GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF C*LARIAS GARIEPINUS* (BURCHELL, 1822) FED WITH VARYING SUBSTITUTION LEVELS OF FISH MEAL WITH CATTLE HOOF MEAL

BY

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DEPARTMENT OF BIOLOGY,
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DECLARATION

I solemnly declare that the work in this dissertation entitled "Growth performance of African catfish, C*larias gariepinus* (Burchell, 1822) fed with varying substitution levels of fish meal with cattle hoofs meal" has been carried out by me in the Department of Biology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

_____ _____

Yusuf Ismaila Abayomi Date

CERTIFICATION

This dissertation entitled "GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) FED WITH VARYING SUBSTITUTION LEVELS OF FISH MEAL WITH CATTLE HOOF MEAL" by Ismaila Abayomi YUSUF meets the regulation governing the award of degree of Master of Science of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to the most-high, most-magnificent and the most-merciful Allah for His mercy and guidance on me and my beloved parents in appreciation of their invaluable parental care especially for the moral and financial support on me.

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All praises be to Allah, the Almighty, for His open and concealed bounties which He bestows to us all times. The creator, sustainer and nourisher Who spared my life and facilitate the overcoming with ease all my previous hardship and distress. He made it possible for me to carry out this research as well as providing the needed strength and wisdom of managing the stress and challenges associated with it. I shall ever be grateful to Him.

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ABSTRACT

Fifteen weeks (105 days) feeding trials were conducted on growth response and economic benefit of replacing fishmeal with cattle hoof meal (CHM) in the diets of African catfish (*Clarias gariepinus*) juveniles. The objectives of the research were to determine the growth performance, feed utilization, apparent digestibility, carcass composition and cost effectiveness of *C. gariepinus* fed with varying substitution levels of fish meal with cattle hoof meal. Five isonitrogenous diets containing 42% crude protein were formulated in which CHM replaced fishmeal at 0, 25, 50, 75 and 100% coded as D2, D3, D4, D5 and D6 respectively. A commercial feed (D1) was used as reference diet. Two hapa partitioned into six treatments of three replicates each embedded in a concrete pond of 5m by 3m by 2m was used for the experiment. The fish were fed at 3% body weight three times daily between the hour of 6.00 and 7.00am, 2.00 and 3.00pm, and 10.00 and 11.00pm. The feeding ration was adjusted every three weeks after weighing. The mean physico chemical parameters of the water in the experimental tank were within the acceptable range for optimum growth of *C. gariepinus*. Cattle hoof meal contained 58.06% crude protein, 2.75% crude fibre, 3.25% crude lipid, 0.08% phosphorus, 0.09% potassium, 0.24% calcium and 19.59% sulphur. Some essential amino acids recorded were leucine (7.15g/100g), lysine (4.29 g/100g), methionine (2.19 g/100g) and tryptophan (0.81g/100g). Feeding trials indicated that all fish consumed the experimental diets actively. The fish fed the control diet with no cattle hoof meal (D2) had lower weight gain compared to the fish fed 25% CHM. The fish fed 50% CHM, 75% CHM and 100% CHM showed a decreasing weight gain (148.60g, 143.73g and 129.00g respectively) as the substitution level of the CHM meal increased. The fish fed with 25% cattle hoof meal (D3) had the best growth performance and feed utilization proficiency (SGR 2.58, RGR 1403, PER 1.67 and FCR 1.47) among the fish

fed the formulated diets (D2, D3, D4, D5 and D6). There was no significant difference ($p>0.05$) in the survival value of fish fed with all the experimental diets. The apparent digestibility coefficient of the feed fed to *Clarias gariepinus* decreased with increasing level of substitution of fish meal with CHM. Carcass composition of the fish revealed that all the fish fed the experimental diets had higher carcass protein, lipid and ash contents but lower nitrogen free extract content than the initial fish sample. The fish fed D3 had the best Net profit (₦46.53) followed by D6 (₦45.63), D1 (₦42.54), D5 (₦41.61), D4 (₦31.49) and D2 (₦20.59) in decreasing order. This experiment revealed that CHM can replace fishmeal at a lower cost and efficient diets for *Clarias gariepinus*. The best result was produced at the substitution level of 25% inclusion of CHM. It is recommended that CHM be included in the feed of *Clarias gariepinus* at 25% for optimum growth performance and reduced cost of production. Field experiment especially the semi intensive system in earthen ponds is suggested. Further studies with CHM using other cultured species are highly recommended.

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LIST OF ABBREVIATIONS

ADC	Apparent digestibility coefficient
ADC_{CP}	Apparent Digestibility Coefficient of Crude protein
ADC_{CL}	Apparent Digestibility Coefficient of Crude Lipid
ADC_{CHO}	Apparent Digestibility Coefficient of Carbohydrate
ADC_{DM}	Apparent Digestibility Coefficient of Dry matter
ADC_{GE}	Apparent Digestibility Coefficient of Gross energy
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
ARA	Arachidonic acid
BCR	Benefit cost ratio
BOD	Biological oxygen demand
CF	Commercial feed
CHM	Cattle hoof meal
D1	Commercial feed
D2	Diet which contained 0% Cattle hoof meal
D3	Diet which contained 25% Cattle hoof meal
D4	Diet which contained 50% Cattle hoof meal
D5	Diet which contained 75% Cattle hoof meal
D6	Diet which contained 100% Cattle hoof meal
DE/P	Digestible energy/Protein
DHA	Docosahexaenoic acid
DO	Dissolved oxygen
EAA	Essential amino acid
EW	Economic weight gain

FAO	Food and Agriculture Organisation
FCR	Feed conversion ratio
IAR	Institute of Agricultural Research
IC	Incidence cost
K	Condition factor
MWG	Mean weight gain
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NAERLS	National Agricultural Extension and Research Liaison Services
NFE	Nitrogen free extract
NP	Net profit
NRC	National Research Council
NSN	No supplement needed
PER	Protein efficiency ratio
PI	Production index
PI	Profit index
RGR	Relative growth rate
SGR	Specific growth rate
SL	Standard length
SR	Survival rate
TAN	Total ammonia nitrogen
TC	Total cost
TL	Total length

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Fish is an important source of proteins, vitamins and minerals (FAO, 2014). It has essential nutrients required for supplementing both infant and adult diet (Abdullahi *et al.*, 2001). Fish is less tough and more digestible when compared with beef, mutton, chicken and bush meat (Idakwo *et al.*, 2016). This is due to the greater ratio of muscles protein to connective tissues in fish in relation to other animals thereby making fish acceptable by infants and adults (Oladipo and Bankole, 2013). Fish have been identified to be the solution to problem of malnutrition due to its availability, general acceptance, nutritional quality and the relatively cheap price as a source of animal protein (Agbabiaka *et al.*, 2013).

More than half of the fish being consumed by humans worldwide are produced by aquaculture (Kiel, 2011). In 2012, aquaculture production overtook global beef production in quantity for the first time (Pucher *et al.*, 2014). Aquaculture, the farming of aquatic organisms including fish, molluscs, crustacean and aquatic plant is growing exponentially in the world today (Diana, 2009).

Recent information by FAO (2016) stated that in Africa, Egypt and South Africa are the first and second (respectively) aquaculture producing countries while Nigeria is the third, but number one in production and consumption of *Clarias gariepinus*. The farming of catfish is important to Nigeria because it is a source of income, creates employment opportunities, contributes towards Gross Domestic Product (GDP), fetches higher price than tilapia due to the fact that it can be sold live at the market (FAO, 2016).

The major aim of fish farming is to have optimum increase in fish production within the shortest interval at minimum cost. The most costly resource in fish farming is the feed which consumes 65-80% of the total cost of production (FAO, 2014). Options for reducing feed costs must focus on formulation strategies to optimize nutrient utilization, particularly Protein source of fish feed which is the most expensive component (Vásquez-Torres *et al.*, 2011; FAO, 2012)

Fish meal is the most widely used protein source in aqua feed due to its highly digestible protein, amino acids and palatability; generally it is the conventional protein source in aquaculture feeds. It supports good fish growth because of its protein quality and palatability. Fish meal is often scarce and expensive, especially good quality brands, thereby contributing to the high cost of fish production and nutrition (Fagbenro and Davies, 2002).

Soybean meal and peanut meal are commonly used as alternatives to fish meal in fish diets (Wu *et al.*, 2016). As the use of soybeans and peanut (groundnut) in human food and fish feed increases, their costs have increased, and the economics of using them in fish feeds may become less favourable. Currently, aquaculture feed use more than 20% of the world supply of fish meal, which is produced at approximately 6mmt per year. The practice of feeding cultured fish with products emanating from finite fishery resources jeopardizes the long term sustainability of aquaculture systems.

Utilization of animal by-product protein in fish feed could be an alternative to expensive fishmeal to decrease production cost. Several animal by-products have been used to supplement/replace fish meal in the diet of *Clarias gariepinus* at different inclusion level with each producing different results. Many studies have been conducted to determine the suitability of animal based ingredients such as poultry by product (Gomaah and El Naggar, 2004),

grasshopper meal (Olaleye, 2015), locust meal (Balogun, 2011), cricket meal (Taufek *et al.*, 2016), recycled dead chicken meal (Agbabiaka *et al.*, 2013), maggot meal (Ogunji *et al.*, 2011), termite meal (Sogbesan and Ugwumba 2008), Mealworm (Ng *et al.*, 2001), silkworm pupae meal (Alegbeleye *et al.*, 2012), black soldier fly meal (St-Hilaire *et al.* 2007), feather meal (Omitoyin and Faturoti 2001; Wei-Kang *et al.*, 2013), to replace fish meal in the diets of different stages of African catfish. Chor *et al.* 2013 reported that 20% inclusion level of feather meal in the diet of *Clarias gariepinus* produced the best promising result. Yue and Zhou (2008) concluded that the substitution of fish meal with feather meal in fish diet should be limited to less than 40% except in the diet of Trout and Tilapia. Animal by-product in the feed of African Catfish produces best result at different substitution level depending on the nutrient composition and digestibility of the product.

Feathers, horns and hooves are composed of keratin protein, consisting of amino acids, and are generally considered as wastes but represent a potential alternative to expensive dietary ingredients for animal feedstuff (Jayapradha *et al.*, 2011). Currently, some industries are involved in feather meal production by steam pressure cooking, and the nutritional value of the feather meal has also been reported (Wei Kang *et al.*, 2013). Horn and hoof are protein rich animal byproducts which are generated in tons in Abattoirs all over the country (Qureshi *et al.*, 1962).

Horn and hooves can be degraded to meals using thermal, solvent or microbial degradation procedure. The meal has been used to replace meat meal as a source of protein rations for growing chicks (Qureshi *et al.*, 1962). The digestibility of horn and hoof meal has been shown to increase progressively as it is ground finer. Cattle hoof is not completely a waste product since it is often used in some cosmetic industries and production of some traditional artifacts. The

utilization of hoof as a protein source for fish feed may be economical because it is widely available and relatively cheap.

The future of aquaculture depends on the development of nutritionally sound and cost-effective feeds based on non-conventional feedstuff that can support increased production levels in both intensive and especially semi-intensive systems (Kirimi *et al.*, 2016). Therefore, the search of alternative high-quality protein sources for the fish feed industry is a key to increase high-quality feeds which will influence fish production to meet the increasing demand. The utilization of by-products from other agricultural activities is advantageous, since it does not require additional space (Krome *et al.*, 2016), but rather, consumption of what is perceived as waste

Catfish of the family Claridae are the most commonly cultivated fishes in Nigeria, particularly *Clarias gariepinus, Heterobranchus bidorsalis, Clarias* × *Heterobranchus* hybrid (*Heteroclarias*) and *Clarias nigrodigitatus* (Chika and Nathaniel, 2014; Omeru and Solomon, 2016). *Clarias gariepinus* is widely appreciated by Nigerian consumers for the quality of its meat, either as fresh or smoked in soups and other meals. The fish is mostly cultured in earthen ponds, but as gained popularity and adapted to culture in other systems such as concrete and plastic tanks and hapas. In the wild and riverine systems, the fish reproduces naturally, but considerable effort is required to induce spawning under culture conditions. *Clarias gariepinus* is an excellent candidate for aquaculture as it is omnivorous, it has high fecundity, grows fast, and tolerates high density and environmental extremes (Amisah *et al.*, 2009). It also accepts wide range of natural and artificial food, and adapts to a variety of feeding modes in expanded niches. However, there is paucity of information on the comparative analysis of the growth performance of *Clarias gariepinus* fed with varying inclusion levels of cattle hoof meal.

1.2 Statement of Research Problem

Inadequate feed input is one of the major constraints to aquaculture in Nigeria and nutrition in fish farming accounts for more than 60% of the production cost (Annune and Oniye, 1993). Feed type and source is one of the factors which affect growth rate and health status of cultured fish. Despite the high potentials in aquaculture, local fish production has failed to meet the country's domestic demand for aquaculture product due to high cost of production which emanate from high cost of feed ingredients (Umar *et al*., 2016).

Some commercially produced feeds are rich but imported, hence very expensive, absolute dependence on such feeds reduces the profit of the farmer. Locally produced feed is faced with the problem of high cost of feed ingredients (Omeru and Solomon, 2016). Protein source is the most expensive component of fish feed (FAO, 2012). Fish meal is a commonly used protein source in fish feed production but it is very expensive and scarce because of its high demand for other purposes.

Cattle hooves are keratinized protein with little economic importance, that can replace expensively imported fish meal in fish feed. Fish feed formulation requires quality raw materials to provide a balanced diet for optimum growth of the fish.

There is therefore a pressing need to search for alternative cheap feed stuffs from unconventional sources to reduce cost of production. Against these constraints, this research intends to explore the potential of using various inclusion levels of hoof meal as a protein source in the diet of *Clarias gariepinus* fingerling.

1.3 Justification

Aquaculture is an important approach in the global fight against hunger, malnutrition and poverty, particularly in developing countries like Nigeria (Annune and Oniye, 1993). Animal by-products such as cattle hooves which are often discarded and considered as waste are cheap protein source which could replace the expensive fish meal in fish feeds.

Cattle hooves are animal byproduct with crude protein of about 88% (Qureshi *et al* 1962). Utilization of cattle hooves as a protein source in fish feed may reduce cost of feed as it is relatively cheap and widely available. Reduction of cost of production, most especially the cost of feed ingredients is expected to boost profit, attract more entrants into fish culture, and increase entrepreneurship thereby increasing employment opportunities in the nation (Umar *et al*., 2016).

The choice of catfish, *Clarias gariepinus*, among other fish species, is due to its high feed conversion ratio, resistance to diseases, low technology farming system, excellent food meat quality/high palatability, ability to tolerate high stocking density and wide range of environmental conditions (Dasuki *et al.*, 2013).

1.4 Aim

The aim of this study was to investigate the growth performance and feed utilization of *Clarias gariepinus* fed varying substitution levels of fish meal with cattle hoof meal in hapas situated in a concrete pond.

1.5 Objectives

The specific objectives of the study were to determine:

I. The proximate and mineral composition and amino acid profile of Cattle hoof meal

II. The growth performance and feed utilization of *Clarias gariepinus* fed with varying substitution levels of fish meal with cattle hoof meal

III. The apparent digestibility of *Clarias gariepinus* fed with varying substitution levels of fish meal with cattle hoof meal.

IV. The carcass composition of *Clarias gariepinus* fed with varying substitution levels of fish meal with cattle hoof meal.

V. The cost effectiveness of replacing fish meal with graded levels of cattle hoof meal

1.6 Hypotheses

I. The proximate and mineral composition and amino acid profile of cattle hoof meal are not suitable for fish formulation.

II. There is no significant difference in growth performance and feed utilization of *Clarias gariepinus* fed with varying substitution levels of fish meal with cattle hoof meal

III. There is no significant difference in apparent digestibility of *Clarias gariepinus* fed with varying substitution levels of fish meal with cattle hoof meal.

IV. There is no significant difference in carcass composition of *Clarias gariepinus* fed with varying substitution levels of fish meal with cattle hoof meal.

