Growth and Haematological Properties of Rats Fed with *Clarias gariepinus* Cultivated in Contaminated Water

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Abstract

This study examines the potential threats posed by contaminated African catfish (*Clarias gariepinus*) by evaluating the biochemical and physiological changes in various tissues, especially the blood, of consumers. African Catfish (*Clarias gariepinus*) were cultured in water contaminated with phthalate, benzene, and cyclohexane (10µg/ml) respectively over a period of 65 days. They were thereafter used as protein source to formulate feed for albino rats. The body weight, haematological properties, and serum lipid profile of the experimental rats were determined. It was observed that body weight gain of rats fed C. gariepinus cultivated in contaminated water was significantly higher than that of controls ($p < 0.05$). Abnormal haematological properties (Hb, PCV, RBC, WBC, etc.) were observed among rats fed *C. gariepinus* cultivated in contaminated water relative to controls. The concentrations of Hb, PCV, and RBC (6.40 ± 0.50, 34.00 ± 3.00, and 4.22 ± 0.52 in that order) of the benzene group of rats in particular, were found to be significantly lower than those of controls (13.70 ± 0.90, 45.00 ± 3.00, and 8.78 ± 1.22) ($p < 0.05$). Generally, concentrations of total cholesterol, triglycerides, and low density lipoprotein (LDL) in the serum of test rats were found to be significantly higher than those of controls ($p < 0.05$), while serum high density lipoprotein (HDL) was found to be significantly lower than that of controls ($p < 0.05$). It follows from this study that consumption of *C. gariepinus* cultivated in contaminated water may have adverse effects, as presented by data on haematology, and there could be a tendency towards increase in risk of cardiovascular diseases.

Keywords: C. gariepinus; haematology; cholesterol; triglycerides; cardiovascular diseases

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1. Introduction

The African catfish, *Clarias gariepinus*, from a biological perspective, is undoubtedly the most ideal aquaculture species in the world. It is widely distributed (70o of latitude), thrives in diverse environments (temperate to tropical), is hardy, adaptable and an ecological pioneer species – principally as a consequence of its air-breathing ability. It feeds on a wide array of natural prey and can adapt its feeding habits depending on food availability [1]. It is able to withstand adverse environmental conditions, is highly fecund and easily spawned under captive conditions.

C. gariepinus is a source of food to Nigerians; it is cultivated by many for commercial and subsistence purposes. Most of the ponds where C. gariepinus is cultivated serve as recipients to runoffs from open dumps where domestic and industrial wastes are deposited [2], while a few others, watered by boreholes, are contaminated by landfill leachate [3]. Components of runoffs and leachate of serious health concern are phthalate, benzene, and cyclohexane [4]. Some species of fish may contain significant levels of these contaminants and other environmental contaminants. These substances are present at low levels in fresh waters and oceans, and they bioconcentrate in the aquatic food chain such that levels are generally highest in older, larger, predatory fish and marine mammals. Fish and seafood are a major source of human exposure to these contaminants [5].

In recognition of the threat posed by contaminated fish to public health, the United States government directed the Environmental Protection Agency (US EPA) to regulate sport-caught fish, while the Food and Drug Agency (FDA) was shouldered with the responsibility of regulating all commercial fish—including farm-raised, imported, and marine fish. US EPA advises women who are pregnant or may become pregnant and nursing

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mothers to limit their consumption of sport-caught fish to one six-ounce meal per week [6].

In Nigeria, little or no attention is given to the issue of contaminated fish. Haematological properties and serum lipid profiles of fish are not taken into consideration as tools for ascertaining safety of consumption. The people, oblivious of this risk, consume fish ad libitum regardless of the source of cultivation. Therefore, all manner of clinical manifestation (such as kidney failure, increased incidences of cardiovascular diseases), which were not rampant before are now common clinical cases. A few studies were conducted on crude-oil contaminated fish [7-9]; however these studies only looked at the contaminant (crude oil) that is well known. However, risks of contaminants from domestic and industrial wastes often go unnoticed – an average Nigerian is potentially on a daily basis. The present study has made an attempt to elucidate the potential threats posed by contaminated *C. gariepinus* by evaluating the biochemical and physiological changes in various tissues, especially the blood.

