Research Article

Effects of Garlic (*Allium sativum*) Supplementation on Growth, Haematological Parameters and Antioxidant Enzyme Status in *Labeo rohita*

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Abstract | Worldwide demands for natural and safe food has triggered the exploration for naturally occurring growth boosters. The role of garlic (*Allium sativum*) delineated for growth promotion, hunger stimulation and immunity melioration so, it is ratified for medicinal applications. Therefore, the experiment was conducted to determine the effect of dietary garlic on growth performance, haematology, and antioxidant enzyme activity of rohu (*Labeo rohita)*. Different percentages of garlic (0-5%) were incorporated in experimental groups (G0-G5) respectively, except in control group. Total 8 fingerlings of *L. rohita* were taken in each aquarium. The duration of experiment was 60-days. Growth parameters including weight gain, Total length, fork length, specific growth rate, condition factor and survival rate (WG, TL and FL, SGR, CF, and SR) were highly significant (P<0.05) and the lowest FCR in G3 (3%) garlic treated groups. Haematological parameters were highly significant (P<0.05) in G3 (3%) garlic diet except for the monocytes, basophils and MCV. Garlic has intrinsic antioxidant properties thus antioxidant activity of enzyme (U mg^{-1} Protein) was also modified by using this natural ingredient, high value was found at G3 diet then followed by G4 diet. Eventually, from experimental results it was recommended that 3% dietary garlic enhanced the growth performance, haematology and the antioxidant enzyme activity of rohu (*L. rohita*) serve as a natural growth promoter.

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Introduction

Fish is an excellent nutrient enriched food source Ibrahim *et al.* (2024). For the first time, aquaculture production of aquatic animals surpassed capture fisheries, reaching 94.4 million tons, which accounted for 51 percent of the global total and 57 percent of production. It supplies good quality protein, source of

omega-3 polyunsaturated fatty acids EPA and DHA (FAO, 2024). The aquatic bodies mostly effected by pathogenic bacteria and to prevent these pathogenic bacteria, aquaculturists use substantial quantities of antibiotics (Elgendy *et al.*, 2024). These antibiotics become accumulated in the muscles of fish, has also led to the emergence of resistant pathogens and hence are consider as deterring factors for their continual use (El-Adawy *et al.*, 2023). The researcher attempted to find out the alternatives of antibiotics for the maintenance of fish health. Phytotherapeutics are the plant derived medicinal products used for curative purposes (E Silva *et al.*, 2024).

Plants are among those natural sources which are affordable and safe, and can be used as growth promoters. Garlic (*Allium sativum*) belongs to the family Alliaceae, according to history it has several therapeutic and dietary roles to treat many diseases (Kanani *et al.*, 2014; Xiong *et al.*, 2015). It is medically important because it provides sulfur compounds like allicin, alliin and ajoene (Valenzuela-Gutierrez *et al.*, 2021). According to previous studies, garlic showed several of biological functions such as antimicrobial, antioxidant, and anti-inflammatory properties in fish (Gabriel *et al.*, 2021; Valenzuela-Gutiérrez *et al.*, 2021). It's a valuable source of various components e.g. carbohydrates, phosphorus, calcium, iodine salts, silicates, and sulfur salts. Allicin is an additional component that have momentous anthelmintic roles (Rodge and Thakare, 2024).

Garlic is an ancient plant used as a spice and herbal treatments. A previous study reported that garlic supplementation has an effect on broiler growth performance and blood biochemistry (Kairalla *et al.*, 2022). Supplementation with garlic powder at 1% and 2% in the diet for crustaceans (*Pontastacus leptodactylus*) increased the growth indices and digestive enzymes activity, 2% elevated lysozyme, nitric oxide synthase, and phenoloxidase activities in the crayfish (Rezaei *et al.*, 2022). In whiteleg shrimp (*Litopenaeus vannamei*), dietary replacement with fish, meat, or bone flour with 3% garlic, improved growth and proximate composition (Gokalp, 2018). For the evaluation of fish health condition normally haematological parameters are considered as worthwhile signals (Nwakpa and Ikwor, 2024). It was observed thatin fish diets inclusion of garlic proliferated the number of erythrocytes, leukocytes, platelets, haematocrit, and haemoglobin content (Guroy *et al.*, 2024). In a crushed garlic, S-allyl cysteine sulfoxide, diallyl thiosulfinate, and other bioactive compounds are present which intensify immune responses and suppress tumor metabolism (Yousefi *et al.*, 2020). Garlic supplementation improve growth performance in seabass (*Lateolabrax japonicas)* (Xu *et al.*, 2020) and to increase body composition in trout (*Salmo caspius*) (Zaefarian *et al.*, 2017).

