



Journal of
**Fisheries and
Aquatic Science**

ISSN 1816-4927



Academic
Journals Inc.

www.academicjournals.com

Effect of Oxytetracycline on Thai Silver Barb (*Barbonymus gonionotus*) and on its Culture Environment

^{1,2}M.J. Islam, ²M.G. Rasul, ²M.A. Kashem, ³M.M. Hossain, ⁴A.A. Liza, ²M.A. Sayeed and ²M. Motaher Hossain

¹Department of Biology, Marine Biology Section, Ghent University, Krijgslaan, 281 S8, B 9000 Gent, Belgium

²Department of Fisheries Technologies and Quality Control, Faculty of Fisheries, Sylhet Agricultural University, Sylhet, 3100, Bangladesh

³Department of Coastal and Marine Fisheries, Faculty of Fisheries, Sylhet Agricultural University, Sylhet, 3100, Bangladesh

⁴Department of Soil Science, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh, 2202, Bangladesh

Corresponding Author: M.J. Islam, Department of Biology, Marine Biology Section, Ghent University, Krijgslaan, 281 S8, B 9000 Gent, Belgium

ABSTRACT

Studies were conducted to investigate impacts of oxytetracycline on *Barbonymus gonionotus* muscle. Oxytetracycline the most widely used antibiotics, was fed to *Barbonymus gonionotus* with 4 g kg⁻¹ rate through diet for 45 days. Growth rate was observed for different concentrations of oxytetracycline. Water quality parameters and bacterial quantification were observed for a period of 21 days. Pond water, sediment and fish muscle were tested before and after oxytetracycline medicated feeding. Before feeding of oxytetracycline medicated feed, there was no oxytetracycline in water, sediment and fish muscles. After 2 days of feeding the initial oxytetracycline accumulation was 5070.0 ppb, which reduced considerably to 1190.0 ppb after 20 days from end of medicated diet feeding and not detected after 42 days. Physico-chemical parameters of pond water were determined. Before treatment dissolve oxygen, pH and total hardness of fish pond was 4.516±0.25 mg L⁻¹, 7.413±0.09 and 822.33±1.52 ppm, respectively. After 21 days of study period, reached to 5.27±0.025 mg L⁻¹, 7.773±0.04 and 769.66±2.08 ppm. It indicates a little change after 21 days. Before oxytetracycline treatment, bacterial load in *Barbonymus gonionotus* pond was 9.417±0.035×10³ CFU g⁻¹ in water, 7.31±0.04×10⁷ CFU g⁻¹ in sediment, 6.51±0.04×10⁶ CFU g⁻¹ in fish gills and 8.52±0.035×10⁷ CFU g⁻¹ in fish intestine, which was significantly reduced to 5.28±0.02×10³ CFU g⁻¹ in water, 2.91±0.02×10⁷ CFU g⁻¹ in sediment, 2.21±0.02×10⁶ CFU g⁻¹ in gills and 2.39±0.02×10⁷ CFU g⁻¹ in intestine.

Key words: Oxytetracycline, *Barbonymus gonionotus*, bacteria, environment, residues

INTRODUCTION

Aquaculture is a fastest growing industry all over the world. Decreasing trend of marine catch has lead to simultaneous intensification of aquaculture techniques, resulting more susceptibility to disease outbreaks and ultimately it triggers the use of the medication and medicated diet (Jerbi *et al.*, 2011). Now, aquaculture is the major protein supplier all over the world. The most hazardous point for aqua industries are that (1) A large proportion of the world's antimicrobial industrial production is used as prophylactics and (2) Growth promoters that far compensate their

use as therapeutics (Bush *et al.*, 2011). Antibiotics are one of the most frequent groups used as feed additives in the form of growth promoter. Aqua farm owners are using various preventive measures to boost up production. To maintain good water quality and appropriate dietary management, antibiotics are mixed with feed and feed ingredients with a sub-therapeutic dose. It has been proved that using antibiotics at low dose mixed with feed remain active for long period rather than direct application to fish pond. These antibiotic medicated diets keep fish healthy and works as safe guard for disease occurrences (Islam *et al.*, 2014). Antibiotics are still used as form of growth promoters in most of the aqua farms in the world (Cabello *et al.*, 2013). The most widely used antibiotics in fish farms is oxytetracycline (Rigos and Troisi, 2005). Oxytetracycline is a tetracycline broad spectrum antibiotic with bacteriostatic action produced by *Streptomyces* spp. fungi, used to treat systemic bacterial infections of fish (Jerbi *et al.*, 2011). Oxytetracycline is more active against some bacteria than chloramphenicol (Burridge *et al.*, 2010). In literature, the oxytetracycline safe dosages for prophylaxis purpose well established but no information about safe dosages to use as growth promoter and feed additives (Viola and DeVincent, 2006). In developing countries generally fish feed producers use to mix antibiotics with aqua feed during formulation in order to make food more effective. This type of practice is more intense mainly for lacking of law enforcement, feed industries and farmers use antibiotics very frequently without any concern from aqua practitioner (Serrano, 2005). Treated fish showed evidence of a considerable increase in weight achieves suggesting a growth promoting action (Sanchez-Martinez *et al.*, 2008). Earlier studies found in the literatures showed the negative effects of antibiotics on the immune system of fish (Lunden and Bylund, 2000; Guardiola *et al.*, 2012). Oxytetracycline interferes with humoral intrinsic immune parameters and increases the cellular parameters (Guardiola *et al.*, 2012). Many studies showed the side effects of extended time antibiotic use on fish which can cause nephrotoxicity and liver damage or malfunctions (Hentschel *et al.*, 2005). Moreover, the use of large quantity of antibiotics may cause the presence of antibiotics residues in fish tissue and products (Samanidou and Evaggelopoulou, 2007). Use of antibiotics in aquaculture has great impacts on aquatic fauna and as well as microbial environment. Entrance of different chemicals through consumption of aquatic food has significant impacts on human health (Kummerer, 2009). Antibiotics used in terrestrial animals are well studied, but information for aquatic animals available only for some worldwide recognized commercial fishes. Application of antibiotics in aquatic environment interfere water quality by different means such as microbial growth control and suppression/control of pathogenic microbes. The antibiotics exposed in the water bodies deposited in organisms, soil sediments and water column. This process ultimately leads antibiotic resistance and threatens human and animal health (Cabello *et al.*, 2013). Thai silver barb (*Barbonymus gonionotus*) is an important commercial species in Asian aquaculture. This species have higher growth rate, food conversion ratio and preferable muscle quality, less pin bones made the species demandable to consumer all over the world especially Asian peoples.

