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# DIETARY TURMERIC (*Curcuma longa*) ENHANCES GROWTH, ANTIOXIDANT DEFENSE, AND IMMUNITY IN *Cirrhinus mrigala*

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**ABSTRACT:**

Aquaculture is pivotal in addressing global food security, yet intensive practices compromise fish health and productivity. This study investigated the efficacy of dietary turmeric (*Curcuma longa*) supplementation as a natural alternative in *Cirrhinus mrigala*, a species critical to South Asian aquaculture. A 60-day feeding trial evaluated four diets: control (0% turmeric), 0.5%, 1.0%, and 1.5% turmeric inclusion. Results demonstrated that 1.0% turmeric significantly enhanced growth performance, with a 28% increase in final body weight (19.6 ± 1.4 g vs. 15.7 ± 1.1 g in control; $p< 0.01$) and improved feed conversion ratio (1.4 ± 0.2 vs. 1.7 ± 0.2; $p= 0.004$). Antioxidant enzyme activities (SOD, CAT, GPx) increased by 34–53%, while lipid peroxidation (MDA) decreased by 29% ($p = 0.001$), indicating robust oxidative stress mitigation. Immunological assays revealed a 65% rise in lysozyme activity and 70% higher phagocytic capacity ($p< 0.01$), underscoring turmeric's immunostimulatory potential. The hepatosomatic index decreased by (1.3 ± 0.2% vs. 1.4 ± 0.2%; $p = 0.038$), suggesting increased metabolic efficiency. These findings highlight 1.0% turmeric as the optimal dosage, offering a sustainable strategy to augment aquaculture productivity while reducing reliance on antibiotics.

**KEYWORDS:** *Curcuma longa*, Growth, Antioxidant Defense, Immunity, *Cirrhinus mrigala*

## 1. INTRODUCTION

Fish farming has emerged as a vital approach to meet the growing global demand for seafood, currently supplying more than 50% of the fish eaten worldwide (Khanjani *et al*., 2024; Nabi *et al*., 2025). The growth of aquaculture is crucial for developing economies, as seafood provides a cost-effective, nutritious protein source (Munguti *et al*., 2024; Rashia *et al*., 2025). Large-scale aquaculture has made significant advancements due to improvements in health and sustainability in potentially in different environmental concerns, while chronic stress and high stocking densities decrease the farm productivity and fish welfare (Hematyar *et al*., 2024; Yadav *et al*., 2024). The use of antibiotics has become a major global health issue in aquaculture because many fish farms rely on antibiotics, and 80% antibiotic resistance genes were found in aquaculture water samples of different fish farms (Hossain *et al*., 2022; Rasul *et al*., 2025). To minimise these emerging current issues, the global regulatory authorities restricted the use of antibiotics in aquaculture and also addressed the alternative health solutions, such as plant-based feed additives, which emerged as a promising alternative that can give numerous health benefits (Mithuna *et al*., 2024; Pitiot *et al*., 2025; Rasheed *et al*., 2024).

*Cirrhinus mrigala* (freshwater carp) is an important aquaculture species found in South Asia, valued for its nutritional benefits, delicious taste, and fast growth (Meitei *et al*., 2025). *C. mrigala* plays a vigorous role in food security and supports the various small-scale fish farmers of different regions of South Asia (Bhattacharya *et al*., 2024; Debnath *et al*., 2025). Moreover, during the intensive farming of *C. mrigala,* especially during the monsoon seasons, it becomes a greater chance of bacterial infections that cause stunted growth in unhealthy water conditions, and these environmental changes disturb the fish's immunity and disrupt the disease patterns (Al Sulivany *et al*., 2024; Kasihmuddin *et al*., 2024).

