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Dr. Muttappa Khavi
Assistant Professor, College of
Fishery Science, PV Narsimha
Rao Telangana Veterinary
University, Pebbair,
Wanaparthy, Telangana, India

Nandini Reddy
College of Fishery Science,
PV Narsimha Rao Telangana
Veterinary University,
Pebbair, Wanaparthy,
Telangana, India

Devanand TN
College of Fishery Science,
PV Narsimha Rao Telangana
Veterinary University,
Pebbair, Wanaparthy,
Telangana, India

Shyam Prasad M
Fisheries Research Station,
PV Narsimha Rao Telangana
Veterinary University,
Fisheries Research Station,
Paleru, Khammam,
Telangana, India

Anusha M
College of Fishery Science,
PV Narsimha Rao Telangana
Veterinary University,
Pebbair, Wanaparthy,
Telangana, India

Sakaram, D
College of Fishery Science,
PV Narsimha Rao Telangana
Veterinary University,
Pebbair, Wanaparthy,
Telangana, India

Corresponding Author:
Dr. Muttappa Khavi
Assistant Professor, College of
Fishery Science, PV Narsimha
Rao Telangana Veterinary
University, Pebbair,
Wanaparthy, Telangana, India

Enzymatic responses in common carp (*Cyprinus carpio*) exposed to oxytetracycline medicated feed

Muttappa Khavi, Nandini Reddy, Devanand TN, Shyam Prasad M, Anusha M and Sakaram, D

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Abstract

The extensive use of antibiotics in aquaculture, particularly oxytetracycline (OTC), has raised concerns regarding fish health and tissue-specific physiological effects. This study evaluated the impact of OTC-mediated feed on Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Lactate Dehydrogenase (LDH) activities in the liver, muscle, and gills of common carp (*Cyprinus carpio*). Fish were fed diets containing 25, 50, 75, and 100 mg/kg OTC for 45 days, and enzyme activities were measured at 0, 15, 30, and 45 days. Results demonstrated tissue and dose-dependent alterations. Hepatic AST and ALT increased during early exposure, indicating acute hepatocellular stress, followed by partial recovery, suggesting adaptive responses. Muscle tissues exhibited initial suppression of transaminase activity, with later elevations, reflecting delayed metabolic stress. LDH activity showed early hepatic elevation, biphasic muscle response, and progressive gill increases, indicating modifications in anaerobic metabolism. Gill tissues revealed cumulative oxidative stress, as evidenced by elevated AST and LDH activities. Collectively, the findings demonstrate that OTC induces hepatotoxicity, disrupts amino acid metabolism, and alters glycolytic pathways in a tissue-specific manner. AST, ALT, and LDH serve as reliable biomarkers for assessing physiological stress under antibiotic exposure, emphasizing cautious use of OTC in aquaculture.

Keywords: Oxytetracycline, common carp, AST, ALT, LDH

Introduction

Aquaculture is one of the fastest growing food production sectors globally, meeting the rising demand for protein and supporting rural livelihoods. However, intensification of aquaculture has increased the risk of bacterial diseases, which cause substantial economic losses and compromise fish welfare (Thakur *et al.*, 2024) ^[1]. Antibiotics, particularly oxytetracycline (OTC), are widely used as prophylactic and therapeutic agents. Despite their benefits, indiscriminate and prolonged application raises concerns regarding environmental contamination, development of antibiotic resistance, and potential risks to fish and human health (Carvalho and Santos, 2016; Balakrishna *et al.*, 2017) ^[3, 2].

OTC, a broad-spectrum tetracycline, is effective against Gram-positive and Gram-negative bacteria, cost-effective, and widely available (Leal *et al.*, 2018) ^[6]. Its persistence in aquatic environments and bioaccumulation in fish tissues can negatively affect non-target organisms and cause sub-lethal stress responses (Daghrir and Drogui, 2013; Thakur *et al.*, 2024) ^[4, 11]. Prolonged exposure has been associated with hepatotoxicity, immunosuppression, oxidative stress, and metabolic disturbances in various fish species (Ambili *et al.*, 2013; Limbu, 2024) ^[1, 7].

