Urea And Creatinine Of Clarias Gariepinus In Three Different Commercial Ponds.

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Abstract: An automated blood serum chemistry analytical system designed for human usage was employed to establish the levels of urea and creatinine parameters present in sera obtained from 90 experimental groups of channel catfish from three commercial ponds ranging 10.2cm-60cm in length and weight ranging 100grams-900grams. The fish *clarias* were divided into three groups with varying length and weight. Group 1 contains 30 catfish 10cm-20cm in length and 100grams-300grams in weight, Group 2 contains 30 catfish 21cm-40cm in length and 301grams-600grams in weight and Group 3 also contains 30 catfish 41cm-60cm in length and 601grams-900grams in weight. The results indicated a significant (p > 0.05) increase in creatinine for all the three groups as the weight increases while there was a significant decrease (p < 0.05) in the urea and creatinine levels of the catfish in group 3 recording 0.00mgdL as the lowest Urea Level and 0.00mgdL as the lowest Creatinine level. The maximum level of Urea and Creatinine was recorded as 10.8mgdL and 2.90mgdL respectively and they are obtained from the catfish in group 3 with weight ranging between 601grams-900grams. Using P < 0.05 level of significance, length (cm) is significant to the weight i.e. P < 0.05 (0.00 < 0.05), likewise creatinine. At a significant level of P < 0.05, it shows that length, Urea and Creatinine of the *C.gariepinus* in group three all depended on their weight. These studies suggest that "normal" values established by any method of sera analysis may be different in the same species depending on the diet, season, and presence of environmental stressors.

[Ajeniyi. S. A. And Solomon. R. J. Urea And Creatinine Of *Clarias Gariepinus* In Three Different Commercial **Ponds.** *Nat Sci* 2014;12(10):124-138]. (ISSN: 1545-0740). <u>http://www.sciencepub.net/nature</u>. 15

Keywords : Urea, Creatine And Clarias Gariapinus.

1. Introduction

Clarias gariepinus of the family claridae is the most common Nigerian fresh water fish species and is prominent in aquaculture practice. They are easily cultured with large economic gains because of their air-breathing and hardy nature, suitable reproductive strategy, nutritional efficiency and attainment of large size in a short time (Fagbenro 1993). The sharptooth catfish (Clarias gariepinus) is one of the most important individuals species in traditional freshwater fisheries in Africa. It is widely attributed in Africa, where it occurs in almost any freshwater habitat but floodplains, large sluggish rivers, lakes and dams. The fish is omnivorous, feeding on fishes, birds, frogs, small mammals reptiles, snails, crabs and other invertebrates. It is also capable of feeding on seeds and fruits. Urea (Blood urea nitrogen) test measures the amount of nitrogen in the blood that comes from the waste product urea, urea is made when protein is broken down in the body. A BUN test is done to see how well the kidneys are working, if the kidneys are not able to remove urea from the blood normally, the BUN level rises. Creatinine is a waste product of muscle turnover. Creatinine also increases as kidney function decreases. Few influences outside the kidney affect creatinine concentration, so it is a better marker of kidney function than BUN. One thing that does affect creatinine is muscle mass, it is measured as a simple blood.Urea and creatinine are nitrogenous end products of metabolism, taken together the BUN and

creatinine levels provide a very accurate estimation of how well the kidneys are working. Both tests are related and are associated with the complete metabolic profile, or CMP. Either test can be run on a blood sample or urine sample. Abnormal levels indicate a kidney or liver-related disease or condition. Any elevation in levels of blood urea nitrogen and/or serum creatinine does not necessarily indicate structural renal disease. Conversely, blood urea nitrogen or serum creatinine values, which appear to be within the range of normal, do not by themselves rule out significant reduction in glomerular filtration rate. Anv interpretation of the blood levels of these two substances must be done with the awareness that a variety of extra renal factors can affect them. The blood urea nitrogen to serum creatinine ratio can be a valuable tool in the determination or renal functional and structural integrity. An increased ratio of BUN to creatinine may be due to conditions that cause a decrease in the flow of blood to the kidneys, such as congestive heart failure or dehydration. It may also be seen with high protein blood levels or from gastrointestinal bleeding. Abnormal levels indicate a kidney or liver related disease or condition.

Experimental Fish Specie.

Clarias gariepinus was chosen because it meets all the requisites conditions for aquaculture namely:-

• It is adaptable to tropical climate conditions and is indigenous to Nigeria

• It can be directly stocked under culture conditions,

- It is highly resistant to disease,
- It has a high growth rate,

• It matures fast and can be induced to produce in captivity,

• It accepts and thrives on cheap diets.

General description of experimental fish.

Catfish of the genus *Clarias* is a prominent freshwater, omnivorous species that are cultured in various parts of the world. They are also known as the "walking catfish" and are distributed throughout Southwest Asia, Africa and Near East, though some are cultured in some European countries.

The sharptooth catfish *Clarias gariepinus* is one of the prominent fish species that has been cultured in Africa and Europe. This specie has been cultured at various levels of intensity in earthen ponds and recirculating water systems. The level of production intensity largely the nutritional regime employed, which ranges from organic fertilization to nutritional complete prepared diets.

It has been recognized that the African catfish (Burchell, 1822) was one of the most suitable species for aquaculture in Africa (Hogendoorn, 1979) and since the seventies, it has been considered to hold great promise for fish farming in Africa. The African catfish having a high growth rate, being resistant to handling and stress, and is being appreciated in a wide number of African countries.

Habitat and feeding habits

C. gariepinus is a nocturnal animal that inhibits calm waters from lakes, streams, rivers, swamps, to floodplains, many of which are subject to seasonal drying. The most common habitats frequented are floodplain swamps and pools in which the catfish can survive during the dry seasons due to the presence of their accessory air breathing organs. They feed on animal matter: either live or dead. Due to a wide mouth, they are able to gulp relatively big prey whole, crawl on dry ground to escape drying pools, search for food or avoid capture and survive in shallow mud for long periods of time, between rainy and dry seasons.

