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Lipid profile and liver marker enzymes in male and female cultured adults *Clarias gariepinus*

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Abstract
Twelve (12) cultured adult *Clarias gariepinus*, six (6) male and six (6) female of average weight 795±179.52g were used to investigate the lipid profile and liver marker enzymes; important and efficient biochemical indices used in determining the general well-being of an organism. Concentrations of cholesterol, triglycerides and phospholipids (lipid profile) as well as the liver marker enzymes, i.e. Alanine Aminotransferase (ALT: EC: 2.6.1.2), Aspartate Aminotransferase (AST: EC: 2.6.1.1) and Alkaline Phosphatase (ALP: EC: 3.1.3.1) were determined using enzymatic procedures while statistical analysis was carried out using Statistical Package for Social Science (SPSS version 20). Plasma cholesterol in female species (81.55±16.25mg/dl) and phospholipids (191.97±53.50 mg/dl) were significant ($P<0.05$) than in male species (49.77±13.91mg/dl) and (153.09±40.08 mg/dl). There was a significant difference ($P<0.05$) between the male (99.44±22.90mg/dl) and female species (85.92±15.08) triglycerides concentrations. The male species are generally significantly ($P<0.05$) higher in High Density Lipoproteins (HDL) than the female. ALT concentration was found to be higher than ALP and AST in both sexes, the overall result was found to be below optimal concentration values thereby making the cultured sampled of adult *Clarias gariepinus* species safe for human consumption.

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1. Introduction

The general well-being of an organism can be determined by various biological indices, one of which is the lipid profile and the liver function tests of the organism. Biochemical characteristics of blood are among the important indices of the status of internal environment of an organism (Edsall, 1999). Changes in the biochemical profile indicates the changes in metabolism and biochemical processes of the organism resulting from the effects of various pollutants and make it possible to study the mechanisms of the effects of these substances. Racicool et al., (1975) related the activities of these enzymes to the presence of pollutants in the water: hence their assay became a routine practice in clinical medicine to diagnose certain diseases and the extent of tissue or organ damage. Since fish blood is sensitive to a variety of environmental changes, it is therefore possible

that certain characteristics of fish blood may register effects on the general wellbeing of the fish.

Enzymes are biochemical macromolecules which control metabolic processes of organisms, thus a slight variation in enzyme activity would affect the organisms (Roy, 2002). Enzymatic activities provide quick screening methods for assessing the health of fish and can be used to determine the incipient lethal concentration of a toxicant. Luskova et al., (2002) and Das et al., (2004) reported the level of transferases in different organs of different fish exposed to toxicants. More so, changes in plasma enzyme activity have been used as indicators of tissue injury, environmental stress, or a diseased condition. The rate of increase of plasma enzyme activity depends on the concentration of an enzyme in cells, the rate of leakage caused by injury and the rate of clearance of the enzyme from plasma (Boyd, 1983).

The lipid profile of an organism is a group of tests that are carried out to determine the risk of coronary heart disease (Hammed et al., 2010). These tests are good indicators of an organism likely to have a cardiovascular attack (heart attack or stroke) caused by the blockage of the blood vessels or hardening of the arteries (atherosclerosis). The lipid profile of an organism includes: the total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides, and sometimes an extended profile may include very low density lipoprotein cholesterol (VLDL-C) and non-HDL-C.

Liver function tests generally refer to a group of blood serum tests that measure the amount of certain enzymes in the blood of an organism. These tests help in detecting, evaluating and monitoring liver disease or damage. It also helps in evaluating the overall health of the liver and sometimes indicates other diseases such as malnutrition or bone diseases in an organism.

Liver function tests (LFT) are a helpful screening tool, which are an effective modality to detect hepatic dysfunction. The protective function of liver is because of its central location with a large number of immunological cells (Pastor et al., 1995). The liver has also been discovered to be the first organ that receive substances absorbed or bacterial translocated from gastro intestinal tract (GIT) after infection/inflammation or in healthy persons (American Gastroenterological Association, 2002).

Foreign compounds are predominantly biotransformed in the liver by the action of metabolizing enzymes including microsomal enzymes, aminotransferases and oxygenases (Stickel et al., 2005). Thus the liver has a high metabolic flexibility in response to enzyme induction to a variety of metabolites as well as the capacity to regulate the expression of genes coding for the biosynthesis of such enzymes. The disruption of the integrity of biochemical and physiological processes in fish has been monitored by determining the changes in the activities of enzymes in plasma/serum and tissues of the gill, brain, liver, muscle and kidney of the fish (de la Torre, et al., 2005, Ayalogu et al., 2001, Gabriel and George, 2005, and Leelanvinonthan and Amali, 2005). The liver marker enzymes of common interest are the transaminases: alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) (Raimi et al., 2012 a and b).

