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Haematology and biochemistry of *Clarias gariepinus* from rivers benue and donga as biomarkers of environmental pollution

Uruku Ndekimbe Mamndeyati * and Mohammed Abubakar Mohammed

Department of Fisheries and Aquaculture, Federal University Wukari, PMB 1020, Taraba State, Nigeria.

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Abstract

The study sought to assess the haematological and biochemical alteration in *Clarias gariepinus* as environmental contamination biomarkers in rivers Benue and Donga. water samples were taken for physiochemical examination while fish blood was obtained for haematological and biochemical analysis using a Haemacytometer. When compared to the reference standard there was a substantial (P>0.05) difference in the RBC of the *C. gariepinus* in both rivers, but no difference in the WBC, HB, PCV, MCV and MCHC. However, there were no statistically significant (P0.05) difference in the ALT, ALP, AST and glucose levels of *C. gariepinus* in either river. The ALT, ALP and glucose levels of *C. gariepinus* in both rivers differed from the reference standard. Temperature, pH, dissolved oxygen and nitrate levels were higher in Benue river than in the Donga River, but ammonia levels were higher in the Donga river. As a result of these finding, it is possible that Benue river is more polluted than the Donga river, and the *C. gariepinus* in Donga river is healthier than those in the Benue river. Hence, adequate water management and pollution control should be implemented to halt the flow of the Benue river.

Keywords: Benue; Biochemical; *Clarias gariepinus;* Donga; Haematology; Rivers

1. Introduction

Like any other aquatic organism, African Catfish *(Clarias gariepinus)* live in direct touch with the aquatic environment, where some changes are swiftly reflected as detectable patho-physiological alterations in exposed fishes [1]. However, such observable alterations are dependent on the biological status of the exposed fish, as well as the type and duration of toxicants exposure in that aquatic habitat. That is why fish are utilized as acceptable bio-indicators of environmental pollution [2], particularly since most aquatic waters are constantly being threatened by pollution [3]. This is due to the fact that normal physiological systems are long disrupted before an organism dies, necessitating the need for physiological and biochemical indicators of health as well as and sub-lethal effects of the toxicants. Water contamination has remained a major issue in Nigeria and other developing countries [4].

Fish are often employed to assess health of aquatic environment and are recognised as bio-indicators of environmental pollution [5]. Fish, in reality, have very close touch with their surrounding and are thus particularly vulnerable to physical and chemical changes that may be reflected in their blood components. It should be emphasized that different environmental conditions and substances have varying sensitivity to haematological indices [6]. The haematological profile is an excellent predictor of physiological dysfunctions [7] thus it not only offers information about the health status of fish and the physical chemical parameters of the water in which they reside, but it also evaluate the link between these factors and correlate them with the health of organisms in relation to environmental conditions [8].

* Corresponding author: Uruku Ndekimbe Mamndeyati

Changes in fish haematology in reaction to stressing agents are indicators of the fish stressful stage, generating essential information to curb any unfavourable state that may influence fish health [9].

The most common criteria used in fish toxicity studies are haematological parameters such as RBC (red blood cells), Hb (haemoglobin), Haematocrit/packed cell volume (HCT/PCV), MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration); and biochemical parameters such as AST (aspartate aminotransferase), ALT (alanine aminotransferase) and ALP (alkaline phosphatase). As a result, the goal of this study was to look at the haematology and biochemical parameters of the African catfish (*C. gariepinus*) from the rivers Benue and Donga as a biomarker of the species health.

