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Effectiveness of oxytetracycline in reducing the bacterial load in rohu fish (*Labeo rohita*, Hamilton) under laboratory culture condition

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PEER REVIEW

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Comments

This paper has been written well and in fact, this is the first comprehensive study on the effects of antibiotic on bacterial flora in rohu fish in Bangladesh.

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ABSTRACT

Objective: To observe the effectiveness of most widely used antibiotic, oxytetracycline (OTC) in reducing the bacterial load in rohu fish under artificial culture condition in the laboratory.

Methods: The experiment was conducted in the Faculty Fisheries, Bangladesh Agricultural University, Mymensingh–2202. The fish were reared in 8 aquaria where fish in 5 aquaria were used for replication of the treatment (experimental group) and fish in remaining 3 aquaria were considered as a control (Control group). OTC was fed to the fish in the experimental aquarium at the rate of 2 g/kg through diet twice daily whereas fish reared under control condition was given feed without antibiotic for 20 d and bacterial content in the aquarium water, gills, skin and intestine of fish were estimated at every alternative day after onset of the experiment.

Results: Rearing the fish with OTC treated feed resulted in gradual decrease of bacterial load in the aquarium water, gills, intestine and skin of the fish whereas the content remain unchanged or little increased in the control group. Water quality parameters such as dissolved oxygen, pH and total hardness were within the suitable range in the experimental aquarium but not in control aquaria throughout the experimental period.

Conclusions: These results suggest that OTC could be a potential antibiotic to reduce the bacterial load in fish and can be used commercially for maintaining the fish health in aquarium conditions.

KEYWORDS

Antibiotics, Oxytetracycline, Bacterial load, *Labeo rohita*, Water quality parameters

1. Introduction

Aquaculture is the prominent food production sector in the world and provides a significant supplement and substitute to wild fish and plant[1]. It has been estimated that fisheries and aquaculture supplied the world with about 110 million metric tons of food fish per year providing a per capita supply of 16.7 kg[2].

Antibiotics are a group of compounds used annually in large quantities to treat bacterial diseases in fish production. Often, these compounds are partially metabolized and can be resided into the sediments. The presence of antibiotics in such water systems is of concern primarily due to the potential to trigger antibiotic resistant bacteria in the environment. In aquaculture, various antibiotics are used as prophylactic and therapeutic agents

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and as growth promoters for successful fish production^[3]. The uses of chemicals in aquaculture systems for various purposes are widely recognized. Among the chemicals of Bangladesh oxytetracycline (OTC) is widely employed to treat bacterial infections in aquaculture farms. It is even one of the most widely used antibacterial agents in aquaculture worldwide^[4]. This chemical has a substantial effect on the water quality parameters, bacterial population and also the storage condition of fishes. The vast majority of OTC supplied in mediated feed can be released to the culture system via fish excreta and even the portion of uneaten feed^[5]. So indiscriminately use of this chemical have long term effect on fish and also consumers.

Among Indian major carps rohu is one of the most popular fish in Bangladesh. It is a fast growing fish in rivers and even under culture condition in ponds and other closed water bodies. It has better taste and high market price, therefore, in the fish pond rohu is always considered as a major species for culture. Although antibiotics are extensively and indiscriminately used in these culture ponds as a tool for fish health management very little is known about their effect in lowering the bacterial load under artificial condition. Therefore, the present study was carried out to investigate the effect of OTC on bacterial flora in rohu fish with an ultimate view to increase the sustainable aquaculture production.

2. Materials and methods

2.1. Fish rearing and experimental design

Rohu fish fry with body mass ranging from 18 to 20 g were used in this experiment. The fry was collected from the nearby Field Hatchery Complex and carefully brought to the laboratory of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh–2202 for experimental purpose. After acclimation for 2 d in the tank the fish were transferred to 8 glass aquaria (size 37 cm×30 cm×60 cm) equipped with ambient aeration and tap water; the water temperature ranged from 28 to 29 °C. The fish in 5 aquaria were used for replication of the treatment which were marked as experimental aquarium 1, 2, 3, 4, 5 and fish in remaining 3 aquaria were considered as a control and marked as control aquarium 1, 2, 3. OTC was fed to the fish reared in the experimental aquarium at the rate of 2 g/kg through diet twice daily whereas fish reared under control condition was given similar amount of feed without antibiotic for 20 d. The aquarium water was changed daily.