V. The cost effectiveness of replacing fish meal with graded levels of cattle hoof meal is not significantly different.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of C*larias gariepinus*

Clarias gariepinus or African sharp tooth catfish (Figure 2.1) is a species of catfish of the family Clariidae, the air breathing catfishes. Since the last three decades, clariid species have been considered to hold great interest for fish farming in Africa and Nigeria in particular (Momoh and Solomon, 2017). The family Clariidae is the second most important group of farmed fish in the world, Carp production taking the lead (FAO 2016). *Clarias gariepinus* is generally considered to be one of the most important tropical fresh water fish species for aquaculture because of its nutritional quality, ease of culturing under controlled environment and ability to accept artificial feed (Omeru and Solomon, 2016).

According to Skelton (2001) *Clarias gariepinus* is probably the most widely distributed fish in Africa. The native range of *C. gariepinus c*overs most of the African continent, with the exception of Maghreb, Upper and Lower Guinea, and the Cape provinces of South Africa. It has an almost pan-African distribution ranging from the North to West Africa and from Algeria to Southern Africa. They also occur in Asia Minor (Isreal, Syria and South of Turkey). *Clarias gariepinus* is called *Clarias lazera* in Northern and central Africa, *Clarias gariepinus* in South Africa (Viveen, *et al.,* 1977). Since the culture of *Clarias gariepinus* through hypophysation was initiated in Western Nigeria in 1973, the procedure has been widely practiced throughout Nigeria thus leading to increase of farm-raised catfishes from the 80's to date (Chika and Nathaniel, 2014)

Clarias gariepinus can be defined as displaying an eel shape having an elongated cylindrical body with dorsal and anal fins being extremely long with both fins containing only soft fin rays. They have strong pectoral fins with spines that are serrated on the outer side (Teugels, 1986). The dorsal fin has 61-80 soft rays and the anal fin has 45-65 soft rays. The head is flattened, highly ossified, the skull bones forming a casque and the body is covered with a smooth, scale-less skin. The skin is generally darkly pigmented on the dorsal and lateral parts of the body. The colour is uniformly marbled and changes from greyish olive to blackish according to the substrate. On exposure to light, the skin colour generally becomes lighter. Colour varies dorsally from dark to light brown and is often mottled with shades of olive and grey while the underside is a pale cream to white. Adults possess a dark longitudinal lines on either side of the head; however, this is absent in young fish. They can grow very large with a maximum reported length of 1.7m and weight of 60 kg (Froese *et al.*, 2014). The Fish has broad, terminal mouth with four pairs of unbranched barbels, one nasal, one maxillar on the vomer and two mandibulars (inner and outer) on the jaw. Tooth plates are present on the jaws as well as on the vomer. The wide mouth enables it to swallow relatively large prey whole.

A supra-brachial or large accessory breathing organs with modified gill arches composed of a pair pear shaped air chamber containing two arborescent structures is generally present. These arborescent structures located on the fourth branchial arcs are supported by cartilage and covered by highly vascularized tissue which can absorb oxygen directly from the atmosphere. The accessory breathing organ allows the fish to survive out of water for many hours or many weeks in muddy marshes. They secrete mucus which prevents the fish from drying and allows it to crawl on dry ground to escape drying pools (Anoop *et al.*, 2009) and also burrow into the muddy substrate of a drying body of water (Pienaar, 1968; Teugels, 1986; Skelton, 1993).



Plate 2.1 *Clarias gariepinus*

The male and female of *Clarias gariepinus* can be easily recognized as the male has a distinct sexual papilla located just behind the anus. This sexual papilla is absent in the females.

Clarias gariepinus is a nocturnal fish which feeds on living and dead animal matter. They are bottom dwellers and do most of their feeding there; in aquaculture, floating feed is preferred so as to enable easy scooping off of unfed feed. *Clarias gariepinus* can survive in very poorly oxygenated water (Pienaar, 1968).

2.1.1 Nutrient Requirement of *Clarias gariepinus*

The dietary nutrient requirements of all aquaculture species can be considered in five different nutrient groups viz protein, lipids, carbohydrates, vitamins and minerals. The science of aquaculture nutrition and feeding is concerned with the supply of these dietary nutrients to fish either directly in the form of an exogenous artificial diet or indirectly through the increase production of natural live food organisms within the water body in which the fish are cultured (FAO, 1987; Falaye, 1992).

2.1.1.1 Protein Requirement

Clarias gariepinus has a relatively high dietary protein requirement. The best growth rates and food conversions are achieved with diets containing 35-42% crude protein and a calculated digestible energy level of 12 kJg-1 (Eyo and Olatunde, 2001). Fish are fed higher percentage of protein in their diets than land animals but they have lower daily energy requirement (Mangalik, 1986). Dietary crude protein is vital in fish feeds and must be supplied for rapid growth (Jauncey, 1982; Lovell, 1989). Oluyinka *et al,* 2015 reported that increased crude protein levels and caloric content of the diet had a significant effect on the mean weight gain and specific growth rate of *Clarias gariepinus*.

Dietary protein is the only source of nitrogen for constructing amino acids and proteins in fish. The protein requirement of fish depends on the composition and ratio of amino acids. Amino acids are substances derived from products of protein digestion. They are classified into two groups, the dispensable and indispensable amino acids; these are in accordance with the ability of the fish to synthesize any of the amino acid (NRC, 1993).

The indispensable amino acids are those that cannot be manufactured by the fish and therefore must be supplied in the diet, also called the Essential Amino acids (EAA). These EAA include lysine, leucine, arginine, methionine, valine, histidine, isoleucine, phenylalanine, tryptophan and threonine (Nose, 1974). The dispensable amino acids can be manufactured and synthesized by fish and are usually called the non-essential amino acids. They include alanine, aspartic acid, cystine, glutamic acid, glutamine, glycine, proline, serine and tyrosine. Lysine is the most important amino acid for the growth of fish and can be obtained from legumes, meat, fish, meat and bone meal. Its requirement in the diet of fish is about 7.3-8.6% (Fagbenro *et al.*, 1999).Tryptophan is very important for the growth of all cells and normal development. It is used for cell proliferation. It is needed in about 4.5-5.8% in the diet of catfish (NRC, 1993). Arginine and histidine play an important role in maintaining a normal and healthy bloodstream and has other complex functions. The amount of arginine needed by fish is 1.2% and histidine 0.42%. NRC (1993) gave the following amount of amino acid requirement for catfish. Lysine 1.43%, leucine 0.98%, arginine 1.2%, methionine 0.64%, valine 0.84%, histidine 0.42%, tryptophan 0.14%, isoleucine 0.72%, phenylalanine 1.40%, and threonine 0.65%.

Information shows that protein need decreases as fish weight increases (Vasquez-Torres *et al.*, 2011). Protein ingested by fish is used in maintenance and growth, but when they are overfed only one part of this protein is effectively used for synthesis of new proteins and the remaining is

metabolized to produce energy (Wilson, 2002), with an unavoidable increase of nitrogen excretion to the aquatic system (Oishi, 2010). Consequently, when fish are fed with optimal dietary protein levels they retain a proportionally higher amount of protein in the body (growth), decreasing production costs and pollution (Vásquez-Torres *et al.*, 2011).

Excess dietary protein is wasteful and stresses the animal as excess energy means more fatty fish. Conditions where energy intake is inadequate, dietary proteins are used as energy source and the production cost increases. The optimum protein to energy (P/E) ratio in the diet is very important to maintain fish quality and to reduce the dietary cost. Hossain *et al*, (1998) reported that diet containing 33.5% digestible protein and 2.75 kcal/g digestible energy with a protein to energy ratio of 121 .8 (mg protein/kcal) appeared to be best utilized for growth.

2.1.1.2 Energy Requirement

All animals require energy for growth, reproduction, and health, regardless of whether they live on land or in water or whether they are cold-blooded or warm-blooded. While their dietary requirements, food sources, and efficiency of energy utilization may differ, their energy needs are ultimately met from energy released during metabolism of carbohydrates, proteins (amino acids), and fats and oils. Quantitatively, energy is the most important dietary component because feed intake in animals with free access to feed is largely regulated by dietary energy concentration. Therefore, feeding standards for many animals are based on energy needs. (Robinson and Li, 2015).

Energy requirement is one of the most notable differences in fish nutrition compared to other livestock. For example, less energy is required for protein synthesis in fish. Maintenance energy requirements are lower for fish than for warm-blooded animals because fish do not have to

maintain a constant body temperature, and they expend less energy to maintain their spatial position. Energy losses in metabolic waste are lower in fish because most nitrogenous waste is excreted as ammonia instead of urea or uric acid. In addition, the increase in energy cost associated with the assimilation of ingested food (i.e., heat increment) is less in fish. (Robinson and Li, 2015).

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 dietary energy, balance of dietary energy is important when formulating catfish feed (Jantrarotai, 1994).

Moreover, if dietary energy content is too high, 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; Henken *et al.*,1985). Energy requirements for catfish, which have generally been expressed as a ratio of digestible energy (DE) to crude protein range from 31.0 to 50.2kg^{-1}. Based on current knowledge, a DE/P ratio from 35.6 to 39.8kg^{-1} is adequate for commercial catfish feeds, increase in the DE/P ratio of catfish diets above this range will increase fat deposition band, reduce processed yield and in contrast if the energy value is too low the fish will grow slowly (Nematipour, *et al.*, 1992a).

Fish evolved in an aqueous environment where food items typically contained high protein and fat levels and little carbohydrate, they appear to have evolved to utilize protein and fats more efficiently for energy than carbohydrates. However, herbivorous and omnivorous fish typically utilize carbohydrate for energy fairly well. For example, the digestibility coefficient for channel catfish for carbohydrate in cooked corn was 70% and that for purified cornstarch was 66%.

The fact that catfish can utilize carbohydrate relatively well is fortunate, not only because it is an inexpensive energy source, but also because starch is necessary to make a floating feed. Also, fats and oils, which are the most concentrated and most highly digestible energy sources for catfish, must be used sparingly because high levels can be detrimental to fish growth and product quality. Therefore, the predominant energy source in commercial catfish feeds is from carbohydrates contained in grains (primarily corn) and grain milling by-products. (Robinson and Li, 2015).

2.1.1.3 Carbohydrate Requirement

Carbohydrates are in a group of compounds that include sugars, starches, celluloses, and other closely related substances among the most abundant organic compounds found in nature. They are the primary energy form stored in seeds, roots, and tubers, making up 60% to 70% of the dry weight of most grains. For catfish and other simple-stomached animals, carbohydrates can be broadly divided into an indigestible fraction (fiber) and a digestible fraction (sugars and starches). Ability to utilize sugars and starches as an energy source differs among fish species. Most fresh- and warm water fish, including catfish, can utilize higher dietary starch levels more efficiently than cold water fish. This ability may be attributed to the fact that warm water fish have a much higher intestinal amylase concentration necessary for starch utilization than cold water species (Robinson and Li, 2015).

Carbohydrates are included in fish feeds to provide a cheap source of energy and for improving pellet binding properties (NRC, 1993). Carbohydrates are important non-protein energy sources for fish and should be included in their diets at appropriate levels which maximize the use of dietary protein for growth. Excessive carbohydrate in fish diet may lead to fat deposition by

stimulating the activities of lipogenic enzymes (Orire and Sadiku 2014). Barroso *et al.*, (2014) reported that carbohydrate deprivation reduces NADPH-production in fish liver but not in adipose tissue. There is actually no dietary requirement for carbohydrate for catfish because they, as other animals, are capable of synthesizing carbohydrates from lipids and proteins (Robinson and Li, 2015). Report by Craig and Helfrich in 2002 showed that up to about 20% of dietary carbohydrate can be used by *Clarias gariepinus*. Dietary fiber should be maintained at as low a level as practical to minimize indigestible material passing into the culture water via the feces. (Robinson and Li, 2015). Digestibility values for catfish for carbohydrates in cooked corn ranged from 62−78%. (Robinson and Li, 2015).

2.1.1.4 Mineral Requirement

The dietary mineral requirements of channel catfish have been quantified based on specific clinical signs resulting from feeding the fish mineral-deficient feeds. Catfish require minerals for a balance between body fluids and their environment. Channel catfish can obtain part of their mineral requirements directly from rearing water. For example, when the water is rich in calcium, the fish can meet their calcium requirement by absorbing calcium from the water. Fourteen minerals are considered to be essential to catfish. Although mineral studies with fish are difficult to do, mineral amounts they need and signs of mineral of deficiency are known.

Catfish require 0.45% calcium in the diet when raised in calcium-free water. In practice, a calcium supplement is not needed because most pond water contains ample calcium, and commercial catfish feeds generally contain a relatively high calcium concentration from feedstuffs. In most animal feeds, the dietary calcium-to-phosphorus ratio is an important

consideration. However, this ratio apparently is not critical for catfish because calcium is regulated at the gills. (Robinson and Li, 2015).

Phosphorus is particularly important in fish feeds because fish require a fairly large amount of it. Feedstuffs, especially those from plants, are poor sources of phosphorus, and fish do not get enough phosphorus from pond water (Robinson and Li, 2015). As a result, catfish feeds are usually supplemented with phosphorus. Dicalcium and defluorinated phosphates are commonly used as phosphorus supplements in catfish feeds.

Other minerals that do not need to be supplemented include sodium, potassium, chloride, magnesium, and sulfur. These minerals are either absorbed from culture water or are abundant in feedstuffs typically used in catfish feeds.

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. A trace mineral premix may not be needed in catfish feeds that contain four percent or more animal protein (Robinson and Brent, 1989; Yue and Zhou., 2008).

2.1.1.5 Vitamin Requirement

Vitamins are generally necessary for optimum growth and health of Fish in intensive culture systems where limited natural food is available (FAO, 2016). Vitamins vary greatly in structure and function. They are generally defined as organic compounds animals require in small amounts in their diets for normal growth, health, and reproduction. Some vitamins may be made in the body and thus are not required in the diet. Vitamin deficiencies are rarely encountered in natural fish populations or in cultured catfish because vitamins are present in natural food sources, and some vitamins can be synthesized in the intestine. Although microorganisms in the intestines of

certain animals can synthesize some vitamins in quantities sufficient to meet metabolic needs, catfish intestinal microflora are generally not as extensive as the bacteria found in some land animals, therefore, the intestinal-synthesized vitamin contribution to the catfish diet is likely rather small.

Natural food (large zooplankton) present in commercial catfish ponds contains vitamins (some in relatively high concentrations) that can meet most, if not all, vitamin requirements for catfish fry and small fingerlings. Large fingerling and food-sized fish do not readily consume zooplankton, so the tiny invertebrates contribute little, if any, to the vitamin nutrition of catfish. (Robinson and Li, 2015).

In the case of cultured fish, many vitamins are inherent in feedstuffs used in commercial fish feeds. (Robinson and Li, 2015). Catfish feeds are generally supplemented with a vitamin premix that contains enough of all essential vitamins to meet the requirement and make up for losses from feed processing and storage. Excessive vitamin supplementation is not needed in the diet to compensate for losses during feed processing (Robinson and Li, 2015).

Vitamin requirements are affected by numerous factors, including stresses encountered in the culture environment. The concentrations of certain vitamins can be reduced, and some vitamins can be removed from the vitamin premix without affecting fish performance (Robinson and Li, 2015).

2.1.1.6 Lipid Requirement

Lipids (fats and oils) are easily digested source of concentrated energy, having more than twice as much energy as equal amount of carbohydrates. In addition to supplying energy, lipids are a source of essential fatty acids (EFAs) that cannot be synthesized by the animal and thus must be

provided in the diet. Lipids play several important roles in an animal's metabolism, including supplying essential fatty acids (EFA), helping absorb fat-soluble vitamins, and other important functions. Lipids in the diet may increase food intake. Lipids stored in body tissues affect the flavor of the flesh. EFAs are classified based on their chemical structure and are designated as either n-3 (omega-3) or n-6 (omega-6) fatty acids. Since catfish appear to have the ability to synthesize most fatty acids, nutritionally there may be no "best" dietary lipid concentration except that needed to provide EFAs, but, because lipid is a concentrated energy source that can spare the more expensive protein, additional lipid should be included in catfish diets. The type and amount of lipid in catfish diets is based on EFA requirements, economics, constraints of feed manufacture, and quality of fish flesh desired. Even though there may be exceptions, fish generally appear to require n-3 fatty acids.

Catfish require 0.5 to 0.75 percent omega-3 fatty acids in the diet. Lipid levels in commercial feeds for food-sized catfish rarely exceed 5 to 6 percent. About 3 to 4 percent of the lipid is in the feed ingredients naturally, with the remaining 1 to 2 percent being sprayed onto the finished pellets. Spraying feed pellets with lipid increases dietary energy and helps reduce feed dust. Too much dietary lipid may result to too much fat deposited in the body. This may affect processed yield, product quality, and storage of processed products. Weight gain and feed efficiency are depressed when catfish are fed diets containing 15% or more lipids.

