidative stress, measured via malondialdehyde (MDA), showed an inverse trend. MDA levels, a marker of lipid peroxidation, declined significantly from 4.34±0.41 nmol mg^{-1} in Group 1 to 2.38±0.058 nmol mg^{-1} protein in Group 4 ($p < 0.001$) (Table 6 and Figure 5).

TABLE 5 Effects of dietary garlic powder serum enzyme activities in *Catla catla* following a 90-day feeding trial.

Parameters	Group 1	Group 2	Group 3	Group 4	*p*-value
AST (U/L)	77±0.7[a]	63±0.63[b]	58.6±0.50[c]	53.8±0.37[d]	<0.001
ALT (U/L)	41.8±0.96[a]	32.6±0.96[b]	25±0.67[c]	18.2±1.14[d]	<0.001
ALP (U/L)	210±0.89[a]	194.4±0.74[b]	182±0.70[c]	172.6±0.4[d]	<0.001
LDH (U/L)	314±1.58[a]	294.4±0.812[b]	184.4±2.2[c]	170±1.04[d]	<0.001

Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

TABLE 6 Antioxidant enzyme activities and lipid peroxidation in *Catla catla* fed garlic powder-supplemented diets over 90 days.

Antioxidant enzyme	Group 1	Group 2	Group 3	Group 4	*p*-value
Catalase (KU/L)	20.4±0.67[a]	23.2±0.2[b]	24.8±0.2[b]	26.2±0.23[c]	<0.001
Glutathione peroxidase (U/L)	52.8±0.37[a]	58±0.44[b]	63.8±0.58[c]	65.8±0.58[c]	<0.001
Superoxide dismutase (U/mL)	9.4±0.24[a]	12.2±0.37[b]	14.2±0.2[c]	16±0.31[d]	<0.001
MDA (Mmol/L)	4.34±0.41[a]	3.28±0.037[b]	2.84±0.05[b]	2.38±0.058[c]	<0.001

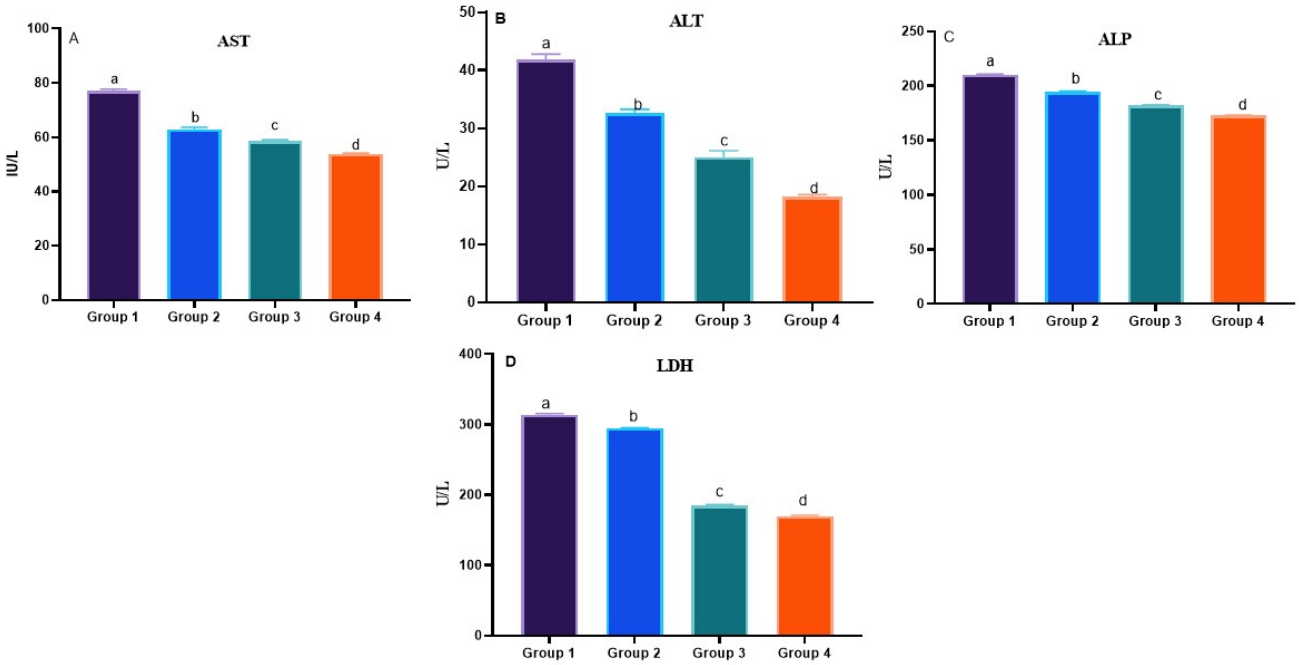

FIGURE 4 Effects of dietary garlic powder supplementation on serum hepatic enzyme activities in *Catla catla* after a 90-day feeding trial. Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