Enzyme biomarkers provide sensitive tools for assessing physiological stress in fish. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reflect liver tissue damage and metabolic disturbances, while lactate dehydrogenase (LDH) indicates shifts in anaerobic metabolism under cellular damage or hypoxic stress (Rodrigues *et al.*, 2017; Hoseini and Yousefi, 2018) ^[9, 5]. Monitoring these biomarkers allows for evaluating OTC-induced biochemical alterations in target tissues.

While previous studies have reported biochemical changes in carp, tilapia, and trout under OTC exposure (Ambili *et al.*, 2013; Nakano *et al.*, 2018; Rodrigues *et al.*, 2019) ^[1, 8, 10] comprehensive investigations comparing tissue-specific responses across liver, muscle, and

gills remain limited. The liver, being the primary detoxification organ, is highly sensitive to xenobiotics. Muscle, the bulk of fish biomass, indicates systemic stress and potential food safety issues, while gills, directly exposed to waterborne contaminants, serve as early indicators of environmental stress (Thakur *et al.*, 2024) ^[11].

Materials and Methods

Juvenile common carp (*Cyprinus carpio*, 5.3±0.7g) were obtained from a certified aquaculture farm in Mangalore, Karnataka, India. Fish were acclimatized for 15 days in 500 L fiberglass tanks under laboratory conditions, with water quality maintained at temperature 25±1 °C, dissolved oxygen 6.5±0.3 mg/L, pH 7.2±0.2, and total hardness 120±5 mg/L. Fish were fed a basal diet at 3% body weight/day in two portions.

Oxytetracycline (OTC) supplemented experimental diets were prepared with four different concentrations at 25, 50, 75, 100 mg/kg diet. The basal feed prepared contains crude protein of 35%. The OTC at required concentrations are mixed with egg albumin, surface coated on pellets and top dressed with sun-flower oil. The medicated feed is stored in refrigerated temperature at 4 °C in order to prevent rapid deterioration in warm environment. The 45-day experiment involved five treatment groups (Control, 25, 50, 75, and 100 mg/kg OTC) with three replicates per group. Fish were fed 3% body weight/day twice daily. Water quality was monitored to avoid confounding stress. Gill, muscle and liver collected every 15 days by percussive stunning and decapitation were stored at -80 °C until analysis.

The test tissues, gills, liver and muscle were homogenised in 0.25 M sucrose solution and centrifuged at 5000 rpm for 10 minutes to estimate lactate dehydrogenase and transaminases. The LDH was assayed in 100 mM phosphate buffer (pH 7.5) and 0.1 mM NADH. The reaction was initiated by adding 0.2 M Na-pyruvate to the supernatant and monitored at 340 nm (Wroblewski and Ladue, 1955) ^[12]. Activities were expressed as IU/L; triplicate assays were performed, and Mean±SD reported.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by a post-hoc multiple comparison Tukey's studentized range (HSD) test at P<0.05 to determine significant difference between individual control and treatment groups

Results and Discussion

Aspartate Aminotransferase (AST) Activity

AST activity showed dose-and time-dependent variations across tissues. Hepatic AST increased markedly at day 15, peaking in the 100 mg/kg group (315.90±1.17 IU/L) versus control (121.82±1.27 IU/L), followed by gradual decline at later stages (Fig 1). The increase in AST indicates leakage of this intracellular enzyme from hepatocytes into circulation, which occurs due to membrane disruption and necrosis. This supports earlier reports where OTC exposure caused hepatotoxicity in *Labeo rohita* and rainbow trout (*Oncorhynchus mykiss*) (Ambili *et al.*, 2013; Hoseini and Yousefi, 2018) ^[1, 5]. Muscle AST initially decreased (31.96±1.45 IU/L in 100 mg/kg at day 15) but rebounded by day 45 (182.56±1.89 IU/L) (Fig. 2). This may be linked to protein catabolism and mobilization of amino acids to meet energy demands under prolonged stress (Limbu, 2024) ^[7]. The delay in enzyme elevation suggests systemic