Natural food organisms found in the pond may also be a good EFA source, but their contribution to the EFA requirement has not been quantified. Highly unsaturated n-3 fatty acid concentration found in zooplankton exceeds the EFA requirements of small catfish fingerlings that prey on these organisms. Lipids also serve as a vehicle for fat-soluble vitamin absorption and are

precursors for steroid hormones and other compounds and also help maintain neutral buoyancy of fish.

The lipid requirements of *Clarias gariepinus* have been studied by a number of authors, with varying results. These requirements depend on lipid source and quality, carbohydrate and protein content of the diet (FAO, 2016). Studies indicate that channel catfish may require higher lipid levels for optimum spawning performance. Sink and Lochmann (2008) found that supplementation of catfish brood stock diets with 10 percent fish oil increased spawning success, fecundity, total egg volume, egg weight, total egg lipid concentration, hatching success and fry survival compared to a control diet with 4 percent fish oil. Quentero *et al.* (2011) evaluated the influence of different lipid sources and n3:n6 ratios on the reproductive performance of female channel catfish. A commercial diet was top coated with 2 percent oil and offered to broodstock females during 70–85 days before spawning season. Four dietary treatments were formulated using the following top-coating ratios: diet 1, soybean oil 0.95 percent and linseed oil 1.05 percent; diet 2, soybean oil 1.75 percent and linseed oil 0.25 percent; diet 3, 2.0 percent linseed oil; and diet 4, 1.0 percent menhaden fish oil supplemented with 0.50 percent arachidonic acid (ARA) and 0.50 percent docosahexaenoic acid (DHA). Quentero found that the fatty acid composition of the eggs reflected the effect of the dietary oils. The diet which was coated with menhaden fish oil supplemented with ARA and DHA produced two to five times the number of fry per female body weight when compared to the effect of feeding diets top coated with vegetable oils.

2.1.2 Digestibility in *Clarias gariepinus*

Digestion is the process by which food in the digestive tract is split into simpler compounds capable of passing through the intestinal wall to be absorbed into the blood stream, this is performed primarily by the digestive enzymes which are secreted into the lumen of the alimentary canal, the digestive enzymes originate from the oesophageal, gastric, pyloric caecal and intestinal mucosa and from the pancreas (De Silva and Anderson, 1995). According to Cho *et al.* (1982), knowing the digestibility of raw materials, is the first step when it is intended to assess their potential for inclusion in fish diets, because the digestibility reveals data about the bioavailability of nutrients and energy of these ingredients that will be used in fish feed (Godoy *et al.*, 2016). The ability of fish to digest nutrients depends on many factors, such as the synthesis of appropriate enzymes, production of digestive enzymes in suitable amounts, their distribution along the gut lumen and the composition of the feed (Godoy *et al.*, 2016).

Digestibility coefficients for protein, lipid, carbohydrate and energy of the major feed ingredients have been determined for channel catfish. Most animal-based and plant-based protein sources are well digested. The total tract apparent digestibility values for protein in protein-rich feedstuffs in channel catfish are usually in the range of 75-95 % (NRC, 1993). Protein digestibility tends to be depressed as the concentration of dietary carbohydrates increases (NRC, 1993). Increased amounts of dietary lipids have been reported to produce increased protein digestibility (Godoy *et al.*, 2016). According to Fagbenro and Davies (1996), the apparent digestibility of protein in feedstuffs of animal origin or of plant origin in catfish (*Clarias gariepinus*) is similar.

The apparent digestibility coefficient of dry matter may be affected by the type of raw material/feed composition of the diet. High level of ash generally affects digestibility of dry

matter and results in high waste outputs and can also produce mineral imbalance (Cho and Bureau, 1998).

Lipids are also highly digestible by channel catfish, and are considered good energy sources for them. Starches are not highly digested by the fish as compared to dietary lipids. Carbohydrate digestion decreases as the dietary level increases (Robinson and Li, 2015). Cooking of feedstuffs during extrusion processing improves the digestibility of most materials, especially those high in carbohydrates (starch). Commercial channel catfish diets contain about 25 percent of digestible carbohydrates. The major sources of carbohydrate in catfish feeds are grains and grain products, which are 60–80 percent digestible, depending on the source, inclusion level and processing.

2.1.3 Carcass Composition

The proximate composition of intensively farmed fish is an integral part of evaluating their nutritional status. The diet composition, metabolic adaptation and variation in fish activity are the main factors responsible for the change in nutritional composition of fish (Adelakun *et al* 2014). The nutritive value of fish feed depends largely on the quality of protein of the ingredients used in the formulation of feed (Laurat *et al.*, 2017). The nutritional value of fish flesh comprises of moisture, dry matter, protein, lipids, vitamins, minerals and caloric value of the fish. The analysis of Carcass composition is an important factor that could be considered in fish feed assessment. Studies on proximate composition are essential for fish and fish products to be utilized efficiently (Odedeyi, 2014).

Studies have been carried out on the carcass composition of *Clarias gariepinus*. Odedeyi (2014) reported that *C. gariepinus* has the following nutrients, moisture 3.97, ash 2.50, carbohydrate 15.92, crude lipid 15.40, crude fibre 1.87 and crude protein 60.34. Jimoh *et al.* (2014) reported

that *C. gariepinus* has the following nutrients, moisture 78.61%, ash 2.77%, carbohydrate 0.01%, crude lipid 3.32 and crude protein 15.29%, result which is contradictory to the report of Odedeyi (2014).

2.1.4 Water Quality Requirement of *Clarias gariepinus*

Water quality is defined in terms of the chemical, physical and biological contents of the water which affect the health and welfare of the aquatic organism. Water quality is as an important factor to the success or failure of a fish culture operation (Momoh and Solomon, 2017). The critical water quality parameters in aquaculture include are temperature, pH, suspended solids and concentrations of dissolved oxygen, ammonia, nitrite, carbon dioxide and alkalinity (Yovita, 2007). The water quality requirement of *Clarias gariepinus* is presented in table 2.1

2.1.4.1 Dissolved Oxygen

Dissolved oxygen (DO) is by far the most important chemical parameter in aquaculture. Low-dissolved oxygen levels are responsible for more fish kills, either directly or indirectly, than all other problems combined (Momoh and Solomon, 2017). Successful fish production depends on good oxygen management. Oxygen is essential to the survival (respiration) of fish, to sustain healthy fish, to propagate bacteria which decompose the waste produced by the fish, and to meet the biological oxygen demand (BOD) within culture system (Yovita, 2007).

Fish is not able to assimilate the food consumed when DO is low. This result to increase in stress, tissue hypoxia, and a decrease in swimming activities and reduction in immunity to diseases. To obtain good growth, fish must be cultured at optimum levels of dissolved oxygen (Yovita, 2007). The amount of oxygen consumed by the fish is a function of its size, feeding rate, activity level, and temperature. Small fish consume more oxygen than do large fish because

of their higher metabolic rate. A good rule of thumb is to maintain DO levels at saturation or at least 5 mg/l. Dissolved oxygen level less than 5 ppm can place undue stress on the fish, and level less than 2 mg/l will result in death. Ending and Kamstra (2001) reported that the standard value of dissolved oxygen for African catfish (*Clarias gariepinus*) is between 2.9 and 6.8mg/l. Federal ministry of environment (2006) reported that the permissible limit of dissolved oxygen for aquatic life is 6.8mg/l. The measurement of dissolved oxygen in an aquatic system can be used not only to define the quality of water but as a means of estimation of the gross photosynthetic and the total community respiratory processes (Odum, 1959).

2.1.4.2 Temperature

Fish are cold-blooded organisms and assume approximately the same temperature as their surroundings (Dhirendra, 2002). The temperature of the water affects the activity, behavior, feeding, growth, and reproduction of all fishes. Metabolic rates in fish double for each -7.8ºC rise in temperature. Temperature also determines the amount of dissolved gases (oxygen, carbon dioxide, nitrogen, etc.) in the water. The cooler the water the more soluble the gas. Temperature plays a major role in the physical process called thermal stratification. Water has a high-heat capacity and unique density qualities (Yovita, 2007). Water has its maximum density at 4ºC. In spring, water temperatures are nearly equal at all pond depths. As a result, nutrients, dissolved gases, and fish wastes are evenly mixed throughout the pond. As the days become warmer, the surface water becomes warmer and lighter while the cooler-denser water forms a layer underneath. Circulation of the colder bottom water is prevented because of the different densities between the two layers of water. Dissolved oxygen levels decrease in the bottom layer since photosynthesis and contact with the air is reduced (Momoh and Solomon, 2017). The already low oxygen levels are further reduced through decomposition of waste products, which settle to

the pond bottom. Localized dissolved oxygen depletion poses a very real problem to the fish farmer. The optimum temperature range for 'cold water' and 'warm water' fishes are 14-18°C and 24-30°C respectively (Degani *et al.*, 1989). Prokesova (2015) reported that temperatures below 17.5 and above 35.1°C may be considered lethal during the embryonic development of *Clarias gariepinus*, and those below 18.9 and above 33.2°C are lethal during the larval period. *Clarias gariepinus* require optimum temperature of 23-32^{o}C for optimum growth (Momoh and Solomon, 2017; FAO, 2006)

2.1.4.3 pH

The hydrogen-ion concentration (pH) of natural waters is an important environmental factor for the growth of fish. Increase in the concentration of H_2 – ions results to lower pH value and low H+ ions concentration brings about higher pH value. The pH of water is the logarithm of the reciprocal of hydrogen ion concentration. PH of water has an important role for enhancement of fish yield (Momoh and Solomon, 2017). Lime fertilizers such as lime stone containing 55-66% CaO, dolomite lime stone containing 42-55% CaO, and dolomite containing 30-32% CaO are generally used for raising the water pH level while urea are used to lower the water pH to required doses. According to Federal Ministry of Environment 2006, the pH value of 6.0-9.0 is considered the permissible limit standard for aquatic life. The average pH value which is sufficiently basic for *Clarias gariepinus* is 6.9 (Ladon, 2000).

2.1.4.4 Nitrite

Nitrite enters a fish culture after fish digest feed and the excess nitrogen is converted into ammonia, which is then excreted as waste into water. The total ammonia nitrogen (TAN; NH_3 and NHS^{+}) is then converted to nitrite (NO_2), which under normal condition is quickly converted

to nontoxic nitrite (NO3) by natural occurring bacteria. Catfish and tilapia for example are fairly sensitive to nitrite (Boyd, 1982). According to Federal Ministry of Environment (2006) the permissible limit standard of nitrite (NO_2) for aquatic life is 0.06 mg/l. Eding and Kamstra (2001) reported that 0.01-0.06 mg/l of nitrite is considered nontoxic for African catfish (*Clarias gariepinus*) culture.

2.1.4.5 Ammonia

Fish excrete ammonia and lesser amounts of urea into the water as wastes. Two forms of ammonia occur in aquaculture systems, ionized and un-ionized. The un-ionized form of ammonia (NH_3) is extremely toxic while the ionized form (NH_4^+) is not. Both forms are grouped together as "total ammonia." Through biological processes, toxic ammonia can be degraded to harmless nitrates. In natural waters, such as lakes, ammonia may never reach dangerous high levels because of the low densities of fish, But the fish farmer must maintain high densities of fish and, therefore, runs the risk of ammonia toxicity. Un-ionized ammonia levels rise as temperature and pH increase(Ladon, 2000). The toxicity of un-ionized ammonia for *Clarias gariepinus* is approximately 6.5 mg/l.

2.1.4.6 Carbon Dioxide

Carbon dioxide (CO_2) is commonly found in water from photosynthesis or water sources originating from limestone bearing rock. Fish can tolerate concentrations of 10mg/l provided dissolved oxygen concentrations are high. Water supporting good fish populations normally contain less than 5 ppm of free carbon dioxide. In water used for intensive pond fish culture, carbon dioxide levels may fluctuate from 0mg/l in the afternoon to 5-15mg/l at daybreak. While in recirculating systems carbon dioxide levels may regularly exceed 20mg/l. Excessively high

levels of carbon dioxide (greater than 20mg/l) may interfere with the oxygen utilization by the fish (Yovita, 2007).

There are two common ways to remove free carbon dioxide. First, with well or spring water from limestone bearing rocks, aeration can "blow" off the excess gas. The second option is to add carbonate buffering material such as calcium carbonate ($CaCO_3$) or sodium bicarbonate (Na_2CO_3). Such additions will initially remove all free carbon dioxide and store it in reserve as bicarbonate and carbonate buffers (Ladon, 2000).

In pond production where photosynthesis is the primary natural source of oxygen, carbonates and bicarbonates are storage area for surplus carbon dioxide. By storing carbon dioxide in the buffering system, it is never a limiting factor that could replace photosynthesis, and in turn, reduce oxygen production. Also by storing carbon dioxide, the buffering system prevents wide daily pH fluctuations (Yovita, 2007).

Table 2.1 Water Quality Requirement of *Clarias gariepinus*

Physical and Chemical features	Desired quantity
Oxygen (O_2)	3-5 mg/l
Temperature	26-28°C
pH	6–9
Ammonia (NH_3-N)	≤8.8mg/l
Nitrite (NO_2-N)	0.01-0.06 mg/l
Nitrate (NO_3-N)	0.0-3.0mg/l
Carbon dioxide (CO_2-C)	0.0-10mg/l
Salinity	10mg/l

(Source; FAO, 2006)

2.2 Description of Cattle Hoof

Hoof is a horny sheath covering the toes or lower part of the foot of mammals of the orders Perissodactyla and Artiodactyla, such as cattle, horse, ox, or deer (Hepworth *et al.*, 2004). The hoof is an extremely important structure which fulfills protective and mechanical functions in the body of cattle (Meulling, 2009).

Cattle hoof is cloven, or divided, into two approximately equal parts (instead of one solid entity like that of a horse), usually called claws or digits. The two digits are analogous to the third and fourth fingers of the human hand. The digits are named by their relative location on the foot. There is the outer, or lateral digit, and the inner, or medial digit (Mülling and Budras, 1998). The lateral digit is slightly larger in the back feet, while the medial digit is the larger digit in the front feet. The space between the two digits is called the interdigital clef; the area of skin is called the interdigital skin. The different surfaces of the digits are named according to their relative position to the interdigital cleft: the abaxial surface is the outer wall of each digit, and the axial surface is the inner wall. The hoof is described from the outside moving in, beginning with the hard outer covering of the hoof, known as the hoof wall, or horn (Tomlinson *et al.*, 2004).

The horn is a hard surface, structurally similar to the human fingernail, but functionally like the epidermis of the skin. The cells that form the horn are produced by the tissue directly beneath the hoof wall, called the corium, at the hoof head. The corium is a nutrient-rich tissue that contains many important blood vessels and nerves inside the hoof. The corium is similar in quick growth to the fingernail in humans because it continuously produces new cells that are then gradually pushed away from the corium (Hepworth *et al.*, 2004). As the cells are pushed away from the corium, they die and produce the hard, new outer growth that is seen in human nails and in hoof

growth. At this point the cells keratinized or cornified. The new growth comes out at the coronary band, the point where the hoof meets the hairy skin on the animal's foot. The soft, new hoof growth that has just come to the surface is referred to as the perioplic horn and is shiny and holds in the moisture of the hoof. As can be seen in the rings that often occur on an animal's hooves, horn is produced at varying rates, because of variation in factors such as nutrition, health, and living conditions. However, as a general rule, bovine hooves grow about 1/5 to ¼ of an inch per month and horse's hooves grow in general ¼ to 3/8 of an inch per month (Hepworth *et al*., 2004).

2.2.1 Economic Importance of Cattle Hoof

Cattle hoof is widely available in every abattoir where Cattles are slaughtered in Nigeria. Jayapradha *et al*. (2011) reported some nutritional and industrial importance of cattle hoof.