The aim of the present research was to determine the effects of dietary supplementation of oxytetracycline for 49 days on *Barbonymus gonionotus* growth performance, the residual period and changes in water quality parameters along with changes in bacterial load in water, sediment, gills and intestine after termination of treatment by 21 days.

MATERIALS AND METHODS

Experimental design: Ninety *B. gonionotus* apparently healthy with an average body weight of 20 ± 0.09 g. Fish were kept in two cement ponds ($2.5 \times 1.25 \times 1.25$ m) equipped with paddle aerator for

14 days to acclimate before starting experiment. Fish were allocated into three groups (30 fish/groups) in duplicate. Group 1 (G1) was fed a basal diet (consisted 26% crude protein and 2885 kcal kg⁻¹ metabolizable energy) as the control group; while group 2 (G2) and 3 (G3) were fed by basal diet with oxytetracycline supplementation at dosage of 100 mg kg⁻¹. The fish of all three groups were fed 5% from biomass regularly three times daily for 7 weeks. Water quality parameters and microbial study of fish muscle, skin, intestine and gills were measured periodically.

Growth performance: Fish were weighed every week to assess the growth performance. The final body weight (g), weight gain (g) and Food Conversion Ratio (FCR) were determined according to Siddiqui *et al.* (1988).

Histopathological examination: Tissues were taken from kidney and liver of fish and fixed on 10% neutral buffered formalin for 24 h, dehydrated in ascending alcohol and cleared in xylene earlier than embedding in paraffin. Five to seven micron sections were prepared according to Bancroft and Gamble (2008).

Detection of oxytetracycline residues using LC-MS/MS: Sample preparation, extraction and determination of oxytetracycline residue were applied using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) analytical assays on an Waters Alliance 2695 series HPLC and Quattro Micro, API mass spectrometer instrumentation-Waters Corporation, USA (Islam *et al.*, 2014).

Microbial analysis: Microbial testing for soil, water, fish muscle, gill, skin and intestine were done followed by APHA (1998) and Clesceri *et al.* (1998).

Determination of water quality parameters: The water quality parameters were recorded with a 7 days interval until 21 days of the study period. Measurements of water quality and sample collections were made between 8.00-10.00 am on each sampling day. Water samples were collected from each pond to a 30 cm depth (Cole and Boyd, 1986). On each and every sampling day, 500 mL of pond water was collected very carefully in a pre-cleaned black plastic bottle with cap, to prevent and minimize any agitation as well as shaking. Each sample was taken with three replicates. Only temperature, dissolved oxygen, hardness and pH were measured. Water temperature was recorded on spot with a centigrade thermometer. Other parameters were measured using HACH Test Kit (Model: FF-2, Cat No. 2430-01; Company: HACH Company World Headquarters, Loveland, Colorado).

Statistical analysis: Statistical analysis of antibiotic contamination for feed and feed ingredient was made using analysis of variance (one-way ANOVA). Differences between means were assessed for significance test by Tukey-HSD test with a significance level of $p < 0.05$.

RESULTS

Changes in water quality parameters: Water quality parameters especially (DO, pH, hardness and temperature) were also determined periodically to know the culture environment conditions for growth and well being of *B. gonionotus*.

Temperature: Temperature fluctuations were studied to understand the growth fish and exact effects of oxytetracycline. Temperatures of the fish ponds were found no significant differences ($p>0.5$). There was no relation between oxytetracycline application and temperature increase. The mean value of water temperature was 26.62°C . In 0 day (before treatment), the lowest pond temperature was recorded $25.87\pm0.015^{\circ}\text{C}$ and highest temperature $27.33\pm0.015^{\circ}\text{C}$ was after 21 days of antibiotic treatment (Fig. 1). These values were also found in control pond (feeding without oxytetracycline medicated feed). Temperature might be increased gradually due to environmental conditions not for oxytetracycline.

Changes in pH: During the day-0 (before treatment), there was lowest pH value (7.413 ± 0.09). After antibiotic treatment, pH values were increased slightly. In the day 7, 14 and 21, the pH values were statistically similar (7.68 ± 0.02) and there were no significant differences of pH among day 7, 14 and 21 (after antibiotic treatments) (Fig. 2).

Changes in dissolve oxygen: From 0-21 days DO values rises from 4.516 ± 0.25 to 5.27 ± 0.025 mg L^{-1} . This increased might be for reduction of bacterial quantity as well as less Biological Oxygen Demand (BOD) (Fig. 2).

Changes in total hardness: During the 0 day, there was highest total hardness value (822.33 ± 1.52 ppm). After antibiotic treatment and at the end of 21 days, the total hardness

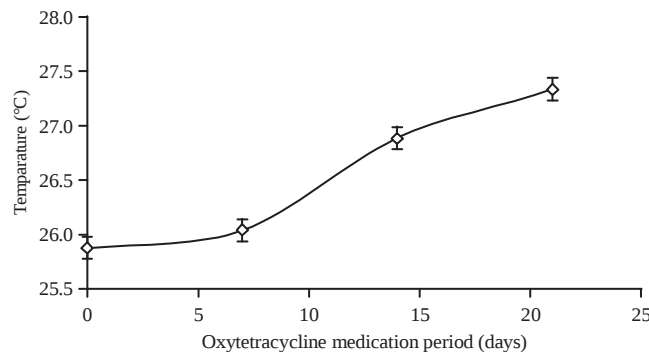


Fig. 1: Water temperature change pattern of *Barbonymus gonionotus*

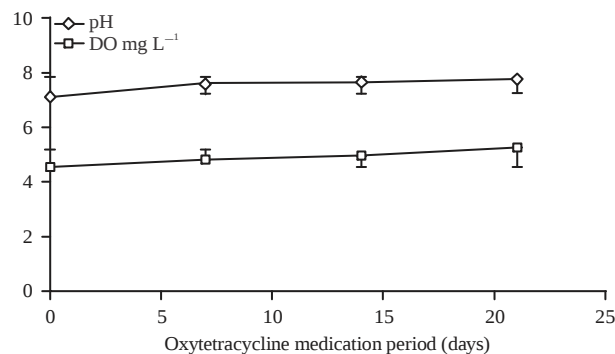


Fig. 2: pH and Dissolve Oxygen (DO) status of *Barbonymus gonionotus* culture pond during the study period

values were decreased to 769.66 ± 2.08 ppm. The changes of hardness in antibiotic treatments in day 0, 7, 14 and 21 were statistically significant ($p < 0.037$) (Fig. 3).

