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EFFECT OF OXYTETRACYCLINE ON MORPHOMETRY AND METABOLIC ACTIVITIES OF POLY-CULTURED TILAPIA AND ROHU

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Abstract

Oxytetracycline, an antimicrobial agent has been playing a potent role in aquaculture to control the bacterial diseases, but it's over dose may pose an undesirable effect. However, the purpose of the experiment was to evaluate the impacts of dietary oxytetracycline on the primary productivity, growth performance, biochemical activity, as well as immunity performance of tilapia (*Oreochromis niloticus*) and rohu (*Labeo rohita*) in a polyculture system. Oxytetracycline was mixed with feed at 100 mg/kg and fed with no antibiotic was denoted as the control. A total of 480 fish were cultured for 70 days, comprising 240 tilapia (with a length of 14.63 cm and weight of 59.85 g in an average) and 240 rohu (with a length of 11.43 cm and weigh of 24.6 g in an average). The findings indicated that there was not any significant (p >0.05) change in the growth of treated fishes in comparison with the control one. Amylase activity increased significantly (p <0.05), while superoxide dismutase activity increased but in a non-significant (p >0.05) way in the both of fish species. Protease and catalase activities were found to decline significantly (p <0.05) in both species. There was no significant increase in plankton abundance in the treatment ponds. Spectrometry study of chlorophyll-a revealed in control and treatment ponds where, there found no significant (p> 0.05) variation among each week. These results suggested that oxytetracycline dosing rate at 100 mg/kg having no significant impact on the growth and enzymatic activity of poly-cultured tilapia and rohu as well as primary productivity of the pond. A therapeutic dose (100 mg/kg) of oxytetracycline is subjected to be safe, polyculture.

Keyword: Antibiotic agent, Bacterial diseases, Enzyme activity, Growth performance.

Introduction

Tilapia (*Oreochromis niloticus*), is a commercially significant food fish for aquaculture in many regions around the world ((El-Sayed, 2020). Rohu (*Labeo rohita*) is known as one of the most important Indian big carp in carp polyculture practice. High growth potential and compatibility coupled with high consumer demand have established tilapia and rohu as the most consumable and delicious freshwater fish cultivated in Bangladesh. Nowadays, intensive and semi-intensive culture techniques have been developed in response to the increased demand for aquaculture products. The fish culture in intensive production systems reduces their immunity, spreading infectious diseases that reduce growth and survival rates which results in considerable economic losses, ultimately diminishing fish production (Abdelsalam et al., 2023). To prevent these losses and increase production farmers often use antibiotics. Antibiotic diets keep fish healthy and protect them from illness outbreaks (Islam et al., 2015). In some studies, researchers have also mentioned the negative consequences of long-term antibiotic usage in fish, including liver damage or dysfunction, nephrotoxicity, metabolic abnormalities, inhibition of key enzymes, growth retardation, and a reduction in the organism's fertility and longevity (Hentschel et al., 2005, Dureja and Rathore 2012). One of the frequently applied antibiotics in aquaculture is oxytetracycline (OTC), which is used to treat bacterial infections, including yersiniosis, vibriosis, and furunculosis. (Oliveira et al., 2013). Nevertheless, it has been documented to have a number of detrimental physiological effects on fish, such as immunosuppression and oxidative stress (Hoseini & Yousefi, 2019; Yonar, 2012; Yonar et al., 2011). The kidneys, liver, gills and brain are the most vulnerable organs in a fish exposed to toxicant-containing media (Lee et al.,

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2019). Enzymatic analysis of various organs in fish, such as muscles, kidneys, liver, heart, and gills, can offer valuable information about the organism's internal environment (Boeger et al., 2003). Few studies have reported the effect of enzymatic activities and immune response against different doses of oxytetracycline on monocultured fishes but no study has been found for the poly-cultured fishes. Therefore, the study was undertaken to assess the effects of oxytetracycline on growth and metabolic activities in Poly-cultured fishes, which is commonly used in agricultural croplands and also observe the effect on the pond's primary productivity.

