

Calculation Of Liver Function Test In *Clarias Gariepinus* Collected From Three Commercial Fish Ponds.

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Abstract: The liver function analysis is an indication of liver fitness of a fish and generally reveals the health of the fish. In this study, the liver function tests of fishes (*Clarias gariepinus*) collected from three different fish ponds designated as A, B, C located in Gwagwalada, Abuja were carried out. A total of ninety (90) live adult *C. gariepinus* were collected from fish ponds of which thirty (30) were collected from each of three selected ponds. The physico-chemical parameters of the ponds (Temperature, pH, Dissolved Oxygen, Alkalinity, Hardness and Transparency) were determined. The Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALP) of the fishes were examined using the standard methods. The results obtained showed that the physico-chemical parameters of Pond A and Pond B within the standard limits set by International Regulatory Authority (FEPA) and the liver enzyme markers (ALP, AST, ALT) were within the standard reference values. Furthermore, physico-chemical parameters of Pond C were higher than the set limits by FEPA and also the liver enzymes (ALP, AST, ALT) assessed were significantly higher ($P < 0.05$) than the standard reference values. The findings of this study would be of assistance in improving policy on quality pond management in order to reduce the incidence of fish liver diseases.

[Dorcas I.K. And Solomon R.J. **Calculation Of Liver Function Test In *Clarias Gariepinus* Collected From Three Commercial Fish Ponds.** *Nat Sci* 2014;12(10):107-123]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 14

1. Introduction

Background to the Study

The liver, he par, is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion (Maton *et al.*, 1993). The liver is necessary for survival; there is currently no way to compensate for the absence of liver function in the long term, although new liver dialysis techniques can be used in the short term (Cotran *et al.*, 2005).

This organ plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification (Kmiec, 2001). It lies below the diaphragm in the abdominal-pelvic region of the abdomen. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Benjamin *et al.*, 2008).

Clarias gariepinus is one of the most widespread catfish genera in the world (Gabriel *et al.*, 2007). They are found throughout Africa and the Middle East and live in freshwater lakes, rivers, swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems (Olaniyi *et al.*, 2009). The African sharp-tooth catfish was introduced all over the world in the early 1980s for aquaculture purposes and is therefore found in countries far outside its natural

habitat like Brazil, Vietnam and India (Faturoti and Akinbote, 2006).

LIVER OF *C. gariepinus*

Liver is an important organ in the body of the fish. The functions of the liver include storing glycogen (fuel for the body) which is made from sugars; helping to process fats and proteins from digested food; making proteins that are essential for blood to clot (clotting factors); helping to remove poisons and toxins from the body of the fish (Anderson, 1974). The liver also makes bile. This is a greenish-yellow fluid that contains bile acids, bile pigments and waste products such as bilirubin. Liver cells pass bile into bile ducts inside the liver. The bile flows down these ducts into larger and larger ducts, eventually leading to the common bile duct (Wales, 1983). The gallbladder is like a 'cul-de-sac' reservoir of bile which comes off the common bile duct. Bile in the gut helps to digest fats (Roberts, 1989).

Functions of the Liver

Liver is a versatile organ, which is involved in metabolism and independently in many other biochemical functions.

A. Metabolic functions: Liver are the key organ and the principal site where the metabolism of carbohydrates, lipids and proteins take place (Vikramjit, 2012).

i) **Carbohydrate Metabolism:** Glucose may be metabolized through glycolysis and then to citric acid cycle and oxidative phosphorylation to yield energy, if the cells are in need of ATP. If ATP is not required, then glucose can be stored as glycogen within the liver

or it can be converted into more stable storage form as triglycerides.

ii) **Lipid Metabolism:** Fatty acids will be catabolized to release acetyl-CoA. It may be used in the TCA cycle and ETC a source of carbon for fatty acid and cholesterol synthesis in healthy individuals.

A small portion of acetyl- CoA is converted to ketone bodies (acetone, acetoacetic acid and beta hydroxybutyric acid). Dietary lipids are repacked and secreted into the systemic circulation as lipoproteins.

The protein parts of the lipoproteins, apoproteins are synthesized by the liver only. Hence, the liver has an important role in the distribution of lipids in the body.

iii) **Protein Metabolism:** Proteins are break down in the intestine and absorbed as a amino acids, which then reach liver by portal vein. There, they may be utilized to form proteins of different kinds. Some of them are produced only in the liver, like albumin, α and β globulins and coagulation factors I, II, V, VII, IX & X. Several proteins of acute phase reactants are produced in the liver for example C-reactive protein.

iv) **Bilirubin Metabolism:** The heme present in the hemoglobin and other proteins/enzymes (cytochromes) are eliminated only through liver. The lysis of red blood cells releases hemoglobin, which splits to release globin and heme. The heme part is catabolized by microsomal heme oxygenase system of reticuloendothelial system produce bilirubin. The bilirubin (unconjugated) thus formed is hydrophobic in nature hence it is transported in the blood by binding with albumin to reach the liver. In the liver, it is conjugated with glucuronic acid to form hydrophilic conjugated bilirubin and is excreted in bile into the intestine. Bacterial action (deconjugation and reduction) forms bilinogen (stercobilinogen, mesobilinogen and urobilinogen). About 20% of the urobilinogen is reabsorbed daily from the intestine to enter enterohepatic circulation to get re excreted into the intestinal lumen (enterohepatic circulation) (Robert *et al.*, 2011).

A small fraction of urobilinogen enters the systemic circulation and gets filtered at the glomerulus and excreted in the urine (Figure-1).

