CHAPTER ONE

1.0 INTRODUCTION

The demand for animal protein in the human diet is increasing due to an increase in world human population, projected to reach 9.3 billion in 2050 (FAO, 2016 and; UN, 2010). Fish is the most-traded food commodities worldwide, supplying on the average, almost 20 per cent of daily protein required by up to 3.1 billion human (FAO, 2016). Global Fisheries and Aquaculture production must be sustained to meet the ever increasing per capital fish supply which reached a record high of 20kg in 2014 (FAO, 2016). On the average, twenty percent of total protein consumed in sub-Saharan Africa comes from fish and, accounts for as high as 80% of animal protein intake in the community around the coast (Adebo and Toluwase, 2014). In Nigeria, Fish contributes to over 40% of total dietary protein consumed due to its relatively cheaper price when compared to other types of animal protein (Akintola and Fakoya, 2017). While captured fisheries has been relatively static since the late 1980s and could not keep pace with human population growth, the growth of Aquaculture has continued to increase and plays crucial role in bridging the supply gap in fish production for human consumption (FAO, 2016).Total capture fisheries landings in 2014 supplied 94.6 million metric tonnes of aquatic product while aquaculture supplied 101.1 million metric tonnes (Tacon and Metian, 2017). Aquaculture production in Sub-Saharan Africa, excluding Nigeria, rose from 174000 tonnes in 1995 to 2437000 tonnes in 2014 and; increased in Nigeria from 16.6 thousand tonnes in 1995 to 3132000 tonnes in the year 2014 according to the record of FAO (2016).

Fish is one of the animal proteins that supply the crucial amino acids, vitamins, minerals and lipids required in the diet of human (FAO, 2014). These nutritional components help reduce the risk of cardiovascular diseases, arteriosclerosis and hypertension, in addition to aiding the adequate development of cells in the brain of a developing foetus and Intelligent Quotient (IQ) in developing children (Domingo, 2007). Fish also plays a significant part in poverty alleviation, food security and the general livelihood of human in most of the developing countries (FAO, 2014). Fish contributes to over forty percent (40%) of total dietary protein consumed in Nigeria due to its relatively cheaper price when compared to other types of animal protein

(Akintola and Fakoya, 2017). However, the total protein consumed in Nigeria is below the 75 gm of the daily per capital intake recommended by United Nations/Food and Agriculture organisation (Oladimeji, 2017). Fifty five percent of fish consumed in Nigeria was from domestic production between 1980 and 2013, while the remaining forty five percent was covered from importation (Kathleen *et al.,* 2017). The statistical survey reported by FAO (2012) and Oladimeji *et al.* (2013) showed that the local fish supply from artisanal fisheries and aquaculture in Nigeria could not meet the 1.5 metric tonnes of fish demand, leading to the annual importation of fish worth US$ 400 million. A recent report by Oladimeji (2017) further revealed that aquaculture sub-sector in Nigeria increased but could not keep pace with the rising demand. While supply of fish from artisanal fisheries is dwindling, aquaculture needs to increase in order to compensate for the reduce catch from over-exploited fisheries (Bosma *et al.*, 2011). However, inadequate input supply (particularly, local feed) is one factor, among many others which hinders sustainable development and profitability of fish farming in Nigeria (Abu *et al.*, 2010).

Feed is a principal operating cost in fish production accounting for over 60% of recurrent expenditure (Gabriel *et al.*, 2007). The demand for feed is increasing alongside increase in intensive aquaculture production (Agbo *et al.*, 2011). Feeds produced for fish are commonly manufactured to have high amount of fishmeal ranging from 32% to 40% (Richard and Chapman, 2007). Over dependency on fishmeal is non-sustainable and profitable feeding strategy. High cost of fishmeal and decline in fish population in the wild for fishmeal production characterise the economic and practical unsustainable utilisation of fishmeal as ingredient for production of fish feed (Naylor *et al*, 2009). Utilisation of fishmeal and other common feedstuffs in fish diets is further restricted by an increase in demand by human as food and other livestock industries (Jimoh *et al.*, 2014). Researchers have therefore realised the necessity to explore the utilisation of cheaper alternative protein ingredients which can replace conventional feed ingredients to produce a low-cost diet, without a compromise on the nutritional quality of feed. Some of these plant protein sources are: Soyabean meal incorporated in the feed of *Clarias gariepinus* as substitute for conventional fishmeal (Fagbenro and Davies, 2001); Lima beans based diet fed to *Oreochromis niloticus* (Adeparusi and Jimoh, 2002) and; the seed of Sesame plant

ground and incooporated in the feed of fish as a replacement for soyabean meal (Jimoh *et al.*, 2014).

Soybean is the most nutritive plant ingredient widely used in the production of livestock feeds (Orire and Ozoadibe, 2015). Tremendous success was recorded by researchers on the fractional or complete substitution of fishmeal for soybean meal (Kalla *et al.*, 2003). Consequently, up to 50% of diet formulated contains soybean protein (NRC, 1999) which supplies high level of balanced amino acids for the growth of livestock (Kopruku and Sertel, 2012). In the past, the increase in the drive to incorporate soybean in the diet of livestock was further strengthened by its low cost and availability (Robinson and Menghe, 2007). However, in recent times, researchers such as Obasa *et al.* (2006) and Alegbeleye *et al.* (2012) advocated for soyabean substitution with other ingredients of plant source in the diet of fish. The reason for the advocate was partly due to a rising price of soyabean resulting from competing demand by other livestock feeds and human nutrition (Bekibele, 2005 and Alegbeleye *et al.*, 2012); reduction in planting area and late season dryness which decline the yield of soybean (IITA, 1990) and; adverse influence of anti-nutritional components on fish (Jindal *et al.*, 2007).

Kenaf (*Hibiscus cannabinus* L.) is a tropical plant from mallow family (*Malvaceae*) (Rajashekher *et al.*, 1993) and native to the east-central Africa (LeMahieu *et al.*, 2003). Kenaf is primarily used as a source of energy (Alexopoulou *et al.*, 2004); pulp which is suitable for the production of rope, twine, sac, and rug (Coetzee *et al.*, 2008); thermal insulated board and; fibre used in reinforcement of thermoplastic composite (Lips *et al.*, 2009). The plant whose core is rich in cellulose is used in animal bedding as absorbent (Lips *et al.*, 2009).

Nigeria has over one million hectares of land suitable for production of kenaf which has the potential to produce 44 million tonnes of kenaf plant per year according to Akubueze *et al.* (2014). This is 5 – 10 times greater than conventional trees that reach maturity between 7 – 10 years (Akubueze *et al.*, 2014). Kenaf, in the recent past was cultivated alongside Jute in Nigeria for the production of bags used for the packaging of agricultural produce (Akubueze *et al.*, 2014). Currently, the increased cost of importation of jute bag as packaging material with 4 billion naira spent on importation

in 2015 (Tide, 2016); the awareness of diverse value chain in the use of kenaf and; foreign exchange earning derived from production of kenaf have led to the increase in the production of kenaf in Nigeria (Akubueze *et al.*, 2014). The foreign monetary earning derived from producing kenaf in Nigeria may have contributed to the advent of Kenaf Development Association of Nigeria (KEDAN) and Kenaf Producers, Processors and Marketers Association of Nigeria (KEPPMAN). According to the Raw Material Research and Development Council of Nigeria, the members of these associations are kenaf farmers and have cultivated kenaf in over 20 states of the Federation (Agronews, 2017).

The seed from kenaf has recently received attention in Nigeria by some researchers to be useful as livestock feed ingredient (Odetola and Eruvbetine, 2012). Kenaf (*Hibiscus cannabinus* L.) seed contains crude protein of 30.88% (Odetola and Eruvbetine, 2012) and a good source of vegetable oil (16 – 22%). The composition of fatty acids in Kenaf is comparable to cotton seed and contains large amount of phospholipids (3.9 – 10.3%), higher than that obtained in Soyabean (1.5 – 3.0%) and cottonseed (< 2.0%) (Patane and Sortino, 2010). Like Soyabean meal – an ingredient from plant origin - which has been extensively researched and successfully used in replacing fishmeal in the diet of culturable fish such as Tiger puffer (Lim *et al.*, 2011), Kenaf seed meal also stands the chance of being used as ingredient in the diet of *Clarias gariepinus* which is majorly cultured in Nigeria.

1.1 Justification of the Study

Prolonged viability of catfish industry relies majorly on operation cost in fish farming operations, of which enormous share are credited to feed (Da *et al.*, 2012). Forty percent of fish farming is dependent on industrial feed and the largest resources for the production of these feeds come from coastal and marine environment (New and Wijkstrom, 2002). When compared with poultry and pig feed, use of fishmeal in aqua feed is increasing (Chamberlain, 2011). Globally in 2009, around sixty three percent of fishmeal and eighty one percent of the oil extracted from fish was reported by Natale *et al.* (2013) to have been used in the production of fish feed.

However, cost of feed rises with an intensification in the utilisation of fishmeal in diet formulated for fish and, it is therefore, not a lasting approach to feed supply in fish farm (Naylor *et al.*, 2009). Researchers around the world have realised the necessity to explore alternate protein feed ingredient suited for fish cultured (Kader *et al.*, 2010). Approximately 20% to 40% fishmeal protein have been successfully substituted by plant proteins in the feed for carnivorous fish (Lim and Lee, 2008; 2009). Soybean is one of ingredients of plant protein origin reported by Halyer and Hardy (2002) to have been used in feed production and the most frequently studied and used in fish and livestock production (Storebakken *et al.*, 2000). However, soybean is beyond fish farmers and fish feed manufacturers reach due to its high cost, scarcity and high competition of use among various livestock farmers (Fasakin *et al.*, 2001). Soybean plant is insufficiently cultivated in tropical regions, in addition, increasing cost of importation and foreign currency exchange fluctuation further strengthen the practical unsustainable utilisation of soybean as ingredient in livestock industries (Ng and Chen, 2002). There are great economic and environmental sustainable incentives to identify and/or develop less expensive protein source to replace soybean in aquaculture feed. This view which will help ameliorate the pressure often experience from soybean shortage in Nigeria is similar to that of Odetola and Eruvbetine (2012). This will aim at reducing feed cost and thereby improve the economic viability of aquaculture industry (Da *et al.*, 2012).

Tremendous successes have been recorded by various researchers such as Fagbenro (2005); Olude *et al.* (2008) and; Alegbeleye *et al.* (2012) in the replacement of soybean with uncommon ingredient of plant origin. The potential of seed from Kenaf (*Hibisciuss canabinus*) plant as protein ingredient in the diet of livestock has also been identified by Odetola and Eruvbetine (2012). Kenaf seed is a novel plant ingredient and it is imperative to verify the viability of the ingredient as dietary component of fish feed. Despite the availability and the low cost of ingredients of plant origin relative to animal protein, their use comes with limitations (Agbo *et al.*, 2011). Research findings revealed that ingredients of plant origin have anti-nutritional components (Liener, 2003). The mineral and amino acid composition of ingredients of plant origin are not always sufficient or are not balanced (Kumar *et al.*, 2017). Moreover, high content of Non-Starch Polysaccharides (NSPs) are expressed in ingredients of plant source which

reduces nutrient digestibility/bioavailability (NRC, 2011) and Ghazalah *et al.*, 2012) and absorbtion (Sinha *et al.*, 2011).

Odetola and Eruvbetine (2012) reported the presence of phytate, tannin and trypsin inhibitor in the seed from kenaf which can reduce its nutritional value. There is need to investigate the processing techniques required to reduce anti-nutritional constituents in kenaf seed. High dietary inclusion of plant ingredients occasioned by high fibre results in improper growth and inefficient utilisation of feed as evidence from the study of Sotolu (2010) and; Adesina (2017). There is therefore a need to establish the optimum dietary inclusion level of Kenaf Seed Meal (KSM) that will support the growth of catfish *Clarias gariepinus*.

1.2 Main aim of the study

The general objective of this study was to evaluate the utilisation of Kenaf (H*ibiscus cannabinus*) seed meal as a replacement for soyabean meal in the diet of Catfish (*Clarias gariepinus*) juvenile.

1.3 Specific Objectives

The specific objectives of this research were to:

1. characterise the chemical composition and nutrient digestibility of raw and differently processed kenaf (*Hibiscus cannabinus*) seed
2. assess the growth performance and nutrient utilisation of African catfish (*Clarias gariepinus*) fed processed kenaf (*Hibiscus cannabinus*) seed meal based diets
3. assess the heamatological, biochemical and histopathological response of African catfish (*Clarias gariepinus*) fed processed kenaf (*Hibiscus cannabinus*) seed meal based diets
4. evaluate the economic benefits of replacing soyabean meal with processed kenaf (*Hibiscus cannabinus*) seed meal in the diet of African catfish (*Clarias gariepinus*).

1.4 Hypothesis

H_0 – There is no significant variation in the chemical composition and nutrient digestibility coefficient of raw and differently processed kenaf (*Hibiscus cannabinus*) seed.

H_0 - There is no significant variation in the growth performance and nutrient utilisation of African catfish (*Clarias gariepinus*) fed graded levels of processed kenaf (*Hibiscus cannabinus*) seed meal based diets.

H_0 -There is no significant variation in the heamatological, biochemical and histopathological response of catfish (*Clarias gariepinus*) fed graded levels of processed kenaf (*Hibiscus cannabinus*) seed meal based diets.

H_0 – there is no significant variation in the economic benefit of replacing soyabean meal with differently processed kenaf (*Hibiscus cannabinus*) seed meal in the diet of African catfish (*Clarias gariepinus*).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.1 Catfish (*Clarias gariepinus*) as Culturable Fish Specie

Catfishes are classified in the order *Siluriformes*. The fish of the family *Clariidae* to which *Clarias gariepinus* belongs are characterised by a huge armored head, without the presence of spine in the dorsal fin. The fish also has a long base, a long anal fin, presence or absence of a long adipose fin and a suprabranchial organ for air breathing. This family consist of three genera in Nigeria freshwater, namely; *Clarias*, *Heterobranchus* and *Gymnallabes* (Adesulu and Syndenham, 2007). Based on the work of Tuegel (1982), the genus *Clarias* in Nigeria Freshwater consists of five sub genera, namely; *Clarias*, *Clariodes, Anguilloclarias, Platycephaloides* and *Brevicephaloides.* The Clariid catfish is mostly found in quiet water, lakes and pools (Teugel, 1986). They may also occur in fast flowing rivers (Seegers, 2008). *Clarias gariepinus* is known to have good tolerance for environmental extremes (Gunder, 2004). They are very adaptive to pH range of 6.5 – 8.0, temperature of 23 – 30 degree Celsius and can tolerate high turbidity (Teugels, 1986). *Clarias gariepinus* are bottom dwellers and are obligate air breathers (Gunder, 2004). *Clarias gariepinus* feeds on various food organisms from plankton to fish (Burgess, 1989). It can efficiently graze on zooplankton and phytoplankton and algae and will also consume higher plants and dentritus (Gunder, 2004). The variety of feeding habits which have been ascribed to this fish do substantiate the notion that *Clarias gariepinus* although euryphagic is an opportunistic omnivore (Hecht and Andrew, 2008), some authors are in agreement, however that *Clarias gariepinus* although euryphagic, is predominantly a predator (Gunder, 2004).

2.1.2 Functional Morphology of the Digestive System of *Clarias gariepinus*

Gross anatomical adaptations of *Clarias gariepinus* for feeding allow it to take prey ranging in size from minute zooplankton to higher organisms such as fish (Bruton, 1979). The wide mouth, large gape and barbells facilitate bottom feeding in turbid water. In addition, the well-developed gill rakers allow efficient filtering of surface scum and mid water organisms. The digestive tract of the fish contains cellular lining with the enterocytes that partakes in the digestion and absorption; the oxynticopeptic

cells which are acid and enzyme-producing cells; the globlet cell (secretes digestive enzymes and mucus) and; endocrine cells that coordinate digestion and absorption processes (Rust, 2002). The oesophagus in fishes functions in transferring eaten food via the mouth and pharynx to the stomach (Stevens and Hume, 1995). The oesophagus is highly elastic and usually, short, wide and straight which has mucus secreting cells to lubricate the flow of materials across the alimentary canal (Stevens and Hume, 1995).

The breakdown of food begins from the stomach containing digestive enzymes and gastric juices (Clements and Raubenheimer, 2006). The oxynticopeptic cells generating pepsin and hydrochloric acid and; the endocrine cells that secrete hormones and mucous cells are present in the gastric mucosa (Rust, 2002). In the stomach, food is crushed and tumbled by the muscular stomach wall. The stomach stops in the pyloric sphincter which monitors the movement of food in the intestine (Rust, 2002). The intestine is a simple, thin walled and relatively short, implying that the fish depends on food rich in protein (Uys, 1989). These adaptations and the opportunistic feeding habit of the fish imply that feed can be presented in a variety of ways and still be utilised efficiently.

2.1.3 Biological Processing of Ingested Feed in Fish

The food eaten by fish undergoes a mechanical and chemical processing (Clements and Raubenheimer, 2006). The oral jaw, pharyngeal apparatus and gizzard-like stomach are involved in the mechanical processing of food in fish. The oral jaw is primarily used for capturing prey before being transferred into the pharynx (Friel and Wainwright, 1999). The pharyngeal apparatus consists of pharyngeal teeth that partake in the secondary mechanical processing of food. The pharyngeal teeth help in tearing and grinding of food eaten by the fish (Xie, 2001) before the food is transferred to the oesophagus. The gizzard-like stomach is used to triturate bacteria, microalgae and macroalgae that are ingested by fish along with inorganic sediments (Lobel, 1981).

The acidity in the stomach and intestine and, the endogenous digestive enzymes in fish perform a vital function in the chemical processing eaten food items in fish. The gastric acidity helps breakdown the cell wall in algae (Horn, 1989). The pH of 3 and 4 rupture the plasma membrane of algae (Zemke-White *et al.*, 1999) while pH 3

increases porosity in algae to encourage the entrance of digestive enzymes into the cells (Zemke-White *et al.*, 2000). Lipid emulsification and, protein and carbohydrate denaturation occur at a low stomach pH, facilitates the activities of digestive enzymes and causes the catabolism of protein in feed to amino acids (Bowen, 1980). Tannin, an anti-nutritional factors in ingredients of plant origin form complex with protein and reduces the bio-availability of protein, high intestinal pH reduces the formation of this protein-tannin complex (Stern *et al.*, 1996).

Carbohydrate eaten in the food of fish is hydrolysed by the endogenous enzyme, polysaccharidase to produce oligosaccharide and monosaccharide (Kuz'mina and Gelman, 1997). The polysaccharidase involved in carbohydrate hydrolysis are amylase, chitinase, and laminarinase (Sabapathy and Teo, 1993). Endoproteases and peptidases are enzymes involved in the hydrolysis of protein (Clements and Raubenheimer, 2006). Endoproteases such as pepsin, chymosin, trypsin, chymotrypsin, elastase and collagenase hydrolyses the peptide ponds within a protein to produce oligopeptides without cleaving the terminal amino acid (Clements and Raubenheimer, 2006). Peptidases however, cleave the termina amino acids or dipeptides off the peptide chain (Kuz'mina and Gelman, 1997). Lipids are emulsified (e.g. by bile salt) before being hydrolysed by lipases which only acts at a liquid-water interface (Stevens and Hume, 1995).

2.1.4 Absorption of Nutrients in Fish

The mucosal lining of the gastrointestinal tract forms a barrier between external and internal environment in fish (Buddington and Krogdahl, 2004), regulating the nature of substance absorbed, amount absorbed and the rate of absorption (Clements and Raubenheimer, 2006). Absorbtion of nutrients takes place in the postgastric region at the pyloric cecea, upper and lower part of the intestine (Clements and Raubenheimer, 2006). Columnar epithelium is the tissue involved in the absorption of nutrients where the nutrients transit from lumen in the gut through the epithelia suface lined with mucous cells to the lymph and blood (Clements and Raubenheimer, 2006). The movement of nutrients across the basolateral and apical membrane can be mediated by specialised membrane-bound carrier molecules or via a passive diffusion (Clements and Raubenheimer, 2006).

The absorption of carbohydrate monomers is transported actively across apical membrane of enterocytes by sodium glucose co-transporters (SGLTs) which are an energy-dependent carrier. Amino acids are also absorbed across the apical membrane through active carrier-mediated transport and passive diffusion cross the apical membrane (Maffia *et al.*, 2003). Intact protein are transported across the apical membrane by the means of enterocytes (Sire and Vernier, 1992). In the case of lipid, the transport of lipid across the apical membrane is by simple diffusion (Clements and Raubenheimer, 2006).

2.1.5 Dietary Requirement of *Clarias gariepinus*

2.1.5.1 Protein Requirement

Uys (1989) reported that the propensity of *Clarias gariepinus* towards a carnivorous feeding habit seems to indicate that the dietary protein required by *Clarias gariepinus* is relatively high. Fish is probably the most important supplier of dietary lipids and, is therefore very likely that its digestive system is geared towards utilizing polyunsaturated fatty acids as source of energy. The animal being able to also feed on plant materials reflects the capability to metabolise proteins in plant and utilize carbohydrate as an energy source. The ideal growth rates and efficiency of converting feed to body flesh in juvenile *Clarias gariepinus* are obtained in a diet having 38 to 42 percent protein in crude form and optimum 10-11% fat (Uys,1989). According to Uys, (1989) no conclusive results are available yet on the dietary vitamin and mineral requirements of *Clarias gariepinus.*

2.1.5.2 Amino Acid Requirement

Ten indispensible essential amino acids are required by fish. The amino acids requirement for channel catfish (*Ictalurus punctatus*) determined by Wilson and Poe (1985) are presented in Table 2.1. The lysine and methionine requirement for *Clarias gariepinus* was estimated to be 5.7 and 3.2% of protein respectively (Fagbenro *et al.*, 1998a and; Fagbenro *et al.*, 1998b).

2.1.5.3 Energy Requirement

Fish requires energy which is essential for growth and reproduction, and this comes from protein, fats and carbohydrate through the process of oxidation. The energy needs of fish are comparable to the warm-blooded animals but, the amount required is also

Table 2.1: Amino acid requirement (% protein) for channel catfish (*Ictalurus punctatus*)

Amino acid	Requirement
Methionine	2.92
Phenylalanine	4.14
Tryptophan	0.78
Leucine	7.40
Valine	5.15
Lysine	8.51
Arginine	6.67
Histidine	2.17
Threonine	4.41
Isoleucine	4.29

(Wilson and Poe, 1985).

influenced by species, sex, age, activity and water quality. Fish preferentially use protein and fat for energy and carbohydrate sparingly. Starter diet for catfish should contain 36-40% with at least one-half coming from fish meal, and 3.0-3.5kcal of digestible energy (DE) per gram, with most coming from protein and fat (Lovell, 1989).

2.1.5.4 Dietary Carbohydrate to Lipid Ratio in Catfish

Fish requires carbohydrates and lipid as energy sources and should be incooporated in the diet at suitable level to maximise the utilisation of protein in the diet for optimum growth. Growth performance, utilisation of nutrients and body composition of fish are influenced by imbalance non-protein energy sources and their inclusion level (Ali, 2001). Further more, level and the forms of dietary non-protein energy required in fish feeds are not fully understood. Interestingly, dietary carbohydrate requirement in fish has not been demonstrated, although, certain fish species display growth retardation when fed diet free of carbohydrate (Ali, 2001). In iso-nitrogen and iso-energetic diet testing, the ability of fish to exhibit sparing effect of protein is reflected in several ratios of carbohydrates to lipid, varying net protein utilisation and protein efficiency ratio. Generally, utilisation of protein and its efficiency peak at a point between extreme of carbohydrate and lipids congregation, sometimes closer to the extremes of carbohydrate and lipid (Ali, 2001). Lipid utilisation in Rainbow trout and Tilapia is better than carbohydrate, while carbohydrate utilisation is better in *Oreochromis niloticus* compared to lipid. The disparity in these results is a reflection of the capabilities of fish to utilise carbohydrate, as well as the various utilisation of ranges of mixture between carbohydrate and lipid and; dietary adequate ratio of carbohydrate to lipid (Ali, 2001).

2.1.5.5 Vitamin and Mineral Requirement

Deficiencies have been found to cause loss of appetite and depressed growth in fish. As observed by Dupree and Huner (1984), discoloration, lack of co-ordination, nervousness, haemorrahages, lesions, and increased susceptibility to bacterial infection are the other symptoms of vitamin deficiency. So far, fish is known to require eleven water soluble and four fat-soluble vitamins. Cho *et al.*, (1985)

Table 2.2: Apparent Digestibility Coefficient (ADC) of some ingredients for rainbow trout.

Ingredient	ADC (%)	
	Crude protein	Energy
Fish meal	77	91
Meat and bone meal	85	80
Feather meal	77	77
Rapeseed meal	77	45
Brewer's dried yeast	91	77
Soyabean protein concentrate	97	84
Soyabean, full-fat, cooked	96	85
Corn yellow	95	39

Adopted from Wilson (2012).

explained that most of the water soluble vitamins function either directly or in a modified form as enzymes. Minerals are necessary for the maintenance of balance between salt and water in the tissue of fish for the metabolism of other nutrients and major structure element. Beveridge (1984) reported that phosphorus is the most essentially required mineral by all fish for adequate growth and bone development, acid-base regulation, metabolism of lipid and carbohydrate. Requirement of phosphorus ranged between 0.29 – 0.9% depending on the species and dietary sources. Some other minerals that may be required by fish are potassium, chloride and sodium and; also minor minerals such as, zinc, aluminum, cobalt and chromium (Cho *et al.*, 1985). Minerals and vitamins are usually included in the feed formulated for catfish as per the requirement, on the assumption that they are the same.

2.1.6 Nutrient Digestibility

Animal must be able to catabolise and utilise the chemical energy deposited in food items for life-sustaining processes (Bureau *et al.*, 2002). Life-sustaining processes includes but no limited to anabolic reactions, muscular contraction and active transport. Feed consumed by fish must be digested and absorbed before it can serve as fuel for life-sustaining processes (Bureau *et al.*, 2002). The components present in feed can resist digestion and move across the digestive tract to be egested as faecal matter (Bureau *et al.*, 2002). The measure of difference between nutrient intake in the feed and nutrient in faecal material gives the digestible nutrients (NRC 1981). The measurement of nutrient digestibility therefore relies on the collection of representative faeces, using indicator to avoid the necessity to determine dietary intake and faecal production (Bureau *et al.*, 2002). The apparent crude protein and energy of some feed ingredients are presented in Table 2.2.

2.1.7 Feed Formulation

Similar to other livestock industries, feed formulation in catfish relies mostly on the use of fixed formular with reduced rate of adoption of least-cost approach. The least-cost computer programming approach in feed formulation requires adequate information on cost of ingredients, nutrient requirement of animal, amount and availability of nutrients in feed ingredient and nutritional and non-nutrient restrictions.

The limitations that come with the use of least-cost feed formulation include: inadequate information on the level of nutrient that could result into a maximum gain and; difficulty in the storage large number of various ingredients in order to obtain wide assortment of feedstuffs at all time.

Feed manufacturers put mixture of feed additives and feedstuffs into a useable form. The main goal in mixing feedstuffs is to increase profit margin in the production of animal by increasing their nutritional value. This process may include the reduction of particles size of ingredients forming feed pellets through extrusion or steam pelleting. Unlike other terrestrial livestock, the mixture of ingredient to form pellets, water stability and ability of feed to float in water characterise the uniqueness of Catfish feeds. Therefore, most of the commercial catfish feeds are produced by extrusion (Jobling *et al*., 2001). In the case where feeds additive cannot withstand extrusion, the steam pelleting methods are adopted to produce feeds that generally sink in water. Dusts coming from manufactured feed are reduced before shipping by spraying with lipids (Jobling *et al*., 2001). The nutrients in the catfish feeds must be adequate and highly digestible in order to enhance the growth of fish.

2.2.0 Agronomic and Nutritional Potential of Kenaf Plant

2.2.1 Botanical Classification

Kenaf (*Hibiscus cannabinus* L.) belongs to the mallow (*Malvaceae*) family. Okro and cotton seed as also categorised under this family. Kenaf is a plant of east-central Africa, tropical and sub-tropical Africa and Asia where it is cultivated for a long time for food and fiber (Le Mahrew *et al.*, 1991). Products such as rope, twine, bagging and rug are manufactured from textile fiber made from kenaf.

2.2.2 Planting

In most areas, April to May are suitable for the planting of Kenaf. It can be planted on a bed or flat ground in a wide range of row spacing. The slated-back of kenaf seed are about 6mm long. One kilogram of kenaf seed contains 33,000 – 40,000 seeds/kg. Relative to size, seed from kenaf plant and grains of sorghum (*Sorghum bicolour* L) are comparable, allowing farmers to use same planting plates for them

in large scale cultivation. The seed of kenaf germinates within two to four days after planting when planted at a depth of 1.25 to 2.5cm (Webber and Bledsoe, 2002).

2.2.3 Planting Population

Maximum desireable yield of kenaf with single stalk having very little or no branching can be achieved with population of 185,000 to 370.000 plants/ha (Cook and Scott 1995). According to Cook and Scott (1995), less than 185,000 plants/ha plan population often results into a decreased stalk yield with multiple branches. Multiple branches in plants make mechanical harvesting difficult.

2.2.4 Fertility

Dempsey (1975) suggested that Kenaf thrives well in wide variety of soil types, ranging from sandy desert soil to high organic peat soil. A well-drained soil with a pH of 7 supports the growth of kenaf. The plant tolerates late season flooding and low soil fertility (Dempsey, 1975). Kenaf can also tolerate a drought condition. According to Webber (1996), optimal Kenaf yield with minimize production cost can be achieved in well fertile soil supplemented with nitrogen. As a result of inherent variability in soil types relative to soil fertility, organic matter, soil texture and pH, diverse reports exist on the response to kenaf plant to fertilizer application. Ching and Webber (1993) reported that nitrogen application did not support the stalk yield in a silty clay soil and on silty clay loam.

2.2.5 Weed Control

Though, kenaf flourishes rapidly and compete favourably with weeds, post-planting weed control is always required. Burnside and Williams (1994) reported that kenaf impedes the growth of weeds by forming a shade on the ground with the leaves. Among the seven tested herbicides by Burnsside and Williams (1994), kenaf was most tolerant to trifluranlin and provides an excellent control of weed. Researchers have consequently adopted the use of Trifluralin as the effective herbicide for Kenaf and has been authorised for controlling weeds in Kenaf cultivated for fibre.

Trifluralin and metolachlor applied at the rate of 0.9 – 1.7 and 3.4 kg ai/hectares, correspondingly provided 90 percent and 80 percent grass control, respectively

(Hickman and Scott, 1989). Metolachlor applied at 3.0kg ai/ha caused unnoticeable damage to the Kenaf, although the herbicide may cause a remarkable reduction in stalk yield (Kurtz and Neill, 1990). Un-branched densely planted Kenaf plants can reach a height of 8 – 14 feet and, under normal condition could reach 20 feet.The stem (outer bark) has soft lengthy best fiber which is a raw material for textile production and cottage industries. Stem of most varieties of kenaf has green colour, but several other varities have red stemmed accessions. Relative to the shapes of the leaf, while the leaves are not lobed in some varieties, others develop leaves that are very deeply lobed at post-juvenile stage of development. The plant has an extensive deep root system.

2.2.6. Nutritional Potential of Kenaf Seed

Kenaf is typically known to be a fiber crop (Webber *et al.*, 2002). The plant stalk (core and bark) and leaves are can be used as livestock feed (Webber et al., 2002), The crude protein in the kenaf leaves and the stalk range from 14 to 34% (Swingle *et al*., 1978) and 2 – 12% (Webber, 1993a), respectively. The digestibility of crude protein in kenaf fodders was reported by Suriyajantratong *et al* (1973) to range from 59 to 71%. The performance of sheep fed rice ration supplemented with kenaf meal compared favourably with Alfalfa meal (Suriyajantratong *et al* (1973). The protein in kenaf seed varies between 24 and 30% on dry matter basis (Rajashekher *et al.*, 1993). The seed from kenaf plant has equally been pointed out by researchers as being a potential source of protein (Rajashekher *et al.*, 1993). This is due to nutritional components (Table 2.3) and the amino acid profile in kenaf seed (Table 2.4). Odetola (2013) reported a positive performance in broiler fed 10% dietary inclusion of kenad seed meal. Kenaf seeds have a relatively high oil content of 29.2% comprising of linoleic (45.9%) as the predominant fatty acid, while palmitoleic (1.6%), linolenic (0.7%), and stearic (3.5%) are the major fatty acid (Webber and Bledsoe, 2002). The oil content in kenaf is high and comparable in quality with that of cotton seed oil which suggests that the oil in kenaf is edible (Webber and Bledsoe, 2002).

Table 2.3: Mean value of the chemical compsotition (% dry matter basis) of kenaf seed and soyabean seed

Parameter	Kenaf seed*	Soybean seed**
Crude protein	25.12	37.08
Crude fat	18.89	18.38
Crude fiber	25.61	5.12
Ash	4.40	4.89
Nitrogen Free Extract (NFE)	19.79	24.00

*Olawepo *et al.* (2014) and ** Banaszkiewicz (2011)

2.3.0 Anti-Nutritional Factors in Plant Seed and Method of Removal

A vast range of primary (nucleotides, lipids, organic acids and amino acids) and secondary metabolites are synthesised by plants (Crozier *et al.*, 2006). The Secondary metabolites or phytochemicals such as saponin, tannin, protease inhibitors, alkaloids and oxalate that are naturally synthesised in most plants function as defence against herbivorous animals, regulator of symbiosis and control of germination processes (Makkar *et al.*, 2007). However, their role as a toxic substance in fish nutrition has been widely studied and documented by various researchers such as Falaye *et al.* (2016), and Olude *et al.* (2016). Anti-nutritional constituent in plants impose adverse effects on the physiology of fish (Table 2.5) and, when present at nontoxic amount, reduce nutrient digestibility and palatability (Makkar *et al.*, 2007). Depending on the amount ingested, some anti-nutritional factors have beneficial effect on animals, such as being an antioxidant and immunostimulatory (Krogdahl *et al.*, 2010). Secondary metabolites have also been useful in the production of flavouring agents, dyes, oils, antibiotics, insecticides and herbicides (Dewick, 2002).

2.3.1 Protease Inhibitor

Protease inhibitors are protein-like substances found in plants, particularly among legumes which inhibit the activities of the proteolytic enzymes (Liener and Kakade, 1969). The inhibitors of protease are found predominantly in the seed of various plants (Liener and Kakade, 1969). They possess polypeptide chain of about two hundred amino acids having 20000 to 250000 Da for Kunitz inhibitor and 6000 to 10000 Da for Bowman-Birk inhibiotors (Liener and Kakade, 1969 and; Makkar *et al.*, 2007). The soybean trypsin inhibitor, for example, has an N-terminal (Sequence: Asp-Phe-Val-Leu-Asp) (Ikenaka *et al.*, 1963) and a leusine residue C-terminal amino acid (Davie and Neurath, 1955).

The interaction of trypsin with the inhibitor produces an inhibitor-enzyme complex detected through modification in the tryptophan and tyrosine residue by physical measurements such as ultraviolet spectra and fluorescence and optical rotations (Edelhoch and Steiner, 1963). The inhibitor-enzymes complex formed reduces the quantity of protease and thus, invokes an adaptive response of hyperactive in pancreas

Table 2.4: Composition of amino acid (g/kg/ protein) in Kenaf seed and soybean seed

Amino acids	Kenaf seed	Soybean
Arginine	96	71
Histidine	27	26
Isoleucine	47	61
Leucine	80	86
Lysine	49	65
Methionine	16	15
Cystine	21	13
Phenylalanine	51	53
Tyrosine	36	39
Threonine	34	40
Tryptophan	13	14
Valine	50	53

Fagbenro, 2005.

to intensify the production of digestive enzymes (Gilani *et al.*, 2011). This abnormal physiological condition leads to enlargement of pancreas and reduction of endogenous amino acids (Friedman and Brandon, 2011) and nutrients excreted unabsorbed or absorbed belatedly to be useful by the animal (Liener and Kakade, 1969). The consequence of such loss of amino acids is reflected in growth depression experienced by animal under the dietary influence of trypsin inhibitor (Gilani *et al.*, 2011). Trypsin inhibitors are inactivated in feedstuff through heat treatment such as boiling in water and toasting (Liener, 2003). This is effective because trypsin inhibitors are among the anti-nutrient components that are unstable to heat (Udensi *et al.*, 2004).

Boiling and roasting reduced the trypsin inhibitor in asparagus beans (Nzewi and Egbuonu 2011). The trypsin inhibitor in cowpea and mung bean significantly reduced when roasted in sand bath at a temperature of 200 ± 2^{0}C for 2 minutes (Bilal *et al.*, 2017). The effectiveness of heat treatment in reducing trypsin inhibitor has also been documented in the research done by Seena *et al.* (2006). Sprouting was reported by Burbano *et al.* (1999) to reduce trypsin inhibitor in plant seed. Sangronis and Machado (2007) recorded a decrease in trypsin inhibitor when *Phaseolus vulgaris and Cajanus cajan* seeds were sprouted.

2.3.2 Phytic acid

Phytic acid is a cyclic compound with the molecular formula 1,2,3,4,5,6-hexakis dihydrogen phosphate myoinositol (Makkar *et al.*, 2007). Phytic acid exists as phytate salt in cereals, legume and oilseeds functioning majorly as phosphorus storage (over 80% composition in legumes and oilseed) in these crops (Reddy and Sathe, 2002). Phytate is found predominantly in protein-rich aleuronic layers of monocotyledon and uniformly distributed in the seeds of dicotyledon plants (Raviridan *et al.*, 1999). Phytate forms a chelate with di-valent minerals such as iron, thereby making them unaccessible for use by the animal for normal physiological processes (Reddy and Sathe, 2002). Phytate also chemically reacts with positively charged proteins and mineral in feeds, making them to be insoluble and difficult to hydrolyse during digestion and absorption (Weaver and Kannan, 2002). Aside the chelation formed between phytate and divalent ions, the insoluble peptide-mineral-phytate complex

Table 2.5: Effect of purified plant anti-nutritional chemicals on fish

Substance investigated	Fish species	Inclusion rate (%)	Biological effect	References
Phytic acid	Rainbow trout	0.5	Depression in growth and food conversion efficiency	Spinelli *et al.*,1983
Tannic acid (hydrolysable tannin) and quebracho tannin (condensed tannine)	Common carp	2	Condensed tannin did not affect the performance of fish but hydrolysable tannin had adverse effects after 28days when it completely supressed feeding	Becker and Makkar, 1999
Soybean protease inhibitors	Rainbow trout	0.37,0.74,1.11 or 1.48	Increased trypsin inhibition with increased inclusion; this was partly compensated by increased enzyme secretion and absorption by the intestine; the compensation was complete at lower levels of the inhibitor	Krogdahl *et al.*,1994
Purified alcohol extract of soybean meal (PAES) or soy protein isolate (SPI) active principle being soy saponins	Chinook salmon	1or 0.3	Suppression of feed intake and growth in both cases	Bureau *et al.*,1998

Excerpt from Francis *et al.* (2001

formed reduces the bio-availability of proteins and enzymatic activities (Deshpande and Cheryan, 1984). Consequentially, the insolubility nature of these complexes may compel the animals to egest faecal dropping containing substantially high amount of the unutilized nutrient components (protein and mineral) of the feed. Phytate needed to degrade the complexes are insufficiently produced in the gastro-intestinal tract of the monogastric animals and adverse physiological effects are expressed in animals consuming feed containing high amount of phytate (Reddy and Sathe 2002). Growth of fish is adversely retarded when fed diets made from ingredients having relatively high phytate content (Francis *et al.*, 2001). Treatment method that increases the activity or amount of reactive phytase may be effective in lowering the level of phytate. Fermentation and germination increase the phytase activity in plant seed and have been reported by Liener (2003) to reduce the phytic acids in legumes. In his opinion, the phytase activity probably increased due to the activity of various molds, bacteria and yeasts involved in fermentation process. Similarly, phytate activities also increase in germinating seed of legumes (Liener, 2003). Fermentation and germination was reported by Bulbula and Urga (2018) to reduce the phytate activity in chickpea. Heat treatment was reported by Hossain and Jauncey (1990) to lessen the phytate in linseed and sesame meal. This reduction may have resulted from the leaching of phytate into the cooking medium (Sidhtraju and Becker, 2001). Similar reduction in phytate was reported by Gemede (2014) when Anchote tubers were boiled for 3 hours.