Materials and Method

Study area

The experiment was conducted for 70 days, in the experimental ponds (22°48'10.2"N 89°31'57.0"E) of Khulna University, Bangladesh. The size of each pond was 0.75 decimal (7.3×3.6×1 m) but the fishes were reared in the hapa whose size was 12 m^3. Initially, the fishes were kept in the rearing pond for 5 days and were shifted in the experimental ponds, where fish were treated with 100 mg/kg oxytetracycline three replication and three control ponds with no antibiotic. After rearing the fish, fish and water samples were collected weekly for next experiment.

Plankton abundance and chlorophyll-a concentration

Plankton abundance was checked using a complex microscope, and the concentration of plankton in ponds per litter was calculated using the following formula.

$$\frac{No.of\ the\ field\ in\ Sedgwick-Rafter\ cells \times ml\ of\ concentration}{No.Of\ Sedgwick-Rafter\ fields\ examined \times total\ ml\ filtered} = \text{Multiplier (Ingram and Palmer, 1952)}$$

At wavelengths 630, 647, 664, and 750 nm, absorbance was used with a spectrophotometer to measure the content of chlorophyll-a. The following formula from Jeffrey and Humphrey (1975) was used to calculate chlorophyll-a concentration: [chl. a] extract = 11.85(A664)/I – 1.54(A647)/I – 0.08(A630)/I

[chl.a] sample = [chl.a] extract * (v/V)

Where, A = corrected absorbance, I= path length of cuvette (10cm), v = volume of extract (5 mL) V = volume of water filtered in (0.025 L)

Proximate composition

Proximate analyses were performed using the AOAC (1984) methodology, including measurements of protein, lipid, and moisture content.

Enzyme activity

Dinitro-salicylic acid (DNS) was used to measure the amylase activity. (Bernfeld 1995). Soluble starch (1%) was employed as the substrate in this method and the absorbance at 540 nm in spectrophotometry machine was recorded to detect the reducing groups that amylase's activity on starch released. Using the Casein technique, protease activity was assessed (Walter 1984). The change in absorbance at 280 nm was used to measure the enzyme activity. Marklund and Marklund (1974)'s method modified by Jing and Zhao (1995) of measuring superoxide dismutase activity. By measuring the spectrophotometer's absorbance at 540 nm, the autoxidation of pyrogallol by O_2 against this radical's dismutation was determined in this process. Using the extinction value of 0.04 mM-1cm-1, the drop in H_2O_2 concentration at 240 nm the catalase activity were calculated (Beers and Sizer 1952).

Statistical analyses

The growth and primary productivity data were examined using a one-way analysis of variance (ANOVA). Enzymatic activity data were evaluated using the t test, and any significant variations between means were observed using the post hoc Duncan's multiple range test. The data was analysed using SPSS (Version 17, Chicago, IL, USA).

Results

In this study, there has been observed an increase in plankton abundance for control ponds which was significant ($p < 0.05$), however, there was no discernible ($p > 0.05$) increase in treatment ponds (**Figure 1 a**). For both the control and treatment ponds, a significant ($p>0.05$) decrease in chlorophyll-a content was observed throughout the duration of the weeks. (**Figure 1 b**).

