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LETHAL AND TOLERANCE CONCENTRATIONS OF CHLORAMPHENICOL TO THE FINGERLINGS OF AFRICAN CATFISH: *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

Fingerlings are often treated with antibiotics. The effects of antibiotics on fingerlings have not been sufficiently reported, therefore, effects of chloramphenicol, a commonly used antibiotic, were investigated on fingerlings of *Clarias gariepinus*. A total of 150 fingerlings of *C. gariepinus* were procured, acclimatized for 7 days and fed with 45% Crude protein diet. They were thereafter randomly assigned to four treatment concentrations of 2.67 mg/L, 3.22 mg/L, 3.88 mg/L, 4.34 mg/L and Control with 0.00 mg/L concentrations of chloramphenicol respectively. Each treatment consisted of 10 fingerlings replicated thrice. They were closely monitored and observed for 96 hours. Data collected was subjected to one-way Analysis of Variance. The fingerlings reacted differently to the concentrations of the chemical and duration of the test. Abnormal behaviour, stress and mortality were observed at higher concentrations. There was significant difference ($p<0.05$) between survivals in test concentrations. Treatment 1 (2.67 mg/L) had the highest survival (100%) while Treatment 4 (4.34 mg/L) had the lowest survival rate (13.30%). Mortality rate increased with increasing duration. Mortality was highest at 96 hours and least at 24 hours. Chloramphenicol affected on survival of the fingerlings. It can induce stress and may not be tolerated by fish at higher concentrations for extended duration, which can cause mortality. The recommended concentration and duration for the treatment of fingelings using Chloramphenicol are 2.67 mg/L or 3.22 mg/L and should not exceed 48 hours, with close monitoring.

Key Words: Chloramphenicol, Stress, Fish mortality, Fingerlings Survival, *Clarias gariepinus*

INTRODUCTION

Fisheries and aquaculture sector is crucial to food security, poverty alleviation and general well-being. Fish plays an important role in improving and sustaining food security and nutritional status; and is critical source of dietary protein and other nutrients for many isolated communities in rural areas (Nwabeze and Erie, 2013). Global fish production has

grown steadily in the last five decades with supply increasing at an average annual rate of 3.2 per cent, outpacing world population growth at 1.6 per cent. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO, 2014). Employment in fisheries and aquaculture has grown faster than the world's population and employment in traditional agriculture (FAO, 2010). About 58.3 million people were engaged in the primary sector of capture fisheries and aquaculture in 2012. Out of which 37 per cent were engaged full time. In 2012, 84 per cent of people employed in the fisheries and aquaculture sector were in Asia and more than 10 per cent in Africa. Between 2010 and 2012, at least 21 million people were capture fishers operating in inland waters (more than 84 per cent in Asia). Overall, women accounted for more than 15 per cent of all people directly engaged in the fisheries primary sector in 2012 the proportion of women exceeded 20 per cent in inland water fishing and up to 90 per cent in secondary activities such as processing. FAO estimates that, overall, fisheries and aquaculture assure the livelihoods of 10–12 per cent of the world's population (FAO, 2014).

However, this production is hampered by unpredictable mortalities that may be due to negative interactions between fish and pathogenic bacteria. Most often, antibiotic compounds are used in fish hatcheries to treat and control disease causing organisms (Cabello, 2006). A wide variety of chemicals are used in aquaculture production; these include disinfectants (hydrogen peroxide and malachite green), antibiotics (Sulphonamides, Chloramphenicol and Tetracyclines) and anthelminitic agents (Pyrethroid insecticides and avermectins). Reasons for the use of drugs in fish hatcheries include treating and preventing diseases, controlling parasites that affect reproduction and growth as well as providing tranquilization when weighing. Only few drugs have been approved for use in aquaculture, which may lead to the inappropriate use of unapproved drugs, general-purpose chemicals, or approved drugs in a manner that deviate from the labelled instructions (Jaime *et al.,* 2012). Chloramphenicol is frequently administered antibiotic to the fish either through the water or included in the fish feed; most of them are not aware of the withdrawal period for the antibiotic or the concentration to administer as well (Isaac and Afisu, 2013).

