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Full Length Research Paper

Hormonal and haematological responses of *Clarias gariepinus* (Burchell 1822) to ammonia toxicity

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This study assessed hormonal and haematological responses of *Clarias gariepinus* to ammonia toxicity. Laboratory study of haematological responses of adult *C. gariepinus* to sub-lethal level of ammonia (2.2 g/l) at different exposure hours (0, 6, 24, 48, 72, 96 h) were carried out. Blood samples of *C. gariepinus* were collected at each exposure hour and evaluated for primary and secondary stress indicators. Data obtained were subjected to simple descriptive analysis and analysis of variance. Plasma cortisol level of *C. gariepinus* increased significantly (P < 0.05) from 0 h (01.0 ± 0.1 ng/dl) to 48 h (154.0 ± 1.0 ng/dl) after which it decreased and stabilized at 72 h (106.0 ± 1.1 ng/dl). Packed cell volume (PCV) and Red blood cell (RBC) 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 ammonia. The mean plasma glucose level recorded at 0 h (95.00 ± 1.00 mM) decreased significantly (P < 0.05) with the lowest mean value of 37.00 ± 1.05 mM obtained at 96 h of exposure. Haematological and hormonal balances of adult *C. gariepinus* were affected under short-term exposure to ammonia toxicity.

Key words: Hormonal, Haematology, *Clarias gariepinus,* ammonia, toxicity.

INTRODUCTION

Stress has been linked as the primary contributing factor of fish disease and mortality in aquaculture (Petric et al., 2006). Any stress in fish causes hormonal changes, which decreases the effectiveness of inflammatory response. Stress also impairs the production and release of antibodies. Fish reared under commercial aquaculture environments are confined to the production unit and are predisposed to and weakened by stress conditions. Of note are chemical stressors from nitrogenous wastes and other metabolic wastes like ammonia, which are of great significance as they are capable of counteracting the improved performance of cultivated fish.

Ammonia is the major end product in the breakdown of proteins in fish. The primary source of ammonia in aquaculture systems is fish feed. Of all water quality parameters, which affect fish, ammonia is the most important after oxygen (Francis – Floyd and Watson, 1996). In small quantity, ammonia causes stress. It is responsible for more unexplained losses in aquaculture than any other water quality parameters. Indepth knowledge fish 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 (Pottinger et al., 1992; Pickering, 1993; Schreck et al., 2001).

Scholars like Das et al. (2004) and Yuen et al. (2005) have worked on the acute toxicity of ammonia and its sub-lethal effects on the haematology of *Cirrhinus mrigala* H and mudskippers, *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti* but dearth information is available on *C. gariepinus* (Burchell 1822).

C. gariepinus is one of the most commonly cultured fish species in the world (FAO, 1977). Its suitability for culture covers almost every part of tropics. This may be linked to its hardy nature and ability to grow and breed under a wide range of culture conditions. The study of responses of *C. gariepinus* to water quality stressors such as ammonia would go a long way in improving the knowledge in the effective production of *C. gariepinus.* This study therefore examined the endocrine and metabolic responses of *C. gariepinus* to water quality stresses due to ammonia.

MATERIALS AND METHODS

Experimental stocks (source and collection)

This experiment was carried out between April and July, 2004 at the Fisheries laboratory of the 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. Test fish were collected from Fishmonger commercial fish farm in Ibadan, Nigeria. The farm from which the fish were collected has no history of pollution. The fish collected were of the same genetic background and were apparently healthy. The fish were collected in the morning between 7.30 and 9.00 am and transported to the laboratory in plastic bowls filled with water from the pond from which they were collected.

Laboratory culture and acclimatization

The test fish were kept in three circular plastic tanks (width – 1.0 m, height – 0.5 m), for three weeks to acclimatize to laboratory conditions (ambient temperature 30°C). The fish were fed twice daily at 3% of their body weight with floating pellets (CHI fish commercial feed) containing 45% crude protein. The water (dechlorinated tap) in the tanks was changed once every other day in order to avoid the accumulation of toxic metabolites and decaying food. 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.

General bioassay techniques

Ammonia was obtained as ammonium chloride. It is of ANALAR grade and was formulated and packaged by Hopkin and Williams Ltd., England. Static renewal bioassay technique was adopted according to Solbe (1995). For the bioassay study, fish (*C. gariepinus*) were kept in 80 x 40 x 40 cm plastic tanks. The toxicants were prepared to obtain a stock solution of known strength as described by Odiete (1999). 96 h LC_{50} value of ammonia for *C. gariepinus* of 4.45 g/l was used based on Ajani (2006). Probit method was used to obtain the LC_{50}. For physiological (haematology and plasma biochemistry) responses of *C. gariepinus* to ammonia toxicity, the test fish were exposed to sub-lethal concentration of the 96 h LC_{50} of ammonia.