2.2.1.1 Nutritional Quality of Cattle Hoof

Formation of hoof in cattle is a complex and structured process of cellular changes that transform living, highly functional epidermal cells into mechanical very stable horn cells, formation that is sensitive to nutritional influences, hormones and environmental influences (Meulling, 2009). Calcium, zinc, copper, Vitamins A, D & E and biotin all play important roles in production and maintenance of good quality hoof (Tomlinson *et al*., 2004). When nutrient supply to hoof producing cells is compromised, hoof of inferior structural and functional integrity is produced and Inferior hoof quality increases the potential for development of claw disease and may ultimately lead to lameness (Meulling, 2009).

Quresh, (1962) reported the nutritional value of cattle hoof when he replaced meat meal as a source of protein ration for growing chicks. The research covered the proximate analysis,

mineral composition and amino acid profile of the hoof. Nutritionally, hooves contain minerals such as phosphorus and calcium, and has 88% crude protein and 23.1kj of energy; while amino acid profile gave the value 4.8 and 2.2 for lysine and methionine respectively (Quresh, 1962).

Isaac Bikorimana of Kibagabaga Hospital in 2001 reported the presence of fibre proteins, collage and elastin which are necessary for proper formation of joints and bones in cattle hoof. Cattle hoof prepared by simmering yield gelatin which contains a colloidal substance which attracts digestive juices which facilitate digestion and absorption, and reduces tendency for stomach related congestion (Klinkenborg and Modica, 2001). Cattle hoof is the best choice for minerals like calcium, silicon, sulphur, magnesium, phosphorous and trace elements because these minerals exist in a form that can easily be absorbed by the body (Muelling, 2009). The cattle hoof diet is often used to treat children with autism and other disorders rooted in gut dysfunction and boiled hooves contain essential amino acids that are necessary in the fight against arthritis (Klinkenborg and Modica, 2001).

2.2.1.2 Industrial Importance of Cattle Hoof

Some of the products industrially produced from cattle hoof include buttons, bone china, piano keys, glues, fertilizer, and gelatin for photographic film, bone meal, cow heel jelly, lubricants, handles, soaps, paper, wallpaper, sandpaper, combs, toothbrushes, and violin string. Cattle hoof is a Keratin type of protein, the keratin when extracted from the cattle hoof is used to create a specialized fire extinguishing foam commonly used in airports to stifle fires caused by jet fuel (Klinkenborg and Modica, 2001).

2.3 Culture of African Catfish in Nigeria

Fish culture production in Nigeria includes stocking of lakes and production in ponds, cages and tanks (Ita, 2005). Nigeria has high potential to develop its fish farming so as to increase the amount of fish production in the country because of its high demand and favourable scale price. Ayinla, (1988) stated that aquaculture provides food of animal protein, generate income and employment as well as farm sustainability, thereby promoting the socio-economic development of Nigerians.

The story of aquaculture in Nigeria is essentially the story of catfish culture and the hope of fish supply in Nigeria hang on its development and culture. The growth of aquaculture in Nigeria now is largely being boosted by a steady rise in catfish culture. Inadequate availability of seed for stocking and feed used to be major problems (Momoh and Solomon, 2017). Since the culture of *Clarias gariepinus* through hypophysation was initiated in Western Nigeria in 1973, the procedure has been widely practiced throughout Nigeria thus leading to increase of farm-raised catfishes from the 80's to date (Adewumi and Olaleye, 2010). The favoured catfish species in Nigeria aquaculture include: *C. gariepinus, Heterobranchus bidorsalis*, *Clarias* × *Heterobranchus* hybrid (Heteroclarias) and *Clarias nigrodigitatus*. *Heterobranchus* sp. is the more commonly cultured fish in the south eastern parts of Nigeria. African catfish is popular in the market and has great potentials to boost the rapidly growing Nigerian aquaculture (Adewumi and Olaleye, 2010).

2.3.1 Feed and Feeding

Feed and feeding of catfishes in grow out ponds are perhaps the most documented in literature (Ayinla, 1988; Adewumi, 2005; Alegbeleye et al., 2008; Oresegun et al., 2007; Olukunle, 2009). Various efforts have been made to establish the crude protein and amino acid requirement of *C. gariepinus*. Ayinla (1988) recommended 35 and 40% crude protein (CP) for raising table size and brood stock respectively. Of the 10 essential amino acids (EAAs) required by warm water fish species, only three EAAs studied have been documented and these are arginine, methionine and lysine. In order to formulate and compound aqua feeds that will meet the nutrient requirements of the catfish at affordable cost, several conventional and non-conventional animal by-products and plant residues have been tested to substitute or replace fishmeal (Fagbenro and Davies, 2002). Feeding development has moved from the use of single ingredient, broadcasting un-pelleted meal to use of pelleted feeds. Fish may be fed sinking or floating pelleted feeds. The sinking pelleted feeds are fairly common and less costly to manufacture than the floating, or extruded, floating feeds. However, the use of pelleted floating feed has made a big difference to aquaculture development in Nigeria as *C. gariepinus* is being raised to maturity within 6 months (Adewumi and Olaleye, 2010). Better feeding conversion ratios (FCR) are obtained in general with floating feeds (Adewumi and Olaleye, 2010).

African catfish is a nocturnal tactile feeder characterized by a distinct crepuscular activity pattern. If food or prey is available only during light hours, *C. gariepinus* adopts a daytime searching and feeding pattern (Momoh and Solomon, 2017).

2.3.2 Feeding Frequency

Fish growth is influenced by feed availability and intake, genetics, age and size, environment and nutrition. Among these factors, feed intake is perhaps the principal factor affecting growth rate of fish (Lee *et al.*, 1997). Feeding frequency is one important consideration as it can affect growth, survival and fillet composition as well as water quality. Feeding also at the optimum frequency can result in tremendous savings in feed cost (Davies et al., 2006). The amount of the daily feed intake, frequency and timing of the feedings and presentation of the predetermined ration are the key factors of feed management strategies, influencing the growth and feed conversion (Jobling, 1995; Goddard, 1995)

Each species has its particular food preferences and feeding behaviours. Feeding and ingesting are the final result of a number of interacting factors between the fish (senses and hormonal systems) and its environment (stock density, size range or variability, season, day length and time of the day). Time of feeding and feeding frequency have been reported to affect feed intake and growth performance in fish (Jamabo *et al.*, 2015).

Arranging the time of feeding according to the natural times affects the feed efficiency, feed conversion rate and intake of nourishment elements in a positive way (Jamabo *et al.*, 2015). However, when the fish is not fed on an optimal frequency, in case of over and long term feeding; the fish develops a larger gut capacity. This situation has been observed in rainbow trout (*Oncorhynchus mykiss*) (Ruohonen and Grove, 1996) and Arctic charr (*Salvelinus alpinus*) (Jobling, 1995). Optimum feeding frequency that fish can show maximum growth rate, is affected by the size of fish and rearing conditions (De Silva and Anderson, 1995). When the fish is fed inadequate or over, growth and feed efficiency can decrease and this situation may

increase the cost of fish production besides, the quality of water may deteriorate in case of over feeding. Some studies show that moderate restriction in ratio had no effect on feed efficiency ((Jamabo *et al*., 2015).

First-feeding fry should be fed a small amount by hand at least ten times per day until all the fish are actively feeding. As the fry grow, frequency of feeding can be gradually decreased to about five times per day. Three times feeding per day and the rate of 3% of the body weight are recommended (Jamabo *et al*., 2015).

2.4 Utilization of Non-Conventional Feedstuff

A wide range of non-conventional feed ingredients could potentially be used to prepare fish feed (Sogbesan and Ugwumba, 2008). Non-conventional feed resources include aquatic and terrestrial plants such as duckweed, azalea and water hyacinth, aquatic animals such as snails, clams frogs, and terrestrial animals such as larvae, earthworms and termites. They may be incorporated into fish feed as protein supplements so as to replace the high-cost conventional protein feedstuffs (Chen and Meyers, 1983).

Catfish culture requires a relatively low entry cost (self-construction of pond, fingerlings for stocking and occasional feeding or fertilization). Non-conventional feed resources are low cost inputs that are affordable to poorer farmers and because the cost of production is low, catfish can be sold at a reasonable and affordable price to poor consumers.

2.4.1 Supplementary Feeds

Fish feed can be prepared locally but the common protein sources used which include fishmeal and soybean meal are expensive following the present market survey. According to Francis *et*

al., (2001), the plant sources such as soybean and some vegetables need to be utilized with caution to avoid negative effects such as growth retardation and death of the fish (FAO, 2016). The price of fishmeal will remain high in the long term because of constant demand and limited supply. Fish biologists have worked on organisms like *Moringa olifera* (Olaniyi, *et al.*, 2013). *Macrotermes subhyalinus* (Sogbesan and Ugwumba, 2008), maggot of *Musca domestica* (Fasakin *et al.*, 2003), *Zonocerus variegatus* (Alegbeleye *et al.*, 2012), to replace fishmeal in fish culture (Banjo, *et al.*, 2003). More research is ongoing on the effectiveness of replacing fish meal and soybean meal with plant and animal by-products in the feed of fish.

2.5 Growth Performance and Nutrient Utilization

Growth performance is the measure of increase in size and weight of fish in response to the feed fed to the fish. Nutrient utilization is the effectiveness of metabolic reactions on the feed in the body of the fish in to bring about growth response. Some parameters commonly used to determine growth performance and nutrient Utilization are weight gain, specific growth rate (SGR), Feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate (SR), condition factor (CF).

Weight gain is the difference between the weight of the fish before experiment and the weight after the experiment which is due to the quality of feed fed to the fish (Adikwu, 2003).

The specific growth rate (SGR) is defined as the increase in cell mass per unit time. The specific growth rate determines the daily rate of growth of the fish. High SGR signifies that the feed fed to the fish is rich and so enhances the growth of the fish. This however denotes that the feed is of high quality (De silver and Anderson, 1995).

Feed conversion ratio is a measure of diet efficiency. The more suitable the diet the less feed is required to produce a unit weight gain i.e. lower feed conversion ratio. Adikwu (2003) reported that the lower the FCR the better the feed utilization by the fish.

DeSilva and Anderson (1995) reported that protein efficiency ratio is a measurement of how well the protein sources in a diet could provide the essential amino acids requirement of the fish fed. The protein efficiency ratio value of above the value one (1) is a demonstration of higher feed quality in terms of the amino acids.

Survival rate is the percentage of the fish that survive at the end of the experiment. Survival rate is affected by factors which include quality of the feed, water quality of the pond, handling process, management and environmental conditions like whether, season and temperature (Akinwole and Faturoti, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This experiment was conducted in the aquaculture unit of National Agricultural Extension and Research Liaison Services (NAERLS), Ahmadu Bello University, Zaria, Kaduna which is on longitude 74º42 E and latitude 11º N and altitude 660m.

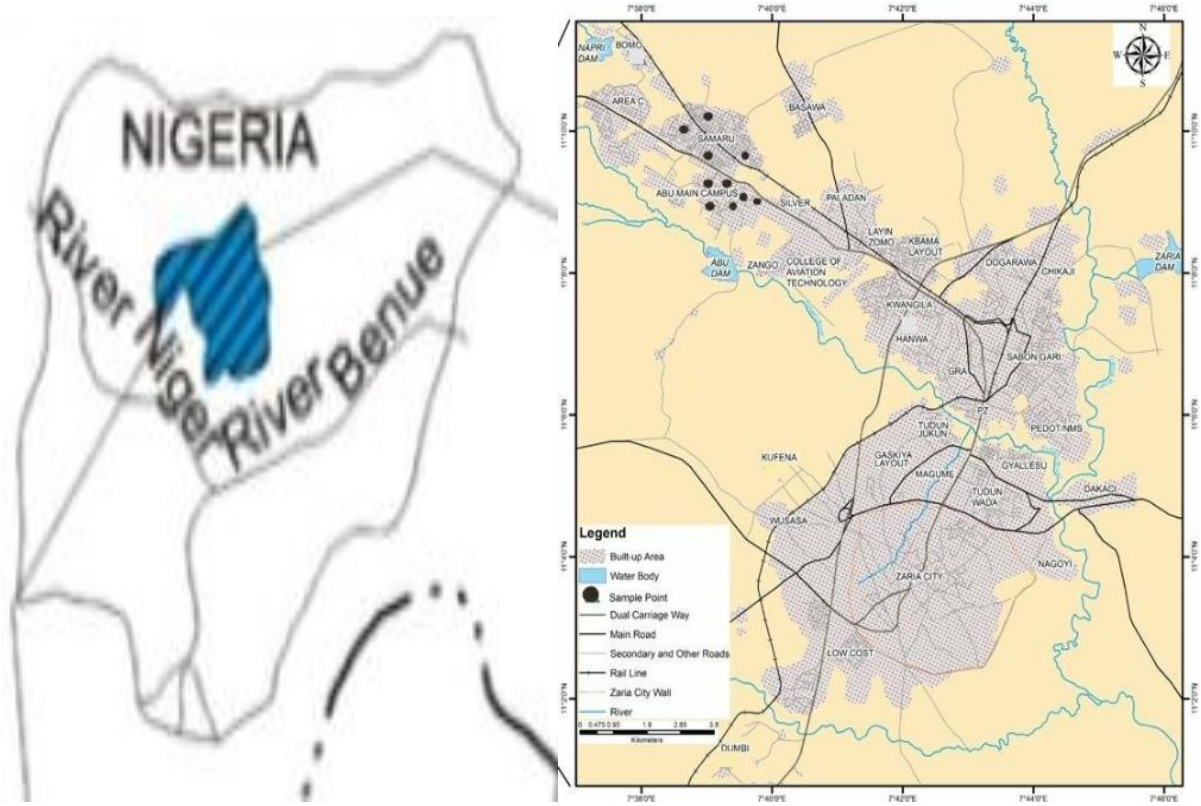


Plate 3.1: Study Area

3.2 Source of *Clarias gariepinus* Juveniles

A total number of 180 juveniles of *Clarias gariepinus* was procured from Arewa farm in Zaria and transported in 50 liter plastic container to the aquaculture unit of NAERLS, Ahmadu Bello University, Zaria. The juveniles were acclimatized for two weeks within the experimental area. The juveniles were fed with vital feed (commercial feed) during the two weeks period of acclimatization.

3.3 Source of Cattle Hooves and Other Feed Ingredients

Cattle hooves were obtained from the abattoir at Zango Area, Zaria, Kaduna State. The other feed ingredients like fish meal, soybean meal, yellow maize, palm oil, salt, methionine, lysine, premix, were all obtained at Sabo market, Zaria.

3.4 Preparation of Cattle Hoof Meal

The Cattle hoof meal was prepared by soaking the hooves in 10 % sodium carbonate for sixty hours at room temperature. Thereafter it was boiled in a big pot (which contained 10% sodium carbonate) on a cooking gas for one hour. When boiling was completed, the hooves were oven dried at 80^0C for 48 hours (Quresh, 1962). This was done to aid the digestibility of the feed. On cooling, the hooves were pulverized into powder using pistil, mortar and a sieve and then stored in an air-tight polythene bag until required.

3.5 Proximate Analysis of Cattle hoof Meal and Experimental diets

Proximate analysis of the prepared cattle hoof meal and experimental diets were carried out according to the Association of Official Analytical Chemist procedures (A.O.A.C., 2005) for dry

matter, crude protein, Crude lipid, ash, crude fibre, carbohydrate and moisture contents. This was done at the Animal Science laboratory, Institute of Agricultural Research (IAR), A.B.U Zaria.

3.5.1 Moisture Content and Dry Matter

Two grams (2g) each of the samples Cattle hoof meal and experimental diets were weighed into silica dish previously dried and weighed. The samples were then dried in an oven at 65^0C for 36 hours, cooled in a desiccator and weighed. The drying and weighing continued until a constant weight was achieved. The dry matter and moisture content were calculated using the formula below.

$$\%\text{Moisture} = \frac{\text{wt of sample + dish before drying - wt of sample + dish after drying}}{\text{Wt of sample}} \times 100$$

%DM = 100 - %Moisture.