The oxidative stress is due to the disproportion in synthesis of reactive oxygen species and defense mechanism of antioxidant enzymes (Nematollahi *et al.*, 2003; Ece *et al.*, 2006). It is authenticated that oxidative stress contributes in the pathogenesis of fish diseases. Tissues of fish are in particular susceptive to oxidative damage (Welker and Congleton, 2005). Garlic has capability to act as catalase enzyme in and blood plasma glucose level is also decreased by using it (Nwabueze, 2012). Therefore, through the current study, we aim to determine the protective effects of garlic on Rohu (*Labeo rohita*), fed with the diet containing different levels of Garlic (0-5%) and access the growth performance, feed utilization, haematology profile, and antioxidant activity in serum of blood.

Materials and Methods

The experiment was performed in research laboratory of Government College Women University, Sialkot to determine the effect of dietary garlic on growth performance, heamatology and antioxidant enzyme activity of rohu (*L. rohita)*.

Experimental design

The 120 days old fingerlings (32.52±0.03 g) of *L. rohita* were obtained from Begowala (District Sialkot) fish farm. Fish were acclimatized for few days. Duration of study was 60 days. In this experiment, total 144 fingerlings of rohu (*L. rohita*) were apportioned into six treatment groups. In total eighteen, aquariums of 50 liters water capacity, fish (n=8) were placed in each. Continuous aeration was supplied to all treatment groups with an air pump by using capillary system. Three replicates were used for each treatment. Among these six treatments, one treatment was fed with basal diet without garlic and was designated as the control group (G0). While other treating five groups contain different percentages of garlic were designated as G1%, G2%, G3%, G4% and G5%. Fish were fed at the rate of 3% (body weight) per day and

fed in two equal portions (08:30 and 04:00). Physico-chemical parameters viz. temperature 30 °C, pH 6.5 and total hardness 200 mg/L were maintained constant throughout the experiment whereas carbon dioxide, dissolved oxygen, calcium, total ammonia and magnesium were measured on regular basis by following the procedures described in (APHA, 2005).The procedures and methods used in the study followed the ethical guidelines of the GC Women University Sialkot, Pakistan.

Experimental diet

Diet containing 30 % digestible protein and 3.10 (K cal/g) digestible energy was provided to fingerlings as shown in Table 1. To formulate experimental diet, garlic was bought from local market of Sialkot, Pakistan. Cloves of garlic were peeled off and ground in mortar and pestle to prepare a well homogenized paste. The dough of basal diet was passed through a meat mincer, then pellets (2 mm) were oven dried and stored at -20 °C in a labeled plastic bag until use. Freshly ground garlic along with basal diet was given to all the treated groups regularly.

Table 1: *Composition of basal diet (%).*

Basal diet ingredients	Ingredient percentages
Fish meal	30
Corn gluten	22
Wheat flour	23
Rice polish	17
Sun flower oil	4
Mixture of vitamin	2
Mixture of mineral	2

DP (Digestible protein) =30 (%), DE (Digestible energy) =3.10 (K cal/g)

Study of growth parameters

In growth studies different parameters were studied including weight gain, forked and total lengths, feed Intake, feed conversion ratio, feed efficiency ratio, specific growth rate, Condition factor, and survival rate. These growth parameters were calculated after every week or after 7 days interval. Following formulas were used for calculation as given by (Lee *et al.*, 2012; Safari *et al.*, 2019; Megbowon *et al.*, 2024).

