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Original article

Growth, Antioxidant Defense, and Immune Response in *Labeo rohita* Fingerlings with the Dietary Vitamin C Supplementation

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ABSTRACT

The study was designed to investigate the vitamin C supplementation effect on growth, oxidative stress, and immune response of *Labeo rohita*. Four diets 0, 200, 400, and 800 mg/kg was designed, 480 fingerlings with the initial weight 6.11 ± 0.08 g in triplicate groups were randomly distributed into 12 fiberglass tanks for 60 days. Growth results showed that final weight (13.87 ± 0.23–19.83 ± 0.34 g), weight gain (152.6 ± 2.7–251.1 ± 3.5%), and specific growth rate (1.91 ± 0.06–2.52 ± 0.03%/day) increased significantly ($p < 0.05$) with increasing dietary vitamin C levels, while feed conversion ratio (1.81 ± 0.04–1.29 ± 0.04) decreased ($p = 0.006$), indicating improved feed utilization.

Survival rates were 91.1 ± 2.4% in the control, whereas they reached 98.4 ± 1.4% in the 800 mg/kg group. Antioxidant enzyme showed a significant ($p < 0.01$) enhancement. Superoxide dismutase (SOD) and catalase (CAT) activities increased from 25.6 ± 1.3 to 36.1 ± 1.3 U/mg protein and 12.4 ± 0.7 to 20.1 ± 0.5 U/mg protein. The malondialdehyde (MDA) levels reduced significantly ($p = 0.005$), while glutathione (GSH) concentration increased significantly ($p = 0.007$) from 3.10 ± 0.13 to 4.41 ± 0.13 µmol/g tissue. Immune response parameters were also positively affected by dietary vitamin C. Lysozyme activity increased significantly ($p = 0.008$) from 15.4 ± 0.3 to 23.4 ± 0.6 U/mL, respiratory burst activity improved from 0.178 ± 0.006 to 0.261 ± 0.006 OD_{630} nm, and phagocytic activity rose from 28.3 ± 1.4 to 41.4 ± 1.4%. Similarly, nitric oxide production increased from 4.13 ± 0.28 to 6.86 ± 0.26 µmol/L. Total protein, albumin, and globulin were also significantly raised ($p < 0.05$). The study concluded that dietary vitamin C can be used to increase growth, support antioxidant defense, and improve immune responses in *L. rohita*

KEYWORDS: *Labeo rohita*, Vitamin C, Growth performance, Oxidative stress, Immune response.

1. INTRODUCTION

Aquaculture in many developing regions fastest-expanding sector, contributing significantly to global food production, economic development, nutritional security, and livelihood support (Irshath *et al*., 2023). In South Asia, aquaculture, predominantly in Pakistan, India, and Bangladesh, relies heavily on inland fish production, including *Labeo rohita*, *Catla catla*,

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and *Cirrhinus mrigala* (Abdul-Qadir *et al*., 2024). Among these, *L. rohita* is recognized as the most commercially valuable freshwater species, with adaptability to semi-intensive and intensive culture systems and a fast growth rate (Gupta *et al*., 2021). However, high stocking densities increased feeding rates, feed efficiency, and survival in aquaculture, but imperfect water exchange disrupted immunity, caused physiological stress, and increased susceptibility to infectious disease (Hossain *et al*., 2022; Namiq *et al.,* 2025). One of the most effective ways to reduce such adverse effects is through nutritional changes, such as integrating micronutrients and antioxidants into the diet, including vitamins and minerals that play critical roles in maintaining metabolic function, immune function, and oxidative balance (Ibraheem et al., 2017; Awuchi *et al*., 2022). Among these, vitamin C (ascorbic acid), due to its multifaceted physiological functions responsible for synthesizing glucose in the liver, maintaining health and performance in cultured fish species, and interpreting them as completely dependent on dietary sources of vitamin C (Jiménez *et al*., 2018). Vitamin C directly interacts synergistically with lipid-soluble antioxidants and reactive oxygen species with free radicals, allowing for the protection of cellular membranes from oxidative damage (Soldado *et al*., 2021). Furthermore, vitamin C increases the activities of antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase, and minimizes oxidative injury in tissues (El-Gendy *et al*., 2010). The disparity between the making of ROS and the antioxidant defense system leads to oxidative stress, subsequent protein denaturation, and lipid peroxidation that impairs growth and biological performance; however, the vitamin C supplementation in fish diets progresses antioxidant enzyme activities, improving oxidative stress in *Oreochromis niloticus* (Surai *et al*., 2019) and *Clarias gariepinus* (Abalaka *et al*., 2021).

