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*Full Length Research Paper*

# Nitrite intoxication of *Clarias gariepinus* at different water temperatures

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***Clarias gariepinus*** **(Burchell, 1822) of mean weight 320±11.2 g and total length 60.0±1.0 cm were exposed to nitrite (0.2 mg/l $NO_2$) for 48 h at 27 and 35°C in order to investigate the effects of nitrite poisoning at these water temperatures. The effects of nitrite exposure on fish was assessed on selected haematological and biochemical indicators of the blood. The test was performed in a semi-static assay for 48 h. Fish were kept in thermostart –controlled water baths each containing 15 L of test solution. Four groups each containing 8 specimens of 3 month old *C. gariepinus* were exposed to nitrite at the different water temperatures. Nitrite exposed fish showed lower haematocrit value (PCV) at both experimental temperatures compared with controls. Significantly higher PCV values were recorded in fish with nitrite at 27°C (36.67±0.57%) when compared with fish at 35°C (33.33±0.57%). A significant difference was also recorded for haemoglobin level and the erythrocyte counts. Leucocyte count in fish with nitrite at $27^0$ C was significantly higher ($P<0.05$) compared to the count at 35°C. For Plasma Biochemical parameters, significant differences were observed between the 2 groups for $K^+$ concentration (27°C, 4.9±0.10 mmol/l; 35°C, 4.1±0.12 mmol/l). Statistically significant differences were observed in plasma $Cl^-$ and $Na^+$ concentrations in fish at both temperatures. It has been established from this study that nitrite is more toxic at higher temperature.**

**Key words:** Haematology, plasma biochemistry*, Clarias gariepinus*, temperature.

## INTRODUCTION

Nitrite is an intermediate product in bacterial nitrification processes. An inbalance in either of these processes can lead to elevated ambient nitrite concentrations (Eddy and William, 1987). Nitrite problems are typically more likely in closed, intensive culture systems due to insufficient, inefficient or malfunctioning of filtration systems (Durborow et al., 1997). High nitrite concentrations in ponds occur more frequently when temperature is fluctuating, which result in the breakdown of the nitrogen cycle due to plankton and bacterial activity. If nitrite levels exceed that which resident bacteria can rapidly convert to nitrate, build-up of nitrite occurs and brown blood disease is a risk. This may result in mass fish mortality (Svobodova et al., 2005a). Nitrite is actively taken up through the gills and enters the blood stream (Margiocco et al., 1983; Jensen et al., 1987). From the blood plasma, nitrite diffuses into red blood cells where it oxidizes the Iron in haemoglobin to produce methaemoglobin which is incapable of oxygen transport. Methaemoglobin turns the blood to a chocolate-brown colour, so nitrite poisoning is often called a brown blood disease.

Nitrite toxicity in fish is influenced by a large number of external and internal factors, among which the chloride concentration in water plays a major role (Lewis and Morris, 1986; Svobodova et al., 2005b; Pistekova et al., 2005). Adeyemo et al. (2003) documented the haemato-

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**Abbreviations:** **$LC_{50}$,** Median lethal concentration; **RBC,** erythrocyte count; **PCV,** haematocrit value; **Hb,** haemoglobin; **WBC,** leucocyte count; **MCV,** mean corpuscular volume; **MCHC,** mean corpuscular haemoglobin; **ANOVA,** analysis of variance.

logical response of *Clarias gariepinus* to changes in acclimation temperature. Kroupova et al. (2006) worked on nitrite intoxication of common carp (*Cyprinus carpio*) at different water temperatures but no information is known about the influence of temperature on nitrite toxicity of the most cultured fish species in Nigeria, *C. gariepinus*.

Generally the water temperature is an important factor that determines chemical toxicity (Cairns et al, 1975), and it has multiple effects on the physiology of fish. Therefore, the aim of this study is to assess the influence of water temperature on the nitrite toxicity in *C. gariepinus*

## MATERIALS AND METHODS

### Experimental fish

*C. gariepinus* of mean weight 320±11.2 g and mean total length of 60.0±1.0 cm were used for the study. The fish were collected in the morning between 8 – 9 a.m. They were obtained from Bowen fish farm and maintained for 2 weeks in 2 circular plastic tanks (300 L) with dechlorinated tap water. They were fed with 3 mm CHI pelleted feed. Feeding was stopped 24 h prior to the commencement of the bioassay. Four days before the start of the experiment, the fish were divided into four groups and acclimated to 27 and 35°C. Temperatures around 27 and 35°C prevail during the growing season in Nigeria. During the experimental period, the fish were not fed.