Urea and Creatinine

Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover. Creatinine is the product of muscle creatine catabolism. Both are relatively small molecules (60 and 113 daltons, respectively) that distribute throughout total body water. In Europe, the whole urea molecule is assayed, whereas in the United States only the nitrogen component of urea (the blood or serum urea nitrogen, i.e., BUN or SUN) is measured. The Urea, then, is roughly one-half (28/60 or 0.446) of the blood urea. BUN stands for Blood Urea Nitrogen, it is a waste product produced from the breakdown of protein. Blood urea is removed from the body via the urine, so BUN levels increase as kidney function decreases. Any increase in protein introduced into the intestines to be digested (such as a very high protein diet of meat or blood proteins from a bleeding ulcer) can increase urea in the blood. Dehydration also increases the urea value. Urea is measured as a simple blood test. It is produced when the livers participates in protein metabolism and it is usually eliminated from the body by the kidneys. Therefore, both the livers and the kidneys must be functioning properly for the body to maintain a normal level of urea in the blood.

Creatinine is a substance the body produces during normal metabolism. The body eliminates creatinine almost exclusively through the kidneys filtration process, so measurement of creatinine is an accurate estimation of how well the kidney filtration processes are working. Anything that alters the ability of kidneys to filter efficiently (such as dehydration) can cause changes in creatinine levels in the blood. Creatinine is a waste product of muscle turnover. Creatinine also increases as kidney function decreases. Few influences outside the kidney affect creatinine concentration, so it is a better marker of kidney function than urea. One thing that does affect creatinine is muscle mass. It is measured as a simple blood test.

Taken together, and usually combined with results of a *urinalysis* (a screening test to evaluate components in the urine), the urea and creatinine levels provide a very accurate estimation of how well the kidneys are working. The urea and creatinine levels are frequently part of a blood test known as *chemistry panel*, so they are often evaluated during routine wellness checkups or pre-surgery screening in healthy pets.

Often, the urea and creatinine levels are evaluated along with other blood tests that screen for the abnormalities involving the kidneys.

Technique

Multiple methods for analysis of urea and creatinine have evolved over the years. Most of those in current use are automated and give clinically reliable and reproducible results.

There are two general methods for the measurement of urea nitrogen. The diacetyl, or Fearon, reaction develops a yellow chromogen with urea, and this is quantified by photometry. It has been modified for use in autoanalyzers and generally gives relatively accurate results. It still has limited specificity, however, as illustrated by spurious elevations with sulfonylurea compounds, and by colorimetric interference from hemoglobin when whole blood is used. In the more specific enzymatic

methods, the enzyme urease converts urea to ammonia and carbonic acid. These products, which are proportional to the concentration of urea in the sample, are assayed in a variety of systems, some of which are automated. One system checks the decrease in absorbance at 340 mm when the ammonia reacts with alpha-ketoglutaric acid. The Astra system measures the rate of increase in conductivity of the solution in which urea is hydrolyzed.

Even though the test is now performed mostly on serum, the term BUN is still retained by convention. The specimen should not be collected in tubes containing sodium fluoride because the fluoride inhibits urease. Also chloral hydrate and guanethidine have been observed to increase BUN values.

The 1886 Jaffé reaction, in which creatinine is treated with an alkaline picrate solution to yield a red complex, is still the basis of most commonly used methods for measuring creatinine. This reaction is nonspecific and subject to interference from many noncreatinine chromogens, including acetone. acetoacetate, pyruvate, ascorbic acid, glucose, cephalosporin's, barbiturates, and protein. It is also sensitive to pH and temperature changes. One or another of the many modifications designed to nullify these sources of error is used in most clinical laboratories today. For example, the recent kinetic-rate modification, which isolates the brief time interval during which only true creatinine contributes to total color formation, is the basis of the Astra modular system.

More specific, non-Jaffé assays have also been developed. One of these, an automated dry-slide enzymatic method, measures ammonia generated when creatinine is hydrolyzed by creatinine iminohydrolase. Its simplicity, precision, and speed highly recommend it for routine use in the clinical Only 5-fluorocytosine laboratory. interferes significantly with the test. Creatinine must be determined in plasma or serum and not whole blood because erythrocytes contain considerable amounts of noncreatinine chromogens. To minimize the conversion of creatinine, to creatinine, specimens must be as fresh as possible and maintained at pH 7 during storage.

Basic Science Of Urea and Creatinine

More than 99% of urea synthesis occurs in the liver. Its primary source is dietary protein. In the gut, the protein is converted into peptides and amino acids, more than 90% of which are absorbed and carried to the liver. In the hepatocyte, the amino acids are deaminated and transaminated. The resulting excess nitrogen feeds into the urea cycle to be incorporated into urea. The protein moieties escaping absorption by the small bowel, plus recycled urea, are converted into ammonia by gut flora predominantly in the colon. The

ammonia diffuses through the portal circulation into the liver to enter the urea cycle. The amount of urea produced varies with substrate delivery to the liver and the adequacy of liver function. It is increased by a high-protein diet, by gastrointestinal bleeding (based on plasma protein level of 7.5 g/dl and a hemoglobin of 15 g/dl, 500 ml of whole blood is equivalent to 100 g protein), by catabolic processes such as fever or infection, and by antianabolic drugs such as tetracyclines (except doxycycline) or glucocorticoids. It is decreased by low-protein diet, malnutrition or starvation, and by impaired metabolic activity in the liver due to parenchymal liver disease or, rarely, to congenital deficiency of urea cycle enzymes. The normal subject on a 70 g protein diet produces about 12 g of urea each day.

This newly synthesized urea distributes throughout total body water. Some of it is recycled through the enterohepatic circulation. Usually, a small amount (less than 0.5 g/day) is lost through the gastrointestinal tract, lungs, and skin; during exercise, a substantial fraction may be excreted in sweat. The bulk of the urea, about 10 gm each day, is excreted by the kidney in a process that begins with glomerular filtration. At high urine flow rates (greater than 2 ml/min). 40% of the filtered load is reabsorbed, and at flow rates lower than 2 ml/min, reabsorption may increase to 60%. Low flow, as in urinary tract obstruction, allows more time for reabsorption and is often associated with increases in antidiuretic hormone (ADH), which increases the permeability of the terminal collecting tubule to urea. During ADHinduced antidiuresis, urea secretion contributes to the intratubular concentration of urea. The subsequent buildup of urea in the inner medulla is critical to the process of urinary concentration. Reabsorption is also increased by volume contraction, reduced renal plasma flow as in congestive heart failure, and decreased glomerular filtration.

