CONVENTIONAL FEEDSTUFFS OF ANIMAL ORIGIN

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MTECH/SAAT/2019/9454

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ABSTRACT

The study was carried out to investigate some chemical and mineral composition of non-conventional feedstuffs of animal origin. One kilogram each of eight (8) feedstuffs namely; crayfish, dry edible hides (*ponmo* or *ganda*), grasshopper meal, poultry viscera, blood meal, bone meal and hydrolysed feather meal were used. They were labelled as treatments; T1, T2, T3, T4, T5, T6 and T7, respectively while fish meal (T8) served as control. The feedstuffs were grinded into powdered form and their proximate compositions, some chemical composition of their oil extract and mineral composition were analysed. The result showed that there was significant ($p<0.05$) difference in the proximate composition of all the treatments. The percentage moisture, crude protein, lipid, crude fibre, nitrogen fixed extract and ash content ranged from 1.2-10.09%, 4.19-84.21%, 0.04-17.46%, 0- 15.81%, 0.57- 7.51% and 1.75- 91.22% respectively. However, T7(hydrolysed feather meal) recorded highest crude protein value (84.21%). There was significant ($p<0.05$) difference in the chemical composition of their oil extract of all the treatments. However, T7(hydrolysed feather meal) and T4(poultry viscera) recorded 8.19 and16.39mgKOH/g in the pH and free fatty acid value respectively. T8(fish meal) recorded highest value in all other parameters (acid value: 12.25mgKOH/g, peroxide value: 20.78meq/kg, iodine value: 140.52g/100g and saponification value: 195.77mgKOH/g). There was significant ($p<0.05$) difference in the mineral composition of all the treatments. T6(bone meal): 222374.9mg/100g and T7 (hydrolysed feather meal): 1988.6mg/100g had higher calcium content compare to T8 (control); 1250.3mg/100g. Also, T5 (blood meal): 266.35mg/100g and T6(bone meal)164.28mg/100g have higher sodium content compare to fish meal: 120.60mg/100g. However, fish meal had higher values (potassium:1355.73mg/100g, magnesium:1350.49mg/100g and phosphorus: 1550.58mg/100g) in other mineral elements. The current results showed that some non-conventional feedstuffs such as blood meal, hydrolysed feather meal, dry edible hides and crayfish have the potentials to be used in fish feed considering their high crude protein level compared to fish meal. Also, bone meal and hydrolysed feather meal could be used as source of calcium in aquafeed. However, all the treatments were not comparable to fish meal in their lipid content. The use of these feedstuffs in fish feed will serve as alternative for fish meal. Further studies need to be carried out on the amino and fatty acid profile of these feedstuffs and on the appropriate quantity to be utilized in feed and their effects on growth and nutrient utilization of various fish species.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Aquaculture is the farming of aquatic animals, including finfish, crustaceans, molluscs, etc. and aquatic plants, mostly algae, using or within freshwater, sea water, brackish water and inland saline water. World aquaculture production attained another all-time (Tacon *et al*., 2008).

Increasing demand and impermanent supply of fish meal (FM) have resulted in tremendous pressure on wild fishery resources, which endanger the marine ecosystem and global fish stock (Lalander *et al.,* 2015). Many studies have shown that plant proteincould be an alternative for fish meal substitution in aquafeeds. However, some nutritional disadvantages of major plant proteins are often associated with nutrient deficiency, weak nutrient absorption, and most importantly, its usage as a primary food source for mankind cannot be overlooked (Gai *et al.*, 2012). Since the major group of commercial fish species in aquaculture are either carnivorous or omnivorous, therefore, it is crucial to seek for a sustainable and eco-friendly protein source with great availability, and efficient in terms of cost and energy conversion potential such as insect protein (Henry 2015; Xiao *et al*., 2018).

Non-Conventional Feed Ingredients are locally available feedstuff that are not standardized and the usage is not widely-spread. In