$$\text{Weight Gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Total Length (cm)} = \text{Final length (cm)} - \text{Initial length (cm)}$$

$$\text{Forked Length (cm)} = \text{Final forked length} - \text{Initial forked length}$$

$$\text{Feed Conversion Ratio (g/g)} = \frac{\text{Feed intake (g)}}{\text{Gain in weight (g)}}$$

$$\text{Specific Growth Rate (\%/day)} = \frac{\ln \text{final weight (g)} - \ln \text{initial weight (g)} \times 100}{\text{Time in days}}$$

$$\text{Condition Factor (g/cm}^3\text{)} = \frac{\text{Weight of fish (g)} \times 100}{(\text{Total length of fish})^3}$$

$$\text{SR \% (Survival Rate)} = \frac{\text{In each replicate final no. of fishes } (N_t) \times 100}{\text{In each replicate initial no. of fishes } (N_0)}$$

Study of haematological parameters

After 60-days of growth trial,with the help of sterilized syringe samples of blood were drawn from caudal vein of each fish and it was stored in vacutainers containing anticoagulant k3 EDTA. Blood samples were analyzed for the examination of different haematological parameters including haemoglobin, total erythrocyte count, mean corpuscular haemoglobin, packed cell volume, total leukocyte count, mean corpuscular haemoglobin concentration and mean corpuscular volume. By employing the following formulas concentration of other haematology was calculated (Alemu *et al.*, 2006).

$$\text{Mean Corpuscular Hemoglobin} = \frac{\text{Hemoglobin} \times 10}{\text{Red blood cells}}$$

$$\text{Mean corpuscular hemoglobin concentration} = \frac{\text{Hemoglobin} \times 100}{\text{Hematocrit}}$$

$$\text{Packed Cell Volume} = \frac{\text{Mean corpuscular hemoglobin} \times \text{Red blood cells}}{10}$$

$$\text{Mean Corpuscular Volume} = \frac{\text{Hematocrit} \times 10}{\text{Red blood cells}}$$

Determination of antioxidant enzyme activity in serum

At the end of 60-days trial, fish were taken from each aquarium and blood samples were collected by venous puncture. Then blood samples were stored in glass tubes and allowed to coagulate at room temperature. For centrifugation, each blood sample was taken in a separate Eppendorf tube. For rapid separation of sera, centrifugation was performed for 10 minutes at 1500 xg. Assay of antioxidant enzyme was performed instantly according to method described by (Goth, 1991).

Enzyme activity measurement

Catalase activity was determined on its ability to reduce the known amounts of hydrogen peroxide

(H_2O_2) at 240 nm as evaluated by Chance and Mehaly (1955). Superoxide dismutase activity (SOD) was assessed based on the nitro blue tetrazole (NBT) methodology (Giannopolitis and Ries, 1977). While glutathione peroxidase activity (GPx) was quantified using the protocol of Flohe and Gunzler (1984).

Statistical analysis

Entire data obtained from the experiment was statistically analyzed (n=3) by using one-way ANOVA (analysis of variance) between the means of two or more unrelated groups (Steel *et al*., 1996).

Results and Discussion

Growth performance of L. rohita

At the end of feeding trial, growth of *L. rohita* was monitored. Experimental results indicated that as compared to the control group in all garlic treated groups from 1% to 5% an increase in growth performance was observed. The effect of dietary garlic on different growth parameters were given in Table 2, 3, 4. According to analysis of variance, there was a highly significant (P<0.05) effect of dietary garlic on total length (cm), forked length (cm), feed intake (g/ Fish), feed conversion ratio (g/g), feed efficiency ratio (g/g), specific growth rate (%/ day), and condition factor CF (g/ cm^3). In group treated with G3 garlic diet, highest values of weight gain (18.58±0.15), forked length gain (0.81±0.01), specific growth rate (0.82±0.00) as compared to other treated groups (Table: 2, 3, 4). Significantly higher total length gain as noticed at G2 diet (2.67±0.01). Maximum value of CF (1.14±0.03) was recorded in G3 treated group and reduced in G0 and G2 treated group.