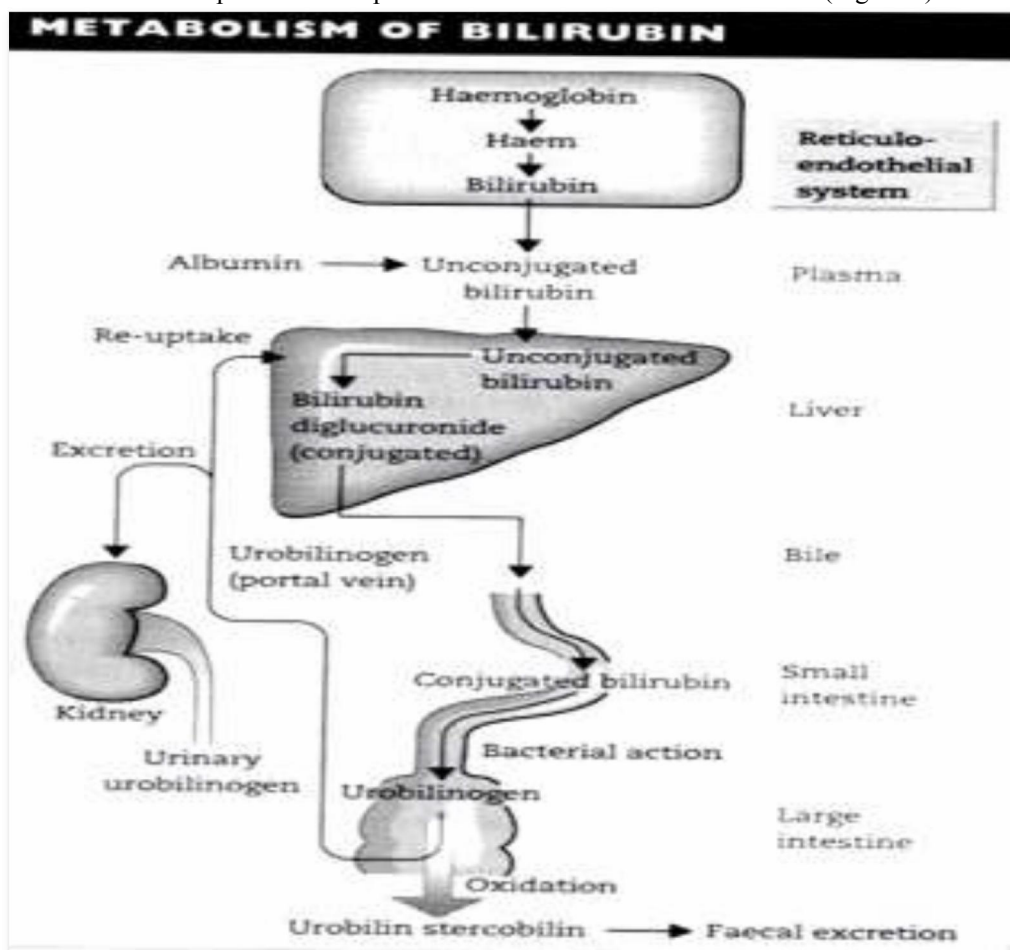


Figure 1: Metabolism of Bilirubin

B. Secretory Functions: Liver is responsible for the formation and secretion of bile in the intestine. Bile pigments –bilirubin formed from heme catabolism is conjugated in liver cells and secreted in the bile (Vasudevan *et al.*, 2013).

C. Excretory functions: Substances detoxified by the liver are excreted through bile. About 3 liters of bile is produced daily and the rest is reabsorbed and out of this 1 liter is excreted and the rest is reabsorbed and circulated in the enterohepatic circulation. The bile contains bile salts, conjugated bilirubin, phospholipids and hormones. Major route of cholesterol excretion is through bile. The bile reaching the intestine facilitates the digestion and absorption of lipids and fat-soluble vitamins (Eve and Bibudhendra, 2008).

D. Detoxification functions: Liver plays a central role in various detoxifying reactions (Godkar, 1994).

i) Exogenous Substances: Toxic substances entering from gut and parental route are mainly detoxified in the liver by different reactions like hydrolysis, hydroxylation, oxidation, carboxylation, reduction and demethylation. The detoxified products are more water soluble and thus easily excreted in urine. The cytochromes P450 enzyme system of hepatocyte is mainly concerned with drug metabolism; conversion of drugs into more soluble forms, which in due course conjugate with compounds like glycine, glucuronic acid and glutathione and finally excreted either in urine or through bile.

ii) Endogenous Substances: Disposal of bilirubin is already above discussed (Figure-1). Ammonia produced from amino acid catabolism is detoxified the liver to form less toxic urea. The key enzymes of urea cycle are located entirely in liver (Rosalki and McIntyre, 1999).

E. Storage functions: Liver stores glucose in the form of glycogen. It also stores Vitamins K, A, D, E, B12 (Postic *et al.*, 2004).

The liver has an indispensable role in the intermediately and energetic metabolism of carbohydrates, lipids and proteins. It provides inactivation and excretion of many endogenous/exogenous substances as well as synthesis of plasma proteins and blood clotting factors as discussed above. Diseases of the liver alter many biochemical parameters in the serum, in turn the assessment of these biochemical parameters are important for the diagnosis of liver diseases. Morphologically, About 72% of the liver tissue is hepatocytes, the Kupffer cells, endothelium and adipose cells together form another 8 %; bile tracts contribute 1% and the rest is extracellular fluid. In an acute liver damage mainly hepatocytes are affected, in other diseases, impairment of bile flow leading to predominant damage to bile capillary cells may prevail (Fialova and Vejrazka, 2003).

Numerous laboratory investigations have been proposed in the assessment of liver diseases.

From among these host of tests, the following battery of blood tests like total, conjugated & unconjugated bilirubin, total and differential proteins & albumin: globulin ratio, and certain enzyme assays such as Aminotransferases, alkaline phosphatase and γ glutamyl transferase. Urine tests for bilirubin and its metabolites, prothrombin time and prothrombin index have become widely known as standard liver function tests (LFTs).

The second generation LFT attempt to improve on this blood battery of tests and to gain a genuine measurement of liver functions like quantitative assessment of functional hepatic mass.

These include the capacity of the liver to eliminate exogenous compounds such as aminopyrine or caffeine or endogenous compounds like bile acids, which have gained much importance recently.

However such investigations are not yet routinely. In this article, only standard LFTs which are routinely done and possible in any standard laboratory are discussed and simplify their interpretation.

Literature Review

Initial Approach to the Evaluation of Abnormal Liver Function Tests

When a patient is identified as having an asymptomatic elevation of one or more liver chemistry test, the physician must decide what initial additional evaluation, if any, is clinically indicated. This will be based, at least in part, on the findings of the history and physical examination. Unfortunately, significant data are lacking on the cost-effectiveness of evaluating patients with asymptomatic abnormalities of liver chemistry tests; nor are there long-term prospective studies to define the natural history of the potential liver disease in these patients. However, given the high prevalence of asymptomatic liver chemistry abnormalities in the United States and the significant costs of an extensive serologic, radiologic, and pathologic evaluation, rational choices must be made based on available data.

In a study of 19,877 presumably healthy Air Force recruits, 99 (0.5%) had confirmed ALT elevations. Of these 99 individuals, only 12% had identifiable causes (4 hepatitis B virus [HBV], 4 HCV, 2 autoimmune hepatitis, 1 cholelithiasis, and 1 gastrointestinal infection). Although this study had a low rate of ALT abnormalities and was performed with less sensitive HCV testing methodology than is available today, it suggests that the majority of asymptomatic individuals with serum ALT abnormalities may not have demonstrable liver disease (Kundrotas and Clement, 1993).

In a Scandinavian study of 151 consecutive patients who were referred for mild to moderately

elevated serum aminotransferase levels (42–300 U/L) continuing for more than 6 months and who subsequently underwent liver biopsy, identifiable causes of liver disease were more common (Mathiesen *et al.*, 1999). Chronic HCV was diagnosed in 15.3% of patients, presumed alcoholic liver disease in 8%, autoimmune hepatitis, hepatitis, and primary biliary cirrhosis (PBC) in 1.3% each, α -1-antitrypsin deficiency in 0.7%, and non-alcoholic steatohepatitis and/or steatosis in 42% (the study began before the availability of HCV serologic testing; thus, the HCV group may be underestimated).

In addition, 36 (24%) patients had chronic hepatitis of unknown origin, with 11 having bridging fibrosis and 16 having moderate fibrosis on liver biopsy.

Cryptogenic cirrhosis was diagnosed in only 1(0.7%) patient. The significant differences between these two studies may be due to the fact that the latter study involved patients with durations of ALT elevations in excess of 6 months, or due to referral bias because these individuals were referred to an academic center. The data from similar studies from the pre-HCV era are difficult to assess, given that the prevalence of HCV infection could not be ascertained. In addition, a study of 92 asymptomatic blood donors with elevated serum ALT levels who were followed for 6 months revealed that ALT elevations were persistent or recurrent in two thirds of the patients (28% persistent, 36% intermittent) and occurred as an isolated elevation in only 33% of patients.

There was no identifiable cause for the ALT elevation in 22 of these 92 patients, although several may have had HCV infection, because testing was not available at the time of this study (Friedman *et al.*, 1987).

Finally, the incidence of hepatic disease in selective high-risk populations is, not surprisingly, significantly higher than in screening populations.

In a large, multicenter screening study for viral hepatitis, the prevalence of HBV or HCV infection was 24.8%³⁷ and asymptomatic liver chemistry abnormalities occurred in over three quarters of patients taking anticonvulsant medication.³⁸ Thus, a decision to observe an asymptomatic patient closely and repeat a liver chemistry test, versus proceeding with an additional evaluation, must be made in the context of the clinical scenario.

