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# Changes in carbohydrate food reserves of African freshwater catfish *Clarias gariepinus* (Burchell, 1822) Exposed to water soluble fraction of bonny light crude oil

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**Abstract**
Three hundred and sixty (360) juveniles of African Freshwater catfish *Clarias gariepinus* were collected from Luis Fish Farm Warri, Delta State and then were brought to the Departmental Laboratory of Fisheries and Aquaculture, Ebonyi State University Abakaliki (latitude $6^0$, 20' 49''N, longitude $8^0$ 06' 11''E), Nigeria. Experimental fish were exposed to 6 treatment concentrations (20, 10, 5, 2.5, 1.25 and 0.00) ml/L of water soluble fraaction of Bonny light crude oil in triplicate replications of 60 fish per treatment for 10 weeks. Lower levels of toxicant led to reduced carbohydrate food reserves in which liver and muscle glycogen were decreased significantly *(P<0.05)* while plasma glucose reduced significantly at 1.25 ml/L of water soluble fraction of Bonny light crude oil and resulted to hypoglycaemia but the same increased significantly *(P<0.05)* from 5 ml/L and resulted to hyperglycaemic condition in exposed fish. WSF of Bonny light crude oil led to dose dependent liver and muscle glycogens and may cause hypoglycaemic or hyperglycaemic conditions at different exposure levels and should be prevented from entry into the aquatic environment.

**Keywords:** Carbohydrate food reserves; crude oil; *Clarias gariepinus*

## 1. Introduction
Changes in carbohydrate food reserve serve as an indicator of stress and important biomarker of toxic effect of environmental contaminants to fish. Hyperglycemia which is a condition of increased plasma glucose level in petroleum exposed fish have been reported [13, 7] following marked reduction in liver and muscle glycogen. They noted that fish under the influence of petroleum effects increase plasma glucose level to meet up with heightened metabolic rate and excretion of the water soluble fraction of the toxicant. Pathological damages on the liver and muscles due to flooding of crude oil may lead to functional disorganization and depletion of their glycogen content [6]. Stressful conditions and environmental pollutants may elicit the secretion of stress hormones, catecholamines and cortisol resulting in hyperglycemic conditions [17]. Omoregie *et al.* (1995) [13] have reported that fish under the effect of crude oil make greater energy available for homeostatic maintenance than storage and as a consequence suffer reduction in growth because energy meant for growth was diverted for homeostasis. Reported cases of growth reduction in fish exposed to crude oil have been documented on Nile tilapia *Oreochromis niloticus* [15], *Menidia beryllina* [2] as well as *Clarias gariepinus* [18]. Bonny Light Crude Oil is a light grade of Nigerian crude oil with high API gravity and low specific gravity, produced in the Niger Delta basin and named after the prolific region around the City of Bonny [16]. With a low sulphur (sweet) content of 0.14% weight, it is a highly desirable grade due to its low corrosion to refinery infrastructures and of lower environmental impact in refinery effluents, a possible reason why American and European refineries are in high demand for it. The high preference for Bonny Light crude oil to other Nigerian crude oils puts pressure on oil companies to produce a greater quantity of the same, coupled with increased vandalization and stealing, engineering failure, leakages, accidents and siphoning, etc, create increased opportunities for spill incidence and possible entry into the aquatic environment. The need therefore to assess its effect on carbohydrate reserves of *Clarias gariepinus* is desirable to assist in informing a safe concentration and risk assessment baseline data of the crude oil to *Clarias gariepinus* juveniles and other fish [11]. The African catfish *Clarias gariepinus* is a teleost of the family Clariidae, easily recognized by its naked skin, four

pairs of unbranched barbels, arborescent organs on the second and fourth gill arches and a spineless dorsal fin. It breeds during the wet season between June and September by laying adhesive eggs which hatch after 20 hours under normal ambient conditions. Their rapid growth, hardy and disease resisting ability coupled with omnivorous feeding habit make them suitable for pond culture [8].

## 2. Materials and Methods

### 2.1 Collection and Acclimation of Experimental Fish

The experiment followed approved method of Enugu State University of Science and Technology Animal Ethics Committee for collection and exposure of animals. Juveniles of *Clarias gariepinus* from same brood stock and age were collected from Luis Farm, Warri, Nigeria and transported in plastic containers to the Departmental (Wet) Laboratory of the Department of Fisheries and Aquaculture, Ebonyi State University, Abakaliki (latitude $6^0$, 20' 49''N, longitude $8^0$ 06' 11''E), Ebonyi State, Nigeria. Thereafter the fish were acclimated to laboratory conditions in 300 liter capacity plastic vats for 14 days and fed at 3% body weight, twice daily using 38% crude protein experimental diet.