Creatinine formation begins with the transamidination from arginine to glycine to form glycocyamine or guanidoacetic acid (GAA). This reaction occurs primarily in the kidneys, but also in the mucosa of the small intestine and the pancreas. The GAA is transported to the liver where it is methylated by S-adenosyl methionine (SAM) to form creatinine. Creatinine enters the circulation, and 90% of it is taken up and stored by muscle tissue. In a reaction catalyzed by creatinine phosphokinase (CPK), most of this muscle creatinine is phosphorylated to creatine phosphate. Each day, about 2% of these stores is converted nonenzymatically and irreversibly to creatinine. Thus, creatinine production essentially reflects lean body mass. Because this mass changes little from day to day, the production rate is fairly constant. Absolute creatinine production declines with

age in line with decreasing muscle mass. Unlike urea, creatinine is largely unaffected by gastrointestinal bleeding or by catabolic factors such as fever and steroids. However, the ingestion of cooked meat can raise the ^sCr because cooking converts the creatine in meat to creatinine. Certain drugs, notably the psychoactive phenacemide, can increase the production rate. Like urea, creatinine distributes throughout total body water. Its concentration in serum is a function of the usually constant production and excretion rates. It may be slightly higher in the evening than in the morning, due most likely to dietary meat intake by day.

In normal subjects, creatinine is excreted primarily by the kidneys. There is minimal extrarenal disposal or demonstrable metabolism. As a small molecule (molecular weight of 113 daltons), it is freely filtered by the glomerulus. Unlike urea, it is not reabsorbed or affected by urine flow rate. It is normally secreted by the tubules in a small but significant amount (up to 10% of total excretion). Excretion of both urea and creatinine is increased during exercise without producing significant change in serum concentration.

Clinical Significance

The Blood urea nitrogen and ^sCreatinine are screening tests of renal function, Because they are handled primarily by glomerular filtration with little or no renal regulation or adaptation in the course of declining renal function, they essentially reflect GFR. Unfortunately, their relation to GRF is not a straight line but rather a parabolic curve. Their values remain within the normal range until more than 50% of renal function is lost. Within that range, however, a doubling of the values (e.g., BUN rising from 8 to 16 mg/dl or ^s Creatinine from 0.6 to 1.2 mg/dl) may mean a 50% fall in the GFR. Therefore, in the early stages of renal disease, these tests could create a false sense of security. Random values above the midrange of normal should be corroborated by a normal ^c Creatinine before one can confidently tell a patient that his or her kidney function is normal. At all stages of renal insufficiency, the Creatinine is a much more reliable indicator of renal function than the BUN because the BUN is far more likely to be affected by dietary and physiologic conditions not related to renal function. For example patients with congestive heart failure and intact kidneys commonly present with a BUN of 50 to 70 mg/dl and an Creatinine below 1.2 mg/dl. Of course, Creatinine may rise under some of these extra renal factors, but seldom will it exceed 3 to 4 mg/dl.

BUN-to-Creatinine Ratio

In medicine, the **BUN-to-creatinine ratio** is the <u>ratio</u> of two serum laboratory values, the blood urea nitrogen (BUN) (mg/dL) and serum creatinine

(mg/dL) (Cr). Outside the United States, particularly in Canada and Europe, the truncated term urea is used (though it is still the same blood chemical) and the units are different (mmol/L). The units of creatinine are also different (μ mol/L), and this value is termed the **urea-to-creatinine ratio**. The ratio may be used to determine the cause of acute kidney injury or dehydration.

The principle behind this ratio is the fact that both urea (BUN) and creatinine are freely filtered by the glomerulus, however urea reabsorbed by the tubules can determine the cause regulated (increased or decreased) whereas creatinine reabsorption remains the same (minimal reabsorption).

When a blood sample is tested, a doctor may evaluate the amount of urea in your blood compared to the amount of creatinine, or the urea/creatinine ratio. This ratio can be used to determine what type of kidney disease is causing abnormal levels of urea or creatinine. Normal urea/creatinine ratios fall between 10 to 1 and 20 to 1. An increased ratio can indicate congestive heart failure or dehydration. A decreased ratio can indicate gastrointestinal bleeding, liver disease or malnutrition.

Normal Kidney Urea and Creatinine Levels

A blood urea nitrogen, or BUN, and creatinine test are usually used in conjunction to measure kidney function, help diagnose kidney disease or monitor kidney status in those who have already been diagnosed with kidney disease. Urea and creatinine tests are often ordered when various signs and symptoms indicate that a kidney disorder might be present. The levels are as follows:-

Normal Blood Urea Nitrogen

When the body metabolizes protein, the liver produces nitrogen. Nitrogen combines with other molecules in the liver to form a waste product called urea. Urea travels through the bloodstream until it reaches the kidneys, where it is filtered out of the blood and deposited into the urine. Normally, the blood and urine both contain small amounts of urea because protein is constantly being metabolized in the body. Normal BUN values range from 7 to 20 mg/dL

Abnormal Blood Urea Nitrogen

High BUN values can indicate kidney disease, congestive heart failure, gastrointestinal bleeding, shock, kidney failure, hypovolemia, a heart attack or a urinary tract obstruction. Low BUN values may occur as a result of liver failure, malnutrition or overhydration, which is a condition in which too much fluid accumulates in the body.

Normal Creatinine

The muscles produce a waste product called creatinine when they break down a compound called creatine, which helps contract the muscles. Most of the creatinine in the body is removed by the kidneys. Because of this, the levels of creatinine in the blood can indicate whether the kidneys are functioning properly. Normal creatinine values range from 0.8 to 1.4 mg/dL. Normal creatinine values for females are usually lower because females usually have a lower muscle mass.

