

Metabolites Alterations in the Muscles and Gills of *Clarias gariepinus* Juveniles on Exposure to Chronic Levels of Detergent (Linear Alkyl Benzene Sulphonate)

¹A.D.I. George and ²B. Uedeme-Naa

¹Department of Fisheries and Aquatic Environment, Faculty of Agriculture,
Rivers State University, Port Harcourt, Nigeria

²Department of Fisheries, University of Port Harcourt, Rivers State, Nigeria

Abstract: Chronic bioassay of a commonly used house hold detergent (linear alkyl benzene sulphonate) was carried out on the juvenile of *Clarias gariepinus* to determine its effect on the metabolites (albumin, total protein, creatinine, total bilirubin and urea) in the muscle and gills. The fish was exposed to varied levels of detergent (10.00, 20.00, 30.00, 40.00 and 50.00 mg/L) for 30 days. In the muscle, albumin, total protein and urea of *C. gariepinus* juveniles decreased with increase in detergent concentration. At 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L: albumin was respectively 51.99, 55.94, 73.27, 73.27 and 68.78% lower than control; total protein was 34.52, 3.18, 55.22, 55.22 and 35.06% lower than control and urea was 5.85% lower than control at 10.00 and 30.00 mg/L. At 40.00 and 50.00 mg/L, urea was respectively 15.38% lower than control but higher at 20.00 mg/L by 20.77%. Creatinine was 14.39, 29.79 and 55.81% less than control at 10.00, 20.00 and 50.00 mg/L. In the gills, albumin was respectively raised beyond control at 10.00, 40.00 and 50.00 mg/L by 96.45, 24.94 and 25.65% and below control at 20.00 mg/L by 22.59%. Total protein was respectively 65.18, 72.45, 80.16, 79.41 and 72.87% less than control at 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L.

Key words: Linear alkyl benzene sulphonate, creatinine, albumin, urea, total bilirubin, juvenile

INTRODUCTION

Lethal and sublethal concentrations of poisons in aquatic environment are known to have toxic effects on fish behaviour, haematology, histopathology, growth, reproduction, feeding, respiration and general physiological processes of exposed organisms (Butler, 1971; Misra *et al.*, 1985). Barbieri *et al.* (2005) reported that alkylphenolics (surfactants) are among the primary ingredients of detergents having grave environmental concerns. Alkylphenolics reduce surface tension in water and make aqueous solutions to spread and penetrate more easily. Grippo and Heath (2003) reported that detergent affects the properties of fish gills and changes the way fish takes in other substances by altering the surface tension of water body.

Low-level mixture of various toxic chemical also has unpredictable effects on fish. Sometimes they are additive, antagonistic or synergistic. Detergents are among those chemicals that can act synergistically with other chemicals in the aquatic environment, making aquatic organisms more vulnerable to other more toxic compounds such as petroleum products, pesticides, chloramines and heavy metals (Jobling and Sumpter, 1993). It should be noted that fish live in equilibrium with

their external environment and must continually adjust to natural changes in the environment through a variety of adaptive physiological and biochemical mechanism to maintain their internal equilibrium (Henderson *et al.*, 1959). The capacity to adapt to human disruptive changes in the environment is limited and the toxic effects of pollutants may affect all levels of animal organization from the cell to entire organism including biochemical processes, cell integrity, organ physiology, growth and reproduction (Stephanuo and Giger, 1982).

MATERIALS AND METHODS

Clarias gariepinus of mean weight, 246.30± 14.12 g SD; mean length 16.15±1.40 cm SD) for juveniles were obtained from a fish farm and held in holding tanks for 30 days prior to experiment. Water with detergent in the holding tanks were renewed daily. Fish feed were fed to fish daily at 2% body weight for juveniles and 1% body weight for adult. Chronic impact of detergent was achieved by renewable startic bioassay. All experiments were conducted in 60 rectangular plastic aquaria (25 × 60 × 25) containing 20 L of fresh water to which varied quantities of detergent was added to achieve different concentrations of the toxicant. The 30 min after the

Corresponding Author: B. Uedeme-Naa, Department of Fisheries, University of Port Harcourt, Rivers State, Nigeria