Growth parameters: The *B. gonionotus* in group 3 (G_3) showed significant increase in final body weight (g), weight gain (g) and significant decrease in Food Conversion Ratio (FCR) followed by *B. gonionotus* in group 2 (G_2) with respect to the values obtained in the control groups (Fig. 4).

Histopathological findings: Liver tissue specimens of the control group show normal hepatocytes and sinusoidal architectures (Fig. 5a). Liver tissue samples had congestion (arrowhead), severe fatty change and vacuolations in the hepatocytes (arrow) (Fig. 5b). Kidney tissue samples of the control group showed normal nephron structure (Fig. 6a). Histopathological alterations in kidney tissue samples of G_2 manifested periglomerular lymphocytes aggregation (arrow) (Fig. 6b).

Oxytetracycline in edible flesh tissue/muscles: Initial oxytetracycline accumulation was 5070.0 ppb which reduced considerably to 1190.0 ppb after 45 days of stopping oxytetracycline

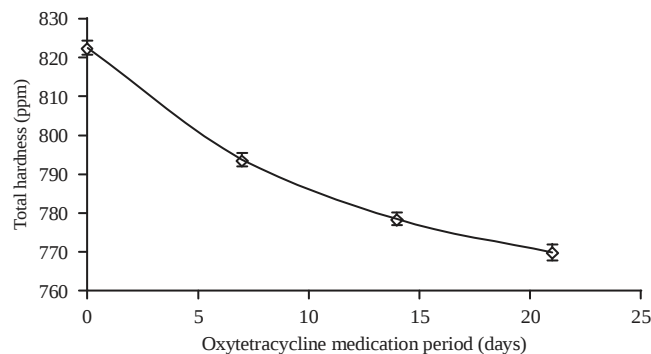


Fig. 3: Total hardness changing trends throughout experiment of *Barbonymus gonionotus* rearing pond

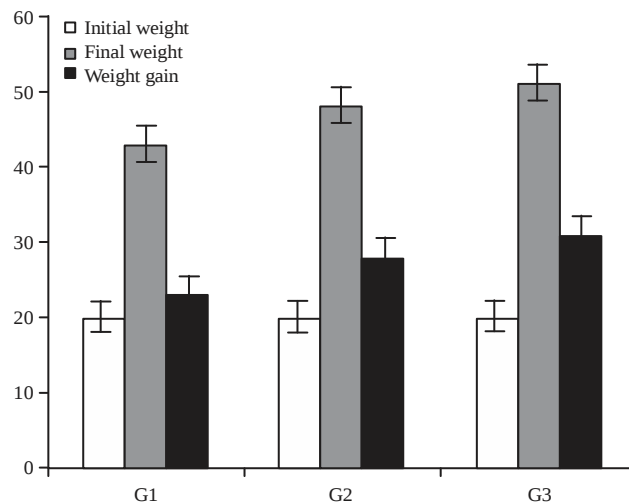


Fig. 4: Effect of oxytetracycline on *Barbonymus gonionotus* growth performance, Data presented as Mean $\pm$ SD

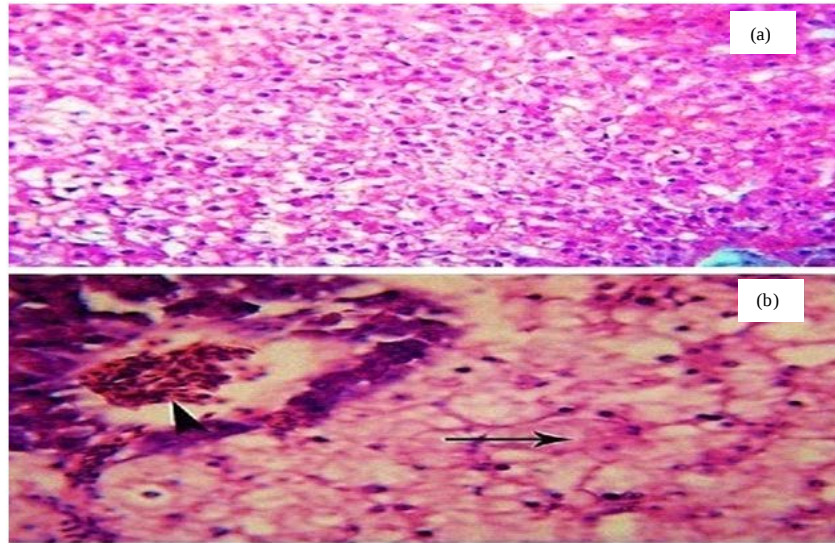


Fig. 5(a-b): Histopathological sections view of liver of *Barbonymus gonionotus* (a) Fed on basal diet and (b) Fed on oxytetracycline at 100 mg kg⁻¹ diet showed congestion (arrow head), severe fatty change and vacuolations in the hepatocytes (arrow)

