EFFECT OF BIOCHAR SUPPLEMENTED DIET ON GROWTH PERFORMANCES AND HAEMATOLOGY OF AFRICAN CATFISH POST JUVENILE

BY

FALAYI CHRISTIANA OPEOLUWA

(FAQ/13/0990)

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF FISHERIES AND AQUACULTURE

IN PARTIAL FULFILLMENT OF REQUIREMENT FOR THE AWARD OF BACHELOR OF FISHERIES AND AQUACULTURE (B.FAQ)

IN THE FACULTY OF AGRICULTURE,

FEDERAL UNIVERSITY OYE-EKITI,

NIGERIA.

CERTIFICATION

This is to certify that FALAYI CHRISTIANA OPEOLUWA with matric no FAQ/13/0990 carried out this project work in the department of Fisheries and Aquaculture of Federal University, Oye-Ekiti.

_______________ 21/03/2019

DR O.S. OKEKE. DATE

SUPERVISOR

_______________ _______________

DR.J.B. OLASUNKANMI DATE

(B. Sc, M. Sc, PGDE, PhD (Ibadan)

HEAD OF DEPARTMENT

DECLARATION

I declare this work entitled **EFFECT OF BIOCHAR SUPPLEMENTED DIET ON THE GROWTH PERFORMANCE AND HEAMATOLOGY OF AFRICAN CATFISH POST JUVINILE (*Clariasgariepinus*)** has been carried out by me in the Department of Fisheries and Aquaculture under the supervision of Dr.O.S. Okeke.

Falayi Christiana Opeoluwa ______________ 23/03/2019

Signature Date

DEDICATION

This project is dedicated to God Almighty, the Helper in ages to come whom in His infinite mercy made this project a successful one, both in sustenance of life, financial provision and also dedicated to my parents.

ACKNOWLEDGEMENT

To my ever faithful Father, the one who knew me from my mother's womb, the author and **the finisher of my faith**, the beginning and the end, the one who is, the one who was and the **one that will continue** to be. I return all the glory, honor and adoration back to Him because **only Him deserves** to be praised.

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ABSTRACT

A 56days (8) weeks trial was conducted to study the effect of dietary inclusion of **BIOCHAR** (Miscanthus) on the growth performance, water quality and hematology of African catfish post juvenile. The graded level of Biochar used were control 0%l (T1), 25% (T2), 50% (T3), 75%(T4) and 100% (T5) which was supplemented for Vitamin/ Mineral premix in the diets 1,2,3,4 and 5 respectively. All diet was iso-nitrogenous (35%) CP. Clariasgariepinus post juvenile (average weight 20.77 to 23.07) were randomly distributed in plastic tanks with 10 fish/ tank in triplicate treatments and were fed twice daily 8:00-9:00am and 4:00-500 pm.

Fish fed 25% BIOCHAR recorded the best weight gain, feed conversion ratio, specific growth and Relative growth rate.

There were no significant differences between fish fed the control diet and 25% biochar supplemented diet in treatments 1 and 2 ($P>0.05$). However, significantly reduced growth and nutritional performance were recorded in diets with elevated inclusion levels of biochar supplemented diet meal especially in treatments 3, 4 and 5. Feed–related mortality was recorded during the feeding trial.

The result of the water quality parameters shows that there were significant differences ($p < 0.05$) in the values of water temperature, dissolved oxygen and Ph. Highest temperature is recorded in tank BM25 and lowest in BM75. Lower and similar ($p > 0.05$) dissolved oxygen were recorded in BM75 and BM100 respectively while the highest was recorded in BM0 Water Ph. were similar ($p > 0.05$) in BM50 and BM75 and the highest Ph. was recorded in BM100 while the lowest was in BM25.

The result of the heamatology parameters showed that the packed cell volume (PCC), Red blood cell, hemoglobin (Hb), mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values were significantly ($p<0.05$) higher in control (0%) compare to other treatments.

Lowest neutrophils and lymphocytes were recorded in fish fed diets BM100 and BM25 respectively while both parameters were highest in C. gariepinus fed diets supplemented with 25% and 100% biochar inclusion levels. In conclusion This study revealed that biochar supplemented diet meal may be included in the diets of Clariasgariepinus at inclusion levels 25% replacement of vitamin/mineral premix.

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CHAPTER ONE

1.0 INTRODUCTION

The Aquaculture industry has been globally recognized as the fastest growing food producing industry (Food and Agricultural organization 2010). Aquaculture has been driven by social and economic objectives such as nutrition improvement in rural areas, generation of supplementary income, diversification of income activities, and the creation of employment (Osigbo*et al.*, 2014), and putting into consideration of the anthropogenic activities, over fishing, use of unregulated fishing gear, destruction of natural habitat with the use of bottom trawls and disposal of effluent into the natural habitat, this and many more, which have led to the migration and/or loss of some species in such habitat, and putting in mind the high demand of fish which the natural habitat cannot be able to meet and the continuous increasing demand population. Aquaculture production has continued to show strong growth, increasing at an average annual growth rate of 6.5% from 6.8 million in 2002 to 50.3 million metric tons in 2007 while capture fish production remain stagnant since 2001 (FAO, 2010). This increase in aquaculture production further brings about higher growth in the number of Aquaculture which was more than 10 million people in 2005 (FAO, 2010).

Fish is an important and the cheapest source of animal protein and account for about 37% of Nigeria total protein requires (FAO, 2002). However, despite the promising nature of aquaculture to increase productivity of fish in the country significant improvement are yet to be noticed and this could be effect of high cost of feeding which covers the major part of production cost of fish (Olaoye*et al.*, 2016). Catfish has been known to be a prolific an easy to raise fish, which is hardy and can survive in most condition and due to this reason and more, it has been used for major research purpose, and culture for its high return of investment if management and high cost of feed is taken care of, which is the primary target of a business owner and so research are on how to reduce the cost of producing it. However, in order to make this available for all, productivity should meet the target increase population. This can only be achieved if the cost of production is reduced, and if the cost of quality feed is being reduced, as aside water and adequate dissolved oxygen, quality feed remains the most essential requirement in an aquaculture production.(reference)

Fish nutrition is critical in fish farming because feed represents 40-50% of production cost (Craig and Helfrish, 2002). In fish culture, nothing is more important than sound nutrition and adequate feeding. If there is no utilizable feed intake by the fish, there can be no growth/development and death/mortality eventually results. Growth performance and nutrient utilization of fish is determined by gross composition of the feed ingredients, processing and storage of the feed products (Ajiboye*et al.*, 2015). Globally, there is a great decline in aquaculture production, due to fish feed manufacturers substituting vital feed ingredients with alternative feed stuffs that cannot achieve fish nutritional requirements (Ajani *et al.*, 2015). One of the critical challenges faced by aquaculture is the high cost of fish feeds and more than 50% of the total cost of production is intensified in culture system (Ali *et al.*, 2004). Nevertheless, nutrients in fish feeds are optimally utilized when the feed stuffs are acceptable and palatable to the fish (Folarinwa*et al.*, 2015). Cost of production can be reduced if growth performances and efficiency are increased in commercial aquaculture (Dada and Olugbemi, 2013).

However, the culture of African catfish, *Clariasgariepinus* has produced means of livelihood for many people in the local communities and has equally generated revenue for the government.

Clariasgariepinus of the family Clariidae is the most commonly cultivated fish in Nigeria (Omeru, 2016). *Clariasgariepinus*is generally considered to be one of the most important tropical fresh water fish species for aquaculture whose aquaculture potential has been documented (Omeru and Solomon, 2016). *Clariasgariepinus*has high fecundity rate, grows faster, tolerates high density and environmental extremes. It also accepts wide range of natural and artificial food and adapts to a variety of feeding modes in expanded niches (Omeru*et al.*, (2016).

Miscanthusis a C4perennial rhizomatous grass that is more productive than maize (Dohleman and Long 2009) and requires less input (Beale and Long 1997; Christian *et al.* 2008; Miguez*et al.* 2008). As a result of the combination of high productivity and the ability to convert all of the above ground cellulosic biomass to ethanol, Miscanthus is predicted to reduce net carbon dioxide emissions (Stampfl*et al.* 2007) and produce more than double the renewable fuel per unit area than maize (Vanloocke*et al.,* 2010).

1.1 JUSTIFICATION

The African catfish,*Clariasgariepinus* is widely cultivated in Nigeria because of its ability to consume supplemented feeds, good conversion of feed to flesh, resistance to disease, ability to reproduce in captivity, fast growth rate and tolerance to a wide range of environmental conditions (Ayinla, 2013). The economic importance of this species has increased tremendously in recent years as a result of its extensive use in aquaculture (De-Graaf and Janseen, 2011).

The fish farmer in Nigeria spends a lot of money on getting feed for fish, in which if not monitored the farmer can be at loss. Miscanthus is a locally and readily available plant source of vitamin that can be gotten easily without purchasing it with money.