Although a decision regarding the evaluation of abnormalities of liver chemistries must be made based on clinical criteria, the costs of these serologic, radiologic and pathologic evaluations can be significant. In the absence of definitive cost-effectiveness data regarding the appropriate evaluation, the physician must assess both the benefit

and costs (non fiscal and fiscal) associated with further evaluating these laboratory abnormalities.

Given the high incidence of abnormal liver chemistries in asymptomatic individuals, decisions to evaluate these abnormalities by ordering a large number of tests will be quite costly, both in fiscal and non fiscal (i.e., false-positive tests, complications of liver biopsy, etc.) terms. Therefore, a decision regarding the need to perform additional testing, and/or the choice of the appropriate additional evaluations required to determine the potential cause of the liver disease should be individualized and based on historical and physical examination findings. If an observational approach is deemed appropriate (with close clinical observation and repeating serial liver chemistries), the physician must continue to re-evaluate the need for additional testing, realizing that a significant number of patients with persistent liver chemistry abnormalities may develop progressive liver disease.

Evaluation of Abnormalities of the Serum ALT and AST Levels

There are numerous causes of increased serum ALT and AST levels in both symptomatic and asymptomatic patients. As previously mentioned, ALT and AST are enzymes released from damaged hepatocytes into the blood following hepatocellular injury or death, although they can originate from other tissues. Historical information and the physical examination are essential for the initial evaluation to determine whether the liver injury is acute or chronic, the underlying etiology, and associated systemic illnesses. The initial management may include repeating the laboratory value if a laboratory error is strongly suspected or if the ALT and AST elevation is minimal. Elevations of both the AST and ALT may rarely be caused by non hepatic conditions, and although the ALT is considered a “liver-specific” enzyme, this is not always true (Scola *et al.*, 2000; Lin *et al.*, 1999).

Both the magnitude and relative level of elevation of the ALT and AST may be useful in narrowing the differential diagnosis for the cause of the liver injury, especially when the level of elevation is either mild or severe. Therefore, a useful paradigm to categorize elevated serum ALT and AST levels involves ALT and AST elevations of less than 5 times normal, with either a predominant ALT or AST elevation; and ALT and AST elevations greater than 15 times normal. ALT and AST elevations in an intermediate range may be caused by numerous disease processes that fall into both of the above categories and thus are less useful for limiting the differential diagnosis.

Aminotransferase elevations of up to 5 times normal may be seen in numerous chronic liver

diseases as well as in acute hepatic processes. Transaminase elevations usually imply liver cell injury and death, which may or may not be associated with cholestasis. The initial evaluation of all abnormal ALT and AST levels involves a detailed history and physical examination to determine potential causes and chronicity of the liver disease. Lifestyle modifications including discontinuation of medications and alcohol, weight loss, and dietary changes can be recommended.

The patient should be evaluated initially for common causes of liver injury and, therefore, knowledge of the prevalence of specific causes of liver disease may be useful in guiding the initial diagnostic management. However, in patients who have been previously identified as having an abnormal serum liver chemistry test, the probability of having any given type of liver disease is much higher than in the general population with normal liver chemistry tests. If the initial evaluation is unrevealing and if the patients are asymptomatic, appropriate management options include additional diagnostic evaluations versus lifestyle changes and close clinical observation with serial serum liver chemistry testing.

Interpretation of Abnormal Liver Chemistry Values

Normal laboratory values are defined as the mean of the distribution ± 2 standard deviations of the "normal" population (Sox *et al.*, 1988). Therefore, by definition, 5% of normal patients will have abnormalities of any given test (2.5% are above and 2.5% are below the 2 standard deviation level). Although low liver chemistry values are not typically associated with disease states, 2.5% of "normal" individuals will have mild elevations of a given serum liver chemistry test. In addition, the "normal" population may not be reflective of normal values for a given patient. Normal laboratory chemistry values may vary according to age, gender, blood group, and postprandial state as well as other factors. In fact, "normal physiologic" states such as pregnancy may result in markedly elevated levels of the serum alkaline phosphatase (Fishman *et al.*, 1972; Ramslo *et al.*, 1975).

Thus, all laboratory abnormalities must be interpreted within the clinical context of the patient.

One may also falsely assume that a normal laboratory value excludes disease. In fact, any population of patients with a given disease also has a distribution of laboratory values, and some affected individuals may have laboratory values extending into the "normal" range (Sox *et al.*, 1988). Thus, if a liver chemistry test is normal, it does not ensure that the patient is free of liver disease. If a laboratory error is suspected, the laboratory test should be repeated. However, a repeated value in the normal range does

not ensure that the initial value was truly erroneous and may reflect a fluctuating biochemical value. Aminotransferase levels typically may fluctuate in liver diseases such as hepatitis C virus (HCV), including serum alanine aminotransferase (ALT) levels that may intermittently be in the normal range and may not correlate well with liver histology (Haber *et al.*, 1995).

Finally, differing laboratories may use alternative methodologies for a respective assay, which can result in differing normal laboratory values.

The clinical significance of any liver chemistry test abnormality must be interpreted in the context of the clinical situation. Patients with marked abnormalities of liver chemistry tests, or with signs and symptoms of chronic liver disease or hepatic decomposition (i.e., ascites, encephalopathy, coagulopathy, or portal hypertension), should be evaluated and treated in a more expeditious manner than asymptomatic individuals with minimal chronic liver test abnormalities and normal physical examinations. Thus, the initial evaluation of laboratory tests must involve an assessment of the patient's symptoms, risk factors for liver disease, concomitant conditions, medications or drug use, history and physical examination findings, and even the consideration that there may potentially be a laboratory error (Sox *et al.*, 1988).

Based on the pattern of the serum liver chemistry abnormalities, serum liver chemistry tests can be classified to provide a practical approach for the evaluation and diagnosis of hepatobiliary diseases. For the purpose of this document, we have classified the analysis of liver chemistry abnormalities to the interpretation of serum ALT and aspartate aminotransferase (AST) abnormalities (hepatocellular injury) and serum alkaline phosphatase and bilirubin abnormalities (cholestatic pattern). Although it is important to emphasize that liver chemistry test abnormalities frequently occur in overlapping patterns, presenting an obvious limitation to this type of categorized analysis, the division of liver chemistry test abnormalities into "hepatocellular injury" and "cholestatic" patterns allows a commonly used, simplified approach for the interpretation of serum liver chemistries.

In addition, elevations of the hepatic alkaline phosphatase with minimal or no elevations of the serum ALT, AST, or bilirubin also may be indicative of "infiltrative" diseases of the hepatic parenchyma. Blood tests such as serum albumin and prothrombin time are also important determinants of hepatic synthetic function, but are not specific for hepatic disease. Nonetheless, these tests have an essential role in the evaluation of the hepatic function of patients with acute or chronic liver diseases.

Biological Basis of Liver Chemistries

The term "liver chemistry tests" is a frequently used but poorly defined phrase that encompasses the numerous serum chemistries that can be assayed to assess hepatic function and/or injury. Although these also are often termed liver function tests, this term is a misnomer because standard liver chemistry tests do not effectively assess the actual function of the liver. The term liver chemistry tests also implies that the biochemical tests are solely of hepatic origin, but in fact, liver chemistry tests are not always specific for the liver and may encompass numerous biochemical assays that reflect hepatocellular injury, intra- or extrahepatic cholestasis, infiltrating diseases of the liver, impairment of hepatic synthesis, and alterations in liver metabolism.