3.5.2 Ether Extract (Crude lipid)

Anhydrous diethyl ether (150ml petroleum ether) of boiling point of 40-60^0C was placed in the flask. Two grams of the sample was weighed into a thimble and the thimble was plugged with cotton wool. The thimble with content was placed into the extractor; the ether in the flask was then heated. As the ether vapour reached the condenser through the side arm of the extractor, it condensed to liquid form and drop back into the sample in the thimble, the ether soluble substances were dissolved and were carried into solution through the siphon tube back into the flask. The extraction continued for 4 hrs. The thimble was removed and most of the solvent was distilled from the flask into the extractor. The flask was then disconnected and placed in an oven at 65^0C for 4 hrs, cool in desiccator and weighed.

$$\% \text{ Ether extract} = \frac{\text{wt of flask + extract – tare wt of flask x 100}}{\text{wt of sample}}$$

3.5.3 Crude Protein

Digestion: The sample (2g) was weighed into kjeldahl flask. Concentrated sulphuric acid (25mls), 0.5g of copper sulphate, 5g of sodium sulphate and a speck of selenium tablet were added. Heat was applied in a fume cupboard slowly at first to prevent undue frothing, digest continued for 45mins until the digesta became clear pale green. It was left until it was completely cool before 100ml of distilled water was rapidly added. The digestion flask was rinsed 3 times and the rinsing was added to the bulk.

Distillation: Markham distillation apparatus was used for distillation. The distillation apparatus was steamed up and about 10ml of the digest was added into the apparatus via a funnel and allowed to boil. 10ml of sodium hydroxide from the measuring cylinder was added so that ammonia was not lost. It was distilled into 50ml of 2% boric acid containing screened methyl red indicator.

Titration: the alkaline ammonium borate formed was titrated directly with 0.1 NHCl. The titre value which is the volume of acid used was recorded. The volume of acid used was fitted into the formula which becomes

$$\%N = \frac{14 \times VA \times 0.1}{1000 \times 100} \times W \times 100$$

VA = volume of acid used

W= weight of sample

%crude protein = %N x 6.25

3.5.4 Crude Fibre

The organic residue left after sequential extraction of feed with ether was used to determine the crude fibre. The fat free material was transferred into a flask/beaker and 200ml of pre-heated 1.25% H_2SO_4 was added and the solution was gently boiled for about 30mins, maintaining constant volume of acid by the addition of hot water. The buckner flask funnel fitted with whatman filter was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was then filtered hot through the funnel under sufficient suction. The residue was then washed several times with boiling water (until the residue was neutral to litmus paper) and transferred back into the beaker. Then 200ml of pre-heated 1.25% Na2SO4 was added and boiled for another 30mins. It was filtered under suction and washed thoroughly with hot water and twice with ethanol. The residue was dried at 65^0C for about 24hrs and weighed. The residue was transferred into a crucible and placed in muffle furnace (400-600^0C) and ashed for 4hrs, then cooled in desiccator and weighed.

$$\%\text{Crude fibre} = \frac{\text{Dry wt of residue before ashing - wt of residue after ashing x 100}}{\text{wt of sample}}$$

3.5.5 Ash

Two grams (2g) of the sample was weighed into a preheated crucible. The crucible was placed into muffle furnace at 400-600^0C for 4hrs or until whitish-grey ash was obtained. The crucible was then placed in the desiccator and weighed.

$$\%\text{ Ash} = \frac{\text{wt of crucible + ash – wt of crucible}}{\text{wt of sample}}$$

3.5.6 Nitrogen Free Extract (NFE)

Nitrogen free extract was determined by mathematical calculation. It was obtained by subtracting the sum of percentages of all the nutrients already determined from 100.

% NFE = 100 - (% moisture + % CF + % CP + % EE + % Ash)

Nitrogen free extract represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed.

3.6 Mineral Composition and Amino Acid Profile of Cattle Hoof Meal

The mineral composition and amino acid profile of the Cattle hoof meal were carried out according to the procedure of the Association of Official Analytical Chemist procedures (A.O.A.C., 2005). The mineral composition was done at the Soil Science laboratory, Institute of Agricultural Research (IAR), A.B.U Zaria while the amino acid profile was carried out at Dr Ojobe's Lab, Jos, Plateau State.

3.6.1 Mineral Analysis

Phosphorus was quantified using the method outlined by Allen (1989). Approximately 10 mg of cattle hoof meal was extracted overnight in 25mL of concentrated nitric acid. This was followed by digestion in concentrated nitric acid and then perchloric acid on a hot plate. Distilled water (20 mL) was added to the sample and boiled until white fumes appeared. This was followed by addition of 5mL of ammonia solution and further boiling until crystals were formed. The crystals were then dissolved using 20mL of acidified water and 80ml of distilled water followed by 20mL of a mixed reagent for colour development. The mixed reagent consisted of 250mL sulphuric acid–antimony, 250ml sodium molybdate, 500 mL distilled water and 2g/l ascorbic

acid. The sample was left for 15 minutes for colour to develop. The quantity of phosphorus was then determined using a spectrophotometer (Cecil Elegant Technology, UK). Other minerals (potassium, calcium, sulphur, carbon and nitrogen) were quantified using a Thermo Xseries 2 inductively coupled plasma mass spectrophotometer (ICP MS) (Thermo Scientific, USA). Cattle hoof meal weighing approximately 80mg was digested in nitric acid for 1hr in a Mars Xpress microwave (CEM Corporation, USA) and then diluted to 10ml with distilled water ready for quantification.

3.6.2 Determination of Amino Acid Profile

The Amino Acid profile in the cattle hoof meal was determined using methods described by Benitez (1989). The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM).

3.6.2.1 Defatting Sample

500mg of dried sample of cattle hoof meal was weighed into extraction thimble and the fat was extracted with chloroform methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006) the extraction lasted for 15hrs.

3.6.2.2 Nitrogen Determination

One hundred and fifteen miligrams (115mg) of ground cattle hoof meal was weighed, wrapped in whatman filter paper (No.1) and placed in the Kjelcihal digestion flask. Concentrated tetraoxosulphate (vi) acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate

(Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added.

The flask was then placed in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Ten milliliter (10m1) of the diluted solution with 10ml of 45% sodium hydroxide was inserted into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10ml)

14. = Nitrogen constant in mg.

3.6.2.3. Hydrolysis of the Sample

A known weight of the defatted hooves meal was weighed into glass ampoule. Seven milliliter (7ml) of 6N HCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and placed in an oven preset at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. Tryptophan was destroyed by 6N HCL during hydrolysis.

The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

3.6.2.4 Loading of the Hydrolysate into Analyzer

The amount of hydrolysate loaded was 60 microlitre. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 minutes.

3.6.2.5 Method of Calculating Amino Acid Values from the Chromatogram Peaks

An integrator attached to the Analyzer calculated the peak area proportional to the concentration of each of the amino acids. The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half height was measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height. The

norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$NE = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture:

Where Sstd NEstd x Molecular Weight x μMAAstd

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/ 100g protein using the following formula:

Concentration (g/100g protein) = NH x WNH/2 x Sstd x C

$$\text{Where } C = \frac{\text{Dilution} \times 16 + NH \times W(nlc\mu)}{\text{Sample Wt (g)} \times N\% \times 10 \times \text{vol. Loaded}}$$

Where: NH = Net height

W = Width at half height

nlμe = Norleucine

3.7 Feed Formulation

The Cattle hoof meal (CHM) produced was mixed with other feedstuffs (fish meal, soybean, yellow maize, methionine, lysine, bone meal, vitamin premix, salt and palm oil) to produce five isonitrogenous diets (Crude Protein 42%) where Cattle hoof meal replaced Fish meal at 0%, 25%, 50%, 75% and 100% (Table 3.1). The feed which contained 0% Cattle hoof meal and commercial pelleted feed were the control diets. The diets were denoted as D1 (Commercial feed), D2 (0%CHM), D3 (25% CHM), D4 (50%), D5 (75% CHM), and D6 (100% CHM). The

formulated practical diets were pelleted using the locally fabricated pelleting machine to produce die of 2mm, 3mm and 4mm which were fed to the fish as they age. Pelleted diets were sun-dried for 2 days until crispy and then packed in an air-tight polythene bags with appropriate labelings.

Table 3.1 Gross Composition of Experimental Diets Fed to *Clarias gariepinus*

Ingredients	D1 (Commercial) Feed	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Cattle hoof meal	-	-	11.73	17.60	26.39	35.19
Fish meal	-	33.00	24.75	16.50	8.25	-
Soybean meal	-	35.19	35.19	35.19	35.19	35.19
Yellow maize	-	19.62	19.62	19.62	19.62	19.62
Vitamin Premix	-	2.89	0.41	1.79	1.25	0.50
Methionine	-	2.00	2.00	2.00	2.00	2.00
Lysine	-	3.00	3.00	3.00	3.00	3.00
Bone Meal	-	1.00	1.00	1.00	1.00	1.00
Salt	-	0.30	0.30	0.30	0.30	0.30
Palm oil	-	3.00	2.00	3.00	3.00	3.20
TOTAL	-	100	100	100	100	100

3.8 Experimental Setup

The feeding and growth experiment was conducted in the Aquaculture unit of NAERLS, Ahmadu Bello University, Zaria. Two hapas of 2m by 2m by 2m partitioned into eighteen hapas of dimension 0.70×0.70×2m each embedded in a concrete pond of 5m by 3m by 2m were used, three hapas per experimental diet. The three hapas per experimental diet which were randomly distributed represented three replicates per experimental diet. Ten fish were stocked in each hapa/replicate, this sum up to a total of thirty fish per treatment. *C. gariepinus* of uniform sizes were stocked in each hapa to avoid cannibalism.

3.9 Fish Feeding and Culture

The fish were fed thrice daily between 6.00am-7.00am, 2.00pm-3.00pm and 10.00pm-11.00pm at 3% body mass throughout the period of the experiment (105 days). The ration was adjusted every three weeks when new mean weights of fish for the various experimental units were determined. The water in the pond was changed every week.

3.10 Physicochemical Parameters of Water

Water temperature and pH were measured weekly, before and after changing the water using HANNA instrument (model 98129) while dissolved oxygen (DO) was measured using DO meter (model DO-510), Ammonia and nitrate was measured using the comb II strips.

3.11 Weighing and Measuring of Fish

The initial mean weight of fish was determined after the acclimatization. The mean weight per replicate was calculated after taking the bulk weight of the fish in each of the hapa. Mean weight (g) per hapa was taken every three weeks throughout the 105 days of the experiment.

Both standard length (SL) and the total length (TL) of the fish used for the experiment were recorded using a measuring board. Growth performance parameters such as mean weight gain (MWG), feed conversion ratio (FCR), Relative growth rate (RGR), specific growth rate (SGR), protein efficiency ratio (PER) and condition factor (K) were calculated.

3.12 Growth Performance

Growth performance of the fish after the 105 days of the feeding trial was determined using the following parameters:

Mean weight gain (g) (MWG)

$MWG = Wt_2 - Wt_1$ (Adikwu, 2003)

where

Wt_1 = Initial mean weight of fish at T_1

Wt_2 = Final mean weight of fish at time T_2

Feed Conversion Ratio (g) (FCR)

$$\text{Feed Conversion Ratio (g) (FCR)} = \frac{\text{Weight of feed given (g)}}{\text{Fish weight gain (g)}}$$ (De silver and Anderson, 1995)

Specific Growth Rate

$$\text{Specific Growth Rate (SGR)} = \frac{\text{Log } w2 - \text{Log } w1 \times 100}{t}$$ (Hepher, 1988)

W_2 = Final average weight at the end of the experiment

W_1 = Initial average weight at the beginning of the experiment

Log_e = the base of natural logarithm (10)

t = Number of days for the experiment

Relative Growth Rate (RGR)

$$\text{Relative Growth Rate (RGR) (\%)} = \frac{(Wf - Wi)}{Wi} \times 100$$ (Wannigama *et al.*, 1985)

Wf = Final average weight at the end of the experiment

Wi = Initial average weight at the beginning of the experiment

Protein Efficiency Ratio

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Fish Weight Gain (g)}}{\text{Protein intake (g)}}$$ (Wilson, 2002)

Condition Factor (K)

$$K = \frac{100 \times W}{L^3}$$ (Wilson, 2002)

Where: K = Condition factor

L = Length (cm)

W = Body weight of fish (g)

Survival Rate (%)

$$\text{(SR) (\%)} = \frac{\text{Initial number of fish stocked} - \text{mortality}}{\text{Initial number of fish stocked}} \times 100$$ (Akinwole and Faturoti, 2006)

Production Index

$$PI = \frac{\text{Survival rate} \times \text{final weight (g)} - \text{initial weight (g)}}{\text{Duration of rearing period (days)}}$$ (Mohanty, 2004)

3.13 Digestibility Estimation

Apparent digestibility coefficient of the diet was estimated using the indirect method which involves the use of a marker, Chromium III Oxide (Lovell, 1989). Freshly prepared diets D1, D2, D3, D4, D5 and D6 were mixed with 0.5% of the marker separately and fed to the experimental fish after the 105 days of the feeding trial for fourteen days. On the last day, ten hours after the fish were fed, three fish per treatment were dissected and the faecal matters collected from the rectal region of the gastro-intestinal tract without scraping the rectal and intestinal tissues, and stored in a freezer. The proximate analysis of the diet and the faeces were determined according to the procedure of AOAC (2005) as described previously, the gross energy was calculated from the result of the proximate analysis using the formula below

Gross energy = (Crude protein × $23.9kjg^{-1}$) + (Crude Lipid × $39.8kjg^{-1}$) + (NFE × $17.6kjg^{-1}$)

Chromic oxide was quantified following the method of (Furukawa and Tsukahara, 1966). Approximately 50-100mg of sample was digested with 5ml of concentrated nitric acid followed by 3ml of perchloric acid which turned the digesta from green to yellow. This was then diluted to 100 ml of distilled water and absorbance read using Uvikon 860 spectrophotometer (Kontron Instruments) at 350nm. The weight and percentage of chromic oxide was calculated using the following formulae:

Weight of Cr2O3 = Absorbance – 0.0032/0.2089

Digestibility coefficients of diets were estimated to determine bioavailability of nutrients. Apparent digestibility coefficients (ADC) of the feeds were computed using the formula described by Maynard and Loosli (1969).

ADC (%) = 100 - [100 × (%nutrient in faeces/%nutrient in feed) × (%marker in feed/marker in faeces)]

3.14 Economic Analysis

The economic analysis of the production of *Clarias gariepinus* used in this experiment was conducted to assess the cost effectiveness of diets used in the feeding trial. Only the cost of feed and juvenile were used in the calculation with the assumption that all other operating costs remain constant.

Economic Weight Gain

$$\text{Economic Weight Gain (EWG)} = \frac{\text{Cost of feed (N)}}{\text{Weight gain (g)}}$$ (Akinwole *et al.*, 2006)

Profit index

$$\text{PI} = \frac{\text{Weight of fish crop}}{\text{Cost of feed}}$$ (Aderolu *et al.*, 2010)

Incidence cost

$$\text{(IC)} = \frac{\text{Cost of feed}}{\text{weight of fish produced}}$$ (Aderolu *et al.*, 2010)

Net Profit

(NP) = sales – expenditure (Aderolu *et al.*, 2010)

Benefit Cost Ratio

$$\text{(BCR)} = \frac{\text{Total sales}}{\text{Total expenditure}}$$ (Mazid *et al.*, 1997)

Total cost

(TC) = fixed cost (FC) + total variable cost (TVC) (Sogbesan and Ugwumba, 2006)

Where FC= cost of fingerlings

TVC = Cost of producing Different diets

3.14 Data Analysis

Mean values of the water quality parameters measured was calculated. Mean values of the growth measurements and cost benefits were subjected to one way Analysis of variance (ANOVA) and tested for significant difference at 5% level of significance. Differences between Means were determined using Duncan's Multiple Range Test.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Cattle Hoof Meal

The proximate analysis of the cattle hoof meal (CHM) is presented in Table 4.1. The CHM contained 33.75% moisture content and 66% dry matter. Its crude protein was 58.06% while the Crude fibre, Crude lipid, ash content and Nitrogen free extract were 2.75%, 3.25%, 0.91% and 1.78% respectively while the gross energy value was 1548.312kjg^{-1}. Its crude protein and NFE are within the recommended values as suggested by FAO.

4.2 Macro-Mineral Composition of Cattle Hoof Meal

The macro mineral composition of Cattle hoof meal are 0.69% nitrogen, 0.081% phosphorus, 0.0899% potassium, 0.2450% calcium, 22.17% carbon and 19.592% sulphur (Table 4.2). The value of phosphorus obtained in CHM is below the recommended value suggested by FAO.