2. Materials and Methods

2.1 Reagents

Chemicals and solvents used were of analytical grade and most were products of Sigma-Aldrich Inc. in St. Louis, Missouri, U.S.A., while others were products of British Drug House (BDH) in Poole, England.

2.1.1 Experimental Water

The experimental water for the study was collected from the supply of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The water was analyzed using the method described by the American Public Health Association (APHA) [10]. Three experimental water samples were prepared with 10 µg/ml of each chemical and tested individually for their effect on rats, as follows:

A: water collected from the university (control)
B: water contaminated with phthalate (10 µg/ml)
C: water contaminated with benzene (10 µg/ml)
D: water contaminated with cyclohexane (10 µg/ml)

The concentration of each pollutant is about ten times the recommended permissible limit [11] and is representative of the concentration found in a typical runoff from open dumps and leachate from landfills [3].

2.2 Experimental Animals

2.2.1 Clarias gariepinus

Eighty (80) African catfish (*Clarias gariepinus*) of average weight 68.56 ± 6.92g were obtained from the Department of Environmental Biology and Fishery, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. They were housed in transparent plastic containers and kept in a well-ventilated laboratory. These fish were fed (3% w/w) with commercial feeds obtained from Livinco feeds, Jubilee Road, Ikare Akoko, Ondo State, Nigeria. The experimental animals were kept inside a transparent plastic container assigned into eight (8) groups of ten (10) animals each. The first two groups of fish were cultured in uncontaminated water from the university supply and water contaminated with 10 µg/ml of phthalate, respectively. The third and fourth groups were placed in water samples contaminated with 10 µg/ml of benzene and 10 µg/ml of cyclohexane, respectively. A replica of the four groups was set up as the last four groups to permit thorough statistical analysis and computation of feed efficiency ratio. The feeding exercise lasted over a period of 56 days (long-term for C. gariepinus) preceded by a 14-day acclimatization period. Two fish samples were taken from each group (enough to compound feed to last the period of feeding) and dried in the oven at 40oC for six hours daily over a period of seven days. After this, they were used to compound rat feed as shown in Table 1.

Table 1: Rat feed composition using *C. gariepinus* cultivated in water contaminated with phthalate, benzene, and cyclohexane over a period of 56 days as a protein source.

Nutrient	Weight (g)
Protein source (*C. gariepinus)*	200
Fat source (soy oil)	80
Sucrose	60
Cellulose	60
*Vitamin and Mineral premix	50
Corn starch	550

*The vitamin/mineral premix contains: Vit. A 3,200,000.00 iu, Vit. D_5 600,000 iu, Vit. E 2,800 mg, Niacin 6,000 mg, Vit. B_1 800 mg, folic acid 70,000 mg, Cobalt 80 mg, iodine 499 mg, selenium 80 mg, Copper 1,200 mg, Vit. B_{12} 4 mg, Vit. B_6 800 mg, Copper 1,200 mg, Folic acid 70,000 mg, Vit. K_3 600 mg, Panthothenic acid 2,200mg, Vit. B_6 800mg, Vit. B_2 1,000 mg, Iron 8,400 mg, Manganese 16,000 mg.

2.2.2 Rattus novergicus

Twenty albino rats (Rattus novergicus) of mean weight 57.5 ± 3.25g were used for the study. They were obtained from the Animal Holding of the Department of Biochemistry University of Ilorin, Ilorin, Nigeria. They were kept in wooden cages and fed, ad libitum, with the formulated diet over a period of 28 days. They were classified into four groups designated as follows:

Group A -- Rats fed with *C. gariepinus* propagated in water from university supply-based diet (Control)
Group B -- Rats fed with *C. gariepinus* propagated in phthalate-contaminated water-based diet
Group C -- Rats fed with *C. gariepinus* propagated in benzene-contaminated water-based diet
Group D -- Rats fed with *C. gariepinus* propagated in cyclohexane-contaminated water-based diet

The feeding exercise was over a period of 28 days preceded by a seven-day acclimatization period. The rats were anaesthetized by placing them in a jar containing cotton wool soaked with chloroform before being sacrificed by jugular puncture. Blood was obtained through their jugular veins into both heparinized and non-heparinized bottles. The blood samples in non-heparinized bottles were spinned using RC650s at 3,500 rpm for 10 minutes and serum was stored at -8oC until required for use, while the blood samples in heparinized bottles were quickly taken to the lab for haematological analysis.

