COMPARATIVE EFFECT OF COMMERCIAL FEED AND FORMULATED DIETS ON THE GROWTH AND BIOCHEMICAL PARAMETERS OF *HETEROBRANCHUS BIDORSALIS* (CATFISH) FINGERLINGS

ABSTRACT

Fish farming is a major viable solution to the problem of protein inadequacy in Nigeria but the high cost of feed remains a major obstacle. This study evaluated the comparative effect of commercial feed and formulated diets on the growth and biochemical parameters of *Heterobranchus bidorsalis* (catfish) fingerlings. Proximate composition of fish wastes (FW), guinea corn (GC), groundnut cake (GNC) and bone meal (BM) were determined and used to formulate diets. Pearson's square method was used to formulate three diets designated as A (FW and GC at 3:1), B (GNC and GC at 3:1) and C (FW, GNC and GC at 3:3:1). Standard methods were used to determine proximate composition, amino acid composition and mineral content of the formulated diets. A commercial feed (Coppens) was used as control diet. Forty fingerlings of 2 g bodyweight were divided into 4 groups consisting of ten fingerlings each. The first group was fed commercial feed, while the remaining groups were fed with the respective experimental diets. Each group was fed 5 % of their average bodyweight twice daily in an aquarium for 49 days. Changes in weight, length, biochemical and haematological parameters of the fishes were determined over the period of experimentation. The results of proximate composition of the selected ingredients revealed increase in percentage protein level in FW (59.90±0.26 %) than other ingredients (GC, GNC and BM). The results of proximate composition of the formulated diets showed significant rise in percentage protein in formulated diet A (44.12±0.15 %) than other formulated diets (B and C) but lower in percentage protein level than the control diet. Amino acid composition and mineral content of the formulated diets showed significant rise in total amino acid composition and mineral content in formulated diet A than other formulated diets (B and C) but lower than the control diet. Bodyweight and length (21.93±0.13 g; 14.97±0.24 cm) of fingerlings fed formulated diet A was significantly higher ($p<0.05$) than other groups (B and C) but lower than the groups fed the control diet (22.77±0.15 g; 15.07±0.25 cm). The results of biochemical parameters showed significant rise in total serum protein of fingerlings fed formulated diet A (19.52±0.21 g/dl) than other groups of fingerlings fed formulated diets (B and C) but lower than the groups of fingerlings fed the control diet (20.35±0.13 g/dl). ALP, AST and ALT values of the fingerlings fed formulated diet A were significantly lower than the groups of fingerlings fed formulated diets (B and C) but no significant difference with the groups fed the control diet. The results of haematological parameters showed that fingerlings fed formulated diet A was significantly higher in values for RBC, haemoglobin and PCV except for the WBC that was significantly lower than other groups fed formulated diets (B and C) but lower than the groups of fingerlings fed the control diet. Formulated diet A yield a better results in terms of protein level of the formulated diets, biochemical and haematological parameters as well as growth performance of catfish and it is therefore, preferably as an alternative for (catfish) fingerlings rearing.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Nutrition is the sum of all the processes in which living organisms obtain, process and utilize food substances. It is the adequate intake of proteins, carbohydrates and micronutrients for maximum growth, development and maintenance of life (WHO, 2013). Living organisms consume foods that consist of various nutrients such as: carbohydrates, proteins, lipids, minerals and vitamins which are transported to the various part of the body where the nutrients in the foods are converted into body tissues for various metabolic activities. Apart from milk and egg, no single food can adequately provide complete necessary nutrients for the growth, development and maintenance of life. Researches have proven that, the early stage of growth of some organisms such as mammals and fishes depend on milk and eggs for growth, development and maintenance of life. However, when these organisms reach certain stage of development, milk and egg cannot provide adequate nutritional requirement for the organisms. The first stage in the life of a fish is completed at the expense of the reserves, which it receives from the yolk in the egg. The fish can only live on its yolk for a short period after which it goes on completely on natural or supplementary feed (Jiri *et al.*, 2014).

Meeting domesticated animals nutritional requirements is important because consumption of nutritionally unbalanced diet might lead to poor development, increase susceptibility to disease, impaired physical and mental development, stunted growth, sluggishness as well as reduced productivity (Igah, 2008 and Jiri *et al.*, 2014). The Nigerian neonatal mortality estimate 157 of every 1000 children (Oyewale and Adeniyi, 2017) die as a result of insufficient nutrient, most especially protein availability from

plant and animals. One-third of the babies endure changing degrees of lack of healthy sustenance; some are under-developed (33 %) and with (29 %) prevalent for low bodyweight (UNICEF, 2014).The United Nations Children Emergency Fund (UNICEF) in 2017 reported that as high as 2 in 10,000 Nigerian children die of malnourishment per day particularly in the emergency inclined district of the North East.

Guinea corn (*Sorghum vulgare*) belong to the class of the grassland species which can survive in a high temperate region with small amount of rainfall producing food for millions of people (Baker and Terry, 1991). The seeds are small, round and have a diversity of colours; like coffee, dark, white and red. Sorghum is a significant staple in the foods of Nigerians where it is a major food crop. It is one of the ingredient used baking bread, pap making as well as source of food for cattle, horses, diet formulation for fish, birds and also in brewing industries (El-Sayed, 1987). It is a vital source of sugar, protein and nutrient, for example, selenium, calcium, manganese and iron which the bioavailability depends on the degree of association with numerous antinutrients (Nasser, 2011). It is additionally plentiful in B-complex nutrients. Aside from its utilization as nourishment, it contains some chemical compounds that are defensive against malignant growth, coronary illness, substantial menstrual stream, and tumor development. It contains no gluten, making it an incredible supplement for animal feed and diet formulation.

Groundnut cake (GNC) is a meal produced from groundnut which is traditionally called kulikuli. It is a significant wellspring of dietary protein for home animals, with a rough protein content of 45-48 % and is used to supplement animal feeds as a sources of protein to enhance growth and development. Because of it odour, it is acceptable by fish as feed. Groundnuts are valued sources of vitamins E, K and B. It is the richest plant source of thiamine (B1) and also rich in niacin, but it is poor in energy content FAO

(2000). Though, research have shown that Groundnut cake crude protein is deficient in certain amino acids such as lysine and methionine and also has little quantity of threonine and tryptophan but amino acids quality can be enhanced when supplemented with lysine, methionine and tryptophan (Eyo, 2003).

Bone meal is a product of defatted, dried animal bones when grinded into a fine powder. It contain high amount of minerals such as calcium, iron, phosphorus and little amount of trace elements with crude protein content of 19.2 % which is used as a sources of minerals for animal feed formulation (Robinson *et al.*, 1994; Akagbejo and Fasakin, 2008). Bone meal is also used as fertilizer to enhance the fertility of the soil for plants growth. Calcium present in bone meal contributes to the muscle contraction activities, hormones secretion, nerve transmission, blood clotting, as well as improving the stabilizing the membrane cells of animals. It also assist the passing of nutrients and other substances in and out of cells. Phosphorus and trace elements in bone meal promote cell growth; perform vital functions for the heart muscle contraction as well as normal kidney function *(Brigham and Woen, 2012)*

To promote aquaculture production and fish farming in Nigeria, there is need to tackle the challenges faced by aquaculture production. These challenges have render aquaculture production less practices and brought about an increasing gap between demand and supply of catfish farmed in Nigeria. These challenges include; poor management, inadequate availability of good seed, poor data collection, lack of favorable environment and subsequently increase in commercial feed cost which takes about 50 % of the production cost. The price of commercial feed per bag in Nigeria are; coppens (₦10, 000), Top feed (₦ 9,500), Aquamax (₦8,000), blue crown (₦6,500) and Durante (₦ 6,000).

1.2 Statement of the Research Problem

Fish farming are faced with different challenges in many Nations of the world, including Nigeria. These challenges varies from one geographical location to another which reduced maximum fish production. Some of these challenges include; poor management practices, poor funding of fish farming through many institutions like research institutions, Universities, Non-governmental organizations, regional authorities, as well as problem with flooding, poor security, problem of predators, incidence of diseases and good quality water (Mwangi, 2008).

Weak record keeping by farmers and inefficient collection of statistical data have hindered the broadcasting of information on fish farming. In addition, poor funding of sub-sector operations and low private sector investment are major constraints in this sector. Furthermore, these problems are exacerbated by insufficient farmers' entrepreneurship skills and the lack of certified quality seeds (fingerlings) and high commercial feed costs are the key critical challenges facing Nigerian fish farmers. Because of poor facilities and lack of technical skills most fish farmers in Nigeria are yet to embrace the technology for producing high quality seeds and locally formulated diet for fish. Commercial produced feeds are difficult to come by because of it availability and high cost for fish farmers to buy. Researches have shown that most fish farmers in Nigeria relied on commercial feed for the Purpose of expanding aquaculture production. Fish farming poses substantial challenges to fish farmers in Nigeria, due to high cost of commercial feeds which takes over 50 % of the production cost and sustenance in Nigeria (; Gabriel *et al.,* 2007; Ahmad and Ibrahim, 2016).

1.3 Justification of the Study

Reports have shown that Nigeria is the second largest African country that produces local raw materials of plants and animals origin. Some of these raw materials are readily

available and also have been analyzed to contain essential amino acids that are required for growth and other physiological functions of farm animals (Adedeji and Okocha, 2011).

Fish, like other aquatic animals require sugars, amino acids, nutrients, minerals and lipids for typical metabolic capacities, sufficient development and advancement, which is the objective of each fish ranchers. The dry load of fish muscle comprised of more than 60 % protein, so nonstop inventory of supplement protein must be guaranteed so as to meet the body protein necessities. Research shows that catfish species, fingerlings need higher crude protein levels (more than 40 percent) for rapid growth and maturity, whereas juvenile and brood fish need 35-38 percent of crude protein, respectively (Csaba, 2011).

Many locally available raw materials are believed to contain indispensable amino acids that are necessary to promote the growth and maturity of catfish. These local available raw materials are considered to be cheap, easy to access, abundantly available and contain nutritional requirements of catfish when mixed at a right formulation (Heuze *et al.*, 2015). However, some of these local raw materials have been studied for their nutritional values and others have been neglected, underutilized due to lack of adequate information about their nutritional efficacy. Based on the reported proximate composition of groundnut cake, fish waste, bone meal and guinea corn there is need to test for their nutritional values for fish diet formulation in order to ascertain their nutritional efficacy (Nwokocha, and Nwokocha, 2013).

This study focuses on expanding the unique properties of groundnut cake, guinea corn, fish waste and bone meal for fish diet formulation. A significant breakthrough in this research to produce feed that will yield corresponding nutritional value comparable with that of commercial feed will help to lessen the cost of feed, increase fish production and

will also contribute immensely to the economy of the nation as well as contributing to scientific knowledge.

1.4 Aim and Specific Objectives of the Study

1.4.1 Aim

The aim of this study is to produce fish diet from local ingredients for optimum growth and development.

1.4.2 Objectives

The specific objectives are to;

i. Determine the proximate composition of groundnut cake, fish waste, bone meal and guinea corn.
ii. Formulate three diets of 45 % crude protein and determine the proximate composition of the diets.
iii. Determine the amino acid composition of commercial feed (coppens) and formulated diets.
iv. Determine the mineral composition of commercial feed and formulated diets.
v. Compare the effects of locally formulated diets and commercial feed on the growth performance of catfish.
vi. Determine the effects of the formulated diets on some biochemical and haematological parameters in catfish.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fish

Fish are poikilothermic (cold- blooded) vertebrates that possess scaly skin (aside from eels that are scale-less) which breathe with gills. They are mostly distributed in freshwater. Fish are classified into two sets based on their skeletal types; bony fishes (superclass osteichthyes) or cartilaginous fishes (superclass chondrichthyes). Sawfishes, sharks, rays, and ratfishes are regular instances of cartilaginous fishes with features of replaceable teeth, scales which have similar structure like that of human. When needed, cartilaginous fish grow new scales, as the individual scales do not constitutively develop with the fish. Over 1,000 different cartilaginous fish are found, of which 470 are skates, rays and sawfish, and 450 are sharks. Fossil evidence and evolutionary understanding of sharks comes primarily from fossilized teeth, as their skeleton is made of cartilage rather than bone. The fossils found in cartilaginous fish are mainly on a scale estimated to be 405 million years old (Keat-Chuan *et al*., 2017).

Bony fishes are also called osteichthyes which are the greatest abundant and diverse among all vertebrates with more than 24,000 recorded species. They are the largest class of vertebrates that exist today. The Osteichthyes class is classified into ray-finned and lobe-finned fish (Actinopterygii) and lobe-finned fish (Sarcopterygii). The oldest known fossils of bony fish, which are also transitional fossils, are around 420, 000,000 years old, displaying a tooth pattern between the tooth rows of sharks and bony fish. They have skeletons that mostly consist of bone tissues that separate them from cartilaginous fishes. They are also known to live in the deep bottom of the water in their environments with a great diversity of temperatures and have an average life span of approximately 1-120 years (*Ricardo et al., 2017).*

Actinopterygii is also called ray-finned fishes and it consist of a bony fish class or subclass. They have lepidotrichia and their fins are webbed with horny spines protecting the flesh. Their fin rays are directly attached to the basal skeletal elements, the radials, which, for instance, indicate the relation between the fin and the inner skeleton, sacral and pectoral girdles. Actinopterygii is the most inactive group of bony fish, comprising around 99% of the 30,000 fish species found in freshwater and marine ecosystems (Laurin and Reisz, 1995).

Sarcopterygii, which belongs to the class or subclass of bony fish, is also called lobe-finned fish. Coelacanths, lungfish, and tetrapods belong to a few sarcopterygii. The characteristics of fleshy, lobed and paired fins are present. They are distinguished from those of all other fish by the presence of paired fins and each fin is carried on a fleshy, lobe-like, scaly stalk extending from the body. Articulations identical to those of tetrapod limbs characterize both their pectoral and sacral fins. Their fins later evolved into legs that have similar characteristics to amphibians, such as the first stage of tetrapod land vertebrates. In comparison to the single dorsal fin of Actinopterygians, they also have two dorsal fins with different bases (ray-finned fish). The early primitive sarcoptergygians braincase has a hinge line, but this is absent in tetrapods and lungfish. Most early lobe-finned fish have a symmetrical tail and teeth coated with real enamel (Retallack, 2011).

Catfish are commonly called mud fish which is a different set of ray-finned fish that possess conspicuous barbels which looks like a cat's whiskers. Some of them do not possess prominent barbels like whiskers and most of them do not have scales. They are distributed in freshwater as well as shallow running water and the majority of them are bottom feeders. Catfish have a diversity of body shapes, however most have a cylindrical body with a leveled ventrum which is used for bottom feeding. Generally,

because of their low gas bladder and hard bony head they are referred to as negative buoyant, meaning that they will usually sink rather than floating (Ferraris and Reis, 2005). Catfish is the most common cultured fish in African which has contributed greatly to the nation's economy as well as protein availability. The species of catfish that are commonly cultured in Nigeria are species of *Clarias* such as Clarias *isheriensis, Clarias gariepinus, Clarias lazera* and the species of *Heterobranchus* such as *Heterobranchus bidorsalis, Heterobranchus longfiliis* (Fagbenro *et al*., 2012). These species of catfish take a unique position in the commercial fish farming in Nigeria because they are hardy, tasty, withstand poor water quality conditions, highly fecund, grow fast, and possess an efficient feed conversion especially in the male and also due to their high market values (Afia & Ofor, 2016).

2.1.1 Scientific classification of catfish

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygi

Order: Siluriformes

Family: Ariidae

Genus: *Heterobranchus*

Species: *H. bidorsalis*

Fig. 2.1: Catfish (Afia and Ofor, 2016).

2.1.2 Importance of fish in human nutrition

Aquatic animal foods are of high nutritional values which are rich sources of proteins, lipids as nicely as micronutrients. They possess a decrease caloric mass and a high amount of eicosapentaenoic and docosahexanoic acids (essential fatty acids) in contrast to terrestrial dwelling creatures (Tacon and Metian, 2013). Also, aquatic protein are rich in several peptides and with an excellent quality of high digestibility as well as indispensable amino acids that are insufficient in land animal proteins (Tacon and Metian, 2013).

Fish plays significant roles in human nutrition because of the presence of high nutritional value of protein content that contains sufficient indispensable amino acids needed by human body for development, muscle tissue maintenance, and synthesis of enzymes for regulating metabolic process. Studies have revealed that the consumption of fish protein have positive wellbeing impacts particularly with the reduced of coronary heart and cardiovascular sicknesses, reduced provocative infection (joint inflammation), counteraction of metabolic disorder and avoidance of osteoporosis (Rudkowska *et al*., 2010; Lund, 2013). Study shows that a diet of sardine protein reduces insulin obstruction, leptin and tumor necrosis factor (TNFα), increases hyperglycemia and decreases oxidative pressure of fat tissue (Maadamni *et al*., 2012).