WWW.ncbi.nlm.nih.gov/books/NBK305

Literature Review

Different research have been carried out on Clarias gariepinus and other species of fish to know the level of urea and creatinine in their blood. In a research 'Effects of paraquat dichloride on some metabolic and enzyme parameters of Clarias gariepinus' (E.N Ogamba, I.R Invang and I.K Azuma,2010). Juvenile C. gariepinus were exposed to varying sub-lethal concentrations of paraquat dichloride (0.05, 0.10, 0.20, 0.30 and 0.40 ppm) for four days. The results indicated a significant (p < 0.05) decrease in total protein and urea with increased concentration of the toxicant. The lowest value of urea was recorded in the gill (2.33±0.05 SD) at 0.40 ppm compared to the control $(2.37\pm0.10 \text{ SD})$. The fluctuations were not significant (p>0.05). For creatinine, the lowest value was recorded in the muscle $(0.4\pm0.60 \text{ SD})$ at 0.4 ppm concentration of paraguat, while the highest value of creatinine for both muscle and gill tissues (0.6±0.00 SD) and (0.97±05 SD), respectively, were recorded at 0.1 ppm concentration of paraguat.

Total protein, urea, and albumin in the gills decreased with increased concentration of paraquat dichloride. However, creatinine in gills did not vary significantly (p>0.05). Invang (2008) reported similar results in plasma total protein, albumin, glucose and organ's total urea and creatinine of Clarias gariepinus exposed to diazinon. Similarly, Sastry et al. (1982) and Das and Mukharjee (2000), observed that exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism, depletion of total protein in the plasma and serum of fish. Similarly, the decrease in creatinine levels in fish exposed to paraguat dichloride compared to the control suggest that creatinine was completely used up by the muscle as a result of the stress induced by the toxicant.

I.R Inyang, U.U Gabriel and E.N Ogamba (2010) studied the "Sublethal effects of diazinon selected metabolic parameters of *Clarias gariepinus*" the effect of diazinon on *Clarias gariepinus* was evaluated using some metabolic parameters (glucose, creatinine, bilirubin, albumin, and total urea) of the fish. The fish were exposed to varying sublethal levels of the toxicant at different concentrations (1.00ppm, 2.50ppm, 5.00ppm, 7.50ppm and 10.0ppm) for 30 days to access their effects on some metabolic status of the fish. Creatinine and total urea values decreased with increasing concentration of the toxicant. Glucose values increased as the concentration of the toxicant increases. Careless use of chemicals such as pesticides may end up in aquatic environment and alter the physiological properties of aquatic organisms. The toxicity of pesticides to aquatic organisms

are incontestable. Pesticide toxicity to fish has been investigated in several studies e.g. Moore and Waring, (2001), Agbon *et al* (2002), Chindah, (2005), Inyang (2008). Diazinon is a widely used toxicant in a number of organ phosphorous pesticides (Roberts and Hutson, 1998), Urea and creatinine have been used as important indices for the evaluation of the effects of chemical on the kidney using a variety of both *invo and in vitro* methods (Davis and Bernat, 1994). A decreased concentrations of these metabolites suggested that the kidney may not be affected by the toxicant.

In a recent research by Kamal A Amin and Khalid S Hashem (2012) on "Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish" deltamethrin is a class of insecticides being used as as substitutes for organochlorines and organophosphates in pest-control programs because of their low environmental persistence and toxicity. Their study was aimed to investigate the impact of commonly used pesticides (deltamethrin) on the blood and tissue oxidative stress level in catfish (Clarias gariepinus). The catfish were divided into three groups, 1st control group include 20 fish divided into two tanks each one contain 10 fish, 2nd deltamethrin group, where Fish exposed to deltamethrin in a concentration (0.75 µg/l) and 3rd Vitamin E group, Fish exposed to deltamethrin and vitamin E at a dose of $12 \mu g/l$ for successive 4 days.

The effect of deltamethrin on renal function tests were observed, urea levels were significantly elevated in deltamethrin exposed group in comparison with control group, this elevation may be due to correlation between urea and increased protein catabolism or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production. Urea and creatinine levels increased which may result either from increased breakdown of tissue or dietary or impaired excretion or increased synthesis or decreased urinary clearance by the kidney or decreased degradation of these compounds. Their data suggest that polluted fish adept glomerular dysfunction rather than tubular insufficiency as blood levels of urea and creatinine depend largely on glomerular function. In consistent with these explanations of decreased total protein level with deltamethrin administration, urea is the end product of protein catabolism in mammals but in fish ammonia is the end products of protein. So the marked increase in blood urea nitrogen could be attributed to impaired

excretion of urea through kidney and this explanation is supported by increasing blood creatinine level which is more sensitive and specific indicator of impaired kidney function.

Kori Siakpere Ovie (2008) also studied "The effects of sub lethal concentrations of potassium permanganate on nitrogenous waste products of African catfish". Changes in the nitrogenous waste products of the fish (Burchell 1822) subjected to sub lethal concentrations (2.0, 6.0, and 10.0mg/L) of potassium permanganate over a period of 192hrs were studied in a semistatic (renewal) system. The nitrogenous waste metabolism was reflected in the changes in total plasma bilirubin, plasma uric acid, plasma creatinine and plasma urea. Increased creatinine concentration in the blood suggest the inability of the kidney to excrete these products, a manifestation of nephritic damage, decreased plasma urea may be attributable to the depleted protein levels. The effects of potassium on the levels of creatinine were elevated while urea was depleted during their research. Plasma creatinine levels in the treated fish showed a seemingly dose and time-dependent trend with the maximum elevation percentage (40.10) being recorded in 10mg/l KMnO4 exposed fish at 192hrs. There was a progressive decrease with increase in the concentration levels, example, the mean levels in the 10mg/l KMnO4 exposed fish were 419.34, 390.98. 303.94, 244.65 and 215.41mg/dl at 12, 24, 48, 96, and 192hours respectively.

In a research work by I.M Adekunle (2010) on the "Potential nephrotoxicity in African mud catfish following exposure to compost derived humic acid" influence of composed-derived humic acid (HA) on nephrotoxicity in juvenile African mud catfish was evaluated in static water culture. Fish samples were exposed to different HA concentrations (0, 100, 250, 500 and 1000mg L⁻¹) for 45 days at 5 samples per aquarium. Renal functions was assessed spectrophotometrically via levels of creatinine by Jaffe method and urea by Nesserelization method. The results revealed that the mean value of urea in the exposed group (1) at each HA concentration was lower than the value found in 1 relative to II: Significant (p< 0.05) variations for 1 and II were obtained, relative to increasing HA concentrations, decreasing albumin (0.84 to 0.43gdL), urea (5.21 to 1.95mgdL) and increasing creatinine (0.20 to 1.53mgdL) were recorded.

