



Optimizing Efficacy of Commercial Antibiotics against Tail and Fin Rot Disease in Rainbow Trout Fingerlings by Varying Dosage, Quantities and Time

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ABSTRACT

Tail and Fin Rot is one of the most common diseases and fishes are more susceptible to the disease when they are reared in suboptimal rearing conditions resulting stress. It is caused by *Aeromonas*, *Pseudomonas* and *Vibrio*, a Gram-negative bacterium. In this work, commercial antibiotics Doxycycline, Norfloxacin and combined Gentamycin with Tobramycin was tested for its efficacy in decreasing the mortalities caused by naturally-infected Tail and Fin Rot disease in Rainbow trout fingerlings. At start of the experiment each treatment's trout fingerlings (avg. 66.77± 18.78, g) showing eroded fins were quantified and had drug efficacy trial through medicated feed of 7 days with 7 days resting period i.e., a period to have fingerlings innate defense mechanisms and repetition of medicated feed. Trout fingerlings with treatment groups T3 having one cycle of medicated feed i.e., Gentamycin 40 mg + Tobramycin 10 mg/ kg fish had significantly lower Cumulative incidence (CI), 1.63 % and disease prevalence rate (DPR), 16.3± 0.071 % followed by treatment group T2, Norfloxacin 50 mg/ kg fish, 3.46± 0.006 % CI and DPR 25.08± 0.048%; and T1, Doxycycline 80 mg/ kg fish CI 3.95± 0.0012 % and DPR 29.82± 0.006% having two drug cycles respectively. The 35th day daily growth rate (g/day) of trout fingerlings had higher growth rate in treatment groups T3 had significantly higher growth rate 1.93± 0.006 followed by T2 1.82± 0.009 and T1 0.34± 0.009. No significant survival rate (%) was recorded in treatment groups T3 and T2 while lowest survival 91.18 ± 0.048 % was recorded in treatment group T1. Findings of present study highlights combination of antibiotics Gentamycin and Tobramycin had increased efficacy thus reducing health and economic burden associated with tail and fin infections.

Keywords: *Tail and Fin rot, rainbow trout, medicated feed, efficacy*

1. Introduction

Tail and Fin Rot is one of the most common diseases observed in captive as well in natural environment and fishes are more susceptible to the disease when they are reared in suboptimal rearing conditions resulting stress (Latremouille, 2003). It was first reported in Nepal in 1993 (Gurung & Lama 1994) and is one of most predominant occurring diseases in January to September and November (Rayamajhi 1998, Rayamajhi and Dhital 2008, Rayamajhi and Prasad 2010). It is caused by *Aeromonas*, *Pseudomonas* and *Vibrio*, a Gram-negative bacterium (Timalcina et al., 2022).

Doxycycline is a broad-spectrum antibiotic synthetically derived from oxytetracycline (NCBI 2022a). It is second-generation tetracycline used to treat a wide variety of gram-positive and gram-negative bacterial infections. It is well established that tetracyclines inhibit bacterial protein synthesis by preventing the association of aminoacyl-tRNA with the bacterial ribosome (Chopra and Roberts 2001, Schnappinger and Hillen 1996). Norfloxacin is an antibiotic in a group of drugs called fluoroquinolones. Norfloxacin is a derivative of nalidixic acid and is active against gram-negative and gram-positive bacteria (Marble and Bosso 1986). Norfloxacin interferes with DNA gyrase, an enzyme needed in synthesis of bacterial DNA. Gentamicin is a parenterally administered, broad spectrum aminoglycoside antibiotic, isolated from *Micromonospora purpurea* in 1963 and is typically used for gram-positive and moderate to severe gram-negative infections (NCBI 2022 b). Similarly, tobramycin is an aminoglycoside antibiotic that is kanamycin B derived from *Streptomyces tenebrarius* with bacteriostatic activity. Following active transport into the cell, tobramycin binds irreversibly to a specific aminoglycoside receptor on the bacterial 30S ribosomal subunit and interferes with the initiation complex between messenger RNA and the 30S subunit, thereby inhibiting initiation of protein synthesis, consequently leading to bacterial cell death (NCBI 2022c).