2.3.3 Tannin

Tannins are polyphenolic compounds, broadly categorized into hydrolysable and condensed tannins (Makkar *et al.*, 2007). Tannins bind digestive enzymes and dietary protein (Fig 2.1) and mineral components in the feedstuff making them unavailable for physiological needs (Liener, 1989). They also decline the rate of absorption of vitamin B_{12} (Francis *et al.*, 2001). Consumption of feed containing condensed tannins results into decreased nutrient utilisation and intake of feed (Francis *et al.*, 2001). Hydrolysable tannins were also reported by Makker *et al.* (2007) to cause mortality in animals through damage on the liver and kidney. This is because hydrolysable tannins are bio-degradable by animals to form smaller toxic molecule that can enter the liver and kidney (Francis *et al.*, 2001). Condensed tannins in copra bean based diets caused

growth depression in tilapia as reported by Jackson *et al.* (1982). Griffiths (1991) recommended dehulling, autoclaving and treatment with alkali as effective methods of removing tannins from seeds. Mugbabo *et al.* (2017) also reported the effectiveness of sprouting, roasting and cooking in reducing the tannin content in climbing bean flours after the bean was pre-treated with steam blanching, soaking in water or soaking in alkali. The reduction in tannin content of cooked and soaked bean flours was linked to leaching into the treatment medium by these researchers. The authors also attributed the diminution in the tannin content in germinated climbing bean flour to the activation of the hydrolysable enzymes, polyphenolase, which breaks down various compounds in germinating seeds. Similarly, a reduction in tannins was recorded for soaked and sprouted horsegram flour (Handa, 2017). The tannin content in soybean flour also reduced when cooked and roasted (Agume *et al.*, 2017).

2.3.4 Saponin

Saponin is steroid or triterpenoid glycoside present mostly in ingredients of plant derivative (Francis *et al.* 2001) with a characteristic bitter-taste (Shi *et al.*, 2004). Saponins produce foam in aqueous solution and are very toxic to fish due to their ability to destroy the epithelia surface of the gill (Francis *et al.*, 2001) and also disrupt the membrane of red blood cells (which may induce haemolysis), if ingested (Crozier *et al.*, 2006). High mortality was recorded within 5 to 6 hours when tilapia fish were exposed to 100ppm aqeous solution containing *Camellia sinensis* having saponin content of 7 – 8 percent (De *et al.*, 1987). The severe damage was recorded in the intestinal mucosa lining of salmon and trout fed 1.5g/kg *Quillaja* bark (Bureau *et al.*, 1998).

The researchers implicated saponins to have been responsible for the damage through its reaction with the biological membranes. Furthermore, saponin form complexes with protein and lowers the bioavailability of protein for growth (Potter *et al.*, 1993). Digestibility of protein in soybean decreased due to the presence of endogenous saponin (Shimoyamada *et al.* 1998). Sprouting, cooking and soaking are effective in reducing saponins in plant seeds (Jood *et al.*, 1986). These processing methods possibly chemically and enzymatically degrade the saponins in plant seeds or transform saponins to other compound (Fenwick and Oakenfull, 1983). The saponin

content of navy beans significantly reduced when cooked (Shi *et al.*, 2009). Cooking and pressure cooking of lenils significantly reduce the sapogenol level in the plant seed (Srivastava and Vasishtha 2013). Soaking and germination was also reported to be active in reducing the saponin content in pigeon pea.

2.3.5 Alkaloid

Alkaloids, a nitrogen-containing compounds mostly derived from amino acids are secondary metabolite having a low molecular weight, (Crozier *et al.*, 2006). Over 12,000 alkaloids have been detected in plants (Crozier *et al.*, 2006) and exploited as stimulant, depressants, narcotics, hallucinogenic agents and poisons ((Liener and Kakade, 1969). Alkaloids in fresh leaf and fruits material are known to impart bitter taste (Makkar *et al.*, 2007). This bitter taste may probably lower rate of feed cosumption in fish (de la Higuera *et al.*, 1988). Reduction in alkaloid content of seed can be achieved through sprouting (Dagnia *et al.*, 1992). Contrary to the report of Dagnia *et al.* (1992), sprouting of lupin for 96 hours was not effective in reducing alkaloid content in the seed (de la Cuadra *et al.*, 1994). Also, sprouting and fermentation significantly increase alkaloid content in

newly developed lupin seeds but, boiling of the fermented seeds significantly decreased the level of alkaloid (Mohammed *et al.*, 2017). The alkaloid content in *Bosqueia angolensis* seed significantly reduced when cooked (Nwosu, 2011). Soaking in brine, in running water or by scalding was reported by Erbas *et al.* (2005) to be effective in reducing alkaloid content in seed. Similar reduction in alkaloid level was reported by Yeheyis *et al* (2014) for soaked local white lupin seed. Roasting increased the content of alkaloid in *Tamarindus indica* seed when compared to the raw seed of *Tamarindus indica* (Bashir *et al.*, 2016). The authors attributed the reduced quantity of the alkaloid in raw *Tamarindus indica* to the removal of the coat in the seed as a result of soaking in water.

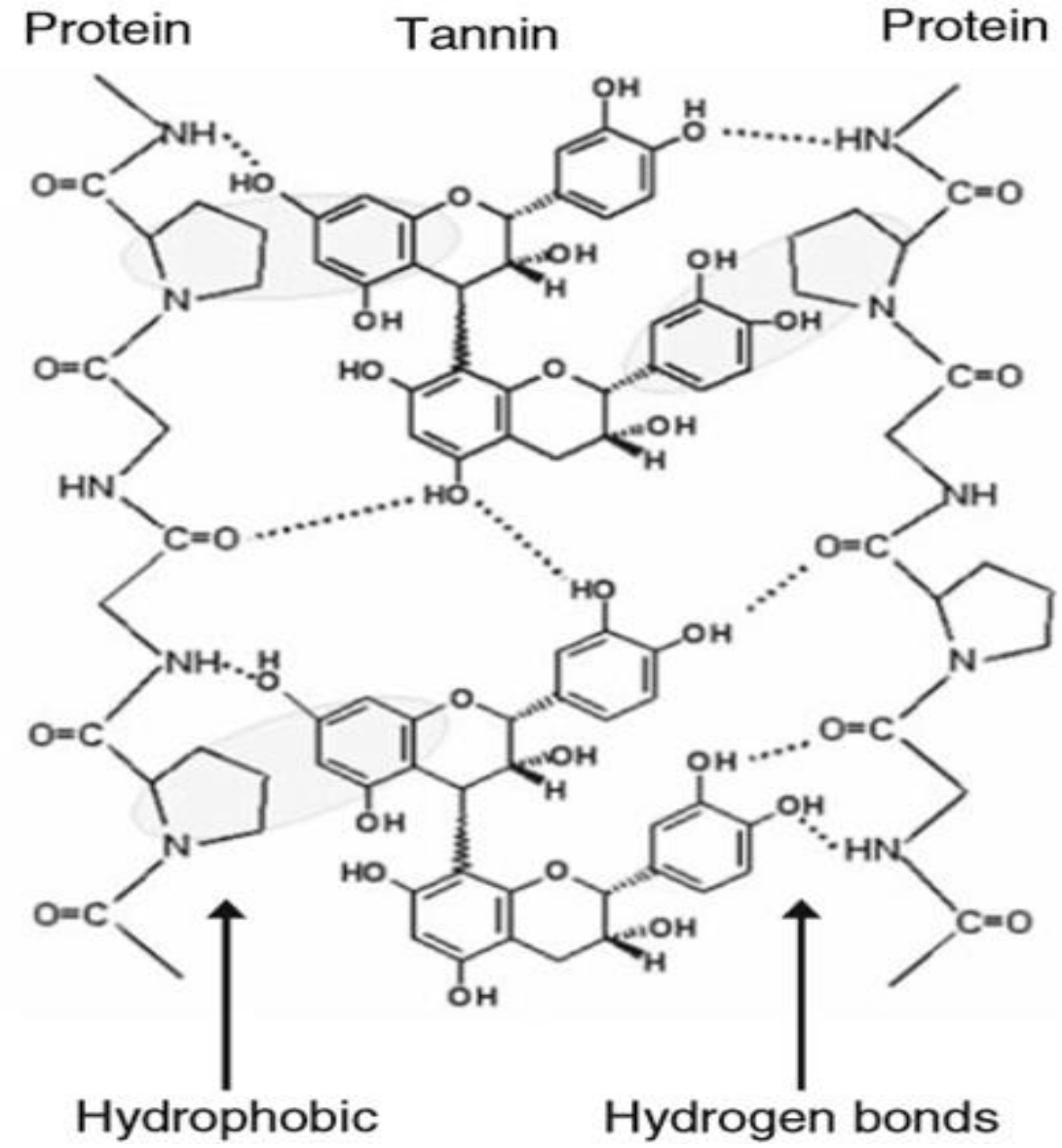

Figure 2.1: Chemical structure of protein-tannin interaction (Santo-Buelga and Freitas, 2009). The benzene rings in tannin interact with proline residues (broken line) in protein. Many proteins can be condensed together with one single large molecule of tannin (Santo-Buelga and Freitas, 2009).

2.3.6 Oxalate

Oxalate, a common constituent of many plant species, accumulates in plant as soluble oxalate, insoluble calcium oxalate or a combination of soluble oxalate and insoluble calcium oxalate (Makkar *et al.,* 2007). Oxalate, being negatively charged (Fig 2.2), binds to minerals such as zinc, magnesium and calcium which affect their bioavailability and metabolism (Makkar *et al.,* 2007). In addition, high dose of soluble oxalate ingested by animal can lead to acute intoxication and death (Bassey *et al.*, 2009). Soaking was reported to effectively decrease the amount of oxalate in yellow maize (Obasi and Wogu, 2008). Soaking and roasting also reduced the oxalate content in *Cyperus esculentus* (Adekanmi *et al.*, 2009). The authors attributed the loss of oxalate in toasted *Cyperus esculentus* to the destruction of oxalate by high temperature and drew a conclusion that oxalate are water soluble and heat labile. Fermentation reduced the oxalate content in sesame seed (Ibukun and Anyasi, 2012). The oxalate content in fermented sandbox seed also reduced (Gbadamosi and Osungbade, 2017). The reduction of oxalate in the fermented sesame and sandbox seed were attributed by the authors to the actions of the microorganisms participating in the fermentation process (Fowomola and Akindahunsi, 2008) and degradation of the anti-nutrients by microbes (Mubarak, 2005).

2.4.0 Haematology, Blood Chemistry and Histology in Fish Health

2.4.1 Haematology

Blood cells are easily accessible for quantitative and qualitative diagnosis in animal under the influence of perturbation (Themel *et al.,* 2004). The cells in the blood are derived from a common stem cell and become differentiated into difference cell lines under the influences of local and humoral factors (Themel *et al.*, 2004). The differentiation gives rise to the formation of erythrocyte, granulocytes and thrombocytes. The first-line of disease diagnosis using blood samples is to make a Full Blood Count (FBC) of the blood cellular components (Adeyemo, 2007). The FBC often consists, but not limited to Erythrocyte count, Leukocytes count, Thrombocyte and differential Leukocytes count. The up or down-regulation in the values of FBC from a reference value gives an indication on the health status of organism (Emiola *et al.*, 2013). Reference values can be obtained from concurrent control, baseline values or historical

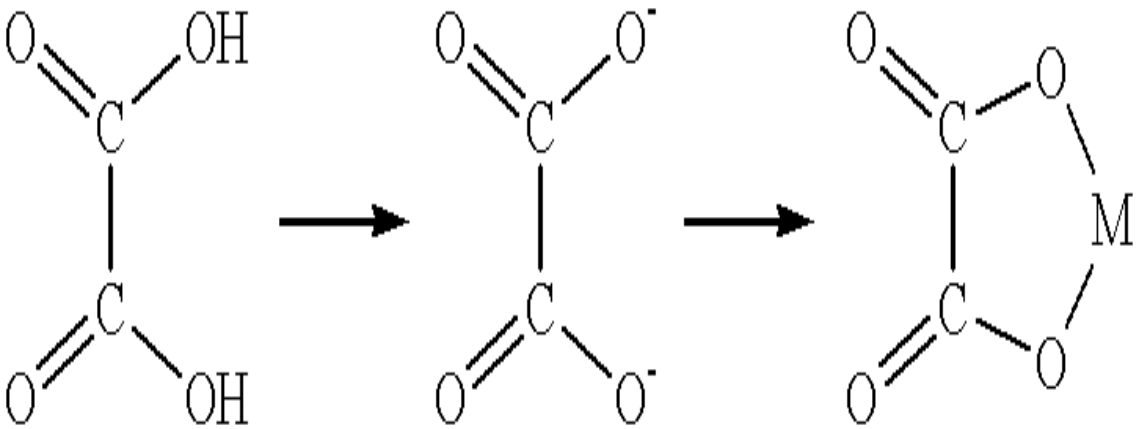

Figure 2.2: Binding of oxalate to metal ion (M). The carboxylic groups lose a proton and form oxalate ion. The oxalate ions functions as a bi-dentate chelate with a metal (M) forming a five-number chelate ring. (Stevens *et al.*, 2002)

values (Whalan, 2015). A down regulation in the RBC, haemoglobin (Hb) and Packed Cell Volume (PCV) for instance, is an indication of anaemia which can result from blood loss, haemolysis and nutritional deficiency in vitamin, mineral and protein (Whalan, 2015). Down regulation with reference to concurrent control in RBC, Hb and PCV was recorded by Osuigwe *et al* (2005) when catfish was fed raw and boiled jackbean meal based diets. An anaemic condition in organism is translated to a physiological impairment in oxygen transportation for aerobic metabolic processes in animal.

Anaemia can also be classified on the basis of the morphology. Leukocytes consists majorly the neutrophils, eosinophils, basophils, monocytes and lymphocytes, and are phagocytic towards foreign materials (Russell *et al.*, 1982). The components of the WBC are cohesive in mode of action but specification exists in their immune reaction. For instance, lymphocytes act as one of the first line of defense and are specialized in removing unwanted and cytotoxic materials (Russell *et al.*, 1982 and Theml *et al.*, 2004) while neutrophil, also a first line of defense, specialises in antibacterial immune response through phagocytic process (Theml *et al.*, 2004). However, the diminution in the WBC count and the peripheral leukocyte percentage has been linked to acute infection and illness, gastrointestinal malabsorption, renal failure and iron deficiency anaemia by Whalan (2015).

2.4.2 Blood Chemistry

Xenobiotic (foreign chemicals) such as environmental pollutants, industrial chemicals and naturally occurring substances are taken up by animals and biotransformed in the liver and kidney (Evans, 2009). Blood biochemistry can be used to evaluate physiological and metabolic function, identify possible target organs, measure impaired function and elucidate the persistence and severity of tissue injury (Evans, 2009). Researchers have engaged the blood enzymes in toxicological study to measure cellular injury, enzyme induction, and activation or inhibition of enzymes. Some of the plasma enzymes often assessed include; Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). ALP, ALT and AST are used to detect liver dysfunction (Campbell, 2012). An increase in these plasma enzymes denotes, but not limited to, the presence of a liver disease, hepatotoxicity

from drugs use and hepatitis, while a decrease in the concentration of these enzymes can be linked to malnutrition, pernicious anaemia, food avoidance and anorexia (Whalan, 2015). In the other hand, Blood Urea Nitrogen (BUN) and creatinine are measurable components in the plasma that gives an indication of kidney dysfunction (Arneson and Brickell, 2007). Creatinine measurement gives the extent of kidney damage caused by disease or toxicity, while BUN is useful for the assessment of reversibility of damage (Whalan, 2015). A diminution of plasma creatinine may result from malnutrition (Braun *et al.*, 2003) and an elevated creatinine can give an indication of impaired glomerular filtration, tubular dysfunction and alteration in renal blood flow (Evans, 2009). Plasma protein can also give an insight into the health status of animal (Allison, 2012). The primary proteins found in the blood include the albumin, fibrinogen and globulin which are produced in the liver (Whalan, 2015). An elevation in the plasma protein can be an indication of a chronic liver diseases, hyperglycemia and marked dehydration while a decrease in plasma protein may indicate a low protein diet, malnutrition, and malabsorption (Allison, 2012 and; Whalan, 2015).

2.4.3 Histology

Histology as a biomarker is used by researchers to study the influence of exogenous and endogenous factor such as xenobiotic on fish. van Dyk *et al.* (2009) engaged the use of histology to examine the health of fish sampled from Okavango Delta in Botswana. Mela *et al.* (2007) also established the dietary effect of methlmercury on neotropical fish using histological examination of liver and kidney. Structural alterations in the histology of tissue and organs from the normal anatomical architecture provide vital facts on the state of health of a fish (Hinton and Couch, 1984). More so, the death of cells without the death of an organism is accompanied by a series of cellular reactions and host responses (Hinton, 1993). Fish under the influence of a xenobiotic at sub-lethal level may display a cellular injury such as necrosis (Hinton, 1993). Cellular injury can be detected by differentiation between the Post-mortem and ante-mortem changes in well-fixed tissue of animal immediately after euthanization (Trump *et al.*, 1980). Histological examination also permits a spatial relationship and synergy in the xenobiotic effect in localized portions of an organ and the subsequent derangements in fluid, tissue or cell in another location (Hinton, 1993). For instance, deregulation of plasma Total Protein (TP) is used as an

indication of impairment in the liver (Coz-Rakovac *et al.*, 2005). Increment in the concentration of TP can result from structural alteration in liver, reducing the activity of amino-transferase, with concurrent reduction in the capacity of the animal to carry out deamination processes (Burtis and Ashwood, 1996). Plasma creatinine and Blood Urea Nitrogen concentration also gives vital information on the physiological status of the kidney.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCE AND PROCESSING OF KENAF (*HIBISCUS CANNABINUS*) SEEDS

Kenaf (*Hibiscus cannabinus*) seeds cultivated according o the agronomic procedures reported by Ajibola and Modupeoluwa (2014) were procured from Institute for Agriculture Research and Training (I.A.R. & T). The kenaf seeds were cleaned off stones, dusts and plant debris by hand-sorting and winnowing. The seeds were then stored in a bag until used.

3.1.1 Raw Kenaf (*Hibiscus cannabinus*) Seed Meal.

Two (2) kilogram of the cleaned kenaf seeds was ground into meal and stored in an air-tight bowl until used.

3.1.2 Cooked Kenaf (*Hibiscus cannabinus)* Seed Meal.

Twenty (20) liters of clean water was boiled to 100^0C in a cooking pot and two (2) kilogram of raw kenaf seed was poured into the boiling water, covered and left to cook for 60 minutes (Hefnawy, 2011). The duration of cooking was taken from the time kenaf seeds were poured into the boiling water. At the end of the 60 minutes cooking, when the seeds became soft, were sieved and air-dried before they were ground into meal. The cooked kenaf seed meal was stored in an air-tight container until used.

3.1.3 Roasted Kenaf (*Hibiscus cannabinus*) Seeds Meal.

Two (2) kilogram of the raw kenaf seeds were roasted at a temperature of 75 – 85^0C for 15 minutes in hot iron pan until the seeds turn brown (Sotolu and Faturoti, 2008) and emitted an aroma similar to that of a roasted groundnut. The roasted kenaf seeds were allowed to cool down before being ground. The meal was stored in an air-tight container until used.

3.1.4 Soaked Kenaf (*Hibiscus cannabinus*) Seeds Meal.

According to the procedures of (ElMaki *et al*.,, 2007), two (2) kilogram of cleaned raw kenaf seeds were soaked in 20 liters of clean water for 48 hours. The seeds

immediately after 48 hours were washed with water and air-dried. The air-dried soaked kenaf seeds were ground and stored until used.

3.1.5 Sprouted Kenaf (*Hibiscus cannabinus*) Seeds Meal.

According to the modified procedures of Sangronis and Machado (2007), two (2) kilogram of raw kenaf seed was rinsed in clean water and evenly spread in-between two layers of a damped cotton cloth. The seeds sprouted in the dark after 3 days. The sprouted seeds were air-dried, thereafter; the non-sprouted kenaf seeds were hand-picked. The air-dried sprouted kenaf seeds were ground and stored in air-tight container until used.

3.2 CHEMICAL ANALYSIS OF RAW AND PROCESSED KENAF (*Hibiscus cannabinus*) SEED MEAL.

Processed and unprocessed kenaf seed meal were subjected to proximate analysis according to the protocol of A.O.A.C (2005). The parameters analysed were crude protein, ether extract, crude fiber, ash, moisture, nitrogen free extract and mineral. The anti-nutritional factors (trypsine inhibitor, Phytic acid, tannin, saponin, alkaloids, oxalate) and the amino acid profile of the raw and processed kenaf seed meal were also analysed.

3.2.1 Crude Protein

Digestion Stage: 1 gram of the sample (w_1) put into a Kjedahl flask was treated with 25 ml of concentrated H_2SO_4 using 1g of copper as catalyst. The mixture was heated in a Kjedahl heating unit until a green colour appeared and digest made up to 50ml by adding distilled water.

Distillation Stage: Three drops of indicator was added to 5ml of boric acid in a conical flask. 5ml of the sample and 10 ml of 40% NaOH was pipetted into the distillation unit and subjected to heating until 50mls of the distilled sample was obtained.

Titration Stage: 50 ml of the distilled sample was titrated completely against 0.01m

HCl. The percentage Nitrogen was calculated as follows:

$$\text{Total Nitrogen } (N_t) = (TV \times Ma \times 1.4x10^{-2} \times L_1 / W_1 \times L_2) \times 10^2$$

Where : T = Titre Value; Ma = acid molarity; W_1 = Weight of sample; L_1 = Initial volume put in distillation unit; L_2 = Final volume obtained from the distillation

$$\text{Percentage (\%) protein (crude)} = N_t \times 6.25.$$

3.2.2 Ether Extract

2 grams each of samples of unprocessed and processed kenaf seed meal were subjected to a continuous extraction with petroleum ether in Soxhilet apparatus according to A.O.A.C (2005). After extraction, the ether was evaporated and the weight determined. The lipid was determined as:

$$\text{Crude Lipid} = (\text{sample weight} - \text{weight of residue} / \text{sample weight}) \times 100.$$

3.2.3 Crude Fiber

The residue from ether extraction was subjected to successive treatment of boiling with diluted acid and diluted base after which it was filtered and ignited in a furnace.

$$\text{\% Crude fibre} = \{(\text{Residue weight} - \text{Ash weight}) / \text{Residue weight}\} \times 10^2.$$

3.2.4 Ash Content

Two grams of dried samples of known weight was heated at 550^0C for 3 hours in a muffle furnace. The samples were transferred into a desiccator to cool down prior to weighing.

$$\text{Ash content of sample (\%)} = (Y_a - X / Y_b - X) \times 10^2$$

X = Crucible weight; Y_a = Crucible weight *plus* sample and; Y_b = Crucible weight *plus* Ash.

3.2.5 Moisture Content

Two grams of the samples from each of raw and processed kenaf seed meals were oven-dried at 80^0C for twenty four hours in evaporating dish of known weight. The samples were cooled in desiccators and weighed.

Moisture Content of Sample = Ya – Yb/Ya – M

Where: Ya = Dish weight *plus* sample weight; Yb = Dish weight *plus* dry weight of sample and; M = Dish weight.

3.2.6 Nitrogen Free Extract

This was determined as follows:

NFE = 10^2 – (% crude lipid + % crude protein + % Ash + % crude fiber).

3.2.7 Determination of Mineral Composition

The mineral component of the unprocessed and processed kenaf seed meal were analysed by dissolving a sample already dry-ashed at 525^0c in volumetric flask. Concentrated hydrochloric acid were added to the solution. Sodium and Potassium content in the solution were obtained using a flame photometer (corning, UK, model 405). Phosphorus was obtained colorimetrically using a spectronic 20 (Gallenkamp, UK). Atomic absorption spectrometer (PYE Unicorn, UK, Model SP9) was used to determine other mineral components.

3.2.8 Determination of Anti-nutritional Factors

3.2.8.1 Trypsine Inhibitor

Trypsin Inhibitory Activity (TIA) was expressed in terms of the degree to which an aqueous extract of the samples repressed the reaction of bovine trypsin on the substrate (Benzoyl – DL – arginine – p nitroanilide hydrochloride, BAPNA). TIA was subsequently determined spectrophotometrically as described by Smith *et al.* (1980).

3.2.8.2 Phytic acid

The iron content in the precipitate of phytate extracted from the sample was analysed by the method of Mackower (1970). Using the method described by Young and Greaves (1940), phytic acid and phytin- phosphorus contents in the samples were obtained. Phitin-Phosphorus was calculated as a percentage of total phosphorus.

3.2.8.3 Hydrolysable Tannin

Hydrolysable tannin was analysed as tannic acid, using the procedure of Makkar and Goodchild (1996).

3.2.8.4 Oxalate, Saponin and Alkaloid

Oxalates in the kenaf seed meal samples was determines using the methods of Henry (1993) while saponin and alkaloid were determined by the method of Harborne (1973),

3.2.9 Determination of Amino acid.

Amino Acids in the kenaf seed meals was determined using Amino Acid Analyser (LKB Biochran Limited, UK), after treating the hydrolysate with 6 mol HCl and reflux for 24 hour at 110^0C.

3.2.10 Estimation of Amino acid Score

The analysed amino acids obtained in the samples were scored according to the formular of FAO/WHO (1991) as adapted from Audu and Aremu (2011):

AMSS = {mg of amino acid (1g of test protein) / mg of amino acid in 1g reference protein} x 100.

3.3 DETERMINATION OF NUTRIENT DIGESTIBILITY COEFFICIENT OF RAW AND PROCESSED KENAF (*Hibiscus cannabinus*) SEED MEAL

A digestibility feeding experiment was carried out using the procedure adapted from Xavier *et al.* (2014) to evaluate the crude protein, lipid and energy digestibility of raw and processed kenaf (*Hibiscus cannabinus*) seed meal for fish.

3.3.1 Experimental System and Fish

The digestibility experiment was carried out in eighteen (18) plastic circular tanks of 100 liters capacity with water supplied from the borehole at the Federal College of Animal Health and Production Technology, Apata, Ibadan. Catfish *(Clarias gariepinus)* of average weight of 8.19±0.17g purchased from GreenSolutions Investments limited fish farm, Ologun-eru, Ido local government, Ibadan, Oyo-state were transported in a plastic container to the experimental site. The fish were acclimated in plastic aquaria tanks for 15 days and fed on commercial pelleted feed during this period.

3.3.2 Experimental Diet for Digestibility Study

A reference diet (RD) (Table 5) and five (5) experimental diets (70% RD and 30% of each of the raw and processed kenaf (*Hibiscus cannabinus*) seed meal) constituted

diets for the experiment. The RD was formulated to meet the nutrient requirement for *Clarias gariepinus* and contained 40% crude protein. Chromic oxide was added to the reference diet at 0.5 percent as external marker. The ingredients were mixed thoroughly and mould into pellet of an average size of 2mm using manually operated machine.

3.3.3 Experimental Design and Procedure for Digestibility Study

The experimental design was a complete randomized design (CRD) containing six (6) treatments and three (3) replicates. After the period of acclimation, ten (10) fish were randomly distributed into each of the 18 circular tanks filled with 70 liters of borehole water (stocking density – 1fish/7liters of water). The fish were fed to apparent satiation with the experimental diets twice daily (between 08.00- 09.00 hours and 16.00-17.00 hours).

Collection of faeces started 15 days after the commencement of feeding the fish with the experimental diets. Fecal matters were retrieved from each tank daily prior feeding in the morning using rubber tube. The fecal dropping siphoned were drained off water using filter paper, transferred to a plastic cup and frozen in a refrigerator at -4^0C. The fecal collection continued for 20 days during which the faeces from each tank were pooled. Uneaten feed particles were siphoned out of water daily, one hour after feeding and 60% of the water in each tank was renewed daily.

3.3.4 Analysis of Experimental Diet and Faeces.

The crude protein, crude fiber and ether extract of the six (6) experimental diets and the eighteen (18) fecal dropping were analysed according to AOAC (2005) as described above. The gross energy in the experimental diets and feces were determined using adiabatic bomb calorimetric method and the Chromium in the diets and feces determined according to the methods of Fenton and Fenton (1979).

3.3.5 Determination of Apparent Digestibility Coefficient

The Apparent Digestibility Coefficients (ADCs) for crude protein, ether extract and gross energy of reference and experimental diets were calculated using the formula of Maynard and Loosli (1969):

$ADCN_{diet}$ (%) = apparent digestibility coefficient of nutrient and energy in the diet

= 100 – 100 x {(% marker in the diet ÷ % marker in the feces)

X (% nutrient in the feces ÷ % nutrient in the diet)}

The Apparent Digestibility Coefficient (ADCs) for crude protein, ether extract and gross energy of raw and the processed kenaf (*Hibiscus cannabinus*) seed meal were calculated according to the formula of NRC (2011):

ADCNi (%)= apparent digestibility coefficient of nutrient in the

$= ADC_{test\ diet} + \{(ADC_{test\ diet} - ADC_{reference\ diet}) \times (0.7 \times D_{reference}/0.3 \times D_{ingredient})\}$

Where

$D_{reference}$ - percentage nutrients or kcal/g gross energy of the reference diet.

$D_{ingredient}$ - percentage nutrients or kcal/g gross energy of the ingredient.

3.4.0 DETERMINATION OF GROWTH PERFORMANCE AND NUTRIENT UTILISATION OF CATFISH (*Clarias gariepinus*) FED RAW AND PROCESSED KENAF (*Hibiscus cannabinus*) SEED MEAL.

A 112 day feeding trail was conducted to assess the growth performance and utilisation of nutrient in raw and differently processed kenaf (*Hibiscus cannabinus*) seed in the diet fed to Catfish (*Clarias gariepinus*).

3.4.1 Experimental Fish and Diets

Clarias gariepinus fish seed produced from the same parent stock at the hatchery of Green Solutions Investment Nigeria Limited, Ibadan were transported to the experimental site at 45 days after spawning. A total of sixteen iso-nitrogenous experimental diets were formulated to contain 40% crude protein as recommended by Faturoti (2000) for *Clarias gariepinus*. Soybean meal was replaced by four (4) graded levels of each of the raw (RA) kenaf meal and processed kenaf (*Hibiscus cannabinus*)

Table 3.1: Gross composition of reference diet and test diet (g/100g)

Ingredient	Reference diet
Fishmeal	31.55
Groundnut cake	21.54
Soybean meal	12.54
Maize	23.20
Cassava	3.97
Lysine	0.50
Methionine	0.70
Vitamin premix	2.00
Palm oil	2.00
Common salt	0.50
Vitamin C	0.50
Bone meal	0.50
Chromic oxide	0.50
Calculated crude protein	40

seed meal at 0%, 10%, 20% and 30%. The processed kenaf (*Hibiscus cannabinus*) seed meal consisted of Roasted (RO), Soaked (SO), Sprouted (SP) and Cooked (CO) kenaf seed meal. The diet containing 0% of kenaf served as the control. Other ingredients included in the diet formulation were fishmeal, maize, starch, palm oil, vitamin premix, vitamin C, Di-calcium phosphate, salt, lysine and methionine (Table 3.2). Each of the ingredients was finely ground, thoroughly mixed together and pelleted using a hand operated pelleting machine. The pellets were sun dried and samples of the feeds were collected for proximate analysis. The dried feeds were packed in a labeled air-tight container and stored in a cool dry place until use.

3.4.2 Experimental Design and Procedure

The design of the experiment was factorial arrangement in a complete randomize design. The two factors considered were the processing methods and replacement levels. The experiment was conducted at the research laboratory of the Department of Fisheries Technology, Federal College of Animal Health and Production Technology, Moor Plantation, Apata, Ibadan. A total of 480 *Clarias gariepinus* transported to the experimental site were randomly allotted in each of the 48 aquaria tanks of 50 liters capacity filled with 40 liters of water at ten (10) fish per tank (stocking density – 1fish/4liters of water). The fish were acclimated for 15 days and fed commercial pelleted fish feed throughout this period. At the end of acclimation period, when the fish weight have attained an average weight of 2.33 ±0.12 g, were fed to apparent satiation with the experimental diets in triplicate for a period of 16 week. Feeding was done twice daily between 08.00- 09.00 hours and 16.00-17.00 hours. The water in each tank was changed every 3rd day and the tanks were cleaned and refilled with water every six (6) days. Fish were weighed fortnightly with the use of sensitive digital scale (Starfrit®, USA). Records of water temperature, pH and dissolved oxygen were taken fortnightly throughout the period of experiment using thermometer (Pometer®) pH-009(III), China), pH test probe (Pometer®) pH-009(III), China), and Dissolved oxygen meter (YSI$^{85®}$ DO meter, USA), respectively. The record of mortality was also taken throughout the experimental period.

3.4.3 Sample Collection

At the end of the 16 weeks of feeding period, one (1) fish per replicates was sacrificed for proximate analysis. Blood samples were collected from one (1) fish per replicate

through caudal puncture and stored in EDTA bottle for heamatological and biochemical analysis. The liver and kidney of the fish in each replicates were also excised and fixed in 10% phosphate buffered formalin for histopathological analysis

3.4.4 Determination of Growth Performance Indices

3.4.4.1 Weight Gain (%) = { [$FW_f - IW_f$] / IW_f} x 10^2

FW_f – Final weight, IW_f – Initial weight

3.4.4.2 Specific Growth Rate (% day) = [$FW_{log} - IW_{log}$] / T.

FW_{log} - Log_e final body weight

IW_{log} - Log_e initial body weight

T – Time (in days)

3.4.4.3 Feed conversion ratio (FCR) = Feed intake / fish weight gain

3.4.4.4 Feed Efficiency Ratio (FER) = {BWG (g)/ WFF (g)} X 10^2

BWG – Body Weight Gain, WFF – Weight of Feed Fed

3.4.5 Determination of Nutrient Utilisation Indices

3.4.5.1 Protein Intake (PI) = FI x % PD

FI – Feed intake. PD – Protein in the Diet

3.4.5.2 Protein efficiency ratio (PER) = Fish weight gain / Protein fed.

3.4.5.3 Protein Productivity Value (PPV) = Increment in body protein of fish / Protein intake

3.4.5.4 Nitrogen Metabolism (NM) = {0.549 x (W_1 + W_2)d}2^{-1}

Where W_1 = initial average weight of fish; W_2 = final average weight of fish

d = period of experiment (in days); 0.549 = metabolism factor/constant

3.4.5.5 Net Protein Utilisation (NPU) = {($N_2 - N_1$)+ Nm}/ Nd

Where: N_1 = Initial nitrogen content of fish

N_2= Final nitrogen content of fish

Nm= nitrogen metabolism

Nd = nitrogen in diet

Nitrogen content = Protein content / 6.25

3.4.5.6 Survival Rate (%) = {(I – M)/I} x 10^2

I – initial stocking density, M - Mortality

Table 3.2: Gross composition of experimental diets containing raw, roasted, soaked, sprouted and cooked kenaf (*Hibiscus cannabinus*) seed meal (g/100g of feed)

	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co
Kenaf seed meal	0.00	9.52	19.04	28.57	8.00	16.00	24.00	6.89	13.79	20.68	7.69	15.38	23.08	6.67	13.33	20.00
Soybean (43.75%)[1]	45.71	41.14	36.57	32.00	41.14	36.57	32.00	41.14	36.57	32.00	41.14	36.57	32.00	41.14	36.57	32.00
Fishmeal(69.65%)[1]	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97	27.97
Corn(13.30%)[1]	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Starch (0.00%)[1]	19.57	14.62	9.67	4.71	16.14	12.71	9.28	17.25	14.92	12.60	16.45	13.33	10.20	17.47	15.38	13.28
Vitamin Premix	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Vitamin C	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DCP	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Palm oil	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Methionine	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Calculated																
Crude protein	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

( )[1] – values in bracket represent the laboratory analysed percentage crude protein. DCP – Di-calcium phosphate

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

3.4.6 Determination of Heamatological Parameters

3.4.6.1 Blood Cell Count

Heamocyteometer was used in blood cell count. The blood diluting fluid was carried out according to Svobodova *et al.* (1991) and the cells in the blood were counted on heamocytometer using compound microscope.

Red blood cell count (RBC) = cells counted (in numbers) x 3 x 10 x ($2x10^2$) (10^6mm^3)

White blood cell count (WBC) = cells counted (in numbers) x 0.25 x 10 x $2x10^1$ (10^4mm^3)

3.4.6.2 Haemoglobin Estimation

Haemoglobinometer was used to estimate the haemoglobin concentration. N/10 HCl was filled up to 20ml mark in a graduated tube. Blood (0.02m) was thoroughly mixed with N/10 HCl and permitted to stay for 5 minutes. Distilled water gradually included in drops and thoroughly mixed until colour corresponds to the standard. The amount of solution in the calibrated tube gave the percentage concentration of haemoglobin.

3.4.6.3 Packed Cell Volume (PCV).

Non-clotted blood drawn by capillary action into microhaematocrit tube was centrifuged in a microhaematocrit centrifuge at 2957xg for 5 minutes. The PVC was read with microhaematocrit reader and recorded as percentage.

3.4.6.4 Mean Haemoglobin Concentration (MCHC)

MCHC = Haemoglobin Content/PCV x 1000 T/l^{-1}

3.4.6.5 Mean Corpscular Volume (MCV)

MCV = PCV x 1000/Er (u^3).

3.4.6.6 Mean Corpscular Haemoglobin (MCH).

MCV = Haemoglobin value / Er (Picogramme)(pg).

3.4.7 Plasma Biochemical Analysis

The serum total protein was estimated by the Biuret method (Reinhold, 1953) with a commercial kit (Randox Laboratories Ltd., UK.). The albumin value was estimated by bromocresol green method (Doumas and Biggs, 1971). The globulin and globulin-albumin ratio was determined according to the method of Coles (1986). The plasma creatinine was estimated by deproteinisation with a kit (Randox Laboratories Ltd., UK.). The plasma enzymes; Alanine Amino-Transferase (ALT) and Aspertate Amino-Transferase (AST) were determined using the Randox Laboratories Ltd. (UK.) test kit. The procedure of King and Armstrong (1934) was used for Plasma alkaline phosphate (ALP). Blood Urea Nitrogen (BUN) was determined according to the method of Harrison (1947).

3.4.8 Histopathological Examination

The liver and kidney removed from each fish were processed for histology using the automated tissue processing machine (Shandon Citadel 2000™, Germany), embedded in paraffin and sectioned at 3.5 µm. The sectioned tissues were stained with haematoxylin and eosin (H&E) to assess histological changes (after Roberts & Powell, 2003; Speare *et al.*, 1997) with the aid of light microscope (Olympus ® BX51, Japan).

3.4.9 Economic Analysis

The economic analysis of incorporating kenaf seed in the diet of catfish was carried out using the following formula:

3.4.6.1 Profit index (PI) = {VF (₦/kg)} / {CF (₦/kg)}

VF – Value of Fish; CF – Cost on Feeding

3.4.6.2 Incidence of Cost (IC) = {Cost on feeding (₦/kg)} / {Total weight of fish (Kg)}

3.5.0 STATISTICAL ANALYSIS

All data were expressed as mean ± Standard Error (SE), except data on amino acid contents of raw and processed kenaf (*Hibiscus cannabinus*) seed meal that was expressed as a score. The data generated were subjected to a two-way Analysis of Variance (ANOVA). A significant level of difference among treatment means was established at probability of 0.05. Tukey multiple-range test was used as post-Hoc test. All statistical analysis was accomplished using IBM ® SPSS ® 22 statistical package while SigmPlot 11® was used for presentation of data in a graphical format. The results of histopathological examination on liver and kidney of the fish were qualitatively analysed and the result presented in a photomicrograph.

CHAPTER FOUR

4.0 RESULTS.

4.1 CHEMICAL CHARACTERISATION OF RAW AND PROCESSED KENAF (*Hibiscus cannabinus*) SEED MEAL.

Based on the statistical analysis, the $\mathbf{H_o}$ (null) hypothesis was rejected. There were significant variations ($p < 0.05$) in the chemical composition and nutrient digestibility coefficient of raw and differently processed kenaf (*Hibiscus cannabinus*) seed.

4.1.1 Proximate Composition of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal

The proximate composition of raw and differently processed Kenaf Seed Meal (KSM) is presented in Table 4.1. The proximate compositions of raw and differently processed KSM significantly varied ($p < 0.05$). Processing techniques adopted significantly ($p<0.05$) improved the crude protein content of the KSM, Cooking has the highest significant increment ($p<0.05$) in the crude protein of KSM (30.45%) followed by soaking (29.14%), sprouting (25.75%) and roasting (25.17%). Raw KSM (21.17%) had the lowest significant crude protein.

Soaking, cooking and sprouting significantly reduced ($P<0.05$) the crude fiber of KSM while roasting significantly increased ($P < 0.05$) the crude fiber relative to the raw (15.28%) KSM. Cooked (9.07%) KSM had the least significant ($p < 0.05$) crude fiber content while, the roasted (16.21%) KSM had the highest significant ($p < 0.05$) content of fiber. No significant variation ($p > 0.05$) was recorded in the fiber content between soaked (13.55%) and sprouted (12.82%) KSM.

The lipid content of KSM significantly reduced ($p < 0.05$) by the methods of processing adopted. Raw (19.60%) KSM had the highest significant ($p < 0.05$) lipid content followed by sprouted (17.55%), cooked (17.02%), roasted (16.94%) and soaked (16.31%) KSM. Soaked KSM had the lowest significant ($p < 0.05$) lipid content and there was no significant difference in the lipid content between roasted and cooked KSM.