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preparation of the test solution, 5 juveniles and 1 adult fish were carefully placed into each replicate tanks of 5 different concentrations (0.00, 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L). Physico-chemical parameters such as ammonia, alkalinity, temperature, pH, conductivity, turbidity and dissolved oxygen were adequately monitored. Ammonia-Nitrogen (NH₃-N) with the phenate method of ammonia determination (American Public Health Association, 1998), temperature measurements was determined with a mercury-in glass thermometer, pH with 291 Mk 2 pH meter, conductivity with Horiba water checker, turbidity with a probe was inserted in water and the turbidity values obtained were read using the Horiba water checker measured by standard methods according to APHA, 1998. Dissolved Oxygen (DO) with Winkler's method (American Public Health Association, 1998)

Fish were killed with a blow on the head after blood collection and dissected in order to collect samples (0.5 g) of muscle and gills tissues with the aid of penknife. Sample was macerated with pestle and mortar. To prepare samples for metabolite, 5 mL of distilled water was used. After the addition of diluent, the samples were centrifuged at the rate of 300 rounds per minutes for 10 min. The supernatants were then removed and stored in plain bottles at -4°C for analysis.

RESULTS AND DISCUSSION

The water quality parameters such as temperature, dissolved oxygen and pH monitored during the exposure period did not differ (p<0.05) within the various concentrations of detergent as well as with the control. Ammonia, Dissolved Oxygen (DO) tended to decrease while alkalinity, turbidity and conductivity increased with

increasing concentration of the test chemical, however values between treatments were not significantly different (p<0.05). pH and temperature values were almost uniform all through the study irrespective of treatment. The limited variation in the physicochemical variables is similar to the trend observed in the wild, which is tolerated by the test organism even in the wild (**Table 1**).

Albumin, total protein and urea in the muscles of *C. gariepinus* juveniles decreased with increase in detergent concentration. At 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L: albumin was respectively 51.99, 55.94, 73.27, 73.27 and 68.78% lower than control; total protein was 34.52, 3.18, 55.22, 55.22 and 35.06% lower than control and urea was 5.85% lower than control at 10.00 and 30.00 mg/L. At 40.00 and 50.00 mg/L, urea was respectively 15.38% lower than control but higher at 20.00 mg/L by 20.77%. Creatinine was 14.39, 29.79 and 55.81% less than control at 10.00, 20.00 and 50.00 mg/L while at 30.00 and 40.00 mg/L it was respectively 39.39 and 49.74% higher than control. Total bilirubin was significantly raised with increase in detergent concentration in that at 10.00, 20.00 and 30.00 mg/L, it was respectively 26.33% higher than control and higher by 52.78% at 50.00 mg/L (**Table 2**).

In the gills, albumin was respectively raised beyond control at 10.00, 40.00 and 50.00 mg/L by 96.45, 24.94 and 25.65% and below control at 20.00 mg/L by 22.59%. Total protein was respectively 65.18, 72.45, 80.16, 79.41 and 72.87% less than control at 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L. Creatinine at 10.00, 30.00, 40.00 and 50.00 mg/L was respectively 27.18, 20.41, 20.21 and 28.21% less than control and higher at 20.00 mg/L by 41.03%. Total bilirubin was 34.47, 17.31, 15.13% less than control at 10.00, 20.00 and 30.00 mg/L and

Table 1: Water quality variables (Mean±SD) in the experimental tanks for juveniles during the exposure period