In pre-horticultural occasions, the foods accessible to people were fish, meat, shellfish, green verdant vegetables, organic products, berries, nectar and nuts (Simopoulos, 2003). These diets contain high measures of essential fatty acids which have shown some useful consequences for human wellbeing (Williams, 2000). The eicosapentaenoic acids are significant in counteraction of arterosclerosis and immune system maladies (Kinsella, 1988; Simopoulos, 1999). Arachidonic acids and eicosanoid produced from n-3 polyunsaturated fatty acids possess the properties of suppressing immune system, while the eicosanoids made from n-6 polyunsaturated fatty acids possess genius provocative characteristics and improve insusceptible response (Calder, 2001). Also, fish that contain essential long chain n-3 PUFA when consumed by human during pregnancy and neonatal period helps in the neural improvement in youngsters. The retina and brain of animals is rich in docosahexanoic acid 22: 6 (DHA) supplied by the consumption of fish (Lauritzen *et al*., 2001)

Fish is an additional major source of vitamin D in addition to lipids and protein sources (Holick, 2008). In individuals with poor vitamin D levels, insufficient vitamin D has induced rickets, osteomalacia, poor bone mineral density (BMD) and also an increased number of instances of decline (Cranney *et al*., 2007). Likewise, the effect of vitamin D insufficiency has been linked to diabetes, increased strength of specific malignancies, and increased immune system disease events as well as strokes (Holick, 2008; Norman, 2008). Vitamin D3 (cholecalciferol), which also produces a 7-degree structure in the skin, is the type of vitamin D found in fish when exposed to ultraviolet light and research have revealed that it have multiple occasions higher strength differentiated to the vitamin D2 (ergocalciferol) which is found in mushroom (Holick, 2008; Norman, 2008).

Seafood like fish are acceptable wellsprings of selenium which is essential minor element in humans. Selenium works as selenoproteins that fill in as cofactor in human metabolic responses and moreover helps in different cancer prevention agent proteins action, as glutathione peroxidases. Notwithstanding, low degrees of selenium have been related with myocardial infarcts and expanded demise rate from cardiovascular ailments. Close to this, poor degrees of selenium have been associated with expanded danger of malignant growth and kidney disease (Holben and Smith, 1999).

Study revealed that fish is a superior sources of phosphorus, with an average range of 204-230 mg/100 g of phosphorus in fish, molluscs and crustaceans, compared to 176 mg/100 g in land animals (Tacon and Metian, 2013). As a constituent of the phospholipids, it plays an important role in the building of membrane lipid bilayer, phosphorus perform a fundamental role in the skeletons as well as in the cellular tissues. In the human body, the absolute substance of phosphorus is about 700 g, of which 80% is bound in the bones, 10.9% in the viscera and 9% in the muscle tissue of the skeleton. Phosphorus insufficiency in the body stimulates muscle damage, metabolic acidosis, encephalopathy and changes in bone mineralization, just as in the heart and respiratory system, metabolic and neurological syndromes (Ghosh and Joshi, 2008).

Fish and other sea organisms are wellsprings of mineral elements, for example, calcium which is a significant mineral element in human sustenance especially for bone formation and thickness. Calcium salts give firmness to the skeleton as well as its ion play a significant role in several metabolic activities (Malde *et al*., 2010)

2.2 Fish Nutrition

Nutrition is the sum of all the processes in which living organisms acquired nutrients and utilize food substances. It is the sufficient intake of vitality, proteins and

micronutrients for greatest development, advancement and backing of life (WHO, 2013). Living organisms consume foods that consist of various nutrients, for examples: sugars, proteins, lipids, minerals and vitamins which are transported to the various part of the body where the supplements in the foods are changed into body tissues for various metabolic processes.

Most living organisms rely on plants and animals as a sources of nutrients for necessary substances needed by the body to play out its daily functions properly. Nutrients are provided through diet intake, for examples, carbohydrates, proteins, fats, minerals and other nutrients. These fundamental substances give energy, forms new body components, and assist in the metabolic activities of the body system (Igah, 2008).

Carbohydrates are the principal sources of energy deposited in roots, stems, tubers, and seeds. Plant uses energy from sunlight to produce sugars from water and carbon dioxide via photosynthesis, a process fundamental to plants since it gives oxygen and energy for life activities. There are limited quantities of starches in animal tissues, most of which are found in the blood as glucose and glycogen in the liver as well as muscle tissues. Blood of animals contain nearly 0.05 percent to 0.1 percent circulating glucose, which is utilized for energy and is replaced from stores of glycogen in the liver. Sugars play several roles in the life of living organisms. In animals, carbohydrates act mainly as energy, form part of tissue constituents most especially liver glycogen, blood glucose, nucleotides and also form metabolic precursors of some intermediates. Similarly, carbohydrate such as starch play a vital roles in serving as binders in feed formulation which aids to bind ingredients together as well as helps to increase feed flow in water so that the feed pellets are water-stable and can glide in the water for aquatic organisms to feed on (Mwangi, 2008).

Among fish species, the capacity to use dietary carbohydrates as a source of vitality varies. Majority of fresh water, warm water fish, like catfish, can utilize a lot more elevated of dietary starches than cold water. This might be ascribed to the way that warm water fish have more elevated gastric amylase activity than cold water types. Proteins for the assimilation and digestion of sugars have been identified in a few fish varieties. Hormonal and metabolic regulation of the digestion of starch in fish, however, remains unclear and can contrast with that of warm blooded animals (Robinson *et al*., 2001)

Study revealed that catfish can use some classes of carbohydrate in fish feed formulation. Conversely, consumption of monosaccharides as well as few complex sugars by catfish is not as proficient. Researches indicated that catfish use glucose in a way like warm blooded animals however at a considerably lower speed. Catfish evidently need compound accomplished for quick digestion of sugar. Catfish feed ought to have satisfactory quantities of cereal by-products that are rich in carbohydrate that will provide the energy requirement of fish (Edwin, 2001).

Lipids (fats and oils) are an exceptionally absorbable wellspring of intense vitality; it encompasses roughly 2.25 times energy compare to the same amount of sugars. Fat and oils have numerous roles, it contributes in metabolic activities of mammals, for example, providing fundamental unsaturated fats, serving as a transporter for fat-soluble nutrients and filling in as forerunners for some chemical substances secreted from ductless gland. It is additionally used to increase palatability in fish feed. Body lipid stores in fish improve the kind of fish just as help to keep up nonpartisan lightness. The lipid type and quantities utilized in catfish depends on fundamental unsaturated fat necessities, financial aspects and quality fish flesh desired (Effiong *et al.*, 2009).

Fundamental unsaturated fats (EFAs) are those that cannot be produced in the body of the animal; they must therefore be present in the food. Essential fatty acids are graded and assigned as either omega-3 (n-3) or omega-6 (n-6) fatty acids according to their chemical configurations. Overall, fish require (n-3) fatty acids, whereas land animals require n-6 fatty acids overall. Some rayfish species, as well as tilapia and carp species also need unsaturated fats for normal body activities. The necessary fatty acids needed for catfish and some other warm water fish have not been adequately described, but a small amount of n-3 unsaturated fats is required for catfish. They also need 1.2-2.4 percent nutritional linoleic acid (18:3 n-3) as well as nutritional acid (18:3 n-3) as well as 0.5–0.75 % extremely unsaturated fatty acids for average growth, since catfish during growth will prolong and de-soak linoleic acid to yield polyunsaturated fatty acids. The indispensable fatty acids requirements can be provided by aquatic oil, for example, oil produced from menhaden. Characteristic nourishment life forms, for example, zooplankton, found in the water are additionally a decent wellsprings of EFAs (Nwokocha and Nwokocha, 2013).

The dry mass of fish muscle contains about 70 % protein which are made from indispensable and dispensable amino acids. These amino acids are present in some plants and animals source of proteins which are synthesized in the system of animals by the consumption of food products made up of protein sources. So as to keep the balance of body protein level of fish, it requires continuous supply of essential amino acids needed for the duration of the life of fish for development and support of health. Fish requires certain amount of indispensable amino acids in regards to their species. Several species of catfish such as *Clarias* spp and *heterobranchus bidorsalis* are flesh-eating and consequently requires more elevated protein level in diet. Age determines the specifications for catfish proteins. As fry and fingerlings need higher protein levels (45

percent) for rapid growth and development, for maintenance, brood fish need 35-38 percent protein. Feedstuffs containing 20 % or extra unrefined protein are taken as protein enhancements, while crude protein less than 20 % is regarded as an energy supplement (Robinson *et al*., 2001; Wurts, 2003; Gabriel *et al*., 2007). Legumes and their by-products, such as soybeans, cottonseed and groundnut, are plant protein supplements commonly available (and their cakes). However, plant protein supplements are less preferable to animal base protein supplements, because some amino acids such as lysine and methionine are deficient in plant base protein, as well as the presence of toxins or anti-nutritional agents or factors that may not be eliminated or deactivated by feed handling and may also interact with other nutrients to prevent their release. This makes it less used in the formulation of animal feed (Robinson *et al*., 2001). The main sources of high quality are animal based protein supplements. They consist of non-edible tissues from the meat processing, milk products and marine supplies industries. These involve blood, hydrolyzed feathers, meat, bone meal, and fish waste (Adeniji and Balogun, 2003; Abowei and Ekubo, 2011). Leftover of fish are a valuable source of protein for the development of animal feed, since they consist of ample and desirable essential amino acids needed for the development and maintenance of fish. It is collected from the remains of fish waste carcass, specifically from canning waste or marine trawlers during fish processing like heads and industrial fish waste (Robinson *et al*., 1994; Effiong *et al*., 2009).

Vitamins are highly diverse organic substances that serve as essential micronutrients that are required in minute quantities in animal nutrition for optimum growth, health and reproduction. Just like other animals, fish also requires vitamin in diet. Their absence leads to conspicuous deficiency signs or diseases in fish. However, vitamin deficiencies in fish are very rare in nature as natural pond organisms are a rich source of vitamins.

Vitamin requirements are subject to variations due to sex, fish size, feed formulation, diseases and environmental factors such as water quality. Vitamin Required by catfish to grow are vitamin A, D, E, K, B_1, B_2, B_5, B_{12}, folic acid, choline, inositol and ascorbic acid (Robinson *et al.*, 2001).

Minerals are essential micronutrients needed by both terrestrial and aquatic organisms. Aquatic species are needed to preserve sufficient osmotic equilibrium between body fluids and the immediate environment. For the metabolism and the optimal development of the skeletal structure, fish required minerals. Depending on the amount needed in the diet, the minerals requested by the fish are categorized into micro- or macro-nutrients. A portion of these minerals can be consumed from the water. Aquaculture production of catfish requires supplementation of diets with trace mineral premix as well as locally available raw materials that are rich in minerals such as bone meal from animal wastes that contains essential dietary mineral requirements by catfish. The mineral requirements of *heterobranchus bidorsalis* to grow outs are; calcium, phosphorus, magnesium, sodium, potassium, chloride, cobalt, iodine, sulphur, selenium, zinc, copper, manganese and iron (Ulene, 2000).

Generally, fish require a lot of protein diets for optimum growth, maturity and health. They required constant supply of essential amino acids from plants and animal's source as well as carbohydrates, fats and oil, and vitamins for energy and health. Studies have shown that out of the ten indispensable amino acids essential for optimum development and maturity of catfish, only 3 have been studied (methionine, Arginine and lysine). Carbohydrates, fats and oil and vitamins are also required for their energy supply and in order to stay healthy.

Fish waste (FW) is an important sources of protein, vitamins, fatty acids, and minerals, mainly valued source of calcium, phosphorus, iron, copper and selenium which is used for diet formulation because of its high nutritive values. It is collected from the leftover

carcass of waste fish, usually from marine trawlers or fish handling trashes, for examples, heads or fish cuttings (Robinson *et al*., 1994; Effiong *et al*., 2009). However, due to the presence of thiaminase, a potent anti-nutrient that destroys thiamine, the use of raw fish as an ingredient in the formulation of fish diets has been shown to be detrimental to optimal fish growth and development (Robinson *et al*., 2001). Crude fish have also been involved in the spread of diseases/pathogens such as mycobacterium and botulism (Robinson *et al*., 1994). Thiaminase is also found in fish, most specifically freshwater fish, and is typically inactivated by heat. Fish waste contains 60-80 % of exceptional quality protein that is very edible to fish, promoting growth and maturity (Effiong *et al*., 2009).

2.2.1 Protein and amino acids in fish nutrition

Of the nutrients, proteins stay one of the most significant. Proteins are amongst the most important bio-molecules as they offer important roles in basically all life processes. Dietary proteins are the wellspring of indispensable amino acids and produce nitrogen for the building of dispensable amino acids. Proteins in the body tissues are synthesized by utilizing about 23 amino acids. Ten of the total amount of amino acids required by the body tissues of fish are fundamental amino acids which must be provided in dietary form. Amino acids derived from protein diets are important for upkeep, development, multiplication and substitution of exhausted tissues. Likewise, there are some amino acids that are specifically transformed to glucose in order to yield basic energy for the immediate need of body tissues and organs activities including the erythrocytes and the brain. Since starch is not common in their natural diet, fish depend more on amino acids as precursors to glucose than every other animals. Thus, a portion of the dietary protein is constantly used as an energy source in fish (Poston, *et al*., 2017). The functions of proteins cannot be over underscored. They function as enzymes where they catalyze

biological reactions. Proteins also serve as transporters in addition to aiding the storage of other substances as well as in provision of structural support along with protection by the immune system. The enhancement of movement of molecules within the body of living organisms is aided by proteins. They aid in the transmission of nerve impulses, aid in control of cell growth as well as differentiation of cells and to some extent control of cell death of which Ceramide, an important signaling molecule (second messenger) regulating pathways including apoptosis (processes leading to cell death) is synthesized in the endoplasmic reticulum from the amino acid serine, for example (Jeremy *et al.,* 2007).

These essential macro-molecules are the result of a series of 20 amino acid micro-molecules. These proteins' tertiary structure and functions depend primarily on the sequence of amino acids and the functional groups present. Amino acids are categorized depending on their functional groups and structure, based on the existence of their side groups. These include amino acids that are simple, acidic, aliphatic, aromatic and hydrophilic (Jiri *et al.*, 2014).

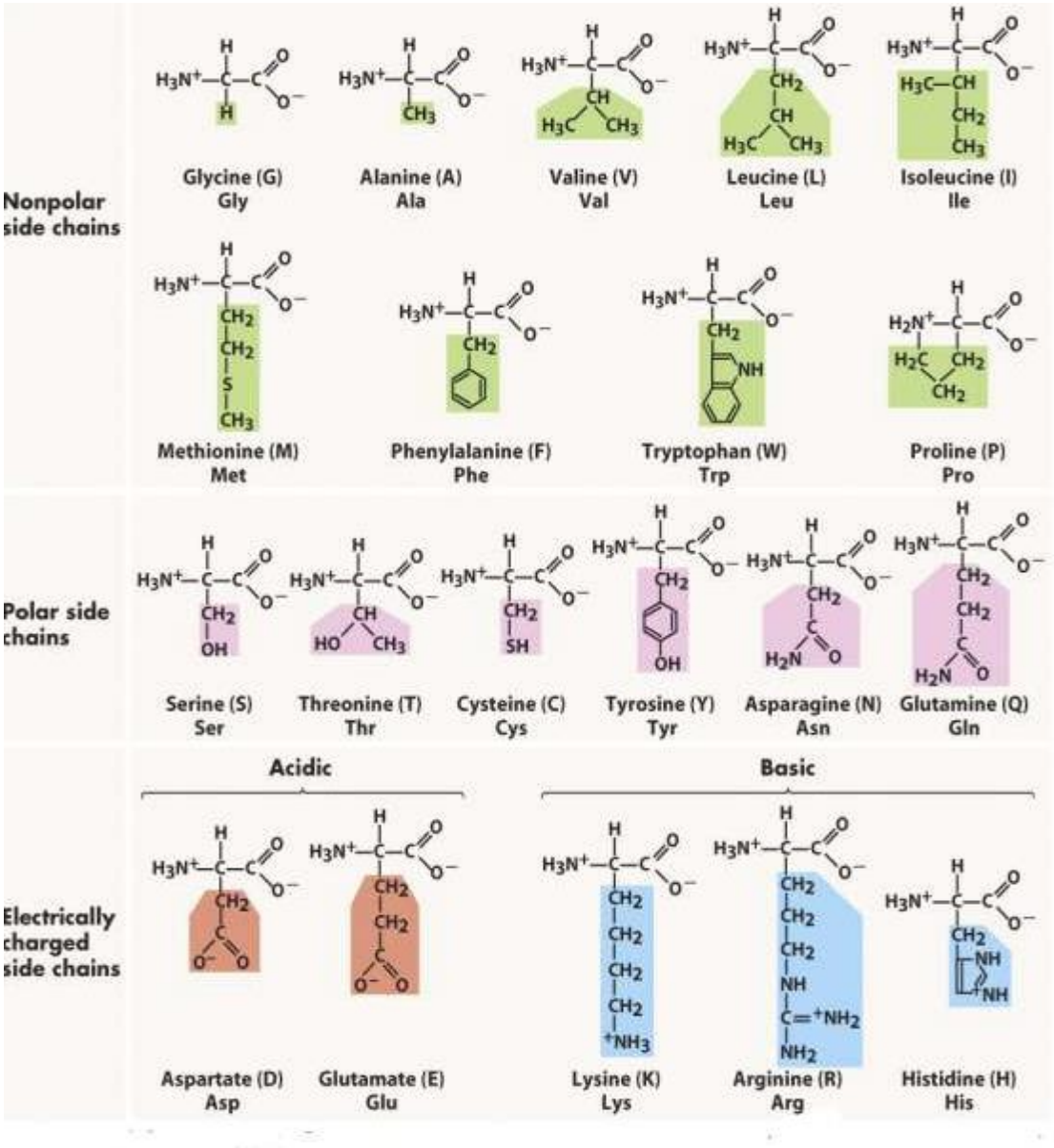

Fig. 2.2: Structures of amino Acids (Jeremy *et al.*, 2007)

Nutritionally, amino acids in fish are also classified as indispensable, semi essential and dispensable amino acids. Indispensable amino acids include Phenylalanine, Tyrosine, Isoleucine, Leucine, Lysine, Methionine, Cysteine, Valine, Threonine and Tryptophan. These are the limiting amino acids in animals. The animal system cannot biosynthesize them and therefore, they must be supplied in diets. The remaining dispensable amino

acids can be produced by animals. Dispensable amino acid like alanine, serine, glutamine, asparagine, glutamic acid, aspartic acid, tryptophan, glycine, are commonly available in the biological system of animal with a normal physiological states (Lucy *et al.*, 2014).