Table 4.1: Proximate composition of raw and processed kenaf (*Hibiscus cannabinus*) seed meal (KSM)

Parameter (g/100g dry matter)	Ra	Ro	So	Sp	Co
Crude protein	21.17±0.10^{e}	25.17±0.10^{d}	29.14±0.28^{b}	25.75±0.09^{c}	30.45±0.22^{a}
Crude fibre	15.28±0.09^{b}	16.21±0.26^{a}	13.55±0.11^{c}	12.82±0.09^{c}	9.07±0.53^{d}
Ether Extract	19.60±0.20^{a}	16.94±0.08^{c}	16.31±0.10^{d}	17.55±0.07^{b}	17.02±0.22^{c}
Ash	5.39±0.09^{b}	6.14±0.16^{a}	2.52±0.04^{d}	5.00±0.03^{c}	2.81±0.11^{d}
Moisture	7.00±0.07^{b}	5.91±0.05^{d}	6.66±0.10^{c}	8.13±0.17^{a}	6.92±0.04^{bc}
Nitrogen Free Extract (NFE)	38.56±0.38^{b}	35.54±0.49^{c}	38.48±0.28^{b}	38.87±0.12^{b}	40.65±0.43^{a}

Mean ±SE value in the same row with the same superscript are not significantly ($p > 0.05$) different.

Legend:

Ra – Raw KSM

Ro – Roasted KSM

So – Soaked KSM

Sp – Sprouted KSM

Co – Cooked KSM

The ash content of KSM increased significantly ($p < 0.05$) when roasted and significantly reduced ($p < 0.05$) when subjected to soaking, sprouting or cooking. The ash contents of
The ash content of KSM increased significantly ($p < 0.05$) when roasted and significantly reduced ($p < 0.05$) when subjected to soaking, sprouting or cooking. The ash contents of roasted (6.14%) KSM was the highest ($p < 0.05$) while, the content of ash in cooked (2.81%) and soaked (2.52%) KSM did not differ significantly ($p > 0.05$) from each other and were significantly lower ($p < 0.05$) than sprouted (5.00%) and raw (5.39%) KSM.

Soaking or sprouting did not significantly influence ($p > 0.05$) the Nitrogen Free Extract (NFE) in the KSM. The NFE among the raw (38.56%), soaked (38.48%) and sprouted (38.87%) KSM did not vary significantly ($p > 0.05$). However, cooking (40.65%) significantly increases ($p < 0.05$) the NFE of KSM while, roasting (35.54%) significantly reduced ($P < 0.05$) the NFE in KSM.

4.1.2 Mineral Composition of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal

The mineral content of raw and differently processed KSM is presented in Table 4.2. The most abundant minerals in the raw and the differently processed KSM were magnesium and phosphorus while copper was the least. There were significant variations ($p < 0.05$) in the mineral content of raw and the differently processed KSM. The calcium content of the raw and processed KSM ranged from 0.01 to 0.28g/100g dry matter. The roasted KSM had the highest significant ($p < 0.05$) calcium content while cooked had the least ($p < 0.05$) calcium content. Magnesium was also the least abundant mineral in cooked KSM and was the highest in soaked KSM. Sprouted KSM had the significant highest ($p < 0.05$) potassium content while, roasted had the least ($p < 0.05$). No significant variation ($p > 0.05$) existed in the potassium content between soaked KSM and cooked KSM. These values were significantly ($p < 0.05$) higher than the raw KSM. Sprouted KSM had the highest significant ($p < 0.05$) sodium content while soaked KSM had the least significant ($p < 0.05$) value. Raw KSM had a sodium content that was significantly ($p < 0.05$) lower than roasted KSM and cooked KSM except soaked KSM. Copper and Iron had the highest ($p < 0.05$) values in cooked KSM and soaked KSM respectively. There was no significant difference ($p > 0.05$) in the copper content between raw KSM and roasted KSM.

Table 4.2: Mineral composition of raw and processed kenaf (*Hibiscus cannabinus*) seed meal (KSM) (g/100g dry matter)

Parameter	RA	RO	SO	SP	CO
Calcium	0.12 ± 0.00^{bc}	0.28 ± 0.01^{a}	0.12 ± 0.00^{b}	0.12 ± 0.01^{b}	0.09 ± 0.00^{c}
Magnesium	1.37 ± 0.01^{ab}	1.12 ± 0.02^{bc}	1.85 ± 0.01^{a}	1.19 ± 0.01^{bc}	0.70 ± 0.34^{c}
Potassium	9.60 ± 0.07^{c}	8.79 ± 0.02^{e}	11.00 ± 0.03^{b}	15.55 ± 0.04^{a}	8.93 ± 0.04^{d}
Sodium	1.32 ± 0.04^{d}	1.73 ± 0.01^{b}	1.06 ± 0.01^{e}	1.82 ± 0.02^{a}	1.60 ± 0.00^{c}
Copper	0.07 ± 0.01^{b}	0.09 ± 0.01^{b}	0.01 ± 0.00^{c}	0.02 ± 0.00^{c}	0.12 ± 0.00^{a}
Iron	1.06 ± 0.00^{b}	0.99 ± 0.02^{c}	1.24 ± 0.01^{a}	0.62 ± 0.03^{d}	0.41 ± 0.00^{e}
Manganese	0.17 ± 0.01^{a}	0.08 ± 0.01^{c}	0.07 ± 0.00^{d}	0.09 ± 0.00^{c}	0.16 ± 0.00^{b}
Phosphorus	6.28 ± 0.05^{c}	7.16 ± 0.01^{b}	7.32 ± 0.02^{a}	7.33 ± 0.00^{a}	7.20 ± 0.02^{b}
Na/K ratio	0.13 ± 0.00^{c}	0.20 ± 0.00^{a}	0.10 ± 0.00^{e}	0.12 ± 0.00^{d}	0.18 ± 0.00^{b}
Ca/P ratio	0.02 ± 0.00^{b}	0.04 ± 0.00^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	0.10 ± 0.00^{c}

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Legend:

Ra – Raw KSM.

Ro – Roasted KSM.

So – Soaked KSM.

Sp – Sprouted KSM.

Co – Cooked KSM.

These values were higher significantly ($p < 0.05$) than soaked and sprouted KSM. The iron content was least ($p < 0.05$) for cooked KSM and, the iron content in the raw KSM

was significantly ($p < 0.05$) higher than roasted and sprouted KSM. The different processing methods adopted in this study significantly reduced ($p < 0.05$) the abundance of Manganese in the KSM. The phosphorus content in soaked and sprouted KSM were the highest ($p < 0.05$) while, the abundance of phosphorus was the least in raw KSM.

The sodium to potassium (Na/K) ratio significantly ($p < 0.05$) differed among raw and the differently processed KSM. The Na/K ranged from 0.10 to 0.20 with roasted KSM having the highest significant ($p < 0.05$) Na/K, and soaked KSM had the lowest significant ($p < 0.05$) ratio. There was significant variation in the Calcium to phosphorus (Ca/P) ratio in the raw and the differently processed KSM. The Ca/P ratio ranged from 0.01 to 0.04 with roasted KSM having the highest significant ($p < 0.05$) Ca/P, and cooked KSM had the lowest significant ($p < 0.05$) Ca/P.

4.1.3 Anti-nutritional Composition of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal

The anti-nutritional composition of raw and differently processed kenaf seed meal is presented in Table 4.3. There were significant difference ($p < 0.05$) in the anti-nutritional content of raw and the differently processed KSM. Except in soaked KSM with the highest significant ($p < 0.05$) content of taninin and trypsine inhibitors, roasting, sprouting and cooking significantly reduced ($p < 0.05$) the tannin and trypsine inhibitor in KSM. The processing methods adopted in this study also significantly reduced ($p < 0.05$) the phytic acid content except roasting which gave the highest significant ($p < 0.05$) phytic acid content. The saponin concentration in the KSM was significantly reduced ($p < 0.05$) by all the methods of processing adopted relative to the raw KSM. Roasting had no significant influence ($p > 0.05$) in the alkaloid concentration of KSM when compared with the raw KSM.

Table 4.3: Trypsin inhibitory activity (TIA) and Anti-nutritional composition of raw and processed kenaf (*Hibiscus cannabinus*) seed meal (KSM) (mg/100g dry matter.)

Parameter	Ra	Ro	So	Sp	Co
TIA	0.12 ± 0.00^{b}	0.08 ± 0.00^{d}	0.15 ± 0.00^{a}	0.10 ± 0.00^{c}	0.03 ± 0.00^{e}
Tannin	0.08 ± 0.00^{b}	0.07 ± 0.00^{c}	0.11 ± 0.00^{a}	0.07 ± 0.00^{c}	0.02 ± 0.00^{d}
Phytic acid	3.91 ± 0.01^{b}	4.21 ± 0.01^{a}	3.40 ± 0.00^{c}	3.06 ± 0.01^{d}	2.95 ± 0.01^{e}
Saponin	2.37 ± 0.01^{a}	2.08 ± 0.01^{b}	2.10 ± 0.01^{b}	1.99 ± 0.01^{c}	2.06 ± 0.02^{b}
Alkaloid	29.87 ± 0.13^{b}	30.17 ± 0.07^{b}	20.46 ± 0.07^{c}	49.97 ± 0.17^{a}	20.43 ± 0.17^{c}
Oxalate	0.20 ± 0.00^{c}	0.15 ± 0.00^{d}	1.54 ± 0.00^{a}	0.22 ± 0.00^{b}	0.09 ± 0.00^{e}

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Legend:

Ra – Raw KSM.

Ro – Roasted KSM.

So – Soaked KSM.

Sp – Sprouted KSM.

Co – Cooked KSM.

The alkaloid concentration was significantly reduced ($p < 0.05$) in soaked and cooked KSM. Sprouting, however, significantly enhanced ($p < 0.05$) the alkaloid concentration of KSM. The oxalate content of the KSM was also significantly influenced ($p < 0.05$) by the different methods of processing adopted in this study.Except in soaked KSM that had a significantly higher ($p < 0.05$) oxalate content when compared to raw KSM, the oxalate concentration in other processing methods were significantly lower ($p < 0.05$).

4.1.4 Amino acid content and amino acid score of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal.

The amino acid contents and the amino acid scores for the essential amino acids of raw and differently processed kenaf seed meal are presented in Table 4.4 and Table 4.5, respectively. The most abundant amino acids in the raw and differently processed KSM were aspartic acid and glutamic acid. The amino acid score showed that Methionine + Cystine were limiting essential amino acid in raw (0.42), roasted (0.47), sprouted (0.60) and cooked (2.39) KSM except in soaked (0.70) KSM that had Threonine as the limiting amino acid. The total amino acid score of the raw and differently processed KSM followed this order of decreasing magnitude: SO (5.83) > CO (5.53) > SP (5.41) > RO (5.35) > RA (4.53).

4.2 APPARENT NUTRIENT DIGESTIBILITY COEFFICIENT OF RAW AND PROCESSED KENAF (*Hibiscus cannabinus*) SEED MEAL

4.2.1 Proximate Composition, Gross Energy and Chromium Content in Reference and Test Diets

The proximate composition, gross energy and chromium content of the reference and test diets are presented in Table 4.6. The crude protein, crude fiber and ether extract of the reference and experimental diets ranged from 33.43 - 36.16%, 2.94 – 3.33% and 6.53 – 7.59%, respectively. The range of ash content, dry matter and gross energy recorded were 8.08 – 9.74%, 91.85 – 92.77% and 3.88 – 3.97%, respectively.

Table 4.4: Amino acid composition (g/100g crude protein) of raw and processed kenaf (*Hibiscus cannabinus*) seed meal (KSM)

Amino Acid	Ra	Ro	So	Sp	Co
Lysine	4.51	5.66	5.11	4.95	5.00
Histidine	1.40	1.46	1.84	1.71	1.78
Arginine	4.25	4.59	4.59	4.42	4.76
Aspartic acid	18.68	23.99	22.22	20.45	22.85
Threonine	2.21	3.07	2.78	2.61	2.73
Serine	3.35	3.00	3.82	3.64	3.70
Glutamic acid	13.22	16.34	14.64	14.07	14.21
Proline	2.89	3.25	4.41	4.17	4.18
Glycine	3.02	4.25	3.58	3.33	3.48
Alanine	3.98	5.39	4.81	4.39	4.56
Cystine	0.76	0.83	1.80	1.31	1.59
Valine	2.92	3.10	4.02	3.41	3.71
Methionine	0.70	0.80	0.86	0.80	0.80
Isoleucine	2.22	2.48	3.26	2.93	3.00
Leucine	4.87	5.99	5.52	5.05	5.46
Tyrosine	2.23	2.81	2.65	2.48	2.48
Phenylalanine	3.17	3.08	3.52	3.26	3.43

Ra- Raw KSM. Ro – Roasted KSM. So – Soaked KSM. Sp – Sprouted KSM. Co – Cooked KSM.

Table 4.5: Amino acid content (g/100 dry matter) and score of raw and processed kenaf (*Hibiscus cannabinus*) seed meal

Parameter	PAAESP									
	Protein	Ra		Ro		So		Sp		Co	
	(g/100g dry matter)	EAAC	AAS	EAAC	AAS	EAAC	AAS	EAAC	AAS	EAAC	AAS
Isoleusine	4	2.22	0.56	2.48	0.62	3.26	0.82	2.93	0.73	3	0.75
Leusine	7	4.87	0.7	5.99	0.86	5.52	0.79	5.05	0.72	5.46	0.78
Lysine	5.5	4.51	0.82	5.66	1.03	5.11	0.93	4.95	0.90	5	0.91
Methionine + Cystine	3.5	1.46	0.42	1.63	0.47	2.66	0.76	2.11	0.60	2.39	0.68
Phenylalanine + Tyrosine	6	5.4	0.9	5.89	0.98	6.17	1.03	5.74	0.96	5.91	0.99
Threonine	4	2.21	0.55	3.07	0.77	2.78	0.70	2.61	0.65	2.73	0.68
Tryptophan	1	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Valine	5	2.92	0.58	3.1	0.62	4.02	0.80	3.41	0.85	3.71	0.74
Total	36	23.59	4.53	27.82	5.35	29.52	5.83	26.8	5.41	28.2	5.53

Essential amino acid with the least score was considered limiting in the raw and processed KSM.

EAAC – Essential Amino Acid Content. AAS – Amino Acid Score. PAASESP – Provisional Amino Acid (Egg) Scoring Pattern. TSAA – Total Sulphur containing Amino Acid. Ra- Raw KSM. Ro – Roasted KSM. So – Soaked KSM. Sp – Sprouted KSM. Co – Cooked KSM. ND – Not determined.

4.2.2 Apparent Digestibility Coefficient (%) of Crude protein, Ether extract and Gross energy of Reference and Experimental Diets for *Clarias gariepinus*.

The apparent digestibility coefficients (ADCs) of crude protein, lipid and gross energy of the reference diet and experimental diets are presented in Table 4.7. The diet containing raw KSM had the significantly lowest ($p < 0.05$) crude protein ADC while, the highest ADC of crude protein was recorded in diet containing sprouted KSM. The ADCs of lipid and gross energy of diet containing roasted, soaked, sprouted and cooked KSM were significantly higher ($p < 0.05$) than the ADC of diet containing raw KSM.

4.2.3 Apparent Digestibility Coefficient (%) of Crude protein, Ether extract and Gross energy of Raw and Processed Kenaf Seed Meal for *Clarias gariepinus*.

The Apparent Digestibility Coefficients (ADCs) of crude protein, lipid and gross energy of raw and differently processed KSM are presented in Table 4.8. No significant variation ($p < 0.05$) in the ADCs of the raw and the differently processed KSM was recorded. The crude protein ADC in the processed KSM was significantly higher ($p < 0.05$) than the raw KSM. There was however, no significant difference ($p > 0.05$) in the crude protein ADC of KSM in all the processing methods adopted in this study. There was no significant variation ($p > 0.05$) in the lipid and gross energy ADC among the soaked, sprouted and cooked KSM. The lipid and gross energy ADC in these processing methods were significantly higher ($p < 0.05$) than the raw KSM. The lipid ADC in the roasted kenaf seed was not significantly different ($p > 0.05$) from the other processing methods but, was significantly higher than the ADC of lipid in the raw KSM. However, the gross energy ADC did not differ significantly ($p < 0.05$) between the roasted KSM (67.19%) and raw KSM (49.12%). No significant difference ($p > 0.05$) was recorded in the gross energy ADC for roasted and raw KSM. The ADCs of gross energy for roasted and raw KSM were significantly lower ($p < 0.05$) than soaked (97.09%), sprouted (97.37%), and cooked (91.75%) KSM whose gross energy ADC did not significantly differ among themselves ($p > 0.05$).

Table 4.6: Proximate composition, gross energy and chromium content of reference diet and test diets

	Reference	Ra	Ro	So	Sp	Co
Crude protein (%)	35.42	34.30	36.16	35.71	35.17	33.43
Crude fibre (%)	3.15	3.14	3.33	2.94	3.41	3.12
Ether extract (%)	6.77	6.93	7.59	6.53	6.73	7.43
Ash (%)	9.74	8.97	8.80	7.91	7.74	8.08
Dry matter (%)	92.77	92.46	92.59	91.93	92.20	91.85
Gross energy (Cal/g)	3.97	3.95	3.94	3.88	3.99	3.86
Chromium (mg/kg)	5.04	3.07	2.67	2.52	2.13	2.45

Legend:

Ra - Raw KSM.

Ro - Roasted KSM.

So - Soaked KSM.

Sp - Sprouted KSM.

Co - Cooked KSM.

Table 4.7: Apparent digestibility coefficient (%) for crude protein, ether extract and gross energy of reference diet and experimental diets

	Reference	Ra	Ro	So	Sp	Co
Crude protein	88.13±1.64^{ab}	80.51±2.21^{c}	85.52±1.65^{b}	89.73±0.60^{ab}	90.40±0.14^{a}	87.19±1.11^{ab}
Ether extract	92.24±0.73^{bc}	90.28±0.64^{c}	92.98±1.07^{ab}	94.93±0.63^{a}	94.82±0.17^{a}	94.98±0.32^{a}
Gross energy	79.04±1.26^{ab}	70.17±3.01^{c}	75.62±2.81^{bc}	84.17±0.65^{a}	84.33±0.29^{a}	82.68±0.29^{a}

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Legend:

Ra - Raw KSM.

Ro - Roasted KSM.

So - Soaked KSM.

Sp - Sprouted KSM.

Co - Cooked KSM.

Table 4.8: Apparent digestibility coefficient (%) for crude protein, ether extract and gross energy of raw and processed kenaf (*Hibiscus cannabinus*) seed meal for *C. gariepinus*

	Ra	Ro	So	Sp	Co
Crude protein	51.45±10.83[b]	77.54±7.07[a]	94.76±2.28[a]	98.29±0.59[a]	85.13±4.14[a]
Ether extract	88.65±1.15[b]	93.61±2.06[a]	97.48±1.25[a]	97.10±0.32[a]	97.48±0.61[a]
Gross energy	49.12±10.04[b]	67.19±9.50[b]	97.09±2.34[a]	97.37±1.01[a]	91.75±1.04[a]

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Legend:

Ra - Raw KSM.

Ro - Roasted KSM.

So - Soaked KSM.

Sp - Sprouted KSM.

Co - Cooked KSM.

4.3 GROWTH PERFORMANCE AND NUTRIENT UTILISATION OF CATFISH (*Clarias gariepinus*) FED RAW AND DIFFERENTLY PROCESSED KENAF (*Hibiscus cannabinus*) SEED MEAL

The $\mathbf{H_o}$ (null) hypothesis was rejected while the alternative hypothesis was accepted because there were significant variations ($p < 0.05$) in the growth performance and nutrient utilisation of African catfish (*Clarias gariepinus*) fed graded levels of processed kenaf (*Hibiscus cannabinus*) seed meal based diets.

4.3.1. Proximate composition of experimental diets fed to Catfish (*Clarias gariepinus*)

Table 4.9 showed the proximate composition of the experimental diets fed to *Clarias gariepinus*. The result of the proximate analysis revealed that the diets were iso-nitrogenous as there was no significant difference ($p > 0.05$) in the crude protein content of the diet. The Nitrogen Free Extract (NFE) which depicts the carbohydrate content of a diet was also not significantly different ($p > 0.05$) among the experimental diets. There were significant variations in the ash, crude fiber, ether extract and moisture contents of the experimental diets. The fish in each treatment groups actively fed on the experimental diets throughout the experimental period.

4.3.2 Whole Body composition of (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The whole body composition of *Clarias gariepinus* fed raw and differently processed KSM is presented in Table 4.10. There was significant variation ($p < 0.05$) in the whole body composition of *C. gariepinus* before and at the end of the feeding period. The lowest crude protein was recorded for the initial (53.36%) body composition of *C. gariepinus* while the highest crude protein was recorded for *C. gariepinus* fed 10% roasted (77.34%) KSM. The crude protein of the control group and *C. gariepinus* fed raw and the other processed KSM based diets were not significantly different ($p > 0.05$) and were all significantly higher ($p < 0.05$) than the initial crude protein content of *C. gariepinus*. Similar trend was recorded for ash, crude fiber, ether extract and moisture content. The highest Nitrogen Free Extract (NFE) was recorded in the initial (31.08%) body composition of *C. gariepinus* and the least recorded for *C. gariepinus* fed RO30 (8.18%) KSM based diet.

Table 4.9: Proximate composition of experimental diets fed to *Clarias gariepinus* for performance study

Parameter (%)	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE±
CP	38.84	38.47	38.63	38.61	38.77	37.82	38.32	39.72	39.72	39.2	38.09	38.32	39.61	38.91	39.31	40.03	0.63
Ash	8.10^{ab}	7.03^{d}	7.40^{bcd}	7.40^{bcd}	7.44^{abcd}	7.11^{d}	8.10^{ab}	7.38^{bcd}	8.05^{ab}	7.90^{abc}	6.96^{d}	7.17^{cd}	7.69^{abcd}	8.20^{a}	8.06^{ab}	8.05^{ab}	0.07
CF	3.24^{abc}	2.98^{c}	3.21^{abc}	3.20^{abc}	3.55^{ab}	3.26^{abc}	3.59^{a}	3.39^{abc}	3.20^{abc}	3.13^{abc}	3.22^{abc}	3.09^{bc}	3.50^{ab}	3.21^{abc}	3.29^{abc}	3.27^{abc}	0.03
EE	6.81^{a}	6.68^{ab}	6.47^{abcd}	6.44^{abcd}	6.19^{bcd}	6.49^{abcd}	6.31^{abcd}	6.19^{bcd}	6.04^{cd}	6.57^{abc}	6.34^{abcd}	5.95^{d}	6.31^{abcd}	6.08^{bcd}	6.08^{bcd}	5.97^{cd}	0.04
MT	8.48^{abcd}	$7.77d^{e}$	8.1^{cd}	7.26^{e}	8.46^{abcd}	8.43^{abcd}	9.18^{a}	7.91^{cde}	8.35^{abcd}	8.99^{ab}	8.44^{abcd}	7.93^{abcd}	8.28^{abcd}	8.27^{abcd}	8.70^{abc}	8.27^{abcd}	0.07
NFE	43.01	44.84	44.29	44.35	44.05	45.32	43.68	43.33	42.99	43.2	45.39	45.47	42.89	43.6	43.26	42.67	0.2

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

LEGEND:

CP – Crude protein. **CF** – Crude Fiber. **EE** – Ether Extract. **MT** – Moisture. **NFE** – Nitrogen Free Extract.

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

4.3.3. Growth performance of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

4.3.3.1 The bi-weekly mean weight gain and survival of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The bi-weekly mean weight gain of *Clarias gariepinus* fed diet containing graded levels of raw and processed KSM are presented in Figures 4.1 to 4.5 while the mean weight gain response of Catfish (*Clarias gariepinus*) to graded dietary inclusion levels of kenaf (*Hibiscus cannabinus*) seed meal based diets are presented in Figures 4.6 to 4.10. The bi-weekly survival of *C. gariepinus* fed graded levels of raw and processed KSM are presented in Figures 4.11 to 4.15. The result showed a steady rate of increment in the mean weight gain over a period of 16 weeks for the control groups and *C. gariepinus* fed 10% of the KSM based diets. While 20% replacement level of raw and processed KSM for soybean were comparable and marginally better than 30% replacement levels, the *C. gariepinus* growth rate in 30% replacement level of raw and processed KSM peaked at 12^{th} weeks of feeding and resulted in a remarkable growth depression thereafter. Results of regression analysis showed that the optimal inclusion level of raw (9.00%), roasted (10.80%), soaked (8.40%), sprouted (13.20%) and cooked (8.40%) kenaf seed meal corresponded to a maximum mean weight gain of 12.13g, 13.06g, 9.11g, 7.24g and 7.44g, respectively.

Except in raw kenaf seed meal based diet that showed reduction in survival at 4^{th} week for 20% and 30% inclusion level, the survival rates of *C. gariepinus* fed the graded levels of processed KSM based diets were comparable up to 8 weeks of culture period. Thereafter, there was remarkable reduction in the survival rate of *C. gariepinus* fed 20% and 30% KSM based diet in the all the processing methods adopted in this study. The survival rate of the control group and the *C. gariepinus* fed 10% kenaf seed meal were comparable for all the processing methods throughout the culture period.

Table 4.10: Proximate composition of *Clarias gariepinus* before and after being fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

Parameter (%)	Initials	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Poo SE ±
CP	58.36^{b}	74.17^{ab}	70.03^{ab}	74.85^{ab}	NR	77.34^{a}	68.90^{ab}	77.93^{a}	72.82^{ab}	NR	NR	72.30^{ab}	76.36^{ab}	NR	67.04^{ab}	65.01^{ab}	67.33^{ab}	5
Ash	2.97^{b}	5.30^{ab}	6.00^{a}	5.25^{ab}	NR	5.70^{ab}	5.15^{ab}	5.40^{ab}	5.05^{ab}	NR	NR	5.10^{ab}	5.10^{ab}	NR	5.40^{ab}	4.96^{ab}	5.80^{a}	0
CF	0.02^{ab}	0.04^{a}	0.04^{ab}	0.02^{ab}	NR	0.01^{ab}	0.03^{ab}	0.02^{ab}	0.04^{a}	NR	NR	0.04^{ab}	0.02^{ab}	NR	0.04^{a}	0.03^{ab}	0.03^{ab}	
EE	7.58^{b}	8.00^{ab}	8.07^{ab}	8.55^{a}	NR	7.90^{ab}	8.30^{ab}	8.48^{a}	8.25^{ab}	NR	NR	8.60^{a}	8.45^{a}	NR	8.64^{a}	8.32^{ab}	8.36^{ab}	0
MT	65.01^{b}	77.63^{ab}	74.44^{ab}	74.15^{ab}	NR	78.92^{a}	76.38^{ab}	73.17^{ab}	76.59^{ab}	NR	NR	76.62^{ab}	76.52^{ab}	NR	70.72^{ab}	68.02^{ab}	67.08^{ab}	5
NFE	31.08^{a}	12.49^{bc}	15.88^{abc}	11.34^{bc}	NR	9.05^{bc}	17.63^{abc}	8.18^{bc}	13.85^{abc}	NR	NR	13.97^{abc}	10.08^{bc}	NR	18.88^{ab}	21.69^{ab}	18.49^{ab}	1

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

LEGEND:

NR – No record due to total mortality

CP – Crude protein. **CF** – Crude Fiber. **EE** – Ether Extract. **MT** – Moisture. **NFE** – Nitrogen Free Extract

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

4.3.3.2. The main effects on growth performance and survival of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The main effect of processing methods and replacement of soybean for kenaf seed meal on the growth performance and survival of *Clarias gariepinus* are presented in Table 4.11. Except the survival that did not significantly vary ($p > 0.05$) among the processing methods and unprocessed (raw) KSM, there was a significant influence ($p < 0.05$) of processing on the growth performance of *Clarias gariepinus* in all the parameter measured. The growth performance of *Clarias gariepinus* fed roasted (RO) KSM based diet was significantly higher ($p < 0.05$) than raw (RA), soaked (SO), sprouted (SP) and cooked (CO) KSM based diet. There was significant "inclusion level"-response variation ($p < 0.05$) in the replacement of soybean meal for KSM in the diet of *C. gariepinus*. *C. gariepinus* fed 30% replacement level of KSM had the lowest significant ($p < 0.05$) growth performance and survival (4.67%). The highest significant performance and survival (76.67%) ($p < 0.05$) were recorded for the control group, followed by those fed 10% KSM based diet.

4.3.3.3 The interaction effects on growth performance and survival of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

The interaction effect of processing methods and replacement of soybean meal for kenaf seed meal on the growth performance and survival of *Clarias gariepinus* are presented in Table 4.12. In all the parameters measured, there were no significant interaction ($p < 0.05$) between the processing methods and the graded level of replacement of soybean meal for KSM. However, C. *gariepinus* fed RO10 had the highest performance ($p < 0.05$) in terms of final weight gain, mean weight gain, specific growth rate and average daily growth rate. Generally, the performance of the fish decreased as the inclusion level of KSM increased for all the processed and unprocessed (raw) KSM based diet for *C. gariepinus*. Similar trend was recorded for survival rate. Values not recorded for the growth performance and survival of *C. gariepinus* were due to total mortality and/or growth retardation.

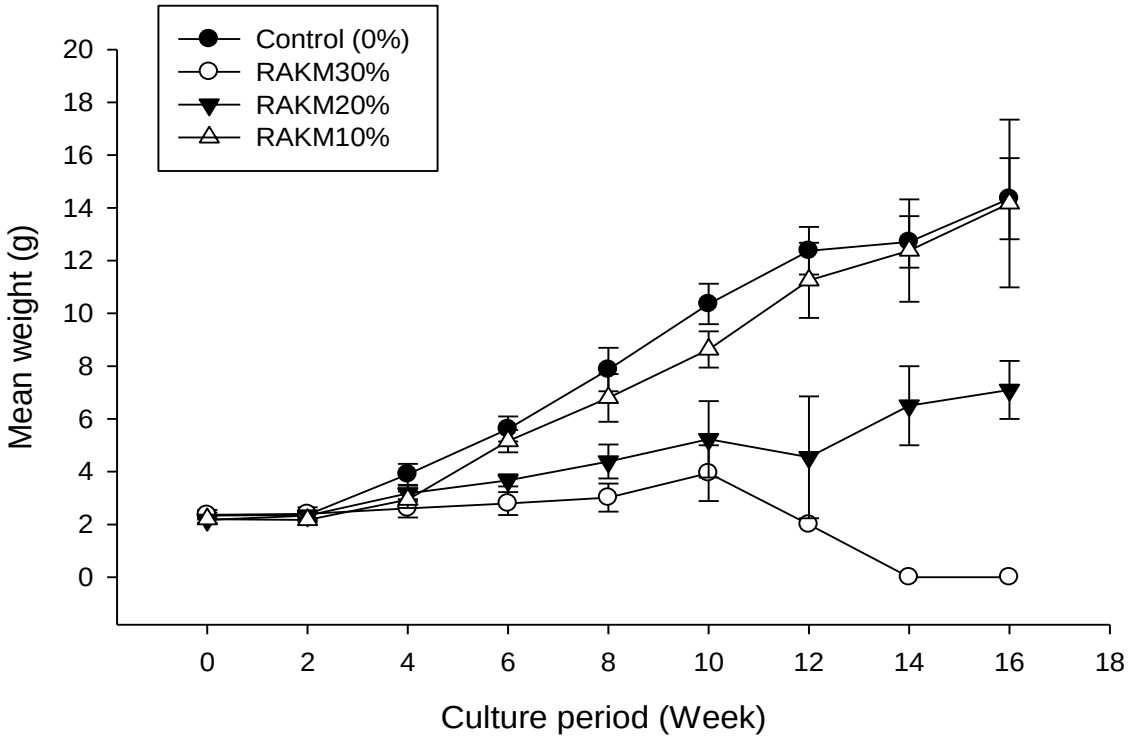

Figure 4.1: Mean bi-weekly growth of Catfish fed graded levels of raw kenaf (*Hibiscus cannabinus*) seed meal based diets

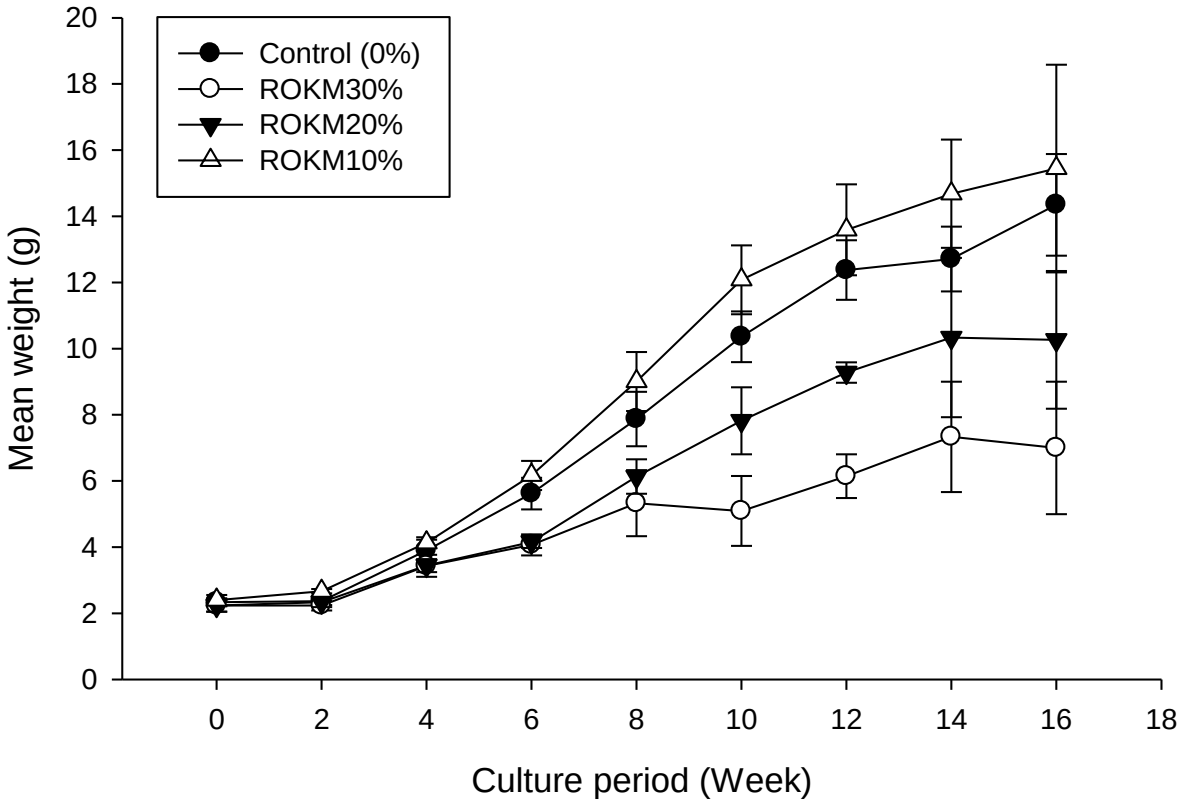

Figure 4.2: Mean bi-weekly growth of Catfish fed graded levels of roasted kenaf (*Hibiscus cannabinus*) seed meal based diets.

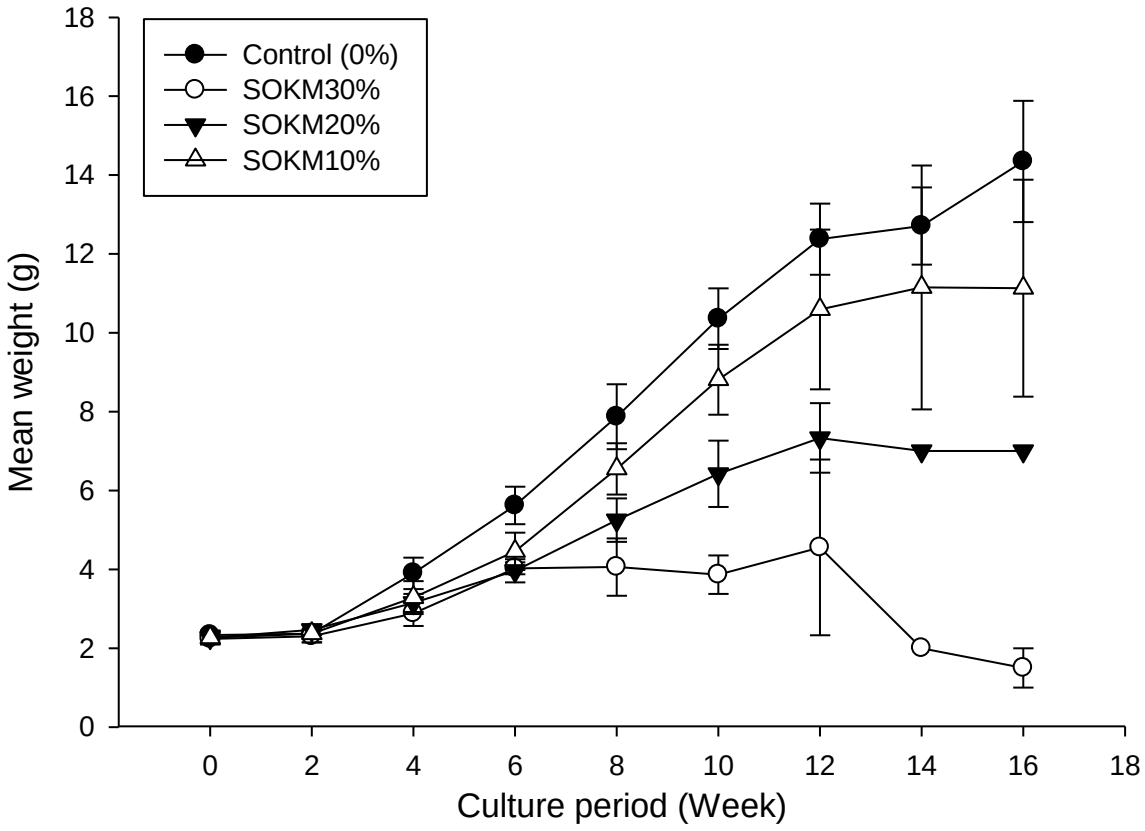

Figure 4.3: Mean bi-weekly growth of Catfish fed graded levels of soaked kenaf (*Hibiscus cannabinus*) seed meal based diets.

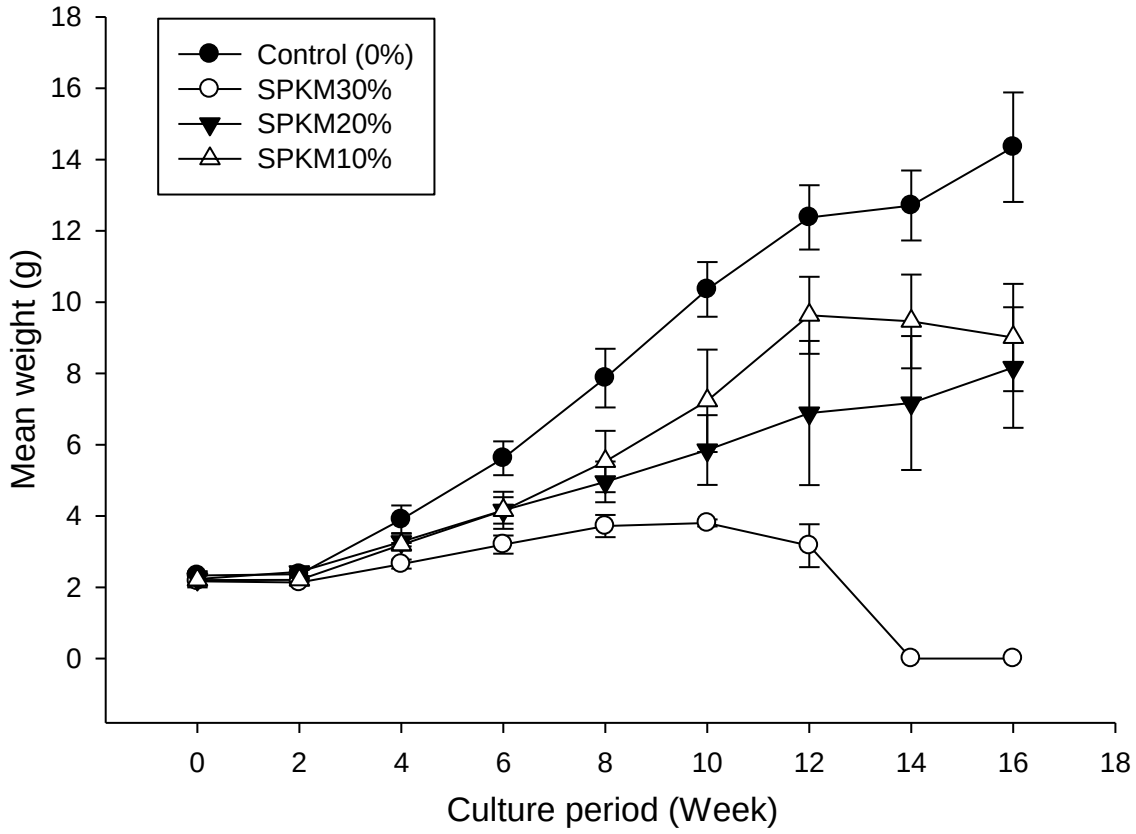

Figure 4.4: Mean bi-weekly growth of Catfish fed graded levels of sprouted kenaf (*Hibiscus cannabinus*) seed meal based diets.

