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# Evaluation of the Haematology and Biochemistry of *Clarias gariepinus* as Biomakers of Environmental Pollution in Tiga dam, Nigeria

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## ABSTRACT

*This study aimed to evaluate the haematological and biochemical changes in* Clarias gariepinus *as biomarkers of environmental pollution in Tiga dam, Nigeria (wild aquatic environment). Water and fishes were sampled twice, a week apart, from the controlled and the wild aquatic environment. There were no significant ($p>0.05$) differences between the temperature, pH and dissolved oxygen contents of both aquatic environments. Similarly, there were no significant ($p>0.05$) changes in the haematological parameters of the reared and wild the* C. gariepinus *except in their white blood cell counts, which were significantly ($p<0.05$) higher in wild* C. gariepinus*. The activities of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (serum enzymes) were significantly ($p<0.05$) higher in the wild* C. gariepinus*. However, the concentrations of serum total triglyceride (serum metabolite) were significantly ($p<0.05$) lower in the wild* C. gariepinus*. The haematological and biochemical alterations in the wild* C. gariepinus*, which were strongly indicative of cellular damages, might have been a consequence of the toxic pollution of Tiga dam, Nigeria.*

**Key words:** Catfish, Serum enzymes, Serum metabolites, Blood values, Aquatic environment

## INTRODUCTION

*Clarias gariepinus*, which is indigenous from Africa (Rahman et al. 1992), is one of the most important tropical catfish species for aquaculture (de Graaf and Janssen 1996) in spite of its commanding presence in the wild. This might have been due to its high growth rate, omnivorous feeding habit, high feed conversion rates and hardiness, including its high resistance to handling and stress (Olaifa et al. 2003; Eyo and Ezechie 2004; Akinsanya and Otubanjo 2006; Ogundiran et al. 2009). *Clarias gariepinus* is known to tolerate harsh aquatic conditions (Hogendoorn 1992; Bruton 1979) in terms of low dissolved oxygen concentrations by utilizing both dissolved and atmospheric oxygen (Okechi 2004), especially in fishes above 12 – 14 days old with functionally developed accessory respiratory organs (Peteri et al. 1992). Though *C. gariepinus* can endure long periods of draughts (Dunn 2000), it cannot survive long in water temperature below 9 – 10$^{o}$C (Peteri et al. 1992). These endearing qualities may have increased the importance of *C. gariepinus* in ecotoxicological studies (Weckler 2000).

*Clarias gariepinus*, like any other aquatic organism, live in direct contact with the aquatic environment where some changes are rapidly reflected as measurable patho-physiological alterations in exposed fishes (Wilson and Taylor 1993; Musa and Omoriegie 1999; Seith and Saxena 2003). However, such measurable changes

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depend upon the biological status of exposed fish as well as upon the type and duration of their exposure to toxicants within that aquatic environment (Brungs 1977). That is why fishes are used as acceptable bio-indicators of environmental pollution (Nussey et al. 1995; Kock et al. 1996; Borkovic et al. 2008; Kim et al. 2008), especially as most aquatic waters are constantly being threatened by the environmental pollution (Biney, et al. 1987; Svensson et al. 1996; Adamu and Kori-Siakpere 2008). This is because normal physiological processes are long affected before the death of an organism thereby creating the need for physiological and biochemical indicators of health and sub-lethal effects of the toxicants (Van Vuren 1986; Van der Merve et al. 1993).

The increasing spates of water pollution have continued to be a major problem in Nigeria and other developing countries (Adelegan 2008). Ayodele and Abubakar (2001) and Sani (2011) have reported the pollution of Tiga dam, Nigeria. However, there is dearth of information on the effects of such pollution on the haematological and biochemical profile of Tiga dam's fish biota. Thus, this study aimed to evaluate the haematology and biochemistry of *C. gariepinus* as biomarkers of environmental pollution in Tiga dam, Nigeria.

## MATERIALS AND METHODS

### Physicochemical Parameters

Water samples were randomly taken from Tiga dam, Nigeria (wild aquatic environment) and from a concrete pond (controlled aquatic environment) within the early hours of the morning (0800 – 0900 h). These were immediately stabilized with manganous sulphate prior to analysis within the first three hours of sampling (0900 – 1200 h). Water samplings were performed twice, a week apart, in April 2010. Temperature and pH was determined using Hanna Instrument hand held meter (Hi 98129, PHK-260-010Y) after calibrations. Dissolved oxygen concentrations were determined using the modified Winkler-Azide method as described by Lind (1979) and APHA (1985).

### Fish Sampling

*Clarias gariepinus* (403.00 ± 26.44 g mean weight and 37.30 ± 0.81 cm mean total length) were purchased from a commercial fish farm (Fannasson Investments Limited, Rano, Kano State, Nigeria). Fish were acclimatized for 14 days in a concrete pond prior to sampling. Fishes were fed 6 mm Coppens® fish feed for aquaculture (Coppens International bv. 5700 AM Helmond, Holland) satiation in two divided portions per day throughout the experimental period. Pond water was changed completely once in every two days. *Clarias gariepinus* (340.40 ± 19.62 g mean weight and 38.75 ± 0.99 cm mean total length) were caught from Tiga dam, Nigeria.