Miscanthussinensis has been analyzed to be very rich in potassium and nitrogen. The objective of this study therefore, is to determine the effect of Biochar supplemented diet on the nutritional composition of fish feed, growth performance and nutrient utilization of African catfish (*Clariasgariepinus).*

1.2 OBJECTIVES OF THE STUDY

The main objective of this study is to investigate the effect of biochar on the nutritional composition and growth performance of post juveniles of *Clariasgariepinus*. Towards this goal, the specific objectives were to:

i. evaluate the effect of treatment diets on the growth performance and nutrient utilization of *Clariasgariepinus*post juveniles;

ii. evaluate the carcass quality of fish fed biochar supplemented diets; and

iii. examine changes in the hematological parameters of post juveniles of African catfish fed biochar supplemented diets.

1.3 HYPOTHESES.

The following null hypothesis would be tested in this work:

Ho_1:Thereisnosignificantdifferenceonthegrowthperformancesandnutrientutilization(feedconversionratio)of*Clariasgariepinus*postjuvenilefedwithfeedsupplementedwithBiochar.

Ho_2: There is a significant difference on the growth performances and nutrient utilization (feed conversion ratio) of *Clariasgariepinus* post juvenile fed biochar supplemented diets.

Ha1:

Ha_3:ThereisnosignificantdifferenceintheproximatecompositionofAfricancatfishfishfedtreatmentdiets.

Ha_3:ThereissignificantdifferenceintheproximatecompositionofAfricancatfishfishfedtreatmentdiets

Ha_2

$Ha_{4:}$ There is no significant difference in the hematological parameters offish fed biochar supplemented diet.

$Ha_{4:}$ There is significant difference in the hematological parameters of fish fed biochar supplemented diet.

CHAPTER TWO

LITERATURE REVIEW

2. 1 DESCRIPTION OF African Catfish (*Clariasgariepinus*)

According to Viveen 1986*Clariasgariepinus*at various geographical locations bears different names. It is called *Clariaslazera*in Northern and central Africa, *Clariasgariepinus*in South Africa. *Clarias gariep7inus* is characterized with nated skin and dougate with fairly long dorsal aid anal fins. The dorsal fin has 61-80 soft rays and anal fin has 45-65 soft rays. They have strong pectoral fins with spines that over serrated on the outer side (Tangels, 1986). Based on research it possesses nasal and maxillary barbells and somewhat smallish eyes, their coloring is dark grey or black dorsally and green colored ventrally. Adult possess a dark longitudinal lies on either side of the head. However, this is absent in young fish the head is large, depressed and heavily boned. The mouth is quiet large and sub-terminals (Shoelton, 1993; Teugels 1986). In *African catfish*, exchange of respiratory gases i.e. oxygen and carbohydrate takes place through the gills. Like other mudfish, it has accessory breathing carborescent organs which enable the fish not only live in stagnant pools but to travel over damp ground. *Clariasgariepinus*is said to differ from other catfish in having an auxiliary breathing organs in this special pochet attached to the second and fourth gill arches and are responsible for the ability of *Clariasgariepinus*to live out of much longer than other catfish (Haylor, 1993.).

According to Sogbesan, 2006, the major Clariasspecies are the most preferred farmed fish species in Africa because of the fast growth rate and higher acceptability of the consumer. *Clariasgariepinus*is very popular to fish farmers for high market price, high fecundity rate, fast growth rate, tolerates high density and environmental extremes, resistance to diseases infection and ability to withstand adverse paid conditions especially low oxygen content and high turbidity, good food conversion ratio in which it accepts wide range of natural and artificial food and then adapts to a variety of feeding modes in expanded niches. According to Bamidele, 2007, the culture of *Clariasgariepinus*as seed for fish production is becoming increasingly essential as the fish is contributing to the food abundance and nutritional benefit to the family health, income generation and employment opportunities. *Clariasgariepinus*is generally considered as one of the most important tropical species of the aquaculture.

2.2 TAXONOMY CLASSIFICATION OF *Clariasgariepinus*

Scientific classification

Kingdom: Animalia

Phylum: Chordate

Class: Actinopterygii

Order: Siluriformes

Family: Clariidae

Genus: Clarias

Species: C. gariepinus

(source:Gruntar and Fink 2004).

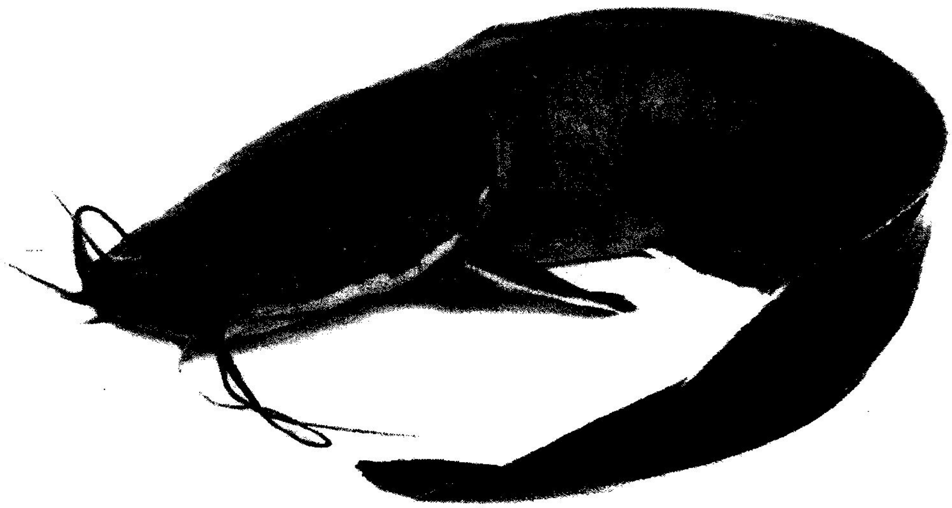

Plate 1: African catfish *(Clariasgariepinus)*

Source: Rutaisire (2005)

2.3 Nutrient Requirements of African Catfish

2.3.1 Energy Requirements of*Clariasgariepinus*

Energy is one of the most important parts of the diet and feeding standard for many animals are based on energy needs, feed intake for catfish may be more a function of how much feed they are allowed to have rather than energy concentration in the strictly regulated by dietary energy, balance of dietary energy is important when formulating catfish feed (Jantrarotai, 1994).

Moreover, if dietary energy content is too high in *Clarias*, catfish may not eat as much as expected resulting in low intake of essentials nutrients. The absolute energy requirement for catfish are unknown, the estimates that are available have been made by measuring weight gain or protein gain of catfish feed diets known contents of energy (Hossain, *et al.,* 1998). Energy requirements for catfish, which have generally been expressed as a ratio of digestible energy (DE) to crude protein range from 31.0 t0 50.2kg-1. Based on current knowledge DE/P ratio from 35.6 to 39.8kg-1 is adequate for used in commercial catfish feeds, increase the DE/P ratio of catfish diets above this range will increase fat deposition and reduce processed yield and in contrast, if the energy value is too low, the fish will grow slowly (Nematipour, *et al.,* 1992).

2.3.2Protein and Amino Acid Requirements of African Catfish

For growth and development to occur in organisms, the nutritional requirement must be met and balanced in accordance to the requirements needed in their feeds. All animals are expected to grow and develop well, for these animals to grow well, protein requirement is needed in every feed to be introduced to animal feed. According to Keremah and Beregha, 2014, Protein requirement study is one of the most important aspects on fish nutrition. Protein provides essential amino acids which are used for tissue repair and growth of the fish. The indispensable amino acids and specific nitrogen are mostly needed in all feeds, so the requirements of fish for indispensable amino acids is expressed as the dietary level (as a percent of the diet) or as a percent of the dietary protein level. To convert an amino acid level from the percent of diet to percent of protein, divide the dietary level of each amino acid by the dietary protein level. These indispensable amino acids include agiline, histamines, methionine, phenylalanine, valine, lysine, leucine, isoleucine, threonine and tryptophan. Dietary protein has significant importance in aquaculture systems because it represents considerable economic investment and a central factor that determines fish growth so long as other physiological requirements needed for growth are fulfilled (De Silva *et al.*, 1989; Buttle*et al.,* 1995). Also, for maximum utilization of dietary protein for growth to occur, the quality of the proteins must also be considered. Catfish requires 40-45% crude protein. Protein comprises about 15-20% of the dry weight of fish muscle (Eyo, 2002).

Anguas –Vélez*et al.*, 2000 reported that food use and fish growth are greatly influenced by some factors such as protein content, feed intake, fish size, salinity, stress and water temperature. According to Watanbe, 1988, it was stated that these factors affect nutritional requirements and optimal dietary levels of nutrients such as protein. During feed formulation, the fish feed should be carefully formulated so as to ensure that the protein fraction does not exceed the optimum level required by the fish in order to avoid or minimize waste. Reports shown by Tucker, 1998 that most of the carnivorous fish studied show a relative increase in the dietary protein requirement in the range of 35-70%. Brett & Groves, 1979 explained this to be that fish being aquatic obtain sufficient energy from chemical breakdown of proteins than animals on land (terrestrial animals).