In 1955, elevations of the serum AST were reported in viral hepatitis as well as other hepatic diseases and subsequently, concomitant ALT elevations were found in similar disorders (De Ritis, 1965; Wilkinson, 1958). The AST and ALT are abundant hepatic enzymes that catalyze the transfer of amino groups to form the hepatic metabolites pyruvate and oxaloacetate, respectively. The ALT is found in the cytosol of liver, whereas two AST isoenzymes are located in the cytosol and mitochondria, respectively. Both the ALT and AST are released from damaged hepatocytes into the blood after hepatocellular injury or death. The AST also is abundantly expressed in several nonhepatic tissues including heart, skeletal muscle, and blood. The ALT is found in low concentrations in tissues other than liver, so it is frequently considered specific for hepatocellular injury. However, this specificity is not absolute because serum ALT elevations can occur in nonhepatic conditions such as myopathic diseases (Scola *et al.*, 2000; Lin *et al.*, 1999).

In addition, the serum ALT has diurnal variation, may vary day-to-day, and may be affected by exercise; the serum AST may be 15% higher in African-American males, in addition to varying day-to-day or with exercise (Dufour, 1998; Siesta *et al.*, 1975). Nonetheless, both the ratio and absolute elevation of the AST and ALT can provide important information regarding the extent and etiology of liver disease. The ratio of mitochondrial to cytoplasmic AST may be useful in the diagnosis of specific liver diseases; however, because isoenzyme activity is not assayed routinely in clinical practice, these data will not be evaluated further.

Bilirubin is a normal heme degradation product that is excreted from the body predominately via secretion into bile. Bilirubin is insoluble in water and requires conjugation (glucuronidation) into the water-soluble bilirubin mono- and di-glucuronide forms before biliary secretion.

In the second decade of the twentieth century, van den Bergh and Muller (1918) used Ehrlich's diazo reagent to determine that two types of bilirubin were present in the serum of jaundiced patients: one that reacted directly with the reagent (direct bilirubin) and a second form that required the addition of alcohol for color development (indirect bilirubin). Four decades later, independent work by Billing and Schmid demonstrated that unconjugated bilirubin was the indirect form, whereas the direct form was a combination of the bilirubin monoand

di-glucuronides (conjugated bilirubin) (Billing, 1977). Although the methodologies in serum bilirubin determination have advanced since this time, the terminology of direct and indirect bilirubin has remained virtually synonymous with conjugated and unconjugated bilirubin, respectively.

To secrete bilirubin into bile, unconjugated bilirubin must be taken up into the hepatocyte and conjugated into the glucuronide form by the endoplasmic reticulum enzyme bilirubin UDP-glucuronyltransferase (bilirubin-UGT), and the water-soluble bilirubin glucuronides must be secreted across the canalicular membrane into bile. The molecular mechanisms of these processes recently have been delineated and reviewed (Trauner *et al.*, 1999) but are beyond the scope of this document.

Bilirubin-UGT, the enzyme that conjugates bilirubin, is expressed shortly after birth. However, once enzyme expression occurs, it continues to be highly expressed and active even in severe liver disease and cirrhosis.^{21,22} Diminished expression of this enzyme is one of the defects causing Gilbert's syndrome, a benign, unconjugated hyperbilirubinemia occurring in up to 5% of the normal population (Beutler *et al.*, 1998; Tukey and Strassburg, 2000). Unconjugated hyperbilirubinemia also may result from hemolysis (increased heme breakdown) or in rare genetic diseases such as the Crigler-Najjar syndrome (Tukey and Strassburg, 2000). After the neonatal period, most hepatic conditions that result in a conjugated hyper bilirubinemia are caused by either extra hepatic obstruction of bile flow, intrahepatic cholestasis, hepatitis, or cirrhosis, with a resultant impairment of hepatocellular bilirubin secretion into bile. Because bilirubin-UGT expression and bilirubin conjugation typically are well preserved, these pathophysiological states usually result in a conjugated hyper bilirubinemia. When conjugated hyper bilirubinemia occurs, significant amounts of bilirubin may also be excreted via the urine.

The alkaline phosphatase families of enzymes are zinc metalloenzymes that are present in nearly all tissues. In liver, the enzyme has been immunolocalized to the microvilli of the bile canaliculus. Under normal conditions, serum alkaline

phosphatase predominantly is caused by liver and bone isoenzymes, with intestinal enzymes contributing up to 20% of total activity. The normal reference range is dependent on a host of factors including the method of determination, patient age and gender, and the postprandial state (Langman *et al.*, 1966). During normal pregnancy, alkaline phosphatase activity begins to rise by the late first trimester (because of placental isoenzymes), may reach levels of twice normal by term, and can remain elevated for several weeks after delivery (Fishman *et al.*, 1972; Ramslo *et al.*, 1975).

Serum alkaline phosphatase levels can be elevated by cholestatic or infiltrative diseases of the liver and by diseases causing obstruction to the biliary system, as well as by bone diseases, numerous medications, and tumors of hepatic and nonhepatic origin. When evaluating serum liver chemistries, the important clinical issue is the determination of whether the alkaline phosphatase abnormality is of hepatobiliary or nonhepatic origin. Liver alkaline phosphatase is more heat stable than bone, and isoenzyme determination can be made based on heat sensitivity; however, this assay may be subject to considerable inaccuracy and therefore its clinical use may be "laboratory-specific." Other isoenzyme determination methodologies may include assays using monoclonal antibodies or wheat germ lectin precipitation (Seabrook *et al.*, 1988; Sorensen, 1988).

Serum assays of 5-nucleotidase or γ -glutamyl transferase activity can be used to confirm the liver-specific origin for an elevation of the alkaline phosphatase. The alkaline phosphatase 5-nucleotidase acts on phosphate groups at the 5' position of the pentose. It is present in many tissues, but serum levels become significantly elevated only in liver diseases. Highest levels are found in cholestatic conditions, but elevations also can occur during hepatitis, cirrhosis, or other hepatocellular conditions.

The glycoprotein γ -glutamyltransferase is located on membranes of cells with high secretory or absorptive activities. It is abundant in liver, kidney, pancreas, intestine, and prostate, but not in bone. Thus, serum levels may be clinically useful for determining whether an alkaline phosphatase elevation is of liver or bone origin (Whitfield *et al.*, 1972).

Serum levels also may be elevated after alcohol consumption (presumably because of enzyme induction) and in almost all types of liver disease (Zein and Discombe, 1970). Elevations of this enzyme are therefore less useful for determining the cause of liver disease.

Liver Function Tests

As the liver performs its various functions, it makes chemicals that pass into the bloodstream and bile. Various liver disorders alter the blood level of

these chemicals. Some of these chemicals can be measured in a blood sample. Some tests that are commonly done on a blood sample are called liver function tests (LFTs). These usually measure the following:

i. Alanine transaminase (ALT):

This is an enzyme that helps to process proteins (Wales, 1983). An enzyme is a protein that helps to speed up chemical reactions. Various enzymes occur in the cells in the body. Large amounts of ALT occur in liver cells (Roberts, 1989). When the liver is injured or inflamed, the blood level of ALT usually rises.

ii. Aspartate aminotransferase (AST):

This is another enzyme usually found inside liver cells. When a blood test detects high levels of this enzyme in the blood it usually means the liver is injured in some way (Wales, 1983). However, AST can also be released if heart or skeletal muscle is damaged (Roberts, 1989). For this reason ALT is usually considered to be more specifically related to liver problems.

iii. Alkaline Phosphatase (ALP):

This enzyme occurs mainly in liver cells next to bile ducts, and in bone. The blood level is raised in some types of liver and bone disease.

iv. Bilirubin

This chemical gives bile its yellow/green colour (Wales, 1983). A high level of bilirubin in your blood will make you jaundiced ('yellow'). Bilirubin is made from haemoglobin. Haemoglobin is a chemical in red blood cells that is released when the red blood cells break down. Liver cells take in bilirubin and attach sugar molecules to it. This is then called 'conjugated' bilirubin which is passed into the bile ducts. A raised blood level of 'conjugated' bilirubin occurs in various liver and bile duct conditions. It is particularly high if the flow of bile is blocked. A raised level of 'unconjugated' bilirubin occurs when there is excessive breakdown of red blood cells.