Reared *C. gariepinus* were randomly netted using locally fabricated hand catching nets. Wild *C. gariepinus* were caught by local fishermen using dug-out canoes and dragging nets. Similarly, the reared and wild fishes were sampled twice, a week apart, in April 2010 as earlier performed for the water. Caught fishes were held in plastic containers with fresh concrete pond and fresh Tiga dam water prior to blood sampling within the first three hours of their capture as suggested by Kurtović et al. (2008).

### Haematological Analysis

Blood samples were collected via caudal vein puncture as described by Kori-Siakpere et al. (2005). Fish was held by the person to collect the blood in a slanting and/or vertical position with the ventral part facing the person. Blood samples were collected with sterile 5 ml syringe and 21G needle. The needle was introduced on the ventral mid line between the anal opening and the beginning of the anal fin to assess the caudal vein beneath the vertebral column. The first portion of the collected blood was dispensed into heparinised tubes for haematological analysis. Red blood cells (RBC) and White blood cells (WBC) counts were estimated using a Neübauer haematocytometer with Hendricks (1952) diluting fluid for RBC counts and Shaw (1930) solution for WBC counts as described by Hesser (1960). Haemoglobin (Hb) concentrations were estimated using the Sahli-Hellige haemoglobin method as described by Hesser (1960). Packed cell volume (PCV) was estimated using a micro-haematocrit method as described by Hesser (1960). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were estimated as follows (Stockham and Scott 2002 ):

$$\text{MCV (fL)} = \frac{\text{PCV}}{\text{RBC}} \times 10$$

$$\text{MCH (pg)} = \frac{\text{Hb}}{\text{RBC}} \times 10$$

$$\text{MCHC (g/dL)} = \frac{\text{Hb}}{\text{PCV}} \times 100$$

**Biochemical Analysis**

The second portion of the collected blood was dispensed into non-heparinised tubes. This was then centrifuged at 1,006 *xg* for five minutes to obtain the serum. Serum glucose concentrations were estimated based on glucose oxidase method as described by Morgan and Iwana (1997). Serum total triglyceride concentrations were estimated based on enzymatic method as described by Tietz (1990) using a commercially available kit (Randox Laboratory Limited, United Kingdom). Serum total protein concentrations were estimated based on Biuret method as described by Henry et al. (1974) using an auto-analyzer (Bayer Express Plus, Model 15950, Germany). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated as described in the Reference method by the International Federation of Clinical Chemistry as described by Schwartz et al. (1985) using the same auto-analyzer as above. Alkaline phosphatase concentrations were estimated based on the enzymatic hydrolysis method as described by King and Armstrong (1934).

**Statistical Analysis**

GraphPad software programme (GraphPad Prism, version 4, San Diego, CA) was used to analyse the data as means ± SEM while student's *t*-test were performed to determine differences between the means at $p<0.05$ statistical significance.

## RESULTS

The temperature, pH and dissolve oxygen of the controlled and wild aquatic environments are as shown in Table 1. Evidently, there were no significant ($p>0.05$) differences between the temperature, pH and the dissolved oxygen concentrations of both aquatic environments.

The haematological parameters of the reared and wild *C. gariepinus* are as shown in Table 2. There were no significant ($p>0.05$) differences between the Hb, PCV, RBC, MCV, MCH and MCHC of the reared and wild C. *gariepinus* except in their WBC counts, which were significantly ($p<0.05$) higher in the wild C. *gariepinus*.

**Table 1** - Physicochemical parameters of the controlled (commercial fish farm) and wild (Tiga dam, Nigeria) aquatic environments.

| Physicochemical parameters | Controlled aquatic environment | Wild aquatic environment | P-value |
|---|---|---|---|
| Temperature ($^oC$) | 25.87 ± 0.03 | 25.83 ± 0.17 | 0.85 |
| pH | 7.34 ± 0.04 | 7.13 ± 0.06 | 0.05 |
| DO (mg/L) | 6.23 ± 0.50 | 5.20 ± 0.07 | 0.11 |

Values with asterisk (*) are statistically significant ($p<0.05$) within the row

**Table 2** - Haematological parameters of the reared (commercial fish farm) and wild (Tiga dam, Nigeria) *Clarias gariepinus.*

| Haematological parameters | Reared Clarias gariepinus | Wild Clarias gariepinus | p-value |
|---|---|---|---|
| Hb (g/L) | 118.00 ± 8.24 | 116.70 ± 7.15 | 0.9036 |
| PCV (%) | 34.07 ± 2.49 | 34.53 ± 2.28 | 0.8911 |
| RBC (x $10^{12}$/L) | 221.30 ± 17.16 | 192.30 ± 13.74 | 0.1979 |
| WBC (x $10^9$/L) | 3.02 ± 0.28 | 4.18 ± 0.28 | 0.0068* |
| MCV (fL) | 1.89 ± 0.14 | 1.64 ± 0.15 | 0.2591 |
| MCH (pg) | 6.42 ± 0.49 | 5.79 ± 0.58 | 0.4220 |
| MCHC (g/dL) | 339.60 ± 4.17 | 348.10 ± 7.37 | 0.3259 |