### Experimental procedure and fish sampling

The test was performed in a semi-static assay for 48 h. Fish were kept in thermostat-controlled water baths, each containing 15 L of test solution. The nitrite concentration was obtained by adding $NaNO_3$ to dechlorinated tap water (Odiete, 1999). The dose of nitrite represented the median lethal concentration ($LC_{50}$) for *C. gariepinus* at a similar chloride water concentration and similar relative weight of fish according to Ajani (2006).

During the acclimation and experimental period, the range of the basic chemical indices of water taken were alkalinity 45mg/l, dissolved Oxygen 8.3mg/l, phosphate 30mg/l, hardness 72.4mg/l, chloride 19.2mg/l, ammonia 55.8mg/l and the pH 7.0 using HACH freshwater aquaculture test kit (FF-1A).

Four groups each containing 8 specimens of three-month old *C. gariepinus* was exposed to nitrite at different water temperatures (27 and 35°C). Each treatment were replicated.

Group $A_1$, (0. 2 mg/l) $NO_2$, t =27±0.4°C
Group $B_1$, (control 1) traces $NO_2$, t =27±0.4°C
Group $A_2$, (0.2 mg/l) $NO_2$, t =35±0.3°C
Group $B_2$, (control 2) traces $NO_2$; t =35±0.3°C

Nitrite and chloride content were checked twice during the test and measured values did not differ from the nominal value by more than 7%. Also, traces $NO_2$ in the control groups were below detection limit.

Forty *C. gariepinus* (5 from each group) with their replicates were examined to determine the haematological and biochemical profiles of blood. The blood samples were taken after 48 h exposure in heparinized bottles by inserting a syringe needle (2 ml) at the ventral midline just posterior to the anal fin at angle 45° until it penetrate the caudal vessels lying between adjacent hermal arches (Morgan and Iwama, 1997). They were centrifuged at 3000G for 5 min and the plasma were separated and analyzed.

The Erythrocyte count (RBC), haematocrit (PCV) haemoglobin (Hb), leucocyte count (WBC), platelets, Mean corpuscular Volume (MCV) and the Mean Corpuscular Haemoglobin (MCHC) were determined in the blood samples according to Jain (1986). Also, white blood cell differential; lymphocytes, neutrophils, monocytes and eosinophils. For plasma biochemistry, plasma $Na^+$, $K^+$ and $Cl^-$ were determined as described by Jain (1986).

### Statistical analysis

Statistical software SPSS package (Version 11) was used to determine differences between the test groups. Two - way analysis of variance (ANOVA) was applied to determine whether there were any significant differences in measured variables between nitrite-exposed fish at different temperatures and control fish.

## RESULTS

### Fish mortality

No mortality was observed in the control groups. High fish mortality was noticed during nitrite poisoning in the first 24 h in groups $A_1$ (35%) and $A_2$ (50%) when compared with the control groups. However, the difference between groups. $A_1$ and $A_2$ were not statistically significant ($P > 0.05$).

### Vital signs

Nitrite – treated *C. gariepinus* at 27 and 35°C showed typical symptoms of methaemoglobinemia which was manifested by erratic and disoriented behaviour and brown colouring of the blood and gills. The intensity of the colouration was temperature dependent. .

### Haematological variables

The result of the haematological parameters is presented in Table 1.

### Haematological variables

The PCV values were significantly lower ($P < 0.05$) in the control of group $B_2$ *C. gariepinus* compared with control group $B_1$. Significantly lower ($P < 0.05$) PCV values were found in fish of $A_1$ compared with $B_1$. No significant difference in PCV values were recorded in fish of $A_1$ compared with $A_2$ .No significant difference was observed in the haemoglobin level ($P < 0.05$) in fish of group $A_2$ compared to the control group $B_2$. A significant decrease was observed ($P > 0.05$) in fish of group $B_1$ compared to $A_1$. Significantly lower Haemoglobin values were obtained in fish of group $B_2$ compared with group $B_1$.

