

SOME SERUM AND GILL METABOLIC WASTE OF CATFISH (*CLARIAS GARIEPINUS*) FED BLOOD MEAL AND MORINGA LEAF SUPPLEMENT DIETS

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ABSTRACT

This work reports the effect of some serum and gill metabolic waste (creatinine, urea and total bilirubin) of catfish fed blood meal and moringa leaf as a supplement diet. Among the other five diets, fish meal was altered from 7%, 5%, 2%, 0%, and 0% of its replacement with blood meal. The control fed had no blood meal and moringa leaf. 0%, 2%, 5%, 7%, 10%, 10%. The fishes were dissected after 8 weeks and the serum and gills were collected for the analysis of creatinine, urea and total bilirubin of the serum and gills using spectrum photometric method. The physico-chemical of each diet for (temperature, pH, dissolved oxygen and conductivity) was monitored. Creatinine shows significant difference ($P < 0.005$) in the fishes from $(0.493 \pm 0.007 \text{ mg/dl})$ to $(0.380 \pm 0.006 \text{ mg/dl})$. Total bilirubin shows no significant difference in gill from $(0.0413 \pm 0.009 \text{ mg/dl})$ to $(0.643 \pm 0.013 \text{ mg/dl})$. While serum shows significant differences from $(6.280 \pm 0.006 \text{ mg/dl})$ to $(5.257 \pm 0.009 \text{ mg/dl})$. Urea shows significant difference on both serum and gills $(6.280 \pm 62.800 \pm 0.058 \text{ mg/dl})$ to $(46.500 \pm 0.115 \text{ mg/dl})$. These metabolic waste activities in the fish's serum and gills demonstrate the impact of 10% blood meal and moringa leaves on fish physiology. The fishes were fed with the experiment diets in triplicates for 8 weeks, after which fish serum and gills from each treatments mean collected for analysis of creatinine, urea and total bilirubin with spectrophotometric method.

Keywords: *Clarias gariepinus*, Serum, Blood Meal, Metabolic Waste.

INTRODUCTION

Local resources that can be included in the diet of hybrid catfish include blood meal and *Moringa oleifera* leaves. These nearby sources of ingredients have the potential to significantly lower production costs. Winter (1929). Consider the digestibility of blood meal, which is a common domestic animal food source. Aladetohun and Sogbesan (2013) employed blood meal supplement feed for Nile Tilapia and received excellent results in their effort to lower production cost of generating hybrid catfish. After further investigation, it is discovered that blood meal is a protein supplement with a high protein content. The catfish would benefit greatly from blood meal as well. Within diet of hybrid catfish, Anyanwu *et al.*, (2013) employed five distinct types of leaves, but they did not include Moringa. A woody and leafy plant that thrives in the tropics is called *Moringa oleifera*. Kathryn (2015) thinks it's important and discusses how different countries' leaf contents might vary within the same range. Evidence on the dietary significance of *Moringa oleifera* leaves was presented in some research work (USDA, 2013; Stadlmayr *et al.*, 2012 & Goplan *et al.*, 1999). They

discovery that dried leaves are less nutrient-dense than fresh leaves, nevertheless dried leaves are simple to use in fish diets. Essential vitamins, minerals, enzymes, as well as some critical amino acids are substantially more abundant in them. Age comparative study on moringa plant nutritional requirements on kids of a year and three was conducted BY FNB from 2005 and 2010. Moringa will be excellent as a premix to the diet of fish because its nutritious content is compared to what newborns need. Nwakocho, (2013) examined Nigeria's high demand for fish as a source of protein as it increased through time from 2010 to 2020. They claimed that as Nigeria's population grows each year, so does the amount of fish exported. Because it requires more feed than other fish, hybrid catfish, a crucial commercial fish in Nigeria, are neglected. The main plant element used in fish feed has traditionally been soybean (Ship ton Hecht, 2005), but as prices have risen, it has become less cost-effective (Hardy 2010). Soybean prices have increased as a result of increased demand from consumer nations like China and other growing economies, as well as its several uses (Hardy, 2010). This highlights the necessity for substitute plant proteins.