However, at certain phases in life the synthesis of some amino acids becomes progressively limiting. Such stages include the period of intensive growth, stress or in some disease conditions. Some amino acids, called semi essential amino acids, must necessarily be included in the diets at such stages. Proline, cysteine, tyrosine and arginine are among others. Thus, suggests that adequate quantity for each essential amino acid are needed in diets-and the more the quantity of the essential amino acids available in a protein diet, the greater the protein diet quality (Ibironke *et al.*, 2012). The amino acids necessities of catfish are shown in the table below

Table 2.1: Dietary Amino Acid Requirements of Catfish

Amino acids	**Dietary amount**
Arginine	4.2
Histidine	2.0
Isoleucine	3.0
Leucine	4.1
Lysine	5.0
Methionine	3.1
Cysteine	3.1
Phenylalanine	4.9
Tyrosine	4.8
Threonine	2.3
Tryptophan	0.6
Valine	3.1

(NRC, 1993)

2.2.3 Protein-energy malnutrition

Malnutrition has been descried by World Health Organization (WHO, 2016) as the cellular imbalance between the supply of nutrients and energy for body's demand that leads to poor growth, improper development, and inability of body to carry out specific functions." It is a disorder that results from consuming a diet in which one or more nutrients are either not adequate or are too much to cause nutritional disease caused by the diet. Insufficient intake of calories, calcium, carbohydrates, vitamins and minerals may be involved. Nutritional diseases are diseases that result in higher than average fish requirements due to excess nutrients or nutritional insufficiency. The lipids, fats, vitamins and mineral salts and proteins are the important nutrients for proper fish growth, development and maintenance of life (Idowu, 2017). The major symptoms of nutritional diseases of fish are diminished fecundity, sluggish growth rate, reduced appetite and increased vulnerability to diseases, illness with clinical signs and pathological lesions and death. Waste produced from artificial feed directly upset the fish production ecosystem and also causes marine contamination. Leftover feeds, fish feces and other metabolic wastes add to the water's particulate nutrient loading, which stresses the development of cultured species and raises the risk of disease occurrence (Joseph and Raj, 2002). Owing to their chronic existence, most nutritional diseases are very difficult to detect but can be avoided by good feeding management practices.

A lack of indispensable amino acids can contribute to low dietary protein use and can lead to stunted growth, poor weight gain, and low feed quality. In extreme cases, amino acid shortage decreases disease tolerance and impairs the immune response mechanism's efficacy. Medical symptoms can also occur from shortages of particular amino acids. Experiments have shown, for example, that tryptophan deficient fish

become scoliotic, displaying a distinctive spine curvature (Kloppel and Post 2015) and a deficiency of methionine is one cause of lens cataracts (Poston *et al*., 2017).

2.2.4 Fish diseases

Similar to other organisms, fish can likewise experience the ill effects of different kinds of illnesses. By and large, two kinds of illnesses are available in fish body; infectious disease and non-infectious diseases. Disease is a primary agent that causes poor growth, development and fish mortality particularly when fish are at the stage of fingerlings (Axelrod & Untergasser, 1989). Fish disease are caused by pathogens, for example viral contamination, bacterial diseases, fungal infections and water mould infection, introduced species from one pond to another, malnutrition, predators as well as fish exposed from different environmental contaminants including antibiotics and chemicals. All fish carry pathogens and parasites that causes malady episode (Koustav and Rimpa, 2018).

2.2.5 Malnutrition diseases

Scurvy in fish is a non-infectious disease that arises as a result of deficiency in some micro-nutrients. When diets are not figured and dependent on the species requirement, it typically occurs once in a while. The main cause of fish scurvy disease is ascorbic acid insufficiency. Replacing a species-specific diet with another can also lead to shortcomings (Sharma, 2012). Fishes are typically impaired in the grow-out processes, but post larval phases occur in spinal deformity. In Thailand, Epinephelus tauvina and Epinephelus malabaricus have been identified (Aly, 2013). Anorexia, fine and opercular erosion, short snout, haemorrhage of the eyes and fins, exophthalmia, abnormal skull, bloated belly, pharyngo-branchial decay, extreme emaciation and abnormality of the spinal column, poor development are signs of fish suffering from Scurvy disease. High

doses of vitamin C intake can help to resolve the problem of scurvy in fish as well as stimulating disease resistance in fish against pathogenic bacterial and virus species that may cause fish disease (Francis-Floyd & Thom'as, 1991).

2.3 Fish Feed

Fish feed is in the form of granules or pellets that provide a stable and concentrated form of nutrition, allowing the fish to feed effectively and grow to their full potential size. Fish feed consists of natural food and rations that are prepared artificially. Live feeds such as rotifers, artemia, microscopic plants (phytoplankton), microscopic animals (zooplankton), insects, crustaceans, copepods and molluscs are also considered natural foods. Live feeds are the primary first feed added daily to fish in the food chain for aquaculture. Feeding fry and fingerlings with Live feed is one of the most important and compulsory matters for the efficient production of fish, since it is one of the most important and compulsory matters for the first few days of fish production, because during the first few days of their life they have no complete develop digestive track and digestive enzymes that can easily digest formulated feed as well as secreting enzymes for breaking down complex molecules (Appelbaum and Damme, 2007). Their development and population can be stimulated in a pond by the process called fertilization. The process of releasing essential nutrients needed for the optimum growth of aquatic food is the fertilization of the pond for natural food for fish. This can be done by submerging poultry droppings, cow dung, and agricultural wastes into the pond for about 5 days before fish are introduced. Fish will only live on natural food if the supply of natural food in the ponds is abundant (Wurts, 2003; Sikiru *et al*., 2009, Faruque *et al*., 2010). However, a high density of fingerlings is stored for large aquaculture fish production and fertilization of natural foods may not be sufficient to provide the optimal level of feed needed for fish growth and maturity. Hence, there is need to for

supplementation of natural feed with artificially formulated feed that contains all essential nutrients needed for fish growth and maturity. Artificially formulated feed provide additional food availability for large aquaculture production. These formulated feed are compounded from plant and animal sources of nutrients.

Fish diets prepared or artificial may be either complete or supplemental. All the ingredients (protein, carbohydrates, fats, vitamins and minerals) are essential for the optimal growth and health of fish are provided by complete diets. Researches shows that most fish farmers use complete diets that is those containing all the needed protein (18-50 percent), lipid (10-25 percent), carbohydrate (15-20 percent), ash (< 8.5 percent), phosphorus (< 1.5 percent), water (< 10 percent) and trace amounts of vitamins and minerals. When fish are reared in a high-density indoor system or confined in cages and are unable to feed freely on natural feed, a complete diet must be provided. Supplementary (incomplete, partial) diets, on the other hand, are intended solely to support the natural feed (insects, algae, small fish) normally available for pond or outdoor fish farming. Supplementary diets do not contain a total supplement of vitamins or minerals, but are used with extra protein, carbohydrates and lipids to help fortify the naturally available diet. Fish, especially when reared in high densities, require a nutritionally balanced diet of high quality to grow rapidly and stay healthy (Houliha *et al*, 2001).

Table 2.2: Some local available materials of plant and animal sources that has been used in fish feed formulation are shown in table 2.2 below

Ingredients	% Protein	% Fat	% Fibre	% Carbohydrate	% Dry matter	% Mineral
Maize (white)	9.4	6.2	1.9	72.0	87.0	1.6
Maize (yellow)	11.6	4.8	6.0	72.3	84	2.0
Guinea corn	11.2	3.0	3.4	70.2	85	2.0
Guinea corn (industrial)	48.0	12.2	8.2	19.8	93	6.3
Palm kernel cake	18.2	6.5	44.0	19.9	-	5.3
Cotton-seed cake	50.0	9.1	32.0	13.4	92	6.0
Rice bran/husk	10.7	5.1	40.3	8.9	92	21.8
Groundnut cake (kuli-kuli)	40.1	23.4	6.0	19.1	91	6.2
Raw soybean	40.2	22.0	6.3	16.3	90	6.4
Fish meal	57.7	1.5	5.2	-	92	33.6
Blood meal	85.0	0.7	2.1	6.8	92	5.0
Millet	10.0	5.0	0.7	83.2	90	2.3
Cassava	5.0	0.4	0.4	94.1	88	2.4
Cassava leaves	15.4	8.4	15.6	45.2	88	16.1
Water leaf	21.1	1.7	11.2	77.8	-	4.2

Source: (Okanlawon and Oladipupo, 2010)

2.3.1 Feed Additives

Substances used to increase the consistency and efficiency of catfish feed are additives. Feed additives are substances that are added in trace quantities to animal diets that provide a method by which dietary shortages can be resolved as they support both the

animal's nutrition and growth. Some of the feed additives include pellet binders, antioxidant and antibiotics (Abdelhadi *et al*., 2010) (Abdelhadi *et al*., 2010).

2.3.2 Pellet binders

One of the important ingredients in fish diets is pellet binders which are added to the diets in order to improve water stability that extend the time pellets remain intact after feeding and it also helps to prevent leaching of nutrient in water. Locally, research shows that beef heart has been used in farm-based feeds as an important binder that also acts as an excellent source of protein. Common binding agents are also carbohydrates (starch, cellulose, pectin) and other different polysaccharides, such as animal extracts or derivatives (gelatin), plants (arabic gum, locust bean) and seaweed (agar, carageenin and other alginates) (Royes and Chapman, 2009).

2.3.3 Antioxidants

Antioxidants are compounds which inhibit certain nutrients from oxidizing. It functions as a preservative and is frequently applied to fish diets in order to prolong the lifespan of fish diets and to decrease rancidity. Nutrients themselves, in some cases, act as biological antioxidants. Examples of antioxidants are Vitamin C and E. Butylated hydroxyanisole and ethoxyquinisole are popular commercial antioxidants (Govind, 2013).

2.3.4 Antiobiotics

Antibiotics are substances used in catfish diets to control microbial diseases. They are incorporated into fish diets during feed production. Just a predetermined number of FDA-affirmed anti-infection agents are accessible for controlling bacterial ailments of catfish. The two anti-infection agents presently available to catfish producers are

oxytetracyclin and a combination of sulfadimethoxine (Romet). Some of this drugs are integrated into feeds to be fed to fish detected with specific disease (Robinson, 2001).

2.4 Fish Feeding

Feeding in fish is the process by which feed are given to fish. It is one of the most important task in catfish production, because it requires an experienced personnel who can tell whether or not the fish are feeding normally or not by observing them during feeding process. There is variation in catfish feeding that depends on catfish stages and the types of feed. Fish feed are produced as floating (extruded) and non-extruded (sinking). Extruded fish feed can withstand such challenges, such as poor water stability, dissipation of feed and waste, pollution of water quality and satiation of fish. Extruded feed makes feeding management simpler and more cost-effective, since aqua feed can float physically on water for a long time and before implementation there is no need to set up feeding stations. Feed can be used by means of a broadcast system and it is easy for farmers to understand the situation of their fish's food intake, thereby changing the quantity of feed as well as monitoring their fish's growth and health status. Non-extruded fish feeding is only valid in some benthic species and in stages in some species of fish. The application process requires fixed position and it is very hard for the fish farmers to know the satiation point of the fish and this leads to wasting of fish feed. Since the fish did not float up on water surface during feeding it is very difficult for the fish farmers to know growth rate and health status of the fish. This method of feeding in fish is not well encouraging because of the disadvantages surrounding it usage (Menghe *et al*., 2001).

2.5 Guinea Corn Varieties

Guinea corn (*Sorghum bicolor*) is a genus of flowering plants in the grass class called poaceae. There are twenty five species worldwide, of which 17 of the twenty five species are common to Australia, with some extending to Africa, Asia, Mesoamerica, and some islands in India and the Pacific Oceans (Sally, 2016). In hot or harsh environments with low rainfall, where other crops grow or yield poorly, sorghum can thrive. They are referred to as rough grain or crops of underprivileged people because they can be cultivated both in areas with minimal water supplies and without fertilizer application. They are small in size, round in shape and they have different varieties of colour: like brown, black, red and white (Baker and Terry, 1991).

Guinaea corns (*Sorghum bicolor*) are called varieties of names in different countries of the world. It is called great millet in South Africa, West Africa, and kafir, mtama in Eastern Africa, dura in Sudan, jowar in India, kaoliang in China and milo-maize in the United States. In Nigeria, it has also been given several local names: it is called oka baba in Yoruba, Okili in Igbo and dawa in Hausa. There are eight groups of guinea corn cultivated in Nigeria and four of them have been identified to be important. The four groups are adapted to the northern guinea savannah region where rain is more than 100 m, the guinea group. The Hausa name fara fara is familiar to local varieties from this group. More suited to the dried region of northern Nigeria is the kaura community. Between the guinea group and the kaura group, the fara fara group is a combination. The earliest group is the Chad group and most of the local species are photosensitive since they flower at the end of the rainy season (October-November) and after the rains have ended, grains ripen. The plants are tall, varying in height from 2.5-4.0m and the tall stalks are needed for a variety of building and fencing purposes (Philips, 1977).

2.5.1 Guinea corn composition

Sorghum is one of the main staple food crops of northern Nigeria. It is a vital source of simple carbohydrate, protein and minerals, for examples, calcium, manganese, selenium, and iron. The bioavailability of the minerals relies on the degree of interactions with various anti-nutritients present such as phytate, tannin, cyanide and flavonoid. It is also rich in B-complex vitamins (FAO, 2001). Generally, *sorghum bicolor* is an important crop world-wide that serve as food for human consumption. It is used for bread making as flour serve as food for cattle, horses, poultry and birds, use in producing akamu (pap) especially for babies. In brewing industries, it is used for production of alcoholic beverages and also serve as cereal; it is used as vitality supplement for animal feed formulation (Fagbenro *et al*., 2003).

2.5.2 Taxonomic classification of guinea corn

Kingdom: plantae

Clade: angiosperms

Clade: monocots

Order: poales

Family: poaceae

Sub-family: parricorideae

Genus: *Sorghum*

Species: *S. bicolor*

Plate 2.1: Guinea corn (Sally, 2016).

2.5.3 Guinea corn and its products

Guinea corn is a multi-use crop; each part of the guinea corn plant is valuable. This is because it is most important cereal crop that is heavily cultivated. The estimated yearly output of guinea corn is about 8.6 million tones (Abdurrahman and Kolewole, 2006). Apart from it been used as a raw material in industries (for beverages, alcohol or biofuel), traditionally, maize had been often utilized as medicine, animal feedstuff and most of all consumed as food (Sule *et al.*, 2014).

2.5.4 Medicinal uses of guinea corn

Apart from the use of *Sorghum bicolor* as food, it also provides medicinal values when consumed. Research shows that *Sorghum bicolor* contains some chemical compounds that provide a protective effect against cancerous growth, heart disease, tumor growth, heavy menstrual flow, high blood pressure, and artheriosclerosis. The presence of mineral element such as iron in guinea corn supports the transport of oxygen in the blood which promotes cell growth and development. Calcium, phosphorus, and potassium present also help in bone formation, strength and maintenance of body fluid.

It makes it an ideal source of nutrients for people with celiac disease because of the absence of gluten in guinea corn (Ulene, 2000). Recent research shows that intake of guinea maize helps prevent diabetes by preventing the body from consuming excessive quantities due to the presence of flavonoid to control the glucose and insulin levels in the body.

2.5.5 Guinea corn as human food and animal feed

Guinea corn and its various products serve mainly as a man's food source. Therefore, in the diet of many species around the world, they serve as the main sources of energy and proteins. Guinea corn is processed into various conventional diets in Nigeria and other developing nations. Almost no portion of the plant for guinea corn is lost. The leaves also get dried into hays and used as animal feed during winter season. As animal feed, various types of guinea maize serve as (Abdurrahaman and Kolawole, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Sample collection

The samples utilized for this study were obtained from Kure International Market, Minna in Niger State between the month of March and April, 2019. They are as follows: Fish waste, bone meal, Groundnut cake, Guinea corn, Cassava starch (Binders) and Vitamin premix. A total of 40 fingerlings catfish were bought from the Department of Fisheries, Ahmadu Bello University, Zaria.

3.1.2 Samples preparation

Fish waste collected was grinded into powder and preserved for further analysis in a plastic rubber bottle.

Groundnut cake collected was grinded into powder and preserved further analysis in a plastic rubber bottle.

Guinea corn collected was grinded into powder and preserved for further analysis in a plastic rubber bottle.

Bone meal used in this study was obtained from Sammy ventures Sabon Kasuwa Minna, Niger State.

3.1.3 Equipment

List of equipment used in the course of this study are as follows:

The Beckman refrigerated centrifuge (model TJ-6, UK),

Amino acid analyzer (Applied Biosystem PTH amino acid analyser model 20A, UK)

Rotary evaporator machine (RE500 Bento Scientific America Incorporation, USA)

Ultraviolet/Visible spectrophotometric machine (model UV-1800, CHINA)

Weighing balance (model A223CN Ohaus Adventurer)

Aquarium air pump (RS-180, CHINA)

Small scale grinding engine (Henry West G160, 5.5 HP)

Liquid extraction oven (Gallenkamp, Size 2, Brainweigh B INDIA) and

Hotplate (Biotec, CHINA).

Pelleting machine

Rubber tank

Soxhlets apparatus (Sigma-Aldrich, SA64826, UK)

3.2 Methods

3.2.1 Specimen Identification

Both the (red) guinea corn (*Sorghum bicolor)* and the groundnut cake samples were identified and authenticated by Dr. A. Saidu in the Department of Plant Biology, Ahmadu Bello University, Zaria. The fish waste and bone meal samples were also identified and authenticated by Prof. S. O. Abubakar in the Department of Zoology, Ahmadu Bello University, Zaria.