No significant decrease was observed in the erythrocyte counts in group $B_1$ compared to group $A_1$. It increased significantly ($P < 0.05$) in group $B_2$ compared to

**Table 1.** Haematological indices of *C. gariepinus* after 48 h nitrite exposure at 27 and 35°C.

| Indices | 27°C | | 35°C | |
|---|---|---|---|---|
| | $A_1$ (Nitrite) | $B_1$ (Nitrite – free) | $A_2$ (Nitrite) | $B_2$ (Nitrite – free ) |
| PCV (%) | 36.67 ± 0.58[a] | 47.67 ± 0.11[c] | 33.33 ± 0.57[a] | 38.33 ± 0.32[b] |
| Hb ( g/dl ) | 12.37 ± 0.82[b] | 15.67 ± 0.06[a] | 11.10 ± 0.10[b] | 11.56 ± 0.05 [b] |
| RBCC ( $10^6$/l) | 3.41 ± 0.02[c] | 3.48 ± 0.11[c] | 3.03 ± 0.01[a] | 3.11 ± 0.04[b] |
| WBC ($10^9$ /l) | 1.70 ± 1.52[c] | 1.58 ± 3.20[b] | 1.6 ± 1.43[ab] | 1.49 ± 0.91[a] |
| Platelets ($10^9$ /l ) | 1.07 ± 1.00 [a] | 1.16 ± 0.61[c] | 1.24 ± 0.21[d] | 1.01 ± 0.57[a] |
| Lymphocyte ($10^3$ /$mm^3$) | 35.00 ± 1.00[a] | 66.33 ± 1.52[b] | 38.00 ± 1.02[a] | 65.00 ± 1.40[b] |
| Neutrophil ( $10^3$ /$mm^3$) | 60.00 ± 0.08[b] | 29.67 ± 0.50[a] | 57.01 ± 0.12[b] | 32.01 ± 2.10[a] |
| Monocyte ($10^3$/$mm^3$) | 2.33 ± 0.57[a] | 3.33 ± 1.52[b] | 2.33 ± 0.10[a] | 1.33 ± 0.57[a] |
| Eoslnophil ($10^3$/$mm^3$ ) | 3.00 ± 2.00[a] | 3.00 ± 2.00[a] | 3.00 ± 1.00[a] | 2.00 ± 1.00[a] |
| MCV (fl) | 107.5 ±10 .00 [a] | 136.9 ± 8.00 [b] | 110.0 ± 1.00 [a] | 123.0 ± 12.00 [b] |
| MCH (pg) | 36.27 ± 3.61[a] | 45.02 ± 0.81[b] | 36.63 ± 2.07 [a] | 37.17 ± 6.34 [a] |
| MCHC(g/l) | 33.73 ± 0.96 [a] | 32.87 ± 1.08 [a] | 33.30 ± 2.11[a] | 30.16 ± 4.21[a] |

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

**Table 2.** Blood plasma indices of *C. gariepinus* after 48 h nitrite exposure at different water temperature.

| | 27°C | | 35°C | |
|---|---|---|---|---|
| | $B_1$ (Nitrite – free) | $A_1$ (Nitrite) | $B_2$ (Nitrite – free) | $A_2$ (Nitrite) |
| $K^+$ (mmol/l) | 3.0 ± 0.2[a] | 4.9 ± 0.10[b] | 3.2 ± 0.24[a] | 4.1 ± 0.12[b] |
| $C1^-$ (mmol/l) | 93.0 ±1.21[a] | 92.0 ± 0.72[a] | 89.0 ± 1.42[a] | 52.0 ± 1.11[b] |
| $Na^+$ (mmol/l) | 129.0 ± 1.00[b] | 135.0 ± 0.86[b] | 131.0 ± 0.72[b] | 90.0 ± 0.64[a] |

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

group $A_2$. A significant decrease was observed in group $B_1$ compared to group $B_2$.

Leucocyte count in group $A_1$ is significantly higher ($P < 0.05$) compared to the count in group $A_2$. Significant differences were observed among groups $A_2$ and $B_2$ and $B_1$ and $B_2$.

Highly significantly lower ($P < 0.05$) platelet counts were found in fish of group $A_1$ compared with groups $A_2$. Also, significant difference ($P < 0.05$) was observed in platelet count among groups $A_2$ and $B_2$; $B_1$ and $B_2$. For the white blood cell differential count, no significant difference were observed in lymphocyte in group $A_1$ compared to the count in group $A_2$ ; and $B_1$ and $B_2$ . However, significant differences were observed among groups $A_1$ and $B_1$; $A_2$ and $B_2$. Also, no significant difference was observed in neutrophils B1 and B2 while significant differences ($P < 0.05$) were observed in groups $A_1$ and $B_1$; $A_2$ and $B_2$.