The substances which are left over after metabolic processes (such cellular respiration) but which the organism cannot utilise because they are poisonous or excessive must be expelled. Water, nitrogen molecules, CO₂, phosphates, sulfates, and animal excretions are among them. Most plants have chemicals that transform some of their beneficial molecules, such as nitrogen compounds, and Brian J. Ford demonstrated that abscissa plants also carry waste materials from the parental plants. Ford also claims that the fallen leaves serve as excretory products. Except for CO₂, which is expelled along with water vapor through the lungs, all metabolic waste products are eliminated in a process of H₂O solutes through the excretory organs (Malpighian tributes, nephridia, and kidneys). The elimination of some of these chemicals helps maintain the chemical equilibrium of the organism.

The purpose of the kidney is to eliminate solutes, water, and toxins from the body for the purpose of maintaining a homeostasis inner environment despite dietary and balance of fluid changes. The osmolality of extracellular fluid (ECF), which is what determines a cell's veracity, controls salt and water excretion through osmo- and volume receptors. The kidneys derive the 20% output of cardiac that each nephron conceive from the plasma ultrafiltrate that enters the renal tubule through the Bowman space. About 99percent of total of the sodium chloride in the purified water is changed to sodium bicarbonate by the renal tubules of the kidneys, which subsequently easily absorb sodium bicarbonate and restore it to the plasma. Its propensity to nearly completely reabsorb sodium can endanger both kidney tissue and function. To do this, the kidneys of vertebrates successively filter blood plasma, reabsorb the majority of the electrolytes filtered water, secrete certain solutes and organic material. The primary function of this kidney is excretion of a variety of metabolic waste products with dietary electrolytes and water. The function of the kidneys is commonly understood to be one nephron scaled up in a similar system. Water re-absorption gradually reduces its volume, and tubule epithelial cells' solute transport alters its shape. The renal cortex, the outermost part of the kidney, and the hypernatremia and volume depletion regions are where the complete glomerular filtration takes place.

CREATININE METABOLIC WASTE

Organ Kidney that produces Guanidine Acetate through arginine, amino acid and glycine but replaced by creatinine in the liver by the aid of S – adenosyl methionine. Its then phosphorylated and conveyed by the blood to the central nervous system, muscles, as well as other organs where it will be converted in to greater energy substances called phosphocreatine. By converting creatinine to phosphocreatine, creatinine kinase catalyzes the body's natural generation of creatinine. The kidneys are the main organs responsible for removing creatinine from the blood, predominantly through glomerular filtration but also through proximal tubular secretion. If the kidneys' filtration is inadequate, there is minimal or absence of tubular creatinine reabsorption of, and blood levels of creatinine increase. Calculating the rate of glomerular filtration (CrCI), which closely related to the glomerular filtration rate, would be done using the amounts of creatinine found in the blood and urine (GFR). Additionally, the expected GFR may be determined purely by blood creatinine levels (eGFR). Since it evaluates renal function, the GFR is essential for clinical applications. The proximal tubules will release more creatinine in situations of severe renal failure, making up a higher proportion of these total creatinine; as a reason, the CrCI rate will overstate the GFR. Particularly in cases of acute renal failure, antacids, cimetidine, and trimethoprim decrease creatinine tubular secretion, increasing the accuracy of the GFR measurement (in the absence of secretion, creatinine behaves like inulin). An alternative assessment of renal function can be made when the blood (plasma) creatinine concentration is assessed along with the urea concentration. Blood urea nitrogen (BUN) to creatinine ratio, which also includes urea. Ratio of blood urea nitrogen (BUN), which also contains urea, to creatinine. The ratio of blood urea nitrogen to creatinine, or BUN-to-creatinine, may indicate problems other than kidney-specific problems. For instance, a prerenal condition like volume depletion may be indicated by urea levels that are elevated relative to creatinine but not proportionally to it.

Creatinine is generated daily within an array of 1% to 2% of muscular creatinine. Men often have higher amounts of creatinine than women because they have more skeletal muscle. Daily creatinine levels may rise as a result of increased dietary creatinine consumption or consuming a lot of protein (example meat). Creatinine is a simple to