The requirements for proteins and amino acids have been studied and researched in catfish for several years, but there is still debate as to which level of dietary level of dietary protein is most cost effective. It is difficult to set a level of protein that is best for all situations because of the factors that affect the dietary protein requirement of catfish. These factors include water temperature, feed allowance, fish size, amount of non-protein energy in the diet, protein quality, natural food available, and management practices. Research showed that for greatest profits, the optimum dietary protein level should be changed as fish and feed prices change. Also, diets containing lower levels of protein are adequate for maximum growth but may increase body fat. It is also stated that catfish fry and small fingerlings require diets with more proteins. Major protein sources include Groundnut cake meal, soybean meal, fish meal, blood meal etc.

2.3.3 Carbohydrate Requirement of African Catfish

Carbohydrates are the main form of energy stored in seeds, roots, and tubers. Carbohydrates have several functions in animals. But since animals can produce carbohydrates from lipid and protein, then the animals do not need carbohydrates in their diet for normal growth and functions. Even though Clariasgariepinus do not need carbohydrates in their diet, their feeds contain considerable carbohydrates supplied from grain or grain products (which include maize grain, wheat grain and wheat middlings) that are rich in starch. Starch is said to aids in feed manufacture, and is also an inexpensive energy source. A typical catfish feed contains 25% or more digestible (i.e. soluble) carbohydrates plus 3 to 6 percent more carbohydrates that are generally present as crude fiber (mainly cellulose). Crude fiber should be kept at a low level as possible due to the fact that catfish is unable to digest crude fiber well. Research showed that commercial catfish feeds typically contain less than 5% crude fiber.

However, carbohydrates present a cheap energy source that would `spare` the catabolism of other component such as protein and lipid to energy. Warm water fish can use much greater amount of dietary carbohydrate than cold water and marine fish species (NRC, 1993). The utilization of carbohydrates by catfish appears to differ depending on the complexity of the carbohydrate. Starch is used more efficiently by catfish than are sugars such as sucrose or glucose (Edwin and Meng, 2012). It has generally been thought that tilapia and certain other fish resemble diabetic animals by having insufficient insulin for maximum carbohydrate utilization (Dupree and Hunter, 2010).

Although catfish use carbohydrates effectively, there is no dietary requirement for carbohydrates. Carbohydrates are important dietary component as an in expensive energy sourced as precursor for various metabolism intermediate such as nonessential amino acids and fatty acids (Dupree and Hunter, 2010) and as an aid in pelleting practical catfish feeds and in reducing the amount of protein used for growth (Dupree and Hunter, 2010).

2.3.4 Minerals Requirements of African Catfish

Most importantly, catfish need the same minerals for bone development and metabolism than other livestock need. Catfish also require minerals for a balance between body fluids and their environment. Catfish can absorb some of the minerals needed in the water. Fourteen minerals are considered to be essential to catfish. Phosphorus as a mineral is particularly important in fish feeds because fish require a fairly large amount of it. Fish do not get enough phosphorus from pond water and feedstuffs, especially those that are plant sources; they are poor sources of phosphorus. Due to this, catfish feeds are supplemented with phosphorus. Examples of phosphorus supplements in catfish feeds are defluorinated phosphates and dicalcium. Also catfish feeds are typically supplemented with a trace mineral premix with enough of all essential trace minerals (minerals required at very low levels) to meet or exceed dietary requirements of catfish. However, a trace mineral premix may not be needed in catfish feeds that contain 4% or more animal protein.

2.3.5 Vitamin Requirements of *Clariasgariepinus*

Vitamins are heterogeneous group of organic compounds essential for the growth and maintenance of animal life. The majority of vitamins are not synthesized by the animal body or at any rate sufficient to meet the animal's needs. They are distinct from the major food nutrients (proteins,lipids and carbohydrate) in that they are chemically related go one another, present in very small quantities within animal and plant foodstuffs and are required by the animal body in trace amount.

In general, all animal display distinct morphological and physiological deficiency signs when individual vitamins are absent from the diet.

Craig and Helfrich (2002) reported that vitamin C is the most important vitamin winces it's a powerful antioxidant and help in the immune system of fishes. The fat soluble vitamins

A,D,E,K perform useful function in fish body, Vitamin A (retinol) is important in vision, vitamin D (cholecaciferols) ensure bone integrity (Craig and Helfrich, 2002).

Fish feeds are generally supplemented with a vitamin premix that contains all essential vitamins in sufficient quantities to meet the requirements and to compensate for losses due to feed processing and storage. Vitamins present in feedstuffs are usually not considered during feed formulation because their availability is not known but they certainly contribute to the vitamin nutrition in catfish.

In the wild, fish obtain food naturally from the aquatic environment, however under culture condition the natural feeds are not adequate for optimum growth therefore there is need for supplementary feeds to help fortify the naturally available diet with extra protein, carbohydrates, lipid,minerals and vitamin (Alatise and Okoye, 2009; Lim and Dominy, 2012).

The quality and quantity off feed used in fish culture are the major factors in determining profitability because feed represents the largest single expenditure in semi-intensive or intensive culture operation. Economic production depended on availability of least-cost nutritionally balanced diet (Lim and Dominy, 2012). In Nigeria high cost of feed inputs are the major problems of fish farmers in intensive and semi-intensive culture system (Ayinla, 2007; Fagbenro and Davis, 2003).

2.4 Biochar (*Miscanthus)*

2.4.1 Nature and Origin of *Miscanthus*

Miscanthus is a perennial rhizomatous grass. The genetic origin of Miscanthus is East Asia, where it is found across a wide climatic range from tropical and subtropical in Southeast Asia to warm temperate in parts of the Pacific Islands. Due to this wide regional spread, there is a large genetic variability in *Miscanthus*. In Europe it can grow as far north as Sweden to the very south of Europe. To cover the large range of climatic conditions in Europe, different genotypes are needed. Miscanthussinensis genotypes are better adapted to more extreme

climates, whereas Miscanthus x giganteus is best adapted to temperate climates. Miscanthus has become a leading candidate crop for production of lignocellulosicfeedstocks due to its rapid biomass accumulation in temperate climates. At present Miscanthus is considered one of the four major promising perennial grasses for the production of biomass for energetic and material use in Europe. The activities leading to these results have received funding from the European Union Seventh Framework Program (FP7/2007-2013) under grant agreement nr. 289159.

2.4.2Description of*Mischanthus*

They have high variable robust perennial grass that can grow to 2-3meters (6.5-10 ft.) in height. It is usually found in large tufts. The branches are very flexible and spread or droop. The leaves are elongated and can measure 1m (3 ft.) in length and 2.5m (1 in.) in width. The leaves have sliver white mid-rib the tips of the leaves are sharp and recurving. The fan-shaped terminal panicle is 15-61 cm (6-24 in.) long and can be silvery to pale pink in color. The branches of the panicles are erect or ascending, these panicles reach full maturity in the fall. The glabrous spikelet's are very small, yellow-brown in color and enriched at the base with white colored hairs.

Miscanthussinensis has the ability to reproduce vegetatively via rhizomes.it can also be dispersed longer distances through it's mechanically or wind-dispersed seed.

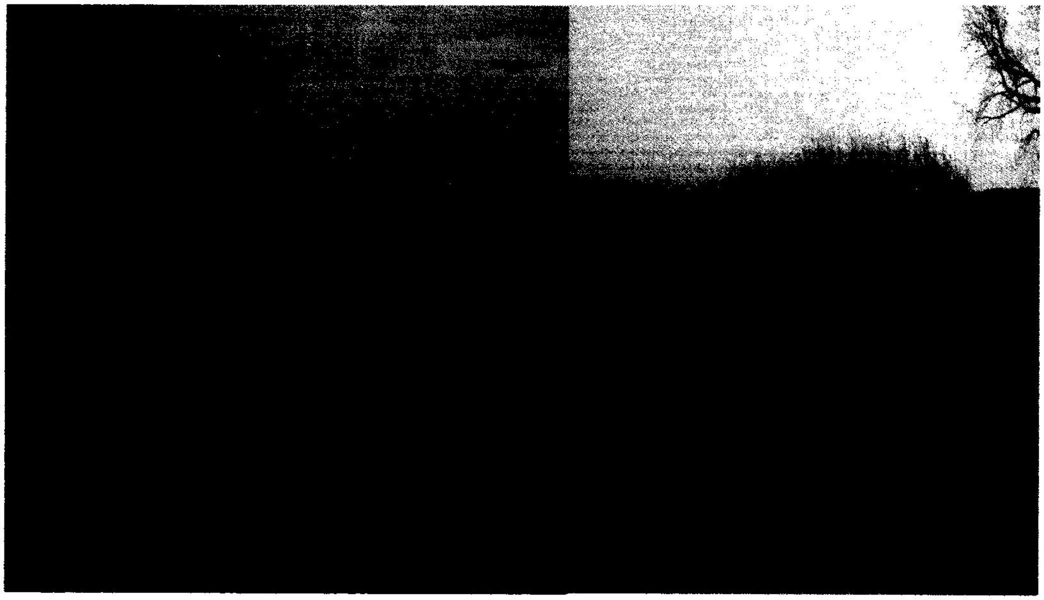

Plate 2a (wet Miscanthus) Plate 2b (dry Miscanthus)

Plate 2: Source of Biochar used ***Miscanthus*** (elephant grass).