*Curcuma longa* L.*,* commonly known as turmeric, contains bioactive compounds such as bisdemethoxycurcumin and demethoxycurcumin (Hafeez *et al*., 2024; Roney *et al*., 2025). It is a powerful natural feed supplement that maintains unhealthy water conditions and offers significant health and growth benefits (Bhatt et al., 2024; Fan *et al*., 2025). Moreover, it is efficiently absorbed by fish through commercial feed (Abdelkarim *et al*., 2025; Naghdi *et al*., 2024). Turmeric also plays a powerful role as an antioxidant, boosting the fish's natural defense systems and neutralizing harmful free radicals through various biological pathways (Bouyahya *et al*., 2024; Esmaealzadeh *et al*., 2024). In aquaculture, it improved the biosynthesis of antioxidant

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enzymes, including Superoxide dismutase and catalase, and reduced cellular oxidative stress markers (Fan *et al*., 2025; Jomova *et al*., 2024).

*C. longa* supplementation in aquaculture enhances various physiological adaptations, such as significant gastrointestinal enhancements, improved intestinal villi architecture, and enterocyte regeneration in specimens receiving dietary turmeric inclusion (Khaldoun *et al*., 2025; Song *et al*., 2024).

Due to wide research on the aquacultural benefits of *C. longa* the optimum application procedures for *C. mrigala* cultivation require further explanation; However, turmeric's effective dosage typically differs, typically ranging from 0.5-2% of feed (Komal *et al*., 2024; Wildhaber *et al*., 2023). The research gaps remain regarding *C. longa* long-term effects on meat quality and fish health (Ringø *et al*., 2023). This experimental study investigates the dietary turmeric supplementation in the diet of *C. mrigala* to check the growth, antioxidant defence, and immunity.

## 2. MATERIALS AND METHODS

The current experimental study was conducted at the Saline Water Aquaculture Research Centre (SWARC), Muzaffargarh. The 216 juvenile experimental fish (*C. mrigala*) with an average weight of 5.3 ± 0.14 g were purchased from the Fish Hatchery, SWARC, Muzaffargarh. Four treatment groups with three replicates were designed: T1 (control, 0% turmeric), T2 (0.5% turmeric), T3 (1.0% turmeric), and T4 (1.5% turmeric). Before the experiment, fish underwent a 15-day acclimation period in a 2000L fiberglass tank with continuous aeration, daily 30% water exchange, and twice-daily feeding of a standard 32% protein diet at 3% body weight. Healthy fish showing active swimming behavior, clear eyes, intact fins, uniform coloration, and bright red gills were selected and randomly stocked into twelve 300L experimental tanks (18 fish per tank). Each tank was equipped with individual water circulation, separate aeration, 1mm mesh covers, and waste collection trays. Water quality was rigorously maintained at 28-30°C (using submersible heaters), dissolved oxygen >5.8 mg/L, pH 7.4-7.8 (adjusted with sodium bicarbonate), and ammonia <0.1 mg/L. All parameters were monitored three times daily using calibrated Hanna HI98194 meters to ensure experimental consistency throughout the 60-day trial period. The research framework enabled precise assessment of *C. longa's* physiological impacts on *C. mrigala* under standardized aquaculture parameters (Hoseini & Al Sulivany, 2024; Owais *et al*., 2024).

**Experimental Diets and Feeding Protocol:**

Four isonitrogenous (32% crude protein) experimental feeds were formulated with graded *C. longa* inclusions (0-1.5%). All diets utilized premium fishmeal and soybean meal protein sources with complete vitamin-mineral fortification. Feed production required accurate ingredient measurement, homogenization, and extrusion into 2mm pellets using 35°C purified water. After 48 hours of drying at 28°C, pellets were vacuum-packed and frozen to maintain nutritional quality. The 60-day experiment involved twice-daily feedings (3% biomass), with rations adjusted weekly. Uneaten pellets were removed after 45 minutes to prevent water contamination. The study employed twelve 300-litre tanks (18 fish/tank), randomly assigned to four treatment groups with three replicates (Owais *et al*., 2023; Al Sulivany *et al*., 2024a; Abdulrahman & Al Sulivany, 2025).