The antioxidative role of vitamin C is closely linked to the enhancement of immune responses against invading pathogens in fish, and its efficacy is also influenced by nutritional status (Ibrahim *et al*., 2021). Vitamin C supplementation modulates immune parameters, including lysozyme activity, respiratory burst, nitric oxide production, and serum protein profiles (Firouzi *et al*., 2022). Furthermore, it also enhances macrophage and neutrophil activity and enhances antibody production (Zhang *et al*., 2023). The dietary intake of vitamin C enhances resistance to bacterial infections, increasing lysozyme and respiratory burst activities in freshwater fish (Xue *et al*., 2022). Moreover, vitamin C plays an essential role in reducing immunosuppression induced by ecological or nutritional stressors by maintaining the functional integrity of the immune system, and it also interacts with other dietary components that help regulate cellular signaling pathways involved in immune parameters (Tang *et al*., 2024). These nutritional formulations with vitamin C are important, especially at the fingerling stage, for the rapid growth, metabolic activity, and greatly enhance survival under intensive culture systems (Xu *et al*., 2022). Despite significant progress in nutritional physiology, the rarity persists in dietary vitamin C data on growth, oxidative stress biomarkers, and immune responses of *Labeo rohita* fingerlings. Thus, the present study was directed to assess the outcome of dietary vitamin C supplementation on growth, oxidative stress biomarkers, and immune response parameters in *L. rohita* fingerlings.

2. MATERIALS AND METHODS

Experimental Design:

This present study was planned to examine the outcome of dietary vitamin C on the growth, oxidative stress biomarkers, and immune response of *L. rohita* fingerlings. 480 total of L. rohita fingerlings with an average initial weight of 6.112± 0.10 g were purchased from the Government Hatchery, Lahore, Punjab, Pakistan. Laboratory conditions for a period of 14 days were provided to the experimental fish, which were fed a basal diet twice daily during this period, and acclimated in fiberglass tanks (400 L capacity of water). The fingerlings, after acclimation, were randomly distributed among 12 fiberglass tanks at a stocking density of 40 fish per tank. Four dietary treatments with triplicate were designed, viz, control diet (0 mg/kg vitamin C) and three diets supplemented with 200, 400, and 800 mg/kg vitamin C, respectively. Siphoning (20% of the water removed from each tank) was performed daily to remove debris and uneaten feed throughout the experiment.

The fish were fed at 5% of their body weight for 60 days, the water parameters were recorded such as temperature; 26.5 ± 0.4°C, dissolved oxygen; 7.12 ± 0.06 mg/L, and pH; 7.98 ± 0.02 and total ammonia nitrogen (TAN) was maintained through regular water exchange below 0.05 mg/L, while nitrite (NO_2^-) levels remained below 0.1 mg/L throughout the experimental period. Continuous aeration was provided using air stones connected to a central air compressor. After the end of the study period, the experimental fish were fasted for 24 hours before sampling. 40 fish from each group were randomly selected for both the biochemical and growth analyses. The clove oil (50 mg/L) was used, and fish were anesthetized, dissection performed, liver tissue was removed and controlled in ice-cold phosphate buffer, and centrifuged at 4°C. Blood samples were collected using sterile heparinized syringes from the caudal vein, centrifuged at 3,000 rpm for 10 min at 4 °C to obtain serum, and stored at −20 °C until analysis of immune response parameters. The protocol was conducted in accordance with subsequent ethical standards for fish nutrition research (Mohamad Hassan *et al*., 2025).

Dietary Preparation:

The study used the following ingredients: fish meal, soybean meal, maize gluten, rice bran, and wheat flour, which were finely crushed and carefully mixed using an automatic mixer. Vitamin C (It was provided in the diet in the form of ascorbyl-2-polyphosphate) at inclusion levels of 0, 200, 400, and 800 mg/kg diet was combined into the basal diet. After the dry mixing, fish oil and tolerable purified water were added to get a consistent dough appropriate for pelleting. The laboratory pellet machine equipped with a 2-mm die was used to produce uniform pellets. The pellets were air-dried at room temperature (28–30°C) for 48 hours, followed by storage in airtight containers at 4°C until further use to preserve nutrient veracity. The comprehensive composition of experimental diets is presented in Table 1.