Classification of Liver Function Test

Liver function test are classified broadly into three groups as follows;

Group-1: Markers of liver dysfunction:

A. Measurement of serum bilirubin (test of excretory function of liver): Bilirubin is an endogenous anion formed by the catabolism of heme.

The measurement of bilirubin as well as detection of bilirubin and urobilinogen in urine is important tests of liver function. The classification of bilirubin into direct and indirect bilirubin is based on the original Van der Bergh method of measuring bilirubin.

According to Van der Bergh, bilirubin in serum forms a purple compound that is azo-bilirubin, where bilirubin in serum is allowed to react with solution of Van der Bergh's diazo-reagent. Conjugated bilirubin is

water soluble and unconjugated bilirubin is not soluble in water and it requires solubilizer that is alcohol. Hence when the reaction is carried out in alcohol then total bilirubin is estimated. Unconjugated bilirubin is estimated by subtracting conjugated bilirubin from total bilirubin (Daniel and Marshall, 1999).

Serum Bilirubin levels:

1. Normal: 0.2 to 0.8mg/dl
2. Indirect/Unconjugated/ Free: 0.2 to 0.7 mg/dl
3. Direct/conjugated: 0.1 to 0.4mg/dl

Latent Jaundice: Above 1mg/dl (patient does not present with jaundice (subclinical jaundice)

Jaundice: Above 2mg/dl (High bilirubin levels are observed in gallstones, acute and chronic hepatitis) (Hasch, 1967).

Bilirubin in body is a careful balance between production and removal of the pigment in body. Hyper bilirubinemia seen in acute viral hepatitis is directly proportional to the degree of histological injury of hepatocytes and the longer course of the disease.

Hyper bilirubinemia: It results from overproduction/impaired uptake, conjugation or excretion / regurgitation of unconjugated or conjugated bilirubin from Hepatocytes to bile ducts.

Increased unconjugated bilirubin: This result from overproduction/impaired uptake of conjugation.

Increased conjugated bilirubin: Impaired intrahepatic excretion / regurgitation of unconjugated or conjugated bilirubin from hepatocytes of bile ducts. Serum bilirubin could be lowered by drugs like salicylates, sulphonamides, free fatty acids which displace bilirubin from its attachment to plasma albumin. On the contrary it could be elevated if the serum albumin increases and the bilirubin may shift from tissue sites to circulation. Bilirubin may be of prognostic value in conditions like fulminant hepatic failure where deep jaundice is associated with increased mortality (Daniel and Marshall, 1999).

Group-II

Markers of hepatocellular injury

1. Alanine aminotransferase (ALT)
2. Aspartate aminotransferase (AST)

Group-III

Markers of Cholestasis

1. Alkaline phosphatase (ALP).
2. γ - glutamyl transferase (GGT)

Hyper bilirubinemia and Hemolysis: Bilirubin itself is not soluble in water and is bound to albumin and thus does not appear in urine. Hemolysis with overproduction of bilirubin and concomitant reduced GFR causes decreased excretion and can lead to high bilirubin levels. Bilirubin levels in excess of 25 mg/dl may be seen in hemolysis in association with liver disease. Other causes of extreme hyper bilirubinemia include severe parenchymal disease, septicemia and renal failure (Rosen and Keefe, 2000).

2. Urine bilirubin

The presence of urine bilirubin indicates hepatobiliary disease.

a. In all cases of jaundice, urine should be examined for the presence of bile pigments (bilirubin), bile salts and urobilinogen.

b. Only conjugated bilirubin is soluble in water and is excreted in urine. Hence, in pre- hepatic jaundice, when the unconjugated bilirubin is increased in blood, it does not appear in urine; hence called acholuric jaundice.

c. But in obstructive jaundice, conjugation of bilirubin is takes place, which cannot be excreted through the normal passage and so, it is regurgitated back into blood stream. This is then excreted through urine. So, in obstructive jaundice, urine contains bilirubin (Choluric jaundice).

Urobilinogen: Most of the urobilinogen is metabolized in the large intestine (into stercobilin and excreted via feces) and small fraction is excreted in urine (less than 4mg/day). An increase in the urobilinogen in urine is a sensitive indicator of hepatocellular dysfunction.

a. In cases of obstruction, bile is not reaching the intestine and so urobilinogen may be decreased or absent in urine.

b. In hepatocellular jaundice, urobilinogen is initially elevated, then decreases or disappears when the obstructive stage sets in and reappears when obstruction is cleared.

c. Urobilinogen is absent in urine, when there is obstruction to bile flow. The first indication of recovery is the reappearance of urobilinogen in urine.

d. In hemolytic anemias, urobilinogen is increased.

e. Bilirubin is detected by Fouchest's test and urobilinogen by Ehrlich's test.

Bile Salts: Normal bile salts (sodium salts of taurocholic acid and glycocholic acid are present in the bile, but are not seen in urine. Bile salts in urine are detected by Hay's sulfur test. Positive Hay's test indicates the obstruction in the biliary passages causing leakage of bile salts into the systemic circulation leading to its excretion in urine.

Obstruction can occur in obstructive jaundice and also in hepatic jaundice due to obstruction of micro biliary channels caused by inflammation.

B. Measurement of Serum proteins (test of synthetic function of liver):

Almost all the plasma proteins except immunoglobulins are synthesized by liver. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of α and β globulins (Schachter and Shafritz, 1988).

1. Albumin: Serum albumin is the most important protein synthesized by the liver. The synthesis of albumin reflects the extent of functioning of liver cell mass. The extent of decrease in serum albumin level is directly proportional to the extent of liver damage.

The half life of serum albumin is as long as 20 days, hence serum albumin levels is not reliable indicator of hepatic protein synthesis in acute liver diseases whereas in all chronic diseases of liver, the albumin level is decreased.

A normal serum level of albumin is 3.5 to 5.0g/dL. Corticosteroids and thyroid hormone stimulate albumin synthesis by increasing the concentration of albumin mRNA and tRNA in hepatocytes (Jefferson, 1985).

The serum albumin levels tend to be normal in diseases like acute viral hepatitis, drug related hepatotoxicity and obstructive jaundice. Albumin levels below 3g/dl in hepatitis should raise the suspicion of chronic liver disease like cirrhosis which usually reflects decreased albumin synthesis. In ascites there may be normal synthesis but the levels may appear reduced because of increased volume of distribution. Hypoalbuminemia is not specific for liver disease and may occur in protein malnutrition, nephrotic syndrome and chronic protein losing enteropathies (Hasch, 1967).

2. Globulin: They constitute immunoglobulin's produced by B lymphocytes as well as α and β globulins synthesized mainly by hepatocytes.

Gamma globulins in the serum are increased in chronic liver diseases like chronic active hepatitis, and cirrhosis. In cirrhosis, antibodies against intestinal bacteria are seen, since the cirrhotic liver cannot clear the bacteria reaching through circulation. IgG is increased in autoimmune hepatitis, IgM is increased in primary biliary cirrhosis and IgA is increased in alcoholic liver disease (Green and Flamm, 2002).

Albumin to Globulin (A/G) ratio: Normal A/G ratio: 1.2/1- 1.5/1.2. Globulin levels increase in hypoalbuminemia as a compensatory mechanism to maintain serum protein, finally result in decreased A/G ratio.