2. Material and methods

2.1. Description of the Study Area

The Niger River principal tributary is the Benue River. The river has a length of about 1,400 Km (870 mi). it serves as a vital transit route in the areas it passes through as a result. It originates in northern Cameroon Adamawa Plateau, from which it runs west via the Nigeria town of Jimeta, Ibi and Makurdi before joining the Niger River at Lokoja. Along the way, it passes past Garoua and Lagdo reservoir in northern Nigeria, south of the Mandara mountain. The Faro River, Gongola River, and Mayo Kébbi, are significant tributaries that, during floods, link it with Logone River (a component of the Lake Chad system). Taraba River and River Katsina Ala are two other tributaries. The Benue is larger than the Niger at the confluence in terms of volume. Before 1960 the average discharge was 3,400 cubic metres per second. The Donga River, on the other hand, originates from the Mambilla Plateau in Eastern Nigeria, forms a portion of the international border between Nigeria and Cameroon, and runs northwest before merging with the Benue River in Nigeria. The Donga watershed covers an area of 20,000 Km^2 (7,700 sq mi). the river produces 1,800 cubic metres (64,000 cubic feet) of water per second at its highest point near the Benue [10].

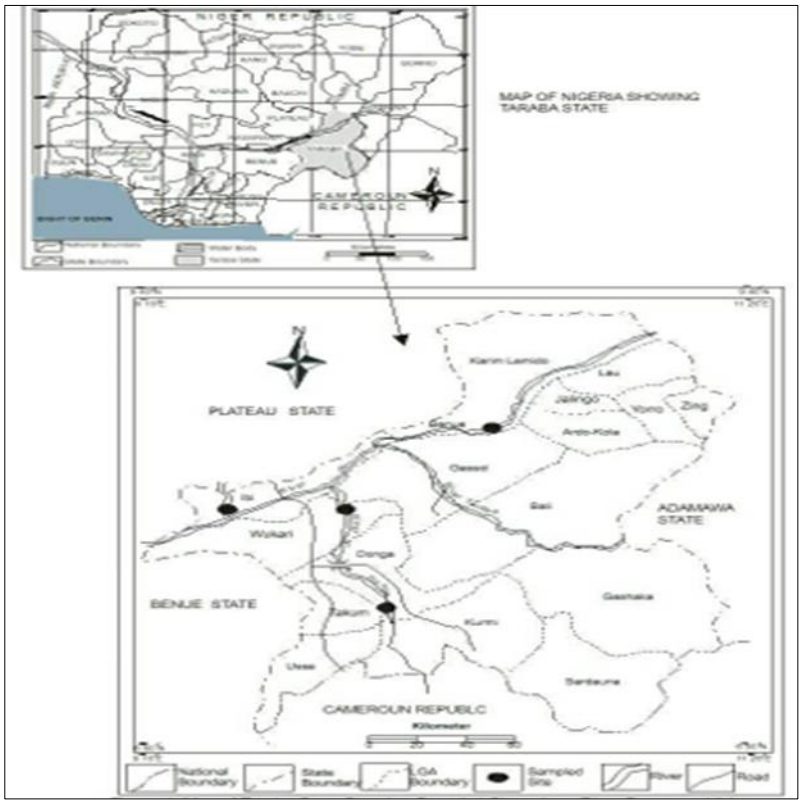

Figure 1 Rivers Benue and Donga sampling sites

2.2. Experimental Site

The experiment was conducted in the medical laboratory of the General Hospital Wukari, Taraba State, Nigeria.

2.2.1. Transportation and Sample Procurement

For these tests, 60 *Clarias gariepinus* from Benue and Donga rivers, each weighing 700 g – 1000 g, were used. These were bought from artisanal fishermen who used drag nets and dug-out canoes between January and June 2021, and they were shipped in plastic jerricans with holes cut out at the top for oxygen exchange. Within the first three hours of capture, fish were kept in plastic containers with fresh water before having their sample.

2.3. Evaluation of Fish Samples Haematological and Biochemical Parameters

2.3.1. Haematological Analysis

Blood analysis: Using disposable 2 mL heparinized syringe treated with EDTA as anti-coagulant, blood samples containing 5-10 mL were drawn from cardiac punctures.