redistribution of energy metabolism rather than immediate damage. Gill AST exhibited minor early fluctuations, followed by significant increases at day 45 (157.19±1.37 IU/L in 100 mg/kg vs. 85.48±0.71 IU/L control) (Fig. 3). As gills are the first organ to encounter waterborne contaminants, the late rise in AST activity suggests cumulative and chronic oxidative damage (Rodrigues *et al.*, 2019) ^[10]. This tissue-specific difference indicates that while the liver responds acutely to xenobiotic stress, the gills manifest damage after prolonged exposure.

Alanine Aminotransferase (ALT) Activity

Hepatic ALT increased up to day 30 (490.75±0.90 IU/L in 100 mg/kg vs. 340.19±1.12 IU/L control), reflecting hepatocellular stress. By day 45, activity declined sharply (240.46±2.50 IU/L) (Fig. 4). This decrease may indicate irreversible tissue damage, enzyme inhibition, or adaptive metabolic responses where the system downregulates aminotransferase activity to conserve energy (Nakano *et al.*, 2018) ^[8]. Muscle ALT showed suppression initially, with recovery and elevations above control by day 45 (80.49±0.64 IU/L in 100 mg/kg) (Fig.5). This suggests a delayed enzyme induction as muscle tissue attempted to compensate for disrupted amino acid metabolism (Limbu, 2024) ^[7]. Given that skeletal muscle constitutes the bulk of fish biomass, such changes are critical as they may affect fish growth and fillet quality, raising concerns for aquaculture productivity and food safety. Gill ALT declined consistently, reaching 30.31±1.28 IU/L in the 15 mg/kg group at day 15 (Fig. 6). This consistent decline suggests that OTC exposure impaired amino acid metabolism and transamination reactions in gill tissues (Thakur *et al.*, 2024) ^[11]. Since gills are highly vascularized and metabolically active, such inhibition could reduce their resilience against stress and impair gas exchange efficiency.

Lactate Dehydrogenase (LDH) Activity

Hepatic LDH increased early (1390.75±1.07 IU/L in 100 mg/kg at day 15 vs. 1045.87±0.55 IU/L control), peaked at day 30 (1756.15±0.21 IU/L), and declined slightly at day 45 (Fig.7), indicating a metabolic shift toward anaerobic glycolysis under antibiotic stress. Similar elevations in liver LDH have been reported in fish exposed to OTC and other pharmaceuticals (Rodrigues *et al.*, 2017; Thakur *et al.*, 2024) ^[9, 11]. The pattern suggests an initial metabolic disruption followed by partial adaptation. Muscle LDH exhibited biphasic response of early suppression (1281.41±0.74 IU/L at 25 mg/kg vs. 1789.6±0.44 IU/L control) at 30 days, followed by dramatic increase at day 45 (2234.54±0.44 IU/L at 100 mg/kg) (Fig.8). The early decline might reflect inhibition of enzyme activity by OTC, while the subsequent spike indicates energy redistribution and elevated anaerobic metabolism in stressed tissues (Ambili *et al.*, 2013) ^[1]. This delayed response is particularly important since muscle tissue directly impacts fish growth performance and market value. Gill LDH increased progressively, peaking at day 45 (1697.98±0.81 IU/L in 100 mg/kg vs. 1192.06±0.26 IU/L control) (Fig.9). This suggests a chronic stress response, possibly linked to oxidative damage, hypoxia, and disruption of membrane integrity (Yonar, 2012; Thakur *et al.*, 2024) ^[13, 11]. As gills are continuously exposed to the external aquatic environment, OTC likely impairs respiratory and osmoregulatory functions, further compounding stress.