2.5 Biochar

2.5.1 Description of Biochar

Biochar is a combination of 'bio' as in 'biomass' and 'char' as in 'charcoal'. It is produced by thermal decomposition of biomass under oxygen-limited condition (pyrolysis) and it has received attention in soil remediation and waste disposal in recent years.

Biochar is a carbon-rich product obtained from the carbonization of biomass. It is commonly used as a soil amendment for crop production and has been shown to increase nutrient availability, soil organic matter, water holding capacity and growth rates of many plants, vegetables (Lehmann 2007; Preston 2015; Bouaravong*et al.*, 2017).

Biochar is a high carbon, fine-grained residue that today is produced through modern 'pyrolysis' process, it is the direct decomposition of biomass in the absences of oxygen which produces a mixture of solids (biochar), liquid (bio-oil), and gas's (syngas) product. The specific yield from a pyrolysis depends on the process condition such as, temperature and can be optimized to produce either energy or biochar (Preston 2015).

Temperature of 400 -500 (752-932) produces more char (charcoal), while temperature above 700) favors the yield of liquid and gas fuel component. Pyrolysis occurs more quickly at the higher temperature, typically requiring seconds instead of hours. High temperature pyrolysis is also known as gasification (Bird *et al.*, 2011).

The characteristics of Biochar are influenced mainly by the preparation temperature and biomass. Biochar has recently been used to remediate soil with both heavy and organic pollutant. Biochar is a charcoal-like material which is applied to a soil or potting mix to improve the structure and growth of plants. Biochar is made by heating recycled organic matter at extreme temperature until most gases and oils are released and recycled to produce green electricity. Majority of Biochar that is produced is derived from terrestrial ligno-cellulosic feedstock's ("woody" plants) that yields Biochar with a high fixed Carbon content (typically in excess of 70%) but low direct nutritive value (Bird *et al.*, 2011).

Biochar differs from charcoal in that the process of carbonization takes place in a more restricted flow of air and at higher temperatures (500 to 1000 °C). These are the conditions found in gasifiers be they updraft (TLUD) stoves for cooking (Olivier, 2010) or downdraft reactors producing gas for internal combustion engines, turbines or simply process heat. By using fibrous waste as feedstock, the gasification system, and the associated production of Biochar, avoids the conflict of using natural resources of soils and water for biofuels instead of food (Preston, 2015). In addition, Biochar also has emerging applications as a source of bioenergy and in the treatment of contaminated waste water (Han *et al.*, 2013).

Aquaculture and irrigation enterprise use Biochar to filter water, the benefits of carbon filters in the water purification process is widely recognized but the effects of Biochar on fishes, if it can result in an increase in growth parameters have not been really dealt with in Nigeria because there is dearth of literature when it comes to the use of Biochar in aquaculture.

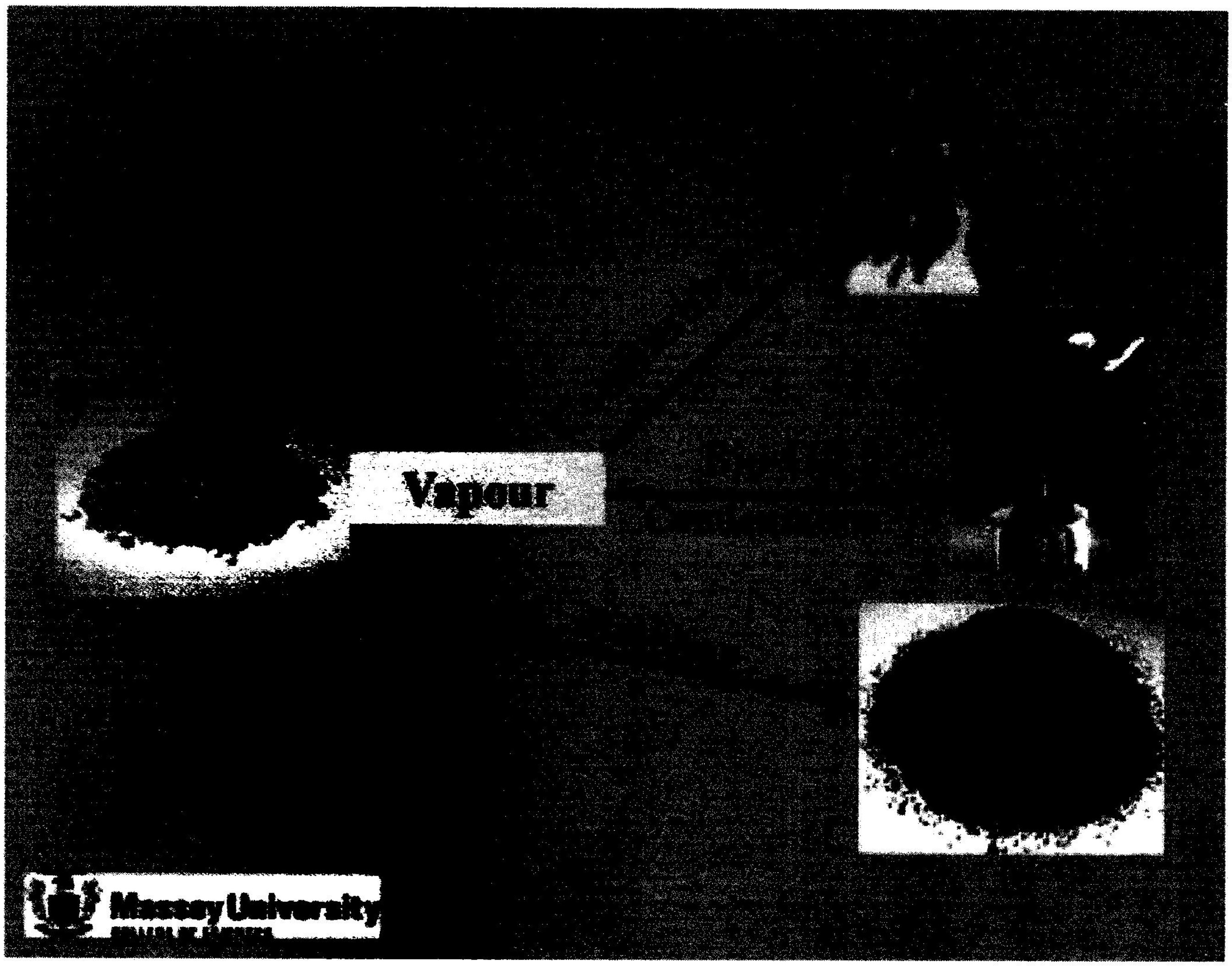

Plate 3: Pyrolysis process of Biomass

Source: Wikipedia (2018).

2.5.2 Sources of Biochar

Biochar can be made from **any of the following materials:**

- Terrestrial feedstock **(woody plant).**
- Seaweeds.
- Micro Algae.
- Plant Biochar (Bamboo, Cocoa pod husk, Rice husk, **Palm bunch, Saw dust**, Wood shaving etc.).
- Agricultural wastes.
- Animal Biochar (Bone, Cow dung, Goat dropping, Pig waste, Poultry dropping etc.).

2.5.3 Production of Biochar

2.5.3.1 Category of Biomasses

BCs are made from range of biomasses that have different chemical and physical properties. The properties of each biomass feedstock are important in thermal conversion processes, particularly the proximate analysis (ash and moisture content), caloric value, fractions of fixed carbon, and volatile components; percentage of lignin, cellulose, and hemicelluloses; percentage and composition of inorganic substance, bulk, true density, particle size, and moisture content. Extensive feedstock biomasses have been used in the production of BC, such as bioenergy crops (willows, Miscanthus and switchgrass), forest residues (sawdust, grain crops, and nut shells), organic waste (green yard waste and animal manure), agricultural waste, kitchen waste, and sewage sludge. In general, a high yield of BC derived from this biomass which has more lignin and less cellulose can be expected. Meanwhile, the porosity of BC increases with the content of lignin in biomass. In addition, the volatile component, water content, and particle size and shape of biomass will affect the property of BC obtained. BCs formed at higher temperatures (lower oxygen to carbon ratio) are expected to be -donors, while BCs formed at lower temperatures are expected to be π-acceptors. BC produced from agricultural waste biomasses does not cause any notable life cycle based greenhouse gas (GHG) emissions.

