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Hormonal and Haematological Responses of *Clarias gariepinus* (Burchell 1822) to Nitrite Toxicity

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Abstract: Study on hormonal and haematological responses of *Clarias gariepinus* to nitrite toxicity was carried out to know the magnitude of the effects of this stressor on fish physiology. Haematological responses of *C. gariepinus* to sub-lethal levels of nitrite (0.1 g L^{-1}) at different exposure h (0, 6, 24, 48, 72 and 96 h) were carried out. Blood samples of *C. gariepinus* were collected at each exposure h and evaluated for primary and secondary stress indicators. Data obtained were subjected to simple descriptive analysis, analysis of variance and Pearson Product Moment correlation analysis. When exposed to nitrite, there was progressive increase in plasma cortisol level of *C. gariepinus* from 0 h (101.00± 0.10 ng dL^{-1}) to 48 h (161.00± 1.20 ng dL^{-1}), this later decreased at 72 h (107.00±1.60 ng dL^{-1}), it increased in 96 h to 136.00±1.00 ng dL^{-1}. PCV and Hb were elevated after 6 h of exposure and decreased significantly ($p<0.05$) at 48, 72 and 96 h of exposure in fish exposed to sub-lethal level of nitrite. From the value of 3.69±0.01 10^6/l recorded at 0 h, RBC level decreased significantly ($p<0.05$) at 6 h, this was followed by significant increase ($p<0.05$) at 24 h. The RBC value later decreased gradually to 2.20±0.56 10^6 L^{-1} at 96 h. The study shows that hormonal and haematology balances of *C. gariepinus* was affected on short term exposure to nitrite toxicity

Key words: Hormonal, haematology, nitrite, toxicity, *Clarias gariepinus*

INTRODUCTION

The performance of cultivated fish is governed not only by its genetic potential and technological manipulation but also by its immediate environmental conditions (Pickering, 1993). Schreck (1981) indicated that the environment further constrains the performance capacity of a fish thereby creating a realized performance capacity against the potential performance capacity. Poor water q uality, physical disturbance and social dominance of one fish by another are very important environmental stresses that affect the performance of fish (Pickering, 1993). Among the researchers and managers, the issue of stress on fish is generating a lot of interest, especially the detrimental effect of these.

Stress is generally described as the response of the body i.e., a physiological cascade of events that occurs when the organism is attempting to resist death or reestablish homeostatic norms in face of an insult (Schreck *et al*., 2001). Stress has been linked as the primary contributing factor of fish disease and mortality in aquaculture (Rottmann *et al*., 1992). Of note are the chemical stressors of the nitrogenous and other metabolic wastes in form of nitrite, which are of great significance as they are capable of counteracting the improved performance of cultivated fish. Nitrite is the product of feeding in aquatic systems and always results from the breakdown of ammonia. Buildup of nitrite in fish culture environment can lead to stress condition in fish and eventually serious production loss in aquaculture (Rottman *et al*., 1992). Good knowledge of ways of response of fish (especially physiological response) to stressors will be of a greater help in improving production of fish and in providing information on ways of effectively controlling and monitoring stress in aquaculture systems.

This study therefore examined the endocrine and metabolic responses of *Clarias. gariepinus* (a widely cultured fish species in Africa and tropics due to its hardiness and fast growth) to water quality stresses due to nitrite.

MATERIALS AND METHODS

The bioassay study of the physiological (Haematology, plasma biochemical and cortisol) responses of *C. gariepinus* to nitrite toxicity was carried

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out in the Fisheries laboratory of Department of Wildlife and Fisheries Management, University of Ibadan, Nigeria.

Two hundred and twenty-five adults of *C. gariepinus* (300-350 g, mean total length of 65.2±0.5 cm) were used for this study. These test fish were collected once from a commercial fish farm in Ibadan. The farm from which the fish were collected has no history of pollution. The fish collected were of the same genetic background and in good health. The fish were collected in the morning between 7.30am and 9.00am by seining

The fish were kept in three circular plastic tanks (Width-1.0 m, Height-0.5 m). They were kept for three weeks in these tanks to acclimatize to laboratory conditions (ambient temperature 30°C). The fish were fed with floating pellets (CHI fish feed) containing 45% crude protein. The proximate composition of this feed is presented in Appendix II. They were fed twice daily at 3% of their body weight. The water (dechlorinated tap) in the tanks was changed once every four days in order to avoid the accumulation of toxic metabolites and decaying food. The feeding was stopped 24 h before the commencement of the bioassay (Solbe, 1995). Only fish of similar size (of mean weight of 310.0±0.86 g) were selected from acclimatization tanks into pre-experiment holding tanks for bioassays.

