Effect of Consuming Fish Caught from Crude Oil-Contaminated Freshwater on the Relative Organ Weights and Carcass Lipid Levels of Rats

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Abstract

The effect of consuming fish caught from crude oil - contaminated freshwater on the relative organ weights and carcass lipid levels of rats were investigated. The polluted water contained 37.33 ppm of crude oil. The total hydrocarbon and ash contents of the polluted fishes were significantly ($p<0.05$) higher than those of the unpolluted fishes. The rats were fed ad libitum for 28 days on diets compounded with crude oil-contaminated tilapia, catfish, knifefish and snakehead fishes at 10% protein level. The relative organ weights of rats fed diets formulated from polluted and unpolluted freshwater fish samples, of the same species, did not vary significantly ($p>0.05$). The carcass lipid content of rats fed polluted freshwater fish diets increased significantly ($p<0.05$) over those of the rats adapted to the unpolluted freshwater fish sample diets, of the same species. The results indicated that though the crude oil polluted freshwater fish diets did not change significantly ($p>0.05$) the relative organ weights of the rats, it increased their carcass lipid contents.

Key words: Crude Oil, Carcass Lipid, Relative Organ Weights, Polluted Fish

Introduction

Fish is a source of protein in our diets. Many of these fishes are caught through the orthodox methods of using fishing nets and hooks. Others are caught by the unscrupulous methods of using dynamites and poisonous chemicals. Fishes killed by crude oil pollution may not be entirely left out in the catches. This way many persons are unsuspectingly exposed to hazardous chemicals. Fishes accumulate hydrocarbons and metals in polluted water (Burns and Teal, 1973), especially the polycyclic aromatic hydrocarbons (PAH's) (Idoniboye and Andy, 1985; Onyeike, 1982) that are incorporated into the fatty layers (Zakrzewski, 1991). The xenobiotics distort enzyme and structural protein functions. With respect to human health, the potential problem is the fact that certain of the larger aromatic hydrocarbons are known to be carcinogenic (NAS, 1975).

In Nigeria, as in many parts of the world, crude oil bearing (or producing) communities are known to suffer massive environmental (land, water and air) degradation due to the exploitation of this precious resource. Non-crude oil producing communities have equally not been spared these forms of environmental degradation (in cases of pipeline bursts) since pipelines carrying this resource to points of refining and export transverse these communities like Adanta-Isiokpo in Ikwerre Local Government Area of Rivers State, Nigeria, whose share of high pressure oil pipelines bear the "Bonny Light" type of crude oil. The study reports a study on the effect of consuming diets formulated with fishes caught from crude oil-contaminated freshwater on the relative organ weights and carcass lipid contents, using rat models.

Materials and Methods

Water sampling: The water samples were collected in plastic containers rinsed 3 times with the water being sampled and stored in the refrigerator to prevent change in volume due to evaporation, after acidification with concentrated HNO_3 (usually 1.5ml HNO_3/ litre sample) to pH < 2 to minimize precipitation and adsorption of certain cations on the container walls. Water samples for biological oxygen demand (BOD_5) and dissolved oxygen (DO) were not stored.

Wide mouthed 500 ml glass sample bottles that were washed with soap, rinsed in water and finally in acid water to remove any residues that may interfere with the analysis, were used for the collection of water for the determination of oil and grease. Physico-chemical analyses were carried out using the procedures of APHA (1985). The pH, temperature and turbidity were measured on site, also using the procedures of APHA (1985).

Fish samples: Four different species of freshwater fish samples [*Tilapia (Hemichromis elongatus)*, knife (*Papynocranus afar*), catfish (*Clariidae Clarias gariepinus*) and snakehead (*Channa obscura*)] were fished from both the polluted and unpolluted sites of the Adanta stream in Adanta – Isiokpo in the Ikwerre Local Government Area of Rivers State, Nigeria. The polluted fish samples were fished 1500 meters from the point of entry of the slick into the stream, downstream, and the unpolluted fish samples were fished 4,000 meters upstream from the point of entry of the oil slick. The samples were separated according to species, washed in double distilled water, labeled in polythene bags and subsequently transferred fresh to the laboratory for analyses. In order to prevent post-mortem changes, which maybe either putrefactive or autolytic in nature, the samples were frozen (-4^{0}C)

till analysis. The samples were thawed at room temperature and prepared by cleaning, descaling and eviscerating before cutting them into slices and subsequently transferring into an oven (80^0C) for drying, which lasted for 24 hours. After drying, the fish samples were ground in a high-speed grinder (a product of Endecotts Limited, London).

Fish total body hydrocarbon (as crude fat) and ash contents were estimated using the methods of AOAC (1984).

Experimental Diets: The ground fish samples (protein sources) were added at the expense of ground corn starch as described by Onyeike *et al* (2000) to give 100g protein per kilogram diet. The diets were stored at 4^0C until required for use.