measure by-product of muscle metabolism that the kidneys eliminate intact, making it a crucial indicator of renal health. Adenosine triphosphate, phosphocreatine, and creatinine are all components of the biological process that generates creatinine. When an ACE inhibitor (ACEI) or angiotensin II receptor antagonist (or angiotensin receptor blocker, ARB) is given, nine excretion creatinine levels may increase. Nephrons will grow if ACEI and ARB are used simultaneously. This test is therefore insufficient for detecting renal disease in its early stages. The estimated glomerular filtration speed can be used to assess kidney performance more precisely (eGFR). The American Diabetes Association (ADA) claims that utilizing blood creatinine concentration and some or all of the following variables—sex, age, weight, and race—it is possible to determine EGFR correctly without collecting urine for 24 hours. When a creatinine test is required, many labs will automatically calculate GFR. The page on renal function discusses formulas for calculating GFR from data such as creatinine levels and other variables. As of late 2010, there are worries about the implementation of a revolutionary analytical methodology and its potential impact on clinical care. Presently, the majority of clinical laboratories compare their measurements of serum creatinine to an advanced, standardized IDMS method. When the serum creatinine levels are very low, like 0.7 mg/dl, IDMS seems to give values that are less accurate than earlier methods. The IDMS technique might result in a relative overestimation of the corresponding computed GFR in some patients with normal renal function. Even with normal renal function, numerous drugs are dosed based on the established GFR. The dose may now be larger than desired, which could lead to additional drug-related harm if it is left unchanged. To lessen the impact of transitioning to IDMS, new FDA guidelines propose limiting carboplatin dosages to particular maximums. A lower serum creatinine level among Japanese men was associated with a higher risk of acquiring type 2 diabetes, according to a 2009 Japanese study. The creatinine concentration is also tested when conducting standard urine drug testing. The creatinine concentration is also tested when conducting standard urine drug testing. Low creatinine levels suggest that the test sample is diluted when compared to normal creatinine levels. Low creatinine levels cause test samples to be suspected of manipulation, which prevents them

from being evaluated and sometimes causes the test to be considered invalid.

TOTAL BILIRUBIN METABOLIC WASTE

When the hemoglobin protein in old red blood cells is broken down in the body, bilirubin is produced. Old cells degenerating is a healthy, natural process. Bilirubin first moves through your bloodstream before arriving at your liver. The liver converts bilirubin into bile, which is then secreted into the bile ducts and stored in the gallbladder. In order to aid in the breakdown of lipids, bile is eventually discharged into the small intestine. Your stool is where it is ultimately eliminated. Total bilirubin has a normal range of 0.3 to 1.0 mg/dL.

Everyone's blood and feces contain the yellow pigment bilirubin. The body's bilirubin can occasionally be too much for the liver to handle. This may be caused by an overproduction of bilirubin, a blockage, or liver inflammation. The whites of your eyes and skin will begin to yellow if your body has an excessive amount of bilirubin. Jaundice is the term for this condition. If you suffer from any of these problems, a bilirubin test can reveal it.

A bilirubin test quantifies the bilirubin levels in a blood sample. A brownish-yellow pigment called bilirubin is present in bile. When the liver degrades old red blood cells, it is generated. After then, bilirubin is eliminated from the body through the feces, giving the stool its usual color. Total and direct bilirubin measurements are used to compute indirect bilirubin levels, whereas blood samples are used to quantify whole and direct bilirubin concentrations.

UREA METABOLIC WASTE

Mammal urine mostly contains urea, which is necessary for animals to process nitrogen-containing compounds. It is a tasteless, colorless compound that is simple to dissolve in water and basically non-toxic, with an LD50 for rats of 15 g/kg. It has a pH that is either alkaline or acidic when dissolved in water. It is utilized by the body for a variety of processes, most notably the removal of nitrogen. As part of something like the urea cycle, the liver creates urea by combining two ammonium (NH₃) molecules with a carbon carbon (CO₂) molecule. In fertilizers, urea is frequently used as a source of nitrogen. Carbamide, a compound with the chemical symbol CO(NH₂)₂, is another name for urea. Due to the presence of two -

NH₂ groups connected by a carbonyl (C=O) substituent, this amide is a crucial natural resource for the chemical industry. Topical dermatological treatments using urea are used to help the skin rehydrate. Urea 40% is advised for the treatment of xerosis, psoriasis, ichthyosis, onychomycosis keratosis, eczema keratoderma, calluses, and corns. If the nail is wrapped by an occlusive bandage, a 40% urea solution may also be utilized for nonsurgical nail debridement. The drug, which has previously been investigated as a diuretic, had no effect on healthy nail plate areas and only damaged or killed diseased or cardiovascular abnormalities nails whenever it "dissolved the interconnecting matrix" of the nail plate. It was initially used by Dr. W. Friendrich in 1892. Urea has been proven to be a straightforward, secure, and affordable therapy for euvolemic hypernatremia in a 2010 RCT of ICU patients. This medication can also be used to get rid of earwax.