2. Material and Methods

2.1 Fish

Rainbow trout, avg. 66.77 ± 18.78 , g body weight, was obtained from a private fish farm at Amare, Nuwakot Bagmati province, Nepal. They were maintained in nursing raceways with flow through system. The fish were fed twice daily with a 45 % Crude Protein commercial pellet diet, 3% of the body weight.

2.2 Isolation and screening of pathogenic bacteria and Antibiotic sensitivity testing

Rainbow trout fingerlings were maintained in the three outdoor nursing raceways, as a regular procedure for accomplishing research work. Signs of fin erosions and surface ulcerations appeared on all reared fish in the raceways, along with gradual mortalities. Timalsina et al (2022) method with slight modifications was adopted for isolation, identification of pathogen in disease lab of National Fishery Research Centre, Godavari, Lalitpur. Five moribund fish from each raceway were sampled for gross examination and kidney swab samples were enriched on Brain Heart Infusion Broth (BHI) broth at 37 for 24 hrs. for microbial isolation. For the selective isolation of suspected pathogen, enriched sample were streaked on Bile Salt Brilliant Green Starch Agar at 30 for 24 hrs. Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS) at 35 for 24hrs & Pseudomonas Agar Base with CFC supplement at 25 for 18-48 hrs. (Hi-media) for selective the isolation of Aeromonas, Vibrio and Pseudomonas Spp. respectively. Vibrio Spp. Yellow and blue centered isolated colony on TCBS agar was inoculated sterile Tryptic Soya Broth (TSB) for further use. Similarly, starch hydrolyzing colony on Bile Salt Brilliant Green Starch Agar (by using Lugol's Iodine) and Fluorescent Colony under the exposure of UV light on Pseudomonas Agar base was inoculated on TSB for further works.

The antibiotics discs with minimum inhibitory concentration used are Ciprofloxacin 5 mcg, Doxycycline 30 mcg, Tobramycin 30 mcg, Gentamycin 10 mcg, Norfloxacin 10 mcg and Ampicillin/Sulbactam 10/10 mcg of Hi-media with minimum inhibitory concentration (MIC) were tested in vitro using Kirby- Bauer disk diffusion method (Hudzicki, 2009). Fresh culture of each isolate was prepared on Tryptic Soya Broth then Spread on Muller Hilton Agar plates then antibiotics paper disc was placed aseptically and incubated at 30°C for 18-24 hrs. Diameter of inhibition was measured using digital Vernier caliper. Independent-Samples T-Test using SPSS was used to comparing the means of antibiotic categories whether it is resistance or sensitive in the variable antibiotics.

2.3 Antibiotics efficacy trial experimental design

Antibiotics efficacy trial experimental was set up after quantifying eroded fins in buffer raceway where fishes were overly stocked (200 number per square meter) along with alternately under fed for a week to create conducive environment for disease outbreak. Then after, homogenous mixture of naturally infected trout fingerlings, (diseased) as well as asymptomatic fishes were stocked in equal number in all three treatments i.e. at the rate of twenty fish per square meter in 12 outdoor nursing raceways. During the study, three commercial antibiotics representing three treatments namely T1, Doxycycline 80 mg/ kg fish; T2 Norfloxacin 50 mg/ kg fish, and T3 Gentamycin 40 mg + Tobramycin 10 mg/ kg fish were compared for efficacy through medicated feed in triplicates in a completely randomized design. Medicated feed was fed to test fishes regularly at its first feeding with respective antibiotic mixing with 45% CP commercial diet. Response of antibiotic efficacy trial through medicated feed was quantified at the end of 7 days for growth, mortality and persistence of fin erosion data. In case the disease sign and symptom persist, after a period of 7 days resting period i.e., a period to have fingerlings innate defense mechanisms, repetition of medicated feed was extended for next 7 days after resting period.