Table 2: *Effect of different concentrations of dietary garlic on the Weight gain parameter of L.rohita.*

Treatment	Initial weight (g)	Final weight (g)	Weight gain (g)	Initial total length (cm)
G0	31.01±0.08^{c}	44.98±0.55^{e}	13.97±0.04^{e}	15.29±0.06^{d}
G1	33.05±0.25^{b}	49.87±0.52^{bc}	16.82±0.06^{c}	17.24±0.06^{a}
G2	34.00±0.45^{b}	51.95±0.47^{a}	17.95±0.04^{b}	17.09±0.08^{a}
G3	30.40±0.38^{c}	48.98±0.20^{c}	18.58±0.15^{a}	17.19±0.11^{ab}
G4	35.50±0.14^{a}	51.51±0.05^{ab}	16.01±0.06^{d}	16.23±0.06^{c}
G5	31.20±0.32^{c}	47.02±0.34^{d}	15.82±0.03^{d}	16.46±0.10^{bc}

Table 3: *Effect of different concentrations of dietary garlic on the total length gain and forked length gain parameters of L. rohita.*

Treatment	Final length (cm)	Total length gain (cm)	Initial forked length (cm)	Final forked length (cm)	Forked length gain (cm)
G0	17.72±0.03^{d}	2.43±0.00^{c}	11.51±0.06^{b}	11.68±0.06^{c}	0.17±0.00^{f}
G1	19.76±0.12^{ab}	2.52±0.00^{b}	11.61±0.00^{ab}	12.07±0.08^{b}	0.46±0.00^{c}
G2	19.76±0.13^{ab}	2.67±0.01^{a}	11.56±0.04^{ab}	12.09±0.02^{b}	0.53±0.00^{b}
G3	19.72±0.03^{a}	2.53±0.00^{b}	11.68±0.06^{ab}	12.49±0.06^{a}	0.81±0.01^{a}
G4	18.64±0.03^{c}	2.41±0.00^{c}	11.76±0.03^{a}	12.14±0.02^{b}	0.38±0.00^{d}
G5	18.88±0.07^{c}	2.42±0.01^{c}	11.58±0.04^{ab}	11.81±0.04^{c}	0.23±0.00^{e}

Table 4: *Effect of different concentrations of dietary garlic on the SGR, CF, FCR and survival rate of L. rohita.*

Treatment	SGR (%/ day)	CF (g/ cm3)	FCR (g/g)	Survival rate %
G0	0.40±0.00^{f}	0.97±0.04^{d}	1.96±0.01^{a}	100.00±0.00^{a}
G1	0.62±0.00^{c}	1.05±0.05^{c}	1.73±0.00^{d}	100.00±0.00^{a}
G2	0.70±0.01^{b}	0.94±0.02^{d}	1.70±0.00^{c}	100.00±0.00^{a}
G3	0.82±0.00^{a}	1.14±0.03^{a}	1.63±0.00^{b}	100.00±0.00^{a}
G4	0.52±0.01^{e}	1.14±0.00^{a}	1.69±0.01^{bc}	100.00±0.00^{a}
G5	0.57±0.00^{d}	1.11±0.01^{b}	1.71±0.00^{c}	100.00±0.00^{a}

Means sharing similar letter in a column are statistically non-significant (P>0.05).

Lowest FCR (1.63±0.00) was examined in G3 dietary group and highest in G0 treated group (1.96±0.01) Survival rate was remained 100% throughout the experiment in control group and all 1-5% garlic treated groups (Table 4). Garlic is widely cultivated for its medicinal properties, including improving fish growth and resistance (Reda *et al*., 2024). Garlic compounds positively impact nitrogen balance in fish by enhancing the proteolytic activity of bacteria in the digestive tract of tested animals (Kamruzzaman *et al*., 2011). Garlic enhances the quality of fish flesh, primarily due to its organosulfur compounds (Shakya *et al*., 2014).

Herbs from many source reported to accelerate the gut secretions or activate the gut microflora to improve animal growth rate and high protein synthesis (Effendi *et al*., 2022). Xiang and Liu. 2002 reported that the growth performance of *pirapitinga fish (Colossoma barchypomum)* improved and then trend to decline with increasing amount of allicin. These results demonstrated that the high content of garlic extract or allicin do not accelerate the fish growth; instead, they are detrimental for the fish health. The reason is the presence of high alkyl sulfide access the intestine, disturbed the normal metabolism, and decreased the mitosis, subsequently results in slow growth and even death. This similar trend was noticed in present study.