3. Prothrombin Time (PT): Clotting is the end result of a complex series of enzymatic reactions that involve at least 13 factors. The liver is the major site of synthesis of 11 blood coagulation proteins (fibrinogen, prothrombin, labile factor, stable factor, Christmas factor, Stuart power factor, Prekallikrein and high molecular weight Kininogen. The estimation of prothrombin is a useful indicator of liver function. The half life of prothrombin is 6 hours only; therefore PT indicates the present function of the liver. The results of this test may be expressed in sec or as a ratio of the plasma prothrombin time to control plasma time. Normal control usually is in the range of 9-11

seconds. A prolongation of more than 2 seconds is considered abnormal. The prolonged PT is not specific for liver diseases and is seen in various deficiencies of coagulation factors, DIC, and ingestion of certain drugs. In acute and chronic hepatocellular disease the PT may serve as a prognostic indicator. In acute hepatocellular disease worsening of PT suggests an increased likelihood of acute hepatic failure (Daniel and Marshall, 1999).

The PT is a predictor of outcome in cases of acetaminophen over dosage and acute alcoholic hepatitis. Prolongation of PT is also suggestive of poor long-term outcome in chronic liver disease. If the PT returns to normal or improves by at least 30% within 24 hr of a single parenteral injection of vitamin K1 (5-10 mg), it may be surmised that parenchymal function is good and that hypovitaminosis K was responsible for the original prolongation of PT. Patients with parenchymal disease by contrast will show only minimal improvement. Most patients with extra hepatic obstruction like EHBA would respond promptly to a single injection of vitamin K1. The PT is particularly important in the management of patients with liver disease. It is important to perform before procedures like liver biopsy and kidney biopsy and it permits an assessment of the tendency to bleed. In many centers the International normalized ratio (INR) is done in place of PT (Daniel and Marshall, 1999).

Use of liver function test

LFTs are useful, and are often the first marker of disease in the liver.

- i. To help diagnose liver disorders. The pattern of the test results may help to say which disorder is causing the problem. For example, depending on which enzyme is highest, it may point to a particular disorder.
- ii. To monitor the activity and severity of liver disorders.
- iii. To check the fitness of the Liver
- iv. To screen for any potential liver disease of the fish.

2. Materials And Methods

This research work was conducted at the Department of Chemistry, Biotechnology Laboratory, Sheda Science and Technology Complex, SHESTCO, FCT, Abuja.

Collection of Fish Samples

A total number of ninety (90) *Clarias gariepinus* were collected alive from fish ponds; Jeremiah Hussein farm, Dagiri farm located in Gwagwalada Area Council and Ajima Farms in Kuje Area Council, Abuja. Three fish ponds were selected of which, Thirty (30) *C. gariepinus* blood samples were collected from each of them in EDTA bottles and then taken to the Laboratory.

Blood samples

Fresh blood samples were collected without anticoagulant from the caudal artery. The needle was run, quite deep, as much as possible through a middle line just behind the anal fin in a dorso-cranial direction till striking the vertebrae. By drawing the needle gently backward, blood was usually sucked into the syringe. The collected blood was centrifuged at 3000 rpm for 10 minutes to separate serum for biochemical analysis.

Determination of Physico-Chemical Parameters of the Water

Such physico-chemical parameters were conductivity, pH, alkalinity, temperature, hardness, dissolved oxygen and transparency.

Temperature

The water temperatures were determined using mercury thermometer. This was done by inserting thermometer about 5cm deep into the pond water and removing it after 5 seconds. The temperature was read almost immediately.

Water pH

The pH was determined in the laboratory using the Hanna pH meter model pH 210 at laboratory temperature. The pH meter was standardized with distilled water at pH 7.0.

Water Hardness

This was determined by diluting 25ml of water sample to 50ml with distilled water 2ml of buffer solution and 0.1g Erichrome-black-T-dye were added. The resultant reddish solutions were titrated with EDTA –titrant drop by drop until a blue colourless end point was observed. Calculations were based on the equation given by Lind (2009).

Alkalinity

100ml of water sample was measured in 250 Erlenmeyer flask and drops of phenolphthalein indicator were added. The sample turned pink, it was then titrated with 0.02NH₂SO₄ until the pink colour disappeared and the ml of acid used was recorded for alkalinity calculation.

Calculation:

$$\text{Total alkalinity} = \frac{B \times N \times 50.000}{\text{ml of sample}} \text{ (as mg/l CaCO}_3\text{)}$$

Dissolved Oxygen

This was determined by the modified Winkler azide method. Duplicated water sample in 250 ml BOD stoppered bottle were fixed in a field with 2 ml manganous sulphate, followed by 2 ml alkaline iodide azide and 2 ml conc. H₂SO₄. The samples were transported in an ice box to the laboratory. 100ml of the sample was titrated with 0.0125N Sodium thiosulphate solution until it turns to a pale straw colour. 1ml of starch solution was added which turned to blue colour. The titration continued until the blue colour disappeared. The volume of the 0.012N Sodium

thiosulphate titrant used is equivalent to the mg of dissolved oxygen per litre.

Liver Function Test

The following liver function tests were carried out to determine the fitness of liver of *C. gariepinus* collected from three commercial ponds at Kuje, Dagiri and Gwagwalada farm, Abuja.

Determination of Aspartate Aminotransferase (AST)

0.5ml of the substrate, sodium azide was added in a test tube plus 0.5ml of the blood or serum. It was then put in a water bath at 37°C for 30 minutes. After this 0.5ml of a 2,4 dinitrophenylhydrazine was added to the mixture, incubated for 20mins and 5ml of sodium hydroxide was added to the mixture which turned brown. It was then placed in a spectrophotometer at 540nm and the results were read on the calibrated graph.

Determination of Alanine Aminotransferase (ALT)

0.5ml of substrate was added into a test tube containing 0.5ml of blood, the mixture is put in a water bath at 37°C for 30minutes, 0.5ml of 2,4-dinitrophenylhydrazine was added to the solution and incubated for 20mins. Then 5ml of sodium hydroxide was added to mixture which turned brown and was placed in a spectrophotometer at 540nm and results were obtained from the calibrated graph.

Calculation = ALT(U/I) = change Abs/mm x 1750

Determination of Alkaline Phosphatase (ALP)

1ml of p-nitrophenylphosphatase was added to 1ml of the serum in a test tube, incubated for 30mins and results were obtained from the spectrophotometer at 410nm and correlated with values on the calibrated graph to give the results.

Statistical Analysis

The results were expressed as Mean ± Standard Deviation. The mean values of the AST, ALP and ALT of the fishes collected from the three ponds were separated using Duncan Multiple Range Test at P=0.05

3. Results

The physico-chemical parameters of the three sampling ponds and the liver function tests of the fish collected from these sampling ponds are shown in this chapter, the ponds are designated as ponds 1 (Ajima farms), 2 (Jeremiah Hussein Farms) and 3 (Dagiri farms).

Physico-chemical Parameters of the Ponds

The mean water temperatures of sampling ponds 1, 2, 3 were 29.3 ±0.20°C, 28.7±0.10°C and 33.10±0.20°C respectively. The mean pH of the sampling ponds 1, 2, 3 were 9.05±0.56, 8.43±0.18 and 10.94±0.52 respectively. However, the dissolved oxygen of the sampling ponds 1, 2, 3 were 3.50±0.04 mg/l, 3.50±0.05 mg/l and 6.50± 0.06 mg/l respectively. The mean alkalinity of the sampling

ponds 1, 2, 3 were 9 ± 2 mg/l, 10 ± 1 mg/l and 13 ± 3 mg/l respectively. The mean hardness of the water of the sampling ponds 1, 2, 3 were 32 ± 4 mg/l, 24 ± 2 mg/l and

60 ± 5 mg/l respectively. The mean transparency of the sampling ponds 1, 2, 3 were 20.30 ± 1.80 cm, 23.0 ± 1.20 cm and 35.3 ± 1.30 cm respectively.