JUSTIFICATION OF THE STUDY

In Nigeria, fish meal supplements may help to lower the high feed costs associated with fish farming. Before using blood meal and moringa leaf as a component in fish feed, it should be confirmed that they have no effect on fish. This research is therefore necessary.

AIM OF THE STUDY

This study aimed to investigate the effects of urea, bilirubin, and creatinine on the metabolic waste of *Clarias gariepinus* fed with blood meal and Moringa oleifera as an enhancement diet.

MATERIALS AND METHOD

Location of Studies

Department of Biological sciences, Ibrahim Badamasi Babangida University, Lapai, Nigeria was the research center. The aquarium laboratory in the Department is where the fish are fetched for the practical.

INVESTIGATIONAL DIETS

Blood meal, leaves, fish meal, soybeans, starch, and vitamin premixes were among the feed items used as dietary supplements. Both the protein % and the lipid percentage of five exogenous diets were created. Dietary I included moringa plant leaves and zero blood meal. Dietary II consists of moringa leaves and 2% blood meal. Dietary III contains moringa leaves and 5% blood meal. Dietary IV consists of moringa leaves and 7% blood meal.

Dietaries V and VI each contained 10% blood meal and moringa leaves. The blood meal in Dieta V was not heated.

INVESTIGATIONAL FISH

Young catfish (*Clarias gariepinus*) were bought from the fish farm owned by Al Hassan Niger state Minna in particular. The study employed the *Clarias* species, whose bodies normally weigh 1.9–2.7 kg and are of standard length. The fish were delivered to the department of biological sciences aquarium at Ibrahim Badamasi Babangida University Lapai, where they were placed in an aerated tank. To prevent starvation, they were acclimated for a few weeks while receiving commercial fish feed (2 mm essential) twice day. Five percent of body weight was fed. To prevent the buildup of ammonia levels, the water and uneaten feed were sucked every two days.

DIETRY EXPERIMENTAL DESIGN

The studies included six treatments, each represented by six (6) replicates, along with the control. Except for the control, every treatment diet included soybean meal replacement. Ingredients were created for the five diets. Analysis was used to identify each diet's primary ingredients. Diets I (Control), II, III, IV, V, and VI, with respective soybean inclusion percentages of 0%, 2%, 5%, 7%, 10%, and 10%, were constructed with varying amounts of soybeans.

MANAGEMENT OF CULTURE

The research fish are fed for 60 days using varied amounts of soybeans after acclimation. Commercial feed was used as the first feed and as a control. Moringa leaves and blood meal are included in the second feed. Blood meal is included in the third feed, whereas moringa is included in the fourth. Blood meal is found in the fish feed, while moringa is found in the fourth feed. In a plastic rubber tank, for 60 days, the fish was given 5percent of the overall of their body mass twice daily. To avoid death, the trash and leftover food were removed, the water was cut in half, and fresh water supplies were made every day. Temperature, conductivity, and pH were the three water quality characteristics that were constantly monitored for the experiment set.

WATER PHYSICOCHEMICAL PARAMETERS

Physicochemical parameters of the water were observed on weekly bases. A thermometer was used

to measure the water's surface temperature in each bowl. After calibrating the thermometer for five minutes, the result was recorded in degrees Celsius (°C). By dipping the conductivity electrode in the plastic tank and measuring the results with a conductivity meter, conductivity was determined. The pH of water is used to determine how much hydrogen is present in it and how acid or base would react.

ORGAN EXTRACTION

BLOOD SAMPLES COLLECTION

Fish were removed from the vessels one at a time using a small hand net. The fish was then placed belly-up, and blood samples were obtained from the caudal circulation using a disposable 21-gauge hypodermic needle and a 2 cm 3 plastic syringe that had been heparinized. This was done after the fish had first been measured for length and weight. Because contact with glass reduces the coagulation time, it is imperative to use plastic syringes when using fish blood. To prevent mucus infection, the puncture site (about 3 to 4 cm from the vaginal hole) was wiped dry with issue paper. The fish's spinal column was penetrated with a needle that was inserted perpendicularly to it. It was then slowly pressed down till blood began to flow as the needle obtained around 1 cm³, at which point the needle was detached and the blood was gently placed into a

tube containing lithium heparin anticoagulant, where it was allowed to coagulate at ambient temperature for 20 to 40 minutes.