Test chemical: Nitrite is obtained as Sodium Nitrite. It is of analar grade and was formulated and packaged by Hopkin and William Ltd., England.

General bioassay techniques: Static renewal bioassay technique was adopted in which the test media (toxicant and dilution water) was renewed at the same concentration once every 48 h. This was done in an attempt to maintain a more constant concentration of test media to which test animals were exposed and to prevent excessive accumulation of toxic metabolites (Solbe, 1995). For the bioassay study, the fish (*C. gariepinus*) were kept in 80×40×40cm plastic tanks.The toxicants were prepared by weighing a pre-determined amount of test chemicals using Electronic digital weighing scale (ACCULAB model meter balance 2000) and this was made up to a given volume to obtain a stock solution of known strength (Odiete, 1999).

96h LC_{50} value of nitrite for *C. gariepinus* of 0.20 g L^{-1} was used based on Ajani (2006). Probit method was used to obtain the LC_{50}. The test fish were exposed to sub-lethal concentration of half (½) of 96-hr LC_{50} of nitrite.

To evaluate the effects of sub-lethal concentrations of this toxicant on adult of *C. gariepinus*, fifteen test fish were stocked per tank (Solbe, 1995) and each treatment was replicated thrice while untreated concentrations served as control. The fish were subjected to photoperiod of 12 h light and 12 h darkness. Blood samples of the test fish were collected at the time interval of 0, 6, 24, 48, 72 and 96 h. The blood samples were collected in heparinized bottles by inserting a syringe needle at the ventral midline just posterior to the anal fin and to continue the insertion at 45° angle until it penetrate the caudal vessels lying between adjacent hermal arches (Morgan and Iwama, 1997). They were centrifuged at 3000 G for 5 min with the aid of Millipore Personal Centrifuge and the plasma were separated and analyzed.

Plasma sodium and potassium were analyzed in the blood samples by using flame emission photometry (Morgan and Iwama, 1997). Plasma chloride was determined by using mercuric nitrate method while Plasma total proteins was determined using the biuret reaction (Sigma test kit no. 541.1) with a certified albumin/globulin standard. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (Morgan and Iwama, 1997). The plasma cortisol level was determined using the radioimmunoassays after extraction with ethyl acetate as described by Pankhurst *et al.* (1992).

The PCV and HaemogloBin (Hb) concentrations were read by the use of Sigma Diagnostics kit no. 525-A. The Red Blood Cell Counts (RBCC) was carried out by using standard haematological techniques (Dacie and Lewis, 1984). Total leucocyte count (WBC) was carried out according to the methods of Schalm *et.al.*,1975. White Blood Cell Differential Count was done according to Wright stain methods of Schalm *et.al.*,1975.Counting was done to determine the percentage leucocyte distribution of the various leucocyte types.

Data analysis: The data from the treatments were subjected to two-way analysis of variance (ANOVA) test to determine the level of interaction among the treatments. All the tests were carried out by using STATISTICA for windows XP 2000 on PC (Linea version).

RESULTS

Table 1 and 2 show variations in the haematological and plasma biochemical levels of *C. gariepinus* on exposure to sub-lethal level of nitrite at different exposure times.

Haematology: The PCV value of *C. gariepinus* increased significantly ($p<0.05$) from 30.67±0.76% at 0 h of exposure to 47.67±0.77% at 6 h, this is followed by significant decrease ($p<0.05$) at 24 and 48 h, this later increased gradually at 72 h followed with sharp decrease at 96 h to 22.83±0.58%. The blood haemoglobin level

Table 1: Haematological parameters of *Clarias gariepinus* subjected to sub-lethal concentration of Nitrite under different exposure time