The objective of the study was to assess the nephrotoxicity potential of humic acid isolated from composted wastes (organic fertilizer) of Nigeria origin to African mud catfish (*C.gariepinus*) grown in static water culture using the three biomarkers, serum creatinine, albumin, and urea. The creatinine level in the control group was $0.36 \pm 0.09 \text{ mgdL}^{-1}$ and ranged

from 0.26 to 1.53mgdL⁻¹ in the test groups. Positive correlation was obtained for creatinine and HA concentrations (+0.704; p<0.10) indicating rising blood levels of creatinine with increasing HA concentration in water. The urea level in the control was group was 5.61 ± 0.07 mgdL⁻¹ in the test groups. Correlations gave negative coefficients for urea versus HA concentration for urea versus HA concentration (-0.568; p>0.10) indicating a decrease trend with increasing HA concentration in water.

2. Materials And Methods Experimental Fish

Ninety catfish (*Clarias gariepinus*) with uniform lengths ranging from 10.2 to 60cm and weights from 100 to 900grams, were collected from three commercial ponds namely Kuje, Abaji, and Gwagwalada (Abuja).

The fishes were divided into three groups with varying length and weight. Group 1 contains 30 catfish ranging from 10 to 20cm in length and 100 to 300grams in weight, Group2 contains 30 fish 21 to 40cm in length and 301 to 600grams in weight and Group 3 contains 30 fish ranging from 41 to 60cm in length and 601 to 900grams in weight. All fish under experimentation were examined individually and recorded for any skin lesions or furunculosis (Foda, 1973)

Collection Of Blood Samples

Fish were caught individually in a small hand net from the containers. After the preliminary investigation of the length and weight, the fish were then placed belly upwards and blood samples obtained from the caudal circulation with the aid of a heparinized 2cm3 disposable plastic syringes and a 21 gauge disposable hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation time. The site chosen for puncture (about 3 - 4cm from the genital opening) was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted perpendicularly to the vertebral column of the fish and gently aspirated during penetration. It was then pushed gently down until blood started to enter as the needle punctured a caudal blood vessel.

Blood was taken under gentle aspiration until about 1cm3 has been obtained, then the needle was withdrawn and the blood gently transferred into lithium heparin anticoagulant tube and allowed to clot at room temperature for 30 - 40 minutes.

Centrifuging of Blood Sample

The blood in the anticoagulant tubes were collected and then transferred into clean dry centrifuge tubes and centrifuged at 4000rpm for 10minutes, followed by serum separation.

Separation of Serum from Blood The serum was separated from the blood after centrifuging for 10minutes by using a pasteur pipette and transferred into a anticoagulant free test-tube and stored in a refrigerator until analysis.

Determination of Urea

Urea was determined via Nesselerization method, described in Pratt, (1996) and Aitken *et al.* (2003). Three test-tubes labeled Blank (B), Standard (S), and Sample (T) were used according to the Centromic Gmbit kit manual (Urea-indicator fluid, German), 1mL of working reagent was transferred to B,S, and T. Exactly 10uL of distilled water was added in each tube and incubated for 10minutes at room temperature. The absorbance values of the sample and standard were read against the reagent blank. Plasma urea was expressed in mg dL⁻¹ and measured at a wavelength of 340nm.

Determination of Creatinine

Creatinine was determined bv Jaffe spectrophotometric method described in Pratt, (1996) and Aitken et al. (2003). The working reagent, samples and standard were prepared at room temperature. Two test-tubes labeled S for standard and T for sample, and 1ml of the working reagent was into both followed by the introduction of 100uL of standard into S and 100uL sample into T. The content of each tube was gently mixed, distilled water was used to zero the automatic chemical analyzer and the absorbance values of the standard and sample were recorded at 500nm after 30 and 90 seconds. All the reagents are used as directed by the manufacturer's manual using Sodium(1+1) fluid (Centromic Gmbit, German). Distilled water was used for blank test, serum creatinine was expressed in mg dL⁻¹ and measured at a wavelength of 340nm.

Statistical analysis

The obtained data were subjected to statistical analysis using one-way analysis of variance

(ANOVA) to test for level of significance between the various levels of Urea and Creatinine of the three ponds. The descriptive statistics mean and standard deviation were also analyzed. All analyses were performed using the software programme (Statistical Package For Social Sciences version 20).

3.Results And Analysis Results

Table 1 shows The results of the physical characteristics and haematological parameters of *C.gariepinus* in pond one. The maximum weight recorded is 300.4g while the minimum weight recorded is 101.7g, the maximum length recorded is 20.3cm while the minimum length is 10.2cm, the maximum level of Urea recorded is 2.65mgdL while the minimum level recorded is 0.04mgdL. The highest level of Creatinine recorded is 0.37mgdL and the lowest level is 0.11mgdL.

Table 2 shows The results of the physical characteristics and haematological parameters of *C.gariepinus* in pond two. The maximum weight recorded is 597.0g while the minimum weight recorded is 305.0g, the maximum length recorded is 40.9cm while the minimum length is 21.0cm, the maximum level of Urea recorded is 6.30mgdL while the minimum level recorded is 0.60mgdL. The highest level of Creatinine recorded is 1.58mgdL and the lowest level is 0.20mgdL.