### 2.2 Preparation of Water Soluble Fraction (WSF) of Crude Oil

The method of preparation of water soluble fraction of crude oil proposed by United Nations Environmental Programme UNEP (1989) [19] was employed in this research. Well water and Bonny light crude oil, obtained from the Nigerian National Petroleum Company (NNPC) Port Harcourt, Nigeria were measured out in ratio of 10:1 into an aspirator and mixed thoroughly with rotator magnetic stirring rod for 20 hours. The mixture was allowed to rest for 12 hours to demarcate layers. Thereafter separating funnel was used to separate out the water soluble fraction, which was corked as stock solution in 50 liter capacity gallons from where 6 treatments 1.25. 2.5, 5, 10.20 and 0 ml/L were prepared in triplicates and exposed 60 fish for ten weeks.

### 2.3 Determination of Reserve Carbohydrate

The determination of plasma glucose and muscle and liver glycogen content were carried out by comparing the absorbance of anthrone portions with those of standard glucose using a colorimeter (Model 605/REV D/01-96) at 620 nm following the method suggested by Wedemeyer and Yasutake (1977) [21].

### 2.4 Plasma glucose

Blood was collected into heparinized micropipette following the routine method of Blaxhall and Daisley (1973) [3] after it was centrifuged at 12000 revolutions per minute for 3 minutes. Thereafter 0.05 ml of supernatant plasma was added to 3.5 ml of anthrone (orthotoluidine colour reagent) in a test tube. Glucose standard was prepared by dissolving 100 mg of reagent grade glucose in small amount of distilled water, diluted up to 100 ml. Equal amount of the standard glucose and distilled water (blank) were added to 3.5 ml of anthrone as described above. The 3 test tubes were heated for 10 minutes in a water bath and their absorbance were then read at 620 nm after cooling in a colorimeter. Plasma glucose was derived by comparism with the standard glucose.

$$\text{Plasma glucose (mg/ml)} = \frac{A_u \times C_s}{A_s}$$

Where,

$A_u$ = Absorbance of unknown

$C_s$ = Concentration of standard

$A_s$ = Absorbance of standard

### 2.5 Estimation of Liver and Muscle glycogens

Portion (known amount of about (100 mg) from each organ was first boiled for 20 minutes with 3% sodium hydroxide (NaOH) to enhance organ dissolution before Sodium sulphate (5 ml) and 95% ethanol (3.5 ml) were added to dissolve the organs. Dissolved organs were further heated to boiling in a water bath after which they were centrifuged at 12000 rpm for 3 minutes. The residues were re-dissolved in distilled water and precipitated with 95% alcohol. Precipitate obtained was re-centrifuged, hydrolyzed and neutralized with 5 M HCl and 0.5 M NaOH respectively. Neutralized samples were made up to 100 ml with distilled water and 5ml of amount were taken into test tubes and added with 10 ml of anthrone and boiled for 10 minutes. Equal amounts of glucose standard and distilled water were treated the same and all three test tubes were allowed to cool and placed in a colorimeter where their absorbance were read at 620 nm wavelength.

$$\textit{Liver/ Muscle glycogen (mg/g)} = \frac{A_u \times C_s}{A_s}$$

Where,

$A_u$ = Absorbance of unknown

$C_s$ = Concentration of standard

$A_s$ = Absorbance of standard

### 2.6 Statistical Method

Data obtained were expressed as standard mean ± standard error of mean and were analyzed with using Statistical Package (SPSS Inc. Chicago Illinois, USA). Differences in the test concentrations and control were subjected to one way analysis of variance (ANOVA) followed to by Turkey's Multiple Range Test was used to separate differences among means. Differences were considered significant at *($P<0.05$)*.

## 3. Results

**Table 1:** Mean Carbohydrate Food Reserves ±SE of *C. gariepinus* Juveniles Exposed to Subacute Concentrations of WSF of Crude oil for 10 weeks

| | Concentrations(ml/L) | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **20** | **10** | **5** | **2.5** | **1.25** | **0** |
| Plasma (mg/l) | $1.15\pm0.05^{a}$ | $1.07\pm0.04^{c}$ | $1.04\pm0.04^{e}$ | $0.89\pm0.01^{d}$ | $0.96\pm0.02^{b}$ | $1.05\pm0.05^{a}$ |
| Liver (mg/g) | $0.55\pm0.05^{d}$ | $1.05\pm0.24^{d}$ | $0.84\pm0.05^{c}$ | $0.76\pm0.05^{c}$ | $0.78\pm0.05^{b}$ | $1.18\pm0.02^{a}$ |
| Muscle (mg/g) | $0.04\pm5.5x10^{-3e}$ | $0.05\pm3.3x10^{-3a}$ | $0.05\pm9.7x10^{-3d}$ | $0.05x10^{-3}$ | $0.1\pm9.7x10^{-3c}$ | $0.11\pm0.02^{b}$ |