Parameter/h	PCV (%)	Hb (g dL^{-1})	WBC (10^9 L^{-1})	RBC (10^6 L^{-1})	Platelet (10^9 L^{-1})	Lymphocytes (10^3 mm^{-3})	Eosinophils (10^3 mm^{-3})	Monocyctes (10^3 mm^{-3})
0	30.67±0.76^a	10.67±0.76^a	2.54±0.29^a	3.69±0.01^a	4.83±0.54^a	63.33± 1.04^a	35.57 ±0.81^a	1.07± 0.12^a
6	47.67±0.77^b	14.83±0.68^b	1.23±2.89^b	3.33±0.02^b	3.42±0.51^b	44.00± 0.50^b	56.33± 1.14^b	1.17± 0.29^a
24	44.00±3.50^c	12.50±0.01^c	1.03±2.89^c	3.49±0.01^c	2.34±0.01^c	64.33± 0.31^a	35.67± 1.47^a	1.00± 0.10^a
48	14.67±0.76^d	10.67±0.77^a	1.19±0.50^d	2.51±0.03^d	2.22±0.50^d	65.67± 1.53^a	34.33± 0.29^a	0.33± 0.58^b
72	44.67±0.78^c	12.77±0.75^c	1.17±0.29^e	3.28±0.01^e	2.76±0.50^e	55.33± 1.26^c	45.27± 0.46^c	0.67± 0.58^b
96	22.83±0.58^e	06.73±0.21^d	1.14±0.50^f	2.20±0.56^f	3.82±1.04^f	53.33± 0.42^c	23.83± 0.76^d	0.33± 0.58^b

Means followed by the same superscript in each column are not significantly different (p>0.05)

Table 2: Plasma biochemistry of *Clarias gariepinus* subjected to sub-lethal concentration of Nitrite under different exposure time

H/Parameters	Plasma sodium (mg dL^{-1})	Plasma Potassium (mg dL^{-1})	Plasma Chloride (mg dL^{-1})	Total protein (g dL^{-1})	Plasma glucose (mM)	Plasma cortisol (ng dL^{-1})
0	128.33±1.04^a	4.50±0.02^a	96.00±0.50^a	3.60±0.10^a	95.00±1.00^a	101.00±0.10^a
6	128.00±0.01^a	6.60±0.10^b	95.00±0.50^a	2.60±0.15^b	100.00±0.12^b	102.00±0.22^b
24	127.00±0.50^a	5.20±0.15^c	94.00±0.51^a	2.80±0.10^c	62.00±1.00^c	100.00±1.00^a
48	121.00±0.50^b	4.10±0.18^a	90.00±0.18^a	3.60±0.12^a	55.00±1.20^d	161.00±1.20^c
72	130.67±0.76^b	5.30±0.21^c	92.50±1.01^a	3.60±0.10^a	52.00±1.50^e	107.00±1.60^d
96	112.00±0.50^c	2.20±0.01^e	86.00±0.50^b	2.60±0.20^b	40.00±1.10^f	136.00±1.00^e

Means followed by the same superscript in each column are not significantly different (p>0.05)

of *C. gariepinus* exposed to sub lethal level of nitrite at different exposure periods exhibited variation in values. The haemoglobin level increased significantly ($p<0.05$) from value of 10.67±0.76 g dL^{-1} recorded at 0 h of exposure to 14.83±0.68 g dL^{-1}, this was later followed with decrease recorded at 24 h (12.50±0.01 g dL^{-1}) and 48 h (10.67±0.77 g dL^{-1}), this later increase at 72 h to 12.77±0.75 g dL^{-1}. However this was followed by a decrease at 96 h to 06.73±0.21 g dL^{-1}. The white blood cell level in *C. gariepinus* exposed to sub lethal level of nitrite toxicity showed a gradual and significant ($p<0.05$) decrease from value of 25.43±28.87 10^3 mm^{-3} recorded at 0 h of exposure to 10.30±28.88 10^3 mm^{-3} (24 h). This was followed by slight increase to 11.93± 50.00 10^3 mm^{-3} at 48 h and this subsequently decreased to 11.67±28.80 10^3 $mm^{-3}$and 11.37±50.00 10^3 mm^{-3} at 72 and 96 h, respectively.

From the value of 3.69±0.01 10^6/l recorded at 0 h, RBC level decreased significantly ($p<0.05$) at 6 h (3.33±0.02 10^6 L^{-1}), this was followed by significant increase ($p<0.05$) at 24 h (3.49±0.01 10^6 L^{-1}). The RBC value later decreased gradually to 2.20±0.56 10^6 L^{-1} at 96 h. The mean lymphocyte level of *C. gariepinus* on exposure to sub lethal level of nitrite decreased significantly ($p<0.05$) from value of 63.33±1.04 10^3 mm^{-3} (0 h) to 44.00±0.50 10^3 mm^{-3} (6 h), after which it rises to 65.67±1.53 10^3 mm^{-3}at 48 h. This later decreased to 53.33±0.42 10^3 mm^{-3}at 96 h. The mean eosinophils level in the blood samples of the *C. gariepinus* exposed to sub-lethal level of nitrite recorded a significant increase ($p<0.05$) from 35.57±0.8110^3 mm^{-3} at 0 h to 56.33±1.14 10^3 mm^{-3}at 6 h, this later decreased gradually but at significant levels ($p<0.05$) to 23.83±0.76 10^3 mm^{-3} at 96 h. The monocyctes level of the *C. gariepinus* exposed to sub-lethal level of nitrite showed an increase to 1.17±0.29 10^3 mm^{-3} at 6 h from 1.07±0.12 10^3 mm^{-3} recorded at 0 h. This was followed by gradual decrease to 0.33±0.58 10^3 mm^{-3} at 96 h.