Hormonal and haematological responses measurement

To evaluate the effects of sub-lethal concentrations of ammonia on adult of *C. gariepinus,* fifteen test fish were stocked per tank according to (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. All fish were anaesthetized with MS 222 prior to the collection of blood samples. Blood samples of the test fish were collected at the time interval of 0, 6, 24, 48, 72 and 96 h in heparinized bottles according to methods of Morgan and Iwama (1997). Blood samples were centrifuged and the plasma were separated and analysed.

Plasma sodium and potassium were analysed in the blood samples by using flame emission photometry according to Morgan and Iwama (1997). Plasma chloride was determined 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). Plasma cortisol level was determined using the radioimmunoassays after extraction with ethyl acetate as described by Pankhurst et al. (1992). PCV and haemoglobin (Hb) concentrations were determined using Sigma Diagnositics kit no. 525 – A. Red blood cell counts (RBCC) was determined using standard haematological techniques as described by Dacie and Lewis (1984). Total leucocyte count (WBC) was carried out according to the methods of Schalm et al. (1975). Mean cell haemoglobin concentration (MCHC) was calculated from (Hb)/(PCV/100), mean cell volume (MCV) from PCV/RBCC, and mean cell haemoglobin from (Hb/RBCC).

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 using STATISTICA for windows XP 2000 on PC (Linea version).

RESULTS

Haematological parameters

Table 1 shows the haematological parameters of *C. gariepinus* exposed to sub lethal concentration of ammonia under different exposure time. The PCV of *C. gariepinus* exposed to sub lethal level of ammonia at different exposure time showed a significant decrease from 30.67 ± 0.76% recorded at 0 h to 24.68 ± 0.84% at 24 h; this value later rose significantly ($P < 0.05$) at 48 and 72 h followed by a decrease to 22.67 ± 0.76% at 96 h.

Haemoglobin level decreased from 10.67 ± 0.76 g/dl at 0 h to 07.84 ± 0.80 g/dl (24 h). This later increased to 10.70 ± 0.70 g/dl and 12.80 ± 0.01 g/dl at 48 and 72 h, respectively; and this was followed by a significant decrease ($P < 0.05$) at 96 h (06.87 ± 0.12 g/dl). On exposure to sub-lethal level of ammonia, the white blood cell decreased significantly ($P<0.05$) from 2.54 ± 0.29 $10^3/mm^3$ (0 h) to 1.03 ± 0.10 $10^3/mm^3$ (24 h). This value increased significantly ($P < 0.05$) at 48 h (1.93 ± 0.59 $10^3/mm^3$) followed by reduction in the WBC level to 1.17 ± 0.76 $10^3/mm^3$ and 1.14 ± 0.77 from $10^3/mm^3$ at 72 and 96 h, respectively. The RBC value of *C. gariepinus* decreased from 3.69 ± 0.01 $10^6/l$ at 0 hour to 1.31 ± 0.02 $10^6/l$ at 24 h on exposure to sub lethal level of ammonia. There was significant increase ($P < 0.05$) in the RBC level at 48 and 72 h followed by a decrease to 3.33 ± 0.03 $10^6/L$ at 96 h.

The mean lymphocyte level increased from 63.33 ± 1.04 $10^3/mm^3$ recorded at 0 h to 72.90 ± 1.65 $10^3/mm^3$ at 72 h, followed by a decrease to 53.17 ± 0.76 10^3mm^3 at 96 h (Table 2). The mean eosinophil level increased significantly ($P > 0.05$) from 35.57 ± 81 $10^3/mm^3$ at 0 h to 58.67 ± 0.76 $10^3/mm^3$ (6 h). This later decreased to 32. 67 ± 0.76 $10^3/mm^3$ at 72 h followed with an increase at 96 h (45.33 ± 1.04 $10^3/mm^3$).

On exposure to sub-lethal level of ammonia, the mean monocyte level of *C. gariepinus* experienced a significant decrease ($P < 0.05$) from 1.07 ± 0.12 $10^3/mm^3$ at 0 h to 0.38 ± 0.33 $10^3/mm^3$ at 6 h followed by an increase to 2.33 ± 1.04 $10^3/mm^3$ (24 h) later and decreased to 1.17 ±

Table 1. Haematological parameter of *C. gariepinus* subjected to sub-lethal concentration of ammonia under different exposure time.