Table 1: The diet composition for *Labeo rohita* fingerlings

Ingredients (g/kg)	Control (0 mg/kg Vit C)	Vit C (200 mg/kg)	Vit C (400 mg/kg)	Vit C (800 mg/kg)
Fish meal	200	200	200	200
Soybean meal	300	300	300	300
Maize gluten meal	120	120	120	120
Rice bran	150	150	150	150
Wheat flour	150	150	150	150
Fish oil	50	50	50	50
Vitamin–mineral premix[1]	20	20	20	20
Dicalcium phosphate	5	5	5	5
Vitamin C (mg/kg) [2]	–	200	400	800
Total	1000	1000	1000	1000

Note: Vitamin–mineral premix such as vitamin A, vitamin D_3, vitamin E, vitamin K_3, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, folic acid, biotin, calcium pantothenate, choline chloride, Fe, Zn, Mn, Cu, I, and Se.

Growth Performance Metrics:

Growth parameters evaluation performed by using the following standard formulas:

$$WG, \% = [(FBW - IBW) / IBW] \times 100$$

$$SGR, \%/day = [(\ln FBW - \ln IBW) / Duration (days)] \times 100$$

$$FCR = FI (g) / WG (g)$$

$$Survival\ rate (\%) = (Final\ fish\ number / Initial\ fish\ number) \times 100.$$

Antioxidant Enzyme Activities:

The antioxidant enzymatic markers were examined, such as the Superoxide dismutase (SOD) activity recorded by the inhibition of pyrogallol auto-oxidation at 560 nm spectrophotometrically. Catalase (CAT) activity was examined by the rate of hydrogen peroxide decomposition with recorded absorbance at 240 nm. Lipid peroxidation was estimated by malondialdehyde (MDA) levels by (TBARS) assay, at 532 nm. GSH (Reduced Glutathione) concentration was determined according to Ilhan *et al*. (2004). Total Protein content was assessed using the method of Moore et al. (2010).

Immune Response Assays:

Immune parameters were analyzed using serum samples obtained from blood centrifugation and stored at −20 °C until analysis. Lysozyme activity was determined using the turbidimetric method described by Fazal *et al.* (2025) with *Micrococcus lysodeikticus* as the substrate, and results were expressed as U/mL serum based on the reduction in absorbance at 530 nm. Respiratory burst activity was measured following Rothe *et al.* (1991) using the nitroblue tetrazolium (NBT) reduction assay and expressed as optical density at 600 nm. Phagocytic activity was assessed according to Pavlou *et al.* (2017) and expressed as percentage phagocytosis. Nitric oxide (NO) production was measured in serum using the Griess reagent, following Privat et al. (1997), with absorbance read at 540 nm and results expressed as µmol/mL. Serum total protein, albumin, and globulin concentrations were determined using the Biuret method, bromocresol green method, and calculated difference, respectively, following Katsoulos et al. (2017), and expressed as g/dL.

3. RESULTS

The vitamin C had a significant effect on the growth performance of *L. rohita* (Table 2). The IW of fish did not fluctuate significantly ($p > 0.05$) among the treatments. The FW was 13.87 ± 0.23 g in the control, 19.83 ± 0.34 g in the 800 mg/kg vitamin C group, which was significantly increased ($p = 0.007$). Similarly, WG at the highest vitamin C level was 251.1 ± 3.5% and lowest in the control (152.6 ± 2.7%) ($p = 0.008$). The SGR was 1.91 ± 0.06%/day in the control group, which improved to 2.52 ± 0.03%/day in the 800 mg/kg group significantly ($p = 0.007$). The FCR in the control group was 1.81 ± 0.04, whereas a gradual decline was observed in the 400 and 800 mg/kg vitamin C diets, with FCR values of 1.34 ± 0.06 and 1.29 ± 0.04, respectively ($p = 0.006$). The survival rate was 91.1 ± 2.4% in the control group and 98.4 ± 1.4% in the 800 mg/kg vitamin C group, which indicated significant ($p = 0.006$) improvement.

Table 2: The measurements of growth parameters on different grades of vitamin C supplementation in *Labeo rohita* fingerlings.

Parameters	Control (0 mg/kg Vit C)	Vit C (200 mg/kg)	Vit C (400 mg/kg)	Vit C (800 mg/kg)	*p*-value
IW (g)	6.11± 0.08	6.13± 0.06	6.14 ± 0.07	6.12± 0.05	NS
FW (g)	13.87 ± 0.23^{d}	15.73 ± 0.34^{c}	17.83± 0.42^{b}	19.83 ± 0.34^{a}	0.007
WG (%)	152.6± 2.7^{d}	189.7± 3.4^{c}	233.6± 4.6^{b}	251.1 ± 3.5^{a}	0.008
SGR (%/day)	1.91± 0.06^{d}	2.16± 0.05^{c}	2.44± 0.04^{b}	2.52± 0.03^{a}	0.007
FCR	1.81± 0.04^{a}	1.51± 0.06^{b}	1.34± 0.06^{c}	1.29± 0.04^{c}	0.006
SR (%)	91.1± 2.4^{b}	95.4± 1.7^{a}	96.4± 1.8^{a}	98.4 ± 1.4^{a}	0.006