2.4 Tail and Fin Rot Scoring

Kindschi (1987) developed fin factor formula was used to quantify the degree of fin erosion (pectoral, and caudal fins) in given treatments. Statistical analysis of fin factor data was performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test for pairwise comparison.

Fin factor (%) = (Fin length)/(Total fish length) X 100

3. Results and discussion

3.1 Identification of bacteria by biochemical test

Screening of *Aeromonas*, *Pseudomonas*, *Vibrio cholera* and *V. parahaemolyticus* was done based on morphological characteristics and biochemical test. The different biochemical test helps in conformity of the bacteria. Details is shown in Table 1.

Table 1. Biochemical test

Bacteria	Gram staining	SIM test	SCA test	TSI test	Catalase test	Oxidative/fermentative	Nitrate test	Urease test	Slant	Butt	Oxidase test
<i>Aeromonas</i> sps	-ve	+ve	+ve	+ve	+ve	F	+ve	-ve	R	Y	+ve
<i>Pseudomonas</i> sps	-ve	-ve	+ve	-ve	+ve	O	+ve	-ve	R	R	+ve
<i>Vibrio</i> sps	-ve	+ve	+ve	-ve	+ve	F	+ve	+ve	Y	Y	+ve

F- Fermentative, O – Oxidative, (+) = Positive; (-) = Negative reaction; (±) = Variable; R = Red (Alkaline reaction); Y=Yellow (Acid reaction)

3.2 Antibiotic susceptibility test

Antibiotic susceptibility test revealed that the antibiotics tetracycline class antibiotic (Doxycycline) had mixed result of efficacy and varied with bacterial isolates as shown in table 2. This might be due to long duration of use of doxycycline in farmers field, lack of wise use of antibiotics, mutation and antibiotics target modification or horizontal transfer of resistant gene. However, aminoglycoside antibiotic (Gentamicin) and quinolones antibiotic (Ciprofloxacin) were have efficacy sensitive in all the bacteria isolates. Independent- Samples T Test was comparing the means of antibiotic categories whether the antibiotic sensitivity test (ast) results as resistance or sensitive in the variable antibiotics are significantly different to each other at 95% level of confidence shows Ciprofloxacin ast results were not significant different to Gentamicin and Doxycycline ast results whereas it is significant different to Tobramycin ast results. Similarly, Gentamicin ast results were significant different to Doxycycline and Tobramycin ast results. And, No significant different in ast results was observed in-between Doxycycline and Tobramycin.

Table 2. Antibiotic sensitivity test on isolated bacteria from infected rainbow trout

Antibiotic (Cons/Disc)	<i>Aeromonas hydrophila</i>	<i>Pseudomonas fluorescense</i>	<i>Vibrio</i> sps
Ciprofloxacin (5µg)	+++	++	+++
Gentamicin (10µg)	+++	+++	+++
Doxycycline (30µg)	++	+++	++
Tobramycin (30µg)	++	+	+

-: no inhibition, +: inhibitory zone less than 14mm, ++: inhibitory zone between 12-15mm, +++: inhibitory zone equal 18mm or above

3.3 Water quality parameters

Average water quality parameters of different treatment raceways are shown in table 3. No significant difference in parameters and treatment groups were obtained using Independent- Samples T Test.

