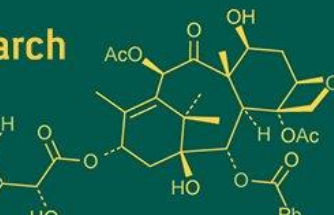
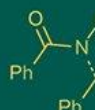


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Physiological responses in common carp (*Cyprinus carpio*) exposed to oxytetracycline

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Abstract

This study investigated the physiological responses of *Cyprinus carpio*, a globally distributed freshwater fish of considerable ecological and economic importance, following dietary exposure to oxytetracycline (OTC), a tetracycline-class antibiotic extensively used in aquaculture. Juvenile fish (5.3 ± 0.7 g) were fed for 45 days with a formulated diet containing 35% crude protein and supplemented with graded concentrations of OTC (25, 50, 75, and 100 mg/kg diet) at a feeding rate of 5% body biomass. Dietary OTC exposure resulted in pronounced alterations in growth performance, respiratory metabolism and ammonia excretion, indicating disruption of normal physiological homeostasis. The dose-dependent responses observed in the present study highlight the sensitivity of these physiological parameters and suggest their potential utility as reliable biomarkers for assessing sublethal effects and environmental risks associated with antibiotic contamination in aquatic ecosystems.

Keywords: *Cyprinus carpio*, OTC, OCR, AER and FCR

Introduction

Antibiotics are widely and often indiscriminately used in human medicine, veterinary practice, aquaculture, and animal husbandry, leading to their continuous release into the environment. Owing to their lipophilic nature, antibiotics readily cross biological membranes and are partially resistant to biotransformation, resulting in toxicological interactions. Environmental contamination arises from hospital effluents, municipal sewage, aquaculture, and livestock operations, with residues frequently detected in rivers and sewage treatment plants at concentrations reaching several $\mu\text{g/L}$, posing risks to non-target aquatic organisms. Aquaculture, a rapidly expanding sector contributing nearly 8% of global animal protein for human consumption, increasingly relies on antibiotics due to intensified farming and heightened disease susceptibility (Jerbi *et al.*, 2011) ^[14]. In food-animal production, antibiotics are used as therapeutants, growth promoters and feed additives (Halling *et al.*, 1998) ^[12]. Tetracyclines alone accounted for over 37 million kg of use in food-producing animals in 2023 (FDA, 2023) ^[9]. As 70-80% of administered antibiotics are released into aquatic environments, they are regarded as significant environmental micropollutants (Christensen *et al.*, 2006) ^[4], contributing to antibiotic resistance (Mathew *et al.*, 2007) ^[18]. Oxytetracycline (OTC), a widely used tetracycline in aquaculture (FDA, 2021; WOA, 2025) ^[8, 29], exhibits broad-spectrum antibacterial activity (Jerbi *et al.*, 2011) ^[14]. Its environmental fate depends on application mode and physicochemical properties; OTC shows low bioavailability (< 10%) in marine systems due to chelation with divalent cations and undergoes photolytic degradation. Pharmaceuticals such as antibiotics can induce physiological disturbances in fish, with responses varying by species and stress intensity (Schyolden *et al.*, 2005; Chavan and Bhilave, 2007) ^[24, 2]. Common carp (*Cyprinus carpio*), a commercially important freshwater species, was selected as a model organism due to its wide distribution and nutritional significance in India (Muttappa *et al.*, 2014) ^[19]. However, limited information exists on the physiological responses of this fish to antibiotic exposure.

Materials and Methods**Experimental Fish and Diet Preparation**

Oxytetracycline dihydrate (99% purity, TLC grade) was used for the experiment. Fingerlings of *Cyprinus carpio* (5.3 ± 0.7 g) were procured from the

College Farm, College of Fisheries, Mangalore, Karnataka, India. This species is recommended for toxicity testing in aquatic organisms as per the guidelines of the Organisation for Economic Co-operation and Development (OECD). Prior to the experiment, fish were acclimatized to laboratory conditions for 15 days.