Parameters/ hour	PCV (%)	Hb (g/dl)	WBC (10^3/mm^3)	RBC (10^6/1)
0	30.67 ± 0.76^a	10.67 ± 0.76^a	2.54 ± 0.29^a	3.69 ± 0.01^a
6	32.67 ± 0.84^b	10.33 ± 0.29^a	1.23 ± 1.00^b	2.19 ± 0.15^b
24	24.68 ± 0.84^c	07.84 ± 0.80^b	1.03 ± 1.00^c	1.31 ± 0.02^c
48	33.71 ± 0.81^a	10.70 ± 0.70^a	1.93 ± 0.59^d	2.24 ± 0.04^d
72	41.33 ± 1.26^d	12.80 ± 0.01^b	1.16 ± 0.76^c	3.38 ± 0.01^e
96	22.67 ± 0.76^e	06.87 ± 0.12^b	1.13 ± 0.76^e	3.33 ± 0.03^e

Mean followed by the same superscript in each column are not significantly different (P > 0.05).

Table 2. Some haematological parameters of *C. gariepinus* subjected to sub-lethal concentration of ammmonia under different exposure time.

Parameters S/ hour of exposure	Lymphocytes (10^3/mm^3)	Eosinophils (10^3/mm^3)	Monocyte (10^3/mm^3)	MCV (fl)	MCH (pg)	MCHC (g/dl)
0	63.33 ± 1.04^a	35.57 ± 0.81^a	1.07 ± 0.12^a	81.67 ± 0.28^a	28.36 ± 1.41^a	33.47 ± 0.13^a
6	42.50 ± 0.50^b	58.67 ± 0.76^b	0.38 ±0.33^b	146.00 ± 0.91^b	45.61 ± 0.57^a	31.27 ± 0.72^a
24	60.83 ± 0.58^a	38.50 ± 0.50^a	2.33 ± 1.04^c	183.57 ± 0.40^c	57.52 ± 0.42^c	30.97 ± 0.30^a
48	72.90 ± 1.65^c	26.80 ± 0.69^a	1.07 ± 0.12^a	146.07 ± 0.90^b	46.57 ± 0.16^b	31.91 ± 0.09^a
72	66.33 ± 1.04^a	32.67 ± 0.76^a	2.17 ± 0.29^c	118.31 ± 0.21^b	37.61 ± 0.57^b	32.30 ± 061^a
96	53.17 ± 0.76^d	45.33 ± 1.04^c	1.17 ±0.29^a	65.02 ± 0.84^a	20.59 ± 0.36^a	31.47 ± 0.89^a

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

Table 3. Plasma biochemistry of *C. gariepinus* subjected to sub-lethal concentration of ammmonia under different exposure time.

Hours/ parameters	Plasma Sodium (mg/dl)	Plasma Potassium (mg/dl)	Plasma Chloride (mg/dl)	Total Protein (g/dl)	Plasma glucose (mM)	Plasma cortisol (ng/dl)
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	147.00 ± 0.50^b	2.60 ± 0.12^b	116.00 ± 0.54^b	3.40 ± 0.15^a	110.00 ± 10.00^b	124.00 ± 1.02^a
24	122.00 ± 0.56^c	2.20 ± 0.15^b	90.33 ± 1.04^c	3.00 ± 0.12^a	70.00 ± 1.20^c	140.00 ± 1.22^b
48	129.00 ± 0.76^a	4.50 ± 0.50^a	93.00 ± 0.52^c	3.50 ± 0.10^a	47.00 ± 1.00^d	154.00 ± 1.00^c
72	127.00 ± 0.53^a	7.77 ± 0.15^b	92.00 ± 0.50^c	3.40 ± 0.15^a	43.00 ± 1.10^d	106.00 ± 1.05^d
96	140.00 ± 0.01^e	5.00 ± 0.50^a	108.00 ± 0.58^d	4.80 ± 0.13^b	37.00 ± 1.05^d	106.00 ± 1.00^d

Mean followed by the same superscript in each column are not significantly different (P > 0.05).

0.29 10^3/mm^3 at 96 h.

A significant rise (P < 0.05) in MCV from 81.67 ± 0.28 fl at 0 h to 183.57 ± 0.40 fl was recorded at 24 h. This was followed by a gradual but significant decrease (P < 0.05) to 65.02 ± 0.84 fl at 96 h. From 0 h value of 28.36 ± 1.41 pg, there was a significant increase (P < 0.05) in MCH to 57.52 ± 0.42 pg (24 h). This later decreased to 20.59 ± 0.36 pg at 96 h. The MCHC level decreased from 33.47 ± 0.13% at 0 h to 31.91 ± 0.90% at 48 h followed by slight increase at 72 h to 32.30 ± 0.61%; this later decreased to 31.47 ± 0.89% at 96 h.