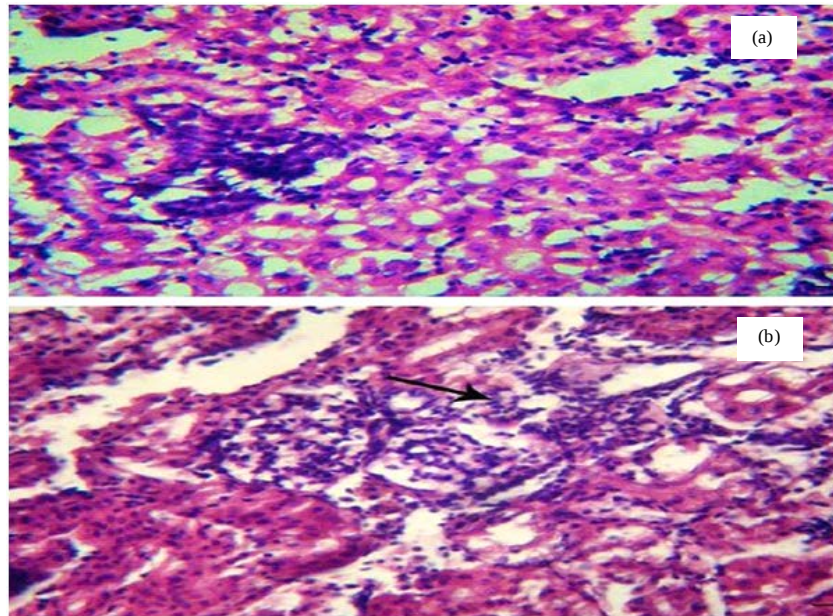


Fig. 6(a-b): Histopathological sections view of kidney of *Barbonymus gonionotus* (a) Fed on basal diet and (b) Fed on oxytetracycline at 100 mg kg⁻¹ diet showed periglomerular lymphocytic aggregation (arrow)

treated diets and this antibiotic was not detected later on. However, the result obtained from the present study indicated that withdrawal period of oxytetracycline in *B. gonionotus* muscle is 45 days (Fig. 7).

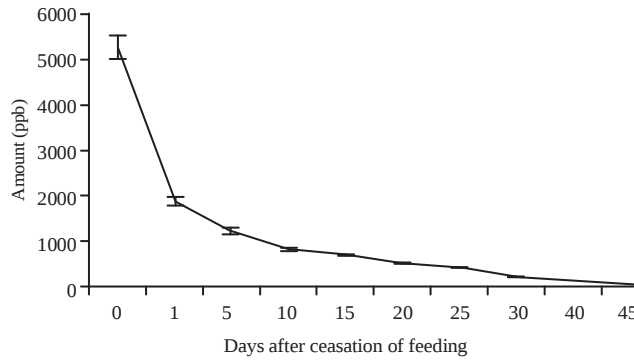


Fig. 7: Residual periods of oxytetracycline in feed after cessation of feeding of *Barbonymus gonionotus*

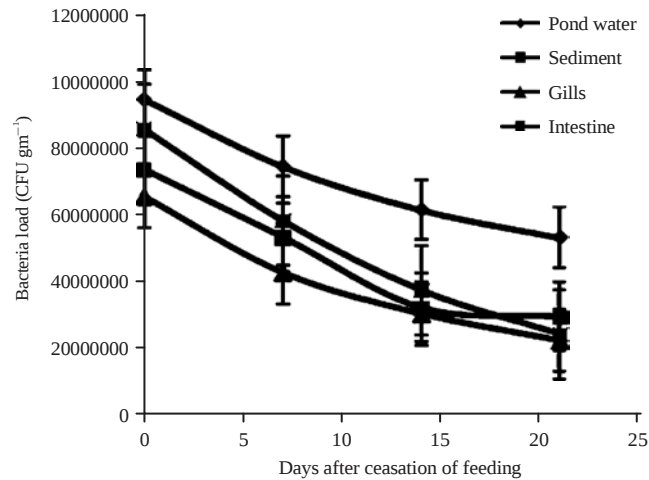


Fig. 8: Bacterial loads in pond water, sediment, gills and intestinal content of *Barbonymus gonionotus*

Bacteriological findings: The results of the quantitative estimation of aerobic heterotrophic bacteria in the experimental pond water, sediment, the gill filaments and intestine of olive barb fish (Fig. 8).

Changes of bacterial quantity in culture environment: For pond water the highest bacterial load was found at 0 days ($9.417 \pm 0.035 \times 10^3$ CFU mL⁻¹). Whereas, it decreases gradually and reached to lowest value ($5.28 \pm 0.02 \times 10^3$ CFU mL⁻¹) at 21-days subsequently. In case of pond sediment the highest quantity was $7.31 \pm 0.04 \times 10^7$ CFU g⁻¹ at 0 days and finally decreased to $2.91 \pm 0.02 \times 10^7$ CFU g⁻¹. There is great significance of reducing bacterial load in water among day 0, 7, 14 and 21.

Changes of bacterial quantity in *Barbonymus gonionotus*: Bacterial load was found highest load at intestine ($8.52 \pm 0.035 \times 10^7$ CFU g⁻¹) followed by gills ($6.51 \pm 0.04 \times 10^6$ CFU g⁻¹) during 0 days of antibiotic mediated feeding. After 21 days of treatment the quantity reduced to $2.21 \pm 0.02 \times 10^6$ and $2.39 \pm 0.02 \times 10^7$ CFU g⁻¹ for gill and intestine, respectively. The bacterial load on the skin was not considered due its close contact with water (Fig. 8).