4.3 Amino Acid Profile of Cattle Hoof Meal

The amino acid (g/100g) profile of Cattle hoof meal is presented in Table 4.3. Among the essential amino acids, leucine (7.15g/100) had the highest concentration while the least was tryptophan (0.81g/100). Glutamic acid (14.61g/100) had the highest concentration among the non-essential amino acids while Cystine (1.03g/100) was the least. The value of Leucin (7.15), Histidine (2.17) and Isoleucin (4.19) in CHM were higher than the values recorded in Fishmeal and those recommended by NRC respectively. Also Threonine (3.8), Valine (4.0) and Tryptophan (0.81) in CHM were above value requirement for *C. gariepinus* as recommended by

Table 4.1 Proximate Composition of Cattle Hoof Meal in Comparison to Fish Meal and *Clarias Gariepinus* Requirement

Composition	Cattle hoof Meal (%)	Fish Meal (%)	FAO *C. gariepinus* Requirement (%)
Moisture	33.75	6.37	-
Dry matter	66.25	93.63	-
Crude lipid	3.25	6.78	4-6
Crude protein	58.06	68.68	26-45
Crude fibre	2.75	1.46	-
Ash	0.91	20.16	-
NFE	1.78	3.73	25-35
Gross energy	1548.31Kj/g	1976.94kj/g	35.56-39.75kj/g

Table 4.2 Macro Mineral Composition of Cattle Hoof Meal in Comparison to Fish Meal and *Clarias gariepinus* Requirement

Composition	Cattle hoof meal (%)	Fish Meal (%)	*C. gariepinus* requirement (%) (FAO, 2006)
Nitrogen	0.69	-	NSN
Phosphorus	0.08	3.35	0.3-0.35
Potassium	0.09	-	NSN
Calcium	0.25	4.40	NSN
Carbon	22.17	-	NSN
Sulphur	19.59	-	NSN

NSN = No supplement needed; abundant in feedstuffs

Table 4.3 Amino Acid Profile of Cattle Hoof Meal in Comparison to Fish Meal and *Clarias gariepinus* Requirement

Amino acid	Cattle hoof meal (g/100g protein)	Fish Meal (g/100g protein)	*C. gariepinus* requirement (% of protein) (NRC, 1993)
Essential Amino Acid			
Leucine	7.15	6.50	3.5
Lysine	4.29	6.90	5.1
Histidine	2.17	2.00	1.5
Isoleucine	4.19	3.70	2.6
Threonine	3.80	3.90	2.0
Phenylalanine	3.99	3.30	5.0
Valine	4.00	4.50	3.0
Arginine	5.85	6.40	4.3
Methionine	2.19	2.60	2.3
Tryptophan	0.81	0.90	0.5
Non-essential Amino Acids			
Proline	3.96	5.30	
Tyrosine	2.75	2.60	
Cystine	1.03	0.90	
Alanine	3.52	6.30	
Glutamic acid	14.61	12.80	
Glycine	4.96	9.90	
Serine	3.24	4.80	
Aspartic acid	8.87	8.50	

NRC. Tyrosin (2.75), Cysteine (1.03), Glutamic acid (14.61) and Aspartic (8.87) in CHM were higher than those in Fishmeal.

4.4 Proximate Composition of Experimental Diet

The proximate composition of the experimental diet had crude protein range of between 40% and 42%. The crude fibre ranged between 2 and 3%, the crude lipids range between 12 and 14 while the gross energy ranged between 1869 and 2048kjg^{-1} (Table 4.4).

4.5 Growth Performance of *Clarias gariepinus* Fed the Experimental Diets

The result of the growth performance of *Clarias gariepinus* fed the experiment diet after 105 days is presented in Table 4.5. The initial weight of all the fish fed the experimental diets before the experiment were similar. Fish fed the commercial feed (D1) had the highest mean final weight and weight gain (217.00g and 204.20g respectively) after the 105 days of the experiment. However, they were not significantly different ($P>0.05$) from fish fed D2 and D3. Significant differences ($P<0.05$) from the fish fed D4 (50%), D5 (75%) and D6 (100%) which had weight gain of 148.60g, 143.73g and 129.00g respectively were recorded. The weight gain of the fish fed D3, D2 and D4 were not different significantly ($P>0.05$) but the weight gain of the fish fed D3 was significantly different ($P<0.05$) from D5 and D6. The weight gain of the fish fed D2, D4, and D5 were not different significantly ($P>0.05$) but D2 was significantly different ($P<0.05$) from D6. The weight gain of the fish fed D4, D5 and D6 were not different ($P>0.05$) significantly.

The Initial standard length and initial total length of all the fish before the experiment were significantly not different ($P>0.05$) from each other. The total length and standard length gain for the fish fed with D4, D5 and D6 were 15.87 and 14.17, 15.10 and 13.37, and 13.67 and 12.30cm

Table 4.4 Proximate Composition of Experimental Diets

Composition	D1 (CF)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Moisture	7.86	10.84	10.36	11.37	13.05	10.27
Dry matter	92.23	89.16	89.64	88.63	86.95	89.93
Crude lipid	14.69	12.54	12.21	12.51	12.35	12.11
Crude protein	42.34	41.09	40.81	40.95	40.38	40.85
Crude fibre	2.80	2.54	2.72	2.83	3.47	1.92
Ash	7.86	10.78	8.03	7.82	7.27	6.60
NFE	25.70	22.34	25.87	24.52	23.48	28.25
Gross Energy	2048.91	1874.33	1916.63	1908.16	1869.86	1955.49

CF**:** Commercial feed; NFE: Nitrogen free extract

Table 4.5 Growth Performance of *Clarias gariepinus* Fed Experimental Diets

Parameter	D1 (Commercial feed)	D2 (0%CHM)	D3 (25%CHM)	D4 (50%CHM)	D5 (75%CHM)	D6 (100%CHM)
Initial Weight(g)	13.07±0.17	13.2±0.31	12.9±0.42	12.83±0.35	13.03±0.29	13.13±0.34
Final weight(g)	217.00±10.33^{a}	189.80±4.90^{abc}	193.9±7.37^{ab}	161.43±8.05^{bcd}	156.77±4.5^{cd}	142.13±20.01^{d}
Weight Gain(g)	204.20±10.23^{a}	176.6±5.02^{abc}	181.00±7.10^{ab}	148.60±8.31^{bcd}	143.73±4.79^{cd}	129.00±20.02^{d}
% Weight Gain	1562±68.7^{a}	1340±59.2^{abc}	1403±43.55^{ab}	1162±98.9^{bcd}	1105±61.20^{cd}	983±158.06^{d}
Initial Total Length (cm)	13.07±0.07	13.07±1.33	13.13±0.09	12.97±0.12	13.07±0.15	13.00±0.15
Final Total Length (cm)	31.00±0.29^{a}	30.13±0.09^{ab}	30.43±0.19^{ab}	28.83±0.60^{abc}	28.17±0.60^{bc}	26.67±1.69^{c}
Total Length Gain (cm)	17.93±0.30^{a}	17.07±0.22^{ab}	17.30±0.12^{ab}	15.87±0.62^{abc}	15.10±0.74^{bc}	13.67±1.72^{c}
% Total Length Gain	135.98±1.24^{a}	130.67±3.03^{a}	131.73±0.74^{a}	122.41±5.14^{ab}	115.71±6.86^{ab}	105.23±13.66^{b}
Initial Standard Length (cm)	11.53±0.03	11.40±0.10	11.40±0.06	11.33±0.09	11.47±0.09	11.37±0.09
Final Standard Length(cm)	27.63±0.45^{a}	27.07±0.07^{ab}	27.50±0.20^{ab}	25.50±0.76^{abc}	24.83±0.73^{bc}	23.67±1.67^{c}
Standard Length Gain (cm)	16.10±0.47^{a}	15.67±0.17^{a}	16.10±0.15^{a}	14.17±0.77^{ab}	13.37±0.77^{ab}	12.30±1.65^{b}
% Standard Length Gain	139.62±4.40^{a}	137.47±2.69^{a}	141.22±0.92^{a}	125.03±6.96^{ab}	116.58±6.17^{ab}	108.19±14.40^{b}
Condition Factor	0.73±0.02	0.69±0.01	0.68±0.01	0.67±0.01	0.70±0.03	0.75±0.04

Means with same superscripts along row were not significantly different (P≥0.05)

CHM: Cattle hoof meal

respectively. The total length gain and standard length gain of the fish decreased with increase in the substitution level from 50% substitution level to 100%.

The ranges of condition factor (0.67-0.75) of all the treatments were not significantly different (P>0.05).

Note! After 25% inclusion level of CHM, increased inclusion level resulted to decrease in weight gain, total length and standard length at 50%, 75%, and 100% respectively. Similarly, SGR and RGR also reduced with increase in inclusion level after 25%.

Among the experimental diets, 25% inclusion level gave the best weight gain, total length, standard length, SGR and RGR.

Similarly 25% inclusion level gave a better weight gain, total length, standard length, SGR and RGR than when on fish meal (0% CHM) diet was given.

4.6 Growth Performance and Nutrient Utilization of *Clarias gariepinus* Fed with the Experimental Diets

The highest specific growth rate (2.68) was in fish fed D1 followed by D3 and D2 (2.58 and 2.54 respectively). The specific growth rate of fish fed D4, D5 and D6 were 2.41, 2.37 and 2.25 respectively. The specific growth rate of fish fed D1 was not significantly different (P>0.05) from those fed D2 and D3 but significantly different from D4, D5 and D6. Fish fed D2 and D3 diets were not significantly different (P>0.05) from fish fed D4 and D5 but significantly different from fish fed D6. Fish fed D4, D5 and D6 were not significantly different (P>0.05) from each other (Table 4.6).

The result showed that fish fed D1 had the highest relative growth rate (1562) while fish fed D6 had the least growth rate (983). The relative growth rate of the fish fed D1, D2 and D3 (1562, 1340 and

Table 4.6 Growth Performance and Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets

Parameter	D1 (Commercial feed)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Specific growth rate (g)	2.68±0.04^{a}	2.54±0.04^{ab}	2.58±0.03^{ab}	2.41±0.07^{bc}	2.37±0.05^{bc}	2.25±0.13^{c}
Relative growth rate (%)	1562±68.7^{a}	1340±59.2^{abc}	1403±43.55^{ab}	1162±98.9^{bcd}	1105±61.20^{cd}	983±158.06^{d}
Survival rate (%)	96.67±3.33^{a}	93.33±3.33^{a}	80.00±5.77^{a}	90.00±5.77^{a}	90.00±5.77^{a}	80.00±15.28^{a}
Feed conversion ratio (g)	1.31±0.07^{b}	1.51±0.04^{ab}	1.47±0.06^{ab}	1.65±0.09^{ab}	1.69±0.06^{ab}	1.85±0.27^{a}
Protein efficiency ratio (g)	1.81±0.09^{a}	1.62±0.05^{ab}	1.67±0.06^{ab}	1.49±0.08^{ab}	1.49±0.06^{ab}	1.38±0.22^{b}
Feed Intake (g)	266.69	264.36	264.97	244.20	242.20	228.45
Protein Intake (g)	112.91	108.63	108.13	100.00	97.80	93.32

Means with same superscripts along row were not significantly different ($P \geq 0.05$)

1403 respectively) were not significantly different ($P>0.05$) but significantly different ($P<0.05$) from the relative growth rate of the fish fed D4, D5 and D6 (1161, 1105 and 983 respectively). Fish fed D3, D2 and D4 were not significantly different ($P>0.05$) but D3 was significantly different ($P<0.05$) from D5 and D6 while D2 was significantly different from ($P<0.05$) D6. The fish fed D4, D5 and D6 were not significantly different ($P>0.05$).

The fish fed with the commercial feed (D1) had the best survival rate (96.67%), the least survival rate (80%) was in the fish fed with D6 and D4. There was no significant difference ($P>0.05$) in the survival rate of the fish fed with all the experimental diets.

The feed conversion ratio (FCR) ranged from 1.31 to 1.85. The fish fed D1 had the least FCR while D6 had the highest. There was no significant difference ($P>0.05$) between the FCR of Fish fed D1, D2, D3, D4 and D5, but there was significant difference ($P<0.05$) between fish fed D1 and D6.

The protein efficiency ratio (PER) of the fish fed the experimental diets ranged from 1.38 to 1.81. Fish fed D1 had the highest PER (1.81) while D6 had the least (1.38). There was no significant difference ($P>0.05$) in PER between the fish fed D1, D2, D3, D4 and D5, but there was a significant difference ($P<0.05$) between the fish fed D1 and D6

The triweekly weight increase of the experimental fish fed diets with varying inclusion levels of the cattle hoof meal is presented in Fig.4.1. There was a uniform growth pattern of fish fed 0% 25%, 50%, 75% and 100% CHM for the first three weeks while fish fed diets containing CF displayed the fastest growth pattern. Fish fed the commercial feed showed a remarkable increase in weight above the other treatments from the first week to the end of the feeding period. This was followed by fish fed diet 0% and 25% while fish fed diet 50-100% lagged behind after the.

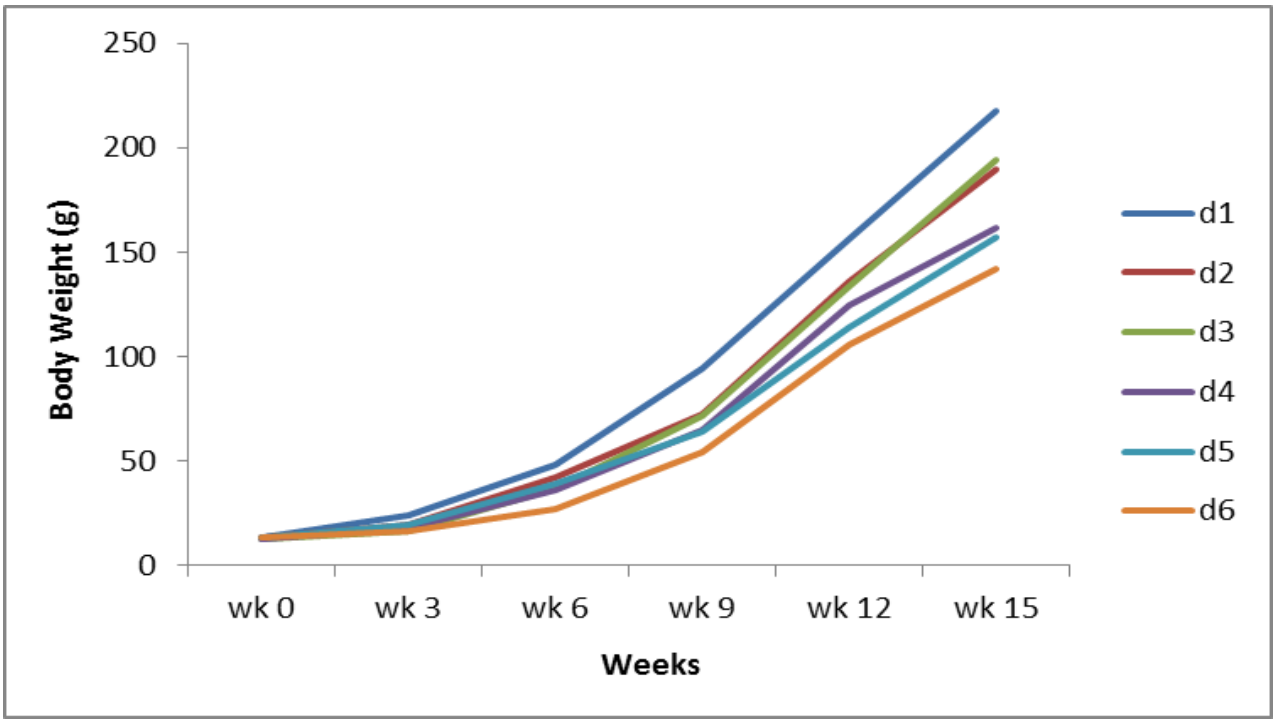


Figure 4.1 Triweekly Growth Pattern of *Clarias gariepinus F*ed Varying Levels of Cattle Hoof Meal

third week. Fish fed diet 100% CHM displayed slowest growth rate after the first three weeks to the end of the feeding trial.