Plasma biochemical parameters

The variations in the blood plasma biochemical parameters of *C. gariepinus* exposed to sub-lethal level of ammonia at exposure hours of 0, 6, 24, 48, 72 and 96 h are presented in Table 3. From 0 h value of 128.33 ± 1.04 mg/dl, the mean plasma sodium level in *C. gariepinus* exposed to sub-lethal level of ammonia toxicity increased significantly (P < 0.05) to 147.00 ± 0.50 mg/dl at 6 h. This was followed by a decrease to 127.00 ± 0.53 mg/dl at 72 h and this level however increased at 96 h to 140 ± 0.01 mg/dl. With value of 4.50 ± 0.02 mg/dl recorded at 0 h, the plasma potassium level was not detectable at 6 and 24 h; this value increased to 7.77 ± 0.15 mg/dl at 72 h and in 96 h, a reduced value of 5.00 ± 0.50 mg/dl was obtained.

This mean plasma chloride level rises from 96.00 ± 0.05 mg/dl (0 h) to 116.00 ± 0.54 mg/dl (6 h), it reduced

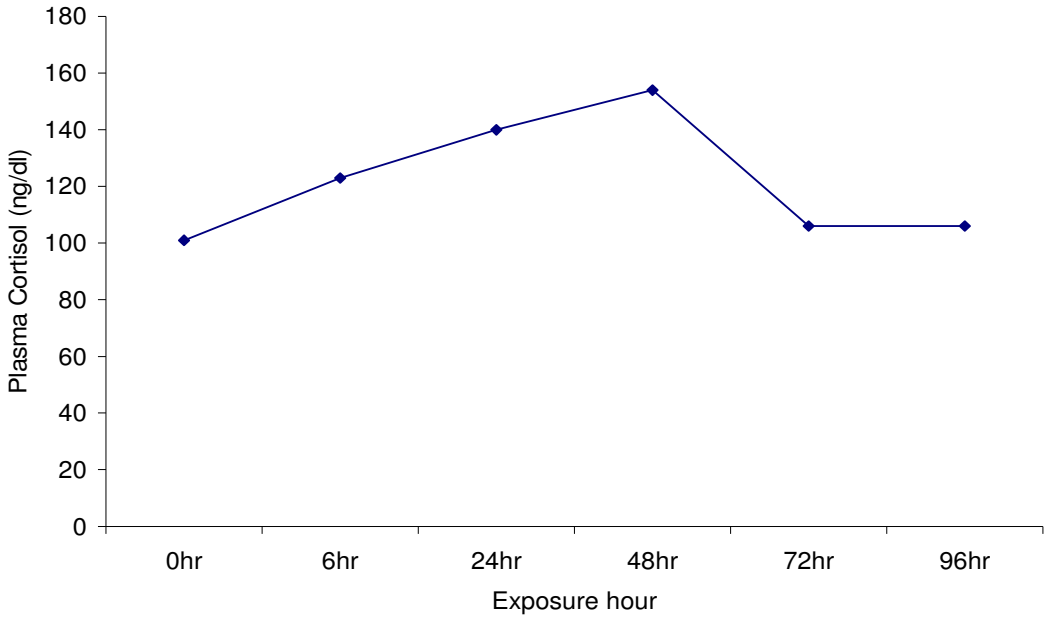

Figure 1. Plasma cortisol concentration in the *C. gariepinus* exposed to sub-lethal level of Ammonia.

to 92.00 ± 0.50 mg/dl at 72 h, then later increased at 96 h to 96.00 ± 0.50 mg/dl. The mean total protein level decreased significantly ($P < 0.05$) from 4.80 ± 0.13 g/dl recorded at 0 h to 3.00 ± 0.12 g/dl (24 h). It increased to 3.50 ± 0.10 g/dl at 48 h and this was followed by a decrease at 72 h (3.40 ± 0.15 g/dl). This value later increased at 96 h of exposure to 3.60 ± 0.10 g/dl.