Yang *et al*. (2010) found the ingestion behavior of larval *Ruditapes philippinarum* that result in aggressive behavior after being fed with garlic (gastrointestinal color became delicate), then recover again after the water was replaced. This showed the inhibitory effect of high dosage of garlic caused the suppression of ingestion when fed to larva and juvenile fish. Therefore, garlic level is not same in all fish species because optimal level is depending on species-specific (Lee and Gao, 2012). Various studies have been carried out to evaluate the effect of garlic in different forms on growth, activity of antioxidant enzymes and haematology of different fish species. In accordance to present results, Ajiboye *et al*. (2016) outlined the effect of garlic containing diet on the growth rate, rate of survival and utilization of nutrients in diet and body constituents of *Tilapia zillii* in a monosex culture. The results indicated that the group fed on 3% of garlic in diet showed the maximum gain in weight, increased rate of survival, considerably increased protein content and lowest FCR value as compared to control.

Garlic inclusion in fish feed improved fish health and increase growth performance in fish (Metwally, 2009). In synchronicity to present results, Maniat *et al*. (2014) noticed the effect of garlic powder on composition of body and growth of *Mesopotamichthys sharpeyi* (Benni fish). The results showed that there was an improvement in growth, feeding rate and efficiency was observed in a treatment fed with 1% of garlic powder in the diet. Shalaby *et al*. (2006) verified that diet consisted of 3% GP (garlic powder) was most effective to enhance the growth rate in *O. niloticus* and this concentration was in line with current study (3%). These positive effects may be related to the presence of bioactive compounds in GP such as ajoene, allicin, and allyl methyl thiosulfate, which positively influenced the processes involved in the somatic growth (Vaseeharan *et al*., 2011). Stimulation of appetite, increase the secretion of digestive enzymes, and modulation of intestinal microflora are due to the garlic diet which ultimately increase growth rate (Zaefarian *et al*., 2017).

The study of Moghazy *et al*. (2021) revealed that garlic inclusion in feed significantly reduced production costs for one kg fish weight gain and enhanced the profit index by 55.76% when fed simultaneously to eels. Garlic inclusion in fish diets accelerates growth, allowing fish to grow more quickly with a lower feed requirement, thereby lowering feed expenses (Guo *et al*., 2012). Garlic supplementation leads to a reduced FCR, which enhances feed utilization efficiency and results in greater economic benefits per unit of feed (Mustafa and Dikel, 2022).

Haematological parameters of L. rohita

Effect of dietary garlic on haematological parameters of *L. rohita* examined in control group and different experimental groups were given in Table 5, 6, 7. Parameters including white blood cells ($\times 10^3/\mu L$), neutrophils (%), lymphocytes (%), eosinophils (%), red blood cells ($\times 10^3/\mu L$), haemoglobin (g/dL), Hct (%), mean corpuscular haemoglobin (pg), mean corpuscular haemoglobin concentration (g/dL), and packed cell volume (%) were recorded significantly higher in G3 diet garlic (3%) treated group. Greater content of monocytes was recorded at G4 diet fed to fish while basophil remain non-significant ($P>0.05$) in all treated groups. On the other hand, MCV (fL) value was higher at G2 diet fed to fish.

Table 5: *Effect of different concentrations of dietary garlic on some haemotological parameters of L .rohita. Mean comparison for different hematological parameters with respect to garlic.*

Treatment	WBCs Count (×10³/µL)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
G0	8.78±0.00^{f}	27.00±0.05^{f}	74.00±0.42^{e}	3.00±0.29^{c}	4.00±0.29^{b}
G1	10.86±0.10^{e}	31.00±0.09^{d}	81.00±0.72^{d}	2.00±0.00^{c}	2.00±0.00^{c}
G2	12.00±0.07^{d}	30.00±0.23^{e}	89.00±1.15^{c}	3.00±0.00^{c}	1.00±0.00^{c}
G3	16.11±0.11^{a}	36.00±0.26^{a}	99.00±0.46^{a}	5.00±0.58^{b}	4.50±0.29^{a}
G4	15.00±0.15^{b}	35.00±0.13^{b}	92.00±0.19^{b}	6.50±0.29^{a}	5.50±0.58^{b}
G5	13.99±0.05^{c}	33.00±0.18^{c}	91.00±0.07^{c}	3.00±0.00^{c}	2.00±0.00^{c}

Table 6: *Effect of different concentrations of dietary garlic on some haemotological parameters of L .rohita. Mean comparison for different hematological parameters with respect to garlic.*

