COMPARATIVE ASSESSMENT OF EFFECT OF SUBLETHAL DOSE OF OXYTETRACYCLINE AND GARLIC ON GROWTH INDICES OF INDIAN CATFISH, CLARIAS BATRACHUS (LINNAEUS)

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ABSTRACT

An initial study was made to assess the effects of sublethal dose of oxytetracycline and garlic on certain growth indices of *Clarias batrachus* (Linnaeus) (55.0±5.0g and 20.0±1.5cm). The acute toxicity tests have also been carried out and data were analyzed using regression analysis to determine 24hr-96hr LC_{50} values. The mean values for 96hr-LC_{50} dose of oxytetracycline and garlic were calculated 648.819 mg l^{-1} and 6662.25mg kg^{-1} respectively. The results indicated that oxytetracycline is moderately/slightly toxic but garlic seems least toxic/practically non-toxic for this fish. *Clarias batrachus* had been fed twice daily with commercial feed at 5% body weight for 60 days. Fish were assigned to seven treatments with three replicates each. The samples have been investigated for final weight, Weight gain, daily weight gain, specific growth rate, feed conversion ratio, feed efficiency ratio and survival rate. The mean values of all growth indices except feed conversion ratio inferred their decline in oxytetracycline but elevation in garlic treated groups compared to base line. All the growth indices had been found increased but feed conversion ratio reduced than base line. ANOVA inferred that 500.0mg/kg garlic adjust the growth parameters more profoundly than 50.0mg/l oxytetracycline. The statistical evaluation also revealed a significant distinction between oxytetracycline and garlic treatments. Therefore, garlic may be used in vicinity of oxytetracycline.

Keywords: *Clarias batrachus*, Oxytetracycline, garlic, toxicity, growth indices.

1.0 Introduction

Over decades, aquaculture has contributed significantly to the arena protein and food production. However, bacterial diseases have affected aquaculture production (Romero *et al.* 2012). In order to check the situation, antibiotics are generally recommended (Cabello 2006). Antibiotics are synthetic compounds successful to inhibit growth of pathogens. The use of antibiotics in aquaculture also treats fish sicknesses and promotes fish growth (Romero *et al.* 2012). The use of oxytetracycline for the prevention of diseases has been stated in aquaculture (Shukla and Pandey 2005). But, essential issues have been raised on the use of antibiotics in aquaculture as in line with its side effects and toxicity on aquaculture (Ramadu and Dash 2013).

Attention has being shifted to phytobiotics as a feasible opportunity to antibiotics and chemotherapeutics (Ramadu and Dash 2013). Garlic (*Allium sativum*, L) is reported to be an appropriate and safe herbal plant (Raa 2000). Garlic also has the faculty to increase resistance to pathogenic infections and stimulate the immune system against diseases. Although garlic has been said to be powerful in dealing with stress incurred by the fish during transportation, sorting and grading (Raa 2000), it seems essential to assess its toxicity and effect on growth indices of the fishes.

Clarias batrachus (Linnaeus) is an air breathing freshwater highly priced catfish within South-East Asian countries due to their taste, salutatory and reproductive prosperities. Therefore, the present study aims to assess the effects of sublethal dose of oxytetracycline and garlic on growth indices of *Clarias batrachus.*

2.0 Materials and Methods

2.1 Maintenance of fish

This work was conducted at Department of Zoology, VKS University, Arrah, Bihar, India from January 2018 to December 2019. *Clarias batrachus* (BW: 55.0±5.0g and TL: 20.0±1.5cm) were procured from local fish market, disinfected and transported to the Departmental laboratory. Fish were acclimated for a fortnight in plastic aquaria before the experiment and fed with fish food.

2.2 Experimental Design

Important physico-chemical parameters of experimental water were measured using standard methods of APHA (2005). Miller and Tainter (1944) regression analysis method was used to determine LC_{50} dose of test substances as there was no partial mortality.

Oxytetracycline (500mg capsule) (Wockhardt Health Care Limited, Chennai, India) was purchased, decapsulated and stored. Fresh bulbs of garlic (*Allium sativum*) were purchased from an open market. The bulbs were dried under shade for one week. The dried bulbs were crushed, homogenized, sieved and stored.