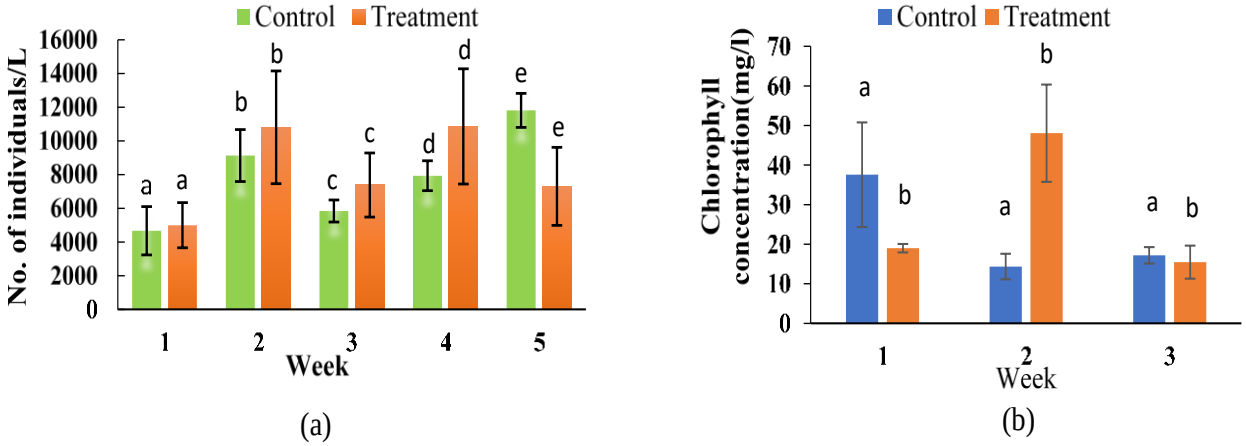

Figure 1. Effect of dietary OTC primary productivity of pond, (a) plankton abundance (b) chlorophyll-a concentration. Significant differences ($p<0.05$) among control and treatment are denoted by different superscript letters.

Table 1. Effect of dietary oxytetracycline exposure on growth of *O. niloticus* and *L. rohita*

Parameters	**Tilapia**		**Rohu**	
	Control	**Treatment**	**Control**	**Treatment**
Initial Weight (g)	58.87±3.03^{a}	55.84±2.93^{b}	25.49±6.76^{a}	29.03±4.90^{b}
Final Weight (g)	70.74±3.99^{a}	78.32±2.92^{b}	26.27±5.63^{a}	29.24±4.53^{b}
Weight Gain (Wg) %	24.382^{a}	30.052^{a}	3.029^{a}	0.73^{b}
Specific growth rate (SGR) (%)	0.752^{a}	0.906^{a}	0.103^{a}	0.025^{b}

Significant changes are represented by various superscript letters in the same row. $^{*}P < 0.05$, $^{\#}P \geq 0.05$.

For metabolic activity of fish, body growth, enzymatic activities, and proximate composition of the body muscle were observed. In the treatment group of tilapias, the weight gain (%) was higher than the control group (**Table 1**). A specific growth rate revealed the fish's development during a given time in the culture condition. The specific growth was clearly higher in the treatment group of tilapias, but statistical analysis did not show any significant ($p>0.05$) increase. In contrast, compared to the Rohu control pond, there was a significant ($p>0.05$) drop in specific growth rate and weight gain (%) in the treatment pond.