Liver disease is a collection of conditions, diseases, and infections that affect the cells, tissues, structures, or functions of the liver. Any abnormality or dysfunction of the liver leads major impairment of the organ function, which, in turn, influences the health of the individual. This can be assessed by biochemical tests that reflect the damage or dysfunction of the liver. However, no single biochemical test can detect the global function of liver. A series of tests employ for initial detection and management of liver diseases and these tests are usually referred as “Liver function test” (LFT) (Friedman et al., 2003). Some of the enzymes and the products of the metabolic pathway that are very sensitive for the abnormality occurred may be considered as biochemical marker of liver dysfunction. These markers are serum bilirubin, albumin, caeruloplasmin, α-fetoprotein, prothrombin time, alanine aminotransferase, and aspartate aminotransferase, ratio of aminotransferases, alkaline phosphatase, γ-glutamyl transferase, and 5-nucleotidase. An isolated or conjugated alteration of biochemical markers of liver damage in patients can challenge the clinicians during the diagnosis of disease related to liver directly or with some other organs.

The aimed towards paucity of published information in Nigeria on the lipid profile and the liver marker enzymes on cultured *Clarias*

gariepinus species brought about this study which was designed to investigate the concentration and range of the liver function enzymes (AST, ALP and ALT) and the lipid profile (Cholesterol, HDL-Cholesterol, Triglycerides, HDL-Triglycerides) in the plasma, erythrocytes (Red blood cells) and tissues (kidney, heart and liver) of male and female cultured *Clarias gariepinus* species.

2. Materials and methods

2.1 Study area

The study area is within the Ojo axis of Lagos, Nigeria, lying between 6º26"N and 3º12'E. The experiment was conducted during the rainy season between May - July 2012.

2.2 Animals and treatments - Specimen collection

Twelve (12) adult *Clarias gariepinus*, six (6) male and six (6) female of average weight 795±179.52g were obtained from Bluefield Fisheries Farm, Ijotun, Oko-Afo, Badagry, Lagos State, Nigeria. The fish were transported to the Department of Fisheries Laboratory, Lagos State University, Ojo, Lagos for further analysis. Weights of the fish were measured to the nearest gram and blood sample was collected into heparinized tubes by cardiac puncture and centrifuged to separate plasma and red blood cells. Liver, kidney and heart were removed from the fish for biochemical analyses. All samples were stored at –70° C until analyzed.

2.3 Biochemical analyses - Plasma liver function test

The activity of both plasma Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) were assayed according to Reitman and Frankel, (1957). Plasma Alkaline Phosphatase was determined by the enzymatic reaction described by the Deutsche Gesellschaft fur Klinische Chemie, (1972).

2.4 Plasma lipid profiles

Plasma concentrations of total cholesterol and triglycerides were determined with (Spin React S.A., Santa Colona, Sant Esteve De Bas, Spain) (Allain et al., 1974, Buccolo and David, 1973) HDL cholesterol and triglycerides were determined in plasma with same commercial kits for total cholesterol and triglycerides after very low density lipoproteins (VLDL) and LDL were precipitated with heparin–$MnCl_2$ solution as described by Gidez et al., (1982). Total phospholipids in plasma were extracted with chloroform–methanol mixture (2:1, v/v) as described by Folch et al., (1957) Phospholipid content was then determined as described by Stewart, (1980). Briefly, an aliquot of the phospholipid extract was evaporated to dryness at 60° C. After cooling, 2 ml of chloroform was added to the dried lipid extract and vortexed. Ammonium ferrothiocyanate (2 ml) was then added and the mixture vortexed for 1min. They were left for 10 min for the phases to separate. The chloroform layer was taken and absorbance read at 488 nm. Phospholipid concentrations were then determined using a phospholipid standard as reference.

2.5 Erythrocyte lipid profile

An improved procedure for the extraction of lipids from erythrocytes using chloroform–isopropanol (7:11, v/v) described by Rose and Oklander, (1965) was employed. For the determination of cholesterol, an aliquot of the chloroform–isopropanol extract was evaporated to dryness at 60° C. Triton X-100/chloroform mixture (1:1, v/v, 20_l) was added to resolve the lipids and again the solvent was evaporated. Then 1ml of commercially available cholesterol kit reagent (Spin React S.A., Santa Colona, Sant Esteve De Bas, Spain) was added and vortexed. After incubation in the dark at room temperature for 30 min, cholesterol content was determined by Determination of total phospholipids in the chloroform–isopropanol extract of the erythrocyte followed the same procedure as described for plasma determination by Stewart, (1980).