3.3 Proximate Determination of Sample

The proximate determination of the grinded samples; (fish waste, groundnut cake, guinea corn, bone meal, commercial feed and formulated diets) were carried out as follows;

3.3.1 Determination of moisture content

Determination of moisture content was carried out by method of Nwosu (2011). This was achieved by oven drying of the sample to a constant weight, and extracting the water content from the sample. The moisture content of the original sample was

expressed as percentage by weight (Okai *et al*., 2015). In a clean crucible, 2 g (grams) of the sample was precisely measured and dried for 3 hours in a liquid extraction oven (Gallenkamp, size 2, Brain-weigh B) at 105 °C. . The dried sample was cooled in a desicator and reweighed. The dried samples were put into desiccators, allowed to cool and reweighed. The whole procedure was repeated until a constant weight was obtained and the difference calculated as a percentage of the original provided sample.

This is given as percentage moisture = $\frac{W2-W1}{W2-W3}$ x 100 (3.1)

Where

W1 = Initial weight of empty dish

W2 = Weight of dish + undried sample

W3 = Weight of dish + dried sample

3.3.2 Determination of ash content

Determination of ash content was carried-out by the method described by Adegunwa *et al.* (2011). Ash is obtained by the complete removal of carbon after combustion and what is left is mainly composed of minerals. The ash content is given as a percentage of the sample's original weight. An empty crucibles were clean and kept for 60 minutes in a Muffle furnace at 550 °C. They are then put in and permitted to cool down in a desiccator. The initial weight was taken from the crucible (W_1). From each sample, 2 g was weighed into the crucible labeled (W_2) and was flamed over a Bunsen flame, until the sample are charred. Then the crucible was taken from the burner and was kept for 3 hours in a muffle furnace equal temperature. The formation of gray white ash indicated full oxidation in the sample of all organic matter. The crucible in the desiccator was then permitted to cool down and weighed (W_3). The obtained weight of the ash was expressed as the sample percentage initial weight.

$$\text{percentage ash} = \frac{\text{Difference in ash weight}}{\text{Original sample weight}} \times 100$$

$$\% \text{ Ash} = \frac{W3-W1}{W2-W1} \times 100 \quad (3.2)$$

W_1 = initial weight of crucible

W_2 = Crucible weight before ashing

W_3 = crucible crucible + (sample) Ash

3.3.3 Determination of crude protein (Kjeldahl distillation method)

10 ml of concentrated tetraoxosulphate (VI) was added to 2 grams of each sample and mixed in a heating tube. 5 g of selenium catalyst was added to the mixture and was heated inside a fume cupboard to obtain a digest. The digest was then removed into 30 ml of distilled water. 45 % of Sodium hydroxide was mixed with 10 ml of the digest and transferred into a Kjeldahl distillation apparatus. After distillation, the distillate was collected into 4 % boric acid solution containing 3 drops of methyl red indicator. 50 ml of distillate was collected and back-titrated with 0.1 M HCl solution.

The above procedure was repeated 3 times and the average of the 3 results was taken. The nitrogen value was calculated by multiplying with the standard conversion factor, 6.25 (Mariotti *et al.*, 2008). This yielded the crude protein given as percentage Nitrogen as indicated by the formula below:

$$\text{Percentage Nitrogen} = \frac{100 \text{ x N x } (14 \text{ x VF}) \text{ T}}{100 \text{ x Va}} \text{ x } 100 \quad (3.3)$$

Where

N= Normality of the titrate (0.1N)

VF= Total volume of the digest= 100ml

T= Titre Value (of Acid used)

Va= Aliquot Volume distilled

3.3.4 Determination of fat content

Soxhlet extraction method as reported by Adegunwa *et al.* (2011) was used. This is a gravimetric method that involves the extraction of liquid with organic solvent. Two grams of the sample was loosely put into the thimble which was fitted to a clean round bottom flask, which has been cleaned, dried and weighed. The flask contained 120 ml of petroleum ether. The sample was heated with a heating mantle and allowed to reflux for 5 hours. The heating was then stopped and the thimbles with the spent samples kept and later weighed. The difference in weight was received as mass of fat and is expressed in percentage of the sample.

The percentage oil content is percentage fat = $\frac{W2-W1}{W2-W3}$ x 100 (3.4)

Where

W1 = weight of the empty extraction flask

W2 = weight of the flask and oil extracted

W3 = weight of the sample

3.3.5 Determination of crude fiber

As described by AOAC, the gravimetry technique was used to evaluate crude fibre (Okai *et al.,* 2015). The indigestible matter mainly of cellulose and other cell wall component of the living matter are primarily referred to as crude fibre. In order to extract the non-fibrous portion, digestion was performed by heating to boil a fixed weight of the sample with a mixture of acid; the residue (un-dissolved) is split and ignited. By measuring the loss in ignition as a percentage, the value of the crude fibre is then obtained. To a 200 ml solution of 1.25 % tetraoxosulphate (VI), was added 2 g of sample and 1g of asbestos and were boiled for 30 minutes. The resulting solution was then transferred into Buchner funnel equipped with muslin cloth secured with an expandable band. This allowed for the filtration of the mixture. The residue was

collected and put into a boiling 200 ml sodium hydroxide solution and the boiling continued for another 30 minutes, then again passed through the Buchner funnel and filtered again. Subsequently, it was washed two times with ethanol and the product obtained was washed three times with petroleum ether. The final residue was placed in dirt free dried pre-weighed crucible and dried in the oven at 105 °C till a steady mass is obtained. The dried crucible was removed, cooled and reweighed. The, difference in weight (that is ignition loss) was recorded as crude fibre and calculated as the percentage crude fibre.

$$\text{Percentage fiber} = \frac{\text{weight of crucible + Sample before ignited – Weight of crucible + Ash}}{\text{Initial weight of sample}} \text{ x } 100 \quad (3.5)$$

3.3.6 Determination of carbohydrate content

The AOAC method as described by Nwosu *et al.* (2011) was used for this analysis. All the proximate contents of the food samples are summed up to a 100 %. Removing the values of the other parameters from 100 gives the value for carbohydrates also called the nitrogen free extract (NFE).The calculated percentage weight of the carbohydrate was derived as weight by difference between 100 and the total sum of the other proximate parameters.

$$\text{NFE} = 100\text{- (Fat + Ash + moisture + crude fibre + Protein)} \quad (3.6)$$

3.4 Mineral Analysis of Samples

The mineral analysis of the commercial feed and the formulated diets (A, B, and C) were determined by the method described by (Olsen *et al*., 2004). In a clean ceramic crucible, 0.48 g of each sample was weighed and kept in a muffle furnace at 500 °C over 2 hours. For a maximum of 12 hours, the samples were permitted to cool down in

an oven. After 12 hours cooling, the samples were removed from the oven and poured into a labeled 50 ml centrifuge tubes. The crucibles were rinsed with 5 ml of distilled water, 5 ml of aqua regia and both were added into the centrifuge tube. All the samples were vortex for proper mixing, after which they were centrifuged for 10 minutes at 3000 rpm. After 10 minutes centrifugation, the samples were removed from the centrifuge and supernatant were decanted into clean vials for macro and micronutrients determination in a flame photometer.

3.5 Amino Acids Analysis of Sample

Amino acid analysis of the samples were analyzed according to the methodology reported by Zygmunt *et al.* (2009).

3.5.1 Principle

The amino acid analyzer was designed with a special ultraviolet (UV) spectrophotometer-HPLC system that can separate and indirectly detect and quantify these amino acids. First the sample is hydrolyzed into amino acid residues. Next is the separation of the individual amino acids using HPLC (High Performance Liquid Chromatography) and post column derivatization and quantification. Derivation using the M otho- phthalaldehyde (OPA) reagent method utilizes the OPA reagent to construct a derivative of an amino acid (isoindole) that had been separated by cation exchange in the column. Isoindole, produced reacts with another reagent embedded in the OPA reagent (Thioflour). The amino acid residue – reagent complex fluoresces under UV light. This is easily detected and measured by

UV/VIS spectrophotometer (Figure 3.1).

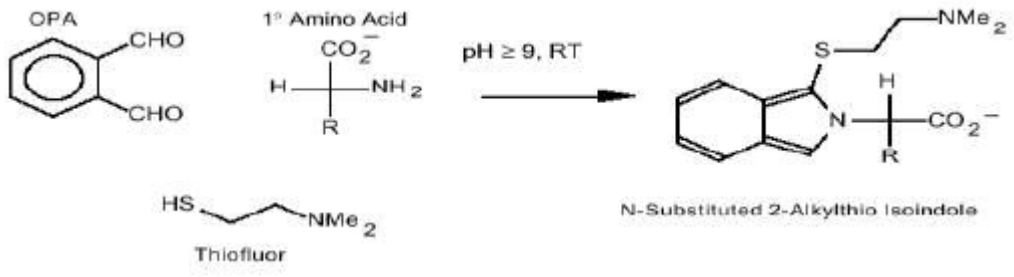

Figure 3.1: Formation of N-substituted 2-Alkylthioisoindole (Zygmunt *et al.*, 2009).

3.5.2 Procedures

The sample (0.49 grams) was placed in a container with 6 M HCl at 150 °C for 6 hours to break down the protein into the constituent amino acids residue. Following the hydrolysis, the acid removed by rotary evaporation (RE500 Yamato Scientific America Inc.). The Sample was again re-suspended on a (2 mL) sodium citrate buffer (pH of 2.2). After the samples were hydrolyzed, the extracts were diluted by adding 50 ml of distilled water. This is then filtered to remove any remaining particulates, and then analyzed by HPLC. A gradient mobile phase of sodium acetate 0.1 M, pH 7.2 and methanol (9:1) elute sample for amino acid separation trough C 18 column reversed-phase octadecyl dymethylsilane particles (100 x 4.6 mm x 1/4" Microsorb 100-3 C18) was done. The HPLC columns separated the various individual amino acids and amino acid standards. The amino acid reference standard consisted of fifteen amino acids (0.05 μmoles mL for each amino acid) and was utilized to determine the retention times for each amino acid. As well, internal standard α amino butyric (0.05 μmoles ml-1) was added to amino acid reference standard. Post column derivatization of the sample was attained by adding 7.5 mM othophthalaldehyde (OPA) to the sample in citrate buffer. The OPA reagent contained thiofluor which fluoresced. Fluorescence detection was realized using an excitation-emission wavelength of 360 and 455 nm. The amount of individual amino acid present in respect to the standards was calculated as below.

Concentration (g/100g protein) = (Net height) X (Width at Net height) x (Sample standard) X C (3.7)

Where Sample standard= (N Elute standard) x (Molecular weight) x (amino acid standard) C=Dilution x16⁄ (sample weight in grams N %) x volume loaded Net height x Weight

(Normal leucine)

Given that

Dilution = x 5

% (fat free) constant =4.06

Volume loaded: 60µL,

Therefore C = 0.009878054

Amino acid composition (g/100g diet) = amino acid concentration (g/100g protein) x percentage protein in diet (3.8)

3.6 Diet Formulation

Three diets (A, B and C) were formulated by Pearson's square method as described by Wagner & Stanton (2016). Fish waste, guinea corn, groundnut cake, bone meal, binders (cassava starch), water and vitamin premix were used for the formulation. The diets were formulated to have the same crude protein of 45 % as the commercial feed (coppens). In the formulation, bone meal and vitamin premix were added as sources of minerals and vitamins and cassava starch (binders) was also added to enhance proper agglutination and water stability of the feed.

Table 3.1: Major Ingredients used as Source of Protein and Energy Supplement for the Diets Formulation

Ingredients	% crude protein
Fish wastes	60
Groundnut cake	48
Guinea corn	11.2

14 steps were followed for the calculation of 45 % crude protein for the diets formulation as discussed below.

3.6.1 Steps for Calculating Diet (A) at 45 % Crude Protein

i. A square was drawn

ii. The percentage crude protein of fish waste (60 %) and guinea corn (11.2 %) were placed at the top and bottom left diagonal of the square

iii. The target crude protein 45 % was placed at the center of the diagonal

iv. The percentage crude protein of fish waste and guinea corn were placed at the top and bottom left diagonal of the square and each were subtracted from the target crude protein at the center of the square neglecting negative sign. The result obtained was placed at the top and bottom right diagonal of the square.

v. The results of the subtractions were added up to get the total ingredient part.

vi. The percentage of each ingredient were calculated using the formula

$$\frac{\text{part of each ingredient}}{\text{total part of ingredient contribution}} \times 100\ \% \qquad (3.9)$$

vii. To obtain intermediate crude protein of 47.37 %, the target crude protein of 45 % was concentrated by multiplying with $\frac{100}{95}$.

viii. The intermediate crude protein 47.37 % was placed at the center of the square.

ix. Step ii, iv, v and vi above was repeated.

x. The result of each ingredient contribution in step xi above was diluted by multiplying with $\frac{95}{100}$ in order to get the percentage of each ingredient contributed to the diet

xi. The percentage sum of each ingredient amounted to 95 % and the remaining 5 % were reserved for additives (vitamin premix, bone meal and cassava starch)

xii. The target crude protein 45 % was checked by multiplying the result of each diluted ingredient by the initial percentage crude protein of each ingredient obtained from proximate composition.

xiii. The percentage crude protein for additives were insignificant and were neglected to be zero.

xiv. The sum of the result of xii and xiii yield 45 % crude protein.

The steps above were repeated for diets (B and C) formulation.

Table 3.2: Ratios of diet formulation

Sample	**Diet A**	**Diet B**	**Diet C**
Fish waste	3	0	3
Groundnut cake	0	3	3
Guinea corn	1	1	1
Cassava (binder)	1	1	1
Bone meal	2	2	2
Vitamin Mix	2	2	2

Table 3.3: Composition of diet A, B and C in grams (per Kg diet)

Sample	**Diet A**	**Diet B**	**Diet C**
Fish waste	701.4	0	401.5
Groundnut cake	0	933.8	401.5
Guinea corn	245.9	16.2	147.2
Cassava (binder)	10	10	10
Bone meal	20	20	20
Vitamin Mix	20	20	20

Composition of Vitamin premix (1.25 kg)

Biotin, Antioxidant (125,000 mg), Vitamin A (600,000 IU), Vitamin D3 (1,5000,000 IU), Vitamin E (5,000 mg), Vitamin K3 (1,200 mg), Folic acid (100 mg), Niacin (15,000 mg), Vitamin B2 (3,000 mg), Vitamin B12 (5 mg), Vitamin B1 (1000 mg), Vitamin B6 (1000 mg), Calpan (3,000 mg).

3.6.2 Mixing of samples

All the samples used for the formulation of each diet were mixed mechanically by (Karankumar and Joshua, 2016) method.

Diet (A): 701.4 g and 245.5 g of grinded fish waste and guinea corn were measured and was mixed with 50 g of additives (20 g of Vitamin premix, 20 g of bone meal and 10 g of cassava starch (binders). 10 ml of warm water was also added. The blend was then moved for proper mixing into a mechanical mixer. The sample was held for further phase after mixing.

Diet (B): 933.8 g and 16.2 g of grinded groundnut cake and guinea corn were measured and was mixed with 50 g of additives (20 g of Vitamin premix, 20 g of bone meal and 10 g of cassava starch (binders). The mixture was moved into a mechanical mixer and 10 ml of warm water was added for proper mixing. After mixing, the sample was kept for further process.

Diet (C): 401.5 g, 401.5 g and 147.7 g of grinded fish waste, groundnut cake and guinea corn were measured and was mixed with 50 g of additives (20 g of Vitamin premix, 20 g of bone meal and 10 g of cassava starch (binders). The mixture was shifted into a mechanical mixer and 10 ml of warm water was added for proper mixing. After mixing, the sample was kept for further process.

3.6.3 Pelleting of feed

Pelleting machine with blade size of 1 mm was used to pellet the formulated diets as described by (Julius *et al.,* 2014). The machine was then put on and the mixed sample was poured into the machine for pelleting. The machine pellet the sample into 1 mm size and the pelleted sample was kept for further process.

3.6.4 Drying of sample

The pelleted sample was air dried at a temperature of 26 – 43 ºC for 192 hours in the department of water Resources and Fishery laboratory, Federal University of Technology, Minna and stored at temperature of 26 ºC for further use.

3.7 Management of Experimental Animals

A total of 40 fingerlings catfish were bought from the Department of Fisheries, Ahmadu Bello University, Zaria. The bodyweight of the fingerlings were within the range of 2-3 grams and were kept under experimental standard laboratory conditions of 23.2 - 34.0 °C Dewan *et al.* (1991).

3.7.1 Experimental Design

The animals were acclimatized for 2 weeks in experimental fish laboratory, following acclimatization; they were divided into 4 groups consisting of 10 fingerlings each. The first group was fed commercial feed (coppens), while the remaining groups were fed with the respective experimental diets; A, B, and C. Each group was fed 5 % of their average bodyweight twice daily in an aquarium for 49 days. Changes in length and weight were monitored and recorded weekly.

3.8 Growth Performance Analysis and Nutrient Utilization of the Experimental Animals

Growth performance analysis were carried out in order to evaluate the parameters of growth efficiency and the use of nutrients according to the following formulas:

3.8.1 Determination of Bodyweight Gain of the Experimental Animals

The bodyweight gain of experimental fish was calculated every week using the Ultra V-bile P055 electronic compact scale (Keri, 2015) method to check the impact on their body weight of the commercial feed and the formulated diets.

Weight gain (g) = Final Weight (g) measured – Initial Weight (g) measured

Average daily weight gain (g) = (Average Final Weight (g) – Average Initial Weight (g)) /Time (Days).

3.8.2 Determination of Body Length Gain of the Experimental Animals

The body lengths of experimental fish were determined using a measuring tape weekly according to (Keri, 2015; Julius *et al*., 2014) method to check the effect of the formulated diets on their length.