Table 3 shows The results of the physical characteristics and haematological parameters of *C.gariepinus* in pond three. The maximum weight recorded is 900.6g while the minimum weight recorded is 501g, the maximum length recorded is 60.0cm while the minimum length is 41.3cm, the maximum level of Urea recorded is 10.8mgdL while the minimum level recorded is 0.00mgdL. The highest level of Creatinine recorded is 2.90mgdL and the lowest level is 0.00mgdL.

s/no	Weight(gm.)	Length(cm)	Urea(mg/dl)	Creatinine(mg/dl)		
1	254.2	15.1	2.23	0.14		
2	153.4	11.3	2.65	0.11		
3	156.2	10.3	2.32	0.12		
4	255.5	13.0	0.43	0.24		
5	255.1	14.1	1.25	0.17		
6	154.1	12.2	0.66	0.22		
7	157.3	12.4	0.14	0.19		
8	287.3	19.5	1.03	0.21		
9	258.5	17.3	0.32	0.32		
10	180.0	11.5	0.46	1.30		
11	295.9	16.1	1.03	0.25		
12	300.4	15.2	0.35	0.13		
13	228.2	12.0	0.46	0.24		

Table 1: Physical and Haematological characteristics of C.gariepinus in group one

14	300.1	16.0	2.12	0.30		
15	289.3	20.3	1.07	0.31		
16	199.4	16.5	2.28	1.29		
17	300.2	17.2	1.03	0.35		
18	101.7	10.2	0.85	0.31		
19	105.5	10.4	0.77	0.28		
20	200.2	14.1	0.98	0.37		
21	220.3	13.4	0.61	0.26		
22	300.1	18.2	1.23	0.34		
23	291.4	20.0	0.85	0.26		
24	154.7	11.1	0.04	0.19		
25	250.6	16.6	0.96	0.24		
26	230.6	14.2	0.32	0.22		
27	223.4	12.1	2.54	0.22		
28	275.3	16.0	3.00	0.28		
29	280.0	18.2	0.10	0.30		
30	143.5	11.3	1.34	1.04		
s/no	Weight(gm.)	Length(cm)	Urea(mg/dl)	Creatinine(mg/dl)		



Figure 1: Physical and Haematological characteristics of C.gariepinus in pond one

Analysis of Variance for the physical and haematological characteristics of *C.gariepinus* in group one

The length, weight, urea and creatinine levels of catfish in group one were tested using Analysis of Variance (ANOVA) at p = 0.05.

From the table above, using P < 0.05 level of significance, Length(cm) is not significant to the

Weight i.e. P>0.05 (P=0.383), the Urea (mg/dl) is not significant to the weight, P>0.05 (P=0.540). Creatinine (mg/dl) is not significant to the weight since 0.072> 0.05, there urea and creatinine levels of catfishes in group one is not significant to their weights. I accept H_o which says There is no significant difference in the levels of Urea and Creatinine of catfishes in relation to their weights.

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Table 2: Physical and Haematological characteristics of C.gariepinus in group two

Figure 2: Physical and Haematological characteristics of *C.gariepinus* in pond two Analysis of Variance for the physical and haematological characteristics of *C.gariepinus* in group two

The length, weight, urea and creatinine levels of catfish in group two were tested using Analysis of Variance (ANOVA) at p = 0.05.

From the above table, using P < 0.05 level of significance, Length (cm) is not significant to the Weight i.e. P>0.05 (P=0.113), the Urea (mg/dl) is not

significant to the weight, P>0.05 (P=0.647). Creatinine (mg/dl) is not significant to the weight since 0.777> 0.05, there urea and creatinine levels of catfishes in group two is not significant to their weights. I accept H_0 which says There is no significant difference in the levels of Urea and Creatinine.

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S/NO	Weight(g)	Length(cm)	Urea(mg/dl)	Creatinine(mg/dl)	
1	605.0	45.3	7.03	1.40	
2	650.1	48.5	5.90	0.39	
3	770.3	52.1	8.50	2.06	
4	620.2	42.4	9.26	1.91	
5	822.1	55.6	8.06	1.40	
6	820.2	57.1	8.00	2.67	
7	900.6	59.6	8.80	2.90	
8	615.2	44.7	1.10	0.00	
9	850.0	51.4	6.60	1.67	
10	864.1	57.3	3.60	1.35	
11	822.2	54.9	2.49	1.32	
12	900.2	58.1	0.90	1.48	
13	625.0	48.0	0.94	2.00	
14	610.1	43.3	0.89	2.14	
15	808.1	50.5	10.4	1.54	
16	810.0	52.8	10.8	0.61	
17	687.1	49.6	8.01	1.27	
18	629.5	41.3	10.0	1.42	
19	611.1	47.6	9.15	0.97	
20	800.4	55.1	1.90	0.61	
21	880.4	60.0	1.57	1.93	
22	610.0	48.3	20.3	0.85	
23	822.0	52.3	4.98	1.05	
24	750.1	53.5	5.05	1.35	
25	720.1	52.3	4.59	0.29	
26	740.0	57.5	6.20	1.15	
27	637.1	51.1	0.00	1.37	
28	665.0	51.7	2.08	1.32	
29	880.0	60.0	0.00	2.89	
30	501.1	55.4	4.80	0.19	



Figure 3 : Physical and Haematological characteristics of C.gariepinus in pond three

Analysis of Variance for the physical and haematological characteristics of *C. gariepinus* in group three

The length, weight, urea and creatinine levels of catfish in group three was tested using Analysis of Variance (ANOVA) at p = 0.05. From the table below, using P < 0.05 level of significance, Length (cm) is significant to the Weight i.e. P < 0.05 (P=0.000), Urea

(mg/dl) is significant to the weight (P=0.000), Creatinine (mg/dl) is also significant to the weight since 0.000 < 0.05. The test statistic is significant in group three, length, urea and creatinine level all depended on the weights of the catfishes. Therefore I accept H₁ which says There is a significant difference in the levels of Urea and Creatinine of catfishes in relation to their weights.

Table 4 shows The descriptive statistics for the physical and haematological characteristics of *C.gariepinus* in pond one. The mean of the physical characteristics, length and weight were 14.53cm and 226.75g respectively. The mean of the haematological parameters, Urea and Creatinine were 1.11mgdL and 0.34mgdL respectively.