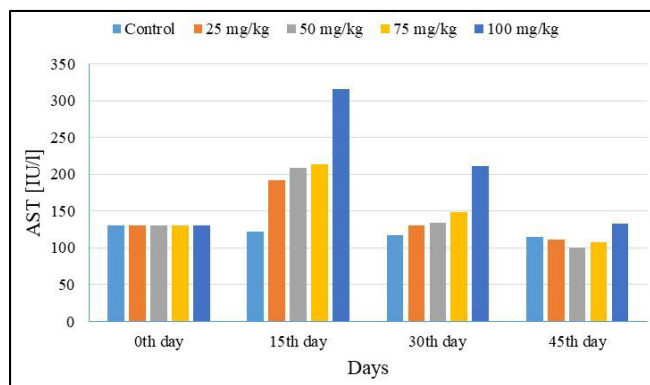


Fig 1: Changes in aspartate aminotransferase (IU/L) activity in liver of common carp exposed to OTC.

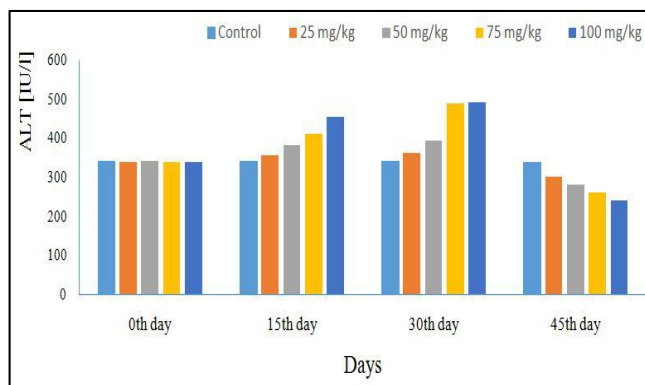


Fig 5: Changes in alanine aminotransferase (IU/L) activity in muscle of common carp exposed to OTC.

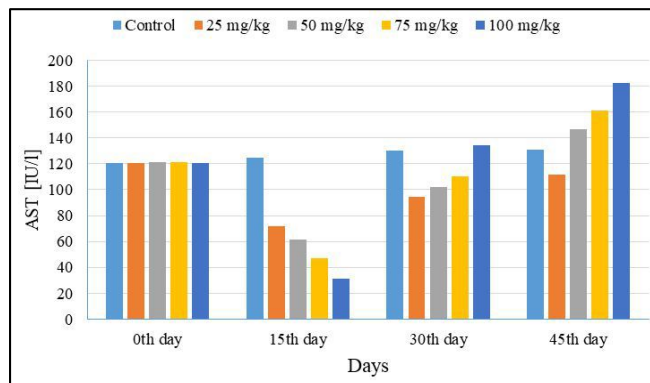


Fig 2: Changes in aspartate aminotransferase (IU/L) activity in muscle of common carp exposed to OTC

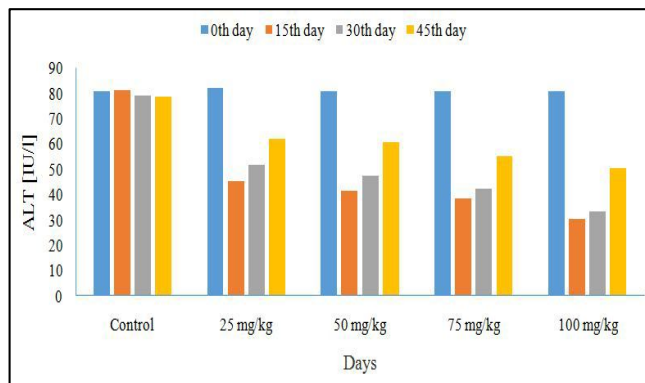


Fig 6: Changes in alanine aminotransferase (IU/L) activity in gill of common carp exposed to OTC.