Length gain (cm) = Final length (cm) – Initial length (cm)

Average daily length gain (cm) = (Average Final length (cm) – Average Initial length (cm) / Time (Days)

3.9 Blood Collection and Serum Preparation for Haematological Analysis

At the end of the experiment, the blood sample was collected according to the method of (MUAWC, 2008). The fish were put on a clean table on their side after mild chloroform anaesthesia of the fish. The fish was carefully handled with hand and a 23-G needle was inserted into the fish caudal fin until blood flow into the syringe. The collected blood sample was transferred into collection tubes containing EDTA or heparin and was gently mixed by carefully turning the tube upside down to mix the contents. For automated haematological analysis, blood samples collected in EDTA bottles were used.

3.9.1 Determination of some Haematological Parameters of the Experimental Animals

Automated haematologic analyzer was used to determine some haematological indices of fish fed commercial feed and experimental diet as described by Dacie and Lewis

(2015). The haematological indices carried out are packed cell volume (PCV), haemoglobin (Hgb), red blood cells (RBC) and white blood cells (WBC).

3.9.1.1 Determination of packed cell volume

3.9.1.2. Principle:

The determination of packed cell volume was carried out by subjecting whole blood to a centrifuge machine. The centrifuge generate a centrifugal force which causes the red blood cell to become packed and the space occupied by the red blood cells is measured. The space occupied measured was expressed as percentage of red blood cell in the whole blood volume.

3.9.1.3 Procedures

The blood collected in an anti-coagulant bottle was properly mixed and allowed to fill capillary tubes into a c haematocrit until approximately 2/3 of the whole sample was filled. This step was applicable to individual tubes and was sealed with plastacine at one end of each tube. Each tube was held in the medial grooves of the haematocrit centrifuge head exactly opposite each other, with the sealed end away from the center of the centrifuge, using the microhaematocrit method. At 12000 resolution per minute, all the tubes were spun for five minutes. After the centrifuge had finished spinning, all the tubes were removed and microhaematocrit were used to take the reading.

3.9.1.4 Calculation of packed cell volume

By calculateing the height of the red blood cell column, packed cell volume was measured using the microhaematocrit reader and this was expressed as a ratio of the total blood column height, as shown below.

$$\text{Percentage packed cell volume} = \frac{\text{Height of cell column in tube}}{\text{Total height of blood colume in the tube}} \times 100 \quad (3.10)$$

3.9.2 Determination of haemoglobin, red blood cells and white blood cells of the experimental animals

3.9.2.1 Principle

Using Dacie and Lewis (2015) techniques, haematological analyses of the sample were carried out. As well as counting the number of cells, haematological analyzers accurately count by detecting and measuring changes in electrical resistance when a particle (such as a cell) moves through a narrow aperture in a conductive liquid. Any cell suspended in a conductive liquid (diluent) acts as an isolator. When each individual cell passes via the opening, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the opening. This generates an electrical pulse which can be determined. The vacuum used to force the diluted cell suspension through the aperture must be at a controlled volume to count in order to count. The number of pulses corresponds to the counting of particles. The electrical pulse height is proportional to the volume of the cell.

3.9.2.2 Procedures

The blood sample collected in EDTA were mixed thoroughly and were kept in haematological racks, the system rack compartments were properly inspect to guarantee that bottles were well arranged, the automated haematological analyzer was made to begin working, the system was allowed to process the sample for five minutes and it generate results through the output compartment. The automated haematological analyzer SYSMEX KX-21 is configured in such a way that operational settings other

than putting the sample in the racks and turning the power button on to run a blood sample are not needed.

3.10 Determination of Some Biochemical Parameters of the Experimental Animals

The method described by Chawla (2003) was used for the determination of biochemical parameters. Tissues was collected carefully from the fish liver into a 15 ml 0.25 N sucrose and then homogenized. The collected tissue was then weighed (1 g) and homogenized using a triston homogenizer in an ice-cold 10 ml tris buffer (pH 7.8). At 2000 rpm for 10 minutes, the homogenates were centrifuged using a refrigerated centrifuge. The supernatant was carefully separated into sample bottles by decantation and held at temperature below 4 ° C for assay using Randox kits (to avoid denaturation of the proteins).

3.10.1 Determination of ALP Activity

3.10.1.1 Principle

Alkaline phosphatase is an enzyme that catalyzes the trans-phosphorylation of p-nitrophenol phosphate (p-NPP) to p-nitrophenol (p-NP) in the presence of a 2-amino-2-methylpropanol (AMP) trans-phosphorylation buffer stimulated by the presence of magnesium and zinc ions, resulting in a transition to absorption of 405 nanometer that is directly proportional to alkaline phosphatase activity.

3.10.1.2 Procedures

A sample of 10 microliters was mounted in a cuvette and mixed with 500 microliters of Randox reagent (a solution containing p-NPP). The initial absorbance was taken at 405 nm and then for 3 minutes afterwards. In the measurement, the mean absorbance per minute was used: alkaline phosphatase activity (IU/l) = 2742 x Δ A 405 nanometer/minute; where: 2742 = Absorbance extinction coefficient; Δ A 405

nanometer/minute = per minute absorbance shift for the homogeneous sample; (Chawla, 2003).

3.10.2. Determination of ALT activity

3.10.2.1 Principle

Alanine transaminase is an enzyme also known as alanine aminotransferase, formerly called glutamate-pyruvate transaminase or serum glutamic-pyruvic transaminase. It catalyzes the conversion of an amino group from L-alanine to alpha-ketoglutarate in the presence of pyridoxal phosphate to yield L-glutamate and pyruvate being the products of this reversible transamination reaction. The pyruvate produced is converted to lactate by the enzyme lactic acid dehydrogenase with the instantaneous oxidation of (NADH). The absorbance change is directly proportional to the activity of alanine transaminase at 340 nm.

3.10.2.2 Procedures

The 50 microliter and 500 microliter samples of the alanine transaminase reagent were combined in a test tube with the initial absorbance read at 340 nanometer after 60 seconds. The timer was started at the same time and after 1, 2, and 3 minutes, more readings of the absorbance were taken and calculations were carried out.

ALT operation (nanometer/minute) = 1746 x Δ A 340 nanometer/minute, Δ A 340 nm/min = Δ homogeneous sample absorbance per minute, 1746 = Molar attenuation coefficient (Chawla, 2003). (3.11)

3.10.3 Determination of AST activity

3.10.3.1 Principle

The inter-conversion of aspartate and alpha-ketoglutarate to oxaloacetate and glutamate is catalysed by aspartate transaminase. The oxaloacetate produced by the enzyme malate

dehydrogenase is converted to malate with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH) to NAD+ in a way that is directly proportional to the activity of aspartate transaminase (at 340 nm).

3.10.3.2 Procedures

About 50 microliter of the sample was measured and mixed in a test tube with 500 microliter of the AST reagent and the initial absorbance was read after 60 seconds at 340 nanometer. The timer was started simultaneously and after 1, 2, and 3 minutes, more readings of the absorbance were taken and calculations were carried out as shown below;

Regulation of aspartate transaminase (nanometer/minute) = 1 746 x Δ A 340 nanometer/minute (3.12)

Where:

Δ A 340 nanometer/minute = per minute absorbance shift for the homogeneous sample,

1746 = Extinction coefficient of NADH and NAD^+ + absorbance of 1 molar (Chawla, 2003).

3.10.4 Determination of total serum proteins

Total protein was determined by method as defined by Randox Diagnostic Kit in serum and tissues (Tietz, 1976) (United Kingdom).

3.10.4.1 Principle

Cupric ions react with protein and polypeptide peptide bonds containing at least 2 peptide bonds to create a violet-coloured complex in an alkaline solution. The absorbance is directly proportional to the protein concentration present in the sample at 546 nm of the complex. Though, this is then changed with the addition of tartarate as a complexing agent (in the kit) in order to inhibit $Cu(OH)_2$ precipitation.

3.10.4.2 Procedures

About 10 microliters of distilled water (blank) and 1 ml of biurette reagent were put into the cuvette and mixed together. After absorbance was taken at 546 nm, it was permitted to stand for 10 minutes. The process was practiced on a standard basis and the technique was repeated three times. In the estimate, average absorbance was utilized in the calculation. $\frac{\text{A sample} \times \text{C standard}}{\text{A standard}} = \text{C sample}$ (3.13)

Where

A =Absorbance, C=concentration

3.11 Statistical Analysis

At the end of the experiment, the data collected were subjected to statistical analysis using Microsoft excel for the determination of mean and standard error of mean values. The statistical kit Multiple Range Evaluation of the Duncan with Statistical Program for Social Statistics version 15.0 tested different determinations for the substantial differences ($p<0.05$) between the mean values (Ajibola *et al*., 2016).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate composition of the selected ingredients

The proximate composition of all the raw selected ingredients for fish wastes, groundnut cake, guinea corn and bone meal are shown in figure 4.1. Moisture content in all the selected ingredients were generally low. Between all the samples, the groundnut cake (2.97±0.10 %) ingredient had the lowest moisture content and bone meal (5.97±0.23 %) had the highest moisture content.

The lipid content of groundnut cake (24.83±0.12 %) and bone meal (16.57±1.09) was significantly higher than fish wastes and guinea corn. Guinea corn (2.83±0.55 %) ingredient had the lowest lipid content.

Protein was found to be lowest in guinea corn (11.80±0.31%) while fish wastes (59.90±0.26 %) and groundnut cake (48.00±0.29 %) were significantly higher in protein content than other selected ingredients.

The crude fibres in all the selected ingredients, bone meal (8.73±0.27 %) was found to be significantly higher ($p<0.05$) than other selected ingredients. There was no significant difference ($p>0.05$) between the crude fibre values of fish waste (2.70±0.16 %) and guinea corn (2.67±0.28 %). This observation is however the same in the ash content. The ash content was found to be significantly higher in bone meal (31.00±0.65 %) than other selected ingredients. Guinea corn (0.90±0.26 %) had the lowest ash content.

The carbohydrate composition of the guinea corn (76.8±0.36 %) was significantly higher (p >0.05) than all other selected ingredients. Of the entire ingredient there were significantly different between the carbohydrate compositions.

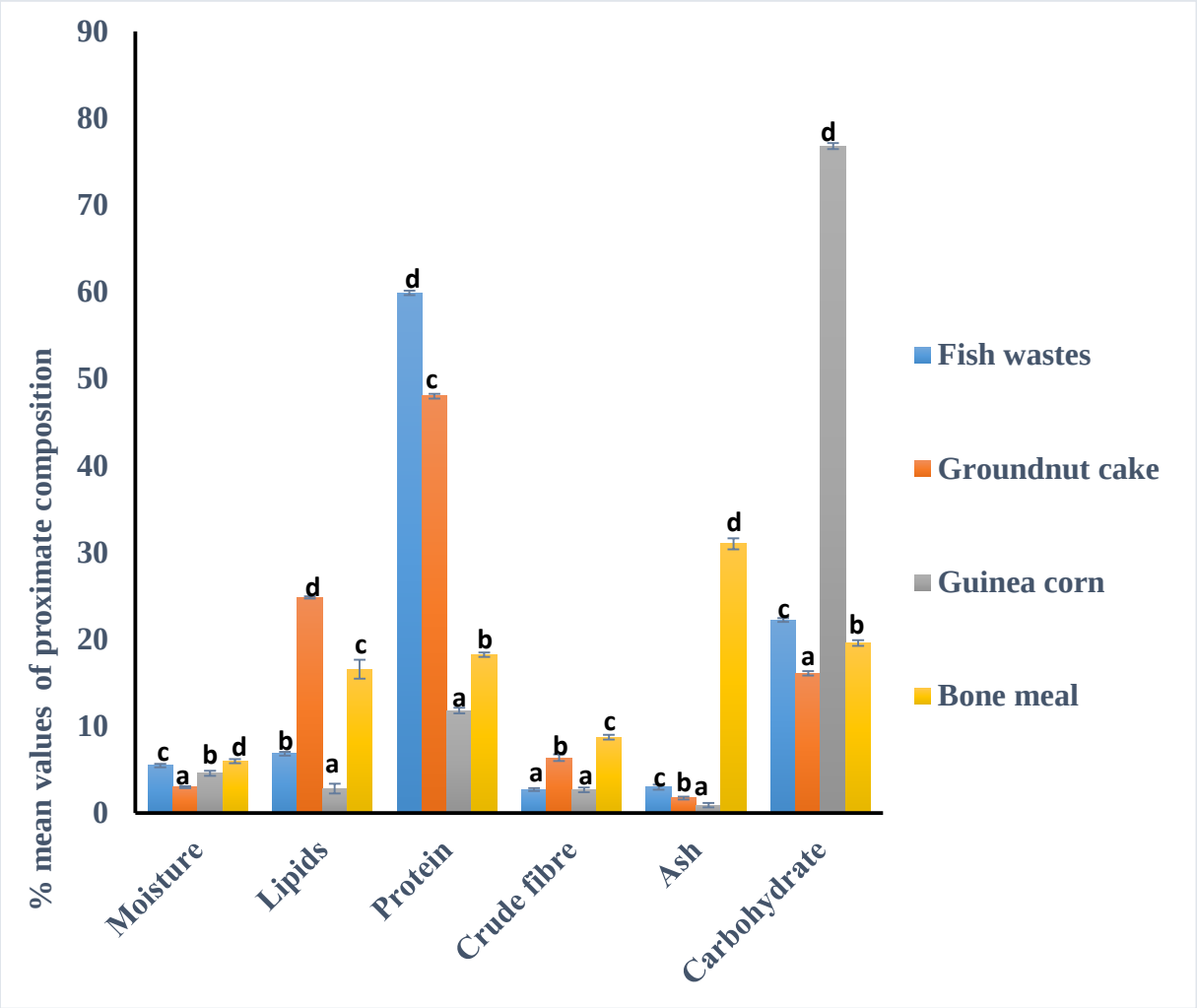

Fig. 4.1: Proximate Composition of Selected Ingredients

Different alphabets on the bars display substantial difference (p<0.05) for the same parameter

4.1.2 Proximate composition of commercial feed (Coppens) and formulated diets.

The proximate composition of the commercial feed and formulated diets (A, B, and C) are shown in figure 4.2.

There was significant differrence ($p<0.05$) between the moisture content of all the formulated diets and commercial feed. Among all the formulated diets, the formulated diet A (2.97±0.27 %) had the lowest moisture content but higher than the commercial feed and formulated diet C (6.07±0.26 %) had the highest moisture content. Lipid content of the formulated diet A (11.67±0.17 %) was significantly higher than the lipid content of formulated diet (B and C) but lower than the commercial feed. Formulated diet C (10.03±0.12 %) had the lowest lipid content.

Protein content was found to be significantly higher in formulated diet A (44.12±0.15 %) than other formulated diets (B and C) but lower than the commercial feed (44.83±0.17 %).

The crude fibre in all the formulated diets was found to be significantly higher in formulated diet A (13.02±0.07 %) than other formulated diet (B and C) and there was no significant difference ($p>0.05$) with the commercial feed (13.07±1.07 %). The same trend was observed for ash content.

Among all the formulated diets, the carbohydrate composition of the formulated diet C (26.33±0.17 %) was found to be significantly higher ($p<0.05$) than other formulated diets (A and B). However, commercial feed (20.20±0.21 %) had the lowest carbohydrate content.

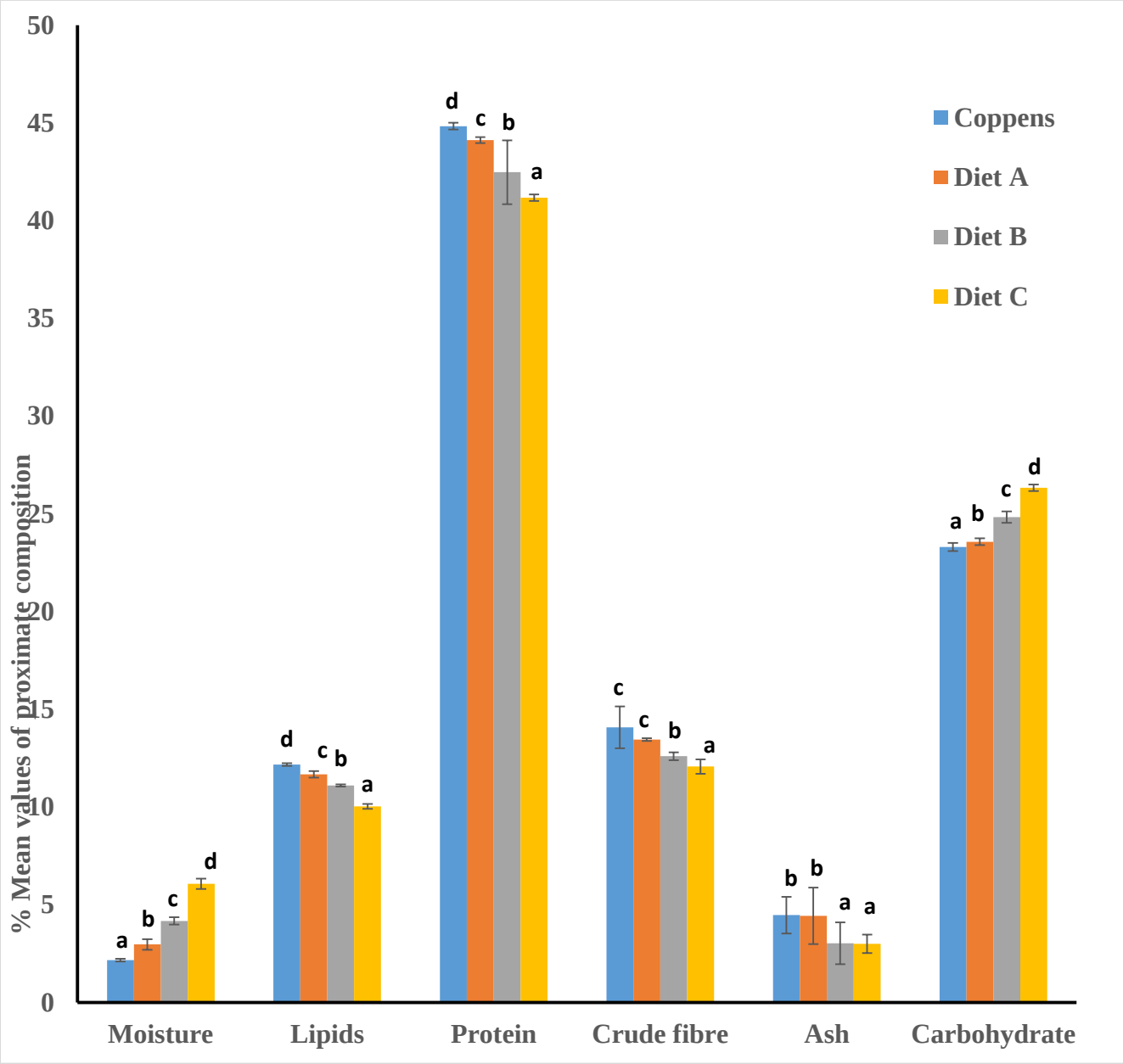

Fig. 4.2: Proximate Composition of Commercial Feed and Formulated Diets

Different alphabets on the bars display substantial difference ($p<0.05$) for the same parameters

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives and Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

4.1.3 Total essential amino acid composition of the commercial feed and formulated diets

The total essential amino acid in the commercial feed and the formulated diets is presented in table 4.1 below. Between all the formulated diets, formulated diet A (32.12±0.31 g/100 g diet) was significantly higher ($p < 0.05$) in total essential amino acid composition than other formulated diets (B and C) but lower than the commercial feed (35.67±0.37 g/100 g diet). However, in some amino acids such as arginine, histidine, leucine and phenylalanine there was no significant difference ($p > 0.05$) in their levels between the formulated diet A and the commercial feed.