Blood Sample Centrifugation

The blood in the anticoagulant tubes was drawn, transferred, and centrifuged at 3000 rpm for 20 minutes with clean, dry tubes before the serum was separated. A Pasteur pipette was used to separate the serum from the blood after centrifuging it for 20 minutes. The serum was then put into an anticoagulant-free test tube and kept in the refrigerated until analysis. The procedures followed: Preceding to the test, If the sample and test strip were refrigerated, they must be brought to room temperature for 5 to 10 minutes before to use. The fundamental steps are; Be careful not to bend the test strip as you remove it.; Using a reflotron pipette, draw 32L of the sample without creating any air bubbles. And with movable covering or flap closed, drop the sample into the center of the treatment zone, taking careful not to contact the area with both the pipette tip.

to confirm that the experiment ferromagnetic information from the testing apparatus in the testing kit has been correctly read, the instrument shows the specific biochemical parameter. After two to three minutes, the outcome is displayed.

SERUM AND GILLS METABOLIC WASTES

	Serum	Gills
Dietary I	0.48, 0.50, 0.50	0.29, 0.30, 0.28
Dietary II	0.44, 0.43, 0.41	0.37, 0.39, 0.38
Dietary III	0.40, 0.41, 0.42	0.43, 0.44, 0.41
Dietary IV	0.39, 0.41, 0.40	0.67, 0.67, 0.69
Dietary V	0.40, 0.38, 0.39	0.71, 0.70, 0.73
Dietary VI	0.39, 0.37, 0.38	0.64, 0.65, 0.62

	Serum	Gills
Dietary I	7.33, 7.34, 7.30	0.40, 0.43, 0.41
Dietary II	7.40, 7.38, 7.39	0.49, 0.46, 0.50
Dietary III	6.52, 6.50, 6.53	0.62, 0.60, 0.63
Dietary IV	6.28, 6.27, 6.29	0.76, 0.72, 0.74
Dietary V	5.76, 5.74, 5.76	0.94, 0.97, 0.95
Dietary VI	5.24, 5.26, 5.27	0.63, 0.63, 0.67

	Serum	Gills
Dietary I	62.8, 62.7, 62.9	46.3, 46.7, 46.5
Dietary II	64.1, 64.0, 64.2	49.6, 49.8, 50.0
Dietary III	60.7, 60.8, 60.7	61.2, 61.4, 61.1
Dietary IV	61.2, 61.4, 61.3	69.3, 69.3, 69.1
Dietary V	57.8, 57.8, 57.9	82.8, 82.6, 82.9
Dietary VI	55.1, 55.4, 55.2	76.4, 76.7, 76.3

ASSESS STANDARD OF CREATININE

Under alkaline conditions, creatinine and picric acid react to generate a yellow-red complex. When measured at a wavelength of 492 nm, the color produced has an absorbance that is directly proportional to the sample's creatinine concentration. Creatinine added to picrate / alkaline pH subtracted from yellow-red complex

The chemical picric acids, which is combustible and possibly explosive in its dry form, is present in just trace amounts in the creatinine picric acid reagent. Before discarding the reagent, drains should be carefully flushed with water to evade material dryness near the reagent plastic hole.

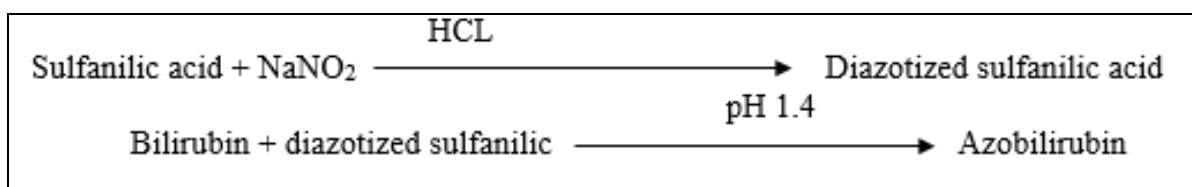
Reagents are namely: Standard (ST) 2mg/dL; Reagent1 (R1); Alkaline pH picric acid 25mmol/L Surfactants; Reagent2 (R2); Sodium hydroxide 0.4mol/L

R36/38 Irritating (xi): irritating to the skin and eyes
S26: If you come in contact with your eyes,

immediately rinse them out with lots of water and get help from a doctor. For more information, see the creatinine Jaffe reagent material safety data sheet. S37/39: Wear appropriate gloves and eye/face protection.