No significant difference was recorded among the various groups for monocyte and eosinophil. Also, no significant difference was observed among the test groups for MCHC and only $B_1$ was significantly higher for MCH.

### Plasma biochemical parameters

The results of the plasma biochemistry of *C. gariepinus* after 48 h nitrite exposure at different water temperatures is presented in Table 2.

Significantly lower values of plasma $K^+$ concentration were obtained for groups $B_1$ and $B_2$ compare with groups $A_1$ and $A_2$ Significantly higher differences were observed in groups A, $B_1$ and $B_2$ compared with $A_2$ for $C1^-$ and $Na^+$. No statistically significant differences were observed in plasma $C1^-$ and $Na^+$ concentrations at the lower temperature

## DISCUSSION

Mortality was observed during nitrite poisoning at both temperatures; 27°C group $A_1$ - mortality 23% and at 35°C group $A_2$ - mortality 30%. However, no significant influence of temperature on mortality in *C. gariepinus* was found. Nitrite - exposed fish showed lower PCV values at both experimental temperatures compared with controls.

The decrease in PCV recorded in fish exposed to nitrite at higher temperature compared to the one at lower temperature may be caused by red cell shrinkage as reported by Jensen et al. (1987). The RBC shrinkage is connected with the efflux of $K^+$ from the red blood cells. The K+ efflux seems to result from activation of a $K^+/Cl^-$ cotransporter that is normally involved in cell volume

regulation (Jensen, 1990). The activation of $K^+/Cl^-$ efflux draws osmotically obligated water out of the cells and hence induces erythrocyte shrinkage (Jensen, 1990). RBC shrinkage is usually followed by the loss of haemoglobin solubility, resulting in haemoglobin crystals and structural damage to erythrocytes (Jensen et al., 1987). Nitrite poisoning caused a marked increase in plasma K concentrations in both test groups compared with controls. The rise in plasma $K^+$ is due to the Nitrite stimulated release of $K^+$ from skeletal muscle and red blood cells (Jensen 1990; Knudsen and Jensen, 1997).

The efflux of $K^+$ from RBC seems to result from the activation of a $K^+/Cl^-$ cotransporter that is normally involved in cell volume regulation (Jensen, 1990, 1992).

A significant decrease in plasma $Na^+$ concentration was observed in group $A_2$ (35°C) compared with group $A_1$ (27°C). This result deviated from the observation of Jensen et al. (1987) and Kroupova et al. (2006), who observed slight decrease between the two groups.

Plasma $Cl^-$ levels in this study was affected by nitrite poisoning. A significant decrease was observed at higher temperature (35°C) and this is in consonance with the findings of Jensen et al. (1987) and Knudsen and Jensen (1997). Nitrite is a competitive inhibitor of chloride uptake and vice versa. Thus, chloride influx is reduced due to the presence of nitrite in ambient water. In freshwater teleosts nitrite exposure is followed by several osmo-regulation responses as hyponatremia, hypochloremia (Jensen et al., 1987), branchial chloride cell failure (Gaino et al., 1984) and inhibition of chloride uptake (Williams and Eddy, 1986).

No significant difference was observed in the $K^+$ and $Cl^-$ between the two control groups at varying temperatures. Adeyemo et al. (2003) corroborated this when she worked on *C. gariepinus* at varying temperatures. A significant difference recorded in the PCV between the control groups (27 and 35°C) was in line with the results of Adeyemo et al. (2003). However, no significant difference was observed between the controls of $Na^+$, $K^+$ and Cl-. It can be suggested that *C.gariepinus* has a high adaptive ability. Nitrite-intoxicated fish at both temperatures recorded significantly lower values for lymphocytes. This may be attributed to its inhibition in producing antibodies. Also, MCV, MCH and MCHC were temperature independent.

## Conclusion

It has been established from this study that mortality during nitrite poisoning are not related to water temperature in *C*. *gariepinus*. This may be due to its hardy nature. However, other haematological and biochemical variables were altered during nitrite exposure at higher temperature.

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