2.2. Water quality parameters analysis

Water quality parameters such as dissolved oxygen, pH

and total hardness were recorded at onset of the experiment and determined at every 4 d interval in both experimental and control aquaria throughout the experimental period. For this purpose, 500 mL water from each aquarium was collected in a clean black plastic bottle at an average depth of 35 cm between 9:00 a.m. to 11:00 a.m. without any agitation. Each bottle was subsequently marked with respective aquarium number with three replicates. Water temperature was also checked and recorded in the laboratory with the help of a centigrade thermometer (div=0.1 °C). HANNA Test kit was used to measure the dissolved oxygen, hardness and pH during experimental period. .

2.3. Microbiological analysis

The bacterial load was estimated in aquarium water, gills, intestine, and skin of the fish. Fish were collected from both experimental and control aquaria at every alternative day after onset of the experiment and samples were prepared for microbiological analysis. Aquarium water from three different locations was collected in sterile glass bottles (250 mL) at a depth of 15–20 cm from the surface and combined to make a composite sample for the same purpose in the laboratory. Appropriate sample dilutions were made (10^{-1} to 10^{-5}) with sterile physiological saline (0.85% w/v NaCl) in deionized water. Aliquots of 0.1 mL of the serial dilutions were inoculated onto nutrient media using the spread plate method as this medium can recover most of the bacteria from the sample. All plates were prepared in duplicate on sterile Petridish using sterile nutrient agar media. In this stage, 0.1 mL of samples from different dilutions were taken by a micropipette and transferred aseptically onto the pre-prepared agar plates for preparing the culture plates by raising the upper lid sufficient enough to enter the tips of the pipette. The samples were then spread homogenously and carefully by sterile flamed L-shaped glass rod throughout the surface of the media until the sample were dried out. All the inoculated plates were incubated at 28 °C for 24–48 h, subsequently taken out and counted total heterotrophic aerobic bacteria for aquarium water, gills, intestine and skin sample of the fish. The colony-forming units (CFU) were counted under a Quebec darkfield colony counter (Leica, Buffalo, NY, USA) equipped with a guide plate ruled in square centimeters. Plates containing 30–300 colonies were used to calculate the bacterial population and recorded as CFU per unit of sample by using following formula:

$$\text{CFU/g} = \frac{\text{No. of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{weight of total sample solution}}{\text{Weight of fish sample (g)}}$$

2.4. Determination of *Escherichia coli* (*E. coli*) and *Salmonella* spp. by selective media

E. coli and *Salmonella* spp. were determined by using

selective media EMB–agar and SS–agar respectively. Bacterial inoculums from the spread plate were transferred into the selective media. Growth of bacterial colony in EMB–agar and SS–agar media indicate the presence of *E. coli* and *Salmonella* spp., concurrently.

2.5. Statistical analysis

The data are expressed as mean±standard error of the means (SEM). They were analyzed by CRD with the aid of the computer software MSTATC program. Differences were accepted as statistically different for $P<0.05$.

3. Results

3.1. Changes in temperature and water quality parameters in aquarium water

Table 1 represents the changes in water temperature (°C), pH, dissolved oxygen (mg/L), and hardness (ppm) of the aquarium water under experimental and control condition during the experimental period. Although a little fluctuation (28–29) °C occurred in temperature, no significant difference was observed in this parameter throughout the experimental period. On the other hand, a change in pH value was observed in the aquarium. At onset of the experiment the pH value was 7.60 before antibiotic treatment which increased to 9.80 after the treatment. An increase in dissolve oxygen was recorded before and after the antibiotic treatment in the aquarium water. Initially the value was 4.00 mg/L which increased to 5.70 mg/L after the antibiotic treatment and remained same throughout the sampling period. The total hardness was recorded 850.00 ppm in aquarium water before the treatment which decreased sharply in the antibiotic treated aquarium but remained high in the aquarium under control conditions.