Treatment	Basophils (%)	RBCs C (×10³/µL)	Haemoglobin (g/dL)	Hct (%)
G0	0.40±0.00^{a}	1.25±0.02^{e}	6.01±0.01^{f}	16.45±0.06e
G1	0.50±0.00^{a}	1.31±0.01^{d}	6.64±0.01^{e}	17.12±0.10^{d}
G2	0.50±0.00^{a}	1.35±0.01^{d}	7.00±0.01^{d}	17.95±0.21^{c}
G3	1.00±0.00^{a}	1.59±0.01^{a}	8.62±0.04^{a}	19.33±0.10^{a}
G4	1.00±0.00^{a}	1.53±0.01^{b}	8.28±0.04^{b}	19.76±0.03^{a}
G5	1.00±0.00^{a}	1.44±0.00^{c}	7.47±0.04^{c}	18.59±0.19^{b}

In the present work effect of dietary garlic on haematological parameters of *L. rohita* was also examined. Haematological parameters in all garlic treated groups were increased (1-5%) by increasing the concentration of dietary garlic as compared to the control group. The results are in agreement with the study performed by Nwakpa & Ikwor, (2024) who analyzed that garlic plays a vital role in improving the number of RBCs, WBCs, value of hematocrit, platelets and concentration of Hb. A study conducted by (Onomu, 2019) outlined that catfish (*Clarias gariepinus*) fingerlings fed with (0.5%) garlic containing diet caused an increase in rate of growth and haematological parameters by supplementation of garlic in fish feed. Pashaki *et al.* (2018) verified that five grams per kilogram GE (garlic extract) supplementation in diet of fingerlings of *Cyprinus carpio* can cause the improvement in parameters of blood, immune system as well as the growth rate.

Determination of antioxidant enzyme activity

At the end of trial, in serum of *L. rohita*, effects of different concentrations of dietary garlic (1%-5%) were observed for analysis of CAT, SOD and GPx enzyme activity (U mg^{-1} Protein) (Figure 1). According to mean square (ANOVA), the effect of garlic on the enzyme activity (U mg^{-1} Protein) showed significant ($P<0.05$) results as compared to control. However highest enzyme activity (U mg^{-1} Protein) was recorded in a group fed with G3 (3%) garlic diet. This, may be due to the garlic has allicin that combine with oxygen and form many compounds such as diallyl disulfide, diallyl trisulfide and diallyl sulfide which increased antioxidant activity.

Garlic functions as a natural antioxidant that helps to reduce oxidative stress in fish. This reduction in oxidative stress contributes to better growth by safeguarding cells from potential damage (Oz, 2018). Khalil *et al.* (2001) found that allicin, a compound in garlic, improves digestion, antioxidant activity and boosts energy utilization. Metwally (2009) carried

Table 7: *Effect of different concentrations of dietary garlic on some haemotological parameters of L .rohita. Mean comparison for different hematological parameters with respect to garlic.*

Treatment	MCH (pg)	MCHC (g/dL)	MCV (fL)	PCV %
G0	48.08±0.19^{c}	36.53±0.39^{c}	131.60±0.76^{ab}	16.45±0.09^{c}
G1	50.68±0.52^{b}	38.78±0.57^{b}	130.69±1.31^{ab}	17.12±0.18^{c}
G2	51.85±0.53^{b}	39.01±0.08^{b}	132.96±1.66^{a}	17.94±0.00^{b}
G3	54.38±0.34^{a}	43.04±0.32^{a}	126.34±0.48^{bc}	19.33±0.04^{a}
G4	51.59±0.10^{b}	40.92±0.00^{b}	124.28±1.50^{c}	18.76±0.09^{b}
G5	51.87±0.22^{b}	40.18±0.09^{b}	129.09±0.92^{abc}	18.59±0.27^{b}

Means sharing similar letter in a column are statistically non-significant ($P>0.05$).