2.6 Organ lipid profiles

Lipids were extracted from liver, kidney and heart as described by Folch et al., (1957). After ashing with 0.05M KCl solution, aliquot of the chloroform–methanol extract was subsequently used for the determination of cholesterol, triglycerides and phospholipids concentrations. Cholesterol was determined in an aliquot of the chloroform–methanol extract of each organ as described for erythrocytes while determination of phospholipids followed the same procedure as described for plasma. Triglyceride concentrations in aliquots of the chloroform–methanol extracts of each organ were determined following the procedure described by Buccolo and David, (1973). Briefly, an aliquot of the chloroform–methanol extract in Eppendorf tubes was evaporated to dryness at 60° C. After cooling, 200 µl of ethanol (97%) was added to the tube to resuspend the triglyceride. Then 1ml of commercially available triglyceride kit (Spin React S. A., Santa Colona, Sant Esteve De Bas, Spain) was added and vortexed. After

incubating in the dark at room temperature for 20 min, triglyceride content was determined spectrophotometrically.

2.7 Statistical evaluation

Results are expressed as mean ± S.E. One-way analysis of variance (ANOVA) was used to analyze the results with $p < 0.05$ considered significant). Where heterogeneity occurred, the groups were separated using Turkey Multiple Range Test (TMRT) and paired t-test for sex variation. All analyses were done using Statistical Package for Social Science (SPSS) version 20.

3. Results

The mean values of the investigated lipids in plasma, HDL and erythrocyte are depicted in Figs. 1–3. There was no significant difference in triglyceride concentrations between male and female animals in plasma, HDL and erythrocyte (Figs. 1 and 2) ($p > 0.05$). In contrast to triglycerides, cholesterol and phospholipid concentrations exhibited different patterns between male and female animals (Figs. 1 and 2). The Plasma cholesterol and phospholipid concentration of female fish was 31% and 38% more than the male fish respectively (figs.1). There was no significant difference in the HDL cholesterol and phospholipid concentrations of both sexes (Figs.1 and 2). The erythrocyte lipid contents of the fish are shown in Fig. 3. The female fish have high phospholipid content of 72% more than male phospholipid in the erythrocytes. The mean values of the investigated lipids in Liver, Kidney and heart are depicted in Figs. 4–6. There was no significant difference in cholesterol and triglyceride concentrations in the liver, kidney and heart of both male and female species (Figs. 4, 5 and 6) ($p > 0.05$). In all the tissues, the cholesterol concentration was lower compare with other lipid (triglyceride and phospholipid concentration). On the contrary, phospholipid present in high concentration in all the tissues more predominantly in the kidney of the animal, although female animals has concentrations than male. Mean concentration of the Liver Marker Enzymes in male and female *Clarias gariepinus* is presented in figure 7. There was a significant difference ($p < 0.05$) in the alkaline phosphatase (ALP) of both male and female fish. There was no significant difference in the Aspartate aminotransferace (AST) and Alanine aminotransferace (ALT) of both male and female species ($p > 0.05$). AST concentration was lower compare to ALT and ALP with male fish having the highest concentration.

Figure 1: Mean concentrations of the Plasma lipid profile in male and female *Clarias gariepinus*

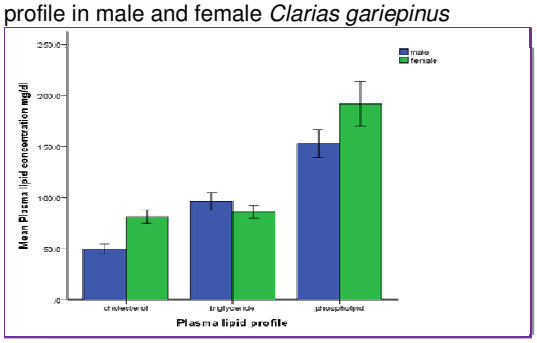


Figure 2: Mean concentrations of the high Density Lipoprotein lipid profile in male and female *Clarias gariepinus*