Table 1: Mean Physio-chemical Parameters of different Ponds.

Parameter	Pond 1	Pond 2	Pond 3	FEPA Standard
Mean Water temperature ($^{\circ}$ C)	29.30 \pm 0.20	28.7 \pm 0.10	33.10 \pm 0.20	28-30
Mean pH	9.05 \pm 0.56	8.43 \pm 0.18	10.94 \pm 0.56	6-9
Mean Dissolved Oxygen (mg/l)	3.50 \pm 0.04	3.50 \pm 0.05	6.50 \pm 0.06	1-4
Mean Alkalinity (mg/l)	9 \pm 2	10 \pm 1	13 \pm 3	7-10
Mean Hardness (mg/l)	32 \pm 4	24 \pm 2	60 \pm 5	20-35
Mean Transparency (cm)	20.3 \pm 1.80	23.0 \pm 1.20	35.30 \pm 1.80	20-25

Results are expressed as Mean of the Triplicates \pm Standard Deviation.

Table 2 shows the mean Concentrations of Alkaline Phosphatase (ALP) of fishes from three different fish pond in Gwagwalada, Abuja. The ALP levels of the fishes collected from Pond 1, 2, 3 were 47.50 \pm 5.30 IU/L, 44.50 \pm 4.60 IU/L and 56.50 \pm 5.30 IU/L respectively.

Table 2: Mean Concentrations of Alkaline Phosphatase (ALP) of Fishes From Three Different Fish Pond in Gwagwalada, Abuja.

Ponds	Concentration (IU/L)
Pond 1	47.50 \pm 5.30 ^a
Pond 2	44.50 \pm 4.60 ^a
Pond 3	56.50 \pm 5.30 ^b
REFERENCE STANDARD	16-50 ^a

Results are Expressed as Mean \pm Standard Deviation (n=50)

Values within the column having different superscripts are significantly different at P<0.05.

Table 3 shows the mean Concentrations of Aspartate Aminotransferase (AST) of fishes from three different fish pond in Gwagwalada, Abuja. The AST levels of the fishes collected from Pond 1, 2, 3 were 53.80 \pm 5.30 IU/L, 58.70 \pm 4.60 IU/L and 38.40 \pm 5.30 IU/L respectively.

Table 3: Mean Concentrations of Aspartate Aminotransferase (AST) of Fishes from Three Different Fish Pond in Gwagwalada, Abuja.

Ponds	Concentration (IU/L)
Pond 1	53.80 \pm 5.30 ^a
Pond 2	58.70 \pm 4.60 ^a
Pond 3	38.40 \pm 5.30 ^b
REFERENCE STANDARD	54-298 ^a

Results are Expressed as Mean \pm Standard Deviation (n=50)

Values within the column having different superscripts are significantly different at P<0.05

Table 4 shows the mean Concentrations of Alanine Aminotransferase (ALP) of fishes from three different fish pond in Gwagwalada, Abuja. The ALP levels of the fishes collected from Pond 1, 2, 3 were 58.70 \pm 12.70 IU/L, 48.90 \pm 9.10 IU/L and 85.60 \pm 11.10 IU/L respectively.

Table 4: Mean Concentrations of Alanine Aminotransferase (ALP) of Fishes from Three Different Ponds in Gwagwalada, Abuja.

Ponds	Concentration (IU/L)
Pond 1	58.70 \pm 12.70 ^a
Pond 2	48.90 \pm 9.10 ^a
Pond 3	85.60 \pm 11.10 ^b
REFERENCE STANDARD	35-80 ^a

Results are Expressed as Mean \pm Standard Deviation (n=50)

Values within the column having different superscripts are significantly different at P<0.05

4. Discussion

The physico-chemical parameters of the ponds is an index of biotic interaction of any water body and it affects biochemistry, hematological and hepatological parameters of the fish which could attributed to individual adaptation patterns, survival life history pattern and reproduction success. The result of this study therefore shows that the physico-chemical parameters (temperature, pH, Dissolved Oxygen, Alkalinity, Hardness and transparency) of sampling fish ponds A and B were within the reference standards set up by Federal Environment Protection Agency (FEPA) for water quality of pond water (Table 1). However, physico-chemical parameters (temperature, pH, Dissolved Oxygen, Alkalinity, Hardness and transparency) of Pond C were higher than the permissible limits of pond water set up by FEPA. Studies have shown that when the water quality is affected, physiological changes will be observed in the values of one or more of the liver function markers.

Alkaline Phosphates (ALP) is a liver enzyme in which high levels of this enzyme in the blood above

the referenced standard values indicates liver is injured in some way. In this present study, the Mean ALP levels of fishes sampled in Pond A and Pond B were not significantly different ($P < 0.05$) from the reference standard values and this indicates that the fishes were healthy. However, the Mean ALP levels of fishes sampled in Pond C were significantly different ($P < 0.05$) from the reference standard values. This can be attributed to the high levels of the assessed physico-chemical parameters (temperature, pH, Dissolved Oxygen, Alkalinity, Hardness and transparency) which were found to be higher than the permissible standards set by FEPA. This correlates with the findings of Oshode *et al.* (2008) that reported high levels of AST in the assessed fish samples as a result of changes in the physio-chemical parameters of the pond water.

Aspartate Aminotransferase (AST) is another enzyme of the liver and high levels of AST always indicate inflammation and liver injury. From the findings of this study, the Mean AST levels of fishes sampled in Pond A and Pond B were not significantly different ($P < 0.05$) from the reference standard values. The Mean AST levels of fishes sampled in Pond C were significantly different ($P < 0.05$) from the reference standard values. The increased levels of AST in Pond C indicate fish liver inflammation, injury, stress and disease and this might be attributed to high levels of temperature, pH and Dissolved oxygen which may have subjected the fish to undue stress and disease. The high values are in accordance with earlier reports of fishes exposed to high levels of ftemperature, pH and Dissolved oxygen (Mazon *et al.*, 2002).

Alanine Aminotransferase is another enzyme that helps to process proteins and large amounts of ALT occur in liver cells (Roberts, 2009). When the liver is injured or inflamed, the blood level of ALT usually rises above this value. As shown in Table 4, the Mean ALT levels of fishes sampled in Pond A and Pond B were not significantly different ($P < 0.05$) from the reference standard values. The Mean ALT levels of fishes sampled in Pond C were significantly different ($P < 0.05$) from the reference standard values. Significant increase in ALT levels of the fishes in Pond C is also an indication of inflammation and disease. The potential of poor pond management to cause damage to the immune system of the fish can lead to increased incidence of liver diseases, hypersensitivity disorders, autoimmune and infectious diseases.

Although some of the possible physico-chemical parameters which affect fish health were analyzed in the present study, it should be recognized that these physio-chemical parameters might not represent all or even the majority of factors that predisposes fishes to injury and liver diseases. The pond water might

contain complex mixture of organic and inorganic chemicals and many unidentified toxicants in the water which may pose risks of unknown magnitude to aquatic biota including fish. Hence, the cause of deviation in the liver enzymes markers (ALP, AST, ALT) of the fishes in Pond C could have been due to one or a combination of known and unknown constituents which have subjected the fish to stress, inflammation and diseases.