The mean plasma glucose level recorded at 0 h (95.00 ± 1.00 mM) increased to 110.00 ± 1.10 mM at 6 h, which was followed by a significant decrease ($P < 0.05$) as the hours of exposure increase, with the lowest mean value of 37.00 ± 1.05 mM obtained at 96 h of exposure to sub-lethal level of ammonia toxicity. The variation in mean plasma cortisol level of *C. gariepinus* exposure to sub-lethal level of ammonia at different exposure is shown in Figure 1. The mean plasma cortisol level in fish exposed to sub-lethal level of ammonia toxicity increased significantly ($P < 0.05$) from 101.00 ± 0.10 ng/dl at 0 h to 154.00 ± 1.00 ng/dl at 48 h. This later reduced with mean plasma cortisol values at 106.00 ± 1.05 ng/dl and 106.00 ± 1.00 ng/dl recorded at 72 and 96 h respectively.

DISCUSSION

The significant decreases in PCV, RBC and Hb levels were in line with observation of Fafioye (2002). He noted significant decreases in these blood cell parameters between the control and experimental specimens of both *C. gariepinus* and *O. niloticus* on exposure to sub-lethal concentrations of the aqueous and ethanolic extracts of *Rafia. vinifera* and *Parkia biglobosa*. The decreased value in PCV may be due to anaemia and haemodilution or haemolysis for RBC (Fafioye, 2002). Saeed, (1997) also recorded a marked reduction in RBC count and Hb concentration after the exposure of *O. niloticus* to acute ammonia concentrations. The significant increase in the value recorded at 48 and 72 h in these parameters might be attributed to the stress – mediated condition which prompts 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 and haemoglobin concentration (Fafioye 2002; Saeed, 1997).

The significant decrease observed in white blood cell level on exposure to sub lethal level of ammonia may be due to the function of the white blood cells. They are the "soldiers" of the body. This is in consonance with the result of Alkahem et al. (1998) where leukocyte counts were decreased after been exposed to an organophosphate pesticide for 96 h. Low leucocyte count in this study is attributed to the reduction in the number of lymphocytes. Maule and Schreck (1990) also noted that stress and cortisol level affect the number of leukocytes in immune organs.

The decrease recorded in total protein values from 0 to 24 h may be due to the severity of the stressor, which causes osmotic imbalance. Alkahem et al. (1998) attributed the reduction in the proteins to its conversion to fulfilling an increased energy demand by fish to cope with detrimental conditions imposed by a toxicant. The increase that was recorded in plasma glucose value in the first six hours may be due to the fact that the fish mobilize energy from all available resources to combat

the stressor (Colombo et al., 1990). The severity of the stressor must have been the reason why there was a significant and sharp decrease as the exposed hour increased. The result obtained in this work is consistent with that of Mahajan and Dheer (1983) when *Channa punctatus* was starved for 10 weeks. The increase observed in the mean lymphocytes values of *C. gariepinus* exposure to sub-lethal level of ammonia initially may be attributed to the production of more antibodies to combat the stressor (Spielman, 2004). The decrease observed from 72 h may be attributed to its failure to salvage the situation due to the severity of the stressor. Also, the significant increase recorded initially in Eosinophil counts follows the normal trend as recorded by Spielman (2004). The dramatic increase may be as a result of the stressor as it plays an important role in inflammatory reactions and other foreign proteins. The significant decrease observed in monocyte in the case of ammonia may be attributed to their movement from the blood immediately they sensed the stressor into the blood tissues where they will mature into cells capable of engulfing and destroying harmful organisms.

The increase in the cortisol level of *C. gariepinus* up to the 48 h is consistent with the result obtained when diploid and triploid Atlantic Salmon were subjected to confinement stress (Sadler et al., 2002). Pickering, (1993) noted that the resultant elevation of plasma cortisol has been widely used as a quantitative measure of the magnitude of the stress response. Pickering and Pottinger (1989) noted that with an acute stress such as handling, cortisol levels are normally only elevated for a few hours before returning to basal values whereas the situation is different with the brown trout (*Salmo trutta*). Under a condition like overcrowding, the plasma cortisol levels may return to basal values despite the continued presence of the stress (Pickering and Stewart, 1984). On the other hand, when fish is faced with continuous social domination by another member of the same species or are stressed by exposure to low pH (Brown et al., 1984; Tabche et al., 1990), plasma cortisol levels may be elevated for many weeks with no sign of returning to basal values.

Conclusion

It was established through this study that haematological and hormonal balances of adult *C. gariepinus* were affected under short-term exposure to ammonia toxicity. Therefore, it is very important that this water quality stressor (ammonia) be monitored regularly and level should be controlled through various management practices when necessary. Uncontrolled level of ammonia 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. If fish is kept at the sub-lethal value of ammonia, it can compromise its well being by jeopardizing its health.

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