2.5.3.2 Preparation of Biochar

Carbonized organic matter can essentially have different physical and chemical properties based on the technology (e.g., torrefaction a pyrolytic process primarily at low temperature), slow pyrolysis, intermediate pyrolysis, fast pyrolysis, gasification, hydrothermal carbonization (HTC), or flash carbonization) used for its production. BC can be produced both in traditional earthen charcoal kilns, where pyrolysis, gasification, and combustion process are carried out in parallel below the earthen kiln layer, and in modern BC retorts, where pyrolysis and combustion processes are physically separated by a metal barrier. Papers have been published on the suitability of BC for the stabilization of organic carbon and on the suitability of BC for the improvement of soil properties and immobilization of contaminants in both soil and solution systems.

Pyrolysis technology can be distinguished by the residence time, pyrolytic temperature of the pyrolysis material (e.g., slow and fast pyrolysis process), pressure, size of adsorbent, and the heating rate and method (e.g., pyrolysis started by the burning of fuels, by electrical heating, or by microwaves).

2.5.3.3 Types of Pyrolysis

a. Slow pyrolysis (heating for seconds or minutes) may be described as a continuous process, where purged (oxygen-free) feedstock biomass is transferred into an external heated kiln or furnace (gas flow removing volatile BC emerging at the other end).

b. Fast pyrolysis on the other hand depends on very quick heat transfer, typically to fine biomass particles at less than 650°C with rapid heating rate (Ca 100–1000). The characteristics of the BC product are heavily affected by the extent of pyrolysis (pyrolytic temperature and residence pressure) and entirely by biomass size and kiln or furnace residence time. The rate at which volatile and gases are removed in a kiln or furnace determines the vapour residence time. Prolonged residence time results in secondary reactions, notably the reactions of tar on BC surfaces and charring of the tar rather than additional combustion or processing outside the kiln or furnace.

c. For gasification in pyrolysis, the biomass feedstock to some extent is oxidized in the gasification chamber at a temperature of about 800at atmospheric or elevated pressure. As already pointed out by its name, the main product of this process is gas; only few or no BCs, liquids, or the likes are formed.

d. The hydrothermal carbonization of biomass is obtained by applying high pyrolytic temperature (200–250°C) to a biomass in a suspension with liquid under high atmospheric pressure for several hours. It yields solid, liquid, and gaseous products. Libra et al. refer to hydrothermal carbonization as a "wet pyrolysis". Because no oxygen is applied to the reactor with the biomass liquid suspension, this explanation is readily accepted.

e. Flash carbonization of biomass, a flash fire is lights up at an elevated pressure (at about 1–3 MPa) at the underneath of a packed bed biomass. The fire travels in an upward direction through the carbonization bed against the downward flow of air supplied to the process. A total of about 0.8–1.5 kg of air per kg of biomass is injected to the process. The residence time of the process is below 30 min, and the pyrolytic temperature in the reactor is in the range of 330–650°C. The process results mainly in gaseous and solid products such as BC. In addition to that, a limited number or no numbers of condensates are formed.

f. While oxygen supplied to the carbonization process is a feature of gasification technologies, both process temperature and the product spectrum (delivery between solid, liquid, and gaseous output) of flash carbonization are exceptional for gasification processes.

It can be observed that typical solid products yields obtained by gasification and fast pyrolysis process are significantly lower as compared to the solid product yields of slow pyrolysis, flash carbonization, hydrothermal carbonization and torrefaction. Recent studies by Dowie et al. revealed the experimental pyrolysis conditions performed at the heating rate of about 10 k/min up to the press cubed pyrolytic temperature ranging from 673 to 973 K. The yields of the resulting BC products are in the range of 25–40 wet%. The yield was observed to decrease slightly at higher pyrolysis temperature, which should be attributed to the volatilization of other volatile products from the component of the biomass. Pellera et al., also deduced that the BC yields of rice husk and compost derived after hydrothermal pyrolysis were about 62.5%, while olive pomace and organic waste were quite lower at 37.5%. Concerning the BCs produced through pyrolysis, the increase of pyrolytic temperature leads to a decrease in the yield for all produced materials. Specifically, the yields at 300°C and 600°C were 32.8% and 31.9% for rice husks, 39.3 and 26.7% for olive pomace, 39.6% and 32.8% for orange waste, and 78.7% and 46% for compost, respectively. The development of integrated systems that produce BC at higher efficiency by slow pyrolysis is still, therefore, mainly on the research scale with technology commercially deployed only at a handful of research centers and locations.

Table 1: proximate composition of Biochar (Miscanthus).

Composition	%
Moisture content	6.6%
Crude protein	2.98%
Crude fiber	0.87%
Ash	65.5%
Crude lipid	11%
NFE	13.05%

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

- 15 White static transparent plastic tanks
- Test fish
- Fish feed
- Water
- Biochar sample
- Head cap
- Small white plastics bowls
- Weighting balance
- Hand gloves
- Meter rule
- Laboratory coat.

3.2 Experimental Site

The experiment was carried out in the Wet Laboratory of the Department of Fisheries and Aquaculture, Federal University OyeEkiti, Nigeria.

3.3Biochar Production and Analysis

Biochar was produced from *Miscanthussinensis* (elephant grass) obtained along the Federal University Oye-Ekiti Campus Hostel at Ikole-Ekiti, Nigeria. It was produced through pyrolysisprocess as described by Almuth (2011). The biochar was taking to the analytical laboratory of the Department of Fisheries & Aquaculture where the proximate compositions (ash content, moisture, crude protein, crude fiber, crude lipids etc.) were analyzed and recorded accordingly.

3.4Diet Formulation

3.4.1 Supplemented Ingredients

The ingredient used to produce the Biochar is *Miscanthussinensis* (elephant grass), the ingredient was gotten along the campus hostel, dried and was decomposed in the presences of little or no oxygen (pyrolysis) to for a solid shaped coal. The coal was smashed with mortal and pestle to form a uniform powdery substance that was used as a supplement for minerals in fish feed.

3.4.2 Minerals sources

The test ingredient (Biochar) served as one of the main sources of minerals in the fish feed, this was used to supplement vitamin/minerals premix at different levels of inclusion.

3.4.3 Fixed Ingredients

The fixed ingredients will consist of cassava starch flour obtained locally from the market and used as the binder for the ingredients for easy pelleting. Other ingredients will include methionine, vitamin and mineral premix, vegetable oil, vitamin C and table salt.

3.4.4Diet Formulation

Five iso-nitrogen diets (35%CP) was formulated using Pearson Square method and comp using Microsoft Excel Sheet. All dietary ingredients were milled to 4mm particle size and integrated into computing that required quantities to make up 100 units of the feed (Table 2). Biochar were substituted at 0% (BM0) 25% (BM25), 50% (BM50), 75% (BM75) and 100% (BM100) levels respectively. The strands were sun dried and packaged in waterproof cellophane during the usage time.

Table 3.1: Composition of Formulated Diets

INGREDIENT (%)	BM0	BM25	BM50	BM75	BM100
Fish meal	19.84	19.84	19.84	19.84	19.84
Soyabeans meal	25.68	25.68	25.68	25.68	25.68
GNC	11.67	11.67	11.67	11.67	11.67
Maize	21.52	21.52	21.52	21.52	21.52
Wheat offal	10.76	10.76	10.76	10.76	10.76
Vegetable oil	5.00	5.00	5.00	5.00	5.00
Binder	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
Vitamin C	0.03	0.03	0.03	0.03	0.03
Vit/mineral premix	2.00	1.50	1.00	0.50	0.00
Biochar	0.00	0.50	1.00	1.50	2.00
Methionine	1.00	1.00	1.00	1.00	1.00
TOTAL	**100**	**100**	**100**	**100**	**100**

*Vitamin premix in the diet contains, *Vitamin 8,000,000 IU, Vitamin D_3 1,600,000 IU, Vitamin E 6,000 IU, Vitamin K 2,000 mg, Thiamine B_1 1,5000 mg, Riboflavin B_2 4,000 mg, Pyriodoxine B_6 1,5000 mg, Niacin 15,000 mg, Vitamin $B_{12 10mg}$, Pantothenic acid 5,000 mg, Folic acid 500 mg, Biotin 20 mg, Choline chloride 200 g, Antioxidant 125 g, Manganese 80 g, Zinc 50 g, Iron 20 g, Copper 5 g, Iodine 1.2 g, Selenium 200 mg, Cobalt 200 mg, P = phytase.