Means on a row with the same superscript did not differ significantly but Means with different superscript differed significantly. Mean separation by Duncan's Multiple Range Test at 5% level of significance

### 3.1 Plasma Glucose

Analysis of variance revealed significant differences in plasma glucose content of juveniles of *Clarias gariepinus* exposed to subacute concentrations of WSF of crude oil for 10 weeks among the various treatment means. Significant differences took place in fish exposed to treatments 1, 2, 3, and 4 but significant difference was not observed in group of fish exposed to treatment 5. Mean values of $T_3$ and $T_4$ were significantly higher than mean control ($P<0.05$) value of 1.05±0.003 mg/l. Plasma glucose increased significantly in $T_3$ to 1.06±0.06 mg/l and to 1.07±0.08 mg/l in $T_4$ but values of $T_1$ and $T_2$ reduced significantly ($P<0.05$) below values of the control of fish. Treatment 1 exposed fish reduced to mean value of 0.96±0.02 mg/l while those in group 2 decreased to a mean value of 0.89±0.009 mg/l. There were significant changes in plasma glucose content of fish exposed to treatments 1, 2, 3 and 4 during the 10 weeks exposure period in which values obtained in weeks 2, 6 and 10 differed with that obtained at start, week 4 and week 8. Significant dose dependent rise in plasma glucose above control in treatment 3 and 4 (Figure 1) exposed fish may have indicated hyperglycaemic conditions while hypoglycaemic condition may have occurred in group 1 and 2 exposed groups (Table 1).

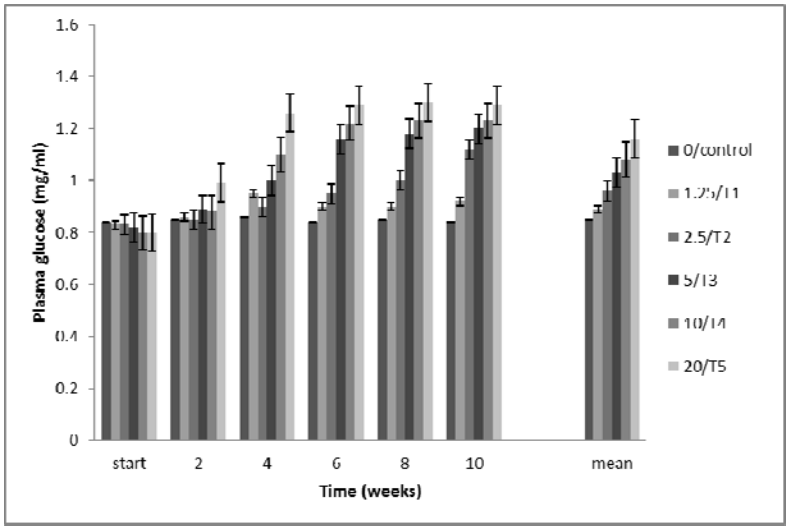


**Figure 1:** Mean Plasma Glucose ±SE (mg/l) of *Clarias gariepinus* Juveniles Exposed to WSF of Crude oil for 10 weeks

### 3.2 Liver Glycogen

One way Analysis of Variance revealed significant difference ($P<0.05$) in liver glycogen in group exposed to treatment 1 and 2 only; but there was no significant difference ($P>0.05$) in fish exposed to treatments 3, 4 and 5. Mean liver glycogen values among fish exposed to treatments 3, 4 and 5 did not also vary along period of exposure, but treatment 2 and 1 exposed fish showed observable differences along the period. Mean glycogen of liver glycogen was statically equal in weeks 2, 6 and 10, but different at start, week 4 and week 8 (Figure 2)