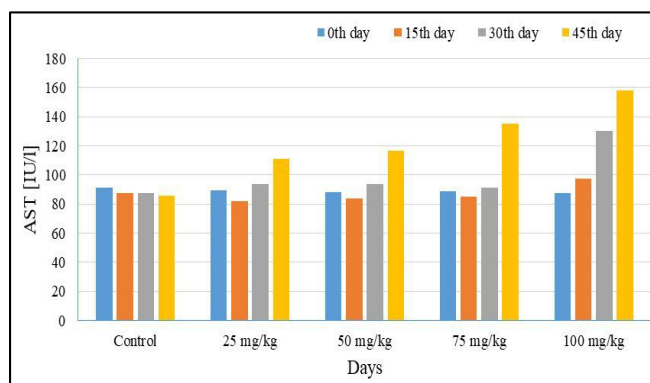


Fig 3: Changes in aspartate aminotransferase (IU/L) activity in gill of common carp exposed to OTC

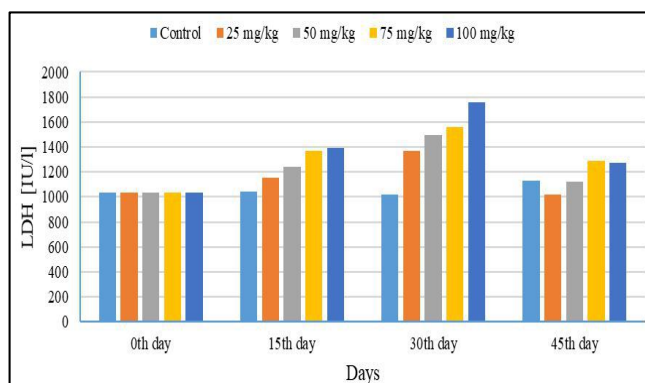


Fig 7: Changes in (IU/L) lactate dehydrogenase activity in liver of common carp exposed to OTC

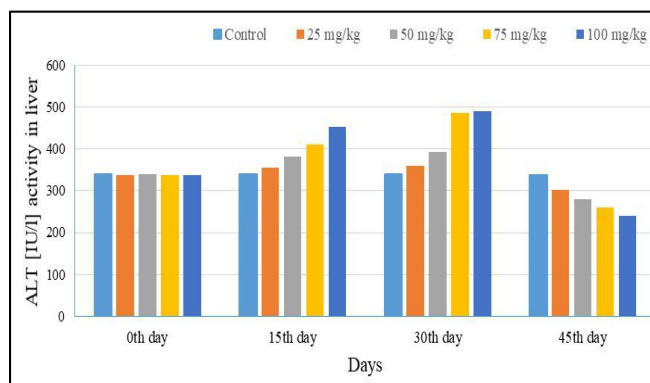


Fig 4: Changes in alanine aminotransferase (IU/L) activity in liver of common carp exposed to.

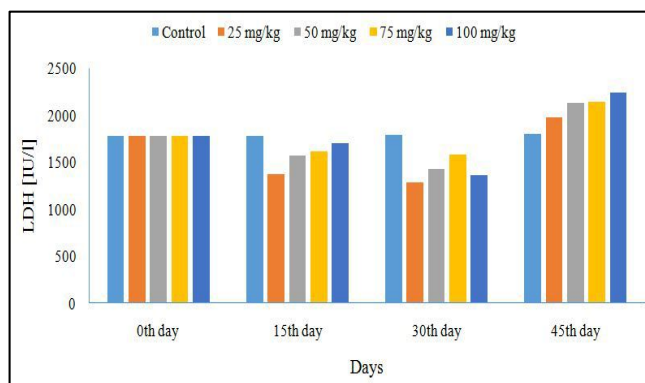


Fig 8: Changes in (IU/L) lactate dehydrogenase activity in muscle of common carp exposed to OTC