**Table 1:** Composition of experimental diets supplemented with graded levels of turmeric powder (g/kg)

| Ingredients | T1 (0%) | T2 (0.5%) | T3 (1.0%) | T4 (1.5%) |
|---|---|---|---|---|
| Fish meal | 190 | 190 | 190 | 190 |
| Soybean meal | 80 | 80 | 80 | 80 |
| Cottonseed cake | 110 | 110 | 110 | 110 |
| Wheat bran | 310 | 308.5 | 307 | 305.5 |
| Wheat pollard | 130 | 130 | 130 | 130 |
| Maize germ | 100 | 100 | 100 | 100 |
| Soybean oil | 18 | 18 | 18 | 18 |
| Vitamin-mineral premix* | 12 | 12 | 12 | 12 |
| Turmeric powder | 0 | 1.5 | 3.0 | 4.5 |
| Total (g) | 950 | 950 | 950 | 950 |

Note: *Vitamin-mineral premix per kg of feed: Vitamin A – 7200 IU, Vitamin $D_3$ – 1600 IU, Vitamin E – 200 IU, Vitamin C – 90 mg, Vitamin $B_1$ – 1.5 mg, Vitamin $B_2$ – 10 mg, Vitamin $B_6$ – 8 mg, Vitamin $B_{12}$ – 0.02 mg, Niacin – 55 mg, Pantothenic acid – 40 mg, Folic acid – 0.08 mg, Biotin – 1.8 mg, Copper – 6 mg, Iron – 110 mg, Zinc – 30 mg, Manganese – 18 mg, Iodine – 0.6 mg, Selenium – 0.2 mg, and Cobalt – 0.12 mg.

**Growth Performance Assessment:**

Following the 60-day feeding period, all *C. mrigala* specimens underwent a 24-hour fasting period to ensure complete gut clearance before final measurements. Individual fish were carefully weighed using a precision digital balance (accuracy ±0.01g) to determine final body weights (Al Sulivany *et al*., 2024b).

Weight gain (g) = Final weight - Initial weight

Specific growth rate (%/day) = [(ln final weight - ln initial weight)/days] × 100

Feed conversion ratio = Total feed intake (g)/Weight gain (g)

Protein efficiency ratio = Weight gain (g)/Protein intake (g)

Survival rate (%) = (Final fish number/Initial fish number) × 100

Hepatosomatic index (%) = (Liver weight/Body weight) × 100

Measurements followed standard aquaculture protocols using calibrated equipment. Three technicians independently verified all calculations to ensure accuracy. The hepatosomatic index was

determined through careful dissection and weighing of liver tissues from sampled specimens.

**Antioxidant Enzyme Activities:**

To evaluate antioxidant and immune responses, blood samples were obtained from sedated fish following standard protocols. For each treatment group, blood was collected from 3 fish per tank, for a total of 9 fish per treatment group (n = 9). Superoxide dismutase (SOD) activity was assessed by measuring pyrogallol oxidation at 560 nm, while catalase (CAT) activity was determined by observing hydrogen peroxide breakdown at 240 nm. Glutathione peroxidase (GPx) activity was assessed by measuring NADPH depletion at 340 nm. Oxidative damage was evaluated by determining malondialdehyde (MDA) levels at 532 nm via thiobarbituric acid assay. All enzyme levels were normalized to total protein concentration, determined using the Bradford assay (595 nm). Adapted for fish tissue analysis, measurements were performed in triplicate with strict quality checks, following standardized protocols (Taysi *et al*., 2024; Verdi *et al*., 2024).

**Innate Immune Parameters:**

Serum lysozyme levels were assessed by measuring the lysis of *Micrococcus lysodeikticus*. A mixture of 25 µL serum and 175 µL bacterial suspension (0.2 mg/mL PBS) was prepared, and absorbance at 450 nm was monitored for 5 minutes. Lysozyme activity units were calculated based on a 0.001 $minute^{-1}$ absorbance decline at 450 nm. Head kidney leukocytes were separated by Percoll gradient centrifugation to distinct feasible phagocytic cells. The purified cells were then incubated with fluorescently labelled bacteria at 25 °C for 30–60 minutes to measure phagocytic activity. After incubation, non-internalized particles were washed off, and the cells were examined using flow cytometry. *cerevisiae* (10% v/v) for 60 minutes at 28°C. Following fixation and staining, phagocytic capacity was quantified as the percentage of cell-positive leukocytes per 100 microscopic fields. All assays were conducted in triplicate with matched controls under aseptic conditions to prevent microbial contamination (Díaz-Puertas *et al*., 2024; Elkhaiat *et al*., 2025; Lulijwa *et al*., 2019).