Clarias batrachus were randomly assigned to seven treatment aquaria. Each aquarium contained ten fish. The fish were divided into group A and B. Oxytetracycline and garlic were given following their 96hr-LC_{50} values. Group 'A' received oxytetracycline (25.0, 50.0 and 75.0 mg l^{-1} of water) while group 'B' garlic (250.0, 500.0 and 750.0mg kg^{-1} of fish) treatment at different concentrations. The control group contained neither oxytetracycline nor garlic. The fish were fed with experimental feed twice daily (8.00 am and 17.00 pm) at 5 % body weight for 60 days.

At the end of experiment, weight of the fish was determined and following growth indices were calculated using the formulae:

a. Daily growth co-efficient (DGC):

$$\frac{(\text{Mean final body weight}^{0.3333} - \text{mean Initial body weight}^{0.3333})}{\text{Time in days}} \times 100$$ (Cowey 1992)

b. Specific growth rate (SGR) (% per day):

$$\frac{\log \text{mean final body weight} - \log \text{mean initial body wieght}}{\text{Time at final sampling} - \text{Time at initial smapling}} \times 100$$ (Castell and Tiews 1980)

c. Feed conversion ratio (FCR): $\frac{\text{Total dry feed consumed (g)}}{\text{Total wet weight gained (g)}}$

2.3 Statistical analysis

Values obtained after the experiment were subjected to F-test using SPSS (version 20 for Windows XP) software.

3.0 Results and Discussion

3.1 Physico-chemical parameters

The physico-chemical parameters of water were found within in range for favourable growth performances as documented by Boyd (1981). Values of carbon-dioxide in both oxytetracycline and garlic were indicative of non suffocative conditions that confirm mortality of fishes occurred due to toxicant. Similarly, the values of pH are not indicative of and acidic or alkaline conditions in aquarium (Table 1). For optimum growth achieved in fishes, there has to be appropriate water parameters along with inherent factors of age and species differences (Milikin 1982).

3.2 LC_{50} dose of oxytetracycline and garlic

The results of acute toxicity of oxytetracycline and garlic are presented in Tables 2 and 3. LC_{50} values were attributed to size of fishes with potentially immune system for biotransformation of test substances from the body. Moreover, the rapid distribution of test substances in the body of fishes lead to faster alterations in behaviour than the normal for the uptake of a toxicant is directly dependent on the size of fishes. The magnitude of the change of dose of oxytetracycline in relation to time was more significant than garlic. The results indicated the oxytetracycline is moderately/slightly toxic but garlic seems least toxic/practically non-toxic for *Clarias batrachus*.

96hr-LC_{50} dose of Oxytetracycline ranged from 62.5-100.0mg/l in different fishes (Brain *et al.* 2004; Ankley *et al.* 2007). Although, Mathew and Ambili (2017) determined 1000mg/l of oxytetracycline as 24hr-LC_{50} dose for *Labeo rohita*. According to Carraschi *et al.* (2011) oxytetracycline cause environmental intoxication risk considering the lowest (50.0 mg/kg) and the highest predicted environmental concentration (1750 mg/kg) because of its the >1 quotient. On the other hand, 96hr-LC_{50} of garlic for *Cyprinus carpio* was estimated to be 253.19 mg/L by Furthermore, Fridman *et al.* (2014) reported that bathing of *Gyrodactylus turnbulli* infected *Poecillia reticulata* in 7.5 and 12.5 ml/L garlic extract significantly reduced the infection in the fish. Garlic oil feeding of 100mg/kg in rats after 24 hr was found lethal by Joseph *et al.* (1989). From this account, it may be inferred that the differences in LC_{50} values in the sensitivity of different fish species to the type of toxicants exposures. It seems to be influenced by the species and age of the fish chosen for the toxicity tests.

3.3 Safe level of oxytetracycline and garlic

A range of safety level of Oxytetracycline of 64.882mg/L and garlic of 666.225mg/kg for *Clarias batrachus* was calculated in this work. Further, maximum growth of fish was observed in 50.0mg/l of oxytetracycline and 500.0mg/kg of garlic, also supports the value of safe level of test materials (https://en.wikipedia.org/wiki/Toxicity).

3.4 Effects of oxytetracycline and garlic on growth indices

Effects of oxytetracycline and garlic on growth indices of *Clarias batrachus* are presented in Table 4. Both the test substances were found to alter the growth parameters of fish, yet oxytetracycline was found to have more adverse effects than garlic. The garlic treated group had the highest mean values for final weight (224.27±6.35g), weight gain (167.62±5.49g and 296.18±6.00%), daily weight gain (2.79±0.09), specific growth rate (2.30±0.022%), feed efficiency ratio (58.72±1.45%) and survival rate (99.33±0.94%) but lowest feed conversion ratio (1.27±0.21) followed by control and lowest for oxytetracycline treated group respectively. Application of 2 way ANOVA considering the effect of oxytetracycline and garlic concludes that both duration of exposure as well as their doses has highly significant effect on final weight and feed efficiency ratio of this fish. On the other hand, that variation in the dose of test materials only has a significant effect on specific growth rate and feed conversion ratio of this fish.