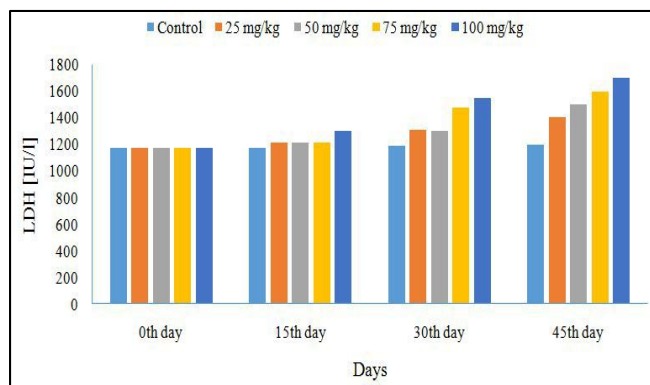


Fig 9: Changes in aspartate aminotransferase (IU/L) activity in gill of common carp exposed to OTC

Conclusion

The enzyme responses observed in this study highlight that OTC exposure, even at dietary concentrations, disrupts key metabolic pathways in fish. Prolonged use of antibiotics in aquaculture not only compromises fish welfare but also raises concerns for environmental sustainability and human health due to the risk of antibiotic residues and resistance development (Carvalho and Santos, 2016; Balakrishna *et al.*, 2017) [3, 2].

The use of biomarkers such as AST, ALT, and LDH thus provides an effective means of assessing sub-lethal toxicity and guiding prudent antibiotic usage in aquaculture. Future work integrating biochemical, histopathological, and molecular endpoints will further enhance our understanding of fish responses to pharmaceutical pollutants.

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References

1. Ambili TR, Saravanan M, Ramesh M, Abhijith DB, Poopal RK. Toxicological effects of the antibiotic oxytetracycline on an Indian major carp, *Labeo rohita*. Arch Environ Contam Toxicol. 2013;64:494-503.
2. Balakrishna K, Rath A, Kumarreddy YP, Guruge KS, Subedi B. A review of the occurrence of pharmaceuticals and personal care products in Indian water bodies. Ecotoxicol Environ Saf. 2017;137:113-120.
3. Carvalho IT, Santos L. Antibiotics in the aquatic environments: a review of the European scenario. Environ Int. 2016;94:736-757.
4. Daghrir R, Drogui P. Tetracycline antibiotics in the environment: a review. Environ Chem Lett. 2013;11:209-227.
5. Hoseini SM, Yousefi M. Beneficial effects of thyme (*Thymus vulgaris*) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*Oncorhynchus mykiss*). Aquac Nutr. 2018;25:298-309.
6. Leal JF, Santos EBH, Esteves VI. Oxytetracycline in intensive aquaculture: water quality during and after its administration, environmental fate, toxicity and bacterial resistance. Rev Aquac. 2019;11:1176-1194.

7. Limbu SM. Dietary low-fat content supplemented with oxytetracycline impairs physiological functions in Nile tilapia (*Oreochromis niloticus*) fingerlings. Anim Res One Health. 2024;3:1-14.
8. Nakano T, Hayashi S, Nagamine N. Effect of excessive doses of oxytetracycline on stress-related biomarker expression in coho salmon. Environ Sci Pollut Res. 2018;25:7121-7128.
9. Rodrigues S, Antunes SC, Correia AT, Nunes B. Rainbow trout (*Oncorhynchus mykiss*) pro-oxidant and genotoxic responses following acute and chronic exposure to the antibiotic oxytetracycline. Ecotoxicology. 2017;26:104-117.
10. Rodrigues S, Antunes SC, Nunes B, Correia AT. Histopathological effects in gills and liver of *Sparus aurata* following acute and chronic exposures to erythromycin and oxytetracycline. Environ Sci Pollut Res. 2019;26:15481-15495.
11. Thakur J, Sharma M, Sharma P, Shah T. Toxic effects of antibiotic oxytetracycline in the fish *Cyprinus carpio* using a multi-biomarker approach. J Environ Biol. 2024;45(3):300-307.
12. Wroblewski F, Ladue JS. Lactic dehydrogenase activity in blood. Proc Soc Exp Biol Med. 1955;90:210-213.
13. Yonar ME. The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 2012;32:994-1001.