THE EFFECT OF COPPER ON SOME LABORATORY INDICES OF C*LARIAS GARIEPINUS* (BURCHELL 1822).

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ABSTRACT

Haematological studies were carried out on the blood of *Clarias gariepinus* broodstock fed different diets. Five isonitrogenous diets, prepared from locally available feedstuffs were fed to 150 broodfish for 10 weeks in triplicate laboratory aquaria tanks at 10 fish per tank. Four of the diets contain different inclusion levels of copper sulphate tagged CSD1 (1.0mg CuSO4/g), CSD2 (2.5mg CuSO4/g), CSD3 (5.0mg CuSO4/g) and CSD4 (7.5 mg CuSO4/g) while the control diet, CSD0 contained no copper sulphate. The haematological parameters measured; haematocrit (HCT), red blood cells, haemoglobin and glucose concentrations were significantly higher ($p<0.05$) in the broodfish fed CSD0 and CSD1 diets than the other diets. Exposure of *Clarias gariepinus* fish to copper in water, at concentrations above 1.0mg CuSO4/g elicits adverse haematological responses and causes homeostatic imbalance. To prevent pollution, it is expedient that there be regulation of the amount of copper sulphate used in cocoa farms, as occurs in south western Nigeria.

Key words: *Clarias gariepinus*, Copper sulphate, Haematological, Fish

INTRODUCTION

In recent years, haematological variables were extensively used when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and disease conditions in fish (Hodson *et al.*, 1978; Dhillion and Gupta, 1983; Benerjec and Kamar, 1988). This, according to Gill and Plant (1981), is as a result of their relationship with energetic (metabolite levels), respiration mechanics (haematological levels) and defence mechanisms (leucocyte levels). Haematological parameters provide an integrated measure of the health status of fishes.

The introduction of toxicants into an environment, where fishes are found, stuns them and/or acts as a stressor of the fish and organisms found in such environment (Olatayo, 2008). The introduction of a toxicant to an aquatic system might decrease the dissolved oxygen concentration, which will impair respiration thus leading to asphyxiation (Warren, 1977). Stickney (1977) also reported that insufficient amount of dissolved oxygen is one of the contributory factors of mortality in some fish species. The darkening of the fish skin, respiratory distress and erratic swimming can be observed when fishes are exposed to acute concentrations of toxicants (De Silva and Ranasingle, 1989; Ayuba and Ofojekwu, 2002).

Copper sulphate ($CuSo_4$) is a popular algicide/fungicide used in cocoa farms. With the recent growing interest in fish farming, a number of earthen ponds are springing up near cocoa farms in the South Western Nigeria. In situations where there are such intercropping with fish farming, unregulated use of copper sulphate can cause leaching into the pond from surrounding farm, which posses toxicological risk to farmed fish and could even lead to massive fish mortality (El-Domiatry, 1987).

Haematological changes shown by fishes exposed to copper include; increase in ammonia (NH_3) levels, antibody and haematocrit values, haemoglobin level and glucose concentration. Sometimes, the changes are permanent while in other times they are temporary (Olurankinse, 2002). Changes in the haemoglobin concentration of the fish, due to the ingestion of copper may obstruct the uptake of oxygen, thus leading to asphyxiation and eventual death of the fish.

This study was carried out to determine the haematological responses of *Clarias gariepinus* broodstock fed with diets containing different concentrations of copper sulphate, in laboratory aquarium tanks for 10 weeks.

MATERIALS AND METHOD

The feed samples were prepared with locally available feedstuffs and different levels of copper sulphate (CuSO4) incorporations. The trial diets were tagged CSD1 (1.0mg CuSO4/g), CSD2 (2.5 mg CuSO4/g), CSD3 (5 mg CuSO4/g) and CSD4 (7.5 mg CuSO4/g) while the control diet, CSD0 has no copper sulphate inclusion (Table 1).

One hundred and fifty male and female *Clarias gariepinus* broodstock were collected from NIFAGOL farms, Yakoyo, Oyo State, and transported to the laboratory in Obafemi Awolowo University, Ile Ife, Nigeria. The fishes were acclimatized and were all fed the control diet for a period of two weeks after which they were randomly distributed in groups of ten into a plastic aquaria tank (60cmx30cmx30cm) in the laboratory. Each of the trial diets was fed to triplicate groups of the fish for a period of 10 weeks. The physico-chemical parameters of the test media, including the control experiment, were monitored before and during the test period. For the purpose of collecting the blood samples to be used for the haematological studies, an anti-coagulant, ethyline diamine tetra-acetic acid (EDTA) was prepared.

Table 1: Experimental Diet Formulations (g/100g).