Plasma biochemistry: The mean plasma sodium level decreased from 0 h with mean value of 128.33±1.04 mg dL^{-1} to 121.00±0.050 mg dL^{-1} at 48 h after which it increased significantly ($p<0.05$) to 130.67±0.76 mg dL^{-1} at 72 h of exposure to nitrite toxicity. This later decreased to 112.00±0.50 mg dL^{-1} at 96 h.

There was a significant increase ($p<0.05$) in plasma potassium level of *C. gariepinus* exposed to sub- lethal level of nitrite toxicity at different exposure times from 0 h (4.50±0.02 mg dL^{-1}) to 6 h (6.60±0.10 mg dL^{-1}). This later decreased to 4.10±0.18 mg dL^{-1} at 48 h followed with an increase at 72 h (5.30±0.21 mg dL^{-1}). The plasma potassium was not detectable after 96-h exposure.

The plasma chloride decreases from 96.00±0.50 mg dL^{-1} at 0 h to 90.00±0.18 mg dL^{-1} in 48 h after which there was a slight but significant increase ($p<0.05$) at 72 h (92.50±1.01 mg dL^{-1}). This later decreased to 86.00±0.50 mg dL^{-1} at 96 h.

The mean total protein decreased significantly ($p<0.05$) from a value of 3.60±0.10 g dL^{-1} recorded at 0 h to 2.80±0.10 g dL^{-1} at 24 h, this value later returned back to 0 h level at both 48 and 72 h and this was followed by a decrease to 2.60±0.20 g dL^{-1} at 96 h.

There was an initial increase from 95mM at 0 h to 100mM at 6 h in mean plasma glucose concentration in the *C. gariepinus* exposed to nitrite toxicity. This was followed by a significant decrease ($p<0.05$) to 40.00±1.10 mM at 96 h.

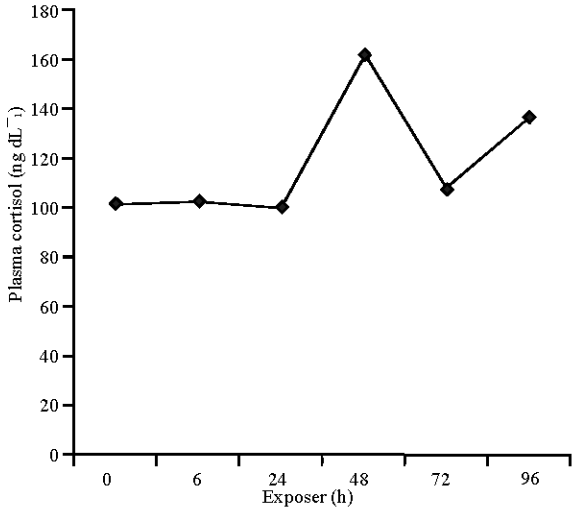

Fig. 1: Variation in blood plasma cortisol of *C. gariepinus* on exposure to sub lethal concentration of nitrite at different exposure h

The mean plasma cortisol level in *C. gariepinus* exposed to sub lethal level of nitrite toxicity at different time of exposure is shown in Fig. 1. The mean plasma cortisol level decreased significantly ($p<0.05$) from 101.00±0.10 ngldl observed at 0 h to 100.00±1.00 ng dL^{-1} at 24 h. This was followed by an increase at 48 h (161.00±1.20-ng dL^{-1}), it reduces to 107.00±1.60 ngldl at 72 h and later increased to 136.00±1.00ng dL^{-1} at 96 h.

DISCUSSION

There was a significant increase ($p<0.05$) in PCV level from 0 h to 6 h in fish exposed to nitrite toxicity. This may be attributed to more release of erythrocytes to combat the stressor (Rottmann *et al*., 1992). This is followed by a significant decrease in PCV level and this may be linked to greater impact of the stressor after prolonged exposure. The blood, hemoglobin and red blood cell levels experienced decrease from 6 h to 48 h and from 72 h to 96 h. A similar decrease was observed in red blood cell count and haemoglobin of both *Oreochromis niloticus* and *Chrysicthyts auratus* on exposure to atrazine (Hussein *et al*., 1996).