The maximum mean increase in final weight of 172.06g after feeding of garlic based diet is due to presence of carbohydrates, vitamins, minerals, fiber and sulphurated compounds (Otunola *et al.* 2010). Garlic has been reported to favour various activities like growth,

enhancement of immune stimulation, maturation of fish species and antipathogenic properties due to the active phytochemicals (Romero *et al.* 2012). Similar results were recorded by Shalaby *et al.* (2006) and Mahmoud *et al.* (2019) in *Oreochromis niloticus*. On the other hand, the better growth performance in fish fed the antibiotic may be attributed to the levels of antibiotic in the diet which might have destroyed pathogenic organisms in the alimentary canal (Rosen, 1995). Such observations based on antibiotics have also been obtained by Lawal *et al.* (2012) and Adewole (2016) in *Clarias gariepinus* with different levels of oxytetracycline, amoxicillin and furasol.

Specific growth rate was developed by Iwama and Tautz (1981) to eliminate the problem with the decline in specific growth ratio with increasing body weight. It is saturation constant and depends on the concentration of limiting substrate. It indicates that if all nutrients are present in a substance, maximum specific growth will be possible. Its maximum mean increase of 2.30% after feeding of garlic based diet in this work is also related with the ingredients. Our results are not in accordance with the findings reported by Mahmoud *et al.* (2019) in *Oreochromis niloticus*. Further, it is in agreement with Shalaby *et al.* (2006) in *Oreochromis niloticus* and Adewole (2016) in *Clarias gariepinus* with antibiotics.

Feed conversion ratio is the ratio of input to output and a function of feed intake. Its value depends on feed intake and temperature. The work inferred that 1.27kg garlic supplemented feed while 1.32kg oxytetracycline supplemented feed can increase 1kg body weight of test fish. On the other hand, Ivlev (1939) proposed feed efficiency ratio as the ratio of output to input and temperature independent. It is a growth parameter related with percentage of utilized percentage of feed. A high feed efficiency ratio with 58.72% garlic supplemented feed in this work shows larger growth rate of fish and a lower excretion rate. The present findings were agreement with Jahanjoo *et al.* (2018) in *Sparidentex hasta* and Mahmoud *et al.* (2019) in *Oreochromis niloticus*. Further, with the same line, Shalaby *et al.* (2006) in *Oreochromis niloticus* and Adewole (2016) in *Clarias gariepinus* with oxytetracycline.

4.0 Conclusion

These results inferred that the ginger stimulate fish growth in relation to garlic supplementation in a dose dependent manner. The findings also revealed that garlic increased growth as compared to oxytetracycline and control group. Therefore, the use of garlic in aquaculture as phytobiotics should be encouraged.

Conflict of Interest

The Authors declare no conflict of interest.

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Table 1: Physico-chemical parameters of experimental water

S. No.	Physico-Chemical Parameters	Value	S. No.	Physico-Chemical Parameters	Value
1.	Temperature	25.0±1.4^{0}C	5.	Chloride	16.7±0.2 mg L^{-1}
2.	pH	7.67±0.19	6.	Hardness	230.67±55.26mg/L
3.	Dissolved oxygen	6.61±0.21 mg/L	7.	Total alkalinity	73.0±4.2mg/L
4.	Free Carbon dioxide	1.43±0.58 mg/L			

Table – 2: Statistical relationship between dose of Oxytetracycline (mg/L) and mortality of *Clarias batrachus* (body weight: 55.0g)

Sl. No.	Exposure period (hours)	Regression equation y =bx +a	Lethal Concentration (mg/L)		Toxicity Factor	t value (df=5)	F value (u = 1; v = 4)	95% Confidence limit	
								Lower	Higher
1	24	y= 0.004643x – 1.429	LC_{10}	523.153	1.000	2.793 (p<0.01)	8.734 (p<0.05)	4.746	1187.622
			LC_{50}	1384.665				23.614	2490.554
			LC_{90}	2246.177				42.482	3793.485
2	48	y = 0.007214x – 1.643	LC_{10}	366.371	1.504	4.84 (p<0.01)	23.53 (p<0.01)	2.054	667.539
			LC_{50}	920.848				10.201	1365.619
			LC_{90}	1475.326				18.348	2063.670
3	72	y = 0.008714x – 1.643	LC_{10}	303.305	1.816	5.351 (p<0.01)	28.47 (p<0.01)	2.673	551.977
			LC_{50}	762.336				13.255	1116.965
			LC_{90}	1221.368				26.482	1681.921
4	96	y = 0.009357x – 1.071	LC_{10}	221.332	2.134	7.925 (p<0.001)	63.91 (p<0.01)	6.147	363.614
			LC_{50}	648.819				30.478	852.015
			LC_{90}	1076.306				54.808	1340.415