The effect of vitamin C on the oxidative stress biomarkers of fingerlings is presented in Table 3. The SOD activity was 36.1 ± 1.3 U/mg protein in the 800 mg/kg vitamin C group, while 25.6 ± 1.3 U/mg protein was recorded in the control group ($p = 0.006$). Similarly, CAT activity was 20.1 ± 0.5 U/mg protein in the 800 mg/kg group, whereas 12.4 ± 0.7 U/mg protein was observed in the control group, which showed a significant ($p = 0.007$) enhancement. In contrast, the MDA concentration was 6.33 ± 0.24 nmol/mg protein in the control group, but 3.27 ± 0.17 nmol/mg protein was recorded in the group receiving 800 mg/kg vitamin C. This decrease in MDA values reflected a reduced oxidative damage and cellular lipid peroxidation. GSH levels increased significantly ($p = 0.007$): in the 800 mg/kg group, 4.41 ± 0.13 µmol/g tissue; and in the control group, 3.1 ± 0.13 µmol/g tissue.

Table 3: The measurement of oxidative stress biomarkers at various vitamin C levels in *L. rohita*.

Parameters	Control (0 mg/kg Vit C)	Vit C (200 mg/kg)	Vit C (400 mg/kg)	Vit C (800 mg/kg)	p-value
SOD, U/mg protein	25.6±1.3^{c}	31.6±1.2^{b}	34.7± 1.4^{a}	36.1±1.3^{a}	0.006
CAT, U/mg protein	12.4±0.7^{c}	16.3±0.6^{b}	17.6 ± 0.6^{a}	20.1±0.5^{a}	0.007
MDA, nmol/mg protein	6.33±0.24^{a}	4.21±0.12^{b}	3.44± 0.16^{c}	3.27±0.17^{c}	0.005
GSH, µmol/g tissue	3.1±0.13^{c}	3.56±0.12^{b}	4.34± 0.02^{a}	4.41±0.13^{a}	0.007

SOD; for Superoxide dismutase (U/mg protein), CAT; For Catalase (U/mg protein), MDA; for malondialdehyde (nmol/mg protein), GSH; Glutathione peroxidase (µmol/g tissue).

Dietary supplementation with vitamin C on *L. rohita* revealed a significant ($p < 0.01$) effect on non-specific immune responses. Lysozyme activity was 15.4 ± 0.3 U/mL in the control group and 23.4 ± 0.6 U/mL in the 800 mg/kg group. The respiratory burst activity was significantly enhanced compared with the control group, from 0.178 ± 0.006 to 0.261 ± 0.006 OD_{630} nm at the highest vitamin C level. The Phagocytic activity was 28.3 ± 1.4% in the control group and 41.4 ± 1.4% in the 800 mg/kg group. Nitric oxide concentration rising from 4.13 ± 0.28 µmol/L in the control to 6.86 ± 0.26 µmol/L in the highest supplemented group ($p = 0.005$). Total protein improved from 3.15 ± 0.03 g/dL in the control up to 4.01 ± 0.05 g/dL, while albumin and globulin also enhanced significantly. The highest albumin level (1.59 ± 0.02 g/dL) and globulin level (2.41 ± 0.06 g/dL) were detected in the 800 mg/kg group.

Table 4: The evaluation of non-specific immune responses and serum biochemical parameters at various levels of vitamin in *L. rohita*.

Parameters	Control (0 mg/kg Vit C)	Vit C (200 mg/kg)	Vit C (400 mg/kg)	Vit C (800 mg/kg)	p-value
Lysozyme activity (U/mL)	15.4± 0.3^{d}	18.6± 0.7^{c}	22.4± 0.6^{b}	23.4± 0.6^{a}	0.008
Respiratory burst activity (OD_{630} nm)	0.178 ± 0.006^{d}	0.211± 0.005^{c}	0.239± 0.007^{b}	0.261± 0.006^{a}	0.007
Phagocytic activity (%)	28.3± 1.4^{d}	31.9± 1.3^{c}	38.7± 1.6^{b}	41.4± 1.4^{a}	0.007
Nitric oxide (µmol/L)	4.13± 0.28^{d}	5.31± 0.21^{c}	6.41± 0.23^{b}	6.86± 0.26^{a}	0.005
Total protein (g/dL)	3.15 ± 0.03^{d}	3.31± 0.05^{c}	3.76± 0.06^{b}	4.01± 0.05^{a}	0.006
Albumin (g/dL)	1.31± 0.03^{d}	1.37± 0.06^{c}	1.55± 0.08^{b}	1.59± 0.02^{a}	0.006
Globulin (g/dL)	1.71± 0.04^{d}	2.23± 0.06^{c}	2.41 ± 0.05^{b}	2.41 ± 0.06^{a}	0.006