Table 4.1: Total Essential Amino Acid Composition of Commercial Feed and Formulated Diets

Amino acids (g/100 g diet)	Feed	Formulated diets		
	Coppens	Diet A	Diet B	Diet C
Methionine	1.32 ± 0.04^{d}	0.50 ± 0.01^{c}	0.23 ± 0.03^{b}	0.20 ± 0.05^{a}
Arginine	5.15 ± 0.01^{c}	5.14 ± 0.04^{c}	5.06 ± 0.02^{b}	4.67 ± 0.03^{a}
Threonine	2.29 ± 0.01^{d}	2.15 ± 0.02^{c}	2.56 ± 0.06^{b}	2.22 ± 0.02^{a}
Tryptophan	0.95 ± 0.08^{d}	0.91 ± 0.01^{c}	0.85 ± 0.06^{b}	0.62 ± 0.02^{a}
Histidine	2.18 ± 0.03^{b}	2.14 ± 0.01^{b}	2.07 ± 0.03^{b}	1.77 ± 0.12^{a}
Isoleucine	3.61 ± 0.06^{d}	3.86 ± 0.02^{c}	3.44 ± 0.04^{b}	3.33 ± 0.01^{a}
Lysine	4.44 ± 0.03^{d}	3.10 ± 0.08^{c}	1.01 ± 0.03^{b}	2.03 ± 0.02^{a}
Leucine	7.20 ± 0.04^{c}	7.10 ± 0.05^{c}	6.91 ± 0.03^{b}	5.25 ± 0.08^{a}
Valine	4.28 ± 0.05^{d}	3.00 ± 0.03^{c}	2.96 ± 0.01^{b}	2.07 ± 0.03^{a}
Phenylalanine	4.25 ± 0.03^{b}	4.22 ± 0.04^{b}	3.87 ± 0.02^{a}	3.79 ± 0.02^{a}
Σ-Total essential amino acid	35.67 ± 0.37^{d}	32.12 ± 0.31^{c}	28.96 ± 0.33^{b}	25.95 ± 0.35^{a}

Values differing in a row of different subscripts are significantly different ($p<0.05$). Values of the formulated diets were obtained in g/100 g proteins and converted to g/100 g

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

4.1.4 Total non-essential amino acid composition of the commercial feed and formulated diets

The non-essential amino acid composition in the commercial feed and formulated diets are shown in table 4.2. The formulated diet A (37.67±0.54 g/100 g diet) was significantly higher ($p<0.05$) in total non-essential amino acid composition than other formulated diets (B and C) but lower than the commercial feed (38.58±0.24 g/100 g diet). The values of some amino acid such as proline, alanine, glutamic acid, aspartic acid, serine, glycine and tyrosine revealed no significant difference ($p>0.05$) in their levels between the formulated diet A and the commercial feed.

Table 4.2: Total non-essential amino acid composition of the commercial feed and formulated diets

Amino acids (g/100 g diet)	Feed	Formulated diets		
	Coppens	Diet A	Diet B	Diet C
Proline	3.25±0.01^{c}	3.23±0.03^{c}	3.12±0.07^{b}	2.41±0.05^{a}
Cysteine	1.16±0.04^{d}	0.29±0.02^{c}	0.21±0.02^{b}	0.19±0.04^{a}
Alanine	4.30±0.02^{c}	4.28±0.01^{c}	4.07±0.04^{b}	3.85±0.03^{a}
Glutamic acid	11.33±0.04^{c}	11.28±0.07^{c}	10.65±0.11^{b}	8.83±0.07^{a}
Aspartic acid	8.86±0.03^{c}	8.83±0.04^{c}	7.46±0.01^{b}	6.47±0.03^{a}
Serine	3.39±0.04^{c}	3.48±0.11^{c}	3.13±0.07^{b}	2.78±0.02^{a}
Glycine	3.03±0.01^{c}	3.05±0.05^{c}	2.92±0.04^{b}	2.52±0.12^{a}
Tyrosine	3.26±0.05^{c}	3.23±0.21^{c}	2.95±0.03^{b}	2.39±0.01^{a}
Σ-Total non-essential amino acid	38.58±0.24^{d}	37.67±0.54^{c}	34.51±0.36^{b}	32.22±0.39^{a}

Values differing in a row of different subscripts are substantially different ($p<0.05$). Values of the formulated diets were obtained in g/100 g proteins and converted to g/100 g diet

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

4.1.5 Mineral composition of commercial feed and formulated diets

The mineral composition of the commercial feed and formulated diets are shown in table 4.3.

The mineral analysis results revealed a significant higher ($p<0.05$) values of calcium (138.85±0.17 mg/kg), copper (0.28±0.01 mg/kg), phosphorus (42.28±0.02 mg/kg), potassium (230.33±0.33 mg/kg), iron (9.84±0.24 mg/kg) and magnesium (80.49±0.04 mg/kg) in formulated diet A than other formulated diets (B and C) but lower than the commercial feed.

Table 4.3: Mineral composition of commercial feed and formulated diets

Mineral composition	**Feed**	**Formulated diets**		
mg/kg	**Coppens**	**Diet A**	**Diet B**	**Diet C**
Ca	150.03 ± 0.01^{d}	138.85 ± 0.17^{c}	130.49 ± 0.03^{b}	120.62 ± 0.01^{a}
Cu	1.28 ± 0.02^{d}	0.28 ± 0.01^{c}	0.07 ± 0.02^{b}	0.02 ± 0.03^{a}
P	60.23 ± 0.02^{d}	42.28 ± 0.02^{c}	39.35 ± 0.03^{b}	30.21 ± 0.01^{a}
K	250.04 ± 0.03^{d}	230.33 ± 0.33^{c}	211.23 ± 0.07^{b}	121.29 ± 0.20^{a}
Fe	12.83 ± 0.07^{d}	9.84 ± 0.24^{c}	5.85 ± 0.08^{b}	3.61 ± 0.02^{a}
Mg	91.27 ± 0.06^{d}	80.49 ± 0.04^{c}	78.39 ± 0.03^{b}	60.58 ± 0.02^{a}

Values were obtained as a result of triplicate determination (means ± Standard error of mean). Values differing in a row of different subscripts are substantially different ($p<0.05$).

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

4.1.6 Growth performance of catfish fed on commercial feed and formulated diets for bodyweight (g) measurement.

The trend of growth performance of catfish (fingerlings) fed commercial feed and formulated diets by bodyweight measurement are shown in the figure 4.3 below. At week 0, there was no significant difference ($p>0.05$) between the bodyweight measurement of groups of fingerlings fed the formulated diets (A, B and C) and the commercial feed. For week 1, the groups of fingerlings fed formulated diet A (6.07±0.20 g) and B (5.93±0.14 g) was significantly higher ($p<0.05$) than the groups of fingerlings fed formulated diet C (4.90±0.15 g) but no significant difference with the groups fed the commercial feed. At week 2, 3, 4, and 5 groups of fingerlings fed formulated diet A was significantly higher ($p<0.05$) in bodyweight measurement than the groups of fingerlings fed the formulated diets (B and C) but there was no significant difference ($p>0.05$) with the groups of fingerlings fed the commercial feed. For week 6 and 7, the groups of fingerlings fed formulated diet A was significantly higher ($p<0.05$) in bodyweight measurement than the groups of fingerlings fed the formulated diets (B and C) but lower than the groups of fingerlings fed the commercial feed.

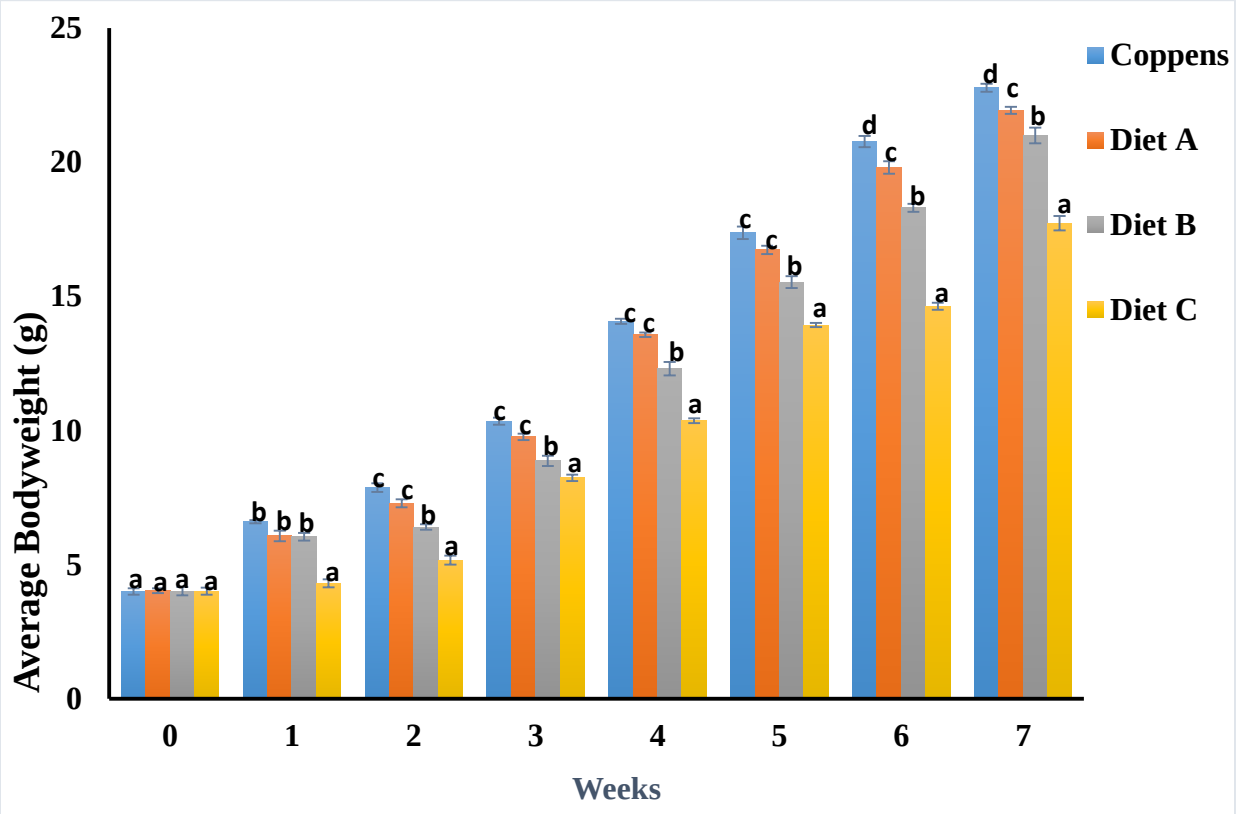

Fig. 4.3: Growth Performance of Catfish Fed on Commercial Feed and Formulated Diets for Bodyweight (g) Measurement.

Different alphabets on the bars for the same week and parameter shows significant difference ($p<0.05$)

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

4.1.7 Growth performance of catfish fed on commercial feed and formulated diets for length (cm) measurement.

The growth performance by length (cm) measurement of the fingerlings fed commercial feed and formulated diets are shown in figure 4.4 below. At week 0 there were no significant difference ($p>0.05$) in growth performance by length (cm) measurement of the groups of fingerlings fed commercial feed and formulated diets. For week 1, 2, 3, and 4 revealed a significant higher ($p<0.05$) in growth performance by length measurement of the groups of fingerlings fed formulated diet A than other groups of

fingerlings fed formulated diets (B and C) but there was no significant difference ($p>0.05$) with the groups of fingerlings fed commercial feed. At week 5, 6 and 7 growth performance of the fingerlings showed a significant higher ($p<0.05$) length (cm) measurement of the fingerlings fed formulated diet A than other groups of fingerlings fed formulated diets (B and C) but significantly lower than the groups of fingerlings fed commercial feed.

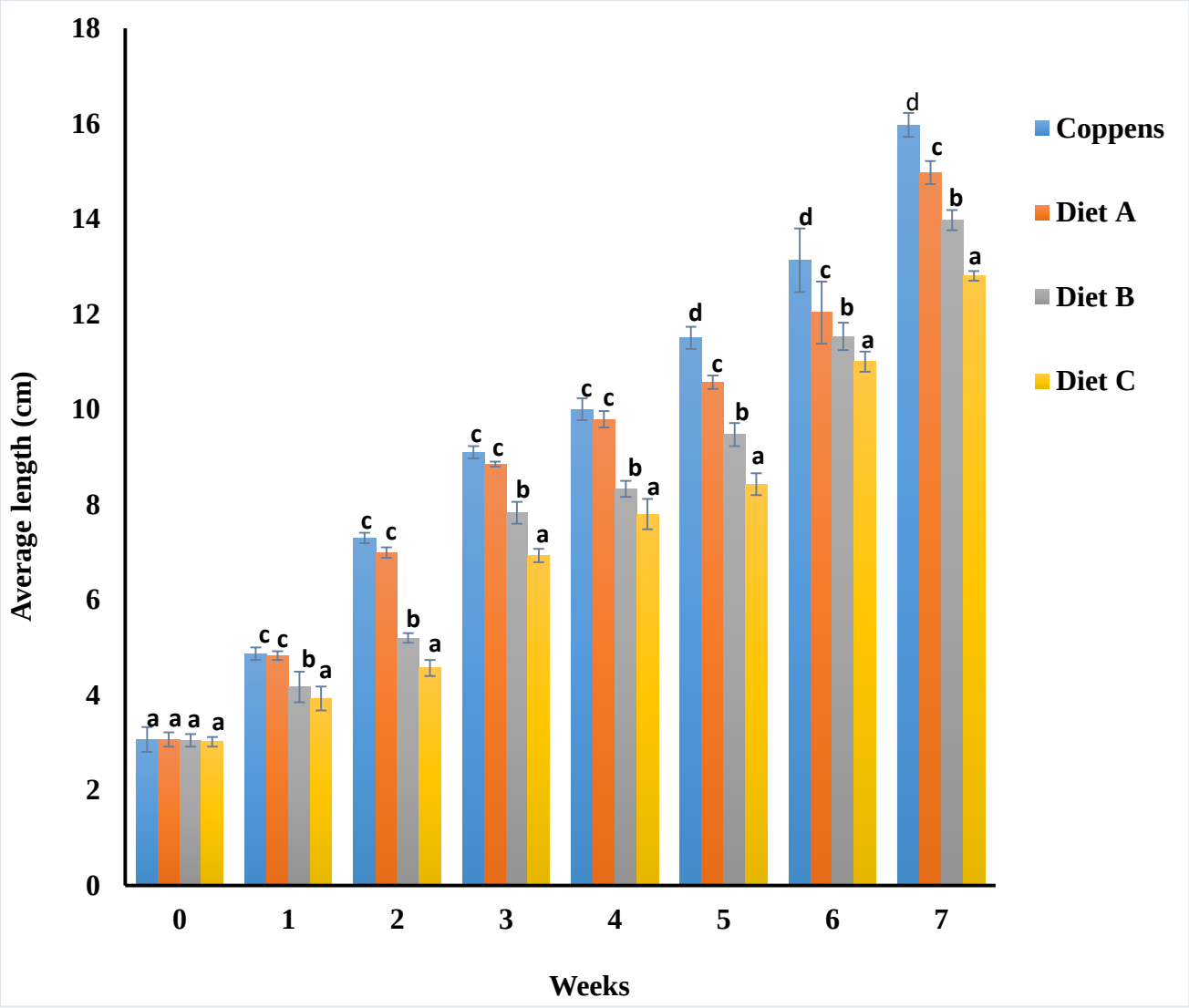

Fig. 4.4: Growth Performance of the Catfish Fed on Commercial Feed and Formulated Diets for Length (cm) Measurement.