Table 1

Changes in water quality parameters in the aquarium water under experimental conditions.

Days after treatment	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Hardness (ppm)
0	28 °C	7.60±0.10	4.00±0.10	850.00±10.00
4	29 °C	8.50±0.20	4.30±0.20	830.00±15.00
8	28 °C	8.70±0.10	4.60±0.20	810.00±20.00
12	29 °C	9.10±0.10	4.80±0.10	780.00±10.00
16	28 °C	9.60±0.10	5.40±0.10	760.00±6.00
20	28 °C	9.80±0.10	5.70 ±0.10	730.00±10.00

Values are expressed as mean±SE.

3.2. Changes in bacterial load in the samples

Table 2 shows the changes in quantitative estimation of aerobic heterotrophic bacteria in the aquarium water, and in the gills, intestine and skin of fish reared under

experimental conditions. At Day 0 before antibiotic treatment highest bacterial load 2.16×10^3 CFU/mL was observed in aquarium water which gradually decreased after the treatment. The lowest bacterial load of water sample was found 1.40×10^3 CFU/mL at Day 16–20. A similar phenomenon in bacterial load was also demonstrated in gill sample 6.70×10^5 CFU/mL which decreased slowly in the antibiotic treated fish. The lowest bacterial load of gill sample found 5.68×10^5 CFU/mL at Day 16–20. The highest bacterial load in intestine of fish sample was 4.90×10^7 CFU/g in the Day 0 (before treatment) and after antibiotic treatment the bacterial load of intestine decreased very slowly. The lowest bacterial load of intestine sample was found 3.57×10^7 CFU/g at Day 16–20 whereas highest bacterial load in skin of fish sample was recorded 79.83×10^5 CFU/g at Day 0 before treatment and after the antibiotic treatment the bacterial load of skin decreased gradually. The lowest bacterial load in the skin sample was found 6.52×10^5 CFU/g at the end of the experimental period at Day 16–20.

Table 2

Changes in bacterial load in aquarium water, gills and intestine of rohu fish (*Labeo rohita*).

Days after treatment	Water (CFU/mL)	Gill (CFU/g)	Intestine (CFU/g)	Skin (CFU/cm ²)
0	$(2.16\pm 0.06)\times 10^3$	$(6.700\pm 0.20)\times 10^5$	$(4.90\pm 0.10)\times 10^7$	$(79.83\pm 3.38)\times 10^5$
4	$(2.03\pm 0.05)\times 10^3$	$(6.630\pm 0.10)\times 10^5$	$(4.76\pm 0.05)\times 10^7$	$(56.95\pm 3.38)\times 10^5$
8	$(1.90\pm 0.01)\times 10^3$	$(6.330\pm 0.20)\times 10^5$	$(4.39\pm 0.10)\times 10^7$	$(30.33\pm 1.53)\times 10^5$
12	$(1.67\pm 0.02)\times 10^3$	$(6.090\pm 0.10)\times 10^5$	$(4.05\pm 0.10)\times 10^7$	$(7.31\pm 1.06)\times 10^5$
16	$(1.53\pm 0.03)\times 10^3$	$(5.910\pm 0.20)\times 10^5$	$(3.89\pm 0.05)\times 10^7$	$(6.90\pm 0.05)\times 10^5$
20	$(1.40\pm 0.03)\times 10^3$	$(5.680\pm 0.20)\times 10^5$	$(3.57\pm 0.05)\times 10^7$	$(6.52\pm 0.01)\times 10^5$

Values are expressed as mean±SE.

3.3. Analysis of pathogenic bacteria in aquarium water

E. coli and *Salmonella* spp. were analyzed as an indicator of sanitary index organism in the sample throughout the experimental period. It was observed that these bacteria were not present in the aquarium water as well as in the fish samples before and after the antibiotic treatment.