BIOASSAY PRINCIPLE OF TOTAL BILIRUBIN

The interaction between diazotized sulphanilic acid and caffeine produces a highly colored diazo dye, which is used to measure the total bilirubin levels in the blood and gills (560-600nm). The amount of this color produced is inversely correlated with the amount of total bilirubin. In the absence of caffeine, direct bilirubin is detected through the direct interaction of red-colored azobilirubin with diazotized sulphanilic acid; the intensity of this color, measured at 546 nm, is proportional to the amount of direct bilirubin present in the sample.



R35 causes severe burns. Consult the reagent material safety data sheet for more details. R41 potential for severe eye injury. S26 If you come into

touch with your eyes, immediately rinse them out with water and get help. Wash carefully with soap and water right away after coming into touch with the skin (828).

Content	Solution Reagent Initial Concentration
Standard (ST)	2mg/dL
Reagent 1 (R1)	177µmol/l
Picric acid	25mmol/l
Surfactants	

Content	Solution Reagent Initial concentration
Reagent 1 (R1)	
Sulfanilic acid	31mmol/l
HCL	0.20
Reagent 2 (R2)	
Sodium nitrate	28.0mm/l
Reagent 3 (R3)	
Caffeine	0.28mol/l
Sodium benzoate	0.55mol/l
Reagent 4 (R4)	
Tartarate	0.99mol/l
Sodium hydroxide	2.0N
Reagents 4 contains caustic material	
Corrosive	

Bioassay Principle of Urea

The assay system's reaction involves the hydrolysis of urea in the presence of water and urease, which results in the production of ammonia and carbon dioxide.

A colorful complex, inversely proportional to the quantity of urea available with in specimen is produced when free ammonia combines with an indicator as well as an alkaline pH.



Table 3.6 Urea Reagent Composition

Content Reagent	Solution Reagent Initial Concentration
Standard urea (ST) aqueous primary standard	50mg/dl 8.33mmol/l
Reagent 1 (R1 buffer)	
Phosphate buffer pH 8.0	100mmol/l
Sodium salicylate	80mmol/l
Sodium nitroprusside	6.0mmol/l
EDTA	30.0mmol/l
Reagent 2 (R2 enzyme)	
Sodium hydroxide	400mmol/l
Sodium hypochlorite	20.0mmol/l

R36/38: itching of the skin and eyes S26: If eye contact occurs, immediately flush the eyes with plenty of water and seek medical help. S37/39: Wear suitable gloves and eye/face protection; for further information, refer to the urea/BUN reagent's material safety data sheet.

Statistical Analysis

Analysis of variance techniques are used in the data analysis for the project (ANOVA). The analysis for assessing the impact on catfish supplied with blood feeding and moringa leaf was carried out using metabolic end testing on the both gills and serum, using graph pad prism 6 fit. The means in the Turkeys numerous comparison tests were compared to (p 0.05).

RESULT

Serum and Gill Metabolic Waste and Monitored Physicochemical Parameter of Water Quality

Physicochemical parameter

Table 4.2 water quality parameters

Water physicochemical Parameter	Diets											
	I		II		III		IV		V		VI	
	M	E	M	E	M	E	M	E	M	E	M	E
Temperature (°C)	26	27	26	26.7	27	28	27	27	27	28	27	28
pH	6.7	6.6	7.3	6.9	6.0	5.8	6.7	6.5	6.3	6.0	6.7	6.3
Dissolved Oxygen (mg/L)	2.37	2.34	2.23	2.25	2.17	3.00	2.17	2.8	2.23	2.23	2.20	2.20
Conductivity	4.6	4.4	4.0	4.1	5.7	5.7	9.3	9.2	4.6	4.8	5.7	5.7

Key: M = Morning and E = Evening

Figure 4.1 shows the creatinine concentration (mg/dL) in catfish (*Claria gariepinus*) fed varied amounts of blood meal and moringa leaves as a

Figure 4.1 showed the physical-chemical water quality characteristics such as temperature, pH, and conductivity; the physicochemical parameters' ranges of values over the course of the experiment's 60 days were as follows.

The physicochemical parameter such as temperature, dissolved oxygen and conductivity range between 26-28°C, 5.8-7.3 and 2.37-2.20mg/L respectively and mean temperature ranges were chosen in order to prevent being influenced by regular feed over the 8weeks of study. The parameters values fell within the average suitable for fish development and health (Mazik *et al.*, 1991 & Boyd 1979)

supplementary diet. All diets' effects on creatinine (mg/dL) serum are significantly different (p0.05), ranging from diet I's (0.4930.007) to diet V's

(0.3900.06) (Appendix), while diet I's effects on the gills are not significantly different.