4.7 Water Quality Analysis of the Experimental Tank

The result of the water analysis of the experimental tanks is presented in Table 4.7. The mean Dissolved oxygen, temperature, pH, ammonia, nitrite, nitrate and bicarbonate are 5.77mg/l, 30.25^{o}C, 7.12, 0.04mg/l, 0.04mg/l, 0.03mg/l, and 5.45mg/l respectively. The water parameters of the experimental tank were not different significantly ($P \geq 0.05$) across the treatments

4.8 Proximate Composition of the Experimental Diets and Concentration of Chromic Oxide

Table 4.8 shows the proximate composition of the experimental diet and concentration of chromic oxide. There was no significant difference ($P > 0.05$) in the protein and carbohydrates contents as the range was within the acceptable range of ±2 as recorded by Agboola, (2004). There was no significant difference ($P > 0.05$) in the chromic oxide concentration in the test diets.

4.9 Proximate Composition of Faecal Samples of *Clarias gariepinus* Fed the Experimental Diets

The result of the proximate composition of faecal samples of *C. gariepinus* fed the experimental diets is presented in Table 4.9. The crude protein, crude lipid and gross energy increased with increasing level of substitution from 25% to 100% cattle hoof meal while the ash content and chromium oxide decreased with increasing level of substitution level. The Carbohydrate and crude fibre showed no definite order of progression.

Table 4.7 Water Quality Parameters of the Experimental Tank

Parameter	D1 (Commercial feed)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Dissolved oxygen (mg/l)	5.77±0.15	5.77±0.15	5.77±0.15	5.77±0.15	5.77±0.15	5.77±0.15
Temperature (^{o}C)	28.25±0.36	28.25±0.36	28.25±0.36	28.25±0.36	28.25±0.36	28.25±0.36
pH	7.12±0.58	7.12±0.58	7.12±0.58	7.12±0.58	7.12±0.58	7.12±0.58
Ammonia (mg/l)	0.04±0.003	0.04±0.003	0.04±0.003	0.04±0.003	0.04±0.003	0.04±0.003
Nitrite (mg/l)	0.04±0.003	0.04±0.003	0.04±0.003	0.04±0.003	0.04±0.003	0.04±0.003
Nitrate (mg/l)	0.03±0.001	0.03±0.001	0.03±0.001	0.03±0.001	0.03±0.001	0.03±0.001
Bicarbonate (mg/l)	5.45±0.66	5.45±0.66	5.45±0.66	5.45±0.66	5.45±0.66	5.45±0.66

Means with same superscripts along row were not significantly different ($P \geq 0.05$)

Table 4.8 Proximate Composition of the Experimental Diet and Concentration of Chromic Oxide

Composition	D1 (CF)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Moisture	7.86	10.84	10.36	11.37	13.05	10.27
Dry matter	92.23	89.16	89.64	88.63	86.95	89.93
Crude lipid	14.69	12.54	12.21	12.51	12.35	12.11
Crude protein	42.34	41.09	40.81	40.95	40.38	40.85
Crude fibre	2.80	2.54	2.72	2.83	3.47	1.92
Ash	7.86	10.78	8.03	7.82	7.27	6.60
NFE	25.70	22.34	25.87	24.52	23.48	28.25
Gross Energy	2048.91	1874.33	1916.63	1908.16	1869.86	1955.49
Chromium Oxide	0.46	0.47	0.45	0.46	0.45	0.47

CF: Commercial feed; NFE: Nitrogen free extract

Table 4.9 Proximate Composition of Faecal Samples of *Clarias gariepinus* Fed the Experimental Diets

Composition	D1 (CF)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Moisture	10.99	11.91	11.01	12.57	14.12	11.99
Dry matter	89.01	88.91	88.99	87.43	85.88	88.01
Crude lipid	6.28	6.58	6.52	7.02	7.24	7.56
Crude protein	13.06	14.27	14.20	18.34	21.43	23.42
Crude fibre	4.82	4.38	4.47	4.89	5.11	4.00
Ash	11.72	12.71	11.61	8.56	8.36	8.01
NFE	53.13	50.97	52.19	48.62	43.74	45.02
Gross Energy	1497.18	1500.01	1517.42	1573.43	1570.15	1652.98
Chromium Oxide	1.30	1.21	1.18	1.14	1.09	1.01

CF**:** Commercial feed; NFE: Nitrogen free extract

4.10 Apparent Digestibility Coefficient of the Feed Fed to *Clarias gariepinus*

The result of the apparent digestibility coefficient of the feed fed to *C. gariepinus* is presented in table 4.10. The result showed that the apparent digestibility coefficient of the feed fed to *C. gariepinus* decreased with increasing level of substitution of fish meal with cattle hoof meal. The fish fed with the commercial feed showed the best digestibility coefficient in all the nutrients. The fish fed 0% (D2) and 25% (D3) cattle hoof were next to the fish fed commercial feed except in carbohydrate digestibility where D2 had the least digestibility coefficient. There was no significant difference ($P>0.05$) in all the nutrient digestibility of the fish fed D2 and D3 except in carbohydrate digestibility. There was a significant difference ($P<0.05$) in the digestibility of crude protein, crude lipid and gross energy among the *C. gariepinus* fed D4, D5 and D6.

Table 4.10 Apparent Digestibility Coefficient of the Feed Fed to *Clarias gariepinus*

Parameter	D1 (Commercial feed)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
ADC_{CP}	89.09±0.34^{a}	86.51±0.30^{b}	86.72±0.21^{b}	81.94±0.16^{c}	78.09±0.10^{d}	73.31±0.26^{e}
ADC_{CL}	84.86±0.32^{a}	79.63±0.19^{b}	79.64±0.30^{b}	77.36±0.15^{c}	75.80±0.13^{d}	71.14±0.19^{e}
ADC_{CHO}	26.84±0.64^{a}	11.34±1.45^{d}	23.05±0.57^{bc}	19.99±0.31^{c}	23.07±0.91^{bc}	25.76±1.67^{ab}
ADC_{DM}	65.51±0.41^{a}	60.90±0.55^{bc}	61.97±0.16^{b}	60.20±0.21^{cd}	59.22±0.26^{d}	54.43±0.73^{e}
ADC_{GE}	74.14±0.23^{a}	68.90±0.41^{b}	69.80±0.17^{b}	66.73±0.19^{c}	65.33±0.11^{d}	60.64±0.55^{e}

Means on the same row having the same superscript are not significantly different (P>0.05)

ADC_{CP}: Apparent Digestibility Coefficient of Crude protein

ADC_{CL}: Apparent Digestibility Coefficient of Crude Lipid

ADC_{CHO}: Apparent Digestibility Coefficient of Carbohydrate

ADC_{DM}: Apparent Digestibility Coefficient of Dry matter

ADC_{GE}: Apparent Digestibility Coefficient of Gross energy

4.11 Carcass Proximate Composition of *Clarias gariepinus* Fed Varying Levels of Cattle Hoof Meal

The moisture, protein, lipid, ash and NFE were 22.22, 55.72, 5.98, 8.64, and 7.44% respectively at the beginning of the experiment. Moisture contents of the fish at the end of the experiment were 17.09, 18.02, 17.70, 17.24, 19.42% and 20.16% respectively for fish fed with commercial feed, 0, 25, 50, 75 and 100% CHM. The initial and final carcass composition of all the fish fed the experimental diets showed significant difference ($P<0.05$) (Table 4.11). The protein contents of the *Clarias gariepinus* carcass were 62.63, 62.19, 62.13, 60.88, 58.56 and 55.72% for fish fed with D1, D2, D3, D4, D5, and D6 respectively. The crude protein of all the fish fed the experimental diets were significantly different ($P<0.05$) from each other. All treatments had higher lipid contents than the initial lipid composition. There was no significant variation ($P>0.05$) in the carcass lipid of the fish fed with D1 in comparison with the initial fish, but a significant difference ($P<0.05$) was found in the carcass lipids of the fish fed with D2, D3, D4, D5, D6. . the fish fed with D4 had the highest crude lipid (7.84%) in their carcass composition

The ash content was highest in the carcass of the fish fed D6 (11.11%) but there were no significant differences ($P>0.05$) in fish fed D1 and D4 (9.62 and 7.84 respectively) and D2 and Initial fish (8.66 and 8.64 respectively). The highest NFE was obtained in the initial fish carcass and was significantly different ($P<0.05$) from all the treatments. The fish fed D6 and D2 were not significantly different ($P>0.05$). The ash content of the fish fed D1, D3 and D5 were not significantly different ($P>0.05$). The least NFE was recorded in the fish fed D4.

Table 4.11 Carcass composition of *Clarias gariepinus* fed with graded levels of cattle hoof meal

composition	Initial	D1 (Commercial feed)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Moisture Content	22.22±0.01^{a}	17.09±0.01^{g}	18.02±0.01^{d}	17.70±0.02^{e}	17.24±0.01^{f}	19.42±0.01^{c}	20.16±0.01^{b}
Dry Matter	77.78±0.01^{g}	82.91±0.01^{a}	81.91±0.01^{d}	82.30±0.02^{c}	82.76±0.01^{b}	80.58±0.01^{e}	79.84±0.01^{f}
Crude Protein	55.72±0.02^{g}	62.63±0.01^{a}	62.19±0.01^{b}	62.13±0.02^{c}	60.88±0.01^{d}	58.56±0.01^{e}	57.44±0.01^{f}
Crude Fibre	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Crude lipid	5.98±0.01^{f}	6.00±0.01^{f}	6.12±0.01^{e}	6.67±0.02^{c}	7.84±0.01^{a}	6.81±0.01^{b}	6.25±0.01^{d}
Ash	8.64±0.01^{e}	9.62±0.01^{c}	8.66±0.01^{e}	8.82±0.02^{d}	9.63±0.01^{c}	10.55±0.01^{b}	11.11±0.01^{a}
NFE	7.44±0.01^{a}	4.65±0.01^{c}	5.01±0.01^{b}	4.68±0.02^{c}	4.41±0.01^{d}	4.66±0.01^{c}	5.04±0.01^{b}

Means with same superscripts along row were not significantly different (P>0.05)

4.12 Cost and Benefit Analyses of Experimental Diets

Table 4.12 presents the cost and benefit analyses of the experimental diets. The commercial feed (D1) had the highest feed cost/kg, feeding cost and total expenditure/cost (₦480, ₦128 and #153/kg respectively) while D6 had the least feed cost/kg, feeding cost and total expenditure (₦207.54, ₦47.29 and ₦82.29). The cost of feed/kg, feeding cost and total expenditure reduced with increase in the inclusion level of cattle hoof meal. The fish fed with D1 had the highest total sales value (#195.54) but not significantly different (P>0.05) from the total sales value of the fish fed with D2 and D3 (₦170.82 and ₦174.51 respectively). The fish fed with D6 had the least total sales value (₦127.92) but not significantly different (P>0.05) from the total sales value of D4 and D5 (₦145.29 and ₦141.09). The total sales value of the fish fed with D1 was significantly different from the fish fed with D4, D5 and D6.

The fish fed D3 had the best Net profit (46.53) followed by the fish fed with D6, D1, D5, D4 and D2 (₦45.63, ₦42.54, ₦41.61, ₦31.49 and ₦20.59 respectively) in the order of decreasing Net profit. The net profit from all the experimental diets was not different significantly (P>0.05).

The economic weight gain was lowest and best in fish fed D6 (0.38g) while D2 (0.65g) had the highest economic weight gain followed by D1 (0.63g). The economic weight gain of fish fed D6 was not significantly different (P>0.05) from D5 (0.45g) but significantly different (P<0.05) from D3, D4, D1 and D2 (0.52g, 0.53g, 0.63g and 0.65g).

The fish fed D6 had the highest profit index (₦3.01) and lowest Incidence cost (₦0.34). The fish fed with D5 had better profit index and incidence cost of 2.43 and 0.41 respectively. Fish fed D2 had the lowest profit index (₦1.65) and highest incidence cost (₦0.61).

Table 4.12 Cost and Benefit Analysis of the Experimental Diets

Parameter	D1 (Commercial feed)	D2 (0%)	D3 (25%)	D4 (50%)	D5 (75%)	D6 (100%)
Cost of Juvenile(₦)	35	35	35	35	35	35
Cost of Feed/Kg(₦)	480	435.89	350.90	322.70	266.24	207.54
Mean feed Intake (g)	266.67	264.36	264.97	244.20	242.20	228.45
Feeding cost (₦)	128.00	115.23	92.98	78.80	64.48	47.29
Total cost (₦)	153.00	150.23	127.98	113.80	99.48	82.29
Cost of fish/kg (₦)	900	900	900	900	900	900
Feed Intake (g)	266.69	264.36	264.97	244.20	242.20	228.45
Protein Intake (g)	112.91	108.63	108.13	100.00	97.80	93.32
Economic Weight Gain (g)	0.63 ± 0.04^{ab}	0.65 ± 0.02^{a}	0.52 ± 0.02^{c}	0.53 ± 0.03^{bc}	0.45 ± 0.01^{cd}	0.38 ± 0.06^{d}
Profit Index (₦)	1.71 ± 0.08^{c}	1.65 ± 0.04^{c}	2.09 ± 0.08^{bc}	2.05 ± 0.10^{bc}	2.43 ± 0.07^{b}	3.01 ± 0.42^{a}
Total Sales (₦)	195.54 ± 9.30^{a}	170.82 ± 4.41^{ab}	174.51 ± 6.63^{ab}	145.29 ± 7.05^{bc}	141.09 ± 4.05^{c}	127.92 ± 18.00^{c}
Incident cost (₦)	0.59 ± 0.03^{a}	0.61 ± 0.02^{a}	0.48 ± 0.02^{b}	0.49 ± 0.03^{b}	0.41 ± 0.01^{bc}	0.34 ± 0.05^{c}
Benefit Cost Ratio	1.28 ± 0.06^{ab}	1.14 ± 0.03^{b}	1.37 ± 0.05^{ab}	1.28 ± 0.06^{ab}	1.42 ± 0.04^{ab}	1.55 ± 0.22^{a}
Net Profit (₦)	42.54 ± 9.3^{a}	20.59 ± 4.41^{a}	46.53 ± 6.63^{a}	31.49 ± 7.25^{a}	41.61 ± 4.05^{a}	45.63 ± 18.01^{a}

Means with same superscripts along row were not significantly different ($p\geq0.05$)

The highest benefit cost ratio was observed in fish fed D6 (1.55) while the highest net profit was observed in fish fed D3 (₦46.53). The least benefit cost ratio and net profit was observed in Fish fed D2 (1.14 and ₦20.59 respectively). The benefit ratio of D6 was significantly different ($P<0.05$) from D2 but not different significantly ($P>0.05$) from D5, D3, D4 and D1. The Net profit for all the experimental diets was not different significantly ($P>0.05$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Nutrient Composition of Cattle Hoof Meal

Cattle hoof meal has high crude protein (58.06%), which is higher than the range (43.10-49.50%) in Termite (*Macrotermes subhyalinus*) meal and in tadpole (*Bufo maculata*) meal (41.30 – 47.80%) but lower than 60- 69% in earthworm and garden snail meals (Odaibo,1997; Seira, 1998; Sogbesan and Ugwumba, 2008). The high crude protein of cattle hoof meal has been proposed to be a good alternate to fish protein (68.68%) in the production of fish feed (FAO, 2006).

The crude lipid of cattle hoof meal (3.25%) obtained in this study is lower than that reported by FAO, (2006) (4.2%). This could be due to the differences in location and availability of food for cattle at the different ecological zones, which may directly affect lipid deposition in the hooves. Olaleye, (2015) and Njidda and Isidahomen (2010) reported little differences in the nutrient composition of feather meal due to difference in location of the experiment. The crude lipid of the cattle hoof (3.25) is lower than the crude lipid of fish meal (6.78%) (FAO, 2006) therefore, there might be the need to supplement cattle hoof meal with fish oil and or other types of lipids.

The mineral composition of cattle hoof meal shows high percentage of sulphur and carbon but low percentage of calcium and phosphorus (0.25 and 0.08 respectively). The finding is in contrast to the report of Quresh (1962) (8.3mg/g and 7.3mg/g for calcium and phosphorus respectively).

Leucine and Arginine are the most available essential amino acid while glutamic acid and aspartic acid are the most non-essential amino acid in the cattle hoof meal. These essential amino acids of cattle hoof meal conform with the NRC, 1993 and FAO/WHO (1990) requirements for the production of *Clarias gariepinus*.