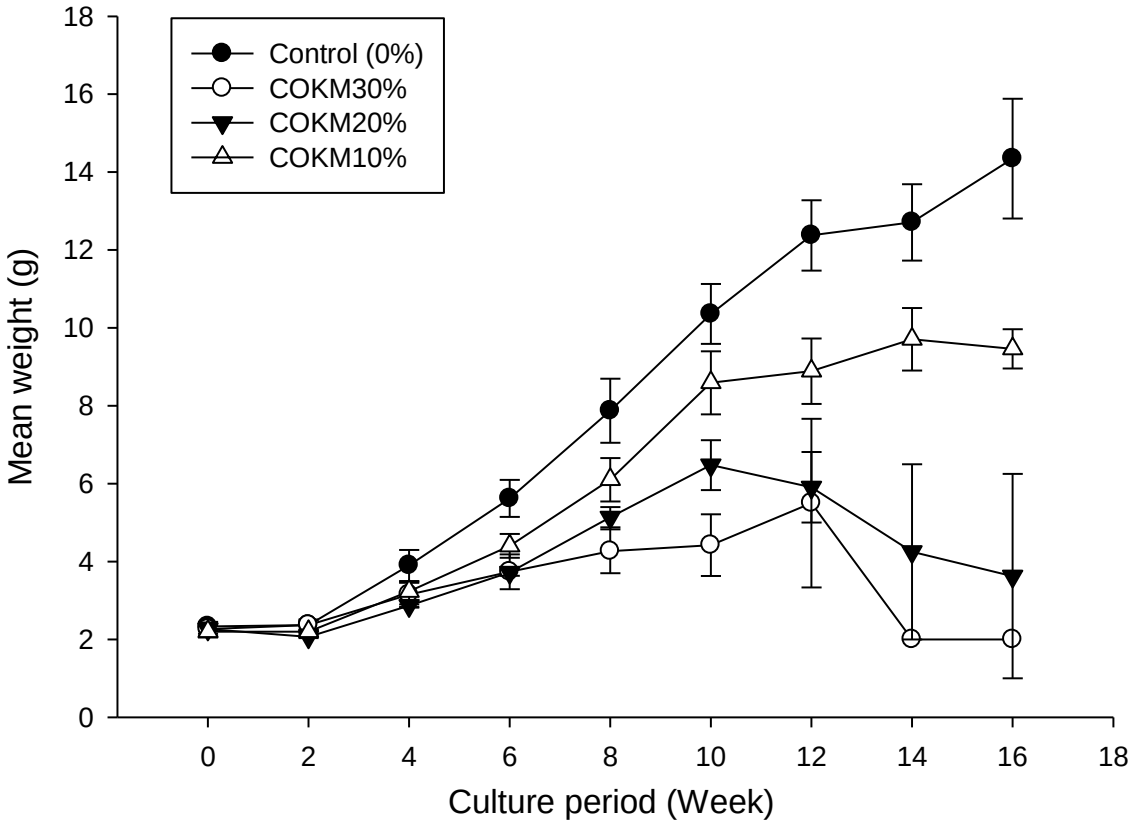

Figure 4.5: Mean bi-weekly growth of Catfish fed graded levels of cooked kenaf (*Hibiscus cannabinus*) seed meal based diets.

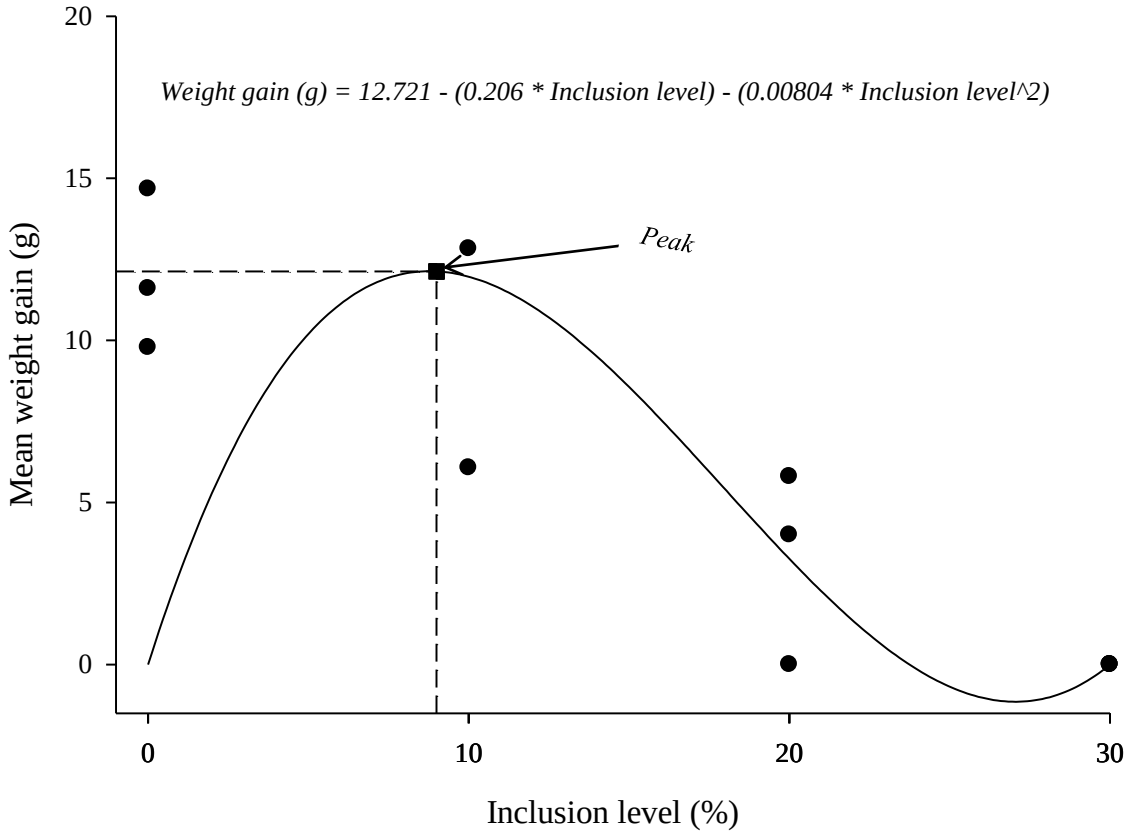

Figure 4.6: Mean weight gain response of Catfish to graded dietary inclusion levels of raw kenaf (*Hibiscus cannabinus*) seed meal based diets. The inclusion level of raw kenaf seed meal to achieve maximum performance was 9.00%. $r^2 = 0.70$

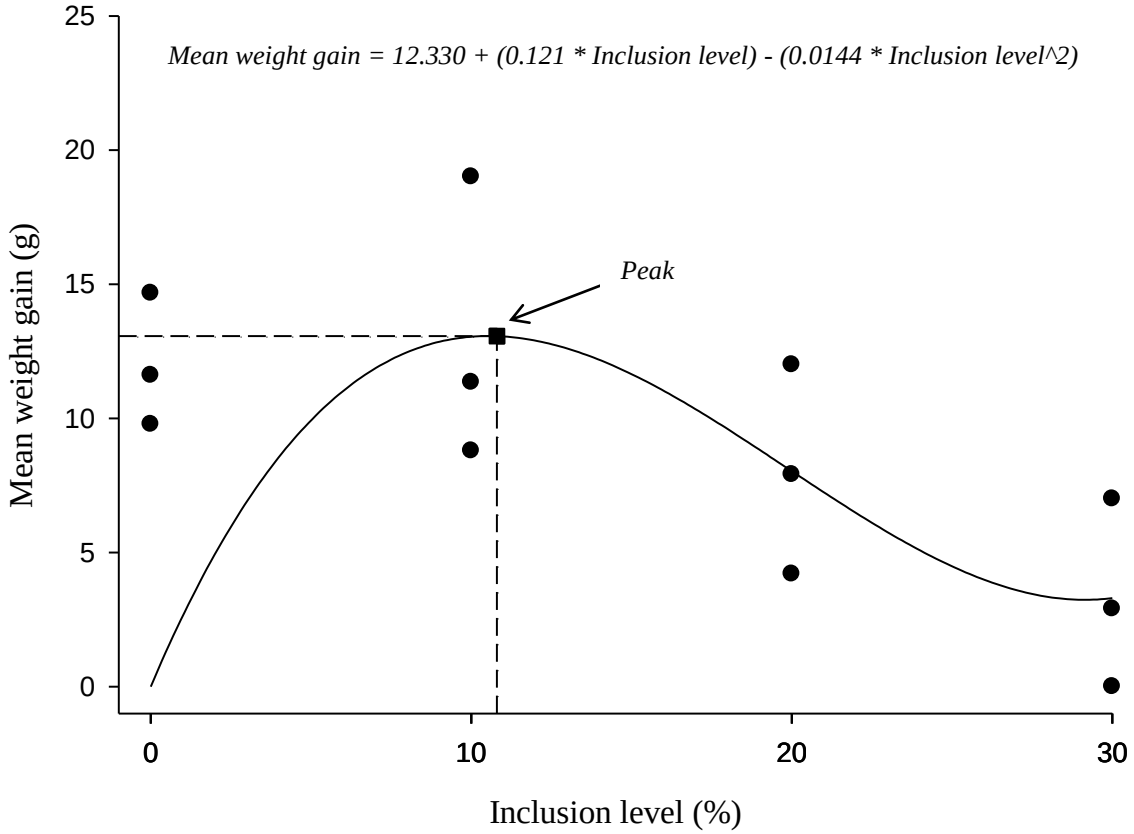

Figure 4.7: Mean weight gain response of Catfish to graded dietary inclusion levels of roasted kenaf (*Hibiscus cannabinus*) seed meal based diets. The inclusion level of roasted kenaf seed meal to achieve maximum performance was 10.8%. $r^2 = 0.49$

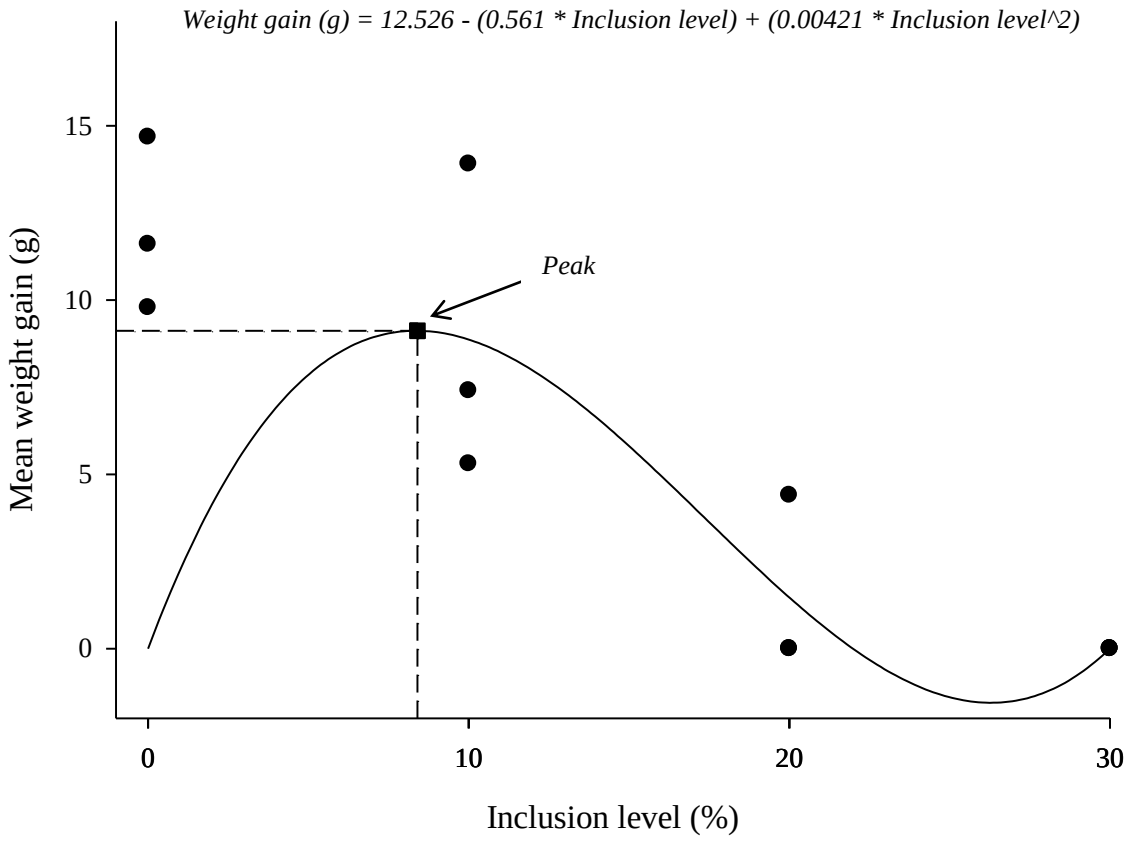

Figure 4.8: Mean weight gain response of Catfish to graded dietary inclusion levels of soaked kenaf (*Hibiscus cannabinus*) seed meal based diets. The inclusion level of soaked kenaf seed meal to achieve maximum performance was 8.40%. $r^2 = 0.77$.

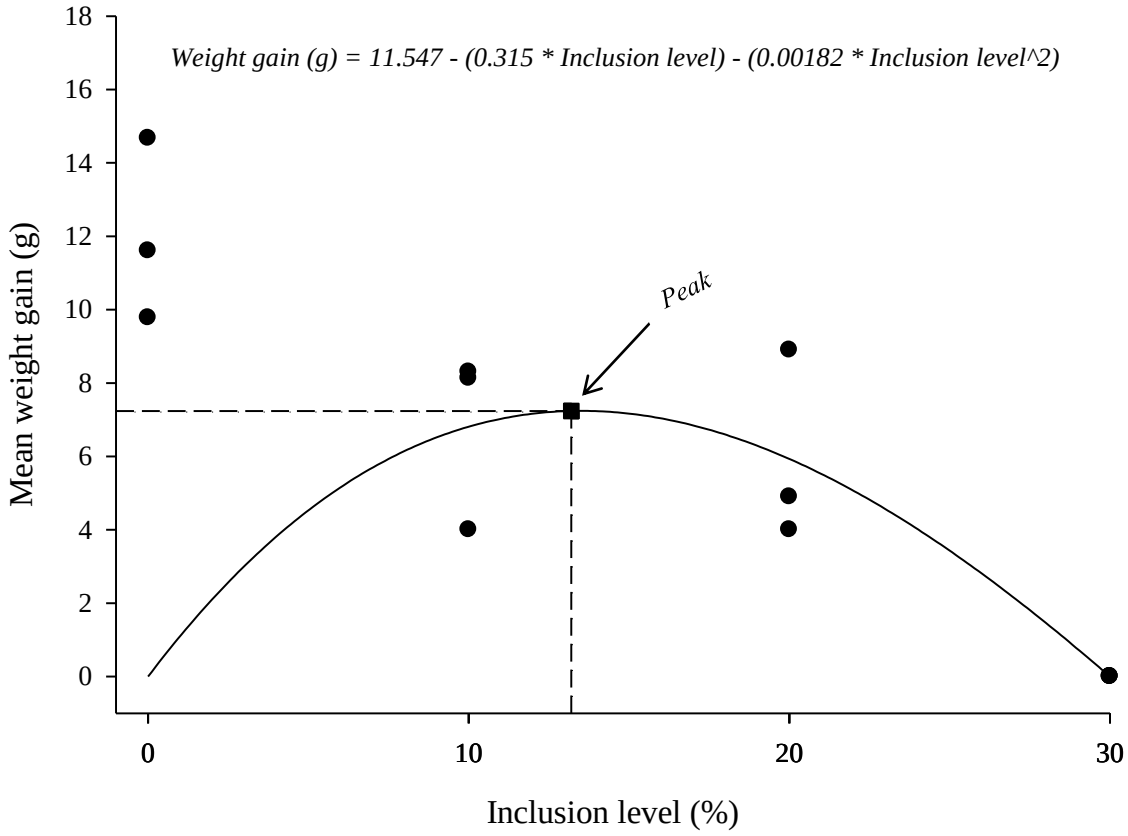

Figure 4.9: Mean weight gain response of Catfish to graded dietary inclusion levels of sprouted kenaf (*Hibiscus cannabinus*) seed meal based diets. The inclusion level of sprouted kenaf seed meal to achieve maximum performance was 13.20%. $r^2 = 0.80$.

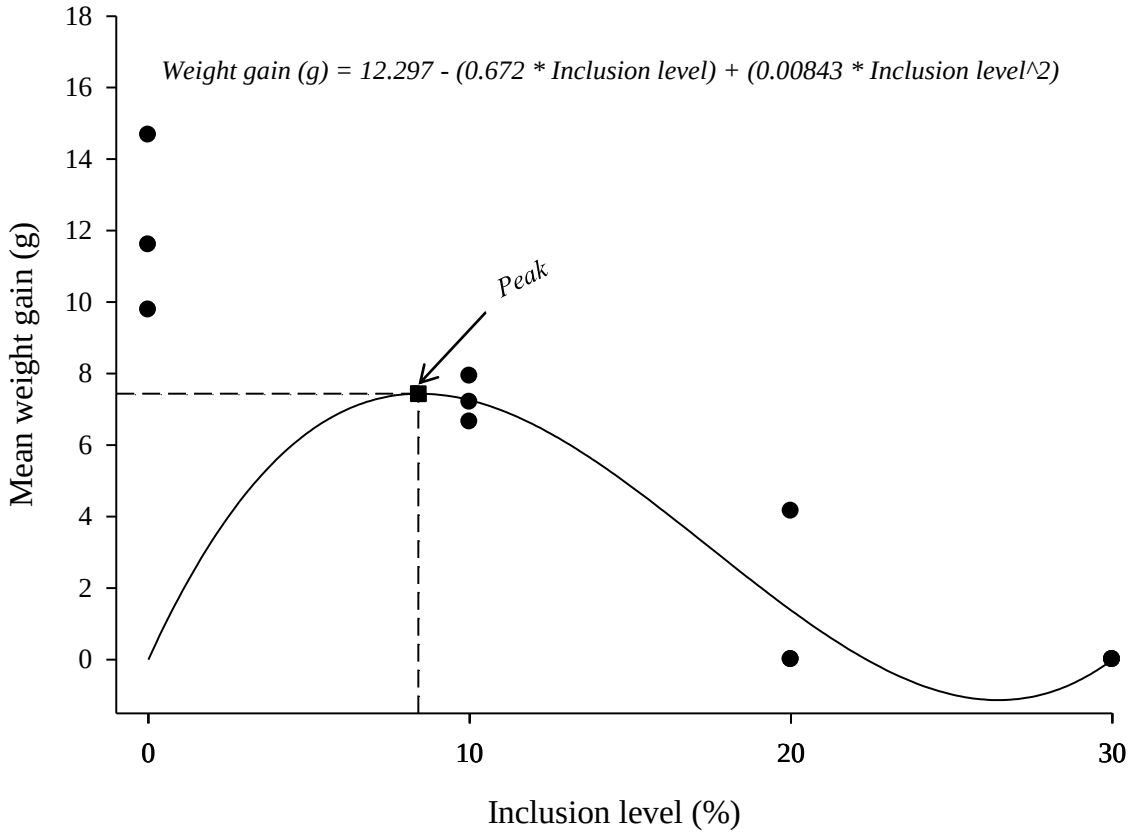

Figure 4.10: Mean weight gain response of Catfish to graded dietary inclusion levels of cooked kenaf (*Hibiscus cannabinus*) seed meal based diets. The inclusion level of cooked kenaf seed meal to achieve maximum performance was 8.40%. $r^2 = 0.88$

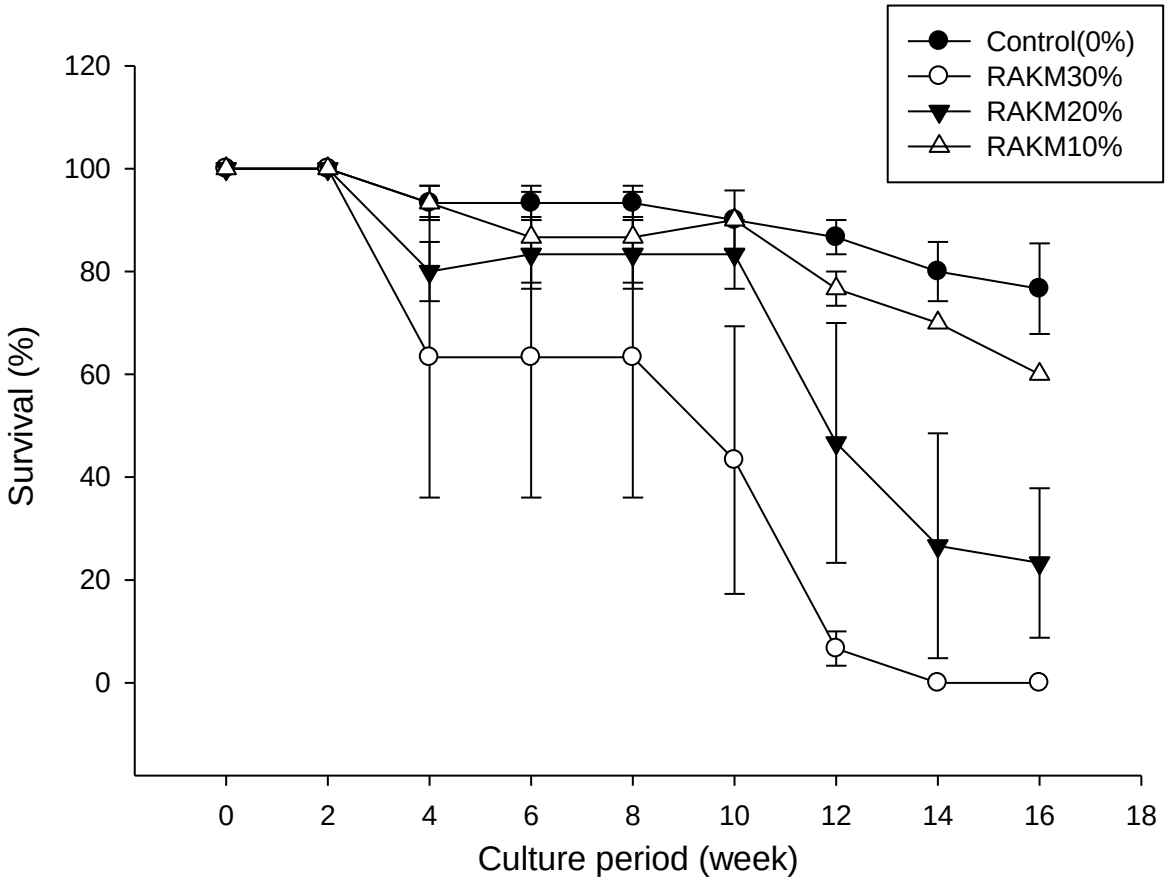

Figure 4.11: Survival rate of Catfish fed graded levels of raw kenaf (*Hibiscus cannabinus*) seed meal based diets.

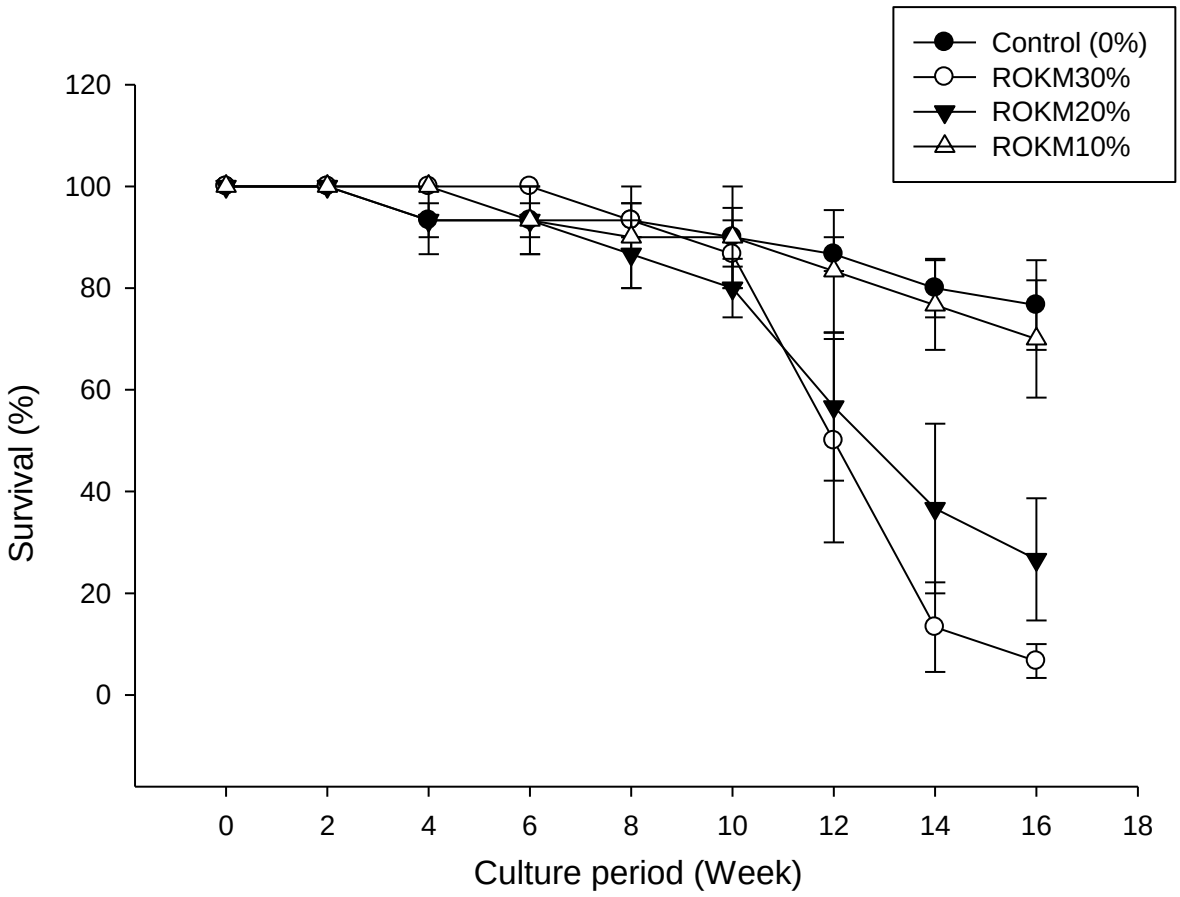

Figure 4.12: Survival rate of catfish fed graded levels of roasted kenaf (*Hibiscus cannabinus*) seed meal based diets.

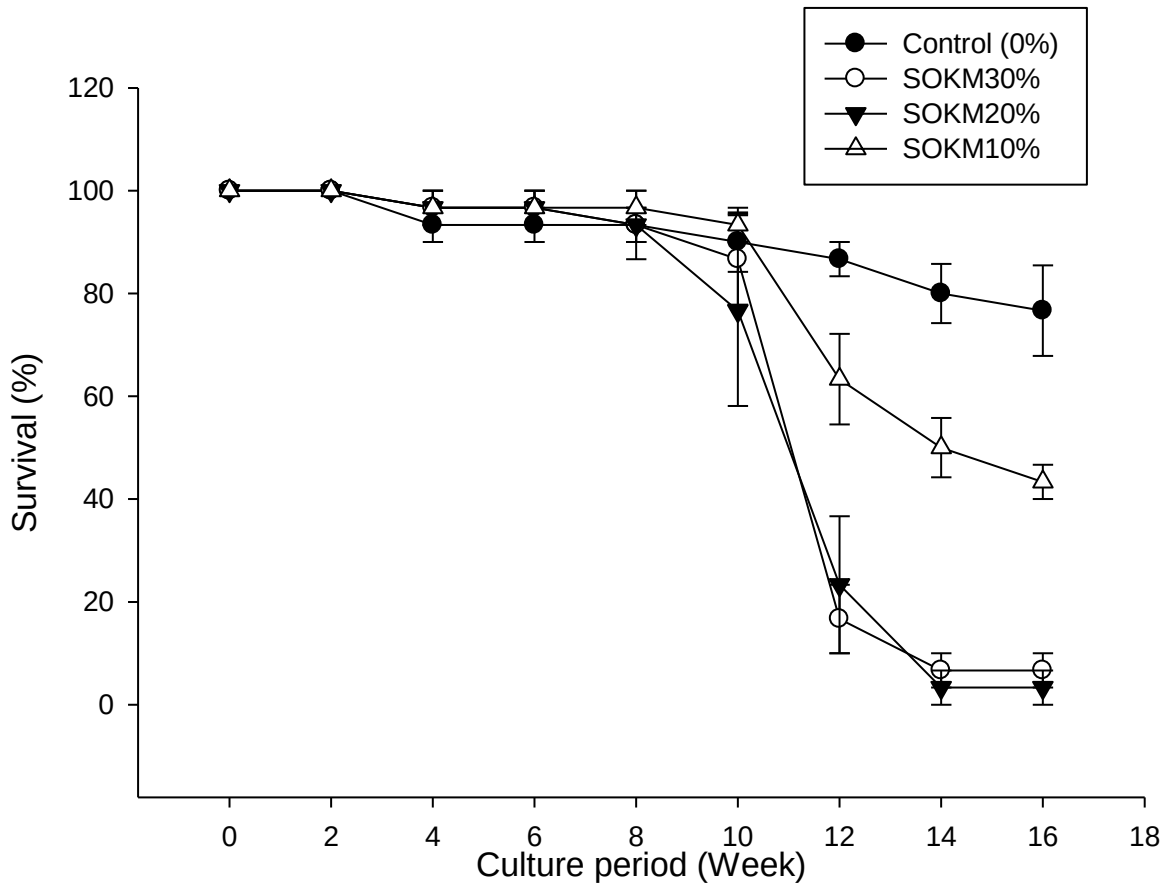

Figure 4.13: Survival rate of Catfish fed graded levels of soaked kenaf (*Hibiscus cannabinus*) seed meal based diets.

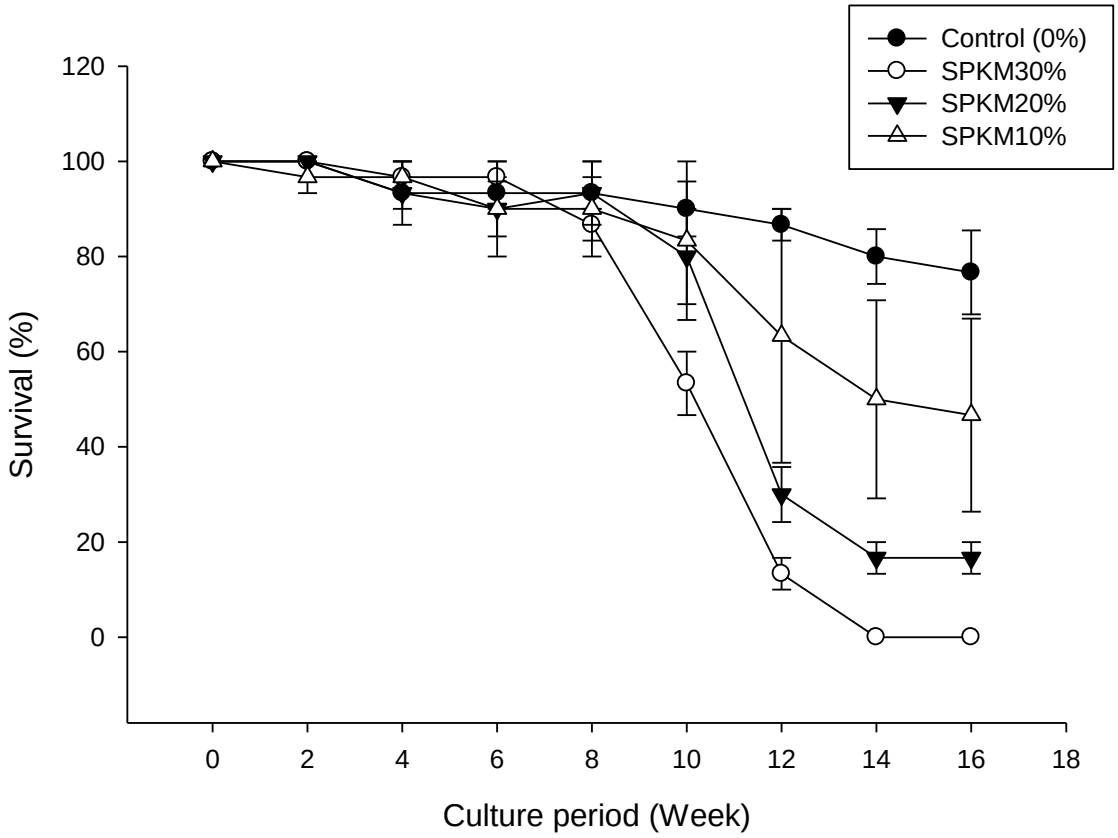

Figure 4.14: Survival rate of Catfish fed graded levels of sprouted kenaf (*Hibiscus cannabinus*) seed meal based diets.

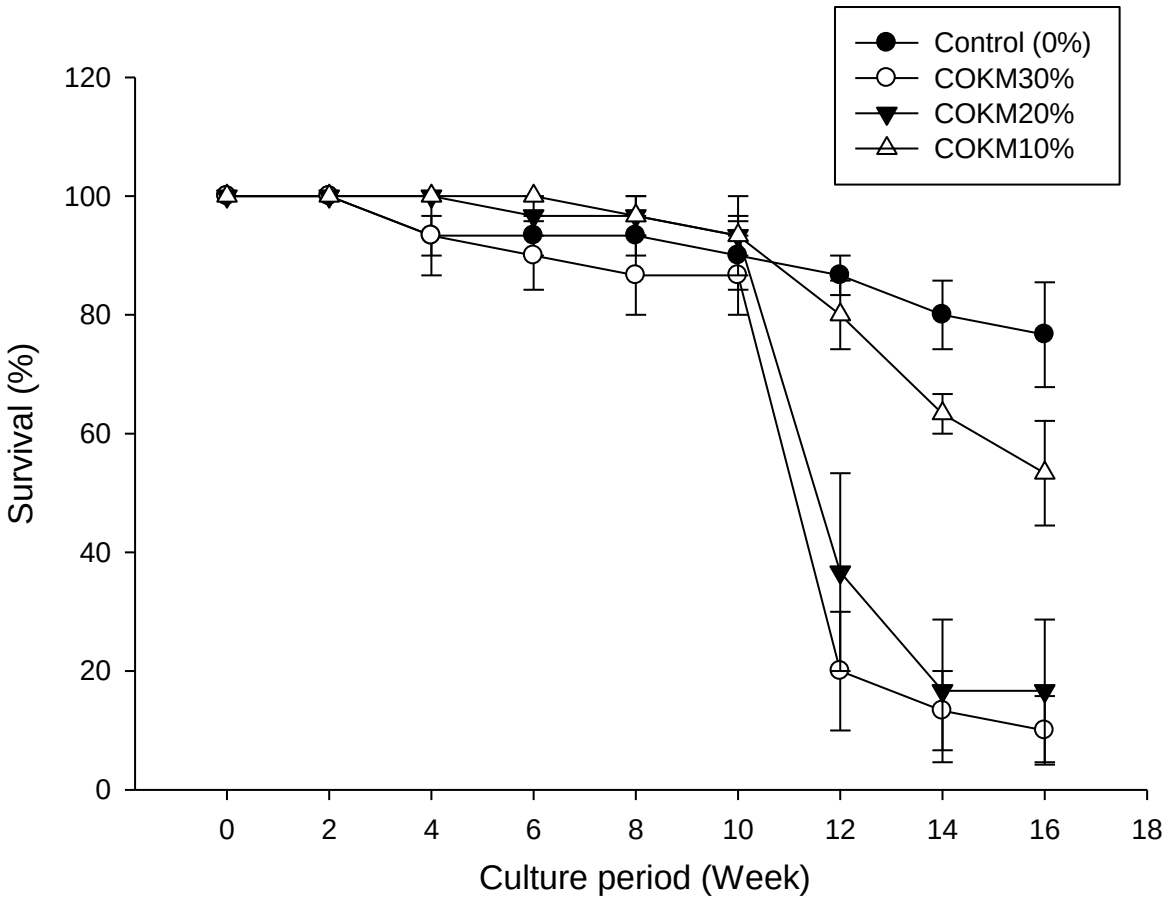

Figure 4.15: Survival rate of Catfish fed graded levels of cooked kenaf (*Hibiscus cannabinus*) seed meal based diets.

Table 4.11: Main effects on Growth performance indices of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

Growth Parameters	Processing						Replacement				
	Ra	Ro	So	Sp	Co	Pooled SE	0%	10%	20%	30%	Pooled SE±
Initial mean weight (g)	2.27	2.30	2.28	2.23	2.27	0.08	2.33	2.25	2.23	2.25	0.07
Final mean weight (g)	8.31^{b}	11.18^{a}	7.20^{b}	7.88^{b}	6.89^{b}	0.95	14.35^{a}	11.84^{b}	5.58^{c}	1.4^{d}	0.85
Mean weight gain(g)	6.81^{ab}	9.10^{a}	5.59^{b}	6.19^{b}	5.16^{b}	0.84	12.01^{a}	9.59^{b}	4.02^{c}	0.66^{d}	0.76
Specific growth rate(%/fish/day)	0.40^{b}	0.54^{a}	0.33^{b}	0.40^{b}	0.33^{b}	0.04	0.65^{a}	0.58^{a}	0.31^{b}	0.06^{c}	0.03
Average daily growth(g)	0.06^{ab}	0.08^{a}	0.05^{b}	0.06^{b}	0.05^{b}	0.01	0.11^{a}	0.09^{b}	0.04^{c}	0.01^{d}	0.01
Relative growth rate (%)	302.44^{ab}	398.64^{a}	237.01^{b}	269.28^{b}	227.24^{b}	34.88	511.82^{a}	421.87^{b}	181.46^{c}	32.54^{d}	31.20
Survival (%)	40.00	45.00	32.00	35.00	39.00	4.47	76.67^{a}	54.67^{b}	17.33^{c}	4.67^{d}	4.00

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Ra – Raw KSM based

Ro – Roasted KSM based

So – Soaked KSM based diet

Sp - Sprouted KSM based diet

Co – Cooked KSM based diet

SE – Standard Error.

Table 4.12: Interaction effects on Growth performance indices of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE
IMW	2.33	2.20	2.17	2.37	2.40	2.33	2.33	2.27	2.27	2.23	2.20	2.23	2.17	2.20	2.27	2.27	0.15
FMW	14.35^{ab}	14.17^{ab}	4.73^{cde}	NR	15.44^{a}	10.27^{abc}	4.67^{cde}	11.13^{abc}	2.33^{de}	1.00^{e}	9.01^{abc}	8.17^{bcd}	NR	9.46^{abc}	2.42^{de}	1.33^{e}	1.9
MWG	12.01^{ab}	11.97^{ab}	3.27^{cd}	NR	13.04^{a}	8.03^{abc}	3.30^{cd}	8.87^{abc}	1.47^{d}	NR	6.81^{bcd}	5.93^{cd}	NR	7.26^{bc}	1.38^{d}	NR	1.69
SGR	0.65^{a}	0.65^{a}	0.23^{bc}	NR	0.66^{a}	0.54^{ab}	0.29^{bc}	0.56^{a}	0.12^{c}	NR	0.50^{ab}	0.46^{ab}	NR	0.53^{ab}	0.13^{c}	NR	0.07
ADG	0.11^{ab}	0.11^{ab}	0.03^{cd}	NR	0.12^{a}	0.07^{abc}	0.03^{cd}	0.08^{abc}	0.01^{d}	NR	0.06^{bc}	0.05^{cd}	NR	0.06^{bc}	0.01^{d}	NR	0.02
RGR	511.83^{a}	550.74^{a}	147.22^{bcd}	NR	540.80^{a}	379.24^{ab}	162.7^{bcd}	379.80^{ab}	56.41^{d}	NR	306.75^{abc}	258.55^{bcd}	NR	331.27^{ab}	65.87^{cd}	NR	69.76
Survival (%)	76.67^{a}	60.00^{ab}	23.33^{cde}	0.00^{e}	70.00^{ab}	26.67^{cdef}	6.67^{e}	43.33^{bcde}	3.33^{e}	6.67^{e}	46.67^{bcd}	16.67^{ef}	0.00^{e}	53.33^{abc}	16.67^{ef}	10.00^{e}	8.94

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

NR – No record due to total mortality and/or growth retardation.

IMW – Initial Mean Weight (g). **FWM** – Final Mean Weight (g). **MWG** – Mean Weight Gain (g). **SGR** – Specific Growth Rate (%/fish/day)

ADG – Average Daily Growth (g). **RGR** – Relative Growth Rate (%).

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

4.3.4 Nutrient utilisation of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

4.3.4.1 The main effects on feed intake and nutrient utilisation of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The main effect of processing methods and replacement of soybean meal for kenaf seed meal on the feed intake and nutrient utilisation of *Clarias gariepinus* are presented in Table 4.13. The processing methods and the replacement levels of soybean for KSM significantly influenced ($p < 0.05$) the feed intake and nutrient utilisation of *C. gariepinus* in this study. While there was no significant variation ($p > 0.05$) in the mean feed intake among the raw, soaked, sprouted and cooked KSM based diets, roasting of KSM significantly increased ($p < 0.05$) the mean feed intake of *C. gariepinus.* There was no significant difference ($p > 0.05$) in the protein intake, feed conversion ratio, and protein productivity values among the processed and unprocessed KSM based diets. Net Protein Utilisation (NPU) and Nitrogen metabolism (Nm) were significantly highest ($p < 0.05$) in *C. gariepinus* fed roasted KSM based diet when compared with the unprocessed (raw) and other processed KSM based diets.