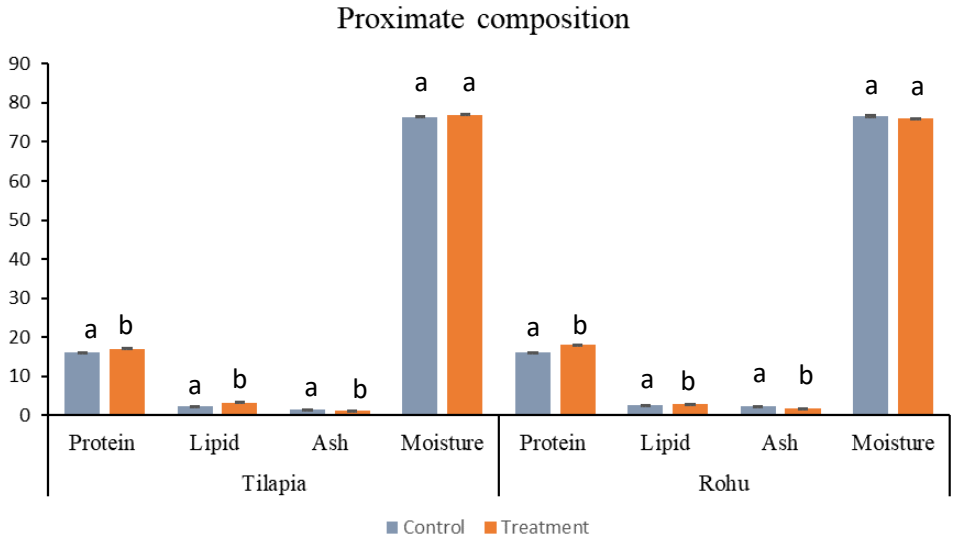

Figure 2. Effect of dietary OTC proximate composition of *O. niloticus* and *L. rohita*. Significant differences ($p<0.05$) among control and treatment are denoted by different superscript letters.

The percentages of ash, moisture, lipids, and all the proteins varied significantly ($p<0.05$) between the treatm ent and control groups. For tilapia, percentage of ash decreased while lipid, protein, and moisture increased. However, in the case of rohu, the average percentages of fat and protein in the treatment group increased and ash and moisture decreased, but only the percentage of moisture was not decreased significantly ($p>0.05$) (**Figure 2**)

For digestive enzyme activity amylase and protease enzyme were examined and to observe immune response, SOD and catalase activity were observed. In the intestines of monosex tilapia, average amylase activity was determined to be 54.02±9.77 µg/ml/min in the control and 66.52±3.52 µg/ml/min in the treatment group. For rohu, there was an average amylase activity of 170.07±11.20 µg/ml/min in the control and 187.91±25.17 µg/ml/min in the treatment group. Each group's, 15 total duplicates were evaluated, and the difference in amylase activity between the control and treatment groups showed to be higher in **Figure 3**. There was found a significant ($p<0.05$) increase of amylase activity in the intestine of both tilapia and rohu.

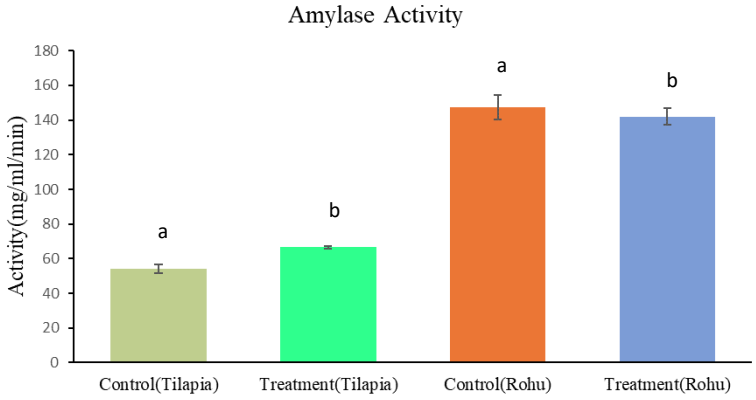

Figure 3. Effect of dietary OTC exposure on amylase activity in intestine of *O. niloticus* and *L. rohita*. Significant differences ($p<0.05$) among control and treatment are denoted by different superscript letters.

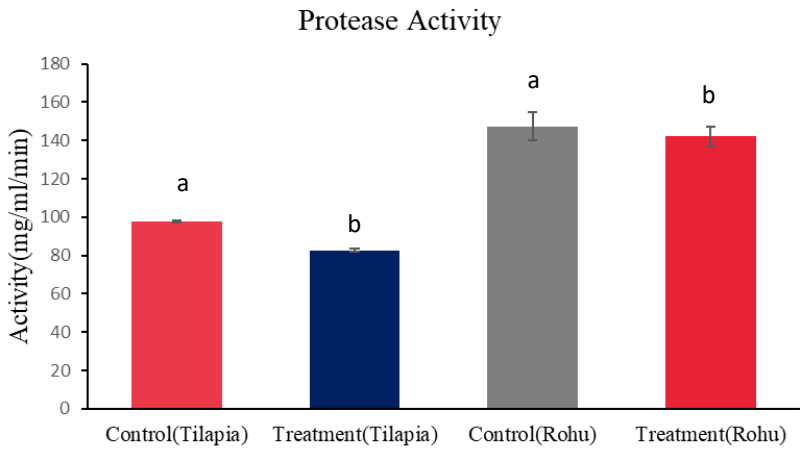

Figure 4. Effect of dietary OTC exposure on protease activity in intestine of *O. niloticus and L. rohita*. Significant differences ($p<0.05$) among control and treatment are denoted by different superscript letters.