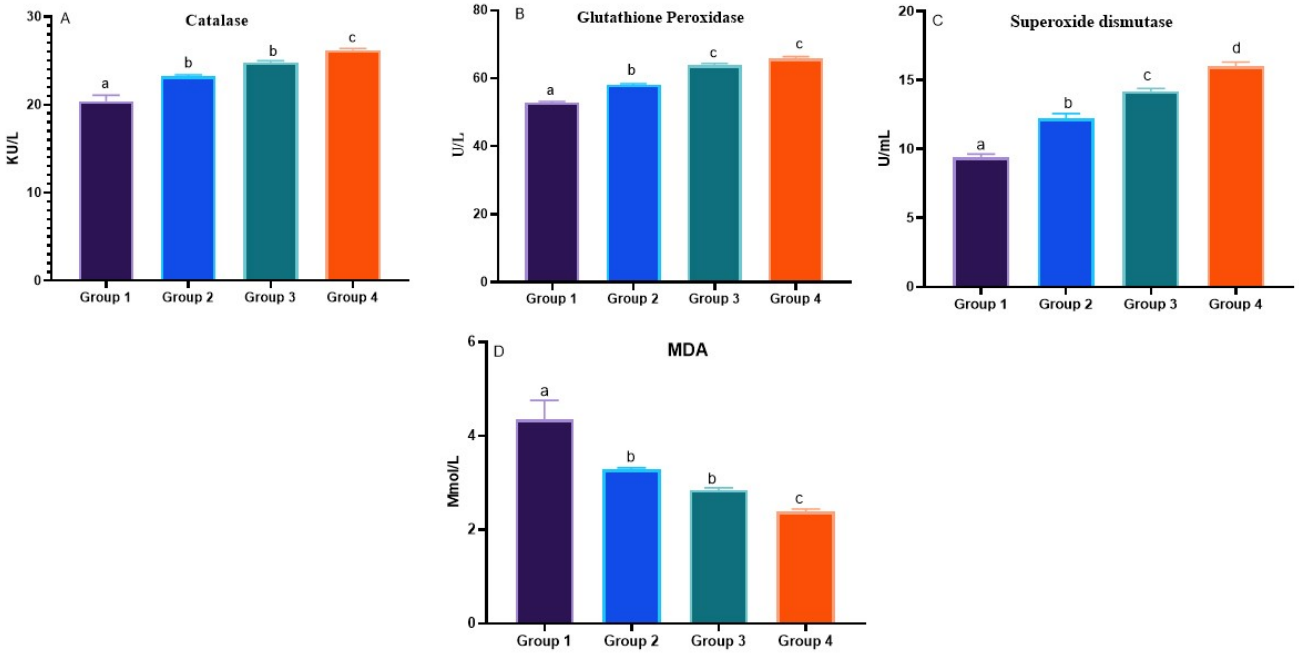

FIGURE 5 Antioxidant enzyme activities and lipid peroxidation in *Catla catla* fed garlic powder-supplemented diets over 90 days. Groups: Control (Group-1), 2% garlic (Group-2), 4% garlic (Group-3) and 6% garlic (Group-4).

4 | DISCUSSION

The studies reveal that dietary supplementation with GP significantly enhanced the growth performance of *C. catla* fingerlings, with the highest final weight, weight gain, and specific growth rate observed in the group receiving 6% GP. This improvement can be attributed to the bioactive compounds in garlic, such as allicin, which enhance feed palatability, stimulate appetite, and improve nutrient absorption (Kaur and Ansal 2020). The progressive enhancement of FCR with increasing GP levels further supports its role in optimizing nutrient utilization, consistent with studies showing that garlic modulates the gut microbiota and enhances digestive enzyme activity (Ashour *et al*. 2025).