DISCUSSION

Oxytetracycline a broad spectrum antibiotic widely used all over the world for human (Sharma and Saxena, 2012), cattle farming, poultry and fish farming (Islam *et al.*, 2014). It's a very cheap and available antibiotics and quickly effective for rapid metabolic characteristics (Samuelsen *et al.*, 1992; Boleas *et al.*, 2005). Fish farmer, aquaculture professionals and aquarium lovers used this antibiotic for the better growth as growth promoter and also for prophylaxis purposes. Oxytetracycline frequently used with fish diet as growth enhancer and administer directly to water (Bjorklund *et al.*, 1991). Farm owner and local farmer always follow maximum dosages limit to ensure their fish are healthy. That's why we used 100 mg kg⁻¹ b.wt. (20 mg extra than the recommended 80 mg). Another reason to use excess amount was our past experiences. Microbes in water have gained significant resistance power against oxytetracycline. Water quality parameters mainly temperature, DO, pH and total hardness were studied to know the quality of culture environment for fish and to compare effect of oxytetracycline and environmental parameters on growth of fish. Temperature ranges between 25.87-27.33°C, pH value ranges from 7.41-7.68 (Fig. 4), whereas DO level ranges from 4.51-2.27 mg L⁻¹. Despite of slight increase in temperate, a little increase in pH and DO values. This might be for decreased bacterial quantity and Biological Oxygen Demand (BOD). We didn't find significant relations between oxytetracycline application and environmental parameters. Further study is needed to make precise understanding about antibiotics effects on water quality parameters. Results was reported for temperature 22-37°C (Del Nery *et al.*, 2013) and 25.8±1.0°C and pH 7.6±0.2 (Castine *et al.*, 2012) and 6.2-9.2 (Losordo and Piedrahita, 1991), DO = 7±2 mg L⁻¹ (Boyd *et al.*, 1978) and 3-5 mg L⁻¹ (Diana *et al.*, 1991). Total hardness ranges from 822.33±1.52-769.66±2.08 mg L⁻¹ (Fig. 5), similar results were recorded by Boyd (1982) and Geiger (1983). However, a lower value of total hardness also reported such as 8-12 mg L⁻¹ (Cole and Boyd, 1986), ratios of hardness from 5-10 mg L⁻¹ (Mandal and Boyd, 1980). We found a high value for total hardness; this was might be application of lime stone for pond preparation before starting of experiment.

The higher dosages of oxytetracycline have a great impact on growth rate of *B. gonionotus* and significantly reduce the Food Conversion Ratio (FCR) (Fig. 4). Oxytetracycline significantly enhanced growth on channel catfish (weight and length), as they were heavier (12.5%) than controls at the end of the trial. In literature prophylaxis dosages were well established for most of commercially important species. About 30-80 mg kg⁻¹ b.wt. of oxytetracycline is frequently prescribed by aquaculture practitioner (Reda *et al.*, 2013). Residual periods of different antibiotics in various species are not well studied and thus have very little information (Islam *et al.*, 2014). The concentrations commonly used in aquaculture, exposure of healthy channel catfish to oxytetracycline enhanced growth (Sanchez-Martinez *et al.*, 2008). The performance effects of oxytetracycline have been known for the food animals e.g. fish, chicken, bovine and swine etc., where it progress weight increase and feed effectiveness (Quigley *et al.*, 1997; Dibner and Richards, 2005). Additionally, significant changes occurred in liver and kidney tissues. Congestion of hepatocytes cells, void space and severe fatty changes were observed in liver whereas, periglomerular lymphocytic aggregation was found in kidney tissues. Similar results also reported for tilapia and channel catfishes (Reda *et al.*, 2013). Applications of higher dosages of antibiotics cause immune disruptions and fish become more susceptible to disease occurrence after antibiotic residual periods (Sanchez-Martinez *et al.*, 2008). Although, the effects of oxytetracycline medicated feed on growth of fish have not been confirmed and the mechanism as growth promoter is not clear

and has not been explicated, it is assumed that it's working function by reducing intestinal bacterial load (Gaskins *et al.*, 2002; Collier *et al.*, 2003).

The maximum effective and safe dosage of oxytetracycline was used (Sarker *et al.*, 2000). However, the amount of dosages and frequency of application, application methods have impacts on efficiency and residual periods (Cabello *et al.*, 2013). The residual periods of oxytetracycline was found until 42 days of experiment (Fig. 7). However, more controlled studies are needed to make a conclusion about safe dosages for treatment purpose and growth promoter. Our study objective was to determine the residual periods for this popular commercial fish. Although, it is easily degradable drugs, but can stay in muscle as metabolites forms and in sediment of culture environment for a certain periods (Burridge *et al.*, 2010; Muhammad *et al.*, 2011). Scientists have investigated the length of time that certain drug residues (sulphamerazine and tetracycline) remain in fish (Wen *et al.*, 2006; Ansari *et al.*, 2012; Pham *et al.*, 2015). They found sulphamerazine and tetracycline residues could still be detected in fish muscle 3-7 days and 17 days after oral treatment, respectively (Pham *et al.*, 2015). The excretion rate of oxytetracycline in trout and were unable to detect residues present in muscle 10 or 35 days after oral administration dependant on the water temperature (Guardiola *et al.*, 2012). Into residues of oxytetracycline in catfish tissues which were undetectable, 2 days after oral administration (Yonar, 2012) and describes an investigation into the length of time drug residues could be detected in the muscle of young rainbow trout after administration by various routes (Fallah and Barani, 2014; Koh *et al.*, 2014).

Histological observations were found for both kidney and liver tissues (Fig. 5-6). Exposure to drug oxytetracycline could result in adaptive alterations, changes in tissue and cellular damages (Nunes *et al.*, 2015). Liver tissue specimens of the control group show normal hepatocytes and sinusoidal architectures (Fig. 5a) whereas, severe fatty change and vacuolations in the hepatocytes (Fig. 5b). Kidney tissue samples of the control group showed normal nephron structure (Fig. 6a) and alterations in kidney tissue samples of G₂ manifested periglomerular lymphocytes aggregation (Fig. 6b). The absorption and elimination period is greatly influenced by temperature (Bjorklund *et al.*, 1992), which was not our study objectives. The distribution profiles of oxytetracycline in tissue was found and tissue absorption decreased in the orderly from liver-bone- kidney to skin muscle at the end of the experiment (Ueno *et al.*, 1989; Namdari *et al.*, 1996). The serum residue of oxytetracycline in fish did not decline until 8 weeks and at end of this time it was found at 3-7 $\mu\text{g mL}^{-1}$ and two days after injection the value was 13 $\mu\text{g mL}^{-1}$. Tow folds higher concentration was determined in liver tissue than body muscles and substantial hepatocytes damage were found in histopathological examination (Islam *et al.*, 2014). Moreover, distribution profiles of oxytetracycline in both liver and kidney were found to be very similar; oxytetracycline concentrations was found decreased after end medication feeding and detection value reached below 0.05 $\mu\text{g g}^{-1}$ at day 8 and 30 post medication (Wang *et al.*, 2004). The oxytetracycline presence in fish muscle showed a dosage and responsive pattern. It was found at 295 $\mu\text{g kg}^{-1}$ after 10 days of continuous medication with the dosage of 75 mg kg^{-1} . So, after 10 days all residues are not eliminated properly and substantial alteration were recorded in both kidney and liver tissues (Elia *et al.*, 2014) and functioned as prooxidant (Nunes *et al.*, 2015). Our finding indicates the same results we found all residues eliminated after 45 days from end of medication feeding (Fig. 7). The values for the detected antibiotics in fish bile, plasma, liver and muscle tissues were at the range of 2.06-4.08, 1.85-3.47, 1.41-3.51 and 0.48-2.70 ppb, respectively (Zhao *et al.*, 2015).