Haemoglobin concentration of the blood of experimental animals was determined following the method described by Mitruka and Rawnsley [12]. The RBC and WBC was done by the method of manual counting, and PCV by the Microhaematocrit method, described by Muthayya [13]. Serum concentrations of cholesterol and triglycerides of experimental fish were determined following the method described by the National Cholesterol Education Programme (NCEP) [14].

2.3 Statistical analysis

All data were analyzed using Analysis of Variance (ANOVA) by employing the method of Steel and Torrie [15]. Significant differences between the treatment means were determined at 5% confidence level using Duncan's Multiple Range Test [16].

3. Results

Experimental rats gained weight in spite of the contaminated fish (Figure 1). Particularly, rats fed a diet formulated with *C. gariepinus* propagated in cyclohexane- and benzene-contaminated water responded significantly better relative to controls ($p < 0.05$). Body weight gain of rats fed with *C. gariepinus* cultivated in water contaminated with phthalate, benzene, and cyclohexane is presented in Table 2. It was found that the gain in body weight of rats fed with contaminated fish was significantly higher than that of controls ($p < 0.05$). Rats fed with *C. gariepinus* cultivated in cyclohexane-contaminated water were found to gain the most weight at the end of the experiment; however, the weight gain was not significantly different from that of rats fed with benzene-contaminated fish ($p > 0.05$) but significantly different from that of rats fed with phthalate contaminated fish ($p < 0.05$).

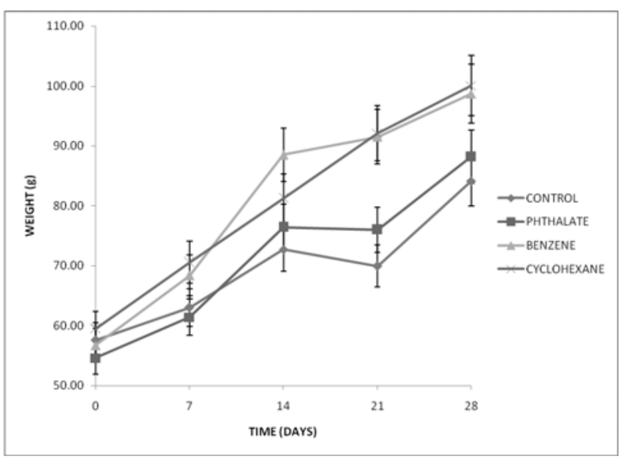

Figure 1: Growth response of rats fed with C. gariepinus cultivated in water contaminated with phthalate, benzene, and cyclohexane. Results are means of five determinations ± SEM.

The haematological properties of experimental rats are presented in Table 3. The concentrations of RBC, HB, and PCV of rats fed with C. gariepinus propagated in contaminated water were found to be significantly lower than those of controls ($p < 0.05$). In contrast, the concentrations of WBC and its differentials for the test rats were found to be significantly higher than those of controls ($p < 0.05$). The concentrations of MCV were observed not be significantly different from controls (53.80 ± 5.20) ($p > 0.05$), except for those of rats fed with C. gariepinus propagated in benzene-contaminated water (80.50 ± 7.50) ($p < 0.05$). The MCH concentrations were observed to be significantly higher in the blood of rats fed with *C. gariepinus* propagated in water contaminated with phthalate and cyclohexane than those of controls ($p < 0.05$). MCHC concentrations in the blood of rats fed with *C. gariepinus* propagated in water contaminated with benzene were found to be significantly lower than those of controls ($p < 0.05$).

Table 2: Gain in body weight of rats fed with *C. gariepinus* cultivated in water contaminated with phthalate, benzene, and cyclohexane. (Results are means of five determinations ± SEM. Values with different superscripts are significantly different, $p < 0.05$).