4. DISCUSSION

The improvement in growth parameters indicates a positive effect on growth and feed efficiency at higher vitamin C levels. Ibrahim *et al*. (2020) studied Oreochromis niloticus and described the effect of vitamin C on growth performance, collagen synthesis, and connective tissue development. Narra *et al*. (2015)
described that vitamin C in *Clarias gariepinus* increases the metabolic processes and growth parameters, with the muscle development and tissue repair. Fasil *et al*. (2021) reported that dietary vitamin C supplementation reduced oxidative stress and improved nutrient utilization, thereby improving growth outcomes. The present findings are also similar to Abdelwahab *et al*. (2020), who described that ascorbic acid controls endocrine and metabolic functions, promotes appetite, and protein combination in sea bass.

This study described the improved feed conversion ratio with vitamin C. Similarly, Magouz *et al*. (2020) also studied the enhanced digestive efficiency, improved FCR by stimulating enzyme activity that leads to better nutrient absorption and energy utilization. Increasing vitamin C supplementation increases survival by strengthening physiological tolerance and reducing stress-induced mortality. This observation agrees with Liua *et al*. (2016), who studied that vitamin C improves the protection of fish resilience against pathogens and stress, with the maintenance of the survival rate. Biswas *et al*. (2020) studied that vitamin C regenerates other antioxidants and neutralizes reactive oxygen species, and increasing the survival rate. In the present study, enhanced enzymatic activities with vitamin C thereby mitigating oxidative damage. Similar trends were reported by Daniel *et al*. (2021), who found that vitamin C supplementation significantly increased SOD and CAT activities in Pangasius pangasius, indicating oxidative stress. Moreover, elevated vitamin C concentrations led to a significant decrease in malondialdehyde levels, indicating reduced oxidative tissue damage and lipid peroxidation in *L. rohita*. This decrease in MDA is consistent with earlier findings in *Oreochromis niloticus* and *Cyprinus carpio*, in which a vitamin C supplement protects membrane lipids from degradation and reduces lipid peroxidation (Sherif *et al*., 2024). The GSH, as a major non-enzymatic antioxidant, increased with the increase of vitamin C, which supports the maintenance of redox balance and detoxification of free radicals. These results agree with Singh et al. (2017), who reported that dietary vitamin C enhances GSH synthesis, thereby supporting the defense of cells against oxidative injury and antioxidant capacity. Vitamin C also improves both humoral and cellular immune mechanisms in *L. rohita* in the present study which is align with findings by Misra *et al*. (2007) who described the enhanced lysozyme and increased respiratory burst activities in fish fed vitamin-supplemented diets. Alberts *et al*. (2025) studied the higher macrophage, oxide production, and neutrophil activity with improved pathogen-killing ability and leukocyte function. Nitric oxide, with a higher level during immune responses, strengthened immune functionality (Gao *et al*., 2021). The improvement in serum biochemical parameters with vitamin C regarded as important indicators of immune and nutritional status. The total protein and globulin levels improved the synthesis of immunoglobulins and improved immune balance. Taalab *et al*. (2022) designated improved serum protein with the metabolic efficiency in *Clarias gariepinus* with vitamin C supplementation.

CONCLUSION

The present findings indicate that dietary vitamin C increases growth performance, decreases oxidative stress, and reinforces the immune system of *L. rohita* fingerlings, and suggest that vitamin C supplementation at 800 mg/kg is necessary to improve growth, health, and immune competence in *L. rohita*.

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Ethical Statement:

The study protocol was approved by the, Lahore College for Women University (Eithic Approval Code No. LCWU: 2025:254).

Author Contributions:

Z. A., D. K., and H. A. Conceptualization, and Methodology. B. S. A., N. H., Data Curation, Formal Analysis and Investigation Z. S., R. H., and M. H. M., Writing – Original Draft Preparation.
Z. S., and B. S. A., Project Administration, Validation, Writing, and Revision.

Declaration of Competing Interest:

The authors declare that they have no competing financial interests.

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