Bacterial load was found highest load at gills ($6.51 \pm 0.04 \times 10^6 \text{ CFU g}^{-1}$) and in intestine ($8.52 \pm 0.035 \times 10^7 \text{ CFU g}^{-1}$) during 0 day of antibiotic medicated feeding. After 21 days of treatment,

the quantity reduced to $2.21 \pm 0.02 \times 10^6$ and $2.39 \pm 0.02 \times 10^7$ CFU g⁻¹ for gill and intestine, respectively (Fig. 8). Seasonal bacterial quantity were studied and result showed that Total Viable Counts (TVC) of bacteria in the fish intestine ranged between $6.8 \pm 1.9 \times 10^6$ to $7.5 \pm 1.4 \times 10^7$ CFU g⁻¹ in early summer, $1.6 \pm 2.0 \times 10^6$ - $5.1 \pm 2.5 \times 10^7$ CFU g⁻¹ in summer, $3.1 \pm 1.4 \times 10^8$ - $1.3 \pm 2.2 \times 10^9$ CFU g⁻¹ in autumn and $8.9 \pm 1.8 \times 10^5$ - $1.3 \pm 0.9 \times 10^7$ CFU g⁻¹ in winter (Al-Harbi and Uddin, 2004). Our study is supported by Al-Harbi and Uddin (2003, 2005). Although, intestinal bacterial quantity varied highly with fasting period of fish, food types and abundances (Margolis, 1935). After 3 weeks of fish meal feeding trial with oxytetracycline shows significant decrease in bacterial count with crucial improvement of cell repairing activities (Smith *et al.*, 1994; Bakke-McKellep *et al.*, 2007). Log cultivable adherent bacteria in the foregut varied between $3.85 (7 \times 10^3)$ /g wet weight for fish when fed soybean meal and $4.85 (7 \times 10^4)$ /g when fed with BPSBM. The quantity of bacteria colonizing the mid-gut were 9×10^3 (log = 3.95)/g (fish fed soybean meal) and 6×10^4 (log = 4.78)/g (fish were fed fish meal). Generally, bacterial load was higher a hind gut region than foregut part of fish intestine (Ringo *et al.*, 2006). At the end of 21 days of oxytetracycline medicated feeding trial, the bacterial quantity reduced significantly in gill, gut and skin (DePaola *et al.*, 1995) as we found similar results. As many as 10^8 viable bacteria per gram wet weight of alimentary tract contents were counted. Total alimentary tract bacteria contents of salmon from 1.4×10^5 - 9.9×10^7 CFU g⁻¹ wet weight of sample (Trust and Sparrow, 1974). Moreover, bacterial content is greatly dependent of water quality, drainage pattern and runoff from catchment area (Islam *et al.*, 2012). Microbes in gut, skin, intestines and gills are significantly impacted by oxytetracycline.

ACKNOWLEDGMENTS

The authors would like to thank Ministry of Science and Technology, Bangladesh for funding under Research and Development (R and D) project. We would also like to express our gratitude to Dr. M. Mahbub Iqbal, Department of Fisheries Biology and Genetics, Sylhet Agricultural University, Sylhet, 3100, Bangladesh. The authors are pleased to say thank to Mr. Shamsul Alam for supplying our required fish seeds.

REFERENCES

- APHA., 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association, Washington, DC., USA., ISBN-13: 9780875532356, Pages: 1270.
- Al-Harbi, A.H. and N. Uddin, 2003. Quantitative and qualitative studies on bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *O. aureus*) cultured in earthen ponds in Saudi Arabia. *J. Aquacult. Res.*, 33: 43-48.
- Al-Harbi, A.H. and M.N. Uddin, 2004. Seasonal variation in the intestinal bacterial flora of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) cultured in earthen ponds in Saudi Arabia. *Aquaculture*, 229: 37-44.
- Al-Harbi, A.H. and N. Uddin, 2005. Bacterial diversity of tilapia (*Oreochromis niloticus*) cultured in brackish water in Saudi Arabia. *Aquaculture*, 250: 566-572.
- Ansari, M., M. Raissy and E. Rahimi, 2012. Determination of florfenicol residue in rainbow trout muscles by HPLC in Chaharmahal va Bakhtiari Province, Iran. *Comp. Clin. Pathol.*, 23: 61-62.
- Bakke-McKellep, A.M., M.H. Penn, P.M. Salas, S. Refstie and S. Sperstad *et al.*, 2007. Effects of dietary soyabean meal, inulin and oxytetracycline on intestinal microbiota and epithelial cell stress, apoptosis and proliferation in the teleost Atlantic salmon (*Salmo salar* L.). *Br. J. Nutr.*, 97: 699-713.