Conclusion

The present findings indicate that liver enzyme markers could be used as an assessment of the liver fitness of the fish and changes in the physio-chemical parameters of the pond water can affect the liver function of the fishes. In the present study, it was concluded that the physico-chemical parameters (temperature, pH, Dissolved Oxygen, Alkalinity, Hardness and transparency) of Pond A and Pond B were within the standard limits set by International Regulatory Authority (FEPA) and the liver enzyme markers (ALP, AST, ALT) were within the standard reference values and this confirms the liver fitness of the fishes collected from the ponds. Furthermore, physio-chemical parameters of Pond C were higher than the set limits by FEPA and also the liver enzymes assessed were higher than the standard reference values. Finally, it can be concluded that fishes in Pond C might have one or more forms of liver diseases and inflammation as attributed by high levels of liver enzymes markers.

Recommendations

- i. Proper pond management in areas of water quality and feed quality can help to reduce the incidence of fish liver diseases
- ii. Other tests such as haematological studies and biomarkers assessment should be carried out on the fishes to ascertain the health status
- iii. Other parameters such as heavy metal analysis should also be conducted in the sampled ponds in order to determine the levels of heavy metals and other toxicants in the water.

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Appendix 1: Weight, Length And Liver Function Test For Fishes For Pond 1

S/N0	Weight(g)	Length(cm)	ALP	AST	ALT
1	45	20.5	44.5	51.7	44.1
2	38	18	44.6	52.8	46.5
3	39	15.5	44.1	53.4	55.8
4	35	15	32.1	52.8	54.7
5	43	18.5	43.2	58.4	58.6
6	37	16.5	36.8	52.7	57.9
7	40	19.5	41.8	54.1	46.9
8	50	22	43.4	56.3	47.4
9	49	21.5	44.1	54.1	48.8
10	40	18.5	36.8	53.4	38.7
11	60	25.5	49.1	58.7	57.9
12	44	20.5	45.7	56.4	69.8
13	45	21.4	46.4	56.2	58.9
14	40	20.3	36.8	55.7	65.1
15	45	21	41.4	66.8	66.6
16	38	17.4	36	68.6	69.7
17	70	28	56.8	60.1	57.6
18	38	19.4	41.3	51.2	54.9
19	45	22.5	44.3	55.6	53.9
20	40	20	36.8	68.7	54.8
21	45	21	44.7	47.7	52.4
22	48	24.2	48.9	76.1	54.3
23	47.5	25.2	45.7	52.1	55.5
24	45.5	21.5	44.3	55.7	46.3
25	45.7	21.8	46.8	55.7	42.7
26	45	21.5	45.8	54.2	47.8
27	50	22.6	44.7	51.3	59.7
28	46	22	39.8	52.6	59.3
29	42	20	41.8	43.7	48.8
30	49.5	23.7	45.7	55.7	49.9

Statistical Analysis for pond A

The analysis is done by comparing the mean of the sampled parameters by using one-way ANOVA.

HYPOTHESIS

H_0 : there is at-least a resemblance among the means.

H_1 :there is no mean resemblance.

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	103655.100	13	7973.469	.759	.702
Within Groups	1219335.527	116	10511.513		
Total	1322990.627	129			

$F_{0.05, 8, 81} = 1.94$

Since $F = 0.759$ is not at least equal $F_{0.05, 8, 81} = 1.94$ at 0.05 level of significance. H_0 is not rejected; hence we accept H_0 and assert that there is mean relationship among the observed parameters at α is 5% level of significance.

Appendix 2: Weight, Length And Liver Function Test For Fishes For Pond 2

S/N0	Weight(g)	Length(cm)	ALP	AST	ALT
1	40	22	41.6	56.5	45.6
2	38	19.5	44.7	67.8	45.8
3	45	22.8	42.4	54.3	46.9
4	50	24.5	41.6	65.1	57.5
5	48.6	23.8	41.9	52.2	48.7
6	39.5	22.1	43.5	52.7	35.8
7	60	25.5	44.7	53.8	36.7
8	65	26.5	35.8	61.6	36.8
9	55	24.8	33.5	53.9	65.9
10	42.5	22.6	54.6	56.4	46.5
11	40.4	22	44.5	64.0	36.4
12	52.3	23.2	54.3	58.7	38.3
13	56.1	24.5	45.5	60.8	39.6
14	50	23.4	46.6	64.1	41.7
15	63.1	25	47.5	54.5	55.8
16	50	23	46.2	52.7	56.1
17	40	20.5	35.0	56.4	54.4
18	40	20	56.5	67.2	45.5
19	41.5	21.5	46.4	54.0	43.6
20	45	22.8	57.5	63.5	47.2
21	48	24	46.7	61.7	49.4
22	39.6	22.5	48.6	57.2	53.8
23	50	24.5	57.8	55.8	45.7
24	60.5	25	44.7	58.3	43.3
25	38.8	19.6	43.6	62.5	61.5
26	40	20	45.9	58.7	47.9
27	50	22.5	46.8	64.1	49.7
28	45	21.0	44.6	55.8	52.8
29	47	21.7	47.8	62.4	45.3
30	55	23.5	48.9	55.6	47.5

Statistical Analysis for pond B

The analysis is done by comparing the mean of the sampled parameters by using one-way ANOVA.

HYPOTHESIS

H_0 : there is at-least a resemblance among the means.

H_1 : there is no mean resemblance.

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	245290.513	13	18868.501	.607	.845
Within Groups	3608235.373	116	31105.477		
Total	3853525.886	129			

$F_{0.05, 8, 81} = 1.94$

Since $F = 0.607$ is not at least equal $F_{0.05, 8, 81} = 1.94$ at 0.05 level of significance. H_0 is not rejected; hence we accept H_0 and assert that there is mean relationship among the observed parameters at α is 5% level of significance.

Appendix 3: Weight, Length And Liver Function Test For Fishes For Pond 3

S/N0	Weight(g)	Length(cm)	ALP	AST	ALT
1	60	23.9	54.2	22.6	84.1
2	40	20	56.3	25.6	86.5
3	40.5	20.3	55.4	26.5	85.8
4	42.5	21	54.6	24.4	84.7
5	38.9	19.5	55.6	25.5	78.6
6	45	22	57.7	26.3	87.9
7	50.3	22.2	51.6	35.7	76.9
8	52.3	22.8	50.7	36.6	87.4
9	40	20	55.6	35.2	78.8
10	40	20.4	57.5	36.5	88.7
11	55.5	22.3	58.6	43.4	77.9
12	62.5	23.9	53.5	36.2	89.8
13	54.5	21.8	54.6	44.4	88.9
14	48.2	20.6	56.4	47.3	85.1
15	50	20	55.5	44.5	86.6
16	45	20.3	57.4	35.3	89.7
17	50	21	53.5	43.2	87.6
18	60	24	55.6	35.5	84.9
19	46.6	21.8	66.7	46.4	83.9
20	45	21.4	62.6	34.7	94.8
21	40	19.5	54.4	25.6	82.4
22	40	20	65.5	23.8	84.3
23	40	20.5	56.6	24.5	85.5
24	50	20.5	45.7	45.4	86.3
25	51.5	21.8	43.8	34.7	82.7
26	55.6	22.5	45.9	33.6	87.8
27	61.3	24.4	47.6	34.4	79.7
28	41	20.5	68.6	41.5	89.3
29	45	21	63.7	32.1	78.8
30	40	20	64.6	42.6	79.9

Statistical analysis for C

The analysis is done by comparing the mean of the sampled parameters by using one-way ANOVA.

HYPOTHESIS

H_0 : there is at-least a resemblance among the means.

H_1 :there is no mean resemblance.

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14107.761	13	1085.212	.448	.948
Within Groups	281020.392	116	2422.590		
Total	295128.153	129			

$F_{0.05, 8, 81} = 1.94$

Since $F = 0.448$ is not at least equal $F_{0.05, 8, 81} = 1.94$ at 0.05 level of significance. H_0 is not rejected; hence we accept H_0 and assert that there is mean relationship among the observed parameters at α is 5% level of significance.

10/2/2014