- **Blood cell count:** A haemocytometer was used to count the blood cells. The fluid that thins blood was ready. Using a compound microscope, the blood cells were counted in the haemocytometer counting chamber.
- **RBC** = No of cells counted x 3 x 10 x 200 (10 mm)
- **WBC** = No of cells counted x 0 x 25 x 10 x 20 (10 mm)

2.3.2. Packed cell volume

After sealing the micro-haematocrit tube, the packed cell volume was calculated and expressed as percentage using micro-haematocrit reader. ESR was calculated using the methods describe in [11]. The difference between 100% and the percentage component presented by the corpuscle volume of ESR during the specified time interval.

Mean corpuscular volume (MCV): MCV was calculated from the haematocrit value (PCV, % and the Erythrocyte count (Er mm) using this formula

$$MCV\ (fL)\ = \frac{PCV}{RBC} \times 100$$

The standard method formula was used by [12] to compute mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

$$MCH\ (pg)\ = \frac{Hb}{RBC} \times 100$$

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

2.4. Biochemical Analysis

2.4.1. Determination of Aspartate Aminotransferase (AST)

In a test tube, 0.5 mL of the substrate, sodium azide, and 0.5ml of the blood or serum were added. After that, it spent 30 minutes in water bath at 37 ^{o}C, following this, the mixture was given 0.5 mL of a 2,4-dinitrophenyl hydrazine, incubated for 20 minutes, and then received 5 mL of sodium hydroxide, which caused the liquid to turned brown. Then, it was put into a spectrophotometer set at 540nm and the outcomes were read off of the calibrated graph.

2.4.2. Determination of Alanine Aminotransferase (ALT)

A test tube containing 0.5 mL of blood had 0.5 substrate added to it. After 30 minutes in a water bath at 37^{o}C and 20 minutes of incubation, the mixture received 0.5 mL of 2,4-dinitrophenylhydrazine. The mixture was then given 5 mL of sodium hydroxide, which caused it turned brown. it was then placed in a spectrophotometer to measure at 540nm, and the results were retrieved from the calibrated graph formula; Calculation = ALT(U/I) = change Abs/mm x 1750

2.4.3. Determination of Alkaline Phosphatase (ALP)

In order to acquire this result, 1 mL of p-nitrophenylphosphatase was added to 1 mL of blood in a test tube. After 30 minutes of incubation, the results were obtained using a spectrophotometer at 410nm and correlated with values on the calibrated graph.

2.4.4. Determination of glucose level

Utilizing the original Accu-Chek Active test stripes and the glucometer Accu-Check Glucotrend 2 from Roche Diagnostics, Germany, fish sample were examined. First, the stripes were placed inside the glucometer. Next each test stripe/sample was given 2 L of plasma, and the measured result were noted.

Due to the tiny sample volume, each sample was measured in monoplicate. This method has 0.6 mmol/L quantitation limit, a working range of 0.6–33.3 mmol/L, repeatability of 2.1%, and the working volume of 1–2 µl.

2.5. Examination of Study River for Water Quality Parameters

pH, temperature, dissolved oxygen, ammonia and nitrate are among the factor used to gauge the quality of the water. Dissolved oxygen and temperature were determined insitu in the field. Using EXTECTH hand held Dissolved Oxygen Meter, Model 407510A, temperature and dissolve oxygen levels were measured in the field. Before taking the reading, the meter was lowered 10cm below the water surface and left there for a few minutes to stabilize. After mixing the substances, Ammonia and Nitrate concentration were measured in the Biological science laboratory, Federal University Wukari using T60 UV- visible spectrophotometer at 470 and 630 wave lengths respectively.