The feed intake, protein intake, protein efficiency ratio, nitrogen metabolism and protein productivity value did no vary significantly ($p < 0.05$) between the control group and *C. gariepinus* fed 10% KSM based diet. These parameters were significantly higher ($p < 0.05$) than those fed 20% and 30% KSM based diet. There was no significant difference ($p > 0.05$) in the feed conversion ratio of *C. gariepinus* at all inclusion levels of KSM in the diet. Net protein utilisation was lowest significantly ($p < 0.05$) in *C. gariepinus* fed 30% KSM based diet and significantly increased ($p < 0.05$) as the level of inclusion of KSM reduced. Similar result was obtained for protein productivity value.

4.3.4.2 The interaction effects on nutrient utilisation of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The interaction effect of processing methods and replacement of soybean meal for kenaf seed meal on the feed intake and nutrient utilisation of *Clarias gariepinus* are

presented in Table 4.14. Processing methods adopted in this study did not significantly interact ($p < 0.05$) with the replacement of soybean of KSM to influence the feed intake and the nutrient utilisation parameters except for the Protein Efficiency Ratio (PER). The highest PER was recorded for RA10 (1.73) which was no significant difference ($p < 0.05$) from the control (1.59), RO10 (1.56), and SO10 (1.32). The lowest significant ($p < 0.05$) PER was recorded for SO20 (0.19) whose value was not significantly different ($p < 0.05$) from RO30 (0.45) and CO30 (0.25). The highest significant ($p < 0.05$) Feed Intake (FI), Net Protein Utilisation (NPU) and Nitrogen Metabolism (NM) was recorded for *C. gariepinus* fed RO10 while the lowest significant values of 6.18 g FI , 5.22% NPU and 88.13 were recorded for RA30, SO30 and CO30 respectively. There was no significant difference ($p < 0.05$) in the feed conversion ratio and protein productivity value among the treatment groups.

4.4 HEAMATOLOGICAL, PLASMA BIOCHEMICAL AND HISTOLOGICAL RESPONSE OF CATFISH (*Clarias gariepinus*) FED RAW AND DIFFERENTLY PROCESSED KENAF (*Hibiscus cannabinus*) SEED MEAL

There were significant significant variations ($p < 0.05$) in the heamatological, biochemical and histopathological response of catfish (*Clarias gariepinus*) fed graded levels of processed kenaf (*Hibiscus cannabinus*) seed meal based diets. The $\mathbf{H_o}$ (null) hypothesis was therefore rejected.

4.4.1 Haematological response of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

4.4.1.1 Main effect on haematological response of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

The main effect of processing methods and replacement of soybean meal for kenaf seed meal on the hematological indices of *Clarias gariepinus* are presented in Table 4.15. The Packed Cell Volume (PCV), Heamoglobin (Hb) concentration, Red Blood Cell (RBC) count, Mean Corpuscular Heamoglobin Ceoncentration (MCHC) and the platelets were not significantly influenced ($p > 0.05$) by the processing methods adopted in this study. White blood cell count, Mean Corpuscular Volume (MCV) and Mean Corpuscular Heamoglobin (MCH) were significantly influenced ($p < 0.05$) by the processing methods.

Table 4.13: Main effects on indices for feed intake and nutrient utilisation of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

Nutrient utilisation parameters	Processing						Replacement				
	Ra	Ro	So	Sp	Co	Pooled SE	0%	10%	20%	30%	Pooled SE (±)
Mean Feed intake (g)	13.34^{b}	18.05^{a}	14.19^{b}	13.48^{b}	13.79^{b}	1.02	19.36^{a}	16.79^{a}	12.78^{b}	9.34^{c}	0.91
Protein intake (g/100g diet/fish	1.52	1.86	1.33	1.09	1.21	0.29	2.38^{a}	2.11^{a}	0.83^{b}	0.29^{b}	0.26
Feed Conversion ratio	1.23	1.85	1.27	1.56	1.20	0.27	1.62	1.92	1.75	2.04	0.24
Protein Efficiency ratio	1.00^{ab}	1.21^{a}	0.78^{b}	0.97^{ab}	0.77^{b}	0.09	1.59^{a}	1.41^{a}	0.70^{b}	0.09^{c}	0.08
Net Protein Utilisation (%)	48.95^{ab}	66.72^{a}	42.14^{b}	40.93^{b}	38.63^{b}	6.50	82.95^{a}	63.39^{b}	33.67^{b}	9.90^{c}	5.81
Nitrogen metabolism	301.65^{b}	407.8^{a}	275.27^{b}	294.29^{b}	269.19^{b}	33.21	512.83^{a}	433.36^{a}	222.07^{b}	70.3^{c}	29.70
Protein Productivity Value	1.54	1.96	1.56	1.45	1.30	0.33	2.22^{a}	1.62^{ab}	1.51^{ab}	0.90^{b}	0.30

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Ra – Raw KSM based

Ro – Roasted KSM based

So – Soaked KSM based diet

Sp - Sprouted KSM based diet

Co – Cooked KSM based diet

SE – Standard Error.

Table 4.14: Interaction effects of indices for feed intake and nutrient utilisation of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co
MFI	19.36^{ab}	17.25^{abc}	10.56^{cdef}	6.18^{f}	21.29^{a}	17.21^{abc}	14.32^{bcd}	16.06^{abcd}	11.51^{cdef}	9.83^{def}	14.22^{bcd}	13.47^{bcde}	6.87^{ef}	15.13^{abcd}	11.17^{cdef}	9.51^{def}
PI	2.38^{abc}	3.15^{a}	0.56^{cd}	NR	3.06^{ab}	1.28^{abcd}	0.71^{cd}	2.42^{abc}	0.45^{cd}	0.08^{d}	1.19^{bcd}	0.77^{cd}	NR	0.70^{cd}	1.10^{cd}	0.66^{cd}
FCR	1.62	1.61	1.7	NR	1.73	2.01	2.05	1.97	1.48	NR	2.20	2.45	NR	2.09	1.10	NR
PER	1.59^{a}	1.73^{a}	0.68^{bc}	NR	1.56^{a}	1.22^{ab}	0.45^{c}	1.32^{a}	0.19^{c}	NR	1.18^{ab}	1.13^{ab}	NR	1.23^{ab}	0.25^{c}	NR
NPU	82.95^{ab}	81.73^{ab}	31.12^{cd}	NR	89.91^{a}	63.78^{abc}	30.24^{cd}	64.62^{abc}	15.79^{d}	5.22^{d}	41.67^{bcd}	39.09^{bcd}	NR	39.00b^{cd}	18.57^{cd}	14.02^{d}
NM	512.83^{a}	503.18^{a}	190.61^{bcd}	NR	548.58^{a}	384.30^{ab}	185.49^{bcd}	411.97^{ab}	98.38^{cd}	77.89^{d}	344.59^{ab}	319.74^{abc}	NR	358.46^{ab}	117.34^{cd}	88.13^{d}
PPV	2.22	1.67	2.28	NR	2.16	1.56	1.99	1.91	0.73	1.36	1.47	2.11	NR	0.91	0.84	1.23

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

NR – No record due to total mortality

MFI – Mean Feed Intake (g) – PI – Protein Intake (g/100g/fish). FCR – Feed Conversion Ratio. PER – Protein Efficiency Ratio

NPU – Net Protein Utilisation (%). NM – Nitrogen Metabolism

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

The WBC count of C. gariepinus fed roasted KSM based diet was significantly higher ($p < 0.05$) than those fed raw, soaked and cooked KSM based diet but, not from those fed sprouted KSM based diet. The MCV and MCH of the fish fed roasted and sprouted KSM based diets were not significantly different ($p > 0.05$) from each other and were significantly higher ($p < 0.05$) than the group of fish fed raw KSM based diet.

C. gariepinus fed 30% inclusion level of KSM had the lowest significant values ($p < 0.05$) of hematological parameters measured in this study. The values of hematological parameters did not significantly differ ($p > 0.05$) between the control group and *C. gariepinus* fed 10% dietary replacement level of soybean meal for KSM. With the exception of RBC count, the values of other hematological parameters of *C. gariepinus* fed 20% inclusion level of KSM in their diet were significantly lower ($p < 0.05$) than the control group and those fed 10% replacement level of soybean meal with KSM in their diet.

4.4.1.2 Interaction effect on haematological response of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

Table 4.16 shows the interaction effect between processing methods and the replacement levels of soybean meal for kenaf seed meal on the hematological parameters of *C. gariepinus*. Significant interactions were only recorded for MCV and MCH. The control groups, RA10, RO10, SO10, SP10, SP20 and CO10 did not differ significantly ($p > 0.05$) in values measured for MCH and MCH. The MCV and MCH parameters for these groups were significantly higher ($p < 0.05$) than RA20, SO20 and CO20. The haematological parameters of fish fed RO10 KSM based diet were not significantly different ($p > 0.05$) from the control group. The general trend showed that the heamatological values of *C. gariepinus* decreased significantly ($p < 0.05$) as the inclusion levels increased for the processed and unprocessed (raw) KSM in their diets. No result was recorded for RA30, SP30 and CO30 due to insufficient blood samples and/or total mortality.

4.4.1.3 Main effect on differential white blood cell count of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

The main effect of processing methods and replacement levels of soybean meal for kenaf seed meal on the differential white blood cell count of *Clarias gariepinus* are presented in Table 4.17. The processing methods did not significantly influence ($p > 0.05$) the neutrophil, eosinophil and basophil counts of *C. gariepinus*. The lymphocyte and monocyte counts of the fish fed roasted KSM based diet were the highest and significantly higher ($p < 0.05$) than those fed raw and cooked KSM based diet but not different significantly ($p > 0.05$) from those fed sprouted KSM based diet. There was no significant difference ($p > 0.05$) in the differential white blood cell count between the control group and *C. gariepinus* fed 10% KSM meal based diet. The differential white blood cell counts in these groups were significantly higher ($p < 0.05$) than *C. gariepinus* fed 20% and 30% KSM based diet. The count significantly reduced ($P < 0.05$) as the quantity of KSM increased in the diet of the fish.

4.4.1.4 Interaction effect on differential white blood cell count of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

Table 4.18 shows the interaction effect between processing methods and the replacement levels of soybean meal for kenaf seed meal on the differential white blood cell count of *C. gariepinus*. No significant interaction ($p > 0.05$) between processing and level of replacement of soybean meal with KSM was recorded in the count for the components of white blood cell in this study. The differential white blood cell count of the control group was not significantly different ($p > 0.05$) from RA10, RO10, SO10, SP10 and CO10. These values tended to decreased with increasing levels of KSM meal in the diet of *C. gariepinus*.

Table 4.15: Main effects on indices for haematological response of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	Processing						Replacement				
	Ra	Ro	So	Sp	Co	Pooled SE	0%	10%	20%	30%	Pooled SE
Packed Cell Volume (%)	16.75	24.67	17.83	22.75	17.92	3.07	30.33^{a}	31.73^{a}	14.53^{b}	3.33^{c}	2.74
Heamoglobin (g/100ml)	5.44	8.03	5.79	7.39	5.84	1.02	9.83^{a}	10.38^{a}	4.71^{b}	1.07^{c}	0.92
Red Blood Cell Count (x 10^{6} µl)	2.46	2.53	1.84	2.16	1.91	0.54	3.00^{a}	3.32^{a}	2.08^{a}	0.33^{b}	0.49
White Blood Cell Count (x 10^{6} µl)	8.04^{b}	12.62^{a}	8.31^{b}	10.8^{ab}	8.29^{b}	1.31	14.77^{a}	13.96^{a}	7.58^{b}	2.14^{c}	1.17
MCHC (g/dl)	18.95	27.06	18.89	24.26	18.98	2.72	32.21^{a}	32.78^{a}	17.27^{b}	4.26^{c}	2.44
MCV (fl)	5.12^{b}	8.34^{a}	5.72^{ab}	8.13^{a}	5.88^{ab}	0.86	10.30^{a}	9.77^{a}	4.98^{b}	1.50^{c}	0.77
MCH (pg/cell)	16.65^{b}	27.06^{a}	18.57^{ab}	26.26^{a}	19.19^{ab}	2.82	33.36^{a}	32.04^{a}	16.03^{b}	4.75^{c}	2.52
Platelets (x 10^{4}/ml)	7.88	10.62	7.84	10.38	7.77	1.14	13.93^{a}	13.24^{a}	6.8^{b}	1.61^{c}	1.02

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

PCV – Packed Cell Volume (%). Hb – Heamoglobin (g/100ml). RBC – Red Blood Cell count (x 10^{6}µl).

WBC count (x 10^{6}µl). MCHC – Mean Corpuscular Heamoglobin Ceoncentration (g/dl). MCV – Mean Corpuscular Volume (fl)

MCH - Mean Corpuscular Heamoglobin (pg/cell)

Ra – Raw KSM bases diet.

Ro – Roasted KSM bases diet.

So – Soaked KSM bases diet.

Sp - Sprouted KSM based diet

Co – Cooked KSM bases diet.

SE – Standard Error.

Table 4.16: Interaction effects on indices for haematology of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE
PCV	30.33^{abc}	28.67^{abcd}	8.00^{e}	NR	31.00^{ab}	20.67^{abcde}	16.67^{bcde}	30.33^{abc}	10.66^{cde}	NR	37.33^{a}	23.33^{abcde}	NR	31.33^{ab}	10.00^{de}	NR	6.13
Hb	9.83^{abc}	9.33^{abcd}	2.60^{e}	NR	10.23^{ab}	6.73^{abcde}	5.33^{bcde}	9.80^{abc}	3.50^{cde}	NR	12.27^{a}	7.47^{abcde}	NR	10.30^{ab}	3.23^{de}	NR	2.05
RBC	3.00	2.94	3.90	NR	3.34	2.19	1.63	3.22	1.12	NR	3.55	2.08	NR	3.54	1.10	NR	1.09
WBC	14.77^{a}	13.77^{ab}	3.63^{c}	NR	14.92^{a}	10.10^{abc}	10.72^{abc}	13.85^{ab}	4.62^{bc}	NR	14.25^{a}	14.17^{a}	NR	13.00^{ab}	5.40^{abc}	NR	2.62
MCHC	32.22^{a}	32.76^{a}	10.83^{b}	NR	33.02^{a}	21.72^{ab}	21.30^{ab}	32.31^{a}	11.04^{b}	NR	32.87^{a}	31.95^{a}	NR	32.92^{a}	10.78^{b}	NR	5.45
MCV	10.3^{a}	9.48^{a}	0.68^{c}	NR	9.28^{a}	6.29^{ab}	7.47^{ab}	9.42^{a}	3.17^{bc}	NR	10.51^{a}	11.71^{a}	NR	10.16^{a}	3.04^{bc}	NR	1.72
MCH	33.36^{a}	31.01^{a}	2.22^{c}	NR	30.64^{a}	20.50^{ab}	23.75^{ab}	30.42^{a}	10.49^{bc}	NR	34.54^{a}	37.13^{a}	NR	33.57^{a}	9.83^{bc}	NR	5.64
Platelets	13.93^{a}	13.90^{a}	3.67^{b}	NR	12.17^{a}	8.33^{ab}	8.03^{ab}	12.83^{a}	4.60^{b}	NR	13.83^{a}	13.73^{a}	NR	13.47^{a}	3.67^{b}	NR	2.29

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

NR – No record due to total mortality and/or insufficient blood sample from fish sample.

PCV – Packed Cell Volume (%). **Hb** – Heamoglobin (g/100ml). **RBC** – Red Blood Cell count (x 10^6µl).

WBC count (x 10^6µl). **MCHC** – Mean Corpuscular Heamoglobin Ceoncentration (g/dl). **MCV** – Mean Corpuscular Volume (fl)

MCH - Mean Corpuscular Heamoglobin (pg/cell)

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

Table 4.17: Main effects on Differential White Blood Cell Counts of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	Processing						Replacement				
	Ra	Ro	So	Sp	Co	Pooled SE	0%	10%	20%	30%	Pooled SE
Lymphocyte(%)	36.83^{b}	54.58^{a}	36.42^{b}	47.25^{ab}	37.42^{b}	5.64	64.67^{a}	62.13^{a}	34.20^{b}	9.00^{c}	5.04
Monocyte(%)	1.42^{b}	2.42^{a}	1.67^{ab}	2.17^{ab}	1.42^{b}	0.31	3.00^{a}	2.40^{a}	1.27^{b}	0.60^{b}	0.28
Neutrophil(%)	17.08	22.67	18.17	22.08	16.75	2.80	28.00^{a}	30.86^{a}	15.13^{b}	3.40^{c}	2.50
Eosinophil(%)	2.75	3.50	2.00	3.33	2.58	0.52	4.00^{a}	4.27^{ab}	2.73^{b}	0.33^{c}	0.47
Basophil(%)	0.25	0.17	0.08	0.17	0.17	0.11	0.33^{a}	0.33^{a}	0.00^{b}	0.00^{b}	0.10

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Ra – Raw KSM bases diet.

Ro – Roasted KSM bases diet.

So – Soaked KSM bases diet.

Sp - Sprouted KSM based diet

Co – Cooked KSM bases diet.

SE – Standard Error.

Table 4.18: Interaction effects on Differential White Blood Cell Counts (%) of catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE
Lymphocyte	64.67^{a}	63.33^{a}	19.33^{c}	NR	60^{ab}	48.67^{abc}	45.00^{abc}	60.33^{ab}	20.67^{bc}	NR	63.33^{a}	61.00^{ab}	NR	63.67^{a}	21.33^{bc}	NR	11.28
Monocyte	3.00^{ab}	2.00^{ab}	0.67^{b}	NR	1.67^{ab}	2.00^{ab}	3.00^{ab}	2.67^{ab}	1.00^{b}	NR	3.67^{a}	2.00^{ab}	NR	2.00^{ab}	0.67^{b}	NR	0.63
Neutrophil	28.00^{abc}	29.33^{ab}	11.00^{bc}	NR	33.00^{a}	12.67^{bc}	17.00^{abc}	34.33^{a}	10.33^{bc}	NR	28.33^{abc}	32.00^{a}	NR	29.33^{ab}	9.67^{c}	NR	5.59
Eosinophil	4.00^{a}	4.67^{a}	2.33^{ab}	NR	5.00^{a}	3.00^{ab}	1.67^{ab}	2.67^{ab}	1.33^{ab}	NR	4.33^{a}	5.00^{a}	NR	4.67^{a}	1.67^{ab}	NR	1.04
Basophil	0.33^{ab}	0.67^{a}	0.00^{b}	NR	0.33^{ab}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	NR	0.33^{ab}	0.00^{b}	NR	0.33^{ab}	0.00^{b}	NR	0.22

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

NR – No record due to total mortality and/or insufficient blood sample collected from fish

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

4.4.2 Plasma biochemical response of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

4.4.2.1 Main effect on plasma biochemistry of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

The main effect of processing methods and replacement levels of soybean meal for kenaf seed meal on the plasma biochemical parameters of *Clarias gariepinus* are presented in Table 4.19. The processing methods and the replacement levels of soybean for KSM significantly influenced ($p < 0.05$) the plasma biochemistry of *C. gariepinus* in this study. There were no significant differences ($p > 0.05$) in all the plasma biochemical parameters measured among *C. gariepinus* fed raw, soaked and cooked KSM based diets. The plasma biochemical parameters for these groups were significantly lower ($p < 0.05$) than *C. gariepinus* fed roasted KSM based diet. The plasma biochemical parameters of catfish fed sprouted KSM based diet were not comparable in relation to other processing methods. In this study, the plasma biochemical parameters significantly decreased ($p < 0.05$) as the quantity of KSM in the diet of *C. gariepinus* increased. There was no significant variation ($p > 0.05$) in the plasma biochemical parameter between the control and those fed 10% KSM based diet except A/G ratio that was significantly lower ($p < 0.05$) in 10% KSM based diet.

4.4.2.2 Interaction effect on plasma biochemistry of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal.

Table 4.20 shows the interaction effect of processing methods and replacement levels of soybean meal for kenaf seed meal on the plasma biochemical parameters of *Clarias gariepinus.* Significant interaction ($p < 0.05$) was recorded only for Aspartate aminotransferase (AST) and Blood Urea Nitrogen (BUN). *C. gariepinus* fed RA20, SO20 and CO20 diet had a significantly lower ($p < 0.05$) AST and BUN when compared to the control groups. The *C. gariepinus* fed RA10, RO10, SO10, SP10 and CO10 treatment diets did not differ significantly ($p > 0.05$) in their measured plasma biochemical parameters from the control group. The values for all these showed a reducing trend with increasing level of inclusion of the processed and unprocessed KSM.

Table 4.19: Main effects on indices for plasma biochemistry of Catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	Processing						Replacement				
	Ra	Ro	So	Sp	Co	Pooled SE	0%	10%	20%	30%	Pooled SE±
Plasma protein(g/dl)	2.71^{b}	4.14^{a}	2.31^{b}	3.41^{ab}	2.63^{b}	0.41	4.97^{a}	4.17^{a}	2.41^{b}	0.61^{c}	0.37
Albumin(g/dl)	0.51^{b}	0.74^{a}	0.48^{b}	0.63^{ab}	0.45^{b}	0.07	0.97^{a}	0.76^{b}	0.42^{c}	0.10^{d}	0.06
Globulin(g/dl)	2.19^{b}	3.39^{a}	2.19^{b}	2.78^{ab}	2.18^{b}	0.31	3.97^{a}	3.71^{a}	1.99^{b}	0.51^{c}	0.28
A/G Ratio	0.13^{b}	0.18^{a}	0.12^{b}	0.16^{ab}	0.11^{b}	0.02	0.27^{a}	0.16^{b}	0.11^{c}	0.02^{d}	0.02
AST(IU/L)	105.92^{b}	167.83^{a}	106.17^{b}	135.92^{ab}	105.25^{b}	13.34	184.33^{a}	179.73^{a}	108.33^{b}	24.47^{c}	11.93
ALT(IU/L)	14.50^{b}	22.00^{a}	14.17^{b}	18.33^{ab}	14.42^{b}	2.16	24.67^{a}	25.40^{a}	13.33^{b}	3.33^{c}	1.93
ALP(IU/L)	128.75^{b}	195.50^{a}	128.58^{b}	160.25^{ab}	114.42^{b}	19.84	232.67^{a}	194.8^{a}	128.13^{b}	26.40^{c}	17.75
BUN(mg/dl)	6.41^{b}	9.73^{a}	6.28^{b}	8.00^{ab}	6.38^{b}	0.78	11.20^{a}	10.63^{a}	6.17^{b}	1.44^{c}	0.7
Creatinine (mg/dl)	0.34^{b}	0.55^{a}	0.35^{b}	0.44^{ab}	0.35^{b}	0.05	0.63^{a}	0.56^{a}	0.35^{b}	0.08^{c}	0.04

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Ra – Raw kenaf seed meal based diet. Ro – Roasted kenaf seed meal based diet. So – Soaked kenaf seed meal based diet

Sp – Sprouted kenaf seed meal based diet Co – Cooked kenaf seed meal based diet. AST- Aspartate aminotransferase.

ALT- Alanine aminotransferase ALP- Alkaline phosphate. BUN - Blood Urea Nitrogen.

Table 4.20: Interaction effects on indices for plasma biochemistry of Catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

Parameter (%)	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE
Plasma protein(g/dl)	4.97^{a}	4.83^{a}	1.03^{b}	NA	4.40^{a}	4.17^{a}	3.03^{ab}	2.87^{ab}	1.40^{b}	NA	4.30^{a}	4.37^{a}	NA	4.47^{a}	1.10^{ab}	NA	0.82
Albumin(g/dl)	0.97^{a}	0.90^{a}	0.17^{b}	NA	0.70^{a}	0.80^{a}	0.50^{ab}	0.73^{a}	0.23^{b}	NA	0.80^{a}	0.73^{a}	NA	0.67^{a}	0.17^{b}	NA	0.14
Globulin(g/dl)	3.97^{a}	3.93^{a}	0.87^{b}	NA	3.70^{a}	3.37^{a}	2.53^{ab}	3.63^{a}	1.17^{b}	NA	3.50^{a}	3.63^{a}	NA	3.80^{a}	0.93^{b}	NA	0.62
A/G Ratio	0.27^{a}	0.20^{abc}	0.03^{e}	NA	0.13^{bcde}	0.23^{ab}	0.10^{cde}	0.13^{bcde}	0.07^{de}	NA	0.20^{abc}	0.17^{abcd}	NA	0.13^{bcde}	0.03^{e}	NA	0.04
AST(IU/L)	184.33^{a}	181.00^{a}	58.33^{b}	NA	179.33^{a}	185.33^{a}	122.33^{ab}	180.67^{a}	59.67^{b}	NA	179.00^{a}	180.00^{a}	NA	178.67^{a}	58.00^{b}	NA	26.67
ALT(IU/L)	24.67^{a}	27.00^{a}	6.33^{b}	NA	25.67^{a}	21.00^{a}	16.67^{ab}	24.00^{a}	8.00^{b}	NA	24.33^{a}	24.33^{a}	NA	26.00^{a}	7.00^{b}	NA	4.32
ALP(IU/L)	232.67^{a}	178.67^{ab}	103.67^{ab}	NA	219.67^{a}	197.67^{ab}	132.00^{ab}	216.33^{a}	65.33^{b}	NA	205.33^{ab}	203.00^{ab}	NA	154.00^{ab}	71.00^{b}	NA	39.68
BUN(mg/dl)	11.20^{a}	11.17^{a}	3.27^{b}	NA	10.20^{a}	10.33^{a}	7.20^{ab}	10.40^{a}	3.50^{b}	NA	10.30^{a}	10.50^{a}	NA	11.07^{a}	3.23^{b}	NA	1.56
Creatinine(mg/dl)	0.63^{a}	0.57^{a}	0.17^{b}	NA	0.53^{a}	0.63^{a}	0.40^{ab}	0.57^{a}	0.20^{b}	NA	0.57^{a}	0.57^{a}	NA	0.60^{a}	0.17^{b}	NA	0.1

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

NR – No record due to total mortality and/or insufficient blood sample collected from fish.

AST- Aspartate aminotransferase. **ALT**- Alanine aminotransferase **ALP**- Alkaline phosphate.

BUN - Blood Urea Nitrogen.

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

4.5.0 Histopathological response of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The histological response that was qualitatively analysed on the liver and kidney from *Clarias gariepinus* fed raw and differently processed kenaf seed meal are presented in Plates 4.1 and 4.2 respectively and the summary of the histopathological changes in the liver presented in Table 4.20. Mild disseminated steatosis (B) was observed in the liver analysed from the control group, *C. gariepinus* fed RO20, RO30, SO10, SO20 and SP10 treatment diets. The periportal track (C) in the liver of *C. gariepinus* fed SO10, SO20, CO10 and CO30 treatment diets showed a mild congestion of the hepatocyte with blood cells. Thrombocytes and lymphocytes were in focus in the portal tract perceived in the catfish fed SO20 and CO30 diets. Mild infiltration of inflammatory cells into the hepatocyte (D) was observed in *C. gariepinus* fed SP10 and CO20 diets. Fibrosis (E) was noticed in catfish fed RA20, SO10 and SO20 diet. The fibrosis was observed around the lining of the portal tract. Necrosis (F) was not observed in the hepatocyte of the control group and *C. gariepinus* fed RA10, RO10, SO20, SP10 and CO10 treatment diets. Sever necrosis was observed in *C. gariepinus* fed RA20 diet. The necrosis was isolated in *C. gariepinus* fed RO20, RO30 and SO10 diet. There was no visible lesion observed in the kidney except mild interstitial congestion. This histopathological change was observed in the control group and the groups of *C. gariepinus* fed the unprocessed (raw) and differently processed KSM based diets at all inclusion levels (G to L).

4.6. Economic Evaluation of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The $\mathbf{H_o}$ (null) hypothesis was rejected at 95% confidence limit. There was significant variation ($p < 0.05$) in the economic benefit of replacing soyabean meal with differently processed kenaf (*Hibiscus cannabinus*) seed meal in the diet of African catfish (*Clarias gariepinus*). The Ho was rejected

4.6.1 Main effects of economic evaluation of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

Table 4.22 shows the main effect of processing methods and replacement levels of soybean meal for kenaf seed meal on the economic indices assessed for *Clarias gariepinus*.

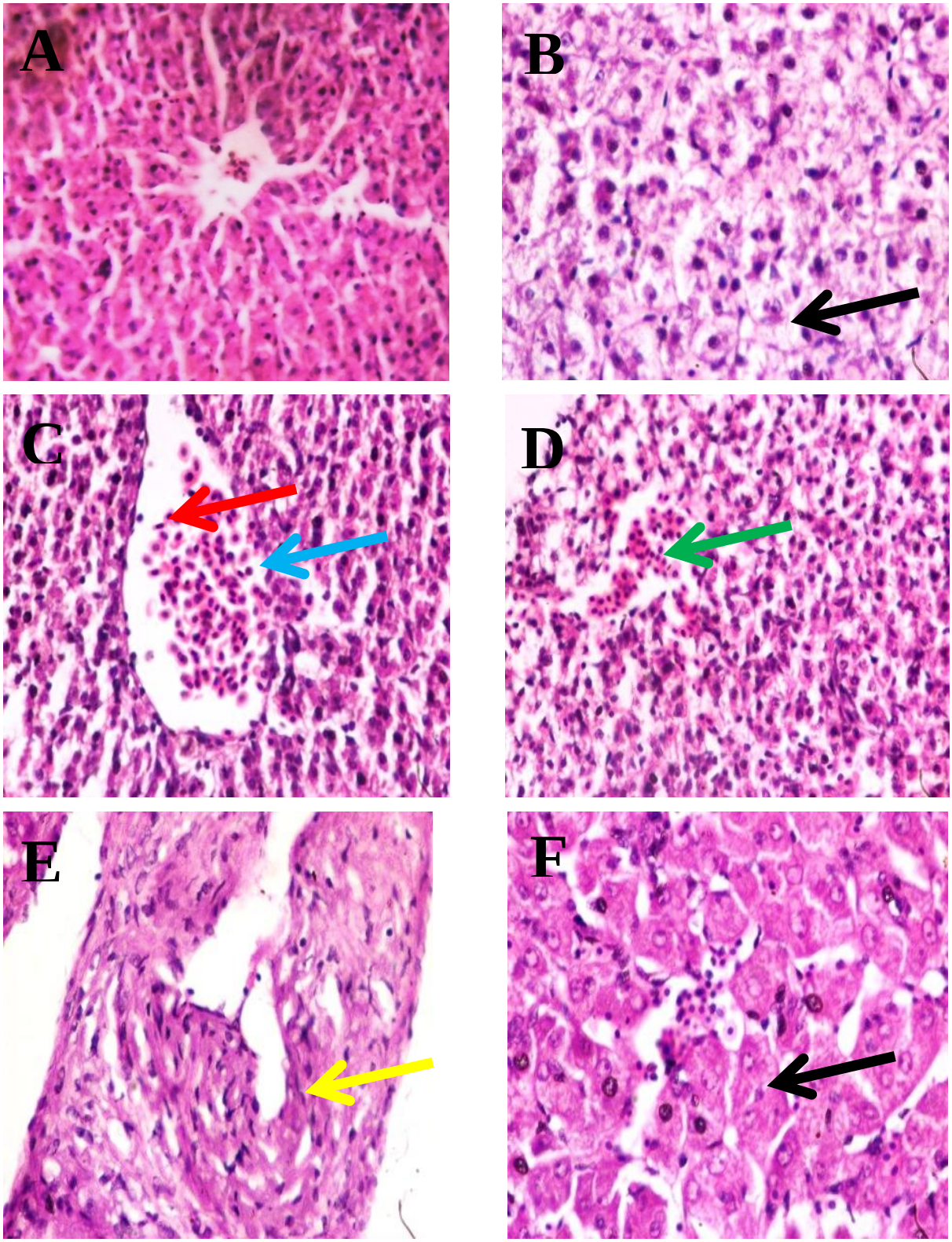

Plate 4.1: Histology section of liver from *C. gariepinus* fed Kenaf (*Hibiscus cannabinus*) seed meal based diet. **A** – Liver with no visible lesion. **B** – Disseminated steatosis (black arrow). **C** – Congestion of hepatic portal (Thrombocyte- red arrow and; Lymphocyte – blue arrow). **D** – Inflammation (infiltration of cellular component into the hepatocyte) green arrow. **E** – Fibrosis around the lining of portal tract (Yellow arrow). **F** – Necrosis, forming individualization of the hepatocyte (black arrow). Mag. X400. Heamatoxylin and Eosin stain.

Table 4.21: Summary of Histopathological changes in the liver from *Clarias gariepinus* fed raw and differently processed kenaf *Hibiscus cannabinus*) seed meal based diet.

Changes	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co
Necrosis (hepatocyte)	0	0	++	NR	0	+	+	+	0	NR	0	+	NR	0	+	+
Inflammation	0	0	0	NR	0	0	0	0	0	NR	+	0	NR	0	+	0
Congestion (portal tract)	0	0	0	NR	0	0	0	+	+(L,T)	NR	0	0	NR	+	0	+
Fibrosis	0	0	+	NR	0	0	0	+	+	NR	0	0	NR	0	0	0
Steatosis/Vacuolation	+	0	0	NR	0	+	+	+	+	NR	+	0	NR	0	0	0

LEGEND:

0, +, ++ - represent the degree

NR – No record due to total mortality and/or difficulty in obtaining organs. L – Lymphocyte. T-Thrombocyte

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

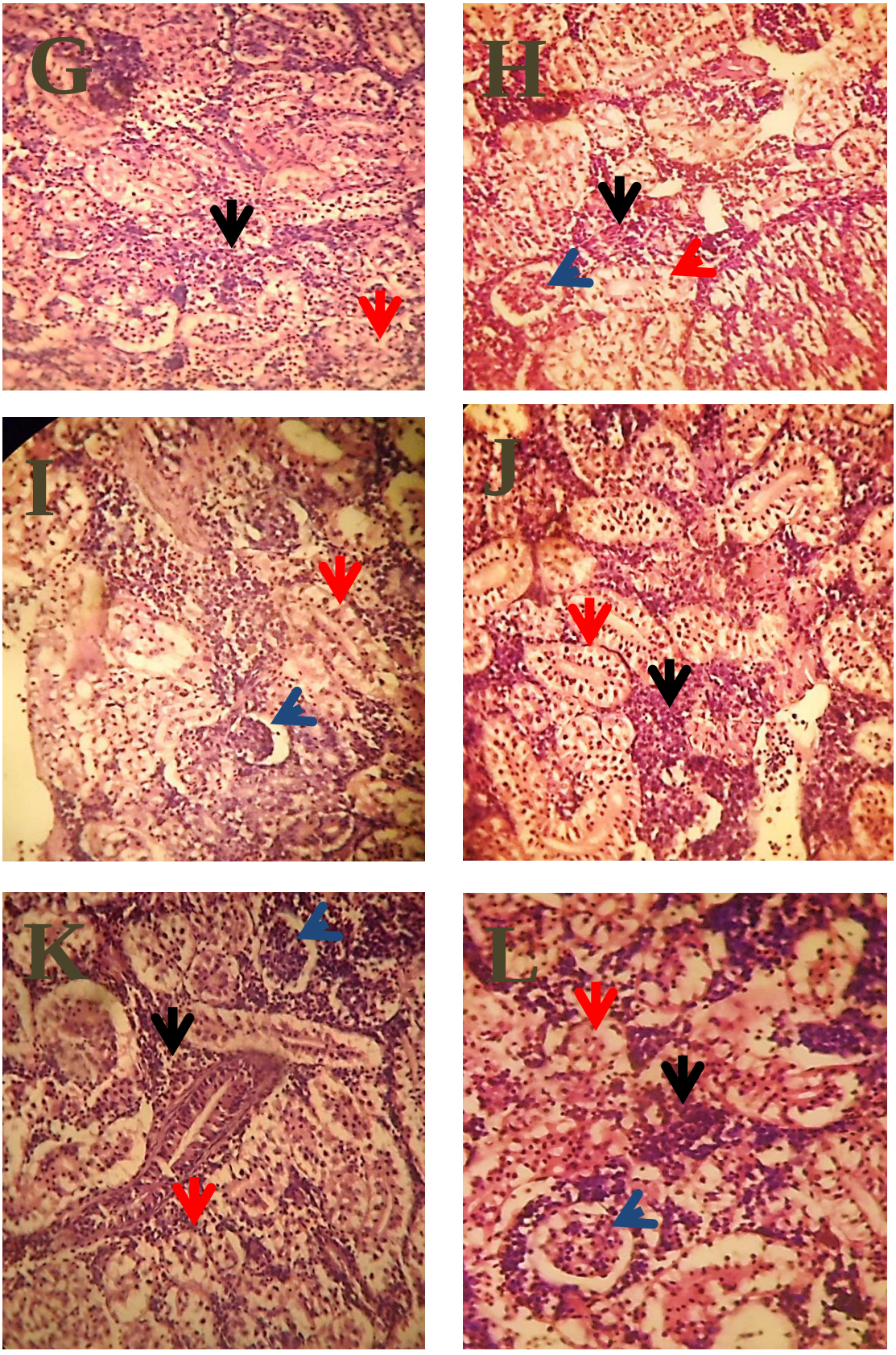

Plate 4.2: Histology section of kidney from *C. gariepinus* fed Kenaf (*Hibiscus cannabinus*) seed meal based diet. **G, H, I, J, K and L** are kidney section from the Control group (0%), RA20, RO20, SO10, SP20 and CO10 respectively. The tubules (red arrow) and the glomerulus (blue arrow) appear to be intact. There was interstitial congestion (black arrow) observed in control and the treatment groups. (Mag. X400. Heamatoxylin and Eosin stain.

The processing methods and the replacement levels of soybean for kenaf seed meal significantly influence ($p < 0.05$) the economic evaluation indices used in this study. Roasted KSM based diet had the highest significant ($p < 0.05$) cost of feed used in the production, value of fish, incidence of cost and profit index when compared to other methods (processed and unprocessed). There was no significant difference ($p > 0.05$) in these parameters within the *C. gariepinus* fed raw, soaked, sprouted and cooked KSM based diet. The economic benefit of replacement of soybean with graded levels of KSM significantly decreased ($p < 0.05$) with increasing level of kenaf seed meal in the diet of *C. gariepinus*. However, there was no significant difference ($p > 0.05$) in the cost of feed used in production, incidence of cost and profit index between the control group and *C. gariepinus* fed 10% KSM based diet. The economic value of *C. gariepinus* fed 10% KSM based diet was significantly lower ($p < 0.05$) than the control group (0%) but higher than those fed 20% and 30% KSM based diet.

4.6.2 Interaction effect on economic evaluation of catfish (*Clarias gariepinus*) fed raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

The interaction effects of processing methods and replacement levels of soybean meal for kenaf seed meal on the economic indices assessed for *Clarias gariepinus* are presented in Table 4.23. There was no significant interaction ($p < 0.05$) in all the economic indices recorded in this study. The highest cost of feed used in production was recorded for RO10. There was no significant difference ($p < 0.05$) in the value of fish and profit index between RO10 and the control group. For all the processing methods, the incidence of cost generally increased as the level of inclusion of processed and unprocessed (raw) KSM increased in the diet of *C. gariepinus*.

Table 4.22: Main effects on indices for economic evaluation of Catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	Processing						Replacement				
	Ra	Ro	So	Sp	Co	Pooled SE	0%	10%	20%	30%	Pooled SE±
Cost of feed per kg (N)	350.22	348.62	349.24	348.50	349.15	0.00	346.45	347.96	350.16	352.02	0.00
Cost of feed used in production(N)	4.66^{b}	6.29^{a}	4.95^{b}	4.69^{b}	4.81^{b}	0.36	6.71^{a}	5.84^{a}	4.48^{b}	3.27^{c}	0.32
Value of fish (N/fish)	4.43^{ab}	5.91^{a}	3.63^{b}	4.02^{b}	3.36^{b}	0.55	7.81^{a}	6.23^{b}	2.61^{c}	0.43^{d}	0.49
Incidence of cost	430.48^{b}	757.39^{a}	679.43^{ab}	545.49^{ab}	419.21^{b}	99.46	562.3^{ab}	670.46^{a}	722.61^{a}	310.23^{b}	88.96
Profit Index	0.72^{ab}	0.87^{a}	0.57^{b}	0.71^{ab}	0.56^{b}	0.06	1.16^{a}	1.03^{a}	0.5^{b}	0.06^{c}	0.06

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

Ra – Raw kenaf seed meal based diet. Ro – Roasted kenaf seed meal based diet. So – Soaked kenaf seed meal based diet

Sp - Cooked kenaf seed meal based diet. Co – Cooked kenaf seed meal based diet

SE – Standard Error

Table 4.23: Interaction effects of indices for economic evaluation of Catfish (*Clarias gariepinus*) fed graded levels of raw and differently processed kenaf (*Hibiscus cannabinus*) seed meal

	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE
Cost of feed per kg (N)	346.45	348.96	351.48	354	346.45	349.92	351.67	348.31	350.17	352.03	347.81	349.18	350.55	348.25	350.05	351.86	0.00
Cost of feed used in production (N)	6.71^{ab}	6.02^{abc}	3.71^{cdef}	2.19^{f}	7.37^{a}	6.02^{abc}	5.04^{abcd}	5.59^{abcd}	4.03^{cdef}	3.46^{def}	4.95^{abcd}	4.71^{bcde}	2.40^{f}	5.27^{abcd}	3.91^{cdef}	3.35^{def}	0.71
Value of fish (N/fish)	7.81^{ab}	7.78^{ab}	2.12^{cd}	NR	8.47^{a}	5.22^{abc}	2.14^{cd}	5.77^{abc}	0.95^{d}	NR	4.43^{bcd}	3.86^{cd}	NR	4.72^{bc}	0.90^{d}	NR	1.10
Incidence of cost	562.30^{ab}	560.70^{ab}	598.92^{ab}	NR	600.02^{ab}	878.38^{a}	988.86^{a}	696.76^{ab}	896.35^{a}	562.30^{ab}	766.03^{a}	853.64^{a}	NR	728.79^{ab}	385.74^{ab}	NR	198.92
Profit Index	1.16^{a}	1.24^{a}	0.49^{bc}	NR	1.12^{a}	0.86^{ab}	0.32^{cd}	0.99^{cd}	0.14^{cd}	NR	0.88^{ab}	0.80^{bc}	NR	0.90^{ab}	0.19^{cd}	NR	0.13

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

4.7.0 Water quality parameters monitored during the feeding trial

The initial and bi-weekly water quality parameter measured during the performance evaluation of *Clarias gariepinus* fed raw and differently processed KSM based diet are presented in Table 4.24. Throughout the period of experiment, the pH, temperature and dissolved oxygen in all the treatment ranged from 7.11 to 8.71, 25.36^{0}C to 27.87^{0}C and 6.65 to 7.94mg/l respectively.