Different alphabets on the bars for the same week and parameter shows significant difference ($p<0.05$)

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

4.1.8 Effects of commercial feed and formulated diets on some biochemical parameters in catfish

Figures 4.5, 4.6, 4.7 and 4.8 below display the impact of commercial feed and formulated diets on serum alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT) and total protein levels. The ALP values of the groups of fingerlings fed formulated diet A (33.00±0.20 U/L) was significantly lower ($p>0.05$) than the groups of fingerlings fed formulated diets (B and C) but there was no significant difference ($p>0.05$) with the groups of fingerlings fed the commercial feed (32.45±0.12 U/L).

The AST values of the groups of fingerlings fed the formulated diet A (34.90±0.18 U/L) was significantly lower ($p>0.05$) than the groups of fingerlings fed the formulated diets (B and C) but there was no signifcant difference ($p>0.05$) with the groups of fingerlings fed the commercial feed (33.45±0.16 U/L)

The ALT activity of the groups of fingerlings fed the formulated diet A (12.13±0.14 U/L) was significantly lower ($p>0.05$) than the groups of fingerlings fed the formulated diets (B and C) but there was no significant difference ($p>0.05$) with the groups of fingerlings fed the commercial feed (12.01±0.13 U/L).

The total protein values of the groups of fingerlings fed formulated diet A (19.52±0.21 g/dL) was significantly higher ($p<0.05$) than the groups of fingerlings fed the formulated diets (B and C) but lower than the groups of fingerlings fed the commercial feed (20.35±0.13 g/dL).

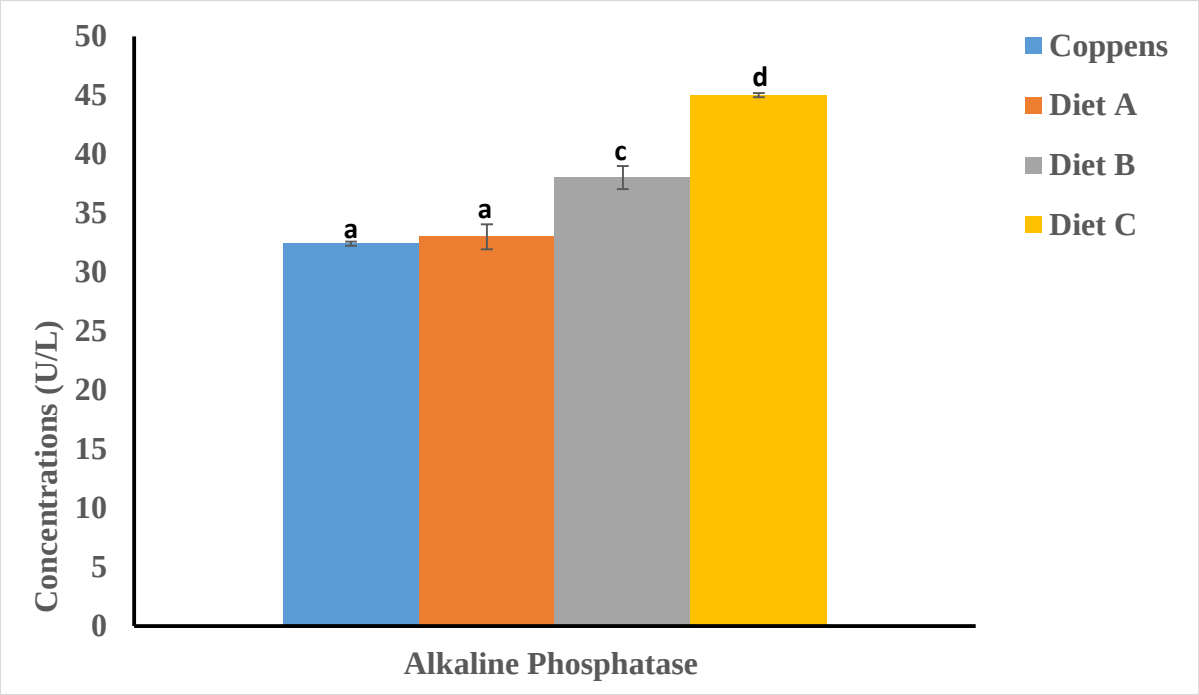

Fig. 4.5: Effects of Commercial Feed and Formulated Diets on Alkaline Phosphatase Activity (U/L))

Different alphabets on the bars for the same week and parameter shows significant difference ($p<0.05$)

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

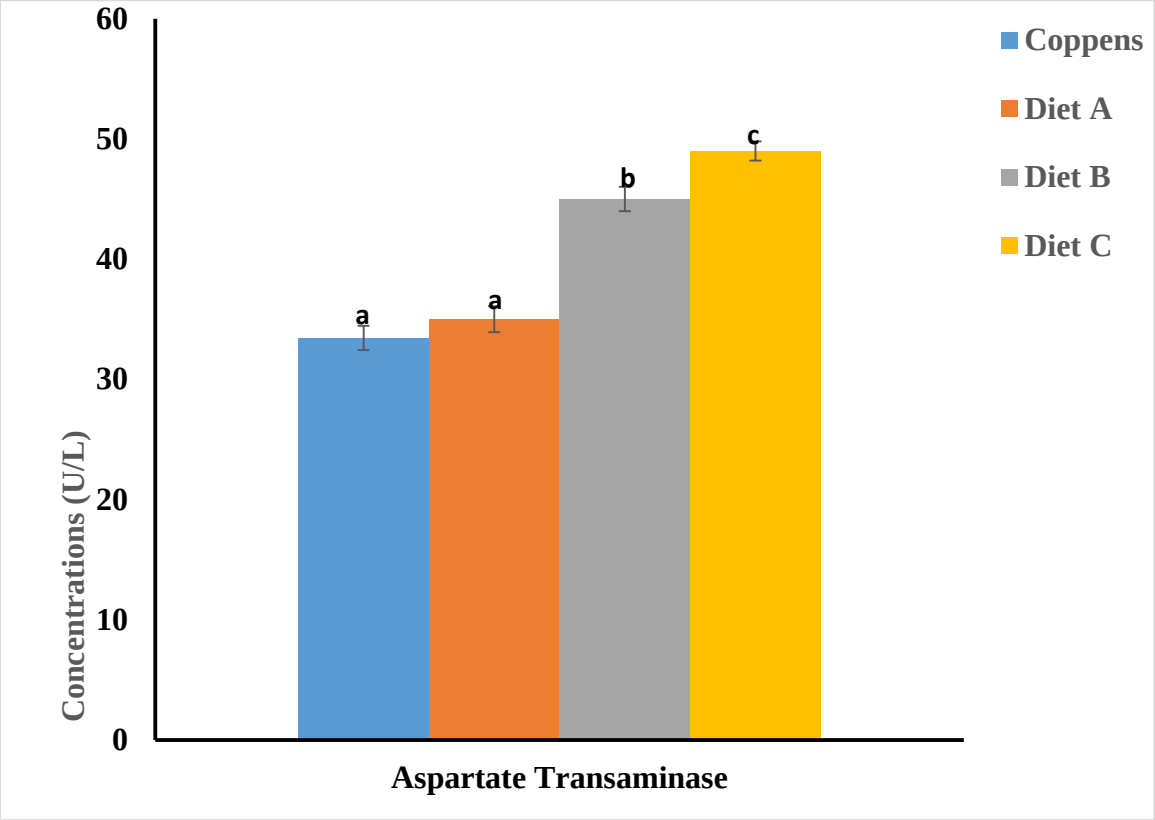

Fig. 4.6: Effects of Commercial Feed and Formulated Diets on Aspartate Transaminase Activity (U/L)

Different alphabets on the bars for the same week and parameter shows significant difference ($p<0.05$)

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

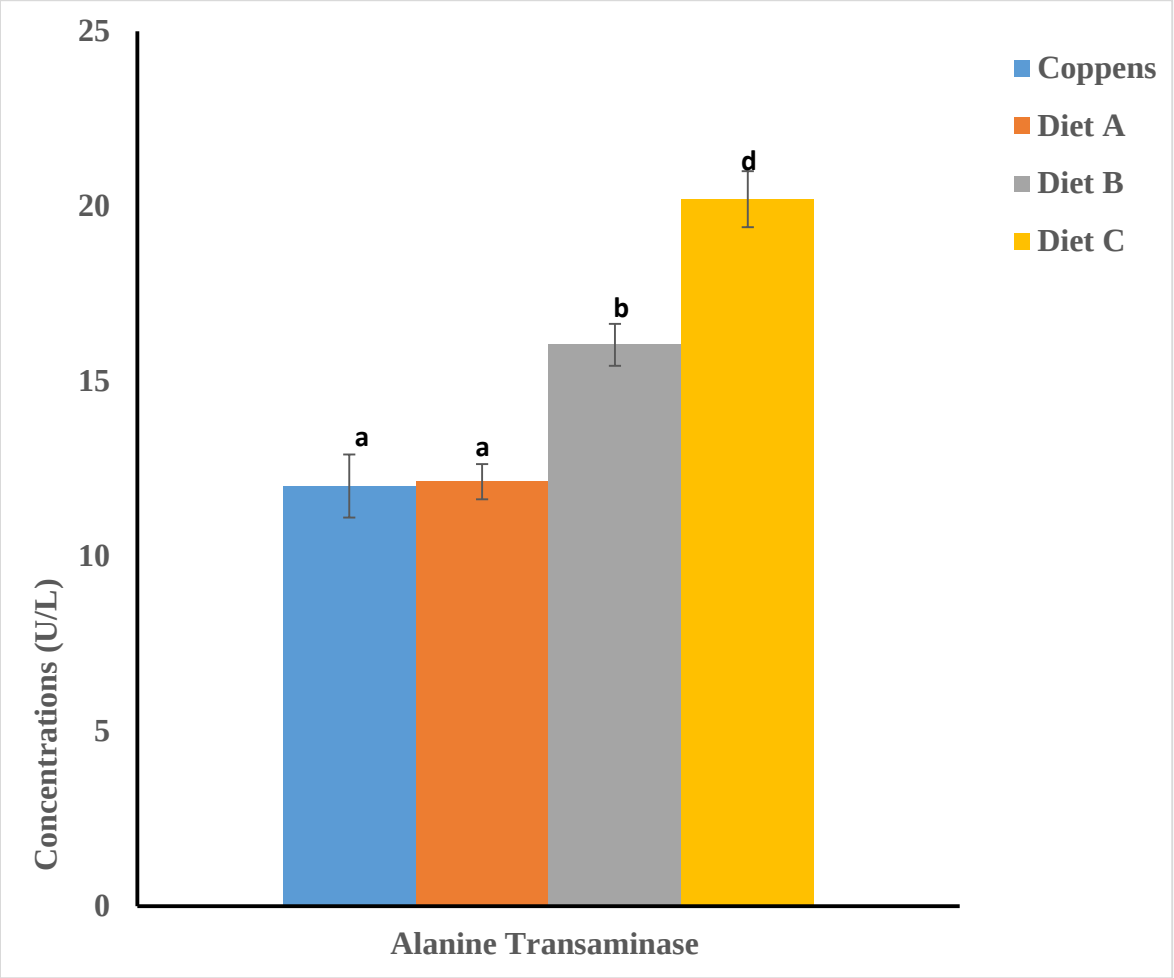

Fig. 4.7: Effects of Commercial Feed and Formulated Diets on Alanine Transaminase Activity (U/L))

Different alphabets on the bars for the same week and parameter shows significant difference ($p<0.05$)

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

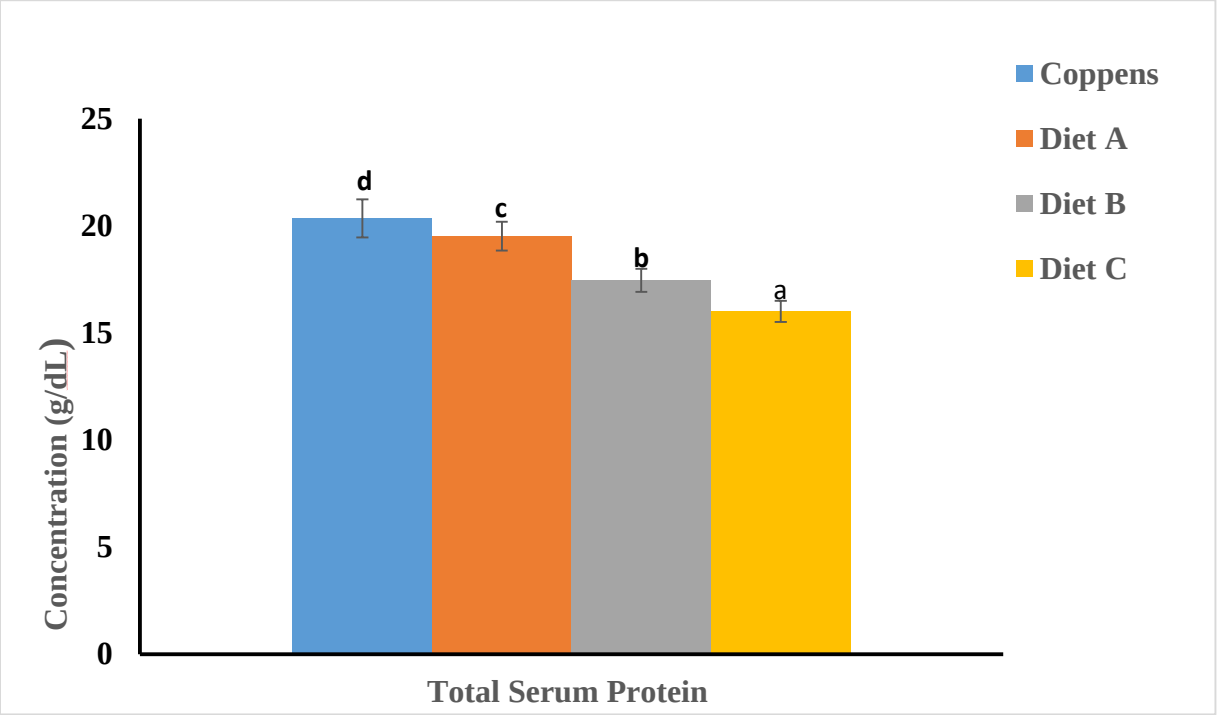

Fig. 4.8: Effects of Commercial Feed and Formulated Diets on Total Serum Protein

Different alphabets on the bars for the same week and parameter shows significant difference ($p<0.05$)

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

4.1.9 Effects of commercial feed and formulated diets on some hematological parameters of catfish.

The effect of commercial feed and formulated diets on some hematological parameters are shown in table 4.4 below. The red blood cell, haemoglobin and packed cell volume for the group of fingerlings fed formulated diet A was higher in values compared to the groups of fingerlings fed formulated diets (B and C) but lower in values than the groups of fingerlings fed the commercial feed. The values of white blood obtained in fingerlings fed formulated diet A ($1.28\pm0.25 \times 10^9$/L) was lower in values than the groups of fingerlings fed formulated diets (B and C) but higher than the group of fingerlings fed the commercial feed ($0.73\pm0.33 \times 10^9$/L).

Table 4.4: Effects of commercial feed and formulated diets on some hematological parameters of catfish.

Haematological parameters	Feed	Formulated diets		
	Coppens	Diet A	Diet B	Diet C
RBC (x 10^9/L)	3.89 ± 0.37^{d}	3.40 ± 0.23^{c}	2.17 ± 0.43^{b}	1.29 ± 0.45^{a}
HAEMOGLOBIN (g/dl)	8.50 ± 0.25^{d}	8.00 ± 0.19^{c}	7.23 ± 0.23^{b}	5.26 ± 0.27^{a}
WBC (x 10^9/L)	0.73 ± 0.33^{a}	1.28 ± 0.25^{b}	2.38 ± 0.18^{c}	3.37 ± 0.21^{d}
PCV (%)	25.67 ± 0.24^{d}	24.45 ± 0.26^{c}	21.65 ± 0.14^{b}	15.67 ± 0.25^{a}

Values were obtained as a result of triplicate determination (means ± Standard error of mean). Values differing in a row of different subscripts are substantially different ($p<0.05$).

Key: Diet A: Fish Wastes + Guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes+ Groundnut cake + Guinea corn + Additives

4.2 Discussion

The carbohydrate content of all the ingredients in figure 4.1 suggests that the ingredients are of different classes of food. The carbohydrate content of the guinea corn is similar and within the range of that reported by Agbebi *et al*. (2009). Generally guinea corn is known to be cereal and all cereals are known to be rich in carbohydrate that meet the minimum amount of carbohydrate required to supplement 20 % diet energy for catfish as reported by Ogunji and Wirth (2001). The lower values of carbohydrate content in other ingredients are expected because they do not belong to the examples of carbohydrate food substances. The moisture and lipid content obtained in this work is in agreement with the findings of Oyetayo and Ogunrotimi (2012).

The protein content of fish waste and groundnut cake obtained in this work is similar in values and within the range reported by Otubusin *et al*. (2009). Their higher values in

protein content suggests that they are good sources of protein. Since the values of protein content obtained for fish waste and groundnut cake are more than 20 % crude protein, they can be considered as a protein supplement for catfish feed formulation as reported by Gabriel *et al*. (2007). The protein of bone meal and guinea corn obtained in this work were not up to 20 % crude protein and therefore they cannot be considered as a protein supplement for diet formulation for catfish rather they are considered to serve as additives and carbohydrate to meet up with the nutrient requirements of catfish as reported by Sikiru *et al.* (2009). The protein content and lipid content of guinea corn obtained in this study is in agreement with the report of Jimoh and Abdullahi (2017).The lower values of protein content and lipid content of guinea corn is expected because it does not belong to a food sources of protein and fatty acids.