2.6. Data Analysis

By measuring three independent replicates, the results were expressed as the mean values standard error of mean (SEM). The experiment data were put via a one-way Analysis of variance (ANOVA). Using SPSS software version 21.0, the Duncan Multiple Range Test was conducted to evaluate whether there was a significant difference between means at (P=0.05)

3. Results

3.1. Haematological parameters of *Clarias gariepinus*

Table 1 shows the impact of environmental pollution in the Benue and Donga Rivers on the haematological parameters of *C. gariepinus*. It shows that the RBC count in the Benue river was lower at (6.5) compared to the Donga River value (13) and the WBC count in the Benue river was higher at (8.85) compared to River Donga value of (5.00). Similarly, to this, the Hb (8.35) in the Benue river was greater than the Donga river (5,15) figure. Benue river PCV of (25) was found to be greater than Donga river (15.5). Study found that the MCV value in the Benue river was (80), which was greater than the MCV value in the Donga river, which was (49). However, it was also noted that the MCH value in the Benue river (12.3) was higher than the value in the Donga river (3.9). Benue and Donga rivers both have the same MCHC value (0.33).

Table 1 Haematological parameters of *C. gariepinus* from Rivers Benue and Donga

River/Standard	RBC	WBC	Hb	PCV	MCV	MCH	MCHC
Benue	6.5±1.5[a]	8.85±1.35[a]	8.35±3.35[a]	25±10[a]	80±10[a]	12.3±2.3[a]	0.33±0.00[a]
Donga	13±1.0[b]	5.00±0.20[a]	5.15±1.85[a]	15.5±5.5	49.15±34.15[a]	3.9±1.1[a]	0.33±0.00[a]
Reference Standard	4.35-6.5[a]	4.5-11.0[a]	11.66-25.47[a]	20-45[a]	80-90[a]	19.42-49.53[a]	33.4-35.4[b]

*Values with different superscripts letters indicate significant difference (P<0.05); **RBC** – Red blood cells; **WBC** – White blood cells; **Hb** – Haemoglobin; **PCV** – Packed cell volume; **MCV** – Mean corpuscular volume; **MCH** – Mean corpuscular haemoglobin; **MCHC** – Mean corpuscular haemoglobin concentration.

3.2. Biochemical parameters of *Clarias gariepinus* from Rivers Benue and Donga

Table 2 display how environmental contamination in Benue and Donga Rivers affected the serum biochemical indicators of *C. gariepinus*. It was found that *C. gariepinus* in the Benue river had a greater AST value (75) than in the Donga river, which only had 1.15. Benue river ALT score (3) was lower than Donga river 4.85. Additionally, it was noted that Benue river ALP value (92.2) was lower than Donga river (96.25) and that Benue river glucose level (0.9) was greater than Donga River (0.6).

Table 2 Biochemical parameters of *Clarias gariepinus* from Benue and Donga Rivers

River/Standard	AST	ALT	ALP	Glucose
Benue	72.1±67.2[a]	3±0.2[a]	92.2±38.1[a]	0.9±0.3[a]
Donga	1.15±0.15[a]	4.85±0.65[a]	96.25±18.05[a]	0.6±0.00[a]
Reference Standard	35 – 80[a]	54 – 80[a]	16 – 60[a]	40 – 90[a]

Means in each column with different superscript are significantly different (P<0.05); **AST** – Aspartate aminotransferase; **ALT** – Alanine aminotransferase; **ALP** – Alkaline phosphatase

3.3. Physical and Chemical Characteristics of Water from Benue and Donga Rivers

Table 3 displays the results of the physicochemical parameters of rivers Benue and Donga. It was noted that the Benue river water temperature, at 30.33 ^{o}C, is greater than the Donga river, at 26.16 ^{o}C. Benue river had a pH of 7.2, while Donga river had a pH 6.76. additionally, it was noted that the Benue river dissolved oxygen level, at 6.26 mg/L, was higher than the Donga river at 5.5 mg/L. Benue river ammonia concentration, 0.24 ppm of was lower than Donga river which was 0.52 ppm. However, the nitrate concentration 0.28 in Benue river was higher than that of 0.08 in the Donga river.