Except in the 6th week and 16th week of cultured period, there was no significant difference ($p > 0.05$) in the water temperature measured in all the treatment. At the 6th week of culture period, the water temperature measured for the control group (25.22^{0}C) and *Clarias gariepinus* fed 30% roasted KSM based diet (25.22^{0}C) had the least significant values ($p < 0.05$) which were not significantly different ($p > 0.05$) from each other. The highest significant ($p < 0.05$) value of water temperature was recorded for *C. gariepinus* fed 30% raw KSM based diet (27.40^{0}C). The lowest significant ($p < 0.05$) water temperature value of 25.36^{0}C was recorded for *C. gariepinus* fed 20% sprouted KSM based diet while the highest was recorded in the culture media containing *C. gariepinus* fed 20% roasted KSM based diet (27.59^{0}C) at the 16th week of culture period.

There was no significant variation ($p > 0.05$) in the dissolved oxygen measured during the experimental period except measurements taken at the 6th week and 14th week of culture period from the culture media. Dissolved oxygen recorded in the culture medium containing *C. gariepinus* fed 10% raw KSM based diet (7.67mg/l) had the highest significant ($p < 0.05$) value while the control group had the lowest (6.67mg/l) dissolved oxygen at the 6th week of feeding trial. At the 14th week of culture period, the dissolved oxygen recorded in the culture medium was highest significantly ($p < 0.05$) in *C. gariepinus* fed 10% roasted KSM based diet with value of 7.10mg/l which was not significantly different from the 7.05mg/l recorded in culture medium containing *C. gariepinus* fed 10% raw KSM based diet. The lowest significant value of dissolved oxygen was recorded in culture medium containing *C. gariepinus* fed 10% sprouted KSM based diet at the 14th week of culture period.

The water pH in the culture media varied significantly throughout the culture period except on the 14^{th} and 16^{th} week. The pH ranged from 7.11 to 7.60, 7.11 to 8.01, 7.11 to 8.17, 7.11 to 8.04, 7.11 to 8.16 and 7.11 to 8.13 for the 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12the week of the experiment. The pH in the control groups of *C. gariepinus* fed processed and unprocessed KSM based diets were significantly higher ($p < 0.05$) than the initial water pH value recorded.

Table 4.24: Bi-weekly water quality parameters measured during the performance evaluation.

		Initials	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE
	Ph	7.11^{b}	7.47^{ab}	7.40	7.49^{ab}	7.47^{ab}	7.54^{a}	7.45^{ab}	7.50^{ab}	7.49^{ab}	7.59^{a}	7.54^{a}	7.60^{a}	7.49^{ab}	7.47^{ab}	7.49^{ab}	7.54^{a}	7.48^{ab}	0.02
I	T	26.47	25.70	26.10	26.53	26.60	25.53	26.07	26.63	26.90	27.23	26.97	26.87	26.53	26.60	27.40	26.70	26.53	0.10
	DO	6.93	7.52	7.73	7.13	7.94	7.07	7.05	6.95	6.86	7.03	7.00	6.94	7.13	7.94	6.92	7.31	7.46	0.07
	pH	7.11^{b}	7.64^{ab}	7.50^{ab}	7.47^{ab}	7.54^{ab}	8.01^{a}	7.79^{ab}	7.83^{ab}	7.62^{ab}	7.93^{ab}	7.78^{ab}	7.85^{ab}	7.63^{ab}	7.83^{ab}	7.59^{ab}	7.53^{ab}	7.52^{ab}	0.04
Ii	T	26.47	25.76	26.70	27.23	26.30	27.23	25.91	26.83	27.00	26.63	26.63	26.57	26.60	26.83	27.07	26.50	26.10	0.38
	DO	6.93	6.63	7.17	7.21	7.29	6.61	6.76	6.72	7.08	6.68	6.92	6.86	6.82	6.90	7.02	7.21	7.27	0.36
	pH	7.11^{b}	7.81^{ab}	7.50^{ab}	7.49^{ab}	7.70^{ab}	7.51^{ab}	7.74^{ab}	7.81^{ab}	8.17^{a}	8.16^{a}	7.83^{ab}	7.97^{a}	7.91^{a}	7.96^{a}	7.73^{ab}	8.08^{a}	7.79^{ab}	0.05
Iii	T	26.47^{abcd}	25.22^{d}	26.97^{ab}	27.40^{a}	27.17^{a}	25.48^{bc}	25.58^{bcd}	25.22^{d}	27.29^{a}	27.46^{a}	27.04^{a}	26.88^{abc}	26.57^{abcd}	27.20^{a}	26.84^{abc}	26.59^{abcd}	27.32^{a}	0.12
	DO	6.93^{ab}	6.67^{b}	7.67^{a}	6.92^{ab}	7.17^{ab}	6.71^{b}	6.62^{b}	6.72^{b}	6.74^{b}	6.88^{ab}	6.92^{ab}	6.82^{b}	6.68^{b}	6.83^{b}	6.92^{ab}	6.71^{b}	6.91^{ab}	0.05
	pH	7.11^{ab}	7.76^{ab}	7.64^{ab}	7.50^{ab}	7.95^{a}	7.66^{ab}	7.69^{ab}	7.99^{a}	7.94^{a}	7.86^{ab}	8.00^{a}	8.04^{a}	7.70^{ab}	8.07^{a}	7.77^{ab}	7.74^{ab}	7.91^{a}	0.04
Iv	T	26.47	27.37	26.03	26.63	27.07	26.40	25.48	25.77	27.28	26.90	26.48	27.18	25.99	27.46	26.78	26.90	27.27	0.12
	DO	6.93	6.67	6.92	6.95	6.79	6.71	6.80	6.72	6.74	6.87	6.74	6.89	6.67	6.82	6.87	6.96	6.97	0.02

Table 4.24 continues

		Initials	0%	10%Ra	20%Ra	30%Ra	10%Ro	20%Ro	30%Ro	10%So	20%So	30%So	10%Sp	20%Sp	30%Sp	10%Co	20%Co	30%Co	Pooled SE
	pH	7.11^{c}	8.08^{ab}	7.98^{ab}	7.72^{ab}	7.55^{bc}	8.07^{ab}	7.93^{ab}	7.98^{ab}	7.79^{ab}	7.86^{ab}	7.92^{ab}	7.91^{ab}	8.16^{a}	7.96^{ab}	7.91^{ab}	7.74^{ab}	8.07^{ab}	0.04
V	T	26.47	27.21	27.73	26.67	26.97	27.87	26.76	27.33	25.48	25.87	26.6	26.48	25.8	26.8	26.82	26.31	27.46	0.12
	DO	6.93	6.73	7.03	7.04	7.05	6.89	6.73	6.84	6.85	6.74	6.77	6.83	6.74	6.83	6.8	6.8	6.82	0.03
	pH	7.11^{d}	7.60^{abcd}	7.75^{abc}	7.94^{abc}	7.95^{abc}	7.49^{cd}	7.59^{abcd}	7.54^{bcd}	8.13^{a}	8.02^{abc}	8.01^{abc}	8.02^{abc}	7.54^{bcd}	7.48^{cd}	8.08^{ab}	8.04^{abc}	7.97^{abc}	0.05
Vi	T	26.47	26.87	26.78	27.28	26.5	26.9	27.23	26.97	26.21	27.81	26.75	27.37	26.7	26.53	26.61	27.18	26.38	0.1
	DO	6.93	6.94	6.83	6.74	6.86	6.86	7.03	7	6.8	6.91	6.72	6.81	7.31	7.46	6.75	6.89	6.74	0.04
	pH	7.11	7.65	7.89	7.97	NR	7.59	7.63	7.81	7.89	7.95	7.62	8.03	7.95	NR	7.69	7.62	7.62	0.44
Vii	T	26.47	26.81	28.06	25.79	NR	26.85	27.35	25.72	26.95	26.9	25.5	27.12	27.21	NR	26.9	26.85	25.94	1.5
	DO	6.93^{ab}	6.71^{ab}	7.05^{a}	6.68^{ab}	NR	7.10^{a}	7.00^{ab}	6.81^{ab}	6.76^{ab}	6.72^{ab}	6.76^{ab}	6.46^{b}	6.73^{ab}	NR	6.90^{ab}	6.89^{ab}	6.65^{ab}	0.38
	pH	7.11	7.65	7.7	7.69	NR	8.12	7.99	7.85	7.88	7.85	7.94	7.74	7.85	NR	7.43	7.97	8.07	0.44
Viii	T	26.47^{abc}	26.85^{abc}	26.94^{abc}	26.80^{abc}	NR	26.44^{abc}	27.59^{a}	25.59^{bc}	26.40^{abc}	26.10^{abc}	27.31^{ab}	26.54^{abc}	25.36^{c}	NR	27.25^{ab}	26.87^{abc}	26.78^{abc}	1.5
	DO	6.93	6.78	6.9	6.71	NR	6.7	7.01	6.66	6.72	6.7	6.76	6.8	6.76	NR	7.47	6.88	6.73	0.39

Mean ±SE value in the same row with same superscript are not significantly ($p > 0.05$) different.

i – week 2 ii – week 4, iii – week 6 iv – week 8 v – week 10, vi – week 12, vii – week 14, viii – week 18.

DO – Dissolved oxygen (mg/l)

T – Temperature (^{0}C)

10%Ra – 10% raw kenaf seed meal; 20%Ra - 20% raw kenaf seed meal; 30%Ra – 30% raw kenaf seed meal

10%Ro - 10% roasted kenaf seed meal; 20%Ro - 20% roasted kenaf seed meal; 30%Ro - 30% roasted kenaf seed meal

10%So - 10% soaked kenaf seed meal; 20%So - 20% raw kenaf seed meal; 30%So - 30% soaked kenaf seed meal

10%Sp - 10% sprouted kenaf seed meal; 20%Sp - 20% sprouted kenaf seed meal; 30%Sp - 30% sprouted kenaf seed meal

10%Co - 10% cooked kenaf seed meal; 20%Co - 20% cooked kenaf seed meal; 30%Co - 30% cooked kenaf seed meal

SE – Standard Error.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal

The raw and processed kenaf (*Hibiscus cannabinus*) seed meal (KSM) in this study had a range of 21.17% to 30.45% crude protein. These values are comparable to 30.88% reported in the biological evaluation of whole kenaf (*Hibiscus cannabinus*) seed meal using Albino rat by Odetola and Eruvbetine (2012). The crude protein is also close to 25.20% reported by Al-Wandawi *et al.*, (1984) for Roselle (*Hibiscus sabdariffa*) and a range of 30.11% to 31.02% reported by El-Adawy and Khalil (1994) for three cultivars of Roselle (*Hibiscus sabdariffa*). The crude protein recorded for processed KSM in this study is not inferior to 37.08% crude protein reported for roasted soybean meal by Van Eys *et al.* (2004). The ether extract (9.07% to 16.21%) reported for kenaf seed meal in this study was inferior to the range of 21.60% to 23.26% reported for the cultivars of Roselle (*Hibiscus sabdariffa*) by El-Adawy and Khalil (1994), Pumpkin seed (34.90%) and Kalahari melon (30.50%) (Nyam *et al.*,2009). However, the ether extract recorded for KSM in this study was higher than the ether extract recorded for Bambara groundnut (Yagoub and Abdalla, 2007). The ether extract in roasted soybean meal (18.38%) reported by Van Eys *et al* (2004) is comparable to the ether extract reported for KSM in this study. The ash content in KSM (2.52 to 6.14%) is comparable to Roselle (Hainida *et al.*, 2008) and soybean (Van Eys *et al*, 2004). Also, the carbohydrate in KSM (35.54 to 40.65%) is close to 36.37 to 38.12% reported by El-Adawy and Khalil (1994) for Roselle. Variability in the chemical composition in plant seed can be linked to differences in cultivar (Nestares, 1999) and; agro-climatic condition during cultivation and the storage methods (Mbwile and Uden, 1996). These factors might be responsible for the disparity recorded in crude protein and ether extract in this study and the referenced researchers. These nutritional characteristics qualify KSM as a potential source of fish feed ingredient.

Rajashekher *et al.,* (1993) and; Odetola and Eruvbetine (2012) had similar opinion about KSM.

The processing methods adopted in this experiment influenced the nutrient and mineral composition of the Kenaf seed. The processing methods increased the crude protein content of kenaf seed meal. Boiling, cooking, roasting, sprouting and fermenting also enhanced the crude protein of Red kidney beans as documented by Audu and Aremu (2011). The crude protein content of soybean also increased slightly when soaked, sprouted, cooked and autoclaved (Ramadan, 2012). Similar increment in crude protein was reported by Solomon *et al.* (2017). Reasons for the increment in the crude protein were not stated by these authors. However, according to Khalil and Mansour (1995), crude protein becomes concentrated and increases in value when other components (lipid, crude fiber, moisture and ash) reduce. In addition to dehydration process that increases the protein content, breakdown in food matrix increases the bioavailability of protein in food materials (Minatel *et al.*, 2017). These might be responsible for an increase in the crude protein recorded for processed KSM in this study. Contrary to the result obtained in this study, a decrease in crude protein was reported for processed lima beans (Falaye *et al.*, 2014), Roselle (Hainida *et al.* 2008) and Sunflower seed meal (Adesina *et al.*, 2013). The reduction was attributed to nutrient loss due to leaching of soluble contents of protein, non-protein nitrogen, ash, fat and formation of protein-fiber complex. However, Hefnawy (2011) recorded no significant effect of processing on crude protein.

The range of 9.07% to 16.21% recorded for crude fiber in this study is close to 10.49% to 19.59% recorded for insoluble dietary fiber in Roselle (Hainida, 2008). The crude fiber in processed KSM is also comparable to a range of 14.19% to 23.94% recorded for processed sunflower seed meal and 7.11% to 11.38% for leucaena by Sotolu (2008). However, a lower crude fiber of 2.30% to 4.80% was recorded by Audu and Aremu (2011) for differently processed Red kidney beans. Soaking and cooking reduced the crude fiber of kenaf seed in this trial. Similar reduction in crude fiber was reported by Shah *et al.* (2011) when Pulses consumed in Pakistan was subjected to cooking. Contrary

to these results, the crude fiber of soybean increased significantly when soaked, cooked and sprouted (Ramadan, 2012).

The lipid and ash content of kenaf seed significantly reduced in this experiment. Similar result was documented by Mubarak (2005) when Mung beans seeds were subjected to roasting, cooking, soaking, sprouting and autoclaving. Alajaji and El-Adawy (2006) also reported reduction in lipid and ash content of chickpea when cooked. These researchers attributed the reduction in the lipid and ash to their leaching into the water. This might also be responsible for the result obtained in this study. Leaching may not occur in sprouted kenaf seed but, the reduction in lipid content may be connected to its utilisation during the start of germination process (El-Adawy, 2002).

Nitrogen Free Extract (NFE) comprises of sugar and soluble carbohydrate which are non-volatile and highly concentrated in the cotyledon (Shaahu *et al.*, 2015). Based on this information, the NFE was supposed to reduce in kenaf seed when cooked and soaked, and increased when roasted. There was however, significant increase in the NFE of cooked kenaf seed and reduction in the NFE of roasted kenaf seed. Similar increase in NFE was reported by Shaahu *et al.* (2015) when Lablab seed was subjected to boiling. Sprouting process in seed requires energy which is obtained from stored carbohydrate, reduced sugar and lipid in the seed cotyledon (Khalil and Mansour,1995). The NFE in sprouted kenaf was probably not used during sprouting process as there was no difference in the NFE between the raw and roasted kenaf seed meal. Perhaps, lipid was engaged as a source of energy during the germination process.

5.2 Mineral Composition of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal

Calcium (Ca) and phosphorus (P) play vital role in the blood formation and bone development (Ogunlade *et al.*, 2005). Ca/P ratio less than 0.5 reduced the absorption of calcium in the small intestine (Audu and Aremu, 2011). In this study, the Ca/P ratio for the raw and differently processed kenaf seed were less than 0.5 which indicates that without fortification of feed produced from kenaf seed with calcium, calcium absorption

in the small intestine may be reduced. Body sodium (Na) and potassium (K) also play vital role in balancing of internal osmotic pressure, maintenance of pH to regulate irritability in muscle, control the absorption of glucose and enhances the retention of protein during growth (NRC, 1989). Na/K ratio less than 1 was documented by Audu and Aremu (2011) to prevent high blood pressure. The Na/K ratios for the raw and differently processed kenaf seed were less than 1. This suggests that dietary KSM may not contribute to high blood pressure in fish with respect to Na/K ratio. The Ca/P ratio was higher than 0.5, while the Na/K ratio was lower than 1 when Red kidney beans was subjected to boiling, cooking and fermentation by Audu and Aremu (2011). The mineral content in the processed kenaf (*Hibiscus cannabinus*) seed compared favorably with that documented by Hainida *et al.* (2008) for Roselle (*Hibiscus sabdariffa*) seed.

5.3 Anti-nutritional Composition of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal

Tannin, trypsine inhibitor, phytic acid, saponin, alkaloid and oxalate detected in the KSM conformed to the report of Sgwane *et al.* (2005) that the family of *Malvacaea* contains anti-nutrient component. The reduction of anti-nutritional components in seed has been linked to their leaching into water during soaking and cooking (Soetan and Oyewole (2009). Anti-nutritional factors are catabolized for use as growth factors and enzymatically hydrolysed in germinating seed (Sangronis and Machado, 2007). Some are denatured when subjected to heat treatment without undergoing hydrolysis (Soetan and Oyewole, 2009). In this study, cooking, sprouting, and roasting reduced the tannin content in kenaf seed meal by 75%, 12.5% and 12.5% respectively. While sprouting was less effective in reducing tannin content in chickpea, tannin content reduced by 50.10% when chickpea was subjected to cooking (El-Adawy, 2002). A remarkable reduction in tannin content was also reported by Minaria *et al.* (2012) for roasted and steam treated *Leucaena leucocephala* seed. Conversely, in this study, soaking appeared not to be effective in decreasing the tannin content in kenaf seed, as the content increased by 62.5% relative to the raw kenaf seed. *Dolichos lablab* soaked by Vijayakumari *et al.* (1995) also had a 55% increase in tannin content. The increase in the tannin content was attributed to hydrolysis of high molecular insoluble polymers into small molecular weight polymer or due to

inhibition of the activity of polyphenol oxidase that functions in reducing tannin during heat treatment (Osama, 2007).

Relative to the raw kenaf seed, cooking, sprouting and roasting reduced the Trypsine Inhibitory Activity (TIA) in kenaf seed by 75%, 16.67% and 33.3%, respectively. Soaking increased the TIA content by 25%, similar in trend to the tannin content in kenaf seed. Similarly, soaking alone appeared not to be effective in reducing TIA in beans as a 6.3% reduction was recorded in beans soaked overnight compared to a 66.7% reduction in TIA in the beans subjected to combined soaking and cooking treatment (Soetan and Oyewole, 2009).

Cooking and germination caused 80.50% and 33.95% reduction in TIA chickpea, respectively (El-adawy, 2002), similar to the result obtained in this study. The activity of Trypsine inhibitor also reduced in *Jatropha curcas* when cooked at 121^0C for 25 minutes (Martinez-Herrera *et al.*, 2005). It was opined by the authors that moist heat was highly effective in deactivating the activity of trypsine inhibitor. Application of moist heat (cooking) in this study also confirms the significant efficiency of cooking in reducing TIA in kenaf seed.

Heat treatment alone is relatively not effective in decreasing phytic acid in plant material without the hydrolytic activity of phytase which removes the ion (Ca^{2+} and Fe^{2+}) binding phosphate groups (Liener, 2003). Liener's (2003) findings may have explained the inefficiency of roasting to reduce phytic acid level of kenaf seed in this study. There was a 7.67% increase in the phytic acid content of roasted kenaf seed relative to the raw kenaf seed. The heat applied during roasting may have reduced the activity of phytase in the roasted kenaf seed (Minatel *et al.*, 2017). Conversely, a 60.69% reduction in phytic acid was reported by Osama (2007) for roasted Lablab bean. Roasting also decreased pytic acid in cowpea (Akinyele, 1989) and; black bean (Sievwright and Shipe, 1986). The principle behind roasting in reducing the phytic acid in these seeds was not elucidated by these authors. In the other hand, less than 26% reduction was recorded in kenaf seed subjected to cooking (25.55%), sprouting (21.74%) and soaking (13.04%) relative to the raw kenaf

seed. The level of reduction of phytic acid recorded in this study were lower than 44.85%, 48.94% and 22.19% recorded for cooked, sprouted and soaked lablab bean, respectively, as reported by Osama (2007). Similar result was also reported for sprouted *Phaseolus vulgaris* and *Cajanus cajan* (Sangroni and Machado, 2007) and; cooked lentil (Hefnawy, 2011). Cooking and soaking was opined by Soetan and Oyewole (2009) and; Osama (2007) to cause leaching out of phytic acid in plant seeds (Soetan and Oyewole, 2009 and; Osama, 2007) while, increase in the activity of phytase during sprouting may be accountable for the decrease in the phytic acid recorded for the kenaf seed in this study. Methods designed to reduce anti-nutritonal factors in plant seeds has been attributed to increase in the activities of enzymes that participate in their biodegradation (Morales-de la pena *et al.*, 2011)

Roasting, soaking, sprouting and cooking reduced the saponin content in kenaf seed by 12.24%, 11.39%, 16.03% and 13.08%, respectively. Relative to the raw chickpea, the saponin content in soaked chickpea reduced by 10%; 44% when sprouted and by 15% when cooked (Jood *et al.*,1986). Similarly, soaking and cooking reduced saponin content in Lentils (Ruiz *et al.*, 1996). Reduction in the saponin content of processed kenaf seed may be attributed to hydrolysis which facilitates the breakdown of food matrix, consequently enhancing the leaching of saponing into the soaking and cooking water (Khokhar and Chauhan, 1986). This can also be attributed to the inactivation of phenolic enzymes that facilitates the biosynthesis of saponin (Morales-de la pena *et al.*, 2011).

Soaking in running water and brine reduced the alkaloid content in lupin (Erbas *et al.*, 2005). Roasting led to 10.27% increase in alkaloid while combined processing methods of roasting and soaking led to 91.45% decrease in alkaloid content in white lupin (Yeheyis *et al.*, 2014). Also, the alkaloid content of roasted *Tamarindus indica* increased by 83.78% (Bashir *et al.*, 2016). In this study, relative to the raw kenaf seed, soaking and cooking reduced the alkaloid content in kenaf seed by 31.50% and 31.60%, respectively. The reduction can be attributed to a rupture in the cellular and subcellular compartments of the kenaf seed which might have facilitated the migration of alkaloid and the subsequent release into the processing medium (Minatel *et al.*, 2017). Roasting and sprouting did not

change the alkaloid contents of kenaf seed. The results obtained in this trial conformed to the finding of Erbas *et al.* (2005) and Bashir *et al.* (2016) that aqueous treatment is effective in reducing alkaloid content in plant seed.

Oxalate is highly corrosive at high concentration and can decrease the availability of essential minerals (Bassey *et al.*, 2009). In this experiment, roasting and cooking reduced the oxalate amount in kenaf seed by 25% and 55%, respectively. Isikwenu *et al* (2013) recorded a 33.66% and 38.33% reduction in oxalate content in roasted and boiled Bambara groundnut, respectively. Similar reduction was also recorded in oxalate by Makinde and Akinoso (2013) when sesame seed was roasted and autoclaved. These researchers attributed the reduction in oxalate in the processed seeds to the fact that oxalate was heat labile. The reduction in the oxalate content recorded for kenaf seed in this study confirmed the opinion of Makinde and Akinoso (2013), and Isikwenu *et al.* (2013). Soaking and sprouting however, increased the oxalate content of kenaf seed, relative to raw kenaf seed. This result contradicted a reduction in oxalate content reported for sesame seed subjected to soaking and sprouting by Makinde and Akinoso (2013) and Adebayo (2014) for soaked lima beans. The increase in oxalate content recorded in this study may have resulted from the release of oxalate linked to food matix or other constituent of the kenaf seed due to soaking which improved the oxalate extractability and consequently increased the content (Wang *et al.*, 2014 and; Rodriguez-Roque *et al.*, 2015). Sprouting may have also increased the reaction of enzymes that play vital role in the bio-synthesis of oxalate in kenaf seed Morales-de la pena *et al.* (2011)

5.4 Amino Acid Content and Score of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal

The amino acid profile recorded in this experiment for the raw and differently processed kenaf (*Hibiscus cannabinus*) is comparable to that reported by El-Adawy and Khalil (1994) for Roselle (*Hibiscus sabdariffa*). The sum of Essential Amino Acid Composition (EAAC) and the Amino Acid Scores (AAS) reported for raw, cooked, roasted, sprouted and fermented red kidney beans by Audu and Aremu (2011) are also comparable to that

obtained for raw and differently processed kenaf seed in this study. Methionine (and Cystine) is the essential amino acid that is most often acting in a limiting capacity in feedstuff of plant origin (Aremu and Audu, 2011). Methionine was equally limiting in the raw and differently processed kenaf seed in this study.

5.5 Apparent Nutrient Digestibility of Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal for Catfish (*Clarias gariepinus*).

The protein digestibility in the reference and kenaf seed meal (KSM) based diets in this study ranged from 85.52 – 90.40%, suggesting that protein in these diets were highly digested by catfish. The protein digestibility in the processed KSM ranged from 77.54 to 98.29% while, the protein digestibility in the unprocessed KSM (raw) was 51.45%. This depicts that processing of kenaf seed by the methods adopted in this study highly enhanced the digestibility of protein in the kenaf seed by *C. gariepinus*. The Apparent Digestibility Coefficients (ADCs) in the processed kenaf seed are comparable to a range of 72.8 – 92.3% reported for plant-based feedstuffs in Chinese sucker (Yuan *et al.*, 2010); 87.06 – 88.95% reported for differently processed tropical almond seed meal fed to *C. gariepinus* (Falaye *et al.*, 2016); 80.69 – 85.69% recorded when Adesina *et al.* (2013) fed *C. gariepinus* with differently processed sunflower seed meal-based diet and; 64.18 – 70.18% protein ADC for *C. gariepinus* fed differently processed *Leucaena leucocephala* (Sotolu, 2008). The low ADC of protein recorded for raw KSM (51.45%) is close to 57.87% reported by Falaye *et al.* (2014) for *C. gariepinus* fed unprocessed lima bean seed meal-based diet. The researchers attributed the low crude protein ADC in the lima bean by *C. gariepinus* to anti-nutritional factors. The ADC of protein obtained for raw and processed KSM in this experiment depicts that *C gariepinus* will efficiently utilize the protein in a diet containing processed KSM better than unprocessed KSM.

However, the low ADC of protein in the raw KSM might not only be ascribed to anti-nutritional factor but, also to the relatively high crude fiber in the seed. Fibers, most especially the insoluble fiber, decrease the digestibility of nutrients in feedstuffs (Baer *et al.*, 1997). Also, dietary fiber reduces the transit time of ingested feed at the upper digestive tract and increases it at the lower part, thereby, decreasing the digestibility of all

nutrients in feed (Wenk, 2001). This reduction in the nutrient digestibility occurs through an accelerated movement of the digesta across the digestive tract (Rahman *et al.*, 2016). These might be responsible for the high but non- significant ADC of protein recorded for soaked kenaf seed (94.8%) despite the high content of anti-nutritional factors (tannin and trypsine inhibitor) when compared to the protein ADC in roasted kenaf seed (77.5%), containing high crude fiber (16.21%). The ADCs of lipid (Range: 88.65 – 97.48%) and ADCs of energy (Range: 49.12 – 91.75%) for raw and processed kenaf seeds in this research are generally comparable with that reported by Koprucu and Ozdemir (2005) and; Sotolu (2008).

5.6 Growth Performance and Nutrient Utilisation of Catfish (*Clarias gariepinus*) Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal Based Diets.

The proximate analysis of the experimental diets revealed that the diets were iso-nitrogenous. The marginal difference of ±2 in the crude protein values of the experimental diets conforms to the range (±2) recommended by Agboola (2004). The crude fat (5.95 – 6.81%) and crude fibre (2.98 – 3.55%) obtained in this trial are comparable to that documented by Falaye *et al.* (2016) and, Sotolu and Sule (2008). The ash (7.03 – 8.20%) and Nitrogen Free Extract (NFE) (42.67 - 45.49%) are also comparable to the range of ash (10.36 – 11.41%) reported by Sotolu (2008) and NFE (30.81 – 33.58%) recorded by Falaye *et al.* (2016). Agbabiaka *et al.* (2013) also recorded a similar value of 29.69 – 34.64% for NFE. The moisture content of the experimental diets was less than 10% recommended for catfish diets (Onuoha and Elezuo, 2013).

Weight gain and specific growth rate are considered the very useful measurements of productivity of feed (Falaye *et al.*, 2016). *Clarias gariepinus* in this study utilized the protein in all the experimental diet for growth as observed from the progressive increase in the mean weight gain. The significant increase in the crude protein of the carcass of the *C. gariepinus* at the end of the experiment corroborates with the observation in the progressive increase in weight gain. *Clarias gariepinus* in this study was able to utilize nutrients in the diets to synthesis protein for cell, tissue and organs formation. Similar

result and conclusion was reported by Sotolu and Faturoti (2008), Lim *et al.* (2011) and Adesina *et al.* (2013). However, the performance with respect to growth in *C. gariepinus* depressed when raw and processed KSM replaced soybean meal in their diets at 20% and further depressed when fed diet containing 30% raw and the differently processed KSM. Similar to the result obtained in this study, the growth of carp depressed when fed 15% and 30% cassava meal based diet (Ufodike and Matty, 1983); more than 20% mucunna bean in the feed of catfish depressed their growth (Bekibele, 2005) and; growth depression was also recorded as the inclusion level of Roselle (*Hibiscus sabdariffa*) seed meal increased in the diet fed to fingerlings of *C. gariepinus* (Fagbenro, 2005). The growth of broiler chicken significantly depressed when fed 20% of kenaf (*Hibiscus cannabinus*) seed meal based diet (Odetola, 2013). From the result of regression analysis in this study, it is evident that the optimum dietary inclusion of KSM required to support the growth of *C. gariepinus* was 10.8% (roasted KSM). Similar to the reports of Hussain *et al.* (2018), high dietary plant protein ingredients included in the diet of fish beyond 10% lead to growth depression.

The survival rate of *C. gariepinus* in this trial ranged from 0 – 76.67%. A less than 76.67% survival was recorded by Akinwunmi and Eniade (2014) when *C. gariepinus* were fed tropical banana blossom (*Musa sapietum*). Over 75% mortality was also recorded within 5 weeks when Albino rat was fed detoxified castor bean cake meal based diets (Akande *et al.*, 2012). A survival range of 60 – 90%, with the control group having the least survival rate was also recorded when Alegbeleye *et al.* (2011) fed catfish with feed containing fermented pigeon pea. In this study, similar to the reason provided by these authors, the low survival rate recorded may not be attributed to low water quality. The bi-weekly water quality parameters measured (pH: 7.11 to 8.71, temperature: 25.36^0C to 27.87^0C and; dissolved oxygen: 6.65 to 7.94mg/l) throughout this experiment were within the optimal range which was adequate to support the growth of catfish. Moreover, a three days interval of static renewal of water was expected to guaranty low accumulation of nitrogenous waste. The 60% survival in the work of Alegbeleye *et al.* (2011) was attributed to poor handling. In this study however, there was a steady pattern of increment in the survival of catfish as the dietary inclusion level of KSM reduced. Similar reduction

in survival, attributed to increasing levels of raw soybean meal in the diet of catfish was recorded by Balogun and Ologbobo (1989). The survival rate of catfish is also comparable to a range of 76.70 to 88.30% survival recorded by Adesina *et al.* (2013) who fed *C. gariepinus* with a graded level of boiled sunflower for 105 days. However, this finding contradicted the report by Fagbenro (2005) who recorded high survival rate (93.30 – 96.70%) in *C. gariepinus* when soybean replaced roselle seed in the feed fed to the fish on a 70 days feeding trial. In this study, the survival of *C. gariepinus* fed raw and differently processed KSM based diet progressively decreases and peaked at 70 days. The survival of *C. gariepinus* in this study at 70 days of feeding period surprisingly is comparable to that reported by Fagbenro (2005). In this study, feeding of KSM based diet to *C. gariepinus* beyond 70 days resulted into a remarkable and progressive declined in survival of the fish until the end of the 112 days experimental period. This suggests that *C. gariepinus* may not be able to tolerate high dietary KSM beyond 10 weeks of consumption. Ochefu *et al.* (2011) also cautioned on the prolong feeding of raw kapok seed meal based diet to rabbit at 30% as it could lead to mortality.

Indices such as the final weight, weight gain, relative growth rate, specific growth rate, feed conversion ratio and feed efficiency ratio are used by researchers (Alegbeleye *et al.*, 2001, Nwanna, 2003) to describe growth pattern in fish. For all the growth performance indices, roasted KSM based diet gave the best performance when compared to other processing methods. Likewise, while the growth performance of *C. gariepinus* were similar between those fed non-KSM based diet (control) and 10% KSM based diet, 20% and 30% KSM based diet resulted into a remarkable inferior growth performance. Odetola (2013) concluded that broiler chicken must be fed 10% cooked or fermented kenaf seed meal as a substitute for soybean to obtain a better performance. The growth response of rainbow trout suppressed when given diet containing over 20% inclusion level of soybean meal based diet (Oliver-Teles *et al.*, 1998). However, same soybean included up to 20% in the feed for stinging catfish (*Heteropneustes fossilis*) resulted in a positive growth response (Siddiqui *et al.*, 2014). The variation in the reports by these researchers may be attributed to differences in maximum dietary tolerance of plants protein ingredients to these fish species (Bhosale *et al.*, 2010); the relative amount of anti-nutritional factors

and; insufficient and/or imbalance amino acid profile (Sotolu and Faturoti, 2008, Okomoda *et al.*, 2016 and; Falaye *et al.*, 2016). These stated facts may probably explain the inferior performance of *C. gariepinus* fed KSM-based diet in this study. Kenaf seed contains anti-nutritional factors which bind protein and minerals making them unavailable for use by fish for growth (Olawepo *et al.*, 2014). The characterization of the amino acid indicated that methionine was limiting in the raw and differently processed kenaf seed. Furthermore, imbalance leucine/isoleucine ratio is one of the factors reported by Tiamiyu *et al.* (2013) to limit the large inclusion of ingredients in the feed of fish. High leucine to isoleucine ratio may result in an antagonistic reaction of isoleucine deficiency (Crawshaw, 1994). Leucine in the raw and differently processed kenaf seed was higher in this study than isoleucine. This may have resulted in the deficiency of isoleucine at inclusion level higher enough to trigger an antagonistic response in *C. gariepinus*.

Roasted KSM based diet had the highest significant feed intake. Roasting probably might have enhanced the palatability of the roasted kenaf seed. Roasting was recorded to improve palatability and acceptability of seed meal from mucunna plant incorporated in the feed of carp (Siddiqui *et al.*, 2014). *C. gariepinus* utilized the nutrient in roasted KSM based diet better when compared to other processed KSM based diet and the raw KSM based diet. Roasted *Cajanus cajan* seed meal included at the rate of 400g kg^{-1} of diet significantly enhanced the utilisation of nutrients in the diet by *C. gariepinus* (Solomon *et al.*, 2017). In the other hand, utilisation of nutrient in the diet containing 0% (control) and 10% KSM as a replacement for soybean meal by *C. gariepinus* was comparable with each other and was the best. However, the utilisation of nutrients by *C. gariepinus* fed diet having 20% and 30% KSM was remarkably inferior. This restricted levels of replacement can be attributed to insufficient and/or imbalance in amino acid contents and the residual effect of anti-nutritional constituent in the KSM, which may have interfered with the protein utilisation, mineral utilisation and enzyme activities (Sotolu and Faturoti, 2009). Anti-nutritional factors reduce bioavailability of lysine, reduce appetite and body weight (Fagbenro, 2005). Tannin contents decreases the digestibility and palatability in seeds, forming insoluble enzyme resistance complexes with protein and carbohydrate (Siddhuraju and Becker, 2001). Protease inhibitor adversely affects the utilisation of

protein by changing trypsin to a non-active trypsin-trypsin inhibitor complex and alters the pancreatic secretion in animal (Balogun and Ologbobo, 1989). Similar inferior nutrient utilisation of non-conventional ingredients of plant protein origin in the feed of fish has been documented by various researchers such as Bekibele (2005), Sotolu and Faturoti (2009), and Bello *et al.* (2012).

5.7 Heamatological, Plasma Biochemical and Histopathological Response of Catfish (*Clarias gariepinus*) Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal Based Diets.

Analysis of heamatological and blood chemistry is valuable in the determination of metabolic disturbances and disease conditions in fish (Celik, 2004). Alterations in structures and functions of internal organs of fish such as liver and kidney can be determined through assessment on plasma/serum chemistry (Thrall *et al.*, 2005). In this study, haematological parameters taken were used to assess the general state of well-being of the experimental fish while the plasma chemistry corroborated the results from the liver and kidney histopathology. The Data from the concurrent control were used as the reference range in this study. Concurrent control is applicable as a reference range because data obtained are from the same population and the sampled fish experienced aging, sampling and husbandry processed similar to the treated fish (Whalan, 2015).

The Packed Cell Volume (PCV), the haemoglobin, Red Blood Cell (RBC) count and platelets count of *C. gariepinus* fed processed and raw KSM based diets ranged from 16.78 - 24.67%, 5.44 – 8.03 (g/100ml), 1.84 – 2.53 (x 10^6 µl) and 7.77 – 10.62 (x 10^4 /ml), respectively. These values were not statistically different within the different groups and were not inferior to the values documented by Osuigwe *et al.* (2005) and; Sotolu and Faturoti (2008). Depletion in the haemoglobin and PCV value in fish is an indication of lysing of RBC which can lead to anaemia (Maheswaran *et al.*, 2008). No remarkable alteration in the haematological parameters recorded in this study depicts that raw and processed KSM incorporated in the diet fed to *C. gariepinus* imposed similar health related physiological influence. However, a significant up-regulation in the White Blood

Cell (WBC) amount in *C. gariepinus* fed roasted and sprouted KSM based diets was observed. The KSM may have excited the defence mechanism of the fish (Gabriel *et al.*, 2007). The WBC count form the fish fed roasted and sprouted KSM based diet in this study were comparable to the values reported for the control group in the study of Omitoyin (2006).

The replacement level of raw and processed KSM for soybean meal in the feed of catfish at 10% inclusion level had the value of haematological parameters similar to the concurrent control group (0%). These values significantly reduced as the quantity of KSM increased in the feed of *C. gariepinus*. The haematological indices also increased as the level of raw tallow (*Detarium microcarpum*) increased in the diet of broiler (Obun, 2013). Hybrid catfish fed diet containing graded level of raw and cooked jackbean seed meal-based diet also showed similar trend (Osuigwe, 2005). Physiologically, heamoglobin plays a crucial role in transportation of oxygen in fish for metabolic activities to sustain survival (Osuigwe et al., 2005).