	Total number	Mean	Std. Error	Std. Deviation
Weight (gm)	30	226.75	11.442	62.671
Length (cm)	30	14.53	0.554	3.036
Urea (mg/dl)	30	1.11	0.152	0.834
Creatinine(mg/dl)	30	0.34	0.056	0.305
Valid N (list wise)	30			

Interpretation Of Result

From the descriptive statistics t the mean weight is 226.75 and the standard error is 11.442 and the standard deviation is 62.671, in addition the Length(cm) average mean is given as 14.53 and the standard error is 0.554 and the standard deviation is 3.036, the Urea (mg/dl) average mean 1.11 and standard error 0.152 and the standard deviation is 0.834, lastly the Creatinine(mg/dl) average mean tends to be 0.34 and the standard error is 0.56 while the standard deviation is 0.305

Table 5 shows the ANOVA of the physical characteristics and haematological parameters of *C.gariepinus* in pond one. Using P < 0.05 level of significance, length (cm) is not significant to the weight i.e. P > 0.05 (0.383 > 0.05), the Urea (mg./dl) is also not significant to the weight i.e. P > 0.05 (0.540 > 0.05) and the Creatinine is also not significant to the weight since 0.072 > 0.05. It can be observed from the table that the levels of Urea and Creatinine of *C.gariepinus* in group one does not depend on the weight. The low levels of Creatinine recorded might be related to low muscle build-up in the catfishes, low carbohydrate intake and also dehydration while the significantly low level of Urea might be due to the inability of the kidneys to filter waste.

		Sum of Squares	DF	Mean Square	F	Sig.
Length (cm)	Between Groups	264.839	28	9.459	3.908	.383
	Within Groups	2.420	1	2.420		
	Total	267.259	29			
Urea(mg/dl)	Between Groups	19.789	28	.707	1.785	.540
	Within Groups	.396	1	.396		
	Total	20.185	29			
Creatinine(mg/dl)	Between Groups	2.697	28	.096	120.4	.072
	Within Groups	.001	1	.001	11	
	Total	2.698	29			

Table 6 shows the descriptive statistics for the physical characteristics and haematological parameters of *C.gariepinus* in pond two. The mean of the physical characteristics, length and weight were 30.15cm and 430.03g respectively. The mean of the haematological parameters, Urea and Creatinine were 0.26mgdL and 0.08mgdL respectively.

	Total number	mean	Std. Error	Std. Deviation
Weight (gm)	30	430.03	15.667	85.810
Length (cm)	30	30.15	1.159	6.345
Urea (mg/dl)	30	2.69	.266	1.458
Creatinine(mg/dl)	30	.86	.085	.464
Valid N (list wise)	30			

Interpretation Of Results

From the descriptive statistics the mean weight is 430.03 and the standard error is 15.667 and the standard deviation is 85.810, in addition the Length (cm) average mean is given as 30.15 and the standard error is 1.159 and the standard deviation is 6.345, the

Urea (mg/dl) average mean 2.69 and standard error 0.266 and the standard deviation is 1.458, lastly the Creatinine(mg/dl) average mean tends to be 0.86 and the standard error is 0.85 while the standard deviation is 0.464. of catfishes in relation to their weights.

Table 7 shows: the ANOVA of the physical characteristics and haematological parameters of *C.gariepinus* in pond two. Using P < 0.05 level of significance, length (cm) is not significant to the weight i.e. P > 0.05 (0.113 > 0.05), the Urea (mg/dl) is also not significant to the weight i.e. P > 0.05 (0.647 > 0.05) and the Creatinine is also not significant to the weight since 0.777 > 0.05. It can be observed from the table that the levels of Urea and Creatinine of *C.gariepinus* in group one does not depend on the weight. It can be observed from the table that the levels of Urea and Creatinine in pond two are generally greater than those in group one, this can be related to the efficiency of the kidney, the protein and carbohydrates intake of the catfishes, state of hydration and so on.

		Sum of Squares	DF	Mean Square	F	Sig.
Length (cm)	Between Groups	1117.243	25	44.690	3.545	.113
	Within Groups	50.432	4	12.608		
	Total	1167.675	29			
Urea (mg/dL)	Between Groups	52.001	25	2.080	.863	.647
	Within Groups	9.636	4	2.409		
	Total	61.637	29			
Creatinine	Between Groups	5.014	25	.201	.652	.777
(mg/dL)	Within Groups	1.231	4	.308		
	Total	6.245	29			

Table 8 shows: the descriptive statistics for the physical characteristics and haematological parameters of *C.gariepinus* in pond three. The mean of the physical characteristics, length and weight were 51.91cm and 734.24g respectively. The mean of the haematological parameters, Urea and Creatinine were 5.73mgdL and 1.38mgdL respectively.

	Total number	Mean	Std. Error	Std. Deviation
Weight (gm)	30	734.24	20.530	112.445
Length (cm)	30	51.91	.961	5.265
Urea (mg/dL)	30	5.73	.801	4.387
Creatinine (mg/dl)	30	1.38	.134	.736
Valid N (list wise)	30			

Interpretation Of Result

From the descriptive statistics the mean weight is 734.24 and the standard error is 20.530 and the standard deviation is 112.445, in addition the Length (cm) average mean is given as 51.91 and the standard error is 0.961 and the standard deviation is 5.265, the

Urea (mg/dl) average mean 5.73 and standard error 0.801 and the standard deviation is 4.387, lastly the Creatinine(mg/dl) average mean tends to be 1.38 and the standard error is 0.134 while the standard deviation is 0.736.

Table 9 shows the ANOVA of the physical characteristics and haematological parameters of *C.gariepinus* in pond three. Using P < 0.05 level of significance, length (cm) is significant to the weight i.e. P < 0.05 (0.00 < 0.05), likewise creatinine. At a significant level of P < 0.05, it shows that length, Urea and Creatinine of the *C.gariepinus* in pond three all depended on their weight. Generally, it can be observed from table three that both the lengths and weights of the *C.gariepinus* are larger than those in group one and two. It can also be observed that both the Creatinine and Urea recorded a very high level which is 2.90mgdL and 10.8mgdL respectively and a very low level 0.00mgdL and 0.00mgdL for both the Creatinine. The Creatinine levels in Group three can be referred to has been abnormal, abnormal levels of Creatinine are found in conditions and diseases affecting the kidneys. The Creatinine level measures kidney function will also have very levels of Creatinine. Low level of Creatine are found in conditions that cause decreased muscle mass. Diseases and conditions that result in an elevated abnormal blood urea level can include high-protein diets, chronic diseases, and heart failure, decreased levels of BUN can indicate liver failure and malnutrition.