***BC.** = Biochar

3.4.5 Proximate composition of Experimental Diet

Nutrient composition of formulated diets is presented in Tables 3, moisture content was similar ($p>0.05$) in BM 25, BM 50 and BM75 but however differ significantly ($p<0.05$) from the moisture content of control and BM100. Fat content differed significantly ($p<0.05$) in all the experimental diet with the highest record in BM 100 and lowest in the control. Crude protein were similar ($p>0.05$) in all the experimental diet with the highest recorded in control and BM 25 respectively and the lowest in BM 100.

Fiber content in all the experiment diets differ significantly ($p<0.05$) with the highest in control and lowest in BM 100.Ash content are significantly similar ($p>0.05$) between control and BM 25 and BM 50 and BM 75 but differ significantly ($p<0.05$) from BM100. Nitrogen free extract were similar ($p>0.05$) in all the experimental diet.

Table 3: **Proximate Composition of Dietary Treatment**

(% Dry Matter)	BM0	BM25	BM50	BM75	BM100
Crude Protein	35 ± 0.58^{a}	35 ± 0.58^{a}	34.80 ± 0.06^{a}	34.65 ± 0.01^{a}	34.60 ± 0.01^{a}
Lipid	16.00 ± 0.58^{a}	17.00 ± 0.58^{ab}	$18.03 \pm 0.06b^{c}$	18.74 ± 0.01^{c}	19.06 ± 0.01^{c}
Crude Fiber	8.60 ± 0.06^{e}	6.23 ± 0.03^{d}	5.27 ± 0.03^{c}	4.23 ± 0.03^{b}	3.33 ± 0.33^{a}
Ash	11.90 ± 0.06^{a}	11.93 ± 0.06^{a}	12.33 ± 0.33^{a}	12.53 ± 0.03^{a}	13.33 ± 0.33^{b}
Moisture	7.36 ± 0.01^{a}	8.20 ± 0.01^{c}	8.06 ± 0.01^{b}	8.15 ± 0.01^{b}	9.80 ± 0.06^{d}
Nitrogen Free Extract	21.14 ± 0.01^{b}	21.68 ± 0.01^{b}	21.51 ± 0.01^{b}	21.70 ± 0.01^{b}	20.88 ± 0.01^{a}

Mean ± S.E with different superscripts are significantly different at $p < 0.05$.

3.5Experimental Procedure

One hundred and fifty (150) post juveniles' of *Clariasgariepinus*(20-23g) were procured from a standard farm in IkoleEkiti, Nigeria and transported in plastic container to the Wet Laboratory of the Department of Fisheries & Aquaculture, Federal University Oye-Ekiti, Nigeria.

The experiment was a complete randomized design (CRD) with three replicates. *Clariasgariepinus*post juvenile were distributed randomly into 15 rectangular glass tanks (60 x 35 x 30 cm^3) and there was10 fish per tank representing five dietary treatments. Fish were fed at 3% of their body weight per day in two equal meals at 0800-1000 and 1700 -1800 Hour. Prior to feeding trials, the fish were starved overnight to empty their gut, increase their appetite and reception for the new diets. Initial weights (g) of fish per treatment tank were taken before the commencement of the experiment. Subsequent weight measurements were taken fortnightly. Water temperature, pH, and dissolved oxygen concentration were monitored daily. Temperature was measured using a mercury-in- glass thermometer, pH was measured with a pH meter (Jenway model 9060) while the dissolved oxygen was measured with an oxygen meter (Hanna model H1-9142). The experimental period of this study was 56 day.

3.6 Proximate Composition

Proximate composition (moisture, ash, crude protein, lipid and fiber) of diets and fish at the end of the 56 day feeding trial were determined according to the methods described bythe Association of Official Analytical Chemistry (2005) at the Analytical Laboratory of the Department of Fisheries and Aquaculture, Federal University OyeEkiti, Nigeria. Crude protein was determined by the Kjedahl procedure. Samples were subjected to petroleum ether extraction at 40-60°C using the soxhlet extraction apparatus. Moisture content was determined by oven drying the samples at 105°C for 12 hour. Crude fiber was determined by boiling the samples under flux in weak sulphuric acid (0.255N H2S04), then in a weak sodium hydroxide (0.312N NaOH) for 1 hour. The residues were dried and weighed. The ash content was determined by igniting a weighed sample in a Muffle furnace at 600°C for 4-hours. Nitrogen free extract (NFE) was obtained by the difference after the percentages of the other fractions were subtracted from 100%.

3.7 Growth Performances and Nutrient Utilization

Indices such as weight gain, relative growth rate, specific growth rate and feed conversion ratio (FCR) were used to evaluate the effect of biochar supplemented diets on the growth and nutritional performance of African catfish post juveniles. The formula for the indices are presented below:

a. **Weight gain (WG)**

WG = Final weight of fish – Initial weight of fish

Relative growth rate (RGR)

$$\textbf{RWG (\%)} = \frac{WF_{(g)} \times 100}{W_1}$$

Where

WF = Final average weight of fish

$\mathbf{W_1}$ = Initial average weight at the beginning of the experiment

c. **Specific Growth Rate (SGR) %**

$$\text{SGR (\%/day)} = \frac{\text{Log}_e\text{WF} - \text{Log}_e\text{W}_1}{\text{Time (days)}} \times 100$$

d. **Survival**

$$\text{Survival Rate} = \frac{\text{Total number of fish} - \text{Mortality}}{\text{Total number of fish}} \times 100$$

e. **Feed conversion ratio (FCR)**

$$\textbf{FCR} = \frac{\text{Dry feed consumed (g)}}{\text{Gain in weight (g)}}$$

3.8 Haematological Analysis

Red blood cell count (RBC), Haemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBC) and white cell differential count was determined by the methods described by Baker and Silverton (1985), while the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was determined from RBC, Hb, and PCV (Hamening 1992).

Using

MCV (Fl)= PCV/RBC (10^6µl-1)

MCH (pg)= [Hb(gdl-1) X10] /RBC (106 µl-1)

And MCHC (gl-1) = [Hb(gdl-1)X 10]/PCV

3.8.1 Packed cell volume (PCV)

The heparinized capillary tubes were 3/4 filled with whole blood and one end sealed with plasticine. The tubes was centrifuged for 5 min in a microhaematocrit centrifuge at 12,000 rpm. The PCV was read by the use of hematocrit reader (Kelly, 1979).

3.8.2 Red blood cell (RBC) and white blood cell (WBC) counts

The RBC and total WBC counts were carried out by use of the Neubauer improved counting chamber as described by Kelly (1979). For red blood cell counts, blood was diluted 1:200 with Dacies fluid (99 mL of 3% aqueous solution of sodium citrate; and 1 mL of 40% formaldehyde) which keeps and preserves the shape of the red blood cell for estimation in the counting chamber (Kelly, 1979).

3.8.3 Total white blood cell counts

For White blood cell counts, the dilution was 1:20 using 2-3% aqueous solution of acetic acid to which tinge of Gentian violet was added. Thin blood smears were stained with Wright-Giemsa stain (Schalm*et al.*, 1975). A total of 100 white blood cells were enumerated and differentiated.

3.8.4 Haemoglobin (Hb)

Haemoglobin was determined by cyanmethemoglobin method described by Schalm*et al.* (1975) and Kelly (1979). Well-mixed blood of 0.02 mL was added to 4 mL of modified Dabkin's solution (potassium ferricyanide, 200 mg; potassium cyanide, 50 mg; potassium dihydrogen phosphate 140 mg. The volume was made up to 1 L with distilled water at pH of 7.0. The mixture was allowed to stand for 3 min and the Hb concentration was read photometrically by comparing with a cyanmethemoglobin standard with a yellow-green filter at 625 nm.

3.9 Statistical Analysis

Water quality parameters, growth and nutritional performance and haematological parameters were analyzed by one-way analysis of variance using the Statistical Package for Social Sciences (Version 20.0). Means were separated where significant differences occur using the Duncan's New Multiple Range Test at $p \leq 0.05$.

CHAPTER FOUR

RESULTS

4.1 Water Quality Parameters of Experimental Medium

Table 4 presents the mean of water quality parameters in the experimental tanks. It indicates that there were significant differences ($p < 0.05$) in the values of water temperature, dissolved oxygen and pH. Temperature was highest (28.70 ± 0.06°C) in tank BM25 and lowest (28.13 ± 0.03°C) in BM75. Lower and similar ($p > 0.05$) dissolved oxygen (3.53 ± 0.03mg/L and 3.47 ± 0.03mg/L) were recorded in BM75 and BM100 respectively while the highest (5.74 ± 0.01mg/L) was recorded in BM0. Water pH were similar ($p > 0.05$) in BM50 (7.35 ± 0.01) and BM75 (7.36 ± 0.01). However, the highest pH (7.67 ± 0.01) was recorded in BM100 while the lowest (7.35 ± 0.00) was in BM25.