4. Discussion

It is generally considered that rearing environments as well as different organs of the fish such as gills, intestine and skin are very much susceptible for bacterial infection and disease. The present study clearly shows that the uses of OTC successfully reduced the bacterial loads both in the aquarium water and these organs throughout the experimental period. On the other hand, bacterial loads were comparatively higher in aquarium water and in these organs under control conditions. Moreover, the water quality parameters of the antibiotic treated aquarium were within the suitable ranges whereas it remained high in the control aquarium. These results suggest that OTC is a potential antibiotic in decreasing

the bacterial load in fish and maintaining a suitable environment for its living and survival.

The utilization of antimicrobial drugs in aquaculture has renowned optimistic effects on the control of bacterial infections; however, quite a lot of side effects that affect both the fish and the environment are connected with excessive use. The effects of antibiotics on the environment are mainly due to the overuse of these drugs by the aquaculture industry and the presence of drug residues in fish products. Unfortunately, there are only a few studies that analyzed the side effects of antibiotic use on fish themselves. There is evidence that some antibiotics can induce nephrotoxicity, but the most well documented side effect is immunomodulation.

In present study, it has been demonstrated that the pH values changed in the aquarium water before and after of the antibiotic treatment. A similar relationship between pH and antibiotic treatment was also observed in those of the studies done in the past[6–8]. It has been observed that the pH value in the pond water was 7.58–7.92 before the antibiotic treatment which changed to 7.57–7.69 in the antibiotic treated pond after two weeks[6]. Another experiment also revealed a higher value of pH (7.9 to 8.2) while assessed the water quality using different antibiotics in the aquarium water[7].

The dissolve oxygen from 4 to 8 mg/L is generally considered as a suitable range for fish culture[9]. In this study this parameter was found to be within the suitable range and varied in accordance to the previous study. It was reported that the dissolve oxygen in an untreated antibiotic pond was 5.52 to 5.80 mg/L which changed between 5.44 to 5.86 mg/L after two weeks of the treatment[6]. Although in the pond water the dissolve oxygen content generally fluctuates within 5.98 to 6.53 mg/L it was found to vary from 2.2 to 8.8 mg/L, in a ponds of BAU campus, Mymensingh during the experimental period[10,11]. The other water quality parameter such as hardness also varied with the progress of the experimental period. The lowest hardness value was found 730.00 ppm at the Day 16–20 in the experimental aquarium which was lower from the control aquarium and was closely related to the previous studies[7]. This may mean that the OTC may play an important role in keeping the water quality within the suitable ranges for fish culture artificial condition in aquarium,

The bacterial load in culture ponds were in the range of $(4.9 \pm 1.03) \times 10^3 - (5.75 \pm 1.0) \times 10^3$ CFU/mL in water, $(5.62 \pm 1.0) \times 10^7 - (6.60 \pm 1.02) \times 10^7$ CFU/g in sediments, $(6.77 \pm 1.0) \times 10^6 - (7.57 \pm 1.0) \times 10^6$ CFU/g in rohu gill filaments, $(7.94 \pm 1.01) \times 10^6 - (9.12 \pm 1.0) \times 10^6$ CFU/g in silver carp gill filaments, $(6.02 \pm 1.02) \times 10^7 - (8.32 \pm 1.0) \times 10^7$ CFU/g in rohu fish intestinal content and $(5.12 \pm 1.05) \times 10^7 - (6.60 \pm 1.0) \times 10^7$ CFU/g in silver carp fish intestinal content. After antibiotic treatment total viable counts in culture ponds were $(3.1 \pm 1.19) \times 10^3 - (3.1 \pm 1.20) \times 10^3$ CFU/mL in water; $(4.27 \pm 1.10) \times 10^7 - (3.1 \pm 1.13) \times 10^7$ CFU/g sediment; $(5.37 \pm$