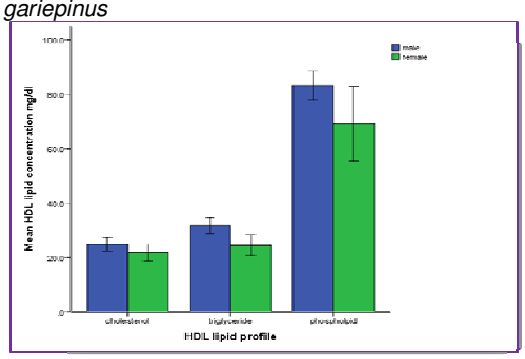


Figure 3: Mean concentrations of the Erythrocyte lipid profile in male and female *Clarias gariepinus*

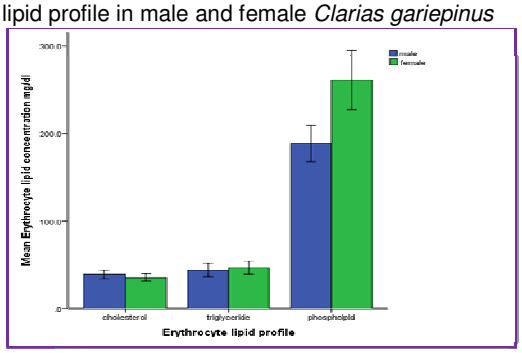


Figure 4: Mean concentrations of the Liver lipid profile of male and female *Clarias gariepinus*

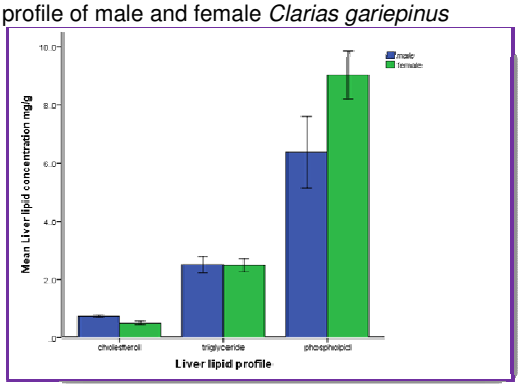


Figure 5: Mean concentrations of the Kidney Lipid Profile of male and female *Clarias gariepinus*

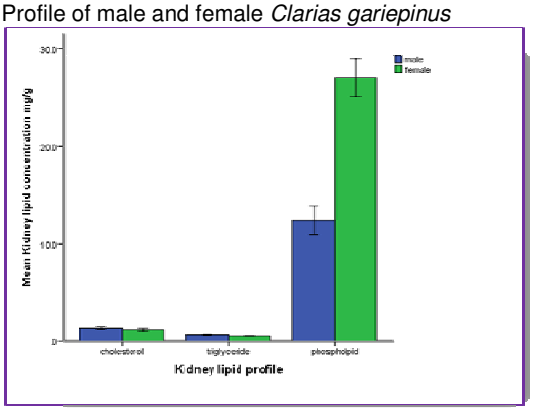


Figure 6: Mean concentrations of the Heart Lipid Profile of male and female *Clarias gariepinus*

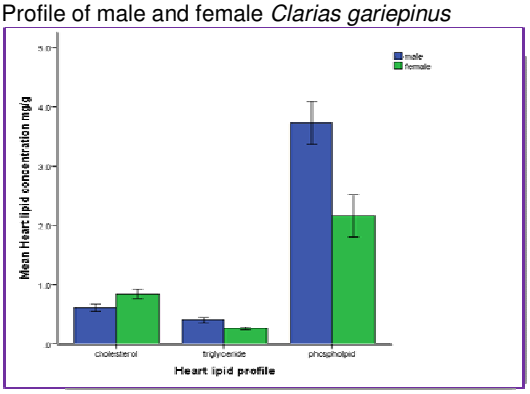
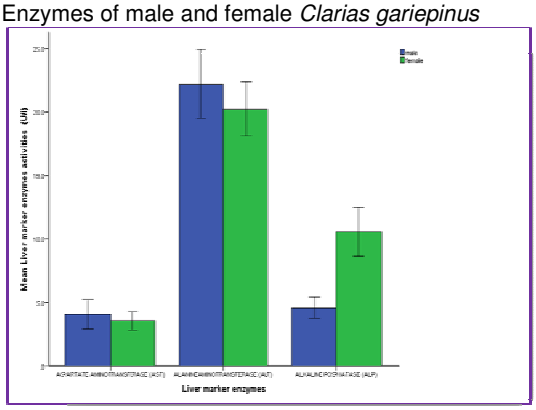