The amount of average protease activity in the digestive tract was found to be 97.61 ± 1.69 µg/min/ml in the Control group and 82.78 ± 2.91 µg/min/ml in the treatment group. In rohu samples, the average amount of protease activity was 147.33±3.51 and 142.04±1.61 µg/min/ml in the control and treatment groups respectively (**Figure 4**). There was a significant ($p<0.05$) decrease in activity between control and treatment group for both tilapia and rohu. The amount of average SOD activity in the liver of the control and treatment groups of tilapias was 5.49±.09 and 5.60±.03 Units/ml respectively. In rohu, the average SOD activity of the control and treatment groups was 5.81±0.068 and 5.85±0.07 Units/ml respectively (**Figure 5**). For, both tilapia and rohu, average SOD activity increased but, in tilapia the activity increased significantly while for rohu the increase of activity was not significant.

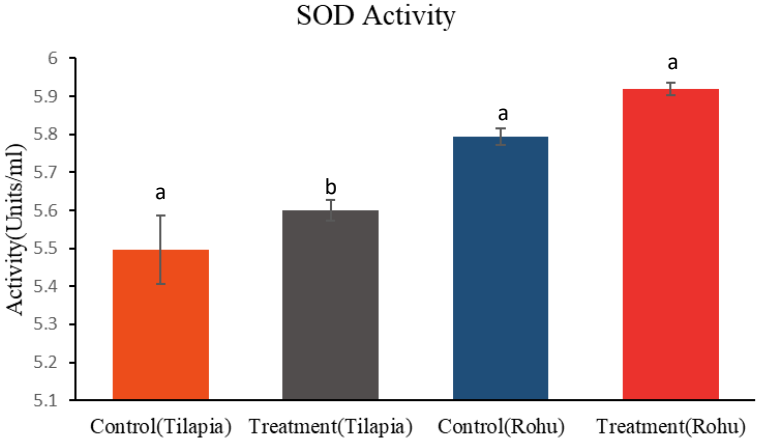

Figure 5. Effect of dietary OTC exposure on SOD activity in intestine of *O. niloticus* and *L. rohita*. Significant differences ($p<0.05$) among control and treatment are denoted by different superscript letters.

The amount of average catalase (CAT) activity in the liver was found 23.51 ± 3.27 µM/min/ml and 16.39 ± 2.39 µM/min/ml respectively in control and treatment samples pf tilapia. For, rohu the average catalase activity was determined to be 16.09 ± 4.25 µM/min/ml and 7.12 ± 2.97 µM/min/ml in the control and treatment groups which indicated that the amount of peroxide reduction decreased consistently from Control to treatment (**Figure 6**). The results reveled that the intestinal peroxide levels in both the control and treatment groups significantly ($p<0.05$)

decreased. The outcome showed a significant ($p<0.05$) decrease in intestinal peroxide level between the treatment and control groups.