The haemoglobin concentration recorded for *C. gariepinus* fed the control diet (9.83g/100ml) and 10% KSM based diet (10.38g/100ml) in this study fell within the normal range (7.98 – 10.63 g/100ml) as reported by Musa and Omoregie (1999) and; Osuigwe *et al.* (2005). In addition, there was no significant difference in the heamoglobin concentration recorded between the control group and the *C. gariepinus* fed 10% KSM based diet. This depicts that the fish in these groups appeared not to be anaemic. However, with reference to the concurrent control group, the significantly low haemoglobin concentration of 4.71 g/100ml and 1.07gldl recorded for *C. gariepinus* fed 20% and 30% kenaf seed meal based diet respective depicts that the fish appeared to be anaemic. Anaemia was linked to deficiency in vitamin, mineral and protein by Whalan (2015). The interference of residual anti-nutrient constituents in the kenaf seed with mineral and protein in the diets may have invoked the anaemic condition in the fish. Similar trend of reduction was recorded for the differential white blood cell count. While low monocyte was stated not to be of a clinical significant, low lymphocytes and neutrophil has been linked to gastrointestinal malabsorption of digested nutrients, renal failure and iron

deficiency anaemia (Whalan, 2015). The anti-nutritional factors in kenaf seed may have also led to the significantly low lymphocyte and neutrophil recorded in *C. gariepinus* fed 20% and 30% kenaf meal-based diet. Phytic acids forms chelate with iron (Muhammad and Oloyede (2009) while tannin impairs the intestinal absorption of iron (Butler, 1989) which reduces the bioavailability of iron needed for haemoglobin formation. A significant reduction in the WBC count recorded in *C. gariepinus* fed 20% and 30% KSM based diet may have depressed the defensive mechanism of the fish to combat environmental perturbation. Very low WBC count was opined by Agrawal and Mahajan (1980) to cause high mortality and poor growth by making animal susceptible to environmental stress and diseases. This abnormal physiological condition can be linked to the poor growth and low survival of *C. gariepinus* fed 20% and 30% KSM based diets in this study.

In this study, there was a significant up-regulation in the blood biochemical parameter of *C. gariepinus* fed roasted KSM based diet when compared to the other processed and raw KSM based diets. The values obtained are comparable to the biochemical parameters recorded for the concurrent control experimental group of Bello *et al.* (2014) who supplemented the diet of catfish with walnut leaf and onion bulb. The plasma total protein and globulin content in this study were higher than that reported for the concurrent control experimental group in Olaifa and Bello (2010) who fed *C. gariepinus* with differently processed yam bean meal based diet but, similar to the albumin content. The plasma biochemical parameters up-regulated with reducing quantity of KSM in the diet of *C. gariepinus*. The result obtained for *C. gariepinus* fed 10% KSM based diet was not inferior to the concurrent control group. However, these parameters reduced remarkably in *C. gariepinus* fed 20% and 30% KSM based diet. This depicts that 20% and 30% KSM based diet fed to *C. gariepinus* may have invoke hypoproteinemia, hypoalbuminemia and hypoglobulinemia which may have been caused by the presence of anti-nutritional factors in the KSM. These physiological abnormalities are connected to protein malnutrition, malabsorption, iron storage diseases, starvation and low protein diet (Whalan, 2015).

Whalan (2015) also attributed elevated total protein level in plasma to chronic liver disease, chronic infection, hepatocyte leakage and necrosis. The results of the histological

examination of the livers of *C. gariepinus* fed non-KSM (control) and KSM based diets were indistinguishable and showed no remarkable histopathological changes that were pathogenically induced. The indication of hypoproteinemia, hypoalbuminemia and hypoglobulinemia in this study may not be directly linked to liver damage but, to nutritional deficiency which may have been caused by anti-nutritional factors. Similar non-significant decrease in these blood biochemical parameters were recorded by Olaifa and Bello (2010) who fed *C. gariepinus* with differently processed african yam bean meal based diet. These authors also attributed the reduction in these parameters to the anti-nutritional components in the african yam bean. Conversely, an elevation in these parameters was recorded by Bello *et al.* (2014). Alanine Aminotransferase (ALT), Alkaline Phosphate (ALP) and Aspartate Aminotransferase (AST) are non-plasma specific enzymes present in the liver, kidney muscle and provide specific information on the dysfunction of the organs (Gabriel and George, 2005). While low level of ALT is suggestive of no organ damage (Whalan, 2015), low level of ALP and AST is an indication of malnutrition, pernicious (Vitamine B_{12} deficiency), anorexia and food starvation (Whalan, 2015). In this study, relative to the concurrent control group, there was no elevation or depression in the ALP, ALT and AST of *C. gariepinus* fed 10% KSM based diet. However, these parameters depressed significantly with increasing amount of KSM in the feed of catfish. The decrease may not be unconnected to the anti-nutrient components which may have led to poor growth and increased mortality. Moreover, this may also suggest that the liver in these fish may not be damaged as only the direction of an elevated ALP, ALT and AST are linked to hepatocyte leakage, necrosis, infectious hepatitis and extrahepatic biliary obstruction (Whalan, 2015). The findings in this study agreed with the results of Bello *et al.* (2014). Assessment of Blood Urea Nitrogen (BUN) and creatinine provide information on kidney dysfunction (Evans, 2009). The values for the BUN and creatinine in *C. gariepinus* fed 10% KSM based diet were not inferior to the control group (0%). The values for BUN and creatinine however, down-regulated as the dietary level of KSM in *C. gariepinus* increased. Down-regulation of creatinine has been linked to malnutrition (Braun *et al.*, 2003) while decrease in BUN can be caused by low protein, high carbohydrate diet and starvation (Whalan, 2015). No visible lesion was observed in the histopathological assessment carried out on the kidney of *C gariepinus* fed

KSM based diets. Sever pathological changes in kidney are associated with impaired glomerulus where an elevated plasma creatinine is noticeable (Evans, 2009). Elevated BUN is also linked to toxic effect on renal tubules and parenchyma and; blockage of urinary outflow by crystalluria, calculi, or other barriers (Evans, 2009). Similar to the findings in this research, Kumar *et al.* (2010) did not record any pathological change in the liver of (*Cyrinus carpio*) fed differently detoxified *Jatropha curcas* kernel seed meal based diet. In their study, the creatinine content of blood plasma of *Cyrinus carpio* fed feed containing *Jatropha curcas* kernel seed down-regulated when related to the control group. The liver and kidney of *C. gariepinus* did not also show histopathological alteration when fed *Chrysophyllum albidum* seed meal based diet by Jimoh *et al.* (2015). More so, Sunflower seed replacing fishmeal up to 30% in the diet of Sharpsnout sea bream caused no pathological defect in the liver (Merida *et al.*, 2010). Adesina (2017) did not also record pathological defect in the liver and kidney of catfish fed up to 20% sunflower seed meal based diet but, *C. gariepinus* displayed sever vacuolization of the hepatocyte and nutritional necrosis at inclusion level greater than 20%. Adesina (2017) linked the pathological change to anti-nutrient constituents, reducing the bio-availability of essential nutrient for growth of the fish. This may also be the reason for the poor response of *C. gariepinus* to dietary KSM in this study. Contrarily, mild congestion, cellular infiltration and necrosis were recorded in Albino rats fed diet having varying quantities of detoxified cake from castor bean (Akande *et al.*, 2012). Similar deleterious effect on liver of cockerel chicks fed full fat raw neem seed meal based diets was recorded by Uko *et al.* (2006), but the effect was less marked in the liver of birds placed on toasted kidney bean based diet. These authors implicated phytotoxins as the cause of the histopathological changes in the Albino rats and birds fed diet having seed from plant. Disparity in results obtained from this study and that reported by Uko *et al.* (2006), Emiola *et al.* (2007) and Akande *et al.* (2012) may have resulted from the differences in species of animal used, species of plant seed, and probably the type and amount of anti-nutrient components in the seeds.

5.8 Economic evaluation of Catfish (*Clarias gariepinus*) Raw and Processed Kenaf (*Hibiscus cannabinus*) Seed Meal Based Diets.

The cost of producing feed using raw and differently processed KSM did not vary. This depicts that no extra cost would be incurred using KSM as ingredient in feed production for *C. gariepinus*. Similar result was observed when Odetola (2013) replaced soybean meal for differently processed KSM in the diet for broiler chicken. The economic indices showed that roasted KSM based diet was more superior in economic benefit than the other processed KSM based diet and raw KSM based diet. However, feeding *C. gariepinus* with 10% kenaf based diet resulted in 20.2% reduction in the value of fish relative to the control (0%). The profit index reduced by 11.2% while, the incidence of cost increased by 20.2%. 20% and 30% KSM based diets were also significantly inferior to the control group. This depicts that no superiority in economic value will be recorded if KSM replaces soybean meal at 10% in the feed of *C. gariepinus*. Amount greater than 10% will also result in a negative economic value. Inclusion of *Leucaena leucocephala* seed meal in amount above 20% in the feed of *C. gariepinus* resulted into a poor economic benefit (Sotolu, 2008). Similar result was also reported by Adesina (2014) who fed *C. gariepinus* with sunflower meal based diet and Aderolu and Akpabio (2010) who also fed C. *gariepinus* with velvet bean. The anti-nutritional constituents in kenaf seed meal which probably led to poor growth performance and utilisation of nutrient in KSM by *C. gariepinus* may be responsible for the negative economic benefit.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

The study investigated the dietary incorporation of kenaf (*Hibiscus cannabinus*) in *Clarias gariepinus* feed. This was considered necessary to reduce cost of feeding catfish by providing alternative cheaper ingredient of plant sources. The research work characterised the chemical constituents of kenaf seed in terms of nutrient and anti-nutrient constituents. The research further investigated the effect of different processing methods on the components of kenaf seed and the apparent digestibility of the nutrients in kenaf seed by *C. gariepinus*. The performance of catfish fed graded dietary levels of raw and differently processed kenaf as a substitute for soybean was also established.

Raw kenaf contains a crude protein of 21.17% which was significantly improved by roasting (25.17%), soaking (29.14%), sprouting (25.75%) and cooking (30.45%). These processing methods resulted in a better mineral retention in kenaf seed and, the Na/K ratio was estimated to be less than 1, which may be an advantage to the balance of internal osmotic pressure in fish. However, a lower Ca/P ratio (less than 0.5) was recorded for raw and differently processed kenaf seed meal, indicating that calcium will be less absorbed in the intestine. Tannin, trypsine inhibitor, phytic acid, saponin, alkaloid and oxalate were detected in the kenaf seed meal. These anti-nutritional factors were reduced in kenaf seed meal by all the processing methods except for tannin, trypsin inhibitor and oxalate that increased when soaked. Raw and processed kenaf (*Hibiscus cannabinus*) seed meal had amino acid content comparable to Roselle (*Hibiscus sabdariffa*) but, was limiting in methionine (+ cyctein). The amino acid score was the lowest in raw KSM (4.53) and increased in processed KSM in the order of: soaked KSM (5.83) > cooked KSM (5.53) > sprouted (5.41) > roasted KSM (5.35). *Clarias gariepinus* digested crude protein, ether extract and gross energy in processed kenaf seed meal to over 77%, 93% and 67% of apparent digestibility coefficient respectively, better than the raw kenaf seed meal.

The survival of *C. gariepinus* fed graded levels (0, 10, 20 and 30%) of raw and differently processed kenaf seed as a substitute for soybean over a period of 112 days ranged from 0% to 76.67%. The control group (0%) had the highest survival followed by *C. gariepinus* fed 10% roasted kenaf seed meal based diet (70% survival). The survival rate reduced with increasing quantity of dietary kenaf seed meal in catfish. Lowest rate of survival was recorded in the highest dietary inclusion of kenaf seed meal in the fish. The retardation in growth with corresponding increase in rate of mortality set in at 10^{th} week of culture period for fish fed 20 and 30% raw and processed kenaf based diet, and persisted all through the period of culture. Performance in terms of growth, utilisation of nutrient in the diet by the fish and indices of economic evaluation followed a similar pattern. Futhermore, the result of regression analysis showed that the optimal dietary inclusion level of KSM that resulted in the best performance was 10.8% in roasted KSM.

The gross pathological examination of the fish could not link the poor performance and high mortality of the fish to the effect of pathogen. The haematological and biochemical parameters assessed revealed a non-significant variation between the control group and those fed 10% roasted kenaf seed meal based diet. These values were significantly superior to those fed 20 and 30% raw and processed kenaf diets having the meal of kenaf seed, including the roasted. The pathological alterations recorded in the kidney and liver of catfish in this study could not be connected directly to effect of dietary treatment. However, result of poor performance on growth and utilisation nutrients, as well as the inferior haematological and plasma biochemical value recorded at high level of inclusion of dietary kenaf seed pointed to the physiological influence of anti-nutritional factors. These factors were opined to interfere with bioavailability of protein and essential minerals needed for life sustaining processes in fish. Consequentially, the residual effect of the anti-nutrients in the kenaf seed meal based diet on the performance of *C. gariepinus* resulted into an inferior economic benefit when assessed against the control (0%).

6.2 CONCLUSION

It can be inferred from this research that:

- The level of crude protein, mineral and amino acid profile qualifies kenaf seed as a potential protein ingredient of plant source in fish feed production. However, it may be necessary to process the raw kenaf seed to enhance protein quality and improve digestibility and; fortified with calcium and methionine when used in feed production.
- Roasting the seeds of kenaf for 15 minutes, ground and incorporated in the feed of catfish promoted the performance and nutrient utilisation better than raw, soaked, sprouted and cooked kenaf seed meal.
- Substitution of soybean meal with dietary roasted kenaf seed meal up to 10% for *C. gariepinus* compared favourably with the control group (0% kenaf). However, inclusion higher than the optimal level of 10.8% in *C. gariepinus* feed resulted into high mortality and growth depression.
- Liver and kidney of catfish fed the treatment diets, even at higher inclusion levels showed no remarkable histopathological alterations. This implied that the residual level of anti-nutritional factors had a chronic effect on *C. gariepinus* by interfering with bioavailability of dietary protein and mineral rather than act as a systemic chemical poison.
- The economic benefit of replacing soyabean with 10% roasted KSM compared favorably with the 100% soyabean (0% KSM) meal based diet. A further increase in the level of replacement led to an inferior economic benefit.
- Soaking, sprouting and cooking, although, enhanced the protein quality and digestibility of raw kenaf seed meal, they appeared not to be suitable as a processing method to positively aid the performance of growing *C. gariepinus*. Their utilisation at all levels of inclusion (10, 20 and 30%) produced high mortality and growth retardation.

6.3 RECOMMENDATION

Fundamental information on the performance of catfish fed kenaf seed meal based diet is provided in this research. While it is not advisable to utilize soaked, sprouted and cooked kenaf seed meal in the diet preparation for *C. gariepinus*, it is recommended that roasted kenaf seed meal should not replace soybean meal beyond 10.8%.

Further research is recommended to use other processing methods in other to explore the nutritional potential of kenaf seed as fish feed ingredient. Investigation is also recommended to explore the performance of other culturable fish species using kenaf seed meal as plant protein ingredient.

REFERENCES

Abu, O. M. G., Sanni, L. O., Erondu, E. S. and Akinrotimi, O. A. 2010. Economic viability of replacing maize with whole cassava root meal in the diet of Hybrid Cat-fish. *Agricultural Journal.* 1:1-5

Adebayo, S. F. 2014. Effect of soaking time on the proximate, mineral composition and anti-nutritional actors of lima bean. *Food Science and Quality Management,* 27: 1 – 3.

Adebo, G. M., and Toluwase S. O. W. 2014. Comparative Analysis of Fresh and Smoked Catfish Marketing in Ekiti and Ondo States of Nigeria. *Journal of Biology, Agriculture, and Healtcare,* 4 (19). 6-11

Adekanmi, O. K., Oluwatooyin, O. F. and Yemisi, A. A. 2009. Influence of processing techniques on the nutrient and antinutrient of Tigernut (*Cyperus esculentus* L.). *World Journal of Dairy & Food Science* 4 (2): 88 – 93.

Adeparusi, E. O. and Jimoh, W. A. 2002. Digestibility Coefficient of Raw and Processed Lima Bean for Nile Tilapia, *Oreochromis niloticus*. *Journal of Applied Aquaculture*, 12, 89-98.

Aderolu, A. Z. and Akpabio, V. M. 2010. Growth and economic performance of *Clarias gariepinus* juvenile fed diets containing vevet bean, *Mucuna pruriens*, seed meal. *African Journal of Aquatic Science,* 34 (2): 131 – 135.

Adesina, S. A. 2014. Growth performance and nutrient utilisation of *Clarias gariepinus* juveniles fed graded levels of differently processed sunflower (*Helianthus annuus*) seed meal-based diets. Ph.D Thesis submitted to the University of Ibadan, Ibadan. Nigeria. p.306

Adesina, S. A. 2017. Assessment of organosomatic indices and histopathological changes in vital organs of *Clarias gariepinus* juvenile fed graded levels of boiled sunflower (*Helianthus annuus*) seed meal. *Ife Journal of Science* Vol. 19 (1): 85 – 98.

Adesina, S. A., Falaye, A. E., Olusola, S. E. and Ajani E. K. 2013. Growth performance and nutrient utilization of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower (*Helianthus annuus* L) seed meal-based diets. *Journal of Agricultural Research*. Vol. 2 (12), pp. 342-351.

Adesulu, E. A. and Sydenham, D. H. J. 2007. *The fresh water fishes and fisheries of Nigeria*. Macmillan Nigeria Publishers Ltd., Lagos, Ibadan. 397 pp.

Adeyemo, O. K. 2007. Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. *Turkish Journal of Fisheries and Aquatic Sciences* 7: 163 – 169.

Agbabiaka, L. A., Madubuike, F. N. and Ekenyem, B. U. 2013. Haematology and Serum Characteristics of African catfish (*Clarias gariepinus* Burchell) fed graded levels of Tiger nut based diet. *American Journal of Experimental Agriculture*. 3(4): pp. 988-995.

Agbo, N. W., Madalla, N. and Jauncey, K. 2011. Effect of dietary cotton seed meal protein levels on growth and feed utilisation of Nile tilapia, *Oreochromis niloticus* L. *Journal of Applied Science and Environmental Management*. 15 (2), 235 – 239.

Agboola, E. O. 2004. The Utilisation of Castor Seed (*Ricinus Communis* L.) by *Clarias gariepinus* (Teugels) and *Oreochromis niloticus* (Trewavas) fingerlings. *In*: Balogun, B.I., Oniye, S.J., Lakpini, C.A.M. and Abeke, F.O. 2016. Growth performance and feed utilisation of *Clarias gariepinus* (Teugels) fed different dietary levels of Soaked *Bauhinia monandra* (Kutz) seed meal. *Journal of Research.* Vol 5(1): 97 – 106.

Agronews 2017. RMRDC receives kenaf production, gives seeds to farmers. Retrieved online at www.agronewsng.com on the 20th of January, 2018, 9:01am.

Agume, A. S. N., Njintang, N. Y. and Mbofung, C.M. 2017. Effect of soaking and roasting on the physicochemical and pasting properties of soybean flour. *Food*: 1 – 10.

Ajibola, A. T. and Modupeoluwa, T. O. 2014. Determination of the optimum weding regime on seed yield of two selected kenaf varieties in South-west Nigeria. *Research Journal of Seed Science* 7: 125 – 131.

Akande, T. O., Odunsi, A. A., Olabode, O. S., and Ojediran, T. K. 2012. Physical and nutrient characterisation of Raw and processed Castor (*Ricinus communis* L.) seeds in Nigeria. *World Journal of Agricultural Science*, 8(1): 89-95.

Akintola, S. L. and Fakoya, K. A. 2017. Small-scale fisheries in the context of traditional post-harvest practice and quest for food and nutritional security in Nigeria. *Agriculture & Food Security* 6 (34), 1 – 17.

Akinwunmi, O. F. and Eniade, A. 2014. Growth performance of African mud catfish, *Clarias gariepinus* (Siluriformes: Clariidae) fed tropical banna blosson *Musa sapietum* (Zingiberales: Musaceae) inclusion. *Journal of Aquaculture Feed Science and Nutrition*. 6(2): 32 – 38.

Akinyele, O. T. 1989. Effect of traditional methods of processing on nutrient content and some antinutritional factors in cowpea (*Vigna unguiculata*). *Food Chemistry* 33: 291 – 299.

Akubueze, E. U., Ezeanyanaso, C. S., Orekoya, E. O., Akinboade, D. A., Oni, F., Muniru, S. O. and Igwe, C. C. 2014. Kenaf fiber (*Hibiscus cannabinus* L): A viable alternative to jute fiber (*Corchorus genus*) for Agro-Sack production in Nigeria. *World Journal of Agricultural Sciences* 10 (6): 308 – 313.

Alajaji, S. A. and El-Adawy, T. A. 2006. Nutritional composition of chickpea (*Cicer arietinum*, L.) as affected by microwave cooking and other traditional cooking methods. *Journal of Food Composition and Analysis*. 1 – 7.

Alegbeleye, W. O., Obasa. S. O, Olude, O. O, Moronkeji, T. and Abdulraheem I. 2012. Growth performance and nutrient utilization of African mud catfish (*Clarias gariepinus*) fingerlings fed different levels of fermented pigeon pea (*Cajanus cajan*) meal. *Israeli Journal of Aquaculture Bamidgeh*, 64 (731). 8 pages.

Alegbeleye, W. O., Oresegun, A. O. and Omitoyin O. 2001. Use of bambara groundnut (*Vigna subterranean*) meal in the diet of *Heteroclarias* fingerlings. *Moor Journal. Agricultural Research*. 2: 54-59.

Alexopoulou, E., Christou, M., Nicholaou, A., Mardikis, M., 2004. BIOKENAF: A network for industrial products and biomass for energy from kenaf. *Proceedings of the 2nd World Biomass Conference,* 10–14 May, Roma, Italy, 2040–2043.

Ali, M. Z. 2001. Dietary protein and energy interactions in African Catfish *Clarias gariepinus* (Burchell, 1822). PhD Thesis, University of Stirling, United Kingdom, 274 pp.

Allison, R. W. 2012. Laboratory evaluation of plasma and serum protein. *Veterinary hematology and clinical chemistry* M. A. Thrall, G. Weiser, R. W. Allison and T. W. Campell, Eds, John Wiley & Sons, Inc. pp. 460 - 475.

Al-Wandawi, H., Al-Shaikhaly, K., and Abdu-Rahman, M. 1984. Roselle seeds: a new source of protein. *Journal of Agricultural and Food Chemistry*, 32, 510–512.

AOAC (Association of Official Analytical Chemist) 2005. *Official methods of analysis* (18th Ed.). Washington, D.C., U.S.A 970pp.

Argrawal, N. K. and Mahajan, C. L. 1980. Hematological changes due to vitamin C deficiency in *Channa punctatus* Bloch. *Journal of Nutrition*, 110: 2172 - 2181.

Arneson, W. and Brickell, J. 2007. Assessment of renal function. *Clinical chemistry: A laboratory perspective,* W. Arneson and J. Brickell (ed). F.A. Davis Company, Philadelphia, pp. 201 – 232.

Audu, S. S. and Aremu, O. M. 2011. Effect of processing on chemical composition of red kidney bean (*Phaseolus vulgaris* L.) flour. *Pakistan Journal of Nutrition* 10 (11): 1069 – 1075.

Baer, D. J., Rumpler, W. V., Miles, C. W., and Fahey Jr., G. C. 1997. Dietary fiber decreases the metabolizable energy content and nutrient digestibility to mixed diets fed to humans. *Journal of Nutrition*. 127, 579–586.

Balogun, A. M. and Ologhobo, A. D. 1989. Growth performance and nutrient utilisation of *Clarias gariepinus* (Burchell) fed raw and cooked soybean diets. *Aquaculture* 76, 119-126.

Banaszkiewicz, T. 2011. Nutritional value of Soybean meal. *Soybean and Nutrition*, H. El-Shamy (Ed.), Assessed online On 2nd of June, 2018 at www.intechopen.com/book/soybean-and-nutrition/nutrition-value-of-soybean-meal.

Bashir, A. Y., Abdullahi, S. A. and Suleiman, B. 2016. Effect of roasting on the proximate, mineral and anti-nutritional components of *Tamarindus indica* seed nuts. *FUW Trends in Science and Technology Journal,* Vol. 1 (2): 493 – 496.

Bassey, O., Nsa, E. E., Anya, M. I., Edets, G. D., Umoren, E. P., and Oko, L. N. 2009. The phytochemistry, vitamin and mineral content of dasheen (*Colocasia esculanta)* leaves. *Proceeding of the 34th Annual Conference of Nigeria Society for Animal Production*, March, 15th – 18th, Uyo – Nigeria, pp: 426 – 428.

Becker, K. and Makkar, H. P. S. 1999. Effects of dietary tannic acid and quebracho tannin on growth performance and metabolic rates of common carp (*Cyprinus carpioL.*). *Aquaculture,* 175: 327–335.

Bekibele, D. O. 2005. The effect of the partial replacement of soya bean meal with Mucuna beans on the growth performance of *Clarias gariepinus* (Burchell, 1822). *Proceedings of the 20th Annual conference of the Fisheries society of Nigeria (FISON).* Port Harcourt, 14th-18th November, 2005. pp.136-139.

Bello, O. S., Olaifa, F. E. and Emikpe, B. O. 2014. Haematological and blood biochemical changes in African catfish, *Clarias gariepinus* fed walnut (*Tetracarpidium conophorum* Mull Arg) leaf and onion (*Allium cepa* Linn) buld supplemented diets. *American Journal of Experimental Agriculture,* 4 (12): 1593 – 1603.

Beveridge, M. C. M. 1984. Cage and pen farming: carrying capacity models and environmental impact. *FAO Fisheries Paper*. 225 FIRI/T255, 131pp.

Bhosale, S. V., Bhilave, M. P., Nadaf, S. B. 2010. Formulation of fish feed using ingredients from plant sources. *Journal of Agricultural Science* 1 (3), 284–287.

Bilal, A., Rakha, A., Butt, M. S. and Shahid, M. 2017. Nutritional and physicochemical attribute of cowpea and mungbean based weaning foods. *Pakistan Journal of Agricultural Science* Vol 54 (3): 653 – 662.

Bosma, R., Verdegem, H. and Marc, C. J 2011. Sustainable aquaculture in Ponds: Principles, Practise and Limits. *Livestock Science*. 139, 58 – 68.

Bowen, S. H. 1980. Detrital non-protein amino acids are the key to rapid growth of Tilapia in Lake Valencia, Venezuela. *Science* 207, 1216 – 1218.

Braun, J. P., Lefebvre, H. P. and Watson, A. D. J. 2003. Creatinine in the dog: A review. *Veterinary Clinical Pathology* 32:162–176.

Bruton, M. N. 1979. The food and feeding behavior of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of Cichlids. *Translational Zoological Society*. London 35, 47-114

Buddington, R. K and Krogdahl, A. 2004. Hormonal regulation of the fish gastrointestinal tract. *Comp. Biochem. Physiol.* 139, 261 – 271.

Bulbula, D. D. and Urga, K. 2018. Study on the effect of traditional processing methods on nutritional composition and anti-nutritional factors in chickpea (*Cicer arietinum*). *Cogent Food Science &Technology* 4: 1 – 12.

Burbano, C., Muzquiz, M., Ayet, G., Cuadrado, C., and Pedrosa, M. M. 1999. Evaluation of antinutritional factors of selected varieties of *phaseolus vulgaris*. *Journal of the Science of Food and Agriculture*, Vol. 79 (11), 1468 - 1472.

Bureau, D. P., Harris, A. M., Cho, C. Y. 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of Chinook salmon (*Oncorhynchus tshawytscha*) and Rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 161, 27–43.

Bureau, D., Kaushik, S. J and Cho, C. Y. 2002. Bioenergetics. *Fish Nutrition*. J. E. Halver and R. W. Hardy (3rd Ed.). Academic Press. U.S.A. Pp 2 – 54.

Burgess, W. E. 1989. *An Atlas of Freshwater and Marine Catfishes*. TFH Publishing Inc., USA, 784 p.

Burnside, O. C. and Williams, J. H. 1968. Weed control methods for kinkaoil, kenaf, and sunn crotalaria. *Agronomy Journal*. 60, 162–164.

Burtis, C. A. and Ashwood, E. R. 1996. *The fundamentals of clinical chemistry*. WB Saunders, Philadelphia. 679pp

Butler L. G. 1989. Effects of condensed tannin on animal nutrition. *Chemistry and significance of condensed tannins*. R. W., Hemingway. And J. J., Karchesy. Eds Plenum Press, New York, 553pp.

Campbell, T.W. 2012. Hematology of fish. *Veterinary haematology and clinical chemistry*. M. A. Thrall, G. Weiser, R. W. Allison, and T. W. Campell (Eds). John Wiley & Sons, Incorporation. pp. 607 – 614.

Celik, E. S. 2004. Blood chemistry (electrolytes, lipoproteins and enzymes) values of black scorpion fish (*Scorpaena porcus*) in the Dardanelles. *Turkey Journal of Biological Science* 4 (6), 716-719.

Chamberlain, T. 2011. A Fish meal and fish oil– the facts, figures, trends and IFFO's responsible supply standard. Accessed online on October, 2013 at www.iffo.net.

Ching, A., Jr. and Webber, C. L. III 1993. Effect of fertilizer applications on kenaf photosysthesis, growth and yield. *Proceeding of the Fourth International Kenaf Conference of the International Kenaf Association*, Ladonia, Texas. p. 17-23.

Cho, C.Y., Cowey, C. B., Watanabe, T. 1985. *Finfish nutrition in Asia: Methodological approaches to research and development*. IFRC, No. 233e, Ottawa/Canada 23pp

Clement, K. D. and Raubenheimer, D. 2006. Feeding and Nutrition. *The physiology of fishes*. D. H. Evans and J. B. Claibprne (3rd Eds.) CRC Press, Florida, United States of America. Pg: 47 – 82.

Coetzee, R., Labuschagne, M.T., Hugo, A., 2008. Fatty acid and oil variation in seed from kenaf (*Hibiscus cannabinus* L.) *Industrial Crop Production*. 27, 104–109.

Coles, E. H. 1986. *Veterinary clinical pathology*. Saunders Company, Philadelphia, USA. 776pp.

Cook, C. G. and Scott, A. W. 1995. Plant population effects on kenaf seed production. *Proceedings of the Conference of the International Kenaf Association*. Irving, Texas. 7: 153–158.

Cos-Rakovac, R., Strunjak-Perovic, I., Hacmanjek, M., Popvic, N. T., Lipej, Z. and Sostaric, B. 2005. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus labrax*) in the North Adriatic sea. *Veterinary Research Communication*, 29, 677 – 678.

Crawshaw, R. 1994. *Blood meal: A review of nutritional qualities for pig, poultry and ruminant animals*. National Renderers Association, London U.K. pp. 1 – 6.

Crozier, A., Clifford, M. N. and Ashihara, H. 2006. *Plant secondary metabolites: Occurrence, structure and role in the human diet*. Blackwell Publishing Ltd. Oxford, UK, p. 383.

Da, C. T., Lundh T. and Lindberg, J. E. (2012). Evaluation of local feed resources as alternatives to fish meal in terms of growth performance, feed utilisation and biological indices of stripped catfish (*Pangasianodon hypophthalmus*) fingerlings. *Aquaculture* 364-365: 150 – 156.

Dagnia S. G., Petterson, G. S., Bell, R. R. and Flanagan, F. V. 1992. Germination alters the nutritional value of lupin seed. *Journal of Science and Food Agriculture*, 60, 419–423.

Davie, E. W. and Neurath, H. 1955. The terminal groups of the soybean trypsin inhibitor. *Journal of Biology and Chemistry*, 212: 507-514.

de la Cuadra, C., Muzquiz, M., Burbano, C., Ayet, G., Calvo, R., Osagie, A. and Cuadrado, C. 1994. Alkaloid, α-galactoside and phytic acid changes in germinating lupin seeds. *Journal of Science Food and Agric*ulture, 66, 357–364

de la Higuera, M., Garcia-Gallego, M., Cardenete, G., Suarez, M.D., Moyano, F.J., 1988. Evaluation of Lupin seed meal as an alternative protein source in feeding of rainbow trout (*Salmo gairdneri*). *Aquaculture* 71, 37–50.

De, D. K., Nath, D., Sen, P. R. 1987. Preliminary studies on tea seed-cake as a fish toxicant. *Indian Journal of Animal Science,* 57 7: 781–783

Dempsey, J. M. 1975. *Fiber Crops*. The University Press of Florida, Gainesville. 457 pp

Deshpande, S. S. and Cheryan, M. 1984. Effects of phytic acid, divalent cations, and their interactions on amylase activity. *Journal of Food Sci*ence 49, 516–519.

Dewick, P. M. 2002. *Medicinal Natural Products: A Biosynthetic Approach*. John Wiley and Sons, Chichester. 453pp

Domingo, J. L., Bocio, A., Falco, G. and Llobet, J. M. 2007. Benefits and risks of fish consumption Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. *Toxicology* 230: 219 – 226.

Doumas, B. T. and Biggs, H. G. 1971. Determination of serumalbumin. *Standard methods of clinical chemistry*. G. R. Copper (ed). Academy press, New York. 175pp.

Dupree, H. K. and Huuer, J. V. 1984. *Third report to the fish farmers.* U.S. Fish and Wildlife Service, Washiogton. 207pp.

Edelhoch, H. and Steiner, R. F 1963. Structural transitions of soybean trypsin inhibitor II. The denatured state in urea. *Journal of Biology and Chem*istry 238: 931-938.

El-Adawy, T. A. 2002. Nutritional composition and anti-nutritional factors of chickpea (*Cicer arietinum* L.) undergoing different cooking methods and germination. *Plant Food and Human Nutrition*, 57, 83 – 97.

El-Adawy, T. A. and Khalil, A. 1994. Characteristics of roselle seed as a new source of protein and lipid. *Journal of Agriculture Food & Chemistry*. 42, 1896 – 1900.

ElMaki, H. B., AbdelRahaman, S. M., Idris, W. H., Hassan, A. B., Babiker, E. E., & ElTinay, A. H. 2007. Content of anti-nutritional factors and HCl-extractability of minerals from white bean (*Phaseolus vulgaris*) cultivars: Influence of soaking and/or cooking. *Food Chemistry*, 100, 363–368.

Emiola, I. A., Ojediran, T. K and Ajayi, J. A 2013. Biochemical and Haematological Indices of Broiler Chickens Fed Differently Processed Legume seed meals. *International Journal of Agriculture and Rural Development* 1 (2), 140-149.

Emiola, I. A., Ologboho, A. D. and Gous, R. M. 2007. Performance and histological responses of internal organs of broiler chickens fed raw, dehulled, Aqueous and dry-heated kidney bean meal. *Poultry Science*. 86, 1234-1240.

Erbas, M., Certel, M. and Uslu, M. K. 2005. Some chemical properties of white lupin seeds (*Lupinus albus* L.). *Food Chemistry*. 89 (3), 341 – 345.

Evans, G. O. 2009. *Animal clinical chemistry: A practical handbook for toxicologists and biomedical researchers*. Taylor & Frabcis Group LLC, USA. pp .346

Fagbenro, O. A. 2005. Soybean meal substitution with Roselle (*Hibiscus sabdariffa* L) seed meal in the dry practical diets for fingerlings of the African Catfish, *Clarias gariepinus* (Burchell 1822). *Journal of Animal and Veterinary Advances* 4 (4): 473 – 477.

Fagbenro, O. A. and Davies, S. J. 2001. Use of soyabean flour (dehulled, solvent-extracted soyabean) as a fish meal substitute in practical diets for African Catfish, *Clarias gariepinus* (Burchell 1822): growth, feed utilisation and digestibility. *Journal of Applied Ichthyology*. 17: 64 – 69.

Fagbenro, O. A., Balogun, A. M. and Fasakm, E. A. 1998a. Dietary methionme requirement of the African catfish. *Clarias gariepinus*. *Journal of Applied Aquaculture*, 8: 47–54.

Fagbenro, O. A., Balogun, A. M., Fasakin, E. A., and Bello-Olusoji, O. A. 1998b. Dietary lysine requirement of the african catfish *Clarias gariepinus Journal of Applied Aquaculture*, 8 (2), 71-77

Falaye, A. E., Elezuo, K. O., Ajani, E. K. and Omoike, A. 2016. Digestibility and nutrient utilisation of differently processed tropical almond (*Terminalia catappa*) kernel meal and cake based diet by *Carias gariepinus* juvenile. *Journal of Agricultural Science and Technology* 17 (1): 42 – 60.

Falaye, A. E., Omoike, A. and Orisasona, O. 2014. Apparent digestibility coefficient of differently processed lima bean (*Phaseolus lunatus* L.) for *Clarias gariepinus* Juvenile. *Journal of Fisheries and Aquatic Science* 9 (2): 75 – 84.

FAO/WHO (1991). *Protein quality evaluation report of joint FAO/WHO expert consultative* FAO, food and Nutrient. 53pp.

Fasakin, E. A., Balogun, A. M. and Fagbenro, A. O. 2001. Evaluation of sundried *Azolla Africana* and duckweed, *Spirodela polyrrhiza* in practical diet for Nile Tilapia, *Oreochromis niloticus* fingerlings. *Journal of Applied Aquaculture*. 11, 83 – 92.

Faturoti, E. O. (2000). *Beneath the ripples and sustainable fish production*. Inaugural lecture, University of Ibadan, p.54

Fenton T. W and Fenton, M. 1979. An improved procedure for the determination of dietary chromic oxide in feed and feces. *Canadian Journal of Animal Science*. 159, 631 – 634.

Fenwick, D. E., & Oakenfull, D. 1983. Saponin content of food plants and some prepared foods. *Journal of the Science of Food and Agriculture*, 34, 186–191.

Food and Agricultural Organisation (FAO). 2014. *The State of World Fisheries and Aquaculture*. FAO. Rome. Pg 1 – 243.

Food and Agriculture Organization (FAO) 2016. *FAO Statistical Pocketbook*– World Food and Agriculture. Rome, Italy. 236p.

Food and Agriculture Organization (FAO). 2012. FAO Country Programming Framework (CPF) Federal Republic of Nigeria. *Fiat Panis*: 1-41.

Fowomola, M. A and Akindahunsi, A. A. 2008. Effects of fermentation on some anti-nutrients and nutrients contents of sandbox (*Hura crepitans*) seed. *Journal of Food, Agriculture and Environment*. 6 (2): 25 – 28.

Fowomola, M. A. and Akindahunsi, A. A. 2007. Nutritional quality of sandbox tree (*Hura crepitans* Linn.). *Journal of Medicinal Food* 10 (1): 159-164.

Francis, G. F., Makkar, H. P. S. and Becker, K. 2001. Antinutritional factors present in plants-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*. 199, 197 – 227.

Friedman, M and Brandon, D. L. 2011. Nutritional and health benefit of soy protein. *Journal of Agriculture and Food Chemistry*. 49, 1069 – 1086.

Friel, J. P., and Wainwright, P. C. 1999. Evolution of complexity in motor patterns and jaw musculature of tetraodontiform fishes. *Journal of Experimental Biology*. 202, 867-880.

Gabriel, U. U. and George, A. D. I. 2005. Plasma enzymes in *Clarias gariepinus* exposed to chronic levels of roundup (glyphosate). *Journal of Environment and Ecol*ogy 23 (2), 271-276.

Gabriel, U. U., Amakiriand, E. U and Ezeri, G. N. O. 2007. Haematology and gill pathology of *Clarias gariepinus* exposed to refined petroleum oil, kerosene under laboratory conditions. *Journal of Animal and Veterinary Advances* 6 (3): 461 – 465.

Gbadamosi, S. O. and Osungbade, O. R. 2017. Effect of cooking and fermentation on the antinutrients, total phenolic content and antioxidant properties of sandbox (*Hura crepitansi*) seeds. *International Food Research Journal* 24 (4): 1621 – 1627.

Gemede, H. F. 2014. Effect of boiling methods on antinutritional factors of anchote (*Coccinia abyssinica* (lam.) tubers grown in western Ethiopia. *Food Science and Quality Management*. pp 39 – 44.

Ghazalah, A. A., Abd- Elsamee, M. O., Abd-ElHakim, A. S. and Ibrahim, M. M. 2012 . Evaluation of using distillers dried grains with solubles (DDGS) in broiler diets. Egypt. *Journal of Poultry Science*. 32, 381-397.

Gilani, O. S., Xiao, C. W. and Cockell, K. A. 2011. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition*. 108, 5315 – 5332

Griffiths, D. W. 1991. Condensed tannins. *Toxic Substances in Crop Plants*. F. J. P D'Mello, C. M., Duffus and J. H. Duffus, Eds. The Royal Society of Chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge, pp. 180–201

Gunder, H. 2004. *Clarias gariepinus*, Animal Diversity. Assessed online on May 20, 2017 at http://animaldiversity.ummz.umich.edu/accounts/Clarias_ gariepinus/.

Hainida Emmy, K. I., Amin, I., Normah, H. and Esa-Mohd, N. 2008. Nutritional and amino acid contents of differently treated Roselle (*Hibiscus sabdariffa* L.) seeds. *Food Chemistry* 111, 906 – 911.

Hainida, E., Ismail, A., Hashim, N., Mohd-Esa, N., & Zakiah, A. 2008. Effects of defatted dried roselle (*Hibiscus sabdariffa* L.) seed powder on lipid profiles of

hypercholesterolemia rats. *Journal of the Science of Food and Agriculture*, 88 (6), 1043-1050.

Halver, J. and Hardy, R. W. 2002. *Fish Nutrition*. Academic Press, California. 828pp

Handa, V., Kumar, V., Panghal, A., Suri, S. and Kaur, J. 2017. Effect of soaking and germination on physic-chemical and functional attributes of horsegram flour. *Journal of Food Science and Technology*. 11pp

Harborne, J. B. 1973. *Phytochemical methods*. Chapman and Hall, Ltd, London. Pp 49-188.