The hematological results indicate that GP supplementation significantly improved the hematological health of *C. catla*. These improvements suggest enhanced oxygen-carrying capacity and overall metabolic efficiency, likely due to the bioactive compounds in garlic, such as allicin, which are known to support erythropoiesis, improve nutrient utilization, and possess well-documented anti-inflammatory properties (Shang *et al*. 2019). These compounds can down regulate pro-inflammatory cytokine production and reduce the activation and proliferation of immune cells in the absence of pathogenic threats (Wang *et al*. 2025). Therefore, the decreased leukocyte, lymphocyte, and neutrophil counts in GP groups may reflect a more regulated immune environment rather than impaired immune function. Similar findings have been reported in *Oreochromis niloticus* fed garlic-supplemented diets, where reduced leukocyte counts were accompanied by enhanced respiratory burst activity and lysozyme levels, indicating improved immune readiness despite lower cell numbers (Ndong *et al*. 2011).

Furthermore, the dietary GP supplementation positively influenced the lipid regulation in *C. catla*. Fish receiving higher GP levels showed reduced triglycerides and LDL cholesterol levels, alongside increased HDL cholesterol, suggesting improved cardiovascular health. These effects may be attributed to garlic's ability to influence lipid-regulating enzymes and the composition of the gut microbiota, which play crucial roles in lipid absorption and metabolism (Elhetawy *et al*. 2025). However, similar results were reported in Atlantic salmon, where garlic showed limited impact on cholesterol levels (Ried *et al*. 2013). The agreement with carp studies supports GP's potential as a functional feed additive, while the differences with salmon research suggest the need for further investigation into the underlying mechanisms across fish species.

The consistent dose-dependent decrease in AST, ALT, ALP, and LDH levels suggests that dietary garlic significantly improved liver function and reduced cellular damage. These beneficial effects are likely mediated through multiple pathways, including enhanced detoxification processes and stabilization of hepatocyte membranes (Almatroodi *et al*. 2020). Similar hepatoprotective effects have been reported in Nile tilapia (*Oreochromis niloticus*), where garlic supplementation reduced serum enzyme levels and improved liver histology (Mahmoud and El-Hais 2017). The reduction in LDH activity indicates that garlic may help maintain cellular energy metabolism and prevent tissue damage during stress conditions (Deniz *et al*. 2011; Abdel-Mageid *et al*. 2018; Abdulla *et al*. 2023).

The increased activities of catalase, glutathione peroxidase, and superoxide dismutase suggest that garlic components may protect these enzymes from oxidative inactivation while potentially enhancing their stability and function (Shang *et al*. 2019). The reduced malondialdehyde levels indicate garlic's ability to prevent lipid peroxidation chain reactions, possibly through direct scavenging of reactive oxygen species (Colín-González *et al*. 2012). These effects are particularly relevant in aquaculture, where fish face constant oxidative challenges from intensive farming conditions. The dose-dependent improvements across all measured parameters suggest that garlic's bioactive compounds, including sulfur-containing molecules and phenolic compounds, work in a concentration-dependent manner to strengthen the antioxidant defense system (Ayala *et al*. 2014).

5 | CONCLUSIONS

The study concluded that dietary GP supplementation significantly improved the overall health and growth of *C. catla* fingerlings, with the highest benefits observed in the group receiving the maximum supplementation level. This group showed superior growth performance. Hematological analysis indicated enhanced blood health, with higher red blood cell counts and hemoglobin levels, suggesting better oxygen transport. Lipid profiles were also optimized, with reduced harmful cholesterol fractions and increased beneficial cholesterol. On the other hand, hepatic enzyme activity stabilized, indicating improved hepatic function, while antioxidant defenses strengthened, reducing oxidative stress. These findings highlight garlic powder as an effective natural feed additive for enhancing growth, metabolic health, and physiology in aquaculture.

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CONFLICT OF INTEREST

There are no competing interests.

AUTHORS' CONTRIBUTION

M.O., B.S.A.A.S., M.M.S and A.L. contributed to the concept and design of the work. R.M.F., B.S.A.A.S., A.S., and

H.A.M. were responsible for the acquisition, analysis, and interpretation of data. M.O., B.S.A.A.S., and H.F. 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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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