Table – 3: Statistical relationship between dose of garlic (mg/fish) and mortality of *Clarias batrachus* (body weight: 55.0g)

Sl. No.	Exposure period (hours)	Regression equation y =bx +a	Lethal Concentration (mg/fish)		Toxicity Factor	t value (df=5)	F value (u = 1; v = 4)	95% Confidence limit	
								Lower	Higher
1	24	y= 0.00774x – 1.237	LC_{10}	289.018	1.000	2.073 (p>0.05)	2.621 (p>0.05)	5.626	342.067
			LC_{50}	805.813				10.952	2030.919
			LC_{90}	1322.609				16.278	3044.770
2	48	y = 0.0128x – 1.624	LC_{10}	205.0	1.557	3.391 (p<0.05)	5.015 (p>0.05)	6.679	144.055
			LC_{50}	517.5				12.027	709.820
			LC_{90}	830.0				17.374	1275.597
3	72	y = 0.0159x – 1.785	LC_{10}	175.157	1.888	3.944 (p<0.05)	6.095 (p>0.05)	6.146	108.294
			LC_{50}	426.729				10.651	532.023
			LC_{90}	678.302				15.155	955.752
4	96	y = 0.0179x – 1.559	LC_{10}	142.961	2.199	3.190 (p<0.05)	10.17 (p<0.05)	6.286	83.084
			LC_{50}	366.424				11.421	407.759
			LC_{90}	589.888				16.556	732.435

Table 4: Growth of *Clarias batrachus* (n = 40 and number of replicates = 3) under different doses of Oxytetracycline and garlic for 60 days

Parameters	Control	Dose of test material					
		Oxytetracycline (mg/L)			Garlic (mg/kg)		
		25.0	50.0	75.0	250.0	500.0	750.0
Initial weight (g)	55.64±1.61	53.79±1.27	54.66±1.02	53.85±0.89	57.81±1.65	56.73±1.61	55.40±1.19
Final weight (g)	212.77±7.47	210.68±4.57	212.96±6.86	196.75±10.92	228.72±9.45	228.79±11.76	215.29±9.94
		F value (c=2 and r=2) c=22.34** and r =16.10**			F value (c=2 and r=2) c=7.029* and r =15.77**		
Weight gain (g)	157.13±6.85	156.89±3.30	158.3±5.64	142.9±9.13	170.91±7.89	172.06±10.15	159.89±7.85
Weight gain (%)	282.40±5.76	291.67±1.67	289.61±4.65	265.37±9.05	296.64±5.21	303.30±9.60	288.61±3.79
Daily weight gain (g)	2.62±0.15	2.61±0.50	2.64±0.90	2.38±0.61	2.85±0.31	2.87±0.81	2.66±0.31
Specific growth rate (%)	2.26±1.90	2.26±1.22	2.27±1.07	2.17±1. 86	2.31±1.08	2.32±1.64	2.27±1.06
		F value (c=2 and r=2) c=0.0057^{NS} and r =36.55**			F value (c=2 and r=2) c=0.030^{NS} and r =46.67**		
Feed Conversion ratio	1.30±0.18	1.30±0.09	1.29±0.49	1.36±0.45	1.26±0.68	1.24±0.45	1.29±0.18
		F value (c=2 and r=2) c=0.0.886^{NS} and r =7.288*			F value (c=2 and r=2) c=0.0803^{NS} and r =9.133*		
Feed Efficiency ratio (%)	56.51±19.0	56.54±7.86	57.25±8.07	53.28±9.68	59.08±9. 37	60.30±10.86	56.79±9.48
		F value (c=2 and r=2) c=13.56* and r =220.5***			F value (c=2 and r=2) c=13.82* and r =426.8***		
Survival rate (%)	98.0±2.0	98.0±2.0	96.0±4.0	96.0±4.0	100.0±2.0	100.0±0	98.0±2.0