Formulations	CSD0	CSD1	CSD2	CSD3	CSD4
Soybean	34.2	34.2	34.2	34.2	34.2
Fishmeal	3.6	3.6	3.6	3.6	3.6
Brewery waste	37.8	36.8	35.3	32.8	30.3
Yellow maize	22.5	22.5	22.5	22.5	22.5
Mineral./Vit.	1.0	1.0	1.0	1.0	1.0
Veg. oil	1.0	1.0	1.0	1.0	1.0
Copper sulphate	0	1.0	2.5	5.0	7.5
Total	100.0	100.0	100.0	100.0	100.0

Baseline data on the haematological status of the fish was carried out in triplicates before the experimental feeding commenced. At the end of the culture period, the haematological data were also collected in triplicates, on the fish fed the experimental diets. To collect the fish blood, an incision was made at the dorsal part of the body just behind the head region. The fish body was then mopped with tissue paper to prevent haemolysis due to dilution of oozing blood with any other body fluid. Blood ($0.5cm^3$) was then collected with a $2cm^3$ glass syringe containing EDTA to give 5mg EDTA per cm^3 of blood properly mixed in a glass tube. The haematological parameters determined include haemoglobin, haematocrit (HCT), red blood cells (RBC), and glucose level.

HCT determination

HCT was determined immediately after blood collection to prevent swelling of the blood cells, which occurs when blood is exposed to the air (Sniezsko, 1960). Extracted blood was released into a micro centrifuge tube ($1.5cm^3$), which was placed in a special micro haematocrit centrifuge operated at a speed of 5000 rev/min for 10 minutes. Centrifuging separates the plasma from the blood cells. After centrifugation, the haemotocrit percentage (HCT) was calculated by measuring the depth of the portion occupied by the red blood cells and dividing by the depth of the whole cell volume.

$$HCT = \frac{\text{depth of red blood cells portion}}{\text{depth of the whole portion}} \times 100$$

RBC determination

The RBC was determined with the aid of a haemacytometer viewed under the microscope to facilitate counting of the red blood cells (Blaxhall and Daisley, 1973). The uncoagulated blood sample was mixed with red blood cell diluting fluid, Dacie's fluid in the ratio of 1:50 (Blood: Dacie's fluid) respectively. The contents were mixed in a tube using an electric mixer after which the contents were allowed to settle for 2-3 minutes. The haemacytometer was covered with a glass tube, which was made to fit it. A centrifuge tube was then filled with the settled blood content and then expelled into the hemacytometer from

the 2 sides. The content was then viewed under the high power (40x) objective of the microscope. The counts from the sides of hemacytometer were totalled separately and averaged.

$$RBC/mm^3 = \frac{\text{averaged cells counted x depth factor (mm) x dilution factor)}}{\text{area covered (mm)}^2}$$

Haemoglobin Determination

The haemoglobin content was estimated using the cyanomethaemoglobin method (Larson and Sniezsko, 1961). Blood sample ($0.01cm^3$) was mixed together with $2.5cm^3$ of the Drabkin's Reagent in a test tube and allowed to settle for 10 minutes at room temperature to allow all the haemoglobin to react with the reagent to form cyanometheamoglobin. The absorbance of the resultant solution was measured at 540nm using Cam spec visible spectrophotometer.

Glucose Estimation

The blood sample was centrifuged at a speed of 5000 rev/minutes to obtain the serum, which was used for the estimation of the glucose content. The serum sample (20µl) was added to 2000µl glucose reagent in a test tube. The content was mixed and incubated for 10 minutes at 37°C. A quantity (20µl) of glucose standard solution was also mixed with 200µl of glucose reagent and incubated for 10 minutes at 37°C. The absorbence of the standard solution and those of the samples were measured against those of the reagent blank in a Cam spec visible spectrophotometer at 546nm (Barham and Trinder, 1972).

RESULTS AND DISCUSSION

With the increasing concentration of the copper sulphate incorporated into the feed fed to the fish, the total hardness, temperature and pH of the culture medium increased while the dissolved oxygen decreased accordingly (Table 2).

The haematological parameters of the fish fed the control diet were higher at the termination of the experiment when compared to the other treatments. The results of the haematological analysis indicated that increasing concentrations of copper sulphate in the feed of *C. gariepinus* broodstock had pronounced depressive effect on the blood (Table 3). An increase in erythrocyte count was recorded when the fish was fed with diet CSD1 (feed containing 1mg $CuSO_4$/g feed). With increasing concentrations of copper in diet of the fish, all the haematological parameters; HCT, glucose, erythrocyte count as well as haemoglobin decreased significantly ($p<0.05$).