4.2 Growth and Nutritional Performance of Fish Fed Biochar Supplemented Diet.

Growth and nutritional performance of African catfish fed Biochar supplemented diets is presented on Table 5. Similar weights ($p > 0.05$) of fish were stocked. Initial weight was highest (23.07 ± 0.31g) in BM100 and lowest (20.77 ± 2.11g) initial weight was stocked in tank BM25. Fish in tanks BM0, BM25 and BM100 consumed similar ($p > 0.05$) quantity (37.27 ± 5.12g, 39.67 ± 2.33g and 35.95 ± 1.48g) respectively. They were however significantly different ($p < 0.05$) from the feed intake (40.02 ± 0.88g and 29.54 ± 1.92g) in BM75 and BM50 respectively. Fish fed BM75 and BM50 consumed the highest and the lowest quantity of diets respectively.

At the end of the 56day experiment, *C. gariepinus* fed 25% Biochar inclusion had the highest final weight (57.68 ± 2.38g) while the least final weight (44.26 ± 2.72g) was recorded in BM50. Final weights were not different ($p > 0.05$) in BM0, BM50, BM75 and BM100. Consequently, fish fed BM25 had the highest (36.91 ± 4.43g) weight gain while the least

(22.01 ± 3.59g) was recorded in fish fed BM50. Weight gained in fish fed diets BM0, BM50, BM75 and BM100 were similar ($p > 0.05$) but were significantly different ($p < 0.05$) from fish fed BM25.

Lowest feed conversion ratio (1.10 ± 0.11) was estimated in fish fed BM25 while the highest (1.75 ± 0.24) was recorded in BM75. Similar ($p > 0.05$) FCR were recorded in fish fed BM0, BM50 and BM100 but differ significantly ($p < 0.05$) from the FCR in fish fed BM25 and BM75. *C. gariepinus* fed BM0, BM50, BM75 and BM100 had similar ($p > 0.05$) specific growth rates but different ($p < 0.05$) from the highest SGR (3.12 ± 0.03) estimated in fish fed BM25. Similar ($p > 0.05$) relative growth rates (201.08 ± 21.11, 204.67 ± 15.86 and 198.00 ± 12.01) were recorded in fish fed BM50, BM75 and BM100 respectively but significantly different ($p < 0.05$) from the RGR values (231.68 ± 40.52 and 286.44 ± 44.04) recorded in BM0 and BM25 respectively. RGR was highest in fish fed BM25 and lowest in fish fed Bm100. Similar ($p > 0.05$) survival rates that ranged between 56.67 ± 3.33 and 63.33 ± 0.33during the study.

Table 4.1: Water Quality Parameters of Experimental Medium

Parameters	BM0	BM25	BM50	BM75	BM100
Temperature (°C)	28.20 ± 0.06^{ab}	28.70 ± 0.06^{d}	28.30 ± 0.06^{b}	28.13 ± 0.03^{a}	28.47 ± 0.03^{c}
Oxygen (mg/L)	5.74 ± 0.01^{d}	5.04 ± 0.01^{c}	3.65 ± 0.00^{b}	3.53 ± 0.03^{a}	3.47 ± 0.03^{a}
Ph	7.37 ± 0.01^{b}	7.35 ± 0.00^{a}	7.35 ± 0.01^{ab}	7.36 ± 0.01^{ab}	7.67 ± 0.01^{c}

Mean ± S.E with different superscripts are significantly different at $p < 0.05$

Parameters	BM0	BM25	BM50	BM75	BM100
Initial Weight (g)	20.79±1.09^{a}	20.77±2.11^{a}	22.25±1.38^{a}	22.71±0.54^{a}	23.07±0.31^{a}
Feed Intake (g)	37.27± 5.12^{b}	39.67 ± 2.33^{b}	29.54 ± 1.92^{a}	40.02 ± 0.88^{c}	35.95 ± 1.48^{b}
Final Weight (g)	48.11± 8.60^{a}	57.68 ± 2.38^{b}	44.26 ± 2.72^{a}	46.43 ± 2.70^{a}	45.85 ± 2.91^{a}
Weight Gain (g)	27.33± 8.55^{a}	36.91 ± 4.43^{b}	22.01 ± 3.59^{a}	23.87 ± 3.27^{a}	22.78 ± 2.82^{a}
Feed Conversion Ratio	1.57 ± 0.42^{b}	1.10 ± 0.11^{a}	1.43 ± 0.27^{b}	1.75 ± 0.24^{c}	1.61 ± 0.12^{b}
Specific Growth Rate	2.96 ± 0.13^{a}	3.12 ± 0.03^{b}	2.91 ± 0.05^{a}	2.95 ± 0.05^{a}	2.94± 0.05^{a}
Relative Growth Rate	231.68±40^{b}	286.44±44.0^{c}	201.08±21.11^{a}	204.67±15.86^{a}	198.00±12.01^{a}
Survival Rate (%)	56.67± 3.33^{a}	60.00 ± 0.00^{a}	63.33 ±3.33^{b}	56.67 ± 3.33^{a}	56.67 ± 3.33^{a}

Table 4.2: Growth and Nutritional Performance of Fish Fed Biochar Meal Diets

Mean ± S.E with different superscripts are significantly different at $p < 0.05$

4.3 Haematological Parameters of *C. gariepinus* fed Biochar Supplemented Diets

Results of blood parameters of fish fed diets with different inclusions of Biochar are presented on Table 4.3. Packed cell volume differ significantly ($p < 0.05$) in all the dietary treatments. Fish fed control 100% biochar supplementation level had the lowest (11.65 ± 0.01%) PCV while the highest (25.57 ± 0.09%) was recorded in fish fed control diet. Similar ($p > 0.05$) levels of PCV (12.13 ± 0.09% and 12.05 ± 0.01%) were recorded in *C. gariepinus* fed BM50 and BM75 respectively.

Haemoglobin differ significantly ($p < 0.05$) in fish fed all dietary treatments. Hb was lowest (3.94 ± 0.04 g/dL) and highest (8.57 ± 0.03g/dL) was recorded in fish fed diet without biochar. Red blood cells were similar ($p > 0.05$) in fish fed BM50 (1.04 ± 0.01), BM75 (1.03 ± 0.01) and BM100 (1.04 ± 0.00). These were however different ($p < 0.05$) from the RBC in fish fed BM25 (1.13 ± 0.01) and control diet (2.27 ± 0.02). White blood cells differ significantly ($p < 0.05$) in fish fed all dietary treatments. African catfish fed 100% biochar inclusion level had more (190.25 ± 0.01) WBC compared with other experimental diets.

Lowest neutrophils and lymphocytes (31.06 ± 0.01 and 29.65 ± 0.01) were recorded in fish fed diets BM100 and BM25 respectively while both parameters were highest (70.35± 0.01 and 68.94 ± 0.01) in C. gariepinus fed diets supplemented with 25% and 100% biochar inclusion levels. Mean corpuscular volume were similar ($p > 0.05$) in fish fed BM50 (46.30 ± 0.06) and BM75 (46.33 ± 0.09) and significantly different ($p < 0.05$) from MCV in fish fed BM0 (99.00 ± 0.06) and BM25 (48.80 ± 0.06). Fish fed BM100 and the control had the highest and least MCV respectively. Mean corpuscular haemoglobin were equally similar ($p > 0.05$) in BM50 (14.36 ± 0.07) and BM75 (14.30 ± 0.01). Mean corpuscular haemoglobin concentration were different ($p < 0.05$) in fish fed all dietary diets. MCHC were more (23.14 ± 0.01) in fish fed BM0 and lowest (10.60 ± 0.60) in fish fed BM100.

Table 4.3: Haematological Parameters of African Catfish fed Biochar Meal

Parameters	BM0	BM25	BM50	BM75	BM100
PCV (%)	25.57 ± 0.09^{d}	12.65 ± 0.01^{c}	12.13 ± 0.09^{b}	12.05 ± 0.01^{b}	11.65 ± 0.01^{a}
Hb (g/dL)	8.57 ± 0.03^{d}	4.23 ± 0.01^{c}	4.10 ± 0.06^{bc}	4.07 ± 0.07^{ab}	3.94 ± 0.04^{a}
RBC (*10^12/L)	2.27 ± 0.02^{c}	1.13 ± 0.01^{b}	1.04 ± 0.01^{a}	1.03 ± 0.01^{a}	1.04 ± 0.00^{a}
WBC (*10^9/L)	141.91 ± 0.01^{c}	134.00 ± 0.58^{a}	150.75 ± 0.01^{d}	151.60 ± 0.06^{d}	190.25 ± 0.01^{e}
Neutrophil (%)	64.33 ± 0.09^{c}	70.35 ± 0.01^{e}	65.62 ± 0.03^{d}	59.70 ± 0.35^{b}	31.06 ± 0.01^{a}
Lymphocyte (%)	35.67 ± 0.09^{c}	29.65 ± 0.01^{a}	34.38 ± 0.03^{b}	40.30 ± 0.35^{d}	68.94 ± 0.01^{e}
MCV (fL)	99.00 ± 0.06^{d}	48.80 ± 0.06^{c}	46.30 ± 0.06^{b}	46.33 ± 0.09^{b}	45.02 ± 0.09^{a}
MCH (Pg)	30.75 ± 0.01^{d}	15.17 ± 0.01^{c}	14.36 ± 0.07^{b}	14.3 ± 0.10^{b}	13.85 ± 0.01^{a}
MCHC g/dL	23.14 ± 0.01^{e}	11.36 ± 0.09^{d}	11.07 ± 0.27^{c}	10.73 ± 0.01^{b}	10.60 ± 0.06^{a}

Mean ± S.E with different superscripts are significantly different at $p < 0.05$.