Experimental diets containing 35% crude protein were prepared and supplemented with oxytetracycline (OTC) at four graded concentrations namely 25, 50, 75, and 100 mg/kg diet. The required quantity of OTC was dissolved in egg albumin, surface-coated onto the feed pellets, and top-dressed with sunflower oil to ensure uniform coating and minimize leaching (Rakesh, 2015) [21]. Medicated feeds were stored at 4 °C and protected from light and moisture to prevent antibiotic degradation. Fish were fed daily at 5% of total biomass, and uneaten feed was siphoned out 30 min after feeding.

Assessment of Physiological Responses

Physiological responses were used as bio-indicators of pollution-induced stress. Growth performance, feed utilization, oxygen consumption rate, and ammonia excretion rate were evaluated fortnightly throughout the 45-day feeding trial.

i. Growth Performance

Fish were weighed at two-week intervals to assess growth performance. Final body weight, weight gain, and feed conversion ratio (FCR) were calculated following standard procedures (Hussein *et al.*, 2016) [13].

ii. Oxygen Consumption Rate (OCR)

Oxygen consumption rate, an indicator of metabolic and toxic stress, was estimated following the method described by Chinni *et al.* (2000) [3].

iii. Ammonia Excretion Rate (AER)

As approximately 85% of ammonia in fish is excreted through the gills (Wilkie and Wood, 1994) [28], ammonia excretion rate was calculated according to the method of Wu and Chen (2004) [30].

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among control and treatment groups. Statistical analyses were performed using SPSS software (IBM, version 25). When significant differences were detected, Tukey's honestly significant difference (HSD) post-hoc test was applied, and group mean differences were considered significant at $p < 0.05$.

Results and Discussion

Final body weight

The final body weight of *Cyprinus carpio* fed oxytetracycline (OTC)-supplemented diets is shown in Fig. 1. The highest weight gain was observed at 50 mg OTC/kg diet (9.63 ± 0.152 g), consistent with reports of enhanced growth in *Oreochromis niloticus* at 40 mg OTC/kg (Soltan *et al.*, 2013) [25] and in *Ictalurus punctatus* fingerlings at 50 mg OTC/kg diet (Sanchez-Martinez *et al.*, 2008) [23]. Improved growth at moderate OTC levels has been attributed to enhanced nutrient absorption, including fatty

acids, rather than alterations in gut microflora (Cravedi *et al.*, 1987) [5]. Fish fed 25 mg OTC/kg diet showed moderate weight gain (7.67 ± 0.015 g).

In contrast, no growth improvement was observed at 75 mg OTC/kg diet (5.52 ± 0.030 g), in agreement with findings in *C. carpio* (Dobsikova *et al.*, 2013) [6], florfenicol-fed fish (Gaikowski *et al.*, 2003) [11], and Atlantic salmon fed with high OTC diets (Toften and Jobling, 1997) [26]. Soltan *et al.* (2013) [25] similarly reported growth suppression at high OTC doses. The increased feed conversion ratio at 75 mg OTC/kg diet indicates reduced feed efficiency, likely due to diversion of metabolic energy towards detoxification, resulting in impaired growth.

Feed conversion ratio

The feed conversion ratio (FCR) closely followed the trend observed in weight gain. Fish fed 75 mg OTC/kg diet exhibited the highest FCR, indicating poor feed utilization, followed by 100 mg OTC/kg diet, whereas the lowest FCR was recorded at 50 mg OTC/kg diet, corresponding to the highest weight gain (Fig. 2). Similar reductions in FCR were reported by Koh *et al.* (2016) [16] in tilapia fed 5 mg florfenicol/kg diet. Reda *et al.* (2013) [22] also observed significantly lower FCR and higher growth in *Oreochromis niloticus* fed florfenicol-supplemented diets compared to oxytetracycline. The reduced FCR at 50 mg OTC/kg diet in the present study confirms improved feed utilization at moderate OTC levels.