GROUP	Weight gained (g)
CONTROL	26.58 ± 3.34^{a}
PHTHALATE	33.58 ± 3.85^{b}
BENZENE	42.00 ± 4.33^{c}
CYCLOHEXANE	40.62 ± 4.10^{c}

abc: values are significantly different ($p < 0.05$).

Table 3: Haematological properties of rats fed with C. gariepinus cultivated in water contaminated with phthalate, benzene, and cyclohexane. (Results are means of five determinations ± SEM. Values in the same row with different superscripts are significantly different, $p < 0.05$).

PARAMETERS	CONTROL	PHTHALATE	BENZENE	CYCLOHEXANE
RBC ($X10^6/mm^3$)	8.78 ± 1.22^a	4.37 ± 0.87^b	4.22 ± 0.52^b	5.78 ± 0.58^c
HB (g/dl)	13.70 ± 0.90^a	8.70 ± 0.70^b	6.40 ± 0.50^c	10.10 ± 0.70^d
PLATELETS ($x10^3/mm^3$)	234 ± 6.00^a	350 ± 9.00^b	345 ± 11.00^{ab}	313 ± 9.00^c
MCV (μ^3)	53.80 ± 5.20^a	61.7 ± 8.9^a	80.50 ± 7.50^b	62.20 ± 7.80^a
MCH (μμg)	15.70 ± 0.80^a	19.9 ± 0.9^b	15.10 ± 0.70^a	17.40 ± 0.80^c
MCHC (%)	30.40 ± 2.30^a	32.20 ± 1.80^a	18.80 ± 2.40^b	28.10 ± 1.70^a
PCV (%)	45.00 ± 3.00^a	27.00 ± 2.00^b	34.00 ± 3.00^c	36.00 ± 3.00^c
WBC ($x10^3/mm^3$)	9.60 ± 0.50^a	12.6 ± 0.8^b	11.80 ± 0.60^c	12.40 ± 0.60^b
NEUTROPHILS(%)	6.20 ± 0.30^a	9.5 ± 0.20^b	9.3 ± 0.20^b	8.80 ± 0.10^c
EOSINOPHILS (%)	0.35 ± 0.01^a	1.2 ± 0.01^b	0.89 ± 0.01^c	0.98 ± 0.01^d
BASOPHILS (%)	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
LYMPHOCYTES (%)	59.40 ± 3.70^a	89.9 ± 5.2^b	99.60 ± 5.80^c	76.7 ± 5.30^d
MONOCYTES (%)	0.24 ± 0.01^a	1.11 ± 0.01^b	0.86 ± 0.01^c	0.45 ± 0.01^d

abc: values are significantly different ($p < 0.05$).

In this study, the concentrations of serum cholesterol, triglycerides, and LDL of rats fed a diet formulated with C. gariepinus propagated in contaminated water were found to be significantly higher than those of controls ($p < 0.05$) (Table 4). Particularly, serum cholesterol concentrations of rats fed a diet formulated with C. gariepinus propagated in contaminated water were observed to be about two-fold those of controls. For instance, concentrations of serum cholesterol of the phthalate group of rats were 225.3 ± 5.1 mg/dl while those of controls were 105.6 ± 3.4 mg/dl. Conversely, serum HDL concentrations in rats fed a diet formulated with *C. gariepinus* propagated in contaminated water were found to be significantly lower than those of controls ($p < 0.05$).

Table 4: Serum lipids of rats fed with *C. gariepinus* cultivated in water contaminated with phthalate, benzene, and cyclohexane. (Results are means of five determinations ± SEM. Values in the same column with different superscripts are significantly different, $p<0.05$)

GROUP	CHOLESTEROL (mg/dl)	TRIGLYCERIDES (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
CONTROL	$105.6±3.4^{a}$	$115.2±0.9^{a}$	$111.76±3.5^{a}$	$42.24±2.1^{a}$
PHTHALATE	$225.3±5.1^{b}$	$119.2±0.7^{b}$	$139.20±6.5^{b}$	$33.93±1.2^{b}$
BENZENE	$258.4±6.8^{c}$	$150.7±2.2^{c}$	$176.00±8.7^{c}$	$36.90±1.8^{b}$
CYCLOHEXANE	$204.5±2.9^{d}$	$114.1±0.8^{a}$	$110.64±2.9^{a}$	$41.81±0.7^{a}$

abc… value are significantly different ($p<0.05$).