4.4 Carcass Quality of African Catfish fed Biochar Supplemented Diets.

Table 4.4 presents data on the proximate composition of *C. gariepinus* fed biochar supplemented diets. Crude protein were similar ($p > 0.05$) in catfish fed BM75 (15.48 ± 0.03%) and BM100 (15.40 ± 0.06%) but different from the crude protein of fish fed other dietary treatments. *C. gariepinus*fed control diet had the highest (16.32 ± 0.08%) crude protein while the least CP (15.40 ± 0.06) was recorded in fish fed BM100. Values of lipid and ash differ significantly for all the treatment diets. Fish fed BM25 and BM100 had the highest lipid and ash while the lowest lipid (4.49 ± 0.01) and ash (6.04 ± 0.01) were recorded in fish fed BM100 and BM0 respectively. Moisture and Nitrogen free extract were similar ($p > 0.05$) in fish fed BM75 and BM100. Catfish juveniles fed BM50the least moisture .Also, *C. gariepinus fed* the control had the highest NFE and moisture content respectively.

Dry Matter)	BM0	BM25	BM50	BM75	BM100
Crude Protein	16.32±0.08[c]	16.21±0.01[bc]	16.12± 0.01[b]	15.48±0.03[a]	15.40±0.06[a]
Lipid	5.13± 0.00[d]	5.16 ± 0.01[e]	4.84 ± 0.01[c]	4.55± 0.01[b]	4.49 ± 0.01[a]
Ash	6.04 ± 0.01[a]	6.35 ± 0.01[c]	6.26 ± 0.01[b]	7.08± 0.01[d]	7.15 ± 0.01[e]
Moisture	7.82± 0.01[d]	5.57 ± 0.01[b]	4.37 ± 0.01[a]	5.97 ± 0.01[c]	5.97 ± 0.01[c]
Nitrogen Free Extract	64.70±0.05[a]	66.51± 0.01[b]	66.60± 0.01[b]	66.93±0.04[c]	66.99±0.07[c]

Table 7:Carcass Quality of African Catfish fed Biochar Supplemented Diets

Mean ± S.E with different superscripts are significantly different at $p < 0.05$.

CHAPTER FIVE

5.1 DISCUSSION

5.1.1 Growth Performances and Nutritional Utilization

The result of this study indicated that growth and nutrient utilization decreased as the level of the biochar increases. *C. gariepinus* fed biochar at 25% supplementation level had desirable growth and nutrient utilization compared to the control and other treatment groups.

There were significant differences in the feed intake with fish fed 25% biochar supplementation level having the highest weight gain (36.91g) and the best feed conversion ratio (1.10). Fish fed 25% biochar had better growth performance compared with the control group and other treatment groups. Previous studies by Thu (2010) and Pirarat*et al.* (2015) reported better performance in aquatic animals supplemented with 10% biochar. The level at which fish fed biochar performed better in this study (25%) was higher (0.2-0.6%) reported by Kutlu*et al.* (2000) Kana *et al.* (2010) Prasai (2013) and Jiya et al. (2013) 0.2-0.6% levels bird, 1% in cattle (Leng*et al.*, 2014). The numerical increase in the value of feed conversion ratio across the treatments fed diet containing biochar supplemented diet may be due to the presence of lipid which may have reduce the utilization of the nutrient and some may have bypassed without being utilized which was in contrast with the report of Durowaye (2015), Mulky and Gandhi(1994). The act and process of supplementing biochar (Black Carbon) in feed has been known to enhance the growth performance of terrestrial and aquatic animal (Mekbungwan*et al.*, 2004; Ven*et al.*2006; Thu *et al.*, 2010).

5.1.2 Proximate Composition of African Catfish Fed Biochar Supplemented Diet

Proximate composition of African catfish fed biochar supplemented diet provides information on the nutrient availability in the fish after 56 days of feed trials. The lipid content in fish fed biochar supplemented diet decreased as the level of biochar diet inclusion increases. This may be due to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy requirements for vital body function (Madalla*et al.*, 2013. Crude protein of the fish decreased as the level of supplementation increases. This is different from the findings of Naren*et al.* (1994) who reported increase in crude protein level. All the experimental feeds were actively accepted by the fish throughout the experimental period which implies incorporation of biochar does not affect the palatability of the diets.

Nitrogen free extract and ash content in the fish increased with the level of supplementation. But NFE is *C. gariepinus* fed 25% supplementation was the least compared with the values in the control and other treatment groups. The ash content is an indication of the levels of minerals which were more in fish fed 100% and lowest in those fed 25% biochar supplementation level.

5.1.3 Haematological Parameters of Post Juvenile African Catfish Fed Biochar Supplemented Diet

The heamatology parameters of this study indicate that heamatological parameters across all treatments differ significantly. Pack cell volume, haemoglobin, Red blood cell, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin and Neutrophil concentration of fish decreased as the level of biochar increases while the white blood cell and lymphocytes increased with increase inbiochar supplementation. All the haematological parameters in test organism were within the recommended physiological ranges a reported for *C.gariepinus*. However, fish fed diet supplemented with 25% biochar had desirable blood.

The decrease in the major blood parameters and increased WBC and lymphocytes most especially in fish fed BM50, BM75 and BM100 respectively could be attributed to high fiber levels or harm substances in the diets. This trend was documented by Bawala*et al.* (2007) who reported increased WBC and lymphocytes as well reduction in other blood parameters in fish fed diets with high levels of fiber and harmful substances could be due to harmful effects of high dietary contents and presences of toxic substances. The result of the present study was different from the findings of Boonanuntanasarn*et al.* (2014), Kana *et al.* (2014) and Majewska*et al.* (2009) whorevealed that dietary supplementation of 0.3% charcoal had no significant effect on hematological indices of tilapia, turkey and broiler respectively.

5.1.4Water Quality Parameters in Experimental Tanks

The result of water quality parametersindicated that the pH (7.37-7.67), dissolved oxygen (3.45-5.74mg/l) and temperature (28.13°C-28.70°C) obtained in this study was within the acceptable limit for catfish growth and health (Omitoyin, 2007). The level of pH and dissolved did not differ among treatments and were within the suitable range for growth of fish (Boyd 1990). Results of this study were similar to the findings of Javan*et al.*(2014) for who reported similar pH and dissolved oxygen in*P.hypophthalmus* fed bamboo charcoal. The values of temperature, pH and dissolved oxygen were with the range recommended by Boyd (1981) for temperature (22 – 27°C), pH (6.5– 9.0) and dissolved oxygen 6.3mg/L-9.6 mg/L) respectively. Balogun, *et al.* (2004) also found that temperature ranged between 23°C and 27°C, pH (6.3-7.8) and dissolved oxygen (6.3 - 9.6mg/l) were adequate for an optimal growth and development of Nile tilapia (*Oreochromisniloticus*) raised under aquaria conditions. The observed water quality parameters were due to constant water change throughout the duration of the experiment. Similarly, the close range of treatment recorded during the experimental period was due to the fact that all treatmentswere indoors.

5.2 CONCLUSION

This study has confirmed that biochar has the potential to be supplemented for vitamin/mineral premix and greatly reduce expenditure on fish feed without reducing growth and nutritional performance of African catfish. Replacing vitamin premix with biochar at 25% and below gives desired nutrient composition, growth and nutritional performance as well as desired blood indices in African catfish, more emphasis should be laid on biocharsupplementation in order to increase its utilization in African catfish and other culturable fish species in Nigeria. Hence, further research and analysis should be carried out or done on the minerals composition of *Miscanthus*biochar.

5.3 RECOMMENDATION

Given the result of the study,I will recommend that mor research should be carried out on the digestability of fish feed with blochar.

More research should be carried out on the economy analysis of blochar as a supplement for mineral in the diet of clarias dariepius.

More emphasizes should be laid on biochar supplementation inorder to increase its ultilization in Africa,cat fish and other culturable fish species in Nigeria.Hence,further research and analysis should be done on the mineral composition of Miscanthus biochar.

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