		Sum of Squares	DF	Mean Square	F	Sig.
Length (cm)	Between Groups	803.787	29	27.717	.000	.000.
	Within Groups	.000	0	.000		
	Total	803.787	29			
Urea (mg/dL)	Between Groups	558.086	29	19.244	.000	.000
	Within Groups	.000	0	.000		
	Total	558.086	29			
Creatinine(mg/dl)	Between Groups	15.691	29	.541	.000	.000
	Within Groups	.000	0	.000		
	Total	15.691	29			

4.Discussion

Blood biochemical values are not commonly used as a diagnostic tool in fish medicine, partly because of the lack of reference intervals for various fish species, and because changes in blood analysis associates with specific diseases and metabolic disorders are not well characterized. With sufficient background data, clinical biochemical analysis could be developed to detect metabolic disorders and sub lethal disease states affecting production efficiency. The present study deals with the Urea and Creatinine levels of catfishes gotten from three different commercial ponds with varying length and weight. There are variations in all the physical characteristics and haematological parameters measured during the study. Similar variations have been reported in the haematological profile of other Catfishes by other researchers. The result of the study showed a slight decrease in the values of haematological parameters of the Clarias gariepinus in pond one and pond two compared to pond three. The increase that was observed in the haematological parameters. Urea and Creatinine in pond three 3 Catfishes fed with normal diet with high carbohydrate concentration is in collaboration with the findings of Joshi et al. (2002) that survival of fish can be correlated with increase in anti-body production which helps in the survival and recovery. Haematological characteristics have been widely used in clinical diagnosis of diseases and pathologies of human and domestic animals. The

applications of haematological techniques have proved valuable for fishery biologists in assessing the health of fish (Fagbenro A.O and E. Adeparusi. 2003) and monitoring stress response (Soivio, A. and A. Oikan. 1976). Some of the values are slightly low due to the condition under which the fishes were kept, the condition based on the fact that the fishes are not in their natural habitat and also because of the small sizes of the fishes, values such as the Creatinine is affected due to size. The Urea level observed in *C.gariepinus* raised in pond effluent water (pond 3) was about two times higher than those reported by Agbede et al. (1999) and Oyelese et al. (1999) for adult catfish. The level of Creatinine in fish raised in pond effluent water was also higher than those reported by Oyelese et al. (1999) for adult catfish.

Both Creatinine and Urea levels were significantly elevated in this investigation. These compounds are the most abundant non-protein nitrogen constituents in the body and their determination are the most commonly ordered tests of the kidney's ability to excrete metabolic wastes (Tresseles, 1988). The result showed significant increases in the levels of Creatinine and Urea in most of the catfish. Since increase in these values are used as indicators of renal failure, it can be postulated that the stress passed through by the fish during capturing or the method of capturing them during the experiment is related to the impairment of the renal. The presence of increasing Creatinine concentration in the blood suggests ac decreases in glomerular filtration rate (GFR). It is reported in literature (National Kidney Literature, 2002) that Creatinine is a more accurate marker of kidney disease than Urea. High Creatinine level implied that many waste products in the fish bloodstream would not be cleared, indicating that the kidneys were not functioning properly. A more complete estimation of renal function can be made when interpreting the blood (plasma) concentration using the ratio. This can indicate other problems besides those intrinsic to the kidney. The ratio of Urea to Creatinine can indicate other problems besides those intrinsic to the kidney; for example a Urea level raised out of proportion to the Creatinine may indicate a pre-renal problem such as volume depletion (Spencer, 1986; National Kidney Foundation, 2012).

The mean Urea and Creatinine level of C.gariepinus obtained in this study is not in conformity with that of other workers. The differences may be due to differences in climatic and environmental factors in the places from where the species of fish were obtained as suggested by Barnhart (1969). Creatinine level greater than 1.5mgdL or lower than 0.8mgdL is considered high or low which means its abnormal while Urea level greater than 7.1mgdL or lower than 1.8mgdL is abnormal. Abnormal Creatinine levels may be due to any of the following conditions that affect the kidneys or muscle, drastic variations in normal Creatinine level may indicate urinaty tract obstruction, kidney failure, dystrophy, reduced blood flow to the kidneys, prerenal azotemia e.t.c. while abnormal Urea level may indicate congestive heart failure, gastrointestinal bleeding, kidney failure or kidney disease.

The result of this experiment revealed that C.gariepinus in pond one and two have Creatinine and Urea levels which is between the normal range. The catfishes in pond three have very high levels Creatinine with 2.90mgdL being the highest level recorded and is said to be abnormal which may be as a result of the muscle built up in the fishes or stress passed through while the lowest Creatinine level was also recorded to be 0.00mgdL which may be due to shock, congestive heart failure, malnutrition etc. The highest level of Urea was recorded to be 6.30mgdL which is still between the normal range while 0.00mgdL is the lowest level which may be due to gastrointestinal bleeding, dehydration, starvation or urinary tract obstruction. Since C. gariepinus is one of the most frequently cultured fish in Nigeria, there is need to carry out more of these haematological studies so that reference intervals can be determined for different population of the fish. A wider range of normal values may be necessary than for terrestrial

animals in view of the fact fish are exposed to extremes of environmental conditions especially oxygen and carbon dioxide.

Conclusion

In conclusion, this study has provided valuable data on the haematology of *C. gariepinus* Gwagwalada and Kuje that could be used as baseline data for future studies and monitoring of the health status as well as production of the fish. The result revealed that weight has an effect in the Creatinine level of C.gariepinus, showing an increase in the haematological parameters of the speci. Creatinine is significant to weight which is visible in group three that have fishes ranging between 601g-900g and using P < 0.05 level of significance, Creatinine depended on the weight where 0.00 < 0.05.

Recommendation

Stress, handling of catfishes and certain kidney diseases have effects on the levels of both urea and creatinine as a result of the kidney functions. Therefore, catfishes should be handled carefully during harvesting or experimentation and they should checked often for diseases affecting catfishes to aid kidney functions. It is also recommended that more research should be made on the haemotological parameters of fishes to provide adequate information on the normal levels of the various parameters for further works in future.

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10/2/2014