5.2 Nutrient Composition of the Experimental Diets

The crude protein of the experimental diets in this study is within the acceptable range (40-45%) for good growth of *Clarias gariepinus* (Agboola, 2004). Fluctuation occurs in the composition of feed ingredients due to seasonal and geographical variations, therefore formulations should be modified accordingly since physical characteristics, milling and composition of the feed ingredients may have significant effect on the quality of finished feed. The crude lipid content of the diets range between 12.11 and 14.69% which is within the acceptable standard lipid requirements for most tropical fishes (Adikwu, 2003).

The quality and quantity of the protein in the experimental diets of this study could be one of the factors responsible for high growth obtained. Faturoti *et al.,* (1986) and Fagbenro *et al.,* (1992) reported that *Clarias gariepinus* and *Heterobranchus bidorsalis* tend to require a minimum of 40% crude protein for normal growth.

5.3 Growth Performance and Nutrient Utilization of *Clarias gariepinus* Fed the Experimental Diets

Clarias gariepinus fed varying inclusion levels of Cattle hoof meal feed showed a positive response to the feed. Fish fed the commercial feed had the best weight gain (204g) followed by the fish fed the experimental diet which contained 25% of Cattle feed meal (181g). The fish fed with the control diet which had no cattle hoof meal (D2) had lower weight gain compared to the

fish fed 25% cattle hoof meal. The higher growth performance observed in the group containing 25% cattle hoof meal might be due to the synergistic effect of combining two biological compounds (i.e fish and cattle hoof) to have superior effect than when applied individually. This observation is in agreement with suggestions that combination of protein sources is better than single protein source in the diet of fish (Ugwumba *et al.*, 2001; Sogbesan *et al.*, 2005; Sogbesan and Ugwumba, 2006). Though the fish fed the commercial feed had the best weight gain, there was no significant difference between the fish fed the commercial feed and the fish fed 0% CHM and 25% CHM. The fish fed 50% CHM, 75% CHM and 100% CHM showed a decreasing weight gain (148.60g, 143.73g and 129.00g) as the substitution level of the cattle hoof meal increases. This could be due to the presence of lower utilizable and digestible protein in the feed as the CHM concentration increase. The feed intake and protein intake by the fish decreased with increasing substitution level, though the crude proteins of the experimental diets were not significantly different, as the difference was within the acceptable range of ±2 (Agboola, 2004). This finding could be due to effective digestibility of the cattle hoof and fish meal, rather than the amino acids only (Massumotu *et al.*, 1996).

The fish fed the commercial diet had the highest specific growth rate (SGR), relative growth rate (RGR) and protein efficiency ratio (PER) and lowest feed conversion ratio. This conferred that the commercial feed produced the best result in comparison to the experimental diets. The fish fed 25% cattle hoof meal (D3) produced the best result SGR, RGR, PER and FCR among the fish fed the experimental feeds. The higher the SGR and the lower the FCR value, the better the feed quality (Omeru and Solomon, 2014). Similarly, Adikwu (2003) also reported that the lower the FCR the better the feed utilization by the fish. The protein efficiency ratio values in this study and in all the experimental feeds were above the value one (1). This is a demonstration of higher

feed quality in terms of the amino acids. DeSilva and Anderson (1995) reported that protein efficiency ratio is a measurement of how well the protein sources in a diet could provide the essential amino acids requirement of the fish fed.

Survival rate was higher in treatment D1 (96.67%) and D2 (93.33%) while the lowest survival rate was recorded in D3 and D6 (80% for both). There was no clear reason for the difference in the survival rate but may be due to stress during handling as most of it occurred after weekly sampling. The high survival rate (80% and above) in the whole treatments may be associated with favorably high water quality of the experimental tanks and possibly, the well processed and preserved feeds administered to the fish.

5.4 Physico Chemical Parameters of the Experimental Tank

The physico chemical parameters of water in experimental tanks were optimum for the culture of *Clarias gariepinus*. Catfish require optimum temperature of 23-32^{o}C, dissolve oxygen of ≥5mg/l, pH of 6-9, Ammonia of <8.8mg/l, Nitrite of <0.06mg/l and bicarbonate of 5mg/l (FAO, 2006; Momoh and Solomon, 2017). There was no significant difference in the physico chemical parameters of the tank across the treatments because the six treatments were held in a large concrete pond but partitioned with two hapas into six treatments of three replicates. The optimum physico chemical parameter of water may have contributed to the growth rate and survival percentages in all treatments.

5.5 Apparent Digestibility Coefficient (ADC) of the Feed Fed to *Clarias gariepinus*

The apparent digestibility coefficient of crude protein in the *Clarias gariepinus* fed with the experimental diets ranged from 89.09% to 73.31% in a decreasing order from D1 to D6. The commercial feed had the best crude protein digestibility coefficient (89.09%). This result agrees

with the finding of Bob-Manuel (2013) who recorded 89.44% crude protein digestibility coefficient in *Clarias gariepinus* fed Coppens. The fish fed 25% cattle hoof meal had the next best crude protein digestibility, this suggests that CHM might be a good protein source for *Clarias gariepinus*. Higher apparent digestibility coefficient for protein of CHM at 25% inclusion than 0%, 50%, 75%, and 100% substitution level in the diets implies that a greater amount of protein was available to the fish from the CHM at 25% inclusion. The protein quality of the dietary ingredients is usually the leading factor affecting fish performance and protein digestibility and is the first measure of its availability for fish (Rahman *et al.*, 2016).

Dry matter apparent digestibility coefficient of between 65.51% and 54.43% for *Clarias gariepinus* fed the experimental diets showed that the feed had high digestible materials. Next to the commercial feed which had the best dry matter ADC (65.51%) is the 25% CHM which had dry matter ADC of 61.97%. The dry matter ADC decreased as the substitution level increased from 50% to 100% CHM. This could be due to the presence of more non digestible material in the diet as the substitution level increased from 50% to 100% CHM. Dry matter apparent digestibility coefficient of various protein feedstuffs offers an estimate of overall digestibility, low value generally indicate that a high level of indigestible material is present in the feedstuff (Li *et al.*, 2013). Dry matter ADC provides the estimate of non-digestible material in the feed stuff in comparison with digestibility coefficient for individual nutrient (Rahman *et al.*, 2016).

The ADC of dietary lipid usually ranged from 85 to 95% in fish (NRC, 1993). The result of this research disagrees with the finding of NRC (1993) as the ADC of dietary lipid recorded in this research ranged from 71.14% to 84.86%. The dietary lipid ADC decreased with increase in the substitution level from 25% to 100% CHM substitution level. The digestibility of lipids is

influenced by factors which include degree of saturation, dietary lipid level, and other constituents (Yuan *et al.*, 2010).

This study shows that the gross energy ADC of *Clarias gariepinus* fed the experimental feed ranged between 60.64 to 74.14%, and the fish fed the commercial feed have the best gross energy ADC (74.14%) followed by the fish fed D3 and D2 (69.80 and 68.90% respectively). The gross energy digestibility decreased with increasing level of substitution from 50% to 100% CHM. The ADC recorded in this research is higher than that recorded by Laining *et al.* (2003) which was 51.1% for fish meal; Gul *et al.* (2007) who reported 52% but lower than the findings of Chor *et al* (2013) that recorded 91% for rainbow trout. Gaylord and Gatlin (1996) reported the efficiency of carnivorous fish in utilizing energy from animal products. Gomes *et al.* (1995) recorded that high ash content of fish meal might reduce energy digestibility.

Carbohydrate had the lowest among the nutrient apparent digestibility coefficient recorded in this research. The carbohydrate ADC of the fish fed the experimental diets ranged between 26.84% and 11.36%. The fish fed D1 had the highest Carbohydrate ADC (26.84%) while the fish fed D2 had the lowest carbohydrate ADC (11.36%). The low carbohydrate ADC recorded in this study is similar to that reported by Jimoh *et al.* (2014) for *Clarias gariepinus* fed sesame seed meal

5.6 Carcass composition of *Clarias gariepinus* fed graded levels of Cattle hoof meal.

At the end of the experiment, the carcass composition of all the fish fed the experimental diets had higher protein, lipid and ash contents but lower nitrogen free extract content in comparison with fish sample at the beginning of the experiment . This result demonstrates that protein synthesis and increased tissue production in *C. gariepinus* occurred which resulted in growth. The chemical composition of fish carcass is a reflection of quality of feed which eventually

affects the final quality of the product, and its processing properties (Lie *et al.*, 1988; dos Santos *et al.*, 1993; Bendiksen *et al.*, 2003; Hemre *et al.*, 2004).

The crude protein of the carcass showed a decreasing trend as the substitution level with cattle hoof meal increases. No clear trend was observed in the crude lipid, ash and nitrogen extract of the experimental fish in relation to the substitution levels of the cattle hoof meal. Similar inconsistences with respect to lipid, ash and nitrogen free extract was reported by Fagbenro *et al.* (2003) in the substitution of soybean meal with roselle seed meal and kenaf seed meal in the diet of *Oreochromis niloticus.*

5.7 Cost and Benefit Analysis of the Experimental Diets

The commercial feed (D1) had the highest cost of feed per Kg, feeding cost and total cost while diet that contained 100% cattle hoof meal (D6) had the least cost of feed/kg, feeding cost and total expenditure/cost. This connotes that D6 is the cheapest feed among the experimental feeds while the commercial feed is the most expensive. The commercial feed had the best total sales as the fish had the highest weight gain, while D6 with least weight gain had the lowest total sales. Next to the commercial feed is the D3 which had the second highest weight gain and the second best total sales value; D3 had the best net profit (₦46.53) among the whole treatments while D2 had the least net profit (₦20.59). The possibility of obtaining a significantly higher value of net profit at 25% substitution level above those of the control and higher substitution levels clearly indicates that more profit would be generated from fish fed D3.

Fish fed D6 had the highest profit index (PI) and benefit cost ratio (BCR) and lowest economic weight gain (EWG) and incidence cost (IC). The higher the profit index and benefit cost ratio, the lower the economic weight gain and incidence cost, the better the feed economically. As a

rule of thumb, project with benefit ratio greater than one, equal to one, or less than one indicate profit break even or loss respectively (Olagunju *et al.*, 2007). Since the whole experimental feeds produced BCR greater than one, it implies that the experimental feeds are good but some are better than the other. In this regard D6 is good economically but the farmer will have to wait for a very long time before the fish could get to table size since it produced the least weight gain in the same time frame of the experiment. Fish in group D3 is the second best when ranked base on the economic weight gain, profit index, incidence cost, and benefit cost ratio. Production estimate base on the gross and net yield for growth are the basis for estimating the economic revenue from the fish culture operation (Umar *et al.*, 2016).

The high net profit, low EWG and good profit index for fish fed 25% CHM could be attributed to good feed quality, acceptance of feed by fish and suitable water quality, which results in better growth performance and high survival rate. In this study, D3 is the best feed because the fish fed in this group have high feed utilization efficiency, digestibility coefficient, carcass composition and the best economic value. This result herein aligns with the report of Agbabiaka *et al.* (2013) that some non-conventional feed resources have better economic value than the costly feeds which contained orthodox feedstuffs.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The nutrient composition of cattle hoof meal is an indication of its suitability in replacing fish meal in the diet of African catfish. The percentage of the crude protein (58.06%) coupled with the amino acid composition of the Cattle hoof meal enhanced the growth performance of the fish fed the experimental diets.

The physico chemical parameters of the water in the experimental pond were all within the standard level for optimum growth of *Clarias gariepinus*. There was no significant difference ($P>0.05$) between the water parameters of the six treatments.

The fish fed the commercial feed had the best weight gain (204g) followed by the fish fed the experimental diet (D3) which contained 25% of Cattle hoof meal (181g). There was no significant difference ($P>0.05$) between the fish fed D1, D2 and D3 but significantly ($P<0.05$) higher than the groups that were fed D4 and D5 while the group that were fed D6 had the lowest mean weight gain (129.00g).

The fish fed the commercial fed (D1) had the best nutrient utilization efficiency (SGR, RGR, FCR and PER) followed by the fish fed D3 (25%). The fish fed the control diet (0%) had the third best nutrient utilization efficiency after the fish fed D1 and D3. The fish fed D6 (100%) had the least nutrient utilization efficiency. The nutrient utilization efficiency reduced as the substitution level of fish meal with cattle hoof meal increased from 50% to 100%.

The apparent digestibility coefficient of the feed fed to *Clarias gariepinus* decreased with increasing level of substitution of fish meal with cattle hoof meal. Aside from the commercial feed, D3 had the best ADC among all the experimental feeds.

The carcass compositions of the fish fed the experimental feed were significantly different ($P<0.05$) from the carcass composition of the initial fish. All the fish fed the experimental diets had higher carcass protein, lipid and ash contents but lower nitrogen free extract content than the initial fish sample. The crude protein showed a decreasing trend with increasing substitution level. This shows that there was protein synthesis and increased tissue production in *C. gariepinus* and that fish growth was not due to the increase in weight alone.

The highest net profit (#46.53) was observed in the fish fed D3 (25%) while least net profit (#20.59) was observed in the fish fed D2 (0%). D6 had the second best net profit but the best economic weight gain, profit index, incidence cost and benefit cost ratio as the feed was relatively very cheap. The benefit cost ratio of D6 was significantly different ($p<0.05$) from D2 but not different significantly ($p>0.05$) from D5, D3, D4 and D1. The Net profit for all the experimental diets was not different significantly ($p>0.05$).

Taking into recognizance the nutrient utilization efficiency, apparent digestibility coefficient and economic cost and benefit, D3 was ranked the best among the six experimental feeds fed to the *Clarias gariepinus*.

6.2 Conclusion

Cattle hoof meal is a rich source of animal protein (58.06%) with essential amino acids and minerals. It can be inferred that cattle hoof meal in the diet of *Clarias gariepinus* as a substitution for fish meal may yield a positive result as both meals have a high crude protein and required amino acid composition as required for optimum growth of *Clarias gariepinus*.

Substitution of fish meal with Cattle hoof meal at 25% substitution level produced the best growth performance and feed utilization (Weight gain 181g). Though the cattle hoof meal can be included up to 100%, since the fish showed good appetite for all the treatment diets, growth performance of *Clarias gariepinus* decreased with increase in substitution level of fish meal with cattle hoof meal in the diet. Inclusion of cattle hoof meal in the diet does not have detrimental effect on *Clarias gariepinus* as revealed by the survival rate.

Substitution of fish meal with cattle hoof meal at 25% had no effect on the apparent digestibility of *Clarias gariepinus*

The carcass composition of the fish fed 25% substitution level produced high crude protein (62.19%) and minimal crude lipid (6.67%). The crude protein of the carcass of the experimental fish showed a decreasing trend with increasing substitution level, the value of crude protein of fish fed D3 (62.13%) was very close to that of D1 and D2 (62.63% and 62.19% respectively).

Substitution of fish meal with cattle hoof meal at 25% produced fish with the best mean net profit (#46.53) which reduced price of fish by 36.34% thereby enhancing profit margin.

6.3 Recommendations

a. It is recommended that cattle hoof meal be included in the feed of *Clarias gariepinus* at 25% for optimum growth performance and reduced cost of production

b. Since this study was carried out under concrete pond experimental conditions, it is recommended that field experiment using the semi intensive system commonly practiced in earthen ponds may be more desirable and can be explored.

c. Further studies on the best substitution level of fish meal with cattle hoof meal should be conducted using culturable indigenous species.

6.4 Contributions to Knowledge

A. This study provides information on the nutritional composition of Cattle hoof meal; 33.75% moisture content, 66% dry matter, 58.06% crude protein, 2.75% crude fibre, 3.25% crude lipid, 0.91% ash content and 1.78% Nitrogen free extract. This serves as baseline information /data to researchers who may want to explore the economic importance of cattle hoof.

B. At 25% substitution level of fish meal with cattle hoof meal, there was better growth performance and nutrient utilization. This implies that cattle hoof meal should be used to substitute fish meal at 25% substitution level for optimum growth performance.

C. The apparent digestibility coefficient of the feed fed to *Clarias gariepinus* decreased with increasing level of substitution of fish meal with CHM. The finding of this study shows that cattle hoof meal is digestible and so, can be included in the diet of livestock.

D. The result of this study shows that it is more economical when fish meal is substituted with cattle hoof at 25% substitution level in the diet of *Clarias gariepinus* as this is the level that produced the best net profit (₦46.53) and weight gain (181g).

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APPENDIX 1



Cattle hooves

APPENDIX II



Experimental setup