Table 3 Physiochemical parameters of Benue and Donga Rivers

River	Parameters				
	Temperature (°c)	pH	Dissolved oxygen (mg/L)	Ammonia (ppm)	Nitrate (ppm)
Benue	30.33 ± 0.88	7.2±0.20	6.26 ± 0.14	0.24 ± 0.02	0.28 ± 0.04
Donga	26.16 ± 1.42	6.76±0.3	5.5 ± 0.28	0.52 ± 0.01	0.08 ± 0.00

Mean values ± standard error mean

4. Discussion

This investigation provided details on the haematological and aspects of biochemical indices for *C. gariepinus* in the Benue and Donga rivers. This is crucial because, as [13] emphasizes, blood parameters play a crucial role in assessing the structural and functional status of fish exposed to pollutants [14]. Haematological assessment is a pathophysiological reflector of the entire body.

4.1. Environmental Pollution impact on *Clarias gariepinus* Haematological Parameters

When the RBC and MCHC of both rivers were compared to the reference standard value, there was a substantial (P>0.05) alteration. This can be attributed to the study rivers interfering with the generation of red blood cells and other haematological parameters (MCHC). This outcome is consistent with [15], which asserts that water body pollution has an impact on fish RBC and other haematological parameters.

Changes in environment and cellular damage in these fish, which may have resulted from toxic pollution of the aquatic environment, may both be responsible for variation in the haematological parameters in these investigations. This is thus because serum enzymes are cytoplasmic in nature and only enter the bloodstream in response to cellular injury [16, 17, 15].

4.2. Biochemical indices of Benue and Donga Rivers *Clarias gariepinus*

Plasma enzymes (AST, ALT, and LDH) are routinely use to assess the toxicity of various contaminants. In neither of the rivers did *C. gariepinus* glucose level fall within the reference standard acceptable range. When compared to the reference standard, there were no detectable changes in the levels of ALT, AST, ALP or glucose (P 0.05). The non-plasma specific enzymes ALT, AST, and ALP enzymes are present in the tissue of the liver, heart, gills, kidney, muscles, and other organs in addition to blood serum. Therefore, their values might offer detailed information on organ malfunction [18]. Fish and other animals' serum ALT, ALP and AST concentrations rising are indicators for forecasting the health and the integrity of the liver.

The ALT, ALP, AST and glucose indices of *C. gariepinus* in the Benue and Donga Rivers were not substantially different from one another in the current study. Compared to the reference standard, the levels of ALT, ALP and glucose were not within ideal limits. This could be explained by the amount of pollution in both rivers, which may have an impact on the fish liver. This is due to the fact that serum enzymes are cytoplasmic in origin and are only released into the bloodstream in response cellular injury [16].

Fish haematological and blood biochemical parameters have been documented to be affected by species, age, sex, stress, and various management approaches [19,20].

4.3. River Physicochemical Characteristics of the Benue and Donga

The physicochemical of water bodies aid in assessing the water quality, which has an impact on aquatic life survival. In the current study, all river parameters; temperature, pH, dissolved oxygen and nitrate were higher in the Benue River than in the Donga River, with the exception of ammonia, which was lower. When compared to Donga river, Benue river may have higher values of these characteristics, which could suggest a higher amount of pollution. The compound nitrification into nitrate may be the cause of the low level of ammonia that was found. This outcome is consistent with [14] findings that fish dispersion is influenced by physicochemical characteristic of water.

Because *C. gariepinus* can survived in a wide range of temperature, low dissolved oxygen levels and high salinities, the physicochemical parameters of both aquatic environments were within acceptable limits [21]. These could be the cause of the modest ($P>0.05$) variations in *C. gariepinus* Hb, PCV, RBC, MCV, MCH and MCHC oxygen transporters in both aquatic habitats.

5. Conclusion

The current research identified haematological and biochemical makers as well as differences in the physiochemical characteristic of aquatic environment that may have an impact on *C. gariepinus* fitness, well-being, and ability to perform. The *C. gariepinus* in the Donga river, however, may be healthier than those in the Benue river because there may be more pollution there than in the Donga river.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Fish used in this study were handled according to the Canadian Council on Animal Care Guide to the care and use of experimental animals (CCAC Guide)

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