Oxygen consumption rate

A reduction in oxygen uptake was observed across treatments, with the greatest decline recorded at 75 mg OTC/kg diet (83.22%), followed by 25 mg (15.92%), 50 mg (14.15%), and 100 mg OTC/kg diet (11.5%) (Fig. 3). The pronounced reduction in oxygen consumption may be attributed to stress, which elevates the critical oxygen concentration and suppresses metabolic respiration (Dube and Hosetti, 2010) [7]. Similar reductions in oxygen uptake were reported in *C. carpio* larvae exposed to heavy metal mixtures (Jezierska and Sarnowski, 2002) [15], possibly due to atrophy of the respiratory epithelium. The marked decline at 75 mg OTC/kg diet in the present study indicates elevated physiological stress at this concentration.

Ammonia excretion rate (AER)

A reduction in ammonia-N excretion rate was observed at all dietary OTC levels throughout the 45-day experimental period (Fig. 4). The greatest reduction occurred at 100 mg OTC/kg diet (49.42%), followed by 25 mg (32.85%) and 75 mg OTC/kg diet (32.38%), while the least reduction was observed at 50 mg OTC/kg diet (5.17%). Ammonia excretion is a key indicator of physiological status and protein metabolism in fish (Fournier *et al.*, 2003) [10]. Similar reductions were reported by Chinni *et al.* (2000) [3] in *Penaues indicus* post-larvae exposed to lead, attributed to disruption of ammonia-producing pathways. As transamination is the primary source of ammonia, alterations in hepatic and extra-hepatic enzyme activity, along with feeding and digestion processes, may account for the observed reductions (Walton and Cowey, 1977) [27].

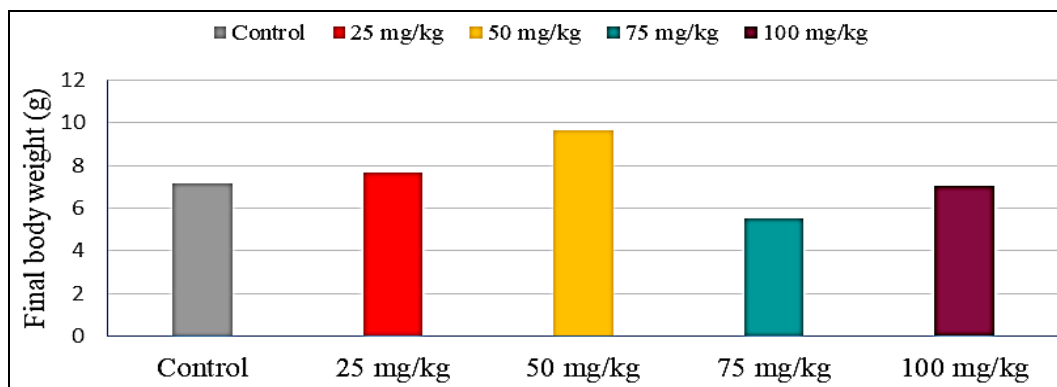


Fig 1: Final body weight (g) achieved by common carp being after fed with oxytetracycline supplemented medicated feed for a duration of 45 days

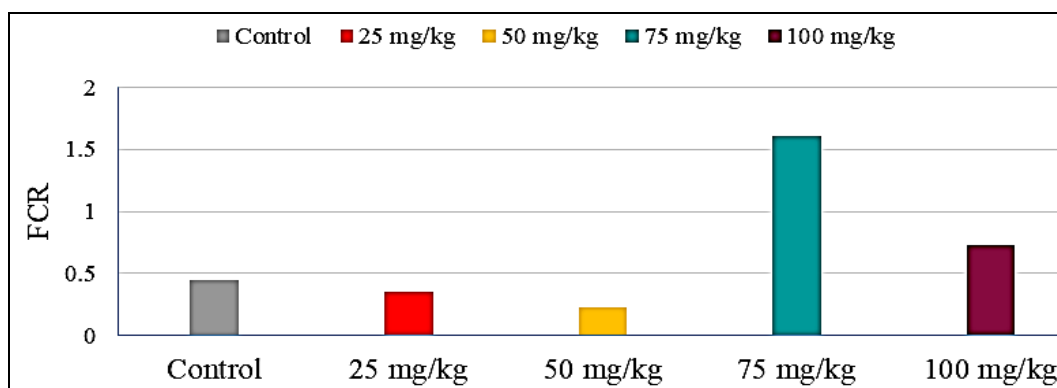


Fig 2: Food conversion ratio (FCR) obtained by common carp after being fed with oxytetracycline supplemented medicated feed for duration of 45 days.