Table 2: Mean Values of the Physico-chemical Parameters of the Water in Which *C. gariepinus* Fed the Different Copper Sulphate-incorporated Diets was Raised.

Parameters	CSD0	CSD1	CSD2	CSD3
Temperature (^0C)	26.0 ± 0.2^a	26.1 ± 0.1^a	26.5 ± 0.2^b	27.0 ± 0.2^b
Dissolved Oxygen (mg/L)	6.0 ± 0.1^a	5.8 ± 0.1^a	3.2 ± 0.2^b	2.1 ± 0.3^b
Total hardness (mg/L)	55.4 ± 0.3^a	58.5 ± 0.2^b	60.3 ± 0.1^b	60.5 ± 0.3^b
pH	6.6 ± 0.1^a	6.7 ± 0.3^b	6.8 ± 0.1^b	6.8 ± 0.3^b

❖Values with the same superscript in each row are not significantly different from each other ($p>0.05$)

Table 3: Haematological Analysis of the Blood of Fish Fed the Different Copper Sulphate- Incorporated Diets

Parameters	CSD0	CSD1	CSD2	CSD3	CSD4
Haematocrit/HCT	34.1 ± 3.0^a	28.3 ± 4.0^b	29.1 ± 3.2^b	20.5 ± 1.4^c	17.7 ± 3.9^c
RBC ($10^6/mm^3$)	2.2 ± 0.5^a	2.5 ± 0.2^a	1.9 ± 0.6^b	2.1 ± 0.3^b	1.5 ± 0.1^c
Haemoglobin (mmol/L)	8.0 ± 0.6^a	6.8 ± 1.1^b	6.8 ± 1.3^b	6.3 ± 0.2^c	5.9 ± 0.3^c
Glucose	7.2 ± 1.0^a	6.8 ± 1.4^b	6.5 ± 2.4^b	5.8 ± 0.9^c	5.3 ± 0.7^c

❖Values with the same superscript in each row are not significantly different from each other ($p>0.05$)

The presence of the copper sulphate as a toxicant in this experiment must have caused the increase in total hardness of the water, thus causing the water to retain heat from the atmosphere, leading to increase in the water temperature (Olatayo, 2008). Water temperature and dissolved oxygen are inversely related. Robert (1978) reported that dissolved oxygen generally decreases in solubility with increase in temperature, which also affects other parameters of the aquatic environment that are important to fish health.

As observed in this experiment, high concentration of heavy metals or long-term exposure of fish to sub-lethal concentrations of heavy metals has been shown to decrease various haematological indices and this could result in an anaemic condition of the fish as a result of plasma volume expansion (Kaori *et al*., 2001). Christenson *et al*. (1972) observed that lower concentrations of copper invariably increase the concentration of haemoglobin in the blood. Increasing levels of copper in the diet is a stressor which will likely cause an osmotic imbalance and change in the fish regulatory system of ionic interchange and which ultimately diminishes the blood pH and decrease the volume of erythrocytes and subsequently lower the percent of haematocrit (Vosyliene, 1999).

The decrease in the haemoglobin of the blood of the fish exposed to increasing concentrations copper in diet, would most probably affect the oxygen uptake and transport. Reduction in the red blood cell count with increasing levels of copper in diet of the fish will cause haemolytic or erythropoietic anaemia in the fish leading to rheological attractions (Kaori *et al*. 2001). Glucose has been shown to be one of the most sensitive indices of the stress state of fish (Vosyliene, 1996; Singh *et al*. 2008). A high concentration of glucose in the blood of the fish fed the control diet, CSD0, is an indication that the fish is not stressed while the decreasing level in the blood of fish fed increasing concentration of $CuSO_4$ is a physiological stress indicator.

A decreasing concentration of blood glucose indicates an exhaustion of energy (glycogen) resources and subsequently, the worsening of the status of the fish condition. This result corroborates Vosyliene (1996) who also observed that during long-term tests, high concentration of copper caused a decrease in the concentration of glucose. El-Domiatry (1987) also reported that exposure of *Clarias lazera* to copper results in haemolysis and anaemia. Van Vuren (1994) also reported that the exposure of *C. gariepinus* to different background copper concentrations induced significant decrease in the erythrocyte count as well as haemoglobin concentrations.

CONCLUSION

From this experiment, it can be concluded that *C. gariepinus* haematological parameters are affected both by exposure to sublethal concentration of copper sulphate and by the duration of exposure. Exposure of the fish to copper in water, at concentrations above 1.0mg/g will elicit adverse haematological responses and can cause reversible and irreversible homeostatic imbalance. To prevent pollution, it is therefore expedient that there be regulation of the amount of copper sulphate used in cocoa farms, as in south western Nigeria, where some fish farms are located.

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