The low moisture content of all the ingredients is likely attributed to drying. This will likely prevent or reduce microbial growth since microbes that spoil food thrive more in moisture environment.

The lipid content of the groundnut cake (in figure 4.1) agrees with the values obtained by Okanlawo and Oladipupo (2010). The high content of lipid in groundnut cake may be attributed to its sources and composition such as groundnut and groundnut oil which are all sources of fats. Bone meal and fish waste also contain fatty acids that contribute to lipid content. The low value of lipid content of guinea may be attributed to low presence of fatty acids. The significant high ash content of bone meal may be attributed to presence of high fibre and minerals.

The carbohydrate content of the commercial feed and the formulated diets in figure 4.2 suggests that the formulated diets are rich in energy and they are able to meet 20 % minimum amount of carbohydrate requirement for catfish feed supplementation as

reported by Menghe *et al*. (2001). The higher carbohydrate content of formulated diet C may be attributed to the ingredients used for the formulation.

The protein content of the formulated diets obtained in this work is not in agreement with what was obtained by Musiba *et al*. (2014). The significant higher protein content of the formulated diet A than other formulated diets (B and C) is most likely attributed to the high crude protein of fish waste used for the formulation. The lower protein content of formulated diet B and C maybe due to the plant base sources of protein used in the formulation of the diet. This is in line with the report by Robinson *et al*. (2001) that the use plant as a source of protein supplements in fish feed formulation may be the deficient and low in some amino acids like lysine and methionine which are the major growth promoter in fish feed and may as well contain anti-nutritional factors that may not be destroyed in the processing of feed.

The significant lower ($p>0.05$) moisture content of the formulated diet A than other formulated diets (B and C) is most likely attributed to drying and the sources of the ingredients used for the formulation. The moisture content of the formulated diet A and B are similar to the findings of Aisha and El-Tinay (2004) who found the moisture value in the range of 4.3-5.1 %

The significant higher value in the lipid content of formulated diet A may be due to the fish waste and bone meal used in the formulation. Fish is not only a source of protein but also serve as a unique source of essential nutrients such as omega 3 and 6 fatty acids. Bone meal also contain a lot of fats and the two of them used for the formulation of diet A may be the reason why there was rise in the lipid level of formulated diet A compared to formulated diets (B and C). This is similar to the work of Ogunji and Wirth (2001).

The ash content of formulated diet B and C in figure 4.2 is low and the non-significant difference ($p>0.05$) between the ash content of the formulated diet B and C is probably due to the low composition of minerals in the formulated diets. The high ash content of formulated diet A may be due to the ingredients used for the formulation. The fish waste and bone meal used for the formulation are good sources of mineral elements and this could contribute to high mineral content of formulated diet A. The non-significant difference between the ash content of formulated diet A and the commercial feed is most likely attributed to similarity in their mineral compositions. The ash content of formulated diet A is within the range reported by Otunola *et al*. (2012). This observation was the same for crude fiber.

The observed consistent higher trend in amino acid values in the formulated diet A over the formulated diet B and C may be due to the high crude protein content of the fish waste used for the formulation in table 4.1. This agrees with the findings of Lacy (2016) that animal sources of protein used in fish feed formulation are better in enhancing protein contents of feed than plant sources of protein. The formulated diet A with the highest total essential and non-essential amino acids than the other formulated diets (B and C) may be the most suitable diet to supply the necessary amino acids required for catfish growth and maturity.

The significant higher values of mineral composition of formulated diet A in table 4.3 may be due to higher mineral compositions of the selected ingredients used for the formulation of the diet. Fish waste is rich in calcium, phosphorus, iron, zinc, iodine and potassium. Bone meal is rich in calcium, phosphorus, iron, magnesium and zinc. All these minerals present in this selected ingredient may be responsible for the high mineral content of formulated diet A. The significant lower values for the mineral compositions of formulated diet B and C may be attributed to low mineral composition

of the selected ingredient used for the formulation of the diet. The non-significant difference ($p>0.05$) between the formulated diet B and C may be that they have similar mineral compositions. However, this findings is not in agreement with the report of Modupe *et al*. (2012).

In this study, the formulated diet A in figure 4.4 and 4.5 supported and enhanced the growth of catfish better than the formulated diet B and C. This suggests that formulated diet A contain more essential growth promoting amino acids and other essential nutrients needed for the growth and development of fingerlings than other formulated diets (B and C). The non-significant difference in growth performance of fingerlings fed commercial feed and formulated diet A suggests that formulated diet A was able to provide the required nutrients for the growth and maturity of catfish (fingerlings). This might also mean that the higher nutritional value maybe from the ingredients used for the formulation of the diet. This is because the total amino acid composition and energy composition were higher in the formulated diet A. The higher growth performance of the catfish that fed on the formulated diet A over the formulated diets (B and C) may be attributed to the animal sources of protein used for the formulation of diet A. This agrees with the findings of Abowei and Ekubo (2011); Adeniji and Balogun (2003). The higher in growth performance of the fingerlings that fed on formulated diet A is also attributed to high concentration of some growth promoting essential amino acids of catfish (fingerlings) such as methionine and lysine. The lower growth performance of catfish that fed on formulated diets (B and C) may be attributed to low concentration of some essential amino acids such methionine and lysine as reported by Robinson *et al.* (2001). Adeniji and Balogun (2003) revealed that animal proteins are known to contain quality amino acids than the plant proteins and it is preferable over plant protein in catfish feed formulation, because plant proteins contain anti-nutritional factors that will

interact with the release of essential nutrients which may leads to mineral deficiency. The lower growth performance of catfish that fed on formulated diets (B and C) may also be linked to the existence of anti-nutritional factor in the selected ingredients used for the formulation of the diets.

Serum enzymes are biomarkers in animals for the toxicity of certain substances. Toxicity may occur during the storage of animal feed or the feeding process, leading to hepatotoxicity in catfish. Although there was no clinical sign, continued intake of formulated diets could lead to toxicity Smiricky *et al*. (2002).

The lower values of ALP, AST and ALT of the groups of fingerlings fed the formulated diet A in figure 4.5, 4.6 and 4.7 than other groups of fingerlings fed the formulated diets (B and C) could be that formulated diet A does not have any cytotoxic effect or did not cause liver damage.

The increased activity of ALP, AST, and ALT in the fingerlings fed the formulated diets (B and C) could be an indication of tissue damage. The tissue damage could be attributed to high fat content of the formulated diets which may leads to fat deposition in the liver. When liver become overloaded with fats, the liver may be become over-whelmed and lead to disease case of the liver. This could result to the linkage of the liver enzymes into the blood circulation. The results of ALP, AST and ALT obtained in this study is lower than the results obtained Zaki *et al*. (2010), but similar to what was reported by Hoseinifar *et al*. (2011).

The low levels of total serum protein in catfish fed with formulated diets (B and C) in Figure 4.8, on the other hand, may be attributable to the low level of protein in the ingredients used in the formulation. In addition, this could also be attributed to the presence of anti-nutritional factors that can bind and prevent the release of essential

nutrients to support the physiological function of the organism. This result is comparable to what was reported in the formulation of fish diets by Van-Huis *et al*. (2013) who used plant protein base.

Haematological parameters are strong clinical markers of the animal's physiological, dietary and pathological status. They are also important in checking feed toxicity and changes in the haematological parameters depend on age, fish species, diseases and the cycle of sexual maturity Olafedehen *et al*. (2010); NseAbasi *et al*. (2014).

The higher values of red blood cell (RBC), haemoglobin (Hb), and packed cell volume (PCV) of the groups of fingerlings fed formulated diet A in table 4.4 than the groups of fingerlings fed the formulated diets (B and C) is an indication that formulated diet A did not have any cytotoxic effect on the fingerlings. The higher values of RBC, Hb and PCV may be due to the high presence of mineral elements such as iron and copper in the formulated diet A. The presence of iron could stimulate the production of red blood cells which will increase the level of RBC, Hb and PCV. Ekpo (2011) published similar findings that diets formulated with animal sources of protein and minerals had no major adverse impact on haematological indices of fish. The lower value of white blood cell (WBC) of the groups of fingerlings fed formulated diet A than the groups of fingerlings fed formulated diets (B and C) may be that formulated diet A did not contain toxic substances which may trigger the production of WBC. WBC are known to be produced whenever a foreign invaders or toxic substances enters the system of an organism.

The lower count for RBC, Hb and PCV observed in the groups of fingerlings fed formulated diet B and C in table 4.4 could be attributed to the decrease level of protein in the formulated diet B and may lead to inadequate nutrient requirements as protein are contributing factors of haemoglobin synthesis. This might have reduced the production

rate of red blood cell or rise its damage. The result of red blood cell count is not in agreement with the findings of Ayoola (2014).

The lower haemoglobin concentration of the groups of fingerlings fed formulated diets (B and C) may be attributed to deficiency of some minerals in the formulated diets. This outcome is similar with the results of Adewolu and Aro (2009). White blood cells are the defense cells of the fish body. The rise in white blood cells of the groups of fingerlings fed formulated diets (B and C) could be attributed to presence of toxic substrate in the formulated diet that may triggered the production of white blood cells in the haematopoietic tissue of the kidney in order to destroy foreign substances. This result is in agreement with the findings of Abalaka (2013). The lower packed cell volume of the groups of fingerlings fed formulated diets (B and C) may be ascribed to the presence of anti-nutritional factors, for example, haemaglutin in the formulated diets which affect blood formation and growth. Packed cell volume of the groups of fingerlings fed formulated diets (B and C) were lower than recommended range (22-38) % for fish as reported by Erondu *et al.* (2003).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The proximate composition of the selected ingredients that were determined in fish waste, groundnut cake, guinea corn and bone meal showed that higher proximate value of protein content was found in fish waste. However, the proximate composition, amino acid compositions and mineral content that was determined in the formulated diets revealed that higher proximate values, amino acid compositions and mineral content were found with formulated diet A compared to the formulated diets (B and C). More so, the groups of fingerlings fed formulated diet A had the highest growth performance for bodyweight and length measurement than the groups of fingerlings fed the formulated diets (B and C). The biochemical parameters result showed that there were significant increase in the level of ALP, AST and ALT values of the groups of fingerlings fed formulated diets (B and C) than the groups of fingerlings fed formulated diet A. The total serum protein of the groups of fingerlings fed formulated diet A were significantly higher than the groups of fingerlings fed formulated diets (B and C). The haematological parameters determined also showed that there was no significant haematological changes in RBC, WBC, PCV and haemoglobin of the groups of fingerlings fed formulated diet A. However, there were significant haematological changes in RBC, WBC, PCV and haemoglobin of the groups of fingerlings fed formulated diets (B and C). Formulated diet A yield a better results in terms of protein level, biochemical and haematological parameters as well as growth performance of catfish and it is therefore preferably as an alternative for (catfish) fingerlings rearing.

5.2 Recommendations

The followings are recommended;

i. Vitamin analysis to tell if they might be need for supplementation with a specific vitamin

ii. Digestibility of the formulated diets by the experimental animals in order to ascertain the amount of not consumed by fish.

iii. Heavy metal analysis in order to ascertain the presence of toxic substrate in the formulated diets.

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APPENDICES

APPENDIX A

Table 5.1: Proximate Composition of the Selected Ingredients

Proximate composition (%)	**Ingredients**			
	Fish waste	**Groundnut cake**	**Guinea corn**	**Bone meal**
Moisture	5.47±0.19^{c}	2.97±0.10^{a}	4.57±0.29^{b}	5.97±0.23^{d}
Lipids	6.83±0.22^{b}	24.83±0.12^{d}	2.83±0.55^{a}	16.57±1.09^{c}
Protein	59.90±0.26^{d}	48.00±0.26^{c}	11.80±0.31^{a}	18.32±0.24^{b}
Crude fibre	2.70±0.16^{a}	6.37±0.39^{b}	2.67±0.28^{a}	8.73±0.27^{d}
Ash	2.97±0.29^{c}	1.73±0.16^{b}	0.90±0.26^{a}	31.00±0.65^{d}
Carbohydrate	22.23±0.23^{c}	16.10±0.25^{a}	76.80±0.36^{d}	19.07±0.32^{b}

Values are means (± Standard error of mean) of triplicate determinations.

Values with different superscript in a row are significantly different from each other ($p<0.05$).

APPENDIX B

Table 5.2: Proximate Composition of Commercial Feed (Coppens) and Formulated Diets.

Proximate composition (%)	Feed	Formulated diets		
	Coppens	Diet A	Diet B	Diet C
Moisture	2.17±0.07[a]	2.97±0.27[b]	4.97±0.19[c]	6.07±0.26[d]
Lipids	12.17±0.07[d]	11.67±0.17[c]	11.10±0.06[b]	10.03±0.12[a]
Protein	44.83±0.17[c]	44.12±0.15[b]	42.47±1.63[a]	41.17±0.17[a]
Crude fibre	13.07±1.07[c]	13.02±0.07[c]	12.60±0.20[b]	12.07±0.37[a]
Ash	4.47±0.94[b]	4.43±1.45[b]	4.03±1.07[a]	4.04±0.47[a]
Carbohydrate	22.20±0.21[a]	23.57±0.17[b]	24.83±0.29[c]	26.33±0.17[d]

Values are means (± Standard error of mean) of triplicate determinations. Values with different superscript in a row are significantly different from each other ($p<0.05$)

Key: Diet A: Fish Wastes + guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

APPENDIX C

Plate III: Formulated diet

Plate IV: Fingerlings before experiment

Plate V: Fingerlings fed control diet

Plate VI: Fingerlings fed formulated diet A

Plate VII: Fingerlings fed formulated diet B

Plate VIII: Fingerlings fed formulated diet C

APPENDIX D

Table 5.3: Growth Performance of Catfish Fed on Commercial Feed and Formulated Diets for Bodyweight (g) Measurement

Weeks	Feed	Formulated diets		
	Coppens	**Diet A**	**Diet B**	**Diet C**
0	4.00 ± 0.12^{a}	4.03 ± 0.09^{a}	4.01 ± 0.16^{a}	4.01 ± 0.13^{a}
1	6.10 ± 0.06^{b}	6.07 ± 0.20^{b}	5.93 ± 0.14^{b}	4.90 ± 0.15^{a}
2	7.87 ± 0.16^{b}	7.85 ± 0.15^{b}	7.49 ± 0.10^{b}	6.17 ± 0.17^{a}
3	9.87 ± 0.13^{c}	9.76 ± 0.12^{c}	8.87 ± 0.19^{b}	8.23 ± 0.12^{a}
4	13.07 ± 0.09^{c}	13.07 ± 0.08^{c}	12.30 ± 0.25^{b}	11.37 ± 0.09^{a}
5	16.77 ± 0.23^{c}	16.73 ± 0.15^{c}	15.53 ± 0.22^{b}	13.93 ± 0.08^{a}
6	19.77 ± 0.21^{d}	18.30 ± 0.23^{c}	17.01 ± 0.15^{b}	14.63 ± 0.13^{a}
7	22.77 ± 0.15^{d}	21.93 ± 0.13^{c}	19.00 ± 0.29^{b}	17.73 ± 0.27^{a}

Values are means (± Standard error of mean) of triplicate determinations.

Values with different superscript in a row are significantly different from each other ($p<0.05$).
Key: Diet A: Fish Wastes + guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish wastes + Groundnut cake + Guinea corn + Additives

APPENDIX E

Table 5.4: Growth Performance of Catfish fed on Commercial Feed and Formulated Diets for Length (cm) Measurement.

Weeks	Feed	Formulated diets		
	Coppens	Diet A	Diet B	Diet C
0	3.07±0.26[a]	3.07±0.15[a]	3.05±0.13[a]	3.06±0.10[a]
1	4.87±0.13[c]	4.83±0.09[c]	4.17±0.32[b]	3.93±0.25[a]
2	6.60±0.11[c]	6.50±0.11[c]	5.20±0.10[b]	4.57±0.17[a]
3	8.27±0.13[c]	8.23±0.05[c]	7.83±0.23[b]	6.93±0.14[a]
4	9.27±0.23[b]	9.23±0.17[b]	8.33±0.17[a]	7.80±0.32[a]
5	10.67±0.23[c]	10.57±0.14[c]	9.47±0.24[b]	8.43±0.23[a]
6	13.53±0.67[d]	12.68±0.65[c]	11.53±0.29[b]	11.00±0.21[a]
7	15.07±0.25[d]	14.97±0.24[c]	13.54±0.21[b]	12.50±0.10[a]

Values are means (± Standard error of mean) of triplicate determinations.
Values with different superscript in a row are significantly different from each other ($p<0.05$).

Key: Diet A: Fish Wastes + guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish waste + Groundnut cake + Guinea corn + Additives

APPENDIX F

Table 5.5: Effects of Commercial Feed and Formulated Diets on Biochemical Parameters

Diet/enzyme activity	Commercial feed	Diet A	Diet B	Diet C
ALP (U/L)	32.45±0.12^{a}	33.00±0.23^{a}	38.01±0.14^{b}	45.01±0.13^{c}
AST (U/L)	33.45±0.16^{a}	34.90±0.18^{a}	45.01±0.12^{c}	49.01±0.14^{d}
ALT (U/L)	12.01±0.13^{a}	12.13±0.14^{a}	16.04±0.04^{b}	20.20±0.15^{c}
Total serum protein (g/dl)	20.35±0.13^{d}	19.52±0.21^{c}	17.46±0.20^{b}	16.01±0.13^{a}

Values are means (± Standard error of mean) of triplicate determinations.
Values with different superscript in a row are significantly different from each other ($p<0.05$).

Key: Diet A: Fish Wastes + guinea corn + Additives, Diet B: Groundnut cake + Guinea corn + Additives Diet C: Fish waste + Groundnut cake + Guinea corn + Additives