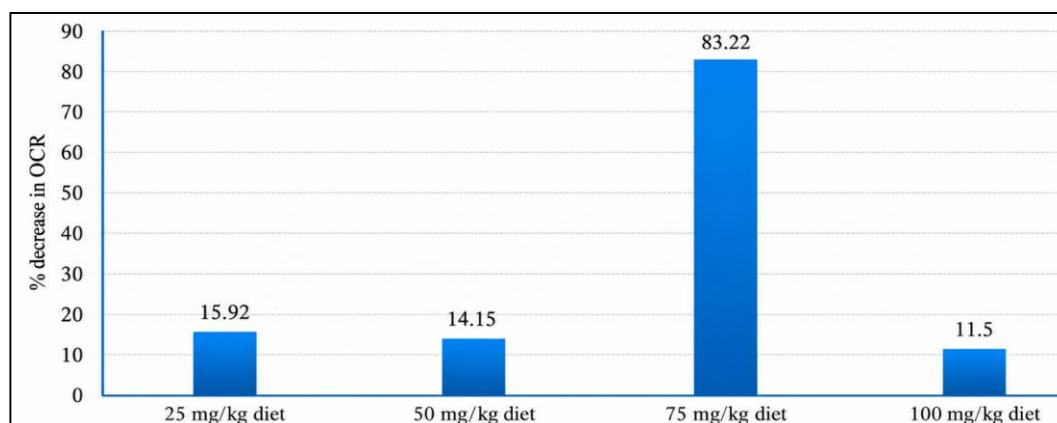


Fig 3: Variation in activity of oxygen consumption rate as percentage over control in common carp fed with oxytetracycline for 45 days.

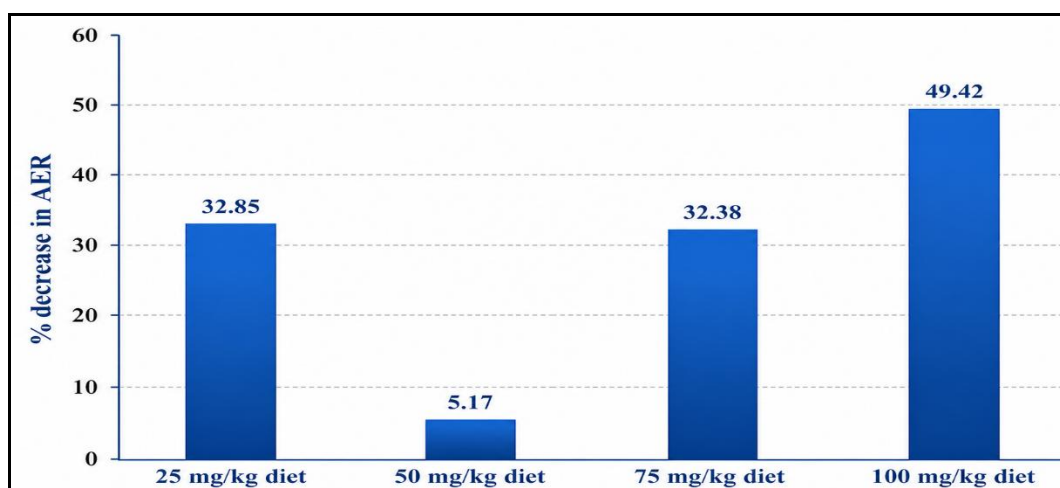


Fig 4: Variation in activity of ammonia-N excretion rate as percentage over control in common carp fed with oxytetracycline for 45 days.

Conclusion

These findings indicate that dietary OTC disrupts normal physiological functions in *C. carpio*. The observed alterations in respiratory activity, growth performance and excretory patterns highlight the potential use of these responses as sensitive biomarkers for assessing environmental risks associated with antibiotic exposure.

Even though the antibiotics at small dosages can be used as growth promoters, further pharmacokinetic studies have to be conducted to evaluate the distribution of antibiotics and analyse residues in fish tissues before consumption.

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