Bioassay: Rats were used as model for humans in the experimental protocol. A total of forty (40) disease-free stock of weanling albino rats of the wistar strain (all males) obtained from the Animal Colony of the Department of Biochemistry, University of Port Harcourt, were used for the rat feeding study. The rats were weighed, allotted to ten (10) groups of 4 rats each, then acclimatized for 4 days. The groupings were done on weight basis and the groups were equalized as nearly as possible with respect to body weight and individually housed in wire-screened battery cages with facilities for food, water and faecal collection. Feed and water were given to the rat *ad libitum* for the 28-day period of study. At the end of the feeding period, the rats were finally weighed, then killed by exposure to chloroform in a closed container (euthanization). Incisions were made into the thoracic and body cavities. The liver, kidneys, heart and spleen of each rat were excised, weighed and returned into the individual carcasses. The organ weights relative to 100g body weights were calculated. Each carcass was dried in an oven (105^0C), ground and stored in a sealed sample bottle in a desiccator. Rat carcass lipid content was determined by the gravimetric method of AOAC (1984).

Statistical Analysis: Data were analysed by the use of the students' t-distribution test of significance at a p-value of 0.05 ($p>0.05$) as described by Onuh and Igwemma (2000) and analysis of variance (ANOVA) as described by Essien (2003).

Results and Discussion

Crude oil contaminated or tainted foods are normally treated with repulsion. Onyeike *et al* (2000) had however argued that the protein qualities of such polluted fishes were not affected. The values of some physico-chemical parameters of the polluted water (Table 1) were higher than those of the unpolluted water and well above the WHO (1983) limits. This pollution increased the total body hydrocarbon and ash (minerals) contents of the polluted fish samples used in the study (Table 2). Crude oil is known to compose of hydrocarbons and dissolved minerals.

Contrary to Payne and Penrose (1975) who reported that compounds of environmental interest, including petroleum products and some crude oils, are capable of elevating mixed function oxidase (MFO) activities leading to hypertrophy and increase in weight of the organs having MFO activities (Guengerich, 1991), our results showed no such increase in weights of these organs, principally the kidney, spleen, liver and heart (Table 3).

Table 1: Physico-chemical Parameters of Water Samples*

Parameter	Polluted water	Unpolluted water	WHO[q]
Oil and grease (ppm)	37.33±23.28	ND	20.00
pH	5.85±0.38	6.61±0.16	6.5-8.5
Temperature (^{0}C)	24.60±0.91	24.30±0.45	NA
Turbidity (NTU)	4.83±0.32	4.20±0.10	5.00
Conductivity (μscm^{-1})	16.35±3.88	12.25±0.92	NA
Total Dissolved Solutes (ppm)	854.60±103.4	38.30±4.50	500.00
DO (mg/L)	1.03±0.32	4.80±1.05	NA
BOD_5 (mg/L)	9.58±2.95	1.85±0.14	NA

**Values are means ± S.D of triplicate determinations; [q]WHO (1983) limit; ND = not detected; NA = not available*

Table 2: Total Body Hydrocarbon and Ash Content of Fish Samples (%)*

Fish type	Total body hydrocarbon	Ash
$Snakehead_p$	$10.02±0.07^c$	$14.72±0.02^a$
Snakehead	$5.75±0.03^f$	$7.62±0.12^f$
$Tilapia_p$	$11.39±0.17^b$	$13.35±0.06^b$
Tilapia	$7.03±0.21^e$	$11.39±0.04^d$
$Knifefish_p$	$7.16±0.09^e$	$9.75±0.20^e$
Knifefish	$4.00±0.10^g$	$5.3±0.09^g$
$Catfish_p$	$13.08±0.01^a$	$12.19±0.05^c$
Catfish	$8.78±0.23^d$	$9.41.0.14^e$

**Values are means ± S.D of triplicate determinations; p = fished from polluted water; values on the same column bearing the same superscript letter are not significantly different at a p-value of 0.05.*

Table 3: Relative Organ Weights (gram of organ per 100g body weight)*

Diet type	Kidney	Organ Spleen	Liver	Heart	Final body weight
$Snakehead_p$	$0.69±0.09^a$	$0.26±0.08^b$	$4.19±0.45^c$	$0.31±0.01^d$	$153.75±59.35^e$
Snakehead	$0.63±0.88^a$	$0.27±0.03^b$	$3.91±0.26^c$	$0.26±0.01^d$	$183.00±15.28^e$
$Tilapia_p$	$0.61±0.10^a$	$0.29±0.05^b$	$3.97±0.26^c$	$0.29±0.02^d$	$190.00±50.99^e$
Tilapia	$0.68±0.12^a$	$0.23±0.04^b$	$4.25±0.65^c$	$0.28±0.02^d$	$188.33±30.14^e$
$Knifefish_p$	$0.67±0.14^a$	$0.22±0.07^b$	$3.33±0.80^c$	$0.25±0.04^d$	$152.50±47.70^e$
Knifefish	$0.69±0.11^a$	$0.26±0.07^b$	$3.63±0.57^c$	$0.29±0.02^d$	$136.25±40.29^e$
$Catfish_p$	$0.65±0.09^a$	$0.28±0.02^b$	$3.40±0.87^c$	$0.27±0.03^d$	$177.50±60.76^e$
Catfish	$0.65±0.16^a$	$0.25±0.08^b$	$3.45±0.68^c$	$0.30±0.05^d$	$145.00±55.08^e$