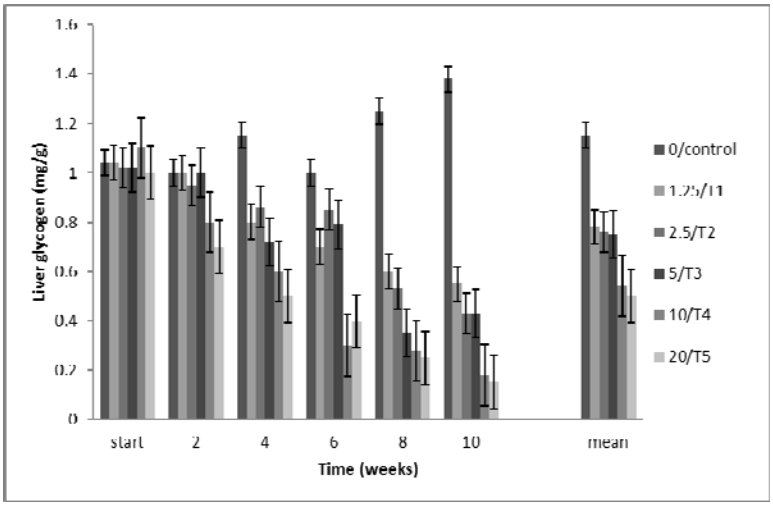


**Fig 2:** Mean Liver Glycogen ±SE (mg/g) of *Clarias gariepinus* Juveniles Exposed to Subacute concentrations of WSF of Crude oil for 10 weeks

### 3.3 Muscle Glycogen

Mean Muscle Glycogen Content of juveniles of *Clarias gariepinus* exposed to subacute concentrations ($T_1$/1.25, $T_2$/2.5, $T_3$/5, $T_4$/10, $T_5$/20 and control/0.00) ml/l of WSF of crude oil revealed significant differences among treatment means. Muscle glycogen was significantly lower ($P<0.05$) than mean control value of 0.11 mg/g in $T_1$, $T_3$ and $T_5$. Muscle glycogen of group of fish exposed to $T_2$ and $T_4$ did not

indicate any evidence of difference. Along period of exposure, evidence of significant difference occurred only in group of fish exposed to treatments: 1, 3 and 5 but no difference took place among those exposed to treatments 2 and 4. Similarly, mean values of fish in treatment 1, 3 and 5 differed significantly from each other. Muscle glycogen reduction was dose dependent with (Figure 3)

### 3.4 Water Quality

Mean water quality (Table 2) of exposed group of fish did not show significant difference with water of control fish.

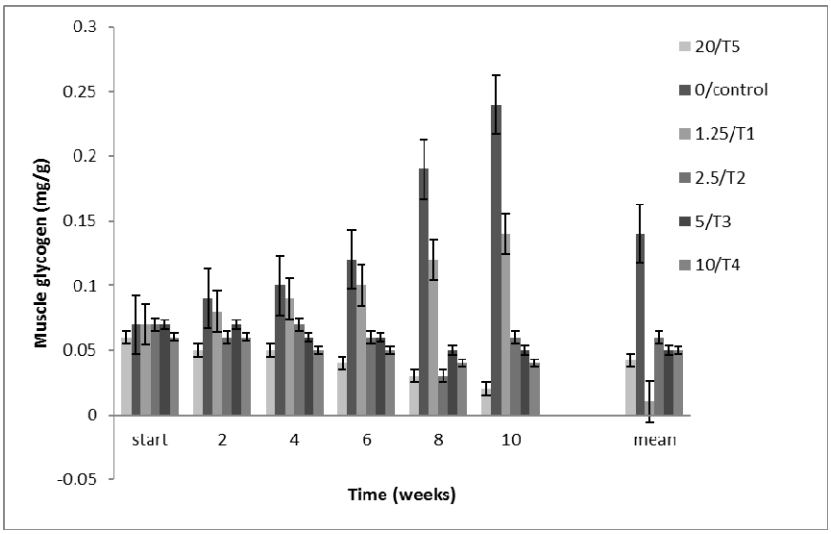


**Fig 3:** Mean Muscle Glycogen ±SE (mg/g) of *Clarias gariepinus* Juveniles Exposed to Subacute concentrations of WSF of Crude oil for 10 weeks

**Table 2:** Mean Water Quality ±SE of Subacute Concentration of WSF of Crude Oil Exposed to *C. gariepinus* for 10 Weeks