MATERIALS AND METHODS

Experimental Location

The experiment was carried out at the Department of Fisheries Technology, College of Agriculture, Science and Technology Gujba, Yobe State, Nigeria.

Experimental Fish and Management

One hundred and fifty fingerlings of African catfish, *Clarias gariepinus* (Burchell, 1822) were procured from Mega Fish Farm, Damaturu, Yobe State and were conveyed to the laboratory in 25 litres jerry-can in the morning hours. They were later transferred to a plastic basin containing clean water of similar temperature (27^oC). They were acclimatized for 7 days and fed with a diet containing 45% crude protein twice a day (at 9am and 5pm), at 5% body weight. During this period, the fingerlings were carefully monitored daily to ensure they are healthy before allocating them to the experimental units.

Experimental Set-Up and Design

The experiment consisted of four treatment groups and a control, each treatment was replicated thrice. The test was conducted in plastic bowls each containing 10 litres of water. The test solution was prepared by dissolving chloramphenicol powder. The Control had 0.00mg/L of chloramphenicol,

while treatments 1, 2, 3 and 4 had 2.76 mg/L, 3.22mg/L, 3.88mg/L and 4.34 mg\L respectively.

The fingerlings were randomly allocated to the experimental units immediately after preparing the test solutions. Each treatment had 10 fingerlings, replicated three times. The fish in each treatment and replicate were closely examined and monitored for a period of 96 hours. They were not fed throughout the test period. This was done to determine their behavioural pattern and reaction to the different concentrations of the chemical and determined at what time and concentration they begin to show abnormal behavioural changes due to stress and when they begin to die. Mortality was the major measure used during the 96 hours of exposure. Observations covered their movement pattern, conditions of their operculum, stress and distress or abnormal locomotion and were compared with those in the control treatment. The number of mortality and survival were recorded in each of the treatments and their replicates during and at the end of the 96 hours test period.

Data Analysis

All data collected were subjected to descriptive statistics to determine the mean values and then subjected to analysis of variance (ANOVA) at 95% probability level where the significant differences were detected. Mean values were separated using Least Significant Difference (LSD) with SPSS (Statistical Package for Social Sciences) version 20.0 statistical package.

RESULTS

Table 1 elucidated the effects of chloramphenicol on the fingerlings of *C. gariepinus* in which the Control (0.00mg\L) and Treatment 1 (2.76mg/L) had a total survival of 30 each from the three replicates, while Treatment 2 (3.22 mg/L) had a total survival of 16 from all its replicates. Treatment 3 (3.88 mg/L) had a total of 5 survival while Treatment 4 (4.34 mg\L) had 4 survival from all replicates.

From Table 2, Mortality rate increased with increase in concentration and duration of the test. At 24 hours, the Control, Treatments 1 and 2 had no mortality while treatments 3 and 4 had mortalities of 4 and 5 respectively. At 48 hours, control and Treatment 1 had no mortality while Treatments 2, 3 and 4 recorded mortalities of 2, 6 and 7 respectively. At 72 hours, the Control and Treatment 1 had no mortality each while treatments 2, 3 and 4 recorded 4, 8 and 6 mortality respectively. Treatment 2, 3 and 4 recorded the highest mortality of 7 each at 96 hours while control and Treatment 1 recorded no mortality. Highest total mortality (26) was recorded in Treatment 4 while there was no recorded mortality in control and Treatment1.

Table 3 elucidated the mean percentage survival and mortality rates at the end of 96 hours of the Test Period. Control and Treatment 1 recorded no mortality of 0% each while Treatment 4 recorded the highest mortality of 86.70%. Percentage mortality increased with increase in concentration of chloramphenicol while survival rate decreased with increase in concentration of Chloramphenicol. Control and Treatment 1 recorded the highest percentage survival of 100% each while Treatment 4 had the lowest percentage survival of 13.30%.