Figure 4.2 displays the UREA activity (mg/dL) in catfish *Claria gariepinus* that were supplemented with moringa leaves and varying volumes of blood. For all diets, UREA (mg/dL) levels in serum and gill are not statistically significant ($p > 0.05$); for serum, these levels range from (62.800 0.058) in diet I to (55.233 0.088) in diet VI (Appendix).

Total bilirubin concentration in catfish (mg/dL) Figure 4.3 displays the total bilirubin (mg/dL) serum findings for *Claria gariepinus* fed varying amounts of blood meal and moringa leaves as a supplement diet. Results for dietary I through III vary between (7.353 0.039) for dietary I and (7.390 0.006) for dietary III, while results for dietaries I through VI in the gills reveal no significant difference ($p > 0.05$).

DISCUSSION

Creatinine

There is no statistically significant change ($p > 0.05$) in creatinine, according to the statistical analysis utilizing the spectrum analysis technique (mg/dL) serum for any of the diets, ranging from dietary I (0.493 0.007) to dietary V (0.393 0.006). (Appendix) Dietary III significantly differs from dietary VI in gills ($p > 0.05$) by (0.427 0.009) and (0.637 0.009), respectively.

When renal insufficiency prevents excretion, the serum removes a nitrogenous waste substance known as creatinine. The creatinine value in this study shows that over the 60-day experimental period, there was no significant variation ($p > 0.05$) in blood, but that significant difference of ($p > 0.05$) was found in diet VI in the gills. Serum creatinine levels may rise as a result of glomerular inefficiency, according to Murray *et al.*, (1990). A spike in creatinine levels is a marker of renal tubular injury, according to Lall *et al.*, (1997). From this study, it is possible to infer that blood meal and moringa leaves are detrimental to fish meal.

Urea

The nitrogenous end product of metabolism is urea. The main metabolic outcome of dietary and tissue protein turnover is it. Over 99% of urea production takes place in the liver. Dietary protein is the main contributor to it. The protein is converted into peptides and amino acids in the gut, and more than 99% of these are drawn to and transported to the serum. These substances are the body's most

prevalent non-protein nitrogen components, and their use as a test of the kidneys' ability to eliminate metabolic waste is frequently requested (Tressels 1988). The outcome demonstrates that there were no differences in serum and gills across all diets ($p > 0.05$).

Total bilirubin

The primary breakdown product as a result of red blood cell injury is total bilirubin. The blood filtered it out by the liver, and makes it a trustworthy sign of the liver's health. Increased total bilirubin synthesis or impaired liver uptake both result in higher blood levels of bilirubin (as a result of liver disease). Total bilirubin levels in serum indicate that all diets had a significant difference ($p > 0.05$) while diets I through VI in the gills showed no significant difference (0.05), indicating that the gills had a high rate of red blood cell death.

The observed rise in bilirubin concentration (figure 4.3) supports the idea that serum dysfunction may be present and may be brought on by the fact that the fish were given several blood meals supplemented with moringa leaves. The water-soluble ingredients in diets have the potential to alter the survival and metabolism of aquatic creatures, including fish, which lends credence to this argument (Cote 1976). Similar to what was seen in this study, the impact might also happen in other animals like rats fed fish with varying blood meal levels and moringa leaves as a dietary supplement. The findings of Ovuru *et al.*, (2004), who observed an increase in total serum bilirubin levels in semi-adult rabbits fed a diet contaminated with crude oil, also support this theory. They linked this finding to a metabolic disturbance in the liver caused by faulty conjugation and/or excretion of total bilirubin. Cheese Borough (1992) noted that as the liver functions as an excretory unit rather than a storage organ, a rise in the concentration of serum bilirubin indicates or predicts liver injury.

CONCLUSION

Creatinine, urea, as well as total bilirubin concentrations show that moringa leaves and 10% blood meal have an effect on *Clarias gariepinus* physiology by increasing the metabolic waste activities of the serum and gills.

RECOMMENDATION

According to this study, moringa leaves and blood meal might be included in fish meals up to 10%, but

if they were to be used more than that, anti-nutrient reduction medicine should be given beforehand. For a better understanding of the effects of blood meal

and moringa leaves added to catfish diets, more research should be conducted.

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