Values with asterisk (*) are statistically significant ($p<0.05$) within the row

Hb – Haemoglobin; PCV – Packed cell volume; RBC – Red blood cells; WBC – White blood cells; MCV – Mean corpuscular volume; MCH – Mean corpuscular haemoglobin; MCHC – Mean corpuscular haemoglobin concentration

The biochemical parameters of the reared and wild *Clarias gariepinus* are as shown in Table 3. The activities of serum enzymes (AST, ALT and ALP) were significantly ($p<0.05$) higher in the wild *C. gariepinus* compared to the reared *C. gariepinus*. The concentrations of serum

triglyceride were significantly ($p<0.05$) lower in the wild *C. gariepinus* compared to the reared *C. gariepinus*. However, there were no significant ($p>0.05$) differences in the concentrations of the other serum metabolites (serum glucose and serum total protein) between the reared and wild *C. gariepinus*.

**Table 3** - Biochemical parameters of the reared (commercial fish farm) and wild (Tiga dam, Nigeria) *Clarias gariepinus.*

| Biochemical parameters | Reared *Clarias gariepinus* | Wild *Clarias gariepinus* | p-value |
|---|---|---|---|
| AST (U/L) | 155.00 ± 12.72 | 463.70 ± 32.90 | <0.0001* |
| ALT (U/L) | 49.17 ± 4.84 | 237.20 ± 27.10 | <0.0001* |
| ALP (U/L) | 24.96 ± 0.70 | 27.62 ± 0.80 | <0.01* |
| Glucose (mmol/L) | 4.36 ± 0.27 | 5.54 ± 0.56 | 0.80 |
| Total protein (g/L) | 32.09 ± 1.79 | 30.12 ± 1.69 | 0.42 |
| Total triglyceride (mmol/L) | 0.04 ± 0.00 | 0.02 ± 0.00 | 0.0001* |

Values with asterisk (*) are statistically significant ($p<0.05$) within the row
AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; ALP – Alkaline phosphatase

## DISCUSSION

The physicochemical parameters of both aquatic environments were within acceptable levels for the survival of *C. gariepinus* as earlier reported by Viveen et al. (1985) and Peteri et al. (1992), especially as the fish has wide tolerance for temperature ranges, low dissolved oxygen and high salinity (Ozmen et al. 2006). These might have accounted for the insignificant ($p>0.05$) changes in the oxygen transport vehicles (Hb, PCV, RBC, MCV, MCH and MCHC) of *C. gariepinus* from both aquatic environments. The method of sampling adopted in this study was to guard against the effect of seasonal fluctuations as suggested by Kurtović et al. (2008).

Significant ($p<0.05$) increase in WBC counts in the wild *C. gariepinus* might be a protective response to stress (Das 1998; Dhanekar et al. 1985) as well as a consequence of tissue damages (Choo and Williams 2003; Adeyemo 2007; Masheswaran et al. 2008). Unacceptable concentrations of lead and cobalt have been reported in Tilapia and catfish tissues sampled from Tiga dam, Nigeria by Sani (2011). The fact that heavy metals pollution of aquatic environments are known to cause physiological, biochemical and cellular alterations in the exposed fishes (Martinez et al. 2004; Al-Attar 2005; Adeyemo 2007; Mohamed 2008; Atamanalp et al. 2010) underscores the presence of these cellular damages. Heavy metals pollution of Tiga dam may have been due to the active mining activities on the Jos highlands, which serves as the originating source of Kano River supplying it.

Increased significant ($p<0.05$) activities of serum enzymes (AST, ALT and ALP) in the wild *C. gariepinus* also resulted from cellular damage in these fish, which might have arisen from the toxic pollution of the aquatic environment. This is because serum enzymes are cytoplasmic in nature and are only released into blood circulation after cellular damage (Sallie et al. 1991; Palanivelu et al. 2005). Significant ($p<0.05$) reduction in serum total triglyceride concentrations of the wild *C. gariepinus* compared to the reared *C. gariepinus* might have been due to liver dysfunction (Kaplan et al. 1988) resulting from liver damage caused by the destructive effects of toxicants in the dam.

In conclusion, the effects of the environmental pollution were more pronounced in serum enzymes activities than in the haematological responses of the exposed *C. gariepinus*. However, significant ($p<0.05$) increase in serum enzymes and WBC counts of the wild *C. gariepinus* were indicative of cellular damages in exposed fishes. Therefore, higher haematological and biochemical values in apparently healthy *C. gariepinus* sampled from Tiga dam, Nigeria might be indicative of toxic pollution of this wild aquatic environment.

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