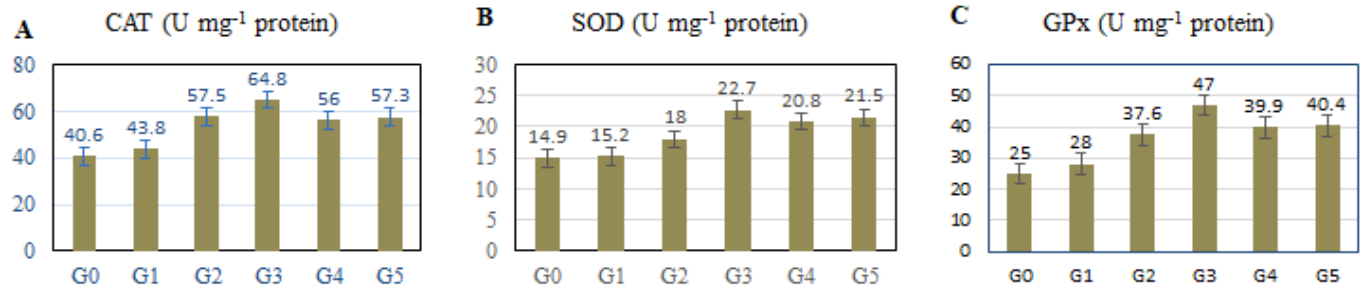

Figure 1: *Mean comparison for antioxidant enzymes activity with respect to garlic fed to L.rohita.*

out study to determine the effects of garlic (*Allium sativum*) on antioxidant system in Tilapia (*Oreochromis niloticus*). The results showed that, antioxidant activity influenced by feeding garlic supplement to fish. According to Zhao *et al.* (2024) garlic has the capacity of increasing antioxidant activity in serum and reduced the levels of plasma glucose in fish. The defense mechanism of tilapia (*Oreochromis niloticus*) has been enhanced by the dietary inclusion of garlic in feed of fish (Diab *et al.*, 2002) as similar with present work, garlic supplementation increased the antioxidant enzymes activity at 3% G3 diet. In agreement with our results, a study carried out by Carmi (2001) reported the role of garlic as a promoter of antioxidant enzymes because they scavenged the ROS and increased the CAT, SOD and GPx enzyme activity.

According to Diab *et al.* (2002) garlic has ability to enhance the antioxidant enzymes activity in fish serum. Moreover, Jahanjoo *et al.* (2018) exhibited high activity of antioxidant enzymes when fish fed with 1% garlic diet and level was low as compared current study. Schulz *et al.* (2004) described that garlic has attributes of antioxidants and it can inhibit or reduce the lipoxygenase while promotes the antioxidant enzymes in hamsters. A study conducted by (Chung, 2006) proved that garlic and extracts of garlic have very conspicuous antioxidant properties. So, they render defense to the body against destructive effects of free radicals.

To cope with multiple and interconnected challenges ranging from the impact of the current economic and financial crisis in a country like Pakistan, the role of garlic is more efficacious as a natural, cheap and secure growth promoter. Nevertheless, under practical conditions, further research is necessary.

Conclusions and Recommendations

It was elucidated that supplementation of garlic in diet of *L. rohita* enhanced the growth performance, proliferated the number of blood cells and due to intrinsic antioxidant properties of garlic catalase enzyme activity in serum was also modified. Hence, garlic has miscellaneous characteristics for fish growth and production as an alternative to chemotherapeutic agents. Finally, treatment group fed with 3% garlic was suggested. Moreover, these results may provide the basis line and novel insights for further study for the improvements in growth performance and fish health by adding garlic powder to promote sustainable aquaculture, as it's a cheap source of additive, especially in developing countries such as Pakistan.

Ethical approval

The study was approved by the ethical committee, Government College Women University, Sialkot, Pakistan. (D/REG/EIRB/4000). The experiment has been performed according to relevant guidelines.

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Novelty Statement

This research showed a novel investigation into the dietary supplementation of *Allium sativum* and its effect on the growth performance, haematology and antioxidant enzymes of *Lebeo rohita*. The outcomes provide valuable insights to a growing body of evidence substantiating the use of natural dietary supplements as garlic to promote the growth of fish and act as sustainable and eco-friendly substitute for

synthetic constituents in aquaculture.

Author's Contribution

Moazama Batool: Supervision, Project Administration, Conceptualization, Funding Acquisition.
Huma Naz & Qurat UL Ain: Data Curation, Visualization, Writing - review & editing, Formal Analysis.
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