4. Discussion

In this study, we aimed to establish the effect of consumption of *C. gariepinus* propagated in contaminated water on physical and haematological properties of rats. Studies have shown that many fish ponds in Nigeria are invaded by runoff and leachate from waste dumps and landfills [2]. Furthermore, Adeyemi et al. [3] reported that phthalate, benzene, and cyclohexane are major components of runoff and leachate. In a separate study, Adeyemi et al. [17] reported that C. gariepinus propagated in contaminated water is unfit for consumption. The general picture is that most fish ponds, streams, and rivers where *C. gariepinus* is cultivated are contaminated. Abnormal haematological properties, cardiovascular diseases, and liver and kidney dysfunction are increasing in Nigeria [18-20], and we are of the opinion that consumption of *C. gariepinus* cultivated in contaminated water may be among the contributing factors.

The increased body weight observed in the present study could be a sign of adverse effects resulting from consumption of the contaminated fish (Figure 1 and Table 2). A number of studies have demonstrated positive growth response to consumption of water and food contaminated with leachate components [19, 21-23]. Our results are in agreement with these earlier findings. It could be inferred that the fish, in response to the contaminants, might have retained water as a result of oedema or impairment in lipid metabolism, and that changes in fatty tissues perhaps occurred.

Several studies have reported changes in haematological parameters routinely used to determine stress associated with environmental, nutritional, and/or pathological factors [19, 24]. The decrease observed in the Hb and RBC of test rats is indicative of anaemia. Reduced PCV observed for the same group of rats may be indicative of excessive fluid retention; this lends credence to the previously mentioned possibility of oedema. Reduced MCHC and increased MCV observed in the blood of rats fed with *C. gariepinus* propagated in water contaminated with benzene could be a condition of hypochromic and macrocytic erythrocytes, respectively. Increased MCH concentration observed in the blood of rats fed with C. gariepinus propagated in phthalate and cyclohexane is suggestive of macrocytic anaemia (Table 3).

Hypercholesterolemia has become a worldwide epidemic and its prevalence continues to increase at a rapid rate in various populations across all age groups [25]. The general belief is that freshwater fish, such as C. gariepinus, are rich in omega 3 (ω3) fatty acids, which lower LDL cholesterol [26]. Conversely, the result of this study showed that consumption of *C. gariepinus* propagated in contaminated water raised serum total cholesterol, LDL cholesterol, and triglycerides, while HDL cholesterol was reduced (Table 4). Four major groups of lipoproteins have been identified that are important physiologically and in clinical diagnosis. These are (1) chylomicrons, derived from intestinal absorption of triglycerides and other lipids; (2) very low density lipoproteins (VLDL, or pre-β-lipoproteins), derived from the liver for the export of triglycerides; (3) low-density lipoproteins (LDL, or β-lipoproteins), representing a final stage in the catabolism of VLDL; and (4) high-density lipoproteins (HDL, or α-lipoproteins), involved in VLDL and chylomicron metabolism and also in cholesterol transport. Triglyceride is the predominant lipid in chylomicrons and VLDL, whereas cholesterol and phospholipids are the predominant lipids in LDL and HDL, respectively [27]. HDL concentrations vary reciprocally with plasma triglyceride concentrations, whereas a positive correlation exists between the incidence of coronary atherosclerosis and the plasma concentration of LDL cholesterol [28]. In addition, the ratio of total cholesterol to HDL cholesterol was also increased; this has been reported to be a powerful indicator of coronary heart disease [20]. Further studies are necessary to establish the relationship between elevated serum cholesterol and triglycerides, as observed in this study, and consumption of *C. gariepinus* cultivated in contaminated water.

5. References

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