values on the same column bearing the same superscript letter are not significantly different at a p-value of 0.05.

It would appear that some organs might withstand a certain amount of injury without any demonstrable symptoms. This maybe as a result of one, a combination of, or, all of the reasons adduced

hereunder: (i) The route of administration (oral route) may have played a role in influencing the bioavailability and/or toxicity of the contaminant (crude oil). In the stomach, compounds are mixed with food, acid, gastric enzymes, and bacteria. All of these can alter the toxicity of the chemical either by influencing absorption or by modifying the compound. It has been demonstrated that there are quantitative differences in toxicity, depending upon whether the compounds are administered with food or directly into the empty stomach (Zakrzewski, 1991). (ii) Although chemicals can bioaccumulate in aquatic and marine species, it is not the sole determinant of the hazard of chemicals to aquatic species themselves. Therefore bioaccumulation and persistence are not necessarily synonymous with toxicity. Some compounds are stored in the body in specific tissues. Such storage effectively removes the material from circulation and thus decreases the toxicity of the compound. Repeated doses of a toxic substance may be taken up and subsequently stored without apparent toxicity until the storage receptors are saturated; then toxicity suddenly occurs (Zakrzewski, 1991). This looked like the most tenable reason. (iii) Another possible reason based on the submission of Parke and Ioannides (1980) that the level of protein in diets play a protective role against toxicity of chemicals (since they are used in synthesizing enzymes involved in the detoxification of xenobiotics) is not tenable because hypertrophy (or increase in weight) of the organs was not noticed.

The increase in the carcass lipid content of rats adapted to the crude oil- contaminated freshwater fish diet over those of the unpolluted fish diet of the same species (Table 4) may be as a result of induction of lipogenesis by crude oil components.

Table 4: Body Weight Gain and Carcass Lipid Content of Rats Fed Experimental Diets*

Diet type	Body weight gain(g)	Total body lipid (%)
Snakehead$_p$	38.75±28.39^a	22.11±0.34^a
Snakehead	40.00±17.32^a	18.22±1.23^b
Tilapia$_p$	70.00±17.80^a	24.13±0.09^a
Tilapia	65.00±15.00^a	19.08±0.30^b
Knifefish$_p$	36.25±10.31^a	25.19±0.32^a
Knifefish	21.25±2.50^a	17.25±0.27^b
Catfish$_p$	65.00±17.32^a	25.49±0.32^a
Catfish	32.80±9.57^a	17.06±0.36^b

**Values are means ± S.D of triplicate determinations. values on the same column bearing the same superscript letter are not significantly different at a p-value of 0.05.*

Prottey (1976) observed that application of surfactants on rat skin was able to induce lipogenesis; ability varying according to the chemical component of the surfactants. This equally suggested that the crude oil constituents in the polluted fish diets were actually taken up from the gastrointestinal tract (GIT) and stored away in tissues, as suggested above, where they may have elicited lipogenesis. Though our study did not look at the lipid profile of the rats placed on the polluted diets, Guengerich, (1991) showed that xenobiotics caused an increase in the levels of lipids, especially phosphatidylcholine, in drug metabolizing organs. This does not imply that ours would follow the same pattern, since there was no hypertrophy, but a further study on the lipid profile is suggested.

The higher carcass lipid content observed in rats fed the polluted fish diets may have accounted for their generally higher [though not statistically significantly ($p>0.05$) different] weight-gain than those adapted to diets based on the unpolluted fish samples (Table 4).

Lipophilic compounds may be stored in fat without apparent harm to the exposed organism (Zakrzewski, 1991). A special danger is that compounds may have escaped detoxifying metabolism while stored in the body, and that their toxicity may be potent and prolonged when released. Another danger is that when fat deposits are mobilized for energy, like in starvation, stored toxins are released causing sickness and death. In addition to a possible lasting inactivation of xenobiotics due to storage in various tissues, living organisms may partially be protected by their reserve functional capacity, as anti-malarials stored in fat are likely to help further protect the body against onset of malaria when such fat deposits are mobilized for energy.

Conclusion: In conclusion, the results indicated that though crude oil pollution did not affect the relative organ weights of the rats, it increased their carcass lipid content.

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