- Bancroft, J.D. and M. Gamble, 2008. Theory and Practice of Histological Techniques. Elsevier Health Sciences, Philadelphia, PA., ISBN-13: 9780443102790, Pages: 725.
- Bjorklund, H.V., C.M.I. Rabergh and G. Bylund, 1991. Residues of oxolinic acid and oxytetracycline in fish and sediments from fish farms. *Aquaculture*, 97: 85-96.
- Bjorklund, H.V., A. Eriksson and G. Bylund, 1992. Temperature-related absorption and excretion of oxolinic acid in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 102: 17-27.
- Boleas, S., C. Alonso, J. Pro, C. Fernandez, G. Carbonell and J.V. Tarazona, 2005. Toxicity of the antimicrobial oxytetracycline to soil organisms in a multi-species-soil system (MS•3) and influence of manure co-addition. *J. Hazard. Mater.*, 122: 233-241.
- Boyd, C.E., 1982. Hydrology of small experimental fish ponds at auburn, Alabama. *Trans. Am. Fish. Soc.*, 111: 638-644.
- Boyd, C.E., R.P. Romaine and E. Johnston, 1978. Predicting early morning dissolved oxygen concentrations in channel catfish ponds. *Trans. Am. Fish. Soc.*, 107: 488-492.
- Burridge, L., J.S. Weis, F. Cabello, J. Pizarro and K. Bostick, 2010. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture*, 306: 7-23.
- Bush, K., P. Courvalin, G. Dantas, J. Davies and B. Eisenstein *et al.*, 2011. Tackling antibiotic resistance. *Nat. Rev. Microbiol.*, 9: 894-896.
- Cabello, F.C., H.P. Godfrey, A. Tomova, L. Ivanova, H. Dolz, A. Millanao and A.H. Buschmann, 2013. Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbiol.*, 15: 1917-1942.
- Castine, S.A., D.V. Erler, L.A. Trott, N.A. Paul, R. de Nys and B.D. Eyre, 2012. Denitrification and anammox in tropical aquaculture settlement ponds: An isotope tracer approach for evaluating N₂ production. *PloS One*, Vol. 7. 10.1371/journal.pone.0042810
- Clesceri, L.S., A.E. Greenberg and D.A. Eaton, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., APHA American Public Health Association, Washington DC., USA.
- Cole, B.A. and C.E. Boyd, 1986. Feeding rate, water quality and channel catfish production in ponds. *Progr. Fish-Culturist*, 48: 25-29.
- Collier, C.T., M.R. Smiricky-Tjardes, D.M. Albin, J.E. Wubben and V.M. Gabert *et al.*, 2003. Molecular ecological analysis of porcine ileal microbiota responses to antimicrobial growth promoters. *J. Anim. Sci.*, 81: 3035-3045.
- DePaola, A., J.T. Peeler and G.E. Rodrick, 1995. Effect of oxytetracycline-medicated feed on antibiotic resistance of gram-negative bacteria in catfish ponds. *Applied Environ. Microbiol.*, 61: 2335-2340.
- Del Nery, V., M.H.Z. Damianovic, E. Pozzi, I.R. de Nardi, V.E.A. Caldas and E.C. Pires, 2013. Long-term performance and operational strategies of a poultry slaughterhouse waste stabilization pond system in a tropical climate. *Resour. Conserv. Recycl.*, 71: 7-14.
- Diana, J.S., C.K. Lin and P.J. Schneeberger, 1991. Relationships among nutrient inputs, water nutrient concentrations, primary production and yield of *Oreochromis niloticus* in ponds. *Aquaculture*, 92: 323-341.
- Dibner, J.J. and J.D. Richards, 2005. Antibiotic growth promoters in agriculture: History and mode of action. *Poult. Sci.*, 84: 634-643.
- Elia, A.C., V. Ciccotelli, N. Pacini, A.J.M. Dorr and M. Gili *et al.*, 2014. Transferability of oxytetracycline (OTC) from feed to carp muscle and evaluation of the antibiotic effects on antioxidant systems in liver and kidney. *Fish Physiol. Biochem.*, 40: 1055-1068.

- Fallah, A.A. and A. Barani, 2014. Determination of malachite green residues in farmed rainbow trout in Iran. *Food Control*, 40: 100-105.
- Gaskins, H.R., C.T. Collier and D.B. Anderson, 2002. Antibiotics as growth promotants: Mode of action. *Anim. Biotechnol.*, 13: 29-42.
- Geiger, J.G., 1983. A review of pond zooplankton production and fertilization for the culture of larval and fingerling striped bass. *Aquaculture*, 35: 353-369.
- Guardiola, F.A., R. Cerezuela, J. Meseguer and M.A. Esteban, 2012. Modulation of the immune parameters and expression of genes of gilthead seabream (*Sparus aurata* L.) by dietary administration of oxytetracycline. *Aquaculture*, 334-337: 51-57.
- Hentschel, D.M., K.M. Park, L. Cilenti, A.S. Zervos, I. Drummond and J.V. Bonventre, 2005. Acute renal failure in zebrafish: A novel system to study a complex disease. *Am. J. Physiol. Renal Physiol.*, 288: F923-F929.
- Islam, M., S. Mian, M.N.A. Khan, M.N. Islam and M. Kamal, 2012. Detection of *Salmonella* in some selected shrimp farms and hatcheries. *Int. J. Anim. Fish. Sci.*, 5: 449-455.
- Islam, M.J., A.A. Liza, A.H.M.M. Reza, M.S. Reza, M.N.A. Khan and M. Kamal, 2014. Source identification and entry pathways of banned antibiotics nitrofurans and chloramphenicol in shrimp value chain of Bangladesh. *EurAsian J. BioSci.*, 8: 71-83.
- Jerbi, M.A., Z. Ouannes, R. Besbes, L. Achour and A. Kacem, 2011. Single and combined genotoxic and cytotoxic effects of two xenobiotics widely used in intensive aquaculture. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, 724: 22-27.
- Koh, C.B., N. Romano, A.S. Zahrah and W.K. Ng, 2014. Effects of a dietary organic acids blend and oxytetracycline on the growth, nutrient utilization and total cultivable gut microbiota of the red hybrid tilapia, *Oreochromis* sp. and resistance to *Streptococcus agalactiae*. *Aquacult. Res.* 10.1111/are.12492
- Kummerer, K., 2009. Antibiotics in the aquatic environment-a review-Part I. *Chemosphere*, 75: 417-434.
- Losordo, T.M. and R.H. Piedrahita, 1991. Modelling temperature variation and thermal stratification in shallow aquaculture ponds. *Ecol. Modell.*, 54: 189-226.
- Lunden, T. and G. Bylund, 2000. The influence of *in vitro* and *in vivo* exposure to antibiotics on mitogen-induced proliferation of lymphoid cells in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.*, 10: 395-404.
- Mandal, B.K. and C.E. Boyd, 1980. Reduction of pH in waters with high total alkalinity and low total hardness. *Progr. Fish-Culturist*, 42: 183-185.
- Margolis, L., 1935. The effect of fasting on the bacterial flora of the intestine of fish. *J. Fish. Res. Board Can.*, 10: 62-63.
- Muhammad, S., Y.A. Adamu, M.A. Umaru, M.B. Abubakar and U.M. Chafe, 2011. A case of hydrated lime (CaOH)₂ toxicity in juveniles. *Sokoto J. Vet. Sci.*, 9: 46-56.
- Namdari, R., S. Abedini and F.C.P. Law, 1996. Tissue distribution and elimination of oxytetracycline in seawater chinook and coho salmon following medicated-feed treatment. *Aquaculture*, 144: 27-38.
- Nunes, B., S.C. Antunes, R. Gomes, J.C. Campos, M.R. Braga, A.S. Ramos and A.T. Correia, 2015. Acute effects of tetracycline exposure in the freshwater fish *Gambusia holbrooki*: Antioxidant effects, neurotoxicity and histological alterations. *Arch. Environ. Contam. Toxicol.*, 68: 371-381.