Harrison, G. A. 1947. *Chemical methods in Clinical Medicine* 3rd edition, Churchill, London. 201pp

Hecht, W. P. and Andrew, T. G. 2008. Does reservoir tropic status influence the feeding and growth of the sharptooth catfish, *Clarias gariepinus* (Teleostei: Clariidae). *African Journal of Aquatic Science*. 33 (2), 149 – 156.

Hefnawy, T. H. 2011. Effect of processing methods on nutritional composition and anti-nutritional factors in lentils (*Lens culinaris*). *Annals of Agricultural Science*. 56 (2), 57 – 61.

Henry, J. 1993. *The plant Alkaloids*. Nabu Press. South Carolina, US. 466pp.

Hickman, M. V. and Scott A. W. 1989. Pre-emergence herbicides for kenaf production. *Proceeding of the Assciation of Advance Indusstrial Crops*. Texax. 68pp

Hinton, D. E, Couch, J. A. 1984. Pathobiological measures of marine pollution effects *Concepts in marine pollution measurements*. L. White (eds.) College Park: University of Maryland, Sea Grant College, 7 – 32.

Hinton, D. E. 1993. Toxicologic histopathology of fishes: a systematic approach and overview. *Pathobiology of Marine and Estuarine Organisms*. J. A. Couch, J. W. Fournie. (Eds.). CRC Press, Boca Raton, Florida, pp. 177–215.

Horn, M. H. 1989. Biology of marine herbivorous fishes. *Oceanogr. Journal of Marine Biolology and Annual Review*. 27, 167 – 272.

Hossain, M. A. and Jauncey, K., 1990. Detoxification of linseed and sesame meal and evaluation of their nutritive value in the diet of carp *Cyprinus carpio* L. *Journal of Asian Fisheries Science* 3, 169–183.

Hossain, M. A., and Jauncey, K. 1990. The effect of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common carp, Cy*prinus carpio*. *Proceedings of International Conference,* Biarritz, France, June 24–27, 1991, pp. 705–715.

Hussain, S. M., Arsalan, M. Z, H., Javid, A., Abdullah Ijaz Hussain, A. I., Aslam, N., Ali., Q., Hussain, M., Rehan, M. M. H., Shahzad, M. M., Khalid, A. and Riaz, D. 2018. Replacement of Fish Meal with *Moringa oleifera* Leaf Meal (MOLM) and its Effect on Growth Performance and Nutrient Digestibility in *Labeo rohita* Fingerlings. *Pakistan Journal of Zooogy.*, 50 (5), 1815 – 1823.

Ibukun, E. O. and Anyasi, O. J. 2012. Changes in antinutrient and nutritional values of fermented sesame (*Sesanum indicum*), musk melon (*Cucumis melo)* and white melon (*Cucumeropsis mannii*). *International Journal of Advanced Biotechnological Research* 4 (1), 131-141

IITA 1990. *Soybean for good health: How to grow and use soybean in Nigeria*. International Institute for Tropical Agriculture, Ibadan, Nigeria, 25 pp.

Ikenaka, T., Shimada, K., and Matsushima, Y. 1963. The N-terminal amino acid sequence of soybean trypsin inhibitor. *Journal of Biochemistry*. 54, 193-195.

Isikwenu, J. O, Nwanbe, R. N, Elechi, F. N. 2013. Effect of some thermal processing techniques on the proximate, mineral and anti-nutrient compositions of Bambara groundnut (*Voandzeia subterranean*, (L) Thour) meal. *Journal of Biology, Agriculture and Healthcare*. 3 (17), 105-109.

Jackson, A. J., Capper, B. S., Matty, A. J. 1982. Evaluation of some plant proteins in complete diets for the tilapia (*Sarotherodon mossambicus*). *Aquaculture* 27, 97–109.

Jimoh, W. A., Sodamola, M. O., Adebayo, M. D., Banjo, O. T., Ayeloja, A. A. and Adeleke, A. B. 2015. Histopathological changes in the liver and kidney of *Clarias gariepinus* fed *Chrysophyllum albidum* seed meal as maize replacer. *International Journal of Zoological Research* 11 (1): 29 – 36.

Jimoh. W. A., Fagbenro, O. A. and Adeparusi, E. O. 2014. Response of African catfish, *Clarias gariepinus* (Burchell 1822), fingerlings fed diets containing differently

timed wet-heat-treated Sesame (*Sesamum indicum*) seedmeal. *Agricultural Science* 5, 1159 – 1171.

Jindal, M., Garg, S. K., Yadava, N. K. and Gupta, R. K. 2007. Effect of replacement of fishmeal with processed soybean on growth performance and nutrient retension in *Channa punctatus* (bloch) fingerlings. *Livestock Research for Rural Development* 19 (11). pp: 165

Jobling, M., Gomes, E and Dias, J. 2001. Feed types, manufacture and ingredients. *Food intake in fish* (eds). D. Houlihan, T. Boujard and M. Jobling. Blackwell Science, Oxford. Pp 25 – 48.

Jood, S., Chauchan, B. M. and Kapoor, A. C. 1986. Saponin content of chickpea and black gram: varietal differences and effect of processing and cooking methods. *Journal of Food and Agriculture*. 37 (11): 1121 – 1124.

Kader, M. A., Koshio, S., Ishikawa, M., Yokoyama, S., Bulbul, M. 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diet for red sea bream, *Pagrus major*. *Aquaculture* 308, 136 – 144.

Kalla A., Garg, S. K., Kaushik, C. P., Arasu, A. R. T. and Dinodia, G. S. 2003. Effect of replacement of fishmeal with processed soybean on growth, digestibility and nutrient retention in *Mugil cephalus* (Linn.) fry. *Indian Journal of Fisheries* 50 (4): 509 – 518.

Kathleen, A. Anne, F. R. and Junning, C. 2017. Nigeria: Faster aquaculture growth needed to bridge fish demand-supply gap. *FAO Newletter*, No: 57 pg 36.

Khalil, A. H and Mansour, E. H. 1995. Effect of cooking, autoclaving and germination on the nutritional quality of faba beans. *Food Chemstry* 54: 177 – 182.

Khokhar, S. and Chauchan, B. M. 1986. Antinutritional factors in moth bean (*Vigna aconitifolia)*: Varietal differences and effects of methods of domestic processing and cooking. *Journal of Food Science*. 51 (3): 591 – 594.

King, E. J. and Amstrong, A. R. 1934. A convenient method for determining serum and bile phosphate activity. *Journal of Canadian Medical Association*. 31: 376-381.

Koprucu, K. and Ozdemir, Y. 2005. Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 250: 308 – 316.

Koprucu, K. and Sertel, E. 2012. The effects of less-expensive plant protein sources replaced with soybean meal in the juvenile diet of grass carp (*Ctenopharyngodon idella*): growth, nutrient utilisation and body composition. *Aquaculture International*. 20: 399 – 412.

Krogdahl, A., Lea, T. B., Olli, J. J. 1994. Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Journal of Comparative Biochemistry and Physiology* 107: 215–219.

Krogdahl, A., Penn, M., Thorsen, J., Refstie, S. and Bakke, M. 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmon. *Aquaculture Research* 41: 333 – 344.

Kumar, S. Zs, J. S., Nagy, Z. Fazekas, G., Havasi, M., Sinha, A. K., Boeck, G. D. and Gal, D 2017. Potential of processed animal protein versus soyabean meal to replace fish meal in practical diets for European catfish (*Silurus glanis*): growth response and liver gene expression. *Aquaculture Nitrition*. Pg: 1 – 11.

Kumar, V., Makkar, H. P. S., Amselgruber, W. and Becker, K. 2010. Physiological, haematological and histopathological responses in common carp (*Cyprinus carpio*) fingerlings fed with differently detoxified *Jatropha carcas* Kernel meal. *Food and Chemical Toxicology*, 48: 2063 – 2072.

Kurtz, M. E. and Neill, S. W. 1990. Possible herbicides for use in kenaf. *Proceeding of the Second Annual conference of Internatona Kenaf Association*. Texas. 210pp.

Kuz'mina, V. V. and Gelman, A. G. 1997. Membrane-linked digestion in fish. *Review of Fisheries Science* 5: 99 – 129.

Le Mahrew, P. J., Oplinger, E. S. and Putnam, D. H. 2003. Kenaf. In: Alternative Field Crop..Manual.http://www.corn.agronomy.wisc.edu/FISC/Alternatives/Kenaf.htm. Accessed October, 2017.

Liener, I. E and Kakade, M. 1969. Protease inhibitor. *Toxic constituents of plant foodstuffs*. I. E. Liener. (ed).. Academic Press, Inc. New York. pp 7 – 53.

Liener, I. E. 1989. Antinutritional factors in legume seeds: state of the art. *Recent Advances of Research in Antinutritional Factors in Legume Seeds*. J. Huisman, A.F.B. Van der Poel, I. E., Liener, Eds Pudoc, Wageningen, pp. 6–14.

Liener, I. E. 2003. *Plant antinutritional factors/Detoxification*. Elsevier Science Ltd. USA. Pg 4587 – 4593.

Lim, S. J. and Lee, K. J. 2008. Supplemental iron and phosphorus increase dietary inclusion of cottonseed and soybean meal in olive flounder (*Paralichthys olivaceus*). *Aquaculture. Nutrition*. 14, 423–430.

Lim, S. J. and Lee, K. J. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish (*Oplegnathus fasciatus*). *Aquaculture* 290, 283–289.

Lim, S., Kim, S, Ko, G., Song, J., Oh, D., Kim J., Kim, J. and Lee, K. 2011. Fish meal replacement by soybean in diet for tiger puffer, *Takifugu rubripes. Aquaculture*, 313 (1 – 4): 165 – 170.

Lips, S. J. J., Niguez de, I, Heredia, G. M., Op den Kamp, R. G. M., van Dam, J. E. G. 2009. Water absorption characteristics of kenaf core to use as animal bedding material. *Journal of Industrial Crop Production* 29, 73–79.

Lobel, P. S. 1981. Trophic biology of herbivorous reef fishes: alimentary pH and digestive capabilities. *Journal of Fish Biology*. 55: 692 – 703.

Lovell, T., 1989. *Nutrition and feeding of fish*. An. AVI Book. Van Nostrand Reinhold, New York. 260 pp.

Mackower, R.V. 1970. Cereal. *Chemistry* 47:288-29

Maffia, M., Rizzello, A., Acierno, R., Verri. T., Rollo, M., Danieli, A, Doring, F., Danieli, H, and Storelli, C. 2003. Characterisation of intestinal peptide transporter of the Antarctic heamoglobinless teleost *Chionodraco hamatus. Journal of Fish Biology* 55: 692 – 703.

Makinde, F. M. and Akinoso, R. 2013, Nutrient composition and effect of processing treatments on anti-nutritional factors of Nigerian sesame (*Sesamum indicum* Linn) cultivars. *International Food Research Journal*, 20 (5): 2293 – 2300.

Makkar, A. O. S. and Goodchild, A. V. 1996. *Qualification of tannins: A laboratory manual*. International Centre for Agriculture Research (ICARDA), Aleppo, Syria, pp: 1 – 25

Makkar, H. P. S., Siddhuraju, P. and Becker, K. 2007. *Plant secondary metabolites*. Human Press. Totowa, New Jersey, p. 129.

Martinez-Herrera, J., Siddhuraju, P., Francis, G., Davila-Ortiz, G. and Becker, K. 2005. Chemical composition, toxic/antimetabolic consitiuents, and effect of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chemistry*, 96 (1): 80 – 89.

Masheswaran, R., Devapaul, A., Muralidharan, S., Velmurugan, B. and Ignacimuthu, S. 2008. Haematological studies of fresh water fish. *Clarias batrachus* (L.) exposed to mercuric chloride. *International Journal of Integrative Biology* 2 (49). Pp 6.

Maynard, I. A. and Loosli, J. K. 1969. *Animal nutrition*. McGraw-Hill Book Company, New York, USA. 778pp.

Mbwile, R. P. and Uden, P. 1996. Effect of age and season on growth and nutritive value of Rhodes grass (Chloris gayana cv. Kunth). *Journal of Animal feed Science and Technology*. 65: 87 -98.

Mela, M., Randi, M. A. F., Ventura, D. F., Carvalho, C. E. V., Pelletier, E. and Ribeiro, O. 2007. Effect of dietary methylmercury on liver and kidney histology in the neotropical fish (*Hoplias malabaricus*). *Ecotoxicology and Environmental Safety*, 68: 426 – 435.

Merida, S. N., Tomas-Vidal, A., Martinez-Llorens, S. and Cerda, M. J. 2010. Sunflower meal as a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diet: Amino acid rentention, gut and liver histology. *Aquaculture* 298: 275 – 281.

Minari, J. B., Odutuga, A. A., Bamisaye, F. A., Dairo, J. O., Fagbohunka, B. S. 2012. Effect of some processing techniques on the proximate and antinutrients composition of *Leucaena leucociphala* seed. *Pakistan Journal of Nutrition*, 11: 310-312.

Minatel, I. O., Borges, C. V., Ferreira, M. I., Gomez, G. H. A., Chen, C, O. and Lima, G. P. P. 2017. Phenolic compound: Functional properties, impact of processing and bioavailability. Accessed online at https://www.intechopen.com/books/phenolic-compounds-biological-activity/phenolic-compounds-functional-properties-impact-of-processing-and-bioavailability. On the 2nd of June, 2018. Pp 1 – 25.

Mohammed, M. A., Mohammed, E. A., Yagoub, A. E. A., Mohamed, A. R. and Babiker, E. E. 2017. Effect of processing methods on alkaloid, phytate, phenol, antioxidant

activity and minerals of newly developed lupim (*Lupinus albus* L). *Journal of Food Processing and Preservation* 41 (1): 12960 – 12967.

Morales-de la Peña, M., Salvia-Trujillo, L., Rojas-Graü, M. A., & Martín-Belloso, O. 2011. Changes on phenolic and carotenoid composition of high intensity pulsed electric field and thermally treated fruit juice-soymilk beverages during refrigerated storage. *Food Chemistry*, 129 (3), 982 – 990.

Mubarak, A. E. 2005. Nutritional composition and anti-nutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chemistry,* 89: 489 – 495.

Mugbabo, E., Afoakwa, E. O., Annor, G. and Rwubatse, B. 2017. Effect of pretreatments and processing conditions on anti-nutritional factors in climbing bean flour. *International Journal of Food Studies* 6: 34 – 43.

Muhammad, N. O. and Oloyede, O. B. 2009. Haematological parameter of broiler chicks fed *Aspergillus niger*-fermented *Terminalia catappa* seed meal-based diet. *Global Journal of Biotechnology and Biochemistry* 4(2): 179 – 183.

Musa, S. O., and Omoregie, E. 1999. Haematological changes in the mudfish, *Clarias gariepinus* (Burchell) exposed to Malachite Green. *Journal of Aquatic Sci*ence 14: 37-42.

Natale, F., Hofherr, J., Fiore, G., and Virtanen, J. 2013. Interactions between Aquaculture and Fisheries. *Marine Policy* 38, 205 – 213.

National Research Council (NRC) 1981. *Nutritional Energetics of Domestic Animals. Glossary of Energy Terms*, (2nd Ed). National Academy Press, Washington, DC. 114pp.

National Research Council (NRC). 1989. *Recommended dietary allowances*. (10th Ed). National Academic Press, Washington DC. 205pp.

Naylor, R., Hardy, R., Bureau, D., Chiu, A., Elliott, M., F. 2009 Feeding aquaculture in an era of finite resources. *Journal of National Academy of Science* 106: 15103–10.

Nestares, T., Barrionuevo, M., Urbano, G. and Lopez-Frias, M. 1999. Effect of processing methods on the calcium, phosphorus and pytic acid contents and nutritive utilisation of Chickpea (*Cicer arietinum* L.). *Journal of Agriculture and Food Chemistry* 47: 2807 – 2812.

New, M. B., and Wijkstrom, U. N. 2002. *Use of Fishmeal and Fish Oil in Aquafeeds: Further Thought on the Fishmeal Trap*. FAO Fisheries Circular No. 975. FAO, Rome, P. 61.

Ng, W. K. and Chen, M. L. 2002. Replacement of soybean meal with palm kernel meal in practical diets of hybrid Asian-African catfish, *Clarias macrocephalus X Clarias gariepinus*. *Journal of Applied Aquaculture*, 12: 67 – 76

NRC 1999. *Nutrient requirement of fish*. National Academy Press, Washington, D. C. Pp 216

NRC 2011. *Nutrient requirements of fish and shrimps*. The National Academy Press, Washington, D. C. Pp 306.

Nwanna, L. C. 2003. Nutritional Value and Digestibility of Fermented Shrimp Head Waste Meal by African Catfish *Clarias gariepinus*. *Pakistan Journal of Nutrition* 2. (6): 339-345.

Nwosu, J. N. 2011. The effect of processing on the anti-nutritional properties of 'Ose' (*Bosqueia angolensis*) seeds. *Journal of American Science.* 7(1): 1 – 6.

Nyam, K. L., Tan, C. P., Lai, O. M., Long, K. and Man Che, Y. B. 2009. Physicochemical properties and bioactive compounds of selected seed oils. *LWT-Food Science and Technology* 42: 1396 – 1403.

Nzewi, D. C. and Egbuonu, A. C. C. 2011. Effect of boiling and roasting on some anti-nutrient factors of asparagus bean (*Vigna sesquipedalis*). *African Journal of Food Science and Technology*. 2 (3) : 075 – 078.

Obasa, S. O., Dada, A. A. and Alegbeleye, W. O. 2006. Evaluation of pigeon pea (*Cajanus cajan*) as a substitute for soybean meal in the diet of Nile tilapia (*Oreochromis niloticus*) fingerlings. *African Journal of Aquaculture*, 23: 51 – 59.

Obasi, N. E. and Wogu, C.O. 2008. Effect of soaking time on proximate, mineral composition and anti-nutritional factors of yellow maize (*Zea mays*). *Nigerian. Food. Journal*, 26 (2): 69-77.

Obun, C. O. 2013. Impact of raw Tallow *Detarium microcarpum* (Guill and Sperr) seed meal on performance and blood parameters in broilers. *Iranian Journal of Applied Animal Science* 3 (2): 289 – 294.

Ochefu, J., Bitto, I. I. and Carew, S. N. 2011. Effect of raw kapok (*Ceiba pentandra*) seed meal on weight gain and carcass yield of rabbit. *Proceedings of the 16th Annual Conference of the Animal Science Association of Nigeria*. September 12 – 15th, 2011. Katsina State University. Ayigba, Nigeria. Pg 391 – 394.

Odetola, O. M. 2013. Protein quality evaluation and utilisation of full fat kenaf (*Hibiscus cannabinus*) seed meal by meat and egg type chickens. Ph.D. Thesis submitted to the Federal University of Agriculture Abeokuta, Abeokuta, Ogun-state, Nigeria. 201pp.

Odetola, O. M. and Eruvbetine, D. 2012. Nutritional evaluation of whole kenaf (*Hibiscus cannabinus* L.) seed meal in Rats. *Journal of Advanced Laboratory Research*. 3 (3) 215 – 220.

Ogunlade, I., Olaofe, O.and Fadare I. 2005. Chemical composition,amino acids and nutritional properties of selected seafoods. Journal of *Food, Agriculture and Environment*, 3: 130-133.

Okomoda, V. T., Tiamiyu, L. O. and Uma, S. G. 2016. Effect of hydrothermal processing on nutritional value of *canavalia ensiformis* and its utilisation by *Clarias gariepinus* (Burchell, 1822) fingerlings. *Aquaculture Report* 3: 214 – 219.

Oladimeji, Y. U. 2017. Trend in fish production parameters in Nigeria and its total estimated demand: Empirical evidence from fish production. *Journal of Animal Production Research.* 29 (1): 410 – 418.

Oladimeji, Y. U., Abdulsalam, Z., and Damisa, M. A. 2013. Socio-Economic Characteristics and Returns to Rural Artisanal Fishery Households in Asa and Patigi LGAs of Kwara State, Nigeria. *International Journal of Science and Nature*. 4 (3): 445-455.

Olaifa, F. E. and Bello, O. S. 2010. Effect of differently processed African yam beans (*Sphenostylis stenocarpa* Harms) on performance of African catfish (*Clarias gariepinus*) juvenile. *Israeli Journal of Aquaculture Bamidgeh.* Pp 7.

Olawepo, K. D., Banjo, O. T., Fawole, W. O., Orisasona, O. and Ojo-Daniel, A. H. 2014. Effect of roasting and cooking on nutritional and anit-nutritional factors in Kenaf (*Hibiscus cannabinus*) seed meal. *Journal of Food Science and Quality Management* . 24:1-5.

Oliva-Teles, A., Pereira, J. P., Gouveria, A., Gomes, E. 1998. Utilisation of diets supplemented with microbial phytase by sea bass (*Dicentrachus labrax*) juveniles. *Aquatic Living Resources* 11: 255–259.

Olude, O. O., Alegbeleye, W. O. A. and Obasa, S. O. 2008. The use of soaked copra meal as a partial substitute for soybean meal in the diet of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Livestock Research for Rural Development* 20 (10) 169 – 170.

Olude, O., George, F. and Alegbeleye, W. 2016. Utilisation of autoclaved and fermented sesame (*Sesamum indicum* L.) seed meal in diets for Til-aqua natural male tilapia. *Animal Nutrition* 2: 339-344.

Omitoyin, B. O. (2006): Haematological changes in the blood of *Clarias gariepinus* (Burchell 1822) juveniles fed poultry litter. *Livestock Research for Rural Development*. Vol (18): 162. 12pp

Onuoha, P. C. and Elezuo, K. O. 2013. *Basic Principles of Pond Management*. Alego Aries Printer and Publisher, Lagos. pp.83

Orire, A. M. and Ozoadibe, T. N. 2015. Evaluation of growth performance and body composition of *Clarias gariepinus* fed graded level inclusion of soybean waste. *World Journal of Fisheries and Aquaculture Sciences Research*. 1 (1), Pp. 1 – 9.

Osama, M. A. 2007. Effect of different processing methods on nutritional composition, anti-nutritional factors, and *In vitro* protein digestibility of Dolichos Lablab bean (*Lablab purpuresus* L Sweet). *Pakistan Journal of Nutrition* 6(4), 299 – 303.

Osuigwe, D. I., Obiekezie, A. I. and Onuoha, G. D. 2005. Some haematological changes in hybrid catfish (*Heterobranchus longifilis x Clarias gariepinus)* fed different dietary levels of rae and boiled jackbean (*Canavalia ensiformis*). *African Journal of Biotechnology*. 4 (9), 1017 – 1021.

Patane', C. and Sortino, O. 2010. Seed yield in Kenaf (*Hibiscus cannabinus* L.) as affected by sowing time in South Italy. *Industrial Crop Production* 32, 381 – 388.

Phengnuam, T. and Suntornsuk, W. 2013. Detoxification and anti-nutrients reduction of *Jatropha curcasseed* cake by Bacillus fermentation. *Journal of Bioscience and Bioengineering*. 115(2), 168 – 175.

Potter, S. M., Jimenez-Flores, R., Pollack, J., Lone, T. A., Berber-jimenez, M. D., 1993. Protein saponin interaction and its influence on blood lipids. *Journal of Agriculture and Food Chemistry* 41, 1287–1291.

Rahaman, M. M., Lee, K. J. and Lee, S. M. 2016. Effect of dietary carbohydrate sources on apparent nutrient digestibility of olive flounder (*Paralichthys olivaceus*) feed. *Fisheries and Aquatic Science,* 19: 1 – 5.

Rajashekher, A. R., Rao, P. V and Reddy, V. R. 1993. Chemical composition and nutritive value of Ambedi (*Hibiscus canabinus*) meant for layers. *Animal Feed Science and Technology* 44: 151 – 166.

Ramadan, E. A. 2012. Effect of processing and cooking methods on the chemical composition, sugars and phytic acids of soybeans. *Food and Public Health* 2(1): 11 – 15.

Ravindran, V., Cabahug, S. and Ravindran, G. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of food stuff for broiler. *Poultry Science* 78: 699 – 706.

Reddy, N. R. and Sathe, S. K. 2002. *Food phytates*. CRC Press. Washington. pp 251.

Reinhold, J. G. 1953. *Standard methods of clinical chemistry*. Academic Press, New York.

Richard, D. M. and Chaoman, F. A. 2007. The concept of ideal protein in formulation of Aquaculture feeds. *NAGA World Fish Center Quarterly Report* 26 (3), Pg 3.

Robbinson, E. H. and Menghe, H. L. 2007. Catfish protein nutrition (Revised). *Bulletin 1153*. *Office of Agricultural Communications*, Mississippi State University, U.S.A. pp.18.

Roberts, S. D. and Powell, M. D. 2003. Reduced total hardness of freshwater enhances the efficacy of bathing as a treatment for amoebic gill disease in Atlantic salmon (*Salmo salar* L.). *Journal of Fish Diseases* 26, 591-599.

Rodriguez-Roque, M. J., De Ancos, B., Sanchez-Moreno, C., Cano, M. P., Elez-Martinez, P., & Martin-Belloso, O. 2015. Impact of food matrix and processing on the in vitro bio-accessibility of vitamin C, phenolic compounds, and hydrophilic antioxidant activity from fruit juice-based beverages. *Journal of Functional Foods*, 14, 33–43.

Ruiz, R. G., Price, K. R., Arthur, A. E., Rose, M. E., Rhodes, M. J. C., and Fenwick, R. G. 1996. Effect of soaking and cooking on the saponin content and composition of chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*). *Journal of Agricultural and Food Chemistry*, 44, 1526–1530.

Russell, N. J., Powell, G. M., Jones, J. G., Winterburn, P. J. and Basford, J. M. 1982. *Blood biochemistry*. Croom Helm. London & Canberra. Pg 184.

Rust, M. B. 2002. Nutritional physiology. *Fish Nutrition*. J. E. Halver and R. W. Hardy (Eds), Academic press. U.S.A. pp388.

Sabapathy, S and Teo, L. H. 1993. A quantitative study of some digestive enzymes in the rabbitfish *Siganus canaliculatus* and the sea bass, *Lates calcarifer*. *Journal of Fish Biology*. 42 (4): 595 – 602.

Sangroni, E. and Machado, C. J. 2007. Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. *LWT* 40: 116 – 120.

Santos-Buelga, C. and de-Freitas, V. 2009. Influence of phenolics on wine organoleptic properties. *Wine chemistry and biochemistry*. M.V. Moreno-Arribas and M. C. Polo (ed). Springer, New York, 529 – 570.

Seegers, L. 2008. *The catfishes of Africa, a handbook for identification and maintenance*. GmbH publisher, Rodgau, Germany, 604 pp

Seena, S., Sridhar, K. R., Arunb, A. B. and Young, C. C. 2006. Effect of roasting and pressure-cooking on nutritional and protein quality of seeds of mangrove legume *Canavalia cathartica* from southwest coast of India. *Journal of Food Composition Analysis*. 19, 284-292.

Sgwane, S. T., Teleni, E. and Gardiner, C. P. 2005. Kenaf seed (*Hibiscus cannabinus* L.) as a protein supplement to sheep. *Proceedingd of the Australian Rangeland Society*. Pg. 1 – 4.

Shaahu, S. N., Carew, S. N. and Ikurior, S. A. 2015. Effect of processing on proximate, energy, anti-nutritional factor, amino acid and mineral composition of Lablab seed. *International Journal of Science and Technology Research*. 4, 1 – 7.

Shah, H. U., Khan, U. L., Alam, S., Shad, A. A., Iqbal, Z. and Parveen, S. 2011. Effect of home cooking on the retention of various nutrients in commonly consumed pulses in Pakistan. *Sarhad Journal of Agriculture*, 27 (2), 279 – 284.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G. and Jiang, Y. M. 2004 Saponins from edible legumes: chemistry, processing, and health benefits. *Journal of Medicine and Food,* 7, 67–78.

Shi, J., Xue, S. J., Ma, Y., Li, D., Kakuda, Y., and Lan, Y. 2009. Kinetic study of saponins B stability in navy beans under different processing conditions. *Journal of Food Engineering,* 93, 59–65.

Shimoyamada, M., Ikedo, S., Ootsubo, R., Watanabe, K., 1998. Effects of soybean saponins on chymotryptic hydrolyses of soybean proteins. *Journal of Agriculture, Food and Chemistry* 46 12 , 4793–4797.

Siddhuraju, P. and Becker, K. (2001). Preliminary nutritional evaluation of mucunna seed meal (*Mucunna pruriens* Var. *Utilis*) in common carp (*Cyprinus carpio* L.): an assessment by growth performance and feed utilisation. *Aquaculture,* Vol 193 (1-2):105 – 123.

Siddhuraju, P. and Becker, K. 2001. Effect of various domestic processing methods on antinutrients and in vitro-protein and starh digestibility of two indigenous varieties of Indian pulses, (*Mucuna pruries*) var *utilis*. *Journal of Agricultural and Food Chemistry* 49(6): 3058–3067.

Siddiqui, M. I., Khan, M. A. and Siddiqui, M. I. 2014. Effect of soybean diet: Growth and conversion efficiencies of fingerling of stingin catfish, *Heteropneustes fossilis*. *Jornal of Kings Saudi University-Science*. 26 (2): 83 – 87.

Sievwright, C. A. and Shipe, W. F. 1986. Effect of storage condition and chemical treatments of firmness, *in vitro* protein digestibility, condensed tannin, phytic acid and divalent cations of the cooked black beans (*Phaselus vuglaris*). *Journal of Food Science.*, 51: 982 – 987.

Sinha, A. K., Kumar, V., Makkar, H. P. S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition - A. Review. *Food Chemistry* 127, 1409–1426.

Sire, M. F. and Vernier, J. M. 1992. Intestinal absorption of protein in teleost fish. *Comparative Biochemistry and. Physiology.* 103: 771 – 781.

Smith, M. V. M., Twaalfhoven, L. and Hitehocock, C. 1980. The determination of trypsin. Inhibitor levels in foodstuffs. *Journal 0f Science, Food and Agriculture* 3:341-350

Soetan, K. O. and Oyewole, O. E. 2009. The need for adequate processing to reduce the anti-utritional factors in plants used as human foods and animal feeds: A review. *African Journal of Food Science*. 3(9): 223 – 232.

Solomon, S. G., Okomoda, V. T. and Oda, S. O. 2017. Nutritional value of toasted pigeon pea, *Cajanus caja* seed and its utilisation in the diet of *Clarias gariepinus* (Burchell, 1822) fingerlings. *Aquaculture Reports* 7:34 – 39.

Sotolu, A. O. 2008. Growth performance and nutrient utilisation of *Clarias gariepinus* (Burchell, 1822) fed *Leucaena leucocephala* (Lam. de Wit) seed meal-based diets. Ph.D Thesis submitted to the University of Ibadan, Ibadan. Nigeria. 154 pp

Sotolu, A. O. 2010. Growth performance of *Clarias gariepinus* (Burchell, 1822) fed varying inclusion of *Leucaena leucocephala* seed meal. *Tropicultura*. 28 (3): 168 – 172.

Sotolu, A. O. and Sule, S. O. 2011. Digestibility and performance of Water Hyacinth meal in the Diets of African catfish (*Clarias gariepinus*; Burchell, 1822).*Tropical and Subtropical Agro-ecosystems*, 14: 245-250

Sotolu, A. O. and Faturoti, E. O. 2008. Digestibility and nutritional values of differently processed *Leucaena leucocephala* (Lam. De Wit) seed meal in the diet of African Catfish (*Clarias gariepinus*). *Middle-East Journal of Scientific Research* 3 (4): 190 – 199.

Sotolu, A. O. and Faturoti, E. O. 2009. Growth performance and haematology of *Clarias gariepinus* (Burchell, 1822) fed varying inclusions of *Leucaena leucocephala* seed meal based-diets. *Revista UDO Agricola* 9 (4), 979-985.

Sotolu, A. O. and Sule, S. O. 2008. Digestibility and performance of Water Hyacinth meal in the Diets of African catfish (*Clarias gariepinus*; Burchell, 1822). *Tropical and Subtropical Agro-ecosystems*, 14, 245-250.

Speare, D. J., Arsenault, G., MacNair, N and Powell, M. D. 1997. Branchial lesion associated with intermittent formalin bath treatment of Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Disease*, 20: 27 – 33

Spinelli, J., Houle, C. R., Wekell, J. C., 1983. The effect of phytates on the growth of rainbow trout (*Salmo gairdneri*) fed purified diets containing varying quantities of calcium and magnesium. *Aquaculture* 30, 71–83.

Srivastava, R. P., & Vasishtha, H. 2013. Soaking and cooking effect on sapogenols of chickpeas. *Current Advances in Agricultural Sciences,* 5(1), 141–143.

Stern, J. L., Hagerman, A. E., Steinberg, P. D. and Mason, F. K. 1996. Phlorotannin-protein interactions. *Journal of Chemistry and Ecology* 22: 1877 – 1899.

Stevens, C. E. and Hume, I. D. 1995.Comparative physiology of the vertebrate digestive system, (2nd ed.). Cambridge University Press. Cambridge. 400pp.

Stevens, W. C., Fairfield, N., Meriden, C. D. and Bethel, B. C. P. 2002. *High temperature pressure swing adsorption system for separation of oxygen-containing gas mixture*. United State Patent, Advance Technology Material Inc. Danbury, USA. Pg 1 – 19.

Storebakken, T., Refstie, S., Ruyter, B., 2000. Soy products as fat and protein sources in fish diets for intensive aquaculture. *Soy in Animal Nutrition* J. K. Drackey, (Ed.),. Federation of Animal Science Societies , Savoy, IL, pp. 127–170.

Suriyajantratong, W., Tucker, R. E., Sigafus, R. E and Mitchell, Jr. G. E. 1973. Kenaf and rice straw for sheep. *Journal of Animal Science.* 37, 1251–1254.

Svobodova D. Ravds, J. and Palackova, J. 1991. Unified method of Haematological examination of fish. *Research Institute of Fish Culture and Hydrobiology*, Vodnany, (2nd Ed), 22, 31pp

Swingle, R. S., Urias, A. R., Doyle, J. C and Voigt, R. L 1978. Chemical composition of kenaf forage and its digestibility by lambs and in vitro. *Journal of Animal Sciencei.* 46, 1346–1350.

Tacon, A., G. J. and Metian, M. 2017. Food Matters: Fish Income, and Food Supply – A Comparative Analysis. *Reviews in Fisheries Science and Aquaculture*. Pg 1 – 14.

Teugel, G. G. 1982. Preliminary results of a morphological study of five Afrcan species of the subgenus *Clarias* (Clarias) (Pisces, Clariidae). *Journal of Natural History* 16: 439 – 464.

Theml, H., Diem, H. and Haferlach, T. 2014. *Colour Atlas of Hematology*. Thieme Stuttgart. New York. 198pg

Thrall, M. A., Weiser, G., Allison, R. W and Campbell, T. W. 2012. *Veterinary hematology and clinical chemistry*. John Wiley & Sons Inc, USA. Pp. 776

Tiamiyu, L. O., Okomoda, V. T. and Iber, B. 2013. Growth response of *Clarias gariepinus* fingerlings fed diet substituted groundnut cake meal and cotton seed meal. Livestock Research for Rural Development. *Brazilian Journal of Aquat Science and Technology*. 20 (2): 12 – 17.

Tide (2016). Nigeria spends N4bn on jute bag importation. Retrieved online at www.thetidenewsonline.com on the 20th of January, 2018, 8:55 am.

Trump, D. L., Allen, H., Olson, J., Wright, J., Humphrey, R. L. 1980. Epidermolysis of *Bullosa aequisita* association with amyloidosis and multiple myeloma. *Journal of American Medical Association* 243, 1461-1462.

Tuegels G. G. 1986. A systematic revision of the African species of the genus *Clarias* (Pisces: Claridae). *Annales du Musée Royal de l'Afrique Centrale* 247, 1-199.

Udensi, E. A., Onwuka, G. I., and Okoli, E. G. (2004). Effect of processing on the levels of some antinutritional factors in *Mucuna utilis*, *Journal of Plant Production resources* 8 (1), 16-21.

Ufodike, E. B. C., and Matty, A. J. 1983. Growth responses and nutrient digestibility in mirror carps (*Cyprinus carpio*) fed different levels of cassava and rice. *Aquaculture* (31), 41-50.

Uko, O. J., Kamalu, T. N., Pindiga, U. H. and Rabo, J. S. 2006. Study on toxicity to serum of pigs given different diet with detoxified castor bean meal. *Nutrition Abstract Review* 52, 21-27.

United Nations (UN). 2010. *World population prospects: The 2010 revision*. Department of Economic and social affairs. Population Division. New York. Pg 30.

Uys, W. 1989. Aspects of the nutritional physiology and dietary requirements of juvenile and adult sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae). Ph.D. Thesis, Rhodes University, South Africa. 190 pp.

Van Dyk., J. C., Marchand, M. J., Smit, N. J. and Pieterse, G. M. 2009. A histological-based fish health assessment of four commercially and ecologically important species from the Okavango Delta panhandle, Botswana. *African Journal of Aquatic Science*, 34 (3): 273 – 282.

Van Eys, J. E., Offner, A., and Bach, A. 2004. Chemical Analysis. Manualof Quality Analysis for Soybean Products in the Feed Industry. American Soybean Association. Accessed online at http//www.asa-europe. org/Library/ library_e.htm. on the 2nd of June, 2018.

Vijayakumari, K., Siddhuraju, P., & Janardhanan, K. 1995. Effects of various water or hydrothermal treatments on certain antinutritional compounds in the seeds of the tribal pulse, *Dolichos lablab* var.vulgaris L. *Plant Foods for Human Nutrition*, 48, 17–29.

Wang, T., He, F., and Chen, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. *Journal of Functional Foods*, 7 (1), 101–111.

Weaver, C. M. and Kannan, S. 2002. Phytate and mineral bioavailability. *Food phytates* N. R. Reddy and S. K. Sathe (ed). CRC Press. Washington 215 – 227.

Webber C. L III. 1993. Yield components of five kenaf cultivars. *Journal of Agronomy* 85, 533–535

Webber, C. L. III and Bledsoe, V. K. 2002. Kenaf yield and plant composition. *Trend in new crops and new uses.* J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria. P. 348 – 357.

Webber, C.L. III. 1996. Response of kenaf to nitrogen fertilization. *Progress in new crops* J. Janick and J.E. Simon (eds.). Wiley, New York. p. 404–408.

Webber, C.L. III., Bhardwaj, H. L., and Bledsoe, V. K. 2002. Kenaf production: Fiber, feed, and seed. *Trends in new crops and new uses*. J. Janick and A. Whipkey (eds.). ASHS Press, Alexandria, VA. p. 327–339.

Wenk, C. 2001. The role of dietary fiber in the digestive physiology of pig. *Animal Feed Science and Technology*, 90 (1), 21 – 33.

Whalan, J. E. 2015. *A toxicologist's guide to clinical pathology in animal*. Springer, New York. pp. 165.

White, C. E., Campbell, D. R. and Combs, G. E. 1999. Effect of moisture and processing temperature on activities of trypsin inhibitor and urease in soybeans fed to swine. *Recent Advances in Antinutritional factors in legume seeds.* T. F. B., Huisman, P. van der, I. E. Liener (Ed). Purdu, Wageningen pp. 230-234.

Wilson, R. P. 2012. Amino acids and proteins. *Fish Nutrition*. J. E. Halver and R. W. Hardy. (Eds). Academic press. United States of America. Pg 143 – 179.

Wilson, R. P. and Poe, W. E. 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus*. *Comparative Biochemistry and Physiology* 80, 385–388.

Xavier, T. O., Michelato, M. and Vidal, L. V. O. 2014. Apparent protein and energy digestibility and amino acid availability of commercial meat and bone meal for Nile Tilapia, *Oreochromis niloticus*. *Journal of the World Aquaculture Society*, 45 (4): 439 – 446.

Xie. P. 2001. Gut content of bighead carp (*Aristichthys nobilis*) and the processing of digestion of algae cells in the alimentary canal. *Aquaculture* 195: 145 – 156.

Yagoub, A. A. and Abdall, A. A. 2007. Effect of domestic processing methods on chemical composition, *In vitro* Digestibility of protein and starch and functional properties of Bambara groundnut (*Voandzeia subterranea*). *Research Journal of Agriculture and Biological Sciences*, 3 (1): 24 – 34.

Yeheyis, L., Kijora, C., Wink, M. and Peters, K. J. 2014. Effect of a traditional processing method on the chemical composition of local white lupin (*Lupinus albus* L) seed in North-Western Ethiopia. *Journal of Biosciences*, 66 (7-8), 403 – 408.

Young, S. M and Greaves, J. E. 1940. Influence of variety and treatment on phytin content of wheat. *Journal of Food Research*, 5, 103-108.

Yuan, Y. C., Song, S. Y., Luo, Z., Yang, H. J., Zhang, G. B. and Chu, Z. J. 2010. Effect of dietary protein to energy ratio on growth and body composition of juvenile chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture nutrition*, 16 (2), 205 – 212.

Zemke-White, W. L., Choat, J. H. and Clement, K. D. 2000. A re-evaluation of the diet feeding hypothesis for marine herbivorous fishes. *Journal of Marine. Biology* 141: 571 – 579.

Zemke-White, W. L., Clement, K. D. and Harris, P. J. 1999. Acid lysis of macroalgae by marine herbivorous fishes: myth or digestive mechanism? *Journal of Experimental Marine Biology and Ecology* 233, 95 – 113.