Figure 7: Mean concentration of the Liver Marker Enzymes of male and female *Clarias gariepinus*



4. Discussions and Recommendations

The lipid profile of an organism, coupled with the liver marker enzymes bio-assay are important and efficient biochemical indices in determining the general well-being of the organism (Edsall, 1999). These biochemical indices have been used in various pollution studies (Gabriel et al., 2009, Inyang, et al., 2010) with results indicating that increase of various aquatic pollutants increases the concentration of liver marker enzymes and lipid profile of the test organisms. The increase in the enzymes in the test organisms results in tissue damages and deviation from the normal body metabolism (functioning) of the organism. High lipid concentration in fish (i.e. cholesterol, triglycerides and phospholipids) above normal values may result in cardiovascular diseases through consumption (Fawole et al., 2009, Hammed et al., 2011a and b: Osibona, 2011).

Mean concentrations of plasma cholesterol in the female species (81.55±16.25mg/dl) and mean phospholipids concentration (191.97±53.50mg/dl) were found to be significantly higher ($p < 0.05$) than the concentration in the male species (49.77±13.91mg/dl) and (153.09±40.08mg/dl) respectively, but the opposite is the case in the triglyceride concentrations, with the male species having a higher mean concentration (99.44±22.90mg/dl) than the female species (Figure 1). The male species are generally high in High Density Lipoproteins, HDL, than the female species (Figure 2). This may be attributed to various physiological factors as female species have been known to use up fat deposits as energy during spawning and reproduction processes (Osibona, 2011).

The mean liver cholesterol and triglycerides concentrations are both higher in the male species (0.71±0.51mg/g and 2.57±0.73mg/g), than their respective concentrations in the female species (0.51±0.14mg/g and 2.49±0.55mg/g), but this is in contrast with the mean liver phospholipids concentration with the female species (9.03±2.02mg/g) having a higher concentration than the male species (6.57±3.53mg/g). Male species also have higher value in the in the concentration of the heart triglycerides (0.36±0.71mg/g) and phospholipids (3.83±1.00mg/g) than the female species, whereas the heart cholesterol concentration is higher in the female species (0.84±0.19mg/g) than in the male species (0.59±0.14mg/g) with no significant difference ($p>0.05$) observed.

The kidney cholesterol (figure 4) (1.45±0.33 mg/g), triglyceride (0.69±0.21 mg/g) and phospholipids (11.27±2.83 mg/g) concentrations were all significantly ($p>0.05$) higher in the male species than in the female species, (1.17±0.39 mg/g, 0.54±0.19 mg/g, 9.03±2.02 mg/g respectively). Furthermore, the higher concentrations in the erythrocytes cholesterol (figure 3) was observed in the male species (39.08±13.91 mg/dl) but the mean erythrocytes triglycerides and phospholipids

concentration were recorded in female species (46.70±18.27 mg/dl and 261.22±82.94).

The phospholipid concentration is generally higher in the Lipid Profiles (Figure 5 and 6) than the cholesterol and triglycerides concentration while Alanine Aminotransferase records the highest concentration in the liver marker enzymes (Figure 7). The lipid profile concentration in the plasma and erythrocytes recorded the highest with the lipid profile of the heart recording the lowest lipid profile concentration, i.e. erythrocytes> plasma> HDL> kidney> liver> heart.

The highest concentration in the lipid profile was mostly recorded in the female fish species, with significant differences observed in relation to sex. These differences may be due to various endogenous and exogenous factors as observed in the proximate composition of various fish species (Fawole et al., 2009, Osibona, 2011, Osibona et al., 2009, Edsall, 1999) and it was discovered that the composition and/concentration of an enzyme (or group of enzymes) and/ or proximate analysis varies with several factors such as: age, sex, specie, environment, temperature, food and feeding habit of the fish species etc.

Alanine Aminotransferase concentration is higher than the ALT and AST concentration. This is in contrast to previous study by Gabriel et al., 2009 and the ALT and AST concentration is below that recorded in the control of previous work by Gabriel et al., 2009: however the concentration of the lipid profile and the metabolic marker enzymes in this study are within the normal values previously recorded in several studies (Obomanu et al., 2009, Adeyemo, 2007 and Orlu, 2011).

The concentration of the lipid profile and liver marker enzymes of the sampled *Clarias gariepinus* shows significant differences ($p<0.05$) with sex. Also, the lipid profile and liver function tests are within the normal limits of concentrations reported by previous studies which might be due to the fish culturing environment and management procedure put in place thereby making the fish safe for consumption.

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