- Pham, D.K., J. Chu, N.T. Do, F. Brose and G. Degand *et al.*, 2015. Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in vietnam. *EcoHealth*. 10.1007/s10393-014-1006-z
- Quigley, III J.D., J.J. Drewry, L.M. Murray and S.J. Ivey, 1997. Body weight gain, feed efficiency and fecal scores of dairy calves in response to galactosyl-lactose or antibiotics in milk replacers. *J. Dairy Sci.*, 80: 1751-1754.
- Reda, R.M., R.E. Ibrahim, E.N.G. Ahmed and Z.M. El-Bouhy, 2013. Effect of oxytetracycline and florfenicol as growth promoters on the health status of cultured *Oreochromis niloticus*. *Egypt. J. Aquat. Res.*, 39: 241-248.
- Rigos, G. and G.M. Troisi, 2005. Antibacterial agents in mediterranean finfish farming: A synopsis of drug pharmacokinetics in important euryhaline fish species and possible environmental implications. *Rev. Fish Biol. Fish.*, 15: 53-73.
- Ringo, E., S. Sperstad, R. Myklebust, S. Refstie and A. Krogdahl, 2006. Characterisation of the microbiota associated with intestine of Atlantic cod (*Gadus morhua* L.): The effect of fish meal, standard soybean meal and a bioprocessed soybean meal. *Aquaculture*, 261: 829-841.
- Samanidou, V.F. and E.N. Evaggelopoulou, 2007. Analytical strategies to determine antibiotic residues in fish. *J. Sep. Sci.*, 30: 2549-2569.
- Samuelsen, O.B., V. Torsvik and A. Ervik, 1992. Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *Sci. Total Environ.*, 114: 25-36.
- Sanchez-Martinez, J.G., R. Perez-Castaneda, J.L. Rabago-Castro, G. Aguirre-Guzman and M. L. Vazquez-Sauceda, 2008. A preliminary study on the effects on growth, condition and feeding indexes in channel catfish, *Ictalurus punctatus*, after the prophylactic use of potassium permanganate and oxytetracycline. *J. World Aquacult. Soc.*, 39: 664-670.
- Sarker, M.A., M.N. Uddin, M.G.A. Sarker and M.B.R. Chowdhury, 2000. Pathogenicity of aeromonas sobria to Thai silver barb (*Barbodes gonionotus* Bleeker) and its sensitivity to some antibiotic agents. *Bangladesh Fish. Res.*, 4: 165-170.
- Serrano, P.H., 2005. Responsible Use of Antibiotics in Aquaculture. Food and Agriculture Organization, Rome, Italy, ISBN-13: 9789251054369, Pages: 194.
- Sharma, A. and R.S. Saxena, 2012. Study of microbial pollution in some rivers of district Kanshi Ram Nagar by MAR indexing. *Indian J. Biol. Stud. Res.*, 2: 34-38.
- Siddiqui, A.Q., M.S. Howlader and A.A. Adam, 1988. Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 70: 63-73.
- Smith, P., M.P. Hiney and O.B. Samuelsen, 1994. Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. *Annu. Rev. Fish Dis.*, 4: 273-313.
- Trust, T.J. and R.A. Sparrow, 1974. The bacterial flora in the alimentary tract of freshwater salmonid fishes. *Can. J. Microbiol.*, 20: 1219-1228.
- Ueno, R., K. Uno, S.S. Kubota and Y. Horiguchi, 1989. Determination of oxytetracycline in fish tissues by high performance liquid chromatography. *Nippon Suisan Gakkaishi*, 55: 1273-1276.
- Viola, C. and S.J. DeVincent, 2006. Overview of issues pertaining to the manufacture, distribution and use of antimicrobials in animals and other information relevant to animal antimicrobial use data collection in the United States. *Prev. Vet. Med.*, 73: 111-131.

- Wang, Q., Q. Liu and J. Li, 2004. Tissue distribution and elimination of oxytetracycline in perch *Lateolabrus janopicus* and black seabream (*Sparus macrocephalus*) following oral administration. *Aquaculture*, 237: 31-40.
- Wen, Y., Y. Wang and Y.Q. Feng, 2006. Simultaneous residue monitoring of four tetracycline antibiotics in fish muscle by in-tube solid-phase microextraction coupled with high-performance liquid chromatography. *Talanta*, 70: 153-159.
- Yonar, M.E., 2012. The effect of lycopene on oxytetracycline-induced oxidative stress and immunosuppression in rainbow trout (*Oncorhynchus mykiss*, W.). *Fish Shellfish Immunol.*, 32: 994-1001.
- Zhao, J.L., Y.S. Liu, W.R. Liu, Y.X. Jiang and H.C. Su *et al.*, 2015. Tissue-specific bioaccumulation of human and veterinary antibiotics in bile, plasma, liver and muscle tissues of wild fish from a highly urbanized region. *Environ. Pollut.*, 198: 15-24.