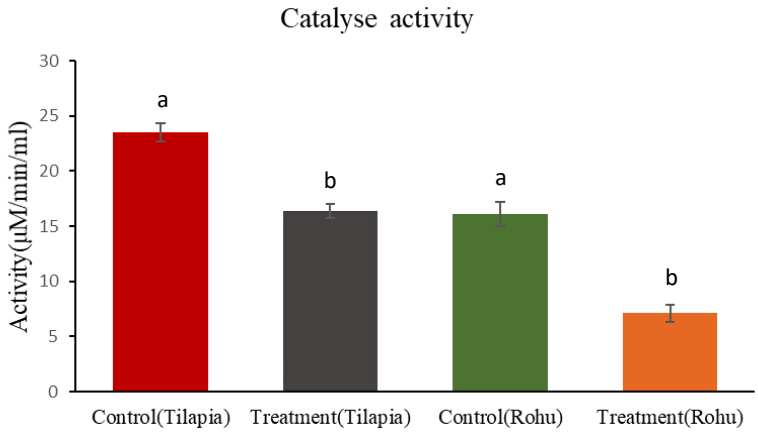

Figure 6. Effect of dietary OTC exposure on catalase activity in intestine of *O. niloticus* and *L. rohita*. Significant differences ($p<0.05$) among control and treatment are denoted by different superscript letters.

Discussion

In the present study, we showed that oxytetracycline residues had no significant impact on plankton growth, but it showed lower chlorophyll-a concentrations in the treatment ponds. This result supports of Seoane et al (2014)., where authors observed the similar trends that cells exposed to oxytetracycline had lower chlorophyll-a. Higher growth performance was observed at dose of OTC (100 mg kg^{-1}) for tilapia in treatment ponds but in Rohu., there was a decreased growth performance in treatment ponds comparing to control ones. Reda et al., (2013) and Sanchez-Martinez et al., (2008) reported in their study that feeding OTC at a dosage of 100 mg kg^{-1} showed a considerable increase in weight gain in *O. niloticus*. While employing a therapeutic dosage of 80 mg/kg b. w./day, Das et al.,2021 found that oxytetracycline had no discernible impact on *Labeo rohita*'s ability to grow.

Numerous factors, especially the accumulation of pollutants and pesticides (Srivastava et al. 2016) and the preservation methods, such as freezing (Siddiqui et al. 2010; Siddique et al. 2011), can affect the biochemical composition of fish. We analyzed the effects of the use of OTC exposure on fish biochemical composition in our study, as this has not been previously reported. Our findings indicated that ash decreased and lipid, protein, and moisture increased statistically significant ($p>0.05$) in Tilapia (Figure 2). Again, in Rohu, lipid and protein in the treatment group increased and ash and moisture decreased in a significant way, but the moisture was not significantly reduced (**Figure 2**). Brown and Murphy (1991) stated that crude protein, ash and moisture found a negative correlation with final relative weight, on the other hand there was inverse relation with crude fat for striped bass.

In the experiment amylase activity was found noticeably higher in the treatment group of the both fish species, whilst substantial reduction in protease activity was observed in both the treatment and control groups. This was in agreement with findings from previous studies of Limbu et al., (2018) and Mog et al., (2021). Oxytetracycline is a substance that causes fish to experience oxidative stress. The most often utilized indicators of oxidative stress are catalase (CAT) activity and superoxide dismutase (SOD) (Nunes et al., 1985). The results of the SOD activity of tilapia and rohu showed that there was an increase in SOD activity in both treatment groups, indicating that the fish experienced less stress under the treatment condition. Xiao-qing Liu (2011) reported that under oxidative stress, CAT breaks down the hydrogen peroxide created when superoxide ions are dismutated by SOD. The catalase results showed that the control group of both tilapias and rohu had greater catalase enzyme activity than the treatment group, indicating that the control group had a stronger adaptive response to OTC than the treatment group and that the treatment group was under stressful conditions.

Conclusion

The findings of this study demonstrated that oxytetracycline residues had no significant impact on growth

performance, enzymatic activity of the Tilapia and Rohu as well as pond primary productivity. It is clear that the therapeutic dose of OTC (100 mg/kg) can be safe for usage when fish species are cultured using the polyculture technique. Further research is required to fully comprehend the dosage level and long-term effects of OTC-induced alterations in the poly-cultured fish species.

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Conflict of Interest
The authors declare no conflict of interest.

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