Variables	Concentrations (mg/L)					
	0.00	10.00	20.00	30.00	40.00	50.00
Ammonia (mg/L)	1.76±0.28 ^a	1.59±0.37 ^a	1.44±0.43 ^a	1.54±0.62 ^a	1.49±0.20 ^a	1.44±0.40 ^a
Alkalinity (mg/L)	77.00±12.76 ^a	87.00±21.64 ^a	96.00±21.14 ^{ab}	115.00±21.66 ^b	133.00±21.12 ^b	141.12±21.11 ^b
Temperature (°C)	30.42±2.33 ^a	30.27±0.48 ^a	30.45±0.63 ^a	30.55±0.59 ^a	30.70±0.73 ^a	30.71±0.84 ^a
pH	6.39±0.57 ^a	6.45±0.65 ^a	6.68±0.30 ^a	6.60±0.14 ^a	6.60±0.16 ^a	6.40±0.21 ^a
Conductivity (S/m)	211.50±16.71 ^a	270.25±18.61 ^{ab}	303.75±28.11 ^b	343.25±19.621 ^b	345.25±14.11 ^b	360.11±28.11 ^c
Turbidity (mg/L)	21.50±6.23 ^a	25.00±7.16 ^b	27.00±6.21 ^b	26.11±7.11 ^b	45.00±7.11 ^c	73.00±8.11 ^d
DO (mg/L)	5.59±0.98 ^c	3.81±0.68 ^{ab}	3.20±0.72 ^{ab}	2.95±0.64 ^b	2.55±0.33 ^b	2.33±0.13 ^b

Means within the same row with different superscripts (a, b, ab, c, d) differ significantly (p<0.05)

Table 2: Selected metabolites in the muscles of *C. gariepinus* juveniles exposed to detergent for 30 days (Mean±SD)

Conc. (mg/L)	Albumin		Total protein		Creatinin		Total bilirubin		Urea	
	(mg/L)	% control	(mg/L)	% control	(mg/L)	% control	(mg/L)	% control	(mg/L)	% control
0.00	23.83±10.10 ^b	100	22.33±14.29 ^{ab}	100	7.50±1.73 ^a	100	9.00±0.00 ^a	100	6.50±0.00 ^a	100
10.00	11.44±5.15 ^b	-51.99	14.62±9.25 ^b	-34.53	5.50±0.00 ^a	-14.39	11.37±4.75 ^b	+26.33	6.12±1.25 ^a	-5.85
20.00	10.50±2.31 ^{ab}	-55.94	21.62±17.76 ^{ab}	-3.18	5.50±0.00 ^a	-29.79	11.37±4.75 ^b	+26.33	7.85±3.01 ^a	+20.77
30.00	6.37±2.45 ^a	-73.27	10.00±0.00 ^a	-55.22	9.00±3.32 ^a	+39.39	11.37±4.75 ^b	+26.33	6.12±1.25 ^a	-5.85
40.00	6.37±2.45 ^a	-73.27	10.00±0.00 ^a	-55.22	7.95±1.65 ^a	+49.74	9.00±0.00 ^a	0.00	5.50±0.00 ^a	-15.38
50.00	7.44±2.12 ^a	-68.78	14.50±5.20 ^b	-35.06	7.00±1.73 ^a	-55.81	13.75±5.48 ^b	+52.78	5.50±0.50 ^a	-15.38

Means within the same column with different superscripts (a,b,ab) differ significantly (p<0.05)

Table 3: Selected metabolites in the gills of *C. gariepinus* Juveniles exposed to detergent for 30 days (Mean±SD)

Conc. (mg/L)	Albumin (mg/L)	% control	Total protein (mg/L)	% control	Creatinin (mg/L)	% control	Total bilirubin (mg/L)	% control	Urea (mg/L)	% control
0.00	4.25±1.00 ^a	100	61.75±21.11 ^c	100	9.75±3.61 ^a	100	13.75±5.41 ^a	100	5.75±0.37 ^a	100
10.00	8.37±5.77 ^b	+96.45	21.50±10.71 ^b	-65.18	7.10±1.73 ^a	-27.18	9.01±1.01 ^a	-34.47	5.62±0.63 ^a	-2.26
20.00	3.29±1.91 ^a	-22.59	17.01±6.81 ^{ab}	-72.45	13.75±5.48 ^{ab}	+41.03	11.37±4.66 ^a	-17.31	5.12±0.25 ^a	-10.96
30.00	4.25±1.01 ^a	0.00	12.25±4.50 ^a	-80.16	7.76±1.70 ^a	-20.41	11.67±3.12 ^a	-15.13	5.18±0.38 ^a	-9.91
40.00	5.31±1.12 ^a	+24.94	12.65±3.50 ^a	-79.41	7.78±1.50 ^a	-20.21	16.12±3.75 ^{ab}	+17.24	5.81±0.71 ^a	+1.04
50.00	5.34±1.21 ^a	+25.65	16.75±4.51 ^{ab}	-72.87	7.00±1.32 ^a	-28.21	21.51. ±4.12 ^b	+56.44	5.25±2.11 ^a	-8.70