The decrease in blood haemoglobin and red blood cell is not unconnected with the presence of the stressor (nitrite), which caused hemodilution to occur due to impaired osmoregulation (Rottmann *et al*., 1992).

The increase observed in RBC count between 6 and 24 h may be due to the release of red blood cell into the blood stream. This agreed with observation of Alkahem *et al*., (1998) and Rottmann *et al*., (1992) when trichlorefon (an organophosphate pesticide commonly used for pest control) was exposed to fish for 96 h. They attributed this to a stress-mediated condition, which trigger the release of new erythrocytes from the erythropoietic tissue to improve the oxygen carrying capacity of exposed fish blood with resultant higher values of erythrocyte count. The decrease in the value of the white blood cells of *C. gariepinus* in this study when exposed to the sub-lethal level of nitrite may be due to the reaction of fish to the effect of the toxicant. Dick and Dixon (1985) reported a significant reduction in leucocyte (leucopenia) of rainbow trout (*Salmo gaidneri*) after acute exposure to 301 μgL^{-1} of copper for 24 h. This was attributed to a generalized stress response resulting from increased pituitary-interrenal activity. Alkahem (1994) also observed a significant decrease in total leucocyte count of *O. niloticus* exposed to sub lethal levels of nickel. This was attributed to reduction in the numbers of circulating thrombocytes and lymphocytes due to a diminution in the delivery of lymphocytes to the circulatory system through a reduced lymphocyte production and a rapid destruction of cells, which leads to an increased rate of peripheral removal of lymphocytes. Ball and Slicher (1962); Ellis (1977); Murad and Houston (1988) attributed such lymphopenias to the lysis of lymphocytes after exposure to toxicants.

Nitrite toxicity caused the mean lymphocyte level to decrease significantly for the first six h before there was an increase and then a decrease. The increase may be as a result of the production of more antibodies to conquer the stressor, which eventually failed with the decrease recorded until the termination of the test.

The significant increase observed for nitrite toxicity in monocytes may be due to recruitment of more cells to combat the stressor, which eventually yielded no positive result.

The plasma chloride level decreased from 0 h to 96 h, when exposed to sub lethal level of nitrite. This decrease suggests that there is an uptake of nitrite. Since nitrite enters the fish through the same route as chloride (Williams and Eddy, 1986). Nitrite is a competitive inhibitor of the active chloride uptake mechanism in fish gills, thus severity of the stressor caused its uptake by the fish. The significant decrease recorded for total protein levels might be due to impaired water balance (Wedemeyer and Yasutake, 1977). Hussein *et al*., (1996) observed that serum total protein tended to decrease significantly ($p<0.05$) with increase in atrazine level and exposure time *O. niloticus* while for *C. auratus,* significant decreases ($p<0.01$) for total protein were observed.

There was an initial increase in plasma glucose from 95 mM (0 h) to 100mM at 6 h before this was followed by

a sharp decrease. The increase recorded was due to the stress response to the toxicant by the fish in order to provide energy to the fish for the 'fight-or-flight' reaction (Wedemeyer *et al*., 1990). Colombo *et al*., (1990) noted that the fish mobilize its energy reserve in attempts to avoid or overcome the immediate threat. The increase/mobilization of energy could not overcome the immediate threat, which is nitrite and so this led to the progressive depletion of energy.

The result obtained for plasma cortisol in adult *C. gariepinus* when exposed to nitrite toxicity followed a haphazard pattern. According to Donaldson, (1981) and Barton and Iwama, (1991), fish species can respond differently to similar stressors. For instance, salmonids respond to handling and crowding stresses with an almost immediate elevation in circulating levels of cortisol (Donaldson, 1981; Barton and Iwama, 1991) but a measurable increase in cortisol level of sea raven, *Hemitripeterus americanus* are apparent only an h after the onset of the stressor (Vijayan and Moon, 1994).

CONCLUSION

Short-term exposure (acute level) of nitrite affects the haematology and the minerals (ions) in the fish's body. Therefore, it is very important that these water quality stressors such as nitrite be monitored regularly and level should be controlled through various management practices when necessary. Uncontrolled level of nitrite in culture environment may not only lead to mortality but may prevent the fish from achieving its full genetic potential in terms of growth and reproductive capability.

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