Table 3. Water quality parameters of treatment raceways

Details	Treatment 1	Treatment 2	Treatment 3
Water temperature, °C	12.7	11.9	12.3
pH	9.4	9.3	9.2
Dissolve oxygen, mg/l	10.4	10.5	10.6
Total Nitrite (NO ₂), mg/l	0.12	0.13	0.11
Total Nitrate (NO ₃), mg/l	0.17	0.15	0.14
Total Ammonical Nitrogen (NH ₄), mg/l	0.01	0.01	0.01
Total Phosphate (PO ₄), mg/l	0.01	0.01	0.33
Total hardness, mg/l	220	229	163
Total Alkalinity, mg/l	29	25	22

3.4 Growth parameters of trout fingerlings

At the end of the study, at 30th day trout fingerlings with treatment groups T3 having one cycle of medicated feed i.e., Gentamycin 40 mg +Tobramycin 10 mg/ kg fish had significantly lower Cumulative incidence (CI), 1.63 % and disease prevalence rate (DPR), 16.3± 0.071 % followed by treatment group T2, Norfloxacin 50 mg/ kg fish, 3.46± 0.006 % CI and DPR 25.08± 0.048%; and T1, Doxycycline 80 mg/ kg fish CI 3.95± 0.0012 % and DPR 29.82± 0.006% having two drug cycles respectively. The 35th day daily growth rate (g/day) of trout fingerlings had higher growth rate in treatment groups T3 had significantly higher growth rate 1.93± 0.006 followed by T2 1.82± 0.009 and T1 0.34± 0.009. No significant survival rate (%) was recorded in treatment groups T3 and T2 while lowest survival 91.18 ± 0.048 % was recorded in treatment group T1.

Table 4. Growth parameters of Trout fingerlings (avg \pm s.e.m) in different treatments

Treatment	Drug cycle	Daily growth rate (g/day)	Survival rate (%)	Disease prevalence rate (%)	Cumulative incidence (%)
1	2	0.34 \pm 0.009c	91.18 \pm 0.048b	29.82 \pm 0.006a	3.95 \pm 0.0012a
2	2	1.82 \pm 0.009b	94.91 \pm 0.006a	25.08 \pm 0.048b	3.46 \pm 0.006b
3	1	1.93 \pm 0.006a	95.75 \pm 0.48a	16.3 \pm 0.071c	1.63 \pm 0.007c

Note: 1 cycle = 7 days with gap of 7 additional days and 2nd cycle begin

3.5 Fin Factor of trout fingerlings

Average fin factor data of different treatment raceways are shown in table 5. No significant difference in respective fin lengths (mm), total fin length (mm) and fin factor (%) parameters of the treatment groups were obtained using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test for pairwise comparison. This could be due to regeneration of fins in response to defensive mechanism of the fish.

Table 5. Fin Factor of trout fingerlings (avg \pm s.e.m) in different treatments

Treatment	Pectoral fin length (mm)	Caudal fin length(mm)	Total fish length(mm)	Pectoral fin factor (%)	Caudal fin factor (%)
1	22.03 \pm 1.74	30.72 \pm 2.83	172.40 \pm 14.12	12.94 \pm 1.01	17.81 \pm 0.77
2	21.00 \pm 0.41	28.47 \pm 0.42	168.81 \pm 5.62	12.50 \pm 0.53	16.95 \pm 0.69
3	18.45 \pm 1.35	27.54 \pm 2.26	155.90 \pm 12.47	11.88 \pm 0.26	17.67 \pm 34

4. Conclusion

The study assessed the efficacy of Doxycycline, Norfloxacin, and Gentamycin with Tobramycin against Tail and Fin Rot in Rainbow trout fingerlings (avg. weight 66.77 \pm 18.78 g) caused by *Aeromonas*, *Pseudomonas*, and *Vibrio*. Fingerlings in Treatment Group T3 (Gentamycin 40 mg + Tobramycin 10 mg/kg fish) for one cycle exhibited the lowest Cumulative Incidence (1.63%) and Disease Prevalence Rate (16.3%). T3 also displayed the highest growth rate (1.93 g/day) on the 35th day, outperforming T2 (Norfloxacin) and T1 (Doxycycline). Notably, T3 and T2 showed significant survival rates, while T1 had the lowest (91.18%). The study concludes that the Gentamycin-Tobramycin combination effectively reduces health and economic burdens associated with Tail and Fin Rot infections in Rainbow trout fingerlings.

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