| | Concentrations (ml/l) | | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **$T_5$/20** | **$T_4$/10** | **$T_3$/5** | **$T_2$2.5** | **$T_1$/1.25** | **control/0** |
| Temp $^oC$ | 27.08±0.29$^a$ | 27.05±0.13$^a$ | 27.50±0.34$^a$ | 27.6±0.21$^a$ | 27.33±0.33$^a$ | 27.05±0.32$^a$ |
| Total Alkalinity (mg/l) | 21.98±0.59$^a$ | 21.88±0.22$^a$ | 22.70±0.45$^a$ | 20.73±0.22$^a$ | 21.90±0.12$^a$ | 21.53±0.68$^a$ |
| $CO_2$ (mg/l) | 4.30±0.19$^a$ | 4.33±0.26$^a$ | 4.40±0.22$^a$ | 4.18±0.16$^a$ | 4.60±0.15$^a$ | 4.3±0.18$^a$ |
| DO (mg/l) | 6.48±0.29$^a$ | 6.63±0.23$^a$ | 6.83±0.19$^a$ | 6.45±0.22$^a$ | 6.60±0.21$^a$ | 7.0±0.2$^a$ |
| pH | 6.43±0.18$^a$ | 6.70±0.18$^a$ | 6.73±0.27$^a$ | 7.10±0.28$^a$ | 6.38±0.07$^a$ | 6.88±12$^a$ |
| $NH_3$ (mg/l) | 01±6.5x10$^{-4a}$ | 0.02±2x10$^{-3a}$ | 0.02±2.5x10$^{3a}$ | 0.02±1.8x10$^{-3a}$ | 0.02±2.2x10$^{-3a}$ | 2.02±2.5x10$^{-3a}$ |

Means on a row with same superscript are not significantly different but means with different superscript are significantly different. Mean separation by Duncan's Multiple Range Test at 5% level of significance ($P$>0.05).

## 4. Discussion

The plasma glucose of test fish to crude oil water soluble fraction subacute concentrations dose dependent significant rise in plasma glucose than control reported in this investigation corroborated with report of Omoregie *et al*. (1994) [12] on fingerlings of Nile tilapia *Oreochromis niloticus* exposed to chronic levels of formalin, in Pacific herring *Clupea pallasi* exposed to water soluble fraction of crude oil (Kennedy and Farell, 2006) [9] and *Clarias gariepinus* fingerlings exposed to petrol (Ezike and Ufodike 2008) [7]. This development according to Wedemeyer and Yasutake (1973) [19] and Ezike and Ufodike (2008) [7] were due to stressful environmental condition occasioned by the toxicant and thus produced hyperglycaemic condition in blood plasma. The evidence of decreased plasma glucose reported at lower concentrations of crude oil suggest that hypoglycaemic condition which agrees with Oladimeji and Ologunmeta (1987) [11], Omoregie *et al.* (1995) and Wade *et al.* (2002) who reported hypoglycaemic conditions in Nile tilapia fish with various toxicants, which suggest that crude oil exposure at varying doses may trigger hyperglycaemic or hypoglycaemic conditions in fish plasma depending on the species, age and condition of exposure.

Dose dependent reduction of glycogen content in liver and muscles agrees with Omoregie *et al*. (1995) [16] on Nile tilapia fingerlings *Oreochromis niloticus* exposed to petroleum effluents; Wade *et al*. (2002) on *Oreochromis niloticus* exposed to cassava *Manihot exculenta* waste water effluent; in Pacific herring *Clupea pallasi* exposed to water soluble fraction of crude oil (Kennedy and Farell, 2006) and *Clarias gariepinus* fingerlings exposed to petrol (Ezike and Ufodike 2008) [7]. Progressive accumulation of plasma glucose was due to depletion of stored glycogen of the liver which suggests that stored glycogen was converted to glucose and emptied into blood circulation, because effects of toxicants hampered absorption of soluble glucose from the intestines (Wade *et al.,* 2002) [20]. Ezike and Ufodike (2008) [7] noted that petroleum impairment of carbohydrate metabolism in test fish was due to allocation of greater energy to the breakdown of aromatic hydrocarbon and excretion of the same which lead to reduction in growth among the exposed fish. Thus lower weight gain and length of fish exposed to water soluble fraction crude oil than in the control group aggress with the reports of Moles and Rice (1983) [10] when they exposed various species of fish to crude oil water soluble fractions and Alaa and Ahmed (2010) [1] when they exposed sub-adults of

*Clarias gariepinus* to ultraviolet radiation. Water soluble fractions of crude oil stimulated hydrocarbon metabolism at the expense of tissue growth, which might have lead to poor growth among exposed group than control fish. Toxicant effect may have hindered food intake thereby resulted to increased rate of food conversion which possibly led to poor growth among groups of fish exposed to water soluble fraction of crude oil. The calculated values for SGR, MGR and FE followed progressive reduction below control, which according to Ugwu *et al.* (2006) [18], Alaa and Ahmed (2010) [1], Esenowo and Ugwumba (2010) [5] and Emeline *et al*. (2012) [4] noted that these reductions were indications of poor growth in fishes.

## 5. Conclusion

The implication of the research show that WSF of BLCO led to dose dependent reduction of plasma glucose and critical levels of liver and muscle glycogens.

It is recommended from this research that the WSF of BLCO be treated alongside the visible surface spill and should be avoided in fisheries and aquaculture ventures.

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