$1.01) \times 10^6 - (3.09 \pm 1.19) \times 10^6$ CFU/g in rohu gill; $(3.16 \pm 1.29) \times 10^6 - (4.07 \pm 1.20) \times 10^6$ CFU/g in silver carp gill; $(2.69 \pm 1.12) \times 10^7 - (4.68 \pm 1.12) \times 10^7$ CFU/g in rohu intestinal content and $(2.95 \pm 1.1) \times 10^7 - (2.63 \pm 1.17) \times 10^7$ CFU/g in silver carp intestinal content. The viable counts significantly varied between the control ponds and treatment ponds[6]. The total viable counts were $(6.7 \pm 2.1) \times 10^3 - (2.7 \pm 1.1) \times 10^3$ CFU/mL in pond water[12]. Total viable counts of bacteria (measured as CFU) were in the range of $(5.6 \pm 0.8) \times 10^3$ to $(2.4 \pm 1.2) \times 10^4$ CFU/mL in pond water; $(9.3 \pm 1.1) \times 10^6$ to $(1.9 \pm 1.5) \times 10^5$ CFU/g in sediment; $(7.1 \pm 0.7) \times 10^5$ to $(8.7 \pm 1.1) \times 10^6$ CFU/g in the gills; and $(3.4 \pm 1.8) \times 10^6$ to $(5.8 \pm 0.4) \times 10^7$ CFU/g in the intestine of tilapia[13]. The organic matter influences the load and composition of microbial population[14]. On the other hand bacterial flora in fish is the reflection of aquatic environments[15]. The bacterial loads were $(9.8 \pm 0.9) \times 10^5$ to $(4.2 \pm 3.1) \times 10^6$ CFU/g in fish gills. The total viable counts of common carp pond waters, sediment, gills and intestine and were $(1.2 \pm 2.9) \times 10^4$ to $(2.5 \pm 3.5) \times 10^5$ CFU/mL; $(9.3 \pm 2.1) \times 10^7$ to $(2.7 \pm 3.5) \times 10^9$ CFU/g; $(4.3 \pm 2.9) \times 10^6$ to $(1.6 \pm 3.9) \times 10^7$ CFU/g; and $(8.7 \pm 4.1) \times 10^9$ to $(5.4 \pm 3.2) \times 10^5$ CFU/g, respectively[16]. The experimented result was closely associated with the above results.

Antibacterial agents are used in aquaculture to prevent the bacterial disease. It may reduce bacteria by killing or inhibiting the growth and both processes reduce the total bacterial content since OTC was reported to very much effective against a wide range of Gram-positive and Gram-negative bacterial[13].

Finally, it can be speculated that the extent of antibiotics in affecting the aquatic habitat may vary. The effects of antibiotics on the environment are mainly due to the overuse of these drugs by the aquaculture industry and the presence of drug residues in fish products. In this study, the bacterial load were usually lower in the fish reared under experimental condition than those of control fish due to an effective action of OTC against the bacterial flora indicating that OTC could be a potential antibiotic to reduce the bacterial load in fish and can be used in commercial purpose while rearing the fish in the aquarium. Further study is necessary to clarify the mode of action of this antibiotic in aquarium condition.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Antibiotics are a group of chemical compounds used annually in large quantities to treat bacterial diseases in fish production. This chemical has effect on the water quality parameters, bacterial population and also the storage condition of fishes. So indiscriminate use of this chemical have long term effect on fish and also consumers. This study investigates the effects of antibiotic on bacterial flora in rohu fish to increase the sustainable aquaculture production.

Research frontiers

Although very little work in this field has been done in the past in Bangladesh, no such studies prevail in the case of rohu fish.

Related reports

Borgstrom G. (1965) in his publication Fish as food processing has stated some related result. This study demonstrates that bacterial load in fish may be reduced by use of OTC.

Innovations and breakthroughs

This study clearly shows that the uses of OTC successfully reduced the bacterial loads both in the aquarium water and different organs of fish throughout the experimental period.

Applications

This study is very important to know the effectiveness of a most widely used antibiotic, OTC in reducing the bacterial load in rohu fish under artificial culture condition in the laboratory.

Peer review

This paper has been written well and in fact, this is the first comprehensive study on the effects of antibiotic on bacterial flora in rohu fish in Bangladesh.

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