Table 1: Effects of Chloramphenicol treatment on Fingerlings of *Clarias gariepinus*

Number of survival at 96 hours

Treatment	Conc. (Mg/l)	Rep. 1	Rep. 2	Rep. 3	Total
Control	0.00	10	10	10	30
Treatment 1	2.76	10	10	10	30
Treatment 2	3.22	5	5	6	16
Treatment 3	3.88	2	1	2	5
Treatment 4	4.34	1	1	2	4

Table 2: Mean Mortality during 96 Hours Test Period

		Time (hours)				
Conc. (Mg/l)	Fish Stocked	24hrs	48hrs	72hrs	96hrs	Total mortality
0.00	30	0	0	0	0	0
2.76	30	0	0	0	0	0
3.22	30	0	2	4	7	13
3.88	30	4	6	8	7	25
4.34	30	5	7	6	8	26

Table 3: Mean Percentage Survival and Mortality at the End of the 96 Hours Test Period

Conc. (Mg/l)	0.00	2.72	3.22	3.88	4.34
No. of Fish Stocked	30	30	30	30	30
% Mortality	0	0	43.30	83.30	86.70
% Survival	100	100	56.70	16.70	13.30
Mean Survival	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	5.67 ± 0.41^{b}	1.67 ± 0.41^{c}	1.33 ± 0.41^{c}

Means in the same row with different superscript are significantly different ($P< 0.05$)

DISCUSSION

This study revealed that fingerlings of *C. gariepinus* reacted differently to varying concentrations of chloramphenicol. The tolerance to the chemical tends to be inversely related to the concentrations of the chemical and duration of the application (test) period as elucidated in Table 1. The number of mortality in all treatments (except control) increased with increasing application (test) period and concentrations of chloramphenicol (Table 2). Treatment 2 (2.88mg/L) had the lowest mortality while Treatment 4 (4.34mg/L) had the highest mortality as elucidated in Table 3. This indicated that mortality is directly related to concentration of chloramphenicol thus percentage mortality increased with increasing concentration and duration of the test whereby Treatment 2 (2.67mg/L) and the control had a survival of 100% while treatment 4 (4.34mg\L) had the lowest mortality of 13.30%, hence, the findings of this study corroborate with the results of Samuelsen (2006); Seyfried *et al.* (2010) and Tamminen *et al*. (2011) who reported that antibiotics are known to suppress immune response to stress in most freshwater fishes. This could be as a result of the increased concentrations and durations which suppressed the immune response to stress and subsequently leading to death of the fingerlings.

Instant abnormal behaviour was observed in all treatments (except control). The behaviour was more aggressive at higher concentrations thus increased with increasing concentrations of the test chemical. Sluggish behaviour was however observed in Treatments 2, 3 and 4 at 72 and 96 hours which were followed by death. The number of mortality increased at higher concentration and duration of test. This study agreed with Isaac and Afisu, (2013) and Jaime *et al.*, (2012) who reported that, antibiotics have cumulative effects on fish; fish in Treatments 1 and 2 showed more distress which tends to reduce at 72 and 96 hours. At 48 hours, fish in treatments 3 and 4 were observed to be jumping and gasping for air.

Same authors, Isaac and Afisu (2013) reported that increase in oxytetracycline concentration increases distress in fish, which is usually indicated by fish gulping for atmospheric oxygen from their habitat. Generally, the fish showed more distress at higher concentrations than at the beginning of the test and that agrees with the findings of Hentschel *et al*. (2005) and Cabello (2006) who reported that when antibiotics are used behavioural responses and higher mortality may be observed at higher concentrations and longer duration which could be as a result of stress imposed on the fish which suppressed their immune response and respiratory activity to a level they can no longer cope and resulted to death.

CONCLUSION

Clarias gariepinus fingerlings can be treated with chloramphenicol at 2.76mg\l for 96 hours, which tends to be ideal based on the findings of this study. However, 2.88mg/l can be used but the treatment period should not exceed 72 hours. 3.22mg\l can also be used for treatment but the withdrawal time should not exceed 48 hours. 4.34mg\l seems to be lethal. However, may be used for dip bath treatment which is usually less than 30 seconds.

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