Means within the same column with different superscripts (a, b, ab, c) differ significantly (p<0.05)

respectively 17.24 and 56.44% higher at 40.00 and 50.00 mg/L. Detergent decreased the urea in fish gill by 2.26, 10.96, 9.91 and 8.70% at 10.00, 20.00, 30.00 and 50.00 mg/L and raised it at 40.00 mg/L by 1.04% when compared with controllable (**Table 3**).

Results obtained from this investigation indicate that chronic concentration of detergent led to alterations of almost all the metabolites in the African catfish *Clarias gariepinus* juveniles at varied concentrations. Plasma proteins, which include globulins, fibrinogens and albumins, serve a vital function in carrying materials from one part of the fish to another via the circulatory system. They have nutritive, transporting, protective, buffering and energetic functions (Cheesborough, 1992). In this study, five metabolites were measured in the muscle and gills. Segen (2002) reported that bilirubin, is an orange-yellow pigment, a waste product of the normal breakdown of red blood cells. Protein and carbohydrate play a major role as energy precursors for fish under stress conditions. Changes in each of these blood components have been employed as useful general indicators of stress in teleosts (Thophon *et al.*, 2003). This is in agreement with this work as the alteration of total protein was seen to be a common denominator in fish muscle and gill. In this research, total protein was 34.52, 3.18, 55.22, 55.22 and 35.06% lower than control at 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L in fish muscle and respectively 65.18, 72.45, 80.16, 79.41 and 72.87% less than control at 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L in the gill due to the impact of detergent (stressor).

Albumin decreased with increased concentration of detergent except in few cases and this agrees with the findings of Ogundiran *et al.* (2010) who observed that necrosis which occurred with increase in concentration of LAS resulted in the reduction of albumin in the liver. In this work, at 10.00, 20.00, 30.00, 40.00 and 50.00 mg/L, albumin was respectively 51.99, 55.94, 73.27, 73.27 and 68.78% lower than control. This could be due to the inability of fish to regenerate new liver cells. It was also observed that the physiological changes in the muscle and gill might have caused metabolic problems.

The creatinine test has been used to diagnose impaired kidney function and to detect renal damage (Hontella *et al.*, 1996). In this research, creatinine at

10.00, 30.00, 40.00 and 50.00 mg/L was respectively 27.18, 20.41, 20.21 and 28.21% less than control and higher at 20.00 mg/L by 41.03%. This is in accordance with the result obtained by Velma *et al.* (2009) in rainbow trout exposed to industrial effluents. They reported that creatinine reduction is typical of organ malfunctions in fish consequent of toxicants.

The increase or decrease in total bilirubin activities as concentration increased in this study may probably cause some stress or damage to the organs concerned and this is in agreement with Huany *et al.* who observed that a rise in serum levels of total bilirubin suggested liver cell damage.

Detergent decreased the urea in fish gill by 2.26, 10.96, 9.91 and 8.70% at 10.00, 20.00, 30.00 and 50.00 mg/L and raised it at 40.00 mg/L by 1.04% when compared with control. This result corroborates with the findings of Parvathi *et al.* (2011) in the organs of *Cirrhinus mrigala* exposed to cypermethrin. The reduction in urea contents in the two organs might be related to the pronounced reduction of transaminases in response to continuous exposure to the toxicant. The high presence of urea in the fish might be due to the vesicular mobilization and translocation in the tissues. Urea is normally refined for the purpose of osmoregulation instead of being excreted under stress condition (Okafor *et al.*, 2008).

CONCLUSION

It is true that the use of detergent for domestic purposes cannot be discontinued but proper disposal of the effluent must be done in such a way that aquatic organisms such *Clarias gariepinus* would not be made to suffer terrible internal damages that could lead to poor or no reproduction at all. The extinction of this good aquaculture candidate would do no man any good.

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