**Statistical Analysis:**

Data were validated through comprehensive statistical testing. Distribution normality was assessed with Shapiro-Wilk ($\alpha$=0.05), and variance uniformity was evaluated via Levene's test. Treatment differences were analyzed by one-way ANOVA, with Tukey's HSD post-hoc tests applied for significant results ($p<0.05$). Analyses used IBM SPSS v26.0 (Armonk, NY), with a significance threshold at $p<0.05$. Values represent mean ± SE (n=3 biological replicates/treatment).

## 3. RESULTS

The experimental investigation evaluating graded levels of dietary turmeric *C. longa* supplementation in *C. mrigala* revealed significant improvements across multiple physiological parameters. The 1.0% turmeric inclusion consistently demonstrated optimal efficacy, establishing a clear dose-response relationship.

Final body weights showed progressive enhancement with increasing turmeric concentrations. The average initial weight was 15. 3± 1.1 g, while the 0.5%, 1.0%, and 1.5% supplemented groups reached 17.4 ± 1.2 g ($p < 0.05$), 19.6 ± 1.4 g ($p < 0.01$), and 17.8 ± 1.2 g ($p < 0.003$), respectively. Weight gain followed an identical pattern, with the 1.0% group achieving 14.3± 1.3 g compared to control values of 10.4 ± 0.9 g ($p = 0.002$). SGR exhibited maximum improvement in the 1.0% treatment (2.11 ± 0.06%/day) versus controls (1.72 ± 0.05%/day; $p = 0.001$). FCR demonstrated similar optimization, decreasing from 1.7 ± 0.2 in controls to 1.4 ± 0.2 in the 1.0% group ($p = 0.004$). PER increased from 2.2 ± 0.2 to 2.7 ± 0.3 ($p = 0.005$) at the optimal supplementation level. Survival rates remained consistently high across all treatments (92-98%), showing no statistically significant variation ($p = 0.213$). The hepatosomatic index (HSI) displayed a modest but significant reduction in the 1.0% group (1.3 ± 0.2%) compared to control (1.4 ± 0.2%; $p = 0.038$), potentially indicating improved hepatic metabolic efficiency (Table 2).

**Table 2:** Effects of Dietary Turmeric Supplementation on Weight Gain, Feed Efficiency, and Hepatosomatic Index in *Cirrhinus mrigala*

| Parameter | Control | 0.5% Turmeric | 1.0% Turmeric | 1.5% Turmeric | p-value |
|---|---|---|---|---|---|
| **Initial Weight (g)** | 5.3 ± 0.14 | 5.3 ± 0.12 | 5.3 ± 0.16 | 5.3 ± 0.13 | 0.06 |
| **Final Weight (g)** | 15.7 ± 1.1 | 17.4 ± 1.2* | 19.6 ± 1.4** | 17.8± 1.2* | 0.003 |
| **Weight Gain (g)** | 10.4 ± 0.9 | 12.1 ± 1.1* | 14.3± 1.3** | 12.5± 1.0* | 0.002 |
| **SGR (%/day)** | 1.72 ± 0.05 | 1.94 ± 0.06* | 2.11 ± 0.06** | 2.04± 0.08* | 0.001 |
| **FCR** | 1.7 ± 0.2 | 1.5 ± 0.3* | 1.4 ± 0.2** | 1.5 ± 0.2* | 0.004 |
| **PER** | 2.2± 0.2 | 2.43± 0.3* | 2.7 ± 0.4** | 2.5 ± 0.3* | 0.005 |
| **Survival Rate (%)** | 92 ± 3 | 97 ± 3 | 98 ± 4 | 95 ± 5 | 0.213 |
| **HSI (%)** | 1.4± 0.2 | 1.4 ± 0.4 | 1.3 ± 0.2* | 1.4 ± 0.1 | 0.038 |

Note: Values represent mean ± SD (n = 3). Asterisks indicate significant differences compared to control: *$p < 0.05$, **$p < 0.01$. SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio; HSI = hepatosomatic index. One-way ANOVA followed by Tukey's test was used for statistical analysis ($p < 0.05$) *

The antioxidant profile showed marked improvements with turmeric administration. SOD activity increased from 13.2 ± 1.4 U/mg in controls to 17.7 ± 1.8 U/mg in the 1.0% group ($p = 0.003$). CAT activity rose from 44.5 ± 3.2 U/mg to 59.6 ± 4.4 U/mg ($p = 0.008$), while (GPx) increased from 17.3 ± 2.2 U/mg to 26.4 ± 2.7 U/mg ($p = 0.005$). These enzymatic enhancements corresponded with a 29% reduction in MDA levels (3.1 ± 0.4

nmol/mg to 2.2 ± 0.2 nmol/mg; p = 0.001), indicating significantly reduced lipid peroxidation.

Lysozyme activity, a crucial component of innate immunity, increased by 65% in the 1.0% group (1.7 ± 0.2 U/mL to 2.8 ± 0.3 U/mL; p = 0.002). Phagocytic capacity showed even greater enhancement, rising by 70% (21.5 ± 2.7% to 36.6 ± 3.8%; p = 0.006). These immunological parameters demonstrated the strongest response at the 1.0% inclusion level, with slightly reduced efficacy at higher concentrations.

**Table 3:** Antioxidant enzyme activities and immune parameters of *C. mrigala* fed turmeric-supplemented diets for 60 days

| Parameter | Control | 0.5% Turmeric | 1.0% Turmeric | 1.5% Turmeric | p-value |
|---|---|---|---|---|---|
| **SOD (U/mg)** | 13.2 ± 1.4 | 15.2 ± 1.6* | 17.7 ± 1.8** | 16.2 ± 1.4** | 0.003 |
| **CAT (U/mg)** | 44.5 ± 3.2 | 51.3 ± 3.2* | 59.6 ± 4.4** | 54.9 ± 3.5** | 0.008 |
| **GPx (U/mg)** | 17.3 ± 2.2 | 22.5 ± 2.3* | 26.4 ± 2.7** | 24.3 ± 2.4** | 0.005 |
| **MDA (nmol/mg)** | 3.1 ± 0.4 | 2.5 ± 0.3* | 2.2 ± 0.2** | 2.4 ± 0.3** | 0.001 |
| **Lysozyme (U/mL)** | 1.7± 0.2 | 2.4± 0.3* | 2.8 ± 0.3** | 2.3 ± 0.4** | 0.002 |
| **Phagocytic activity (%)** | 21.5 ± 2.7 | 27.4 ± 3.2* | 36.6 ± 3.8** | 32.2 ± 3.4** | 0.006 |

Note: Values are means ± SD of three replicates. Significant differences compared to control group are indicated by * ($p < 0.05$) and ** ($p < 0.01$). SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; MDA = malondialdehyde. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD test.

## 4. DISCUSSION

This study examined the effect of *C. longa* 1.0% dietary supplementation in *C. mrigala*, which enhanced growth performance, increased antioxidant capacity, and improved immune responses. These results also align with the previous studies on turmeric's multifunctional advantages in aquaculture nutrition (Asad *et al*., 2025; El-Gogary *et al*., 2025; Khieokhajonkhet *et al*., 2023; Wei *et al*., 2024), which describe that turmeric dietary supplementation in fish improved growth parameters viz, body weight, higher SGR, FCR, nutrient utilization, and metabolic efficiency. Similar growth performance was recorded in Nile tilapia with the dietary supplementation of curcumin through enhanced digestive function and intestinal health (Abdel-Ghany *et al*., 2023; Kusi *et al*., 2025; Sulivany *et al*., 2024b;). The present study describes the reduced HIS in the 1.0% turmeric that indicates enhanced hepatic efficiency. Similarly, Bhatt *et al*. (2024) also studied the reduced HSI in *cyprinids*.

The present study highlights the marked rise in antioxidant properties of critical defense enzymes
Viz: SOD, CAT, and GPx, and reduction in oxidative stress indicators like MDA. These results also align with the previous studies on turmeric, which also caused protective effects, and a marked rise in antioxidant properties was observed in common carp (Bin-Ammar *et al.,* 2024; Eissa *et al.,* 2024). Tahir *et al*. (2024) and Zheng *et al*. (2025) studied the turmeric antioxidant effects mechanism and explained that bioactive constituents directly scavenge reactive oxygen species, simultaneously activating the body's innate antioxidant defenses via the Nrf2 signaling pathway. Metwaly *et al.* (2025) also studied the Turmeric defensive properties in aquaculture systems, especially during ecological stressors such as high stocking densities and fluctuating water parameters that can lead to severe oxidative damage.

Turmeric supported immune function in the studied fish, as seen in improved infection-fighting enzyme levels and more efficient pathogen removal by immune cells, both crucial parts of fish immune defenses. These results align with Alsubaie *et al.* (2025) work showing curcumin-enriched feed increased disease resistance mechanisms in grouper fish. Turmeric boosts fish immunity by activating key defense pathways (NF-κB and MAPK), which increase protective protein production and disease resistance (Shin *et al*., 2024; Wang *et al*., 2025). These findings are especially valuable as fish farmers increasingly need natural alternatives to replace antibiotics and address drug resistance issues (Neil *et al*., 2024).

Our study found the 1.0% turmeric dosage worked best, matching previous fish nutrition research. Komal *et al*. (2024) reported identical results in tilapia, where higher 1.5% doses became less effective - likely because the fish could only use so much of the active compounds. This dosage pattern appears across species, as Jasim *et al*. (2024) observed similar limits in chickens, where too much curcumin disrupted fat processing.
While this study provides valuable insights, some questions remain unanswered. We did not examine how long-term turmeric use affects meat quality or breeding success in fish. Next steps should investigate these areas, along with testing turmeric combinations with other plant-based additives like garlic or thyme oil to create more effective feeds (Antache *et al*., 2025; Khaldoun *et al*., 2025; Rasheed *et al*., 2024). Advanced genetic analysis methods could also help pinpoint exactly how turmeric influences nutrient processing and immune system activation at the molecular level (Zou *et al*., 2025).

## CONCLUSION

Our research shows that adding 1.0% turmeric to feed delivers the best results for boosting growth, fighting oxidative stress, and strengthening immunity in *C. mrigala*. These findings prove turmeric works effectively as a natural replacement for artificial additives in fish farming. While these results are promising, more studies should examine extended use effects and the most effective ways to administer turmeric. This work contributes to more sustainable fish production by enhancing

welfare and yields naturally. Turmeric represents an eco-conscious option for aquaculture operations seeking greener solutions.

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**Ethical Considerations:**

The study strictly adhered to national and institutional ethical standards for aquatic research. The Punjab Fisheries Department's Animal Ethics Committee approved all protocols (APF-188; 2023). All participating researchers provided verbal agreement before experimentation.

**Declarations:**

**Consent for Publication:**

We, the authors of this review article, confirm that the work is original, has not been published elsewhere, and is not under consideration by another journal. All authors have reviewed and approved the final manuscript for submission.

**Competing Interests:**

The authors declare that they have no competing financial or other interests that could influence the work reported in this manuscript.

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**Author Contributions:**

A. A. H., and M. O. contributed to the work's concept and design. K. H. J. and A.S. were responsible for writing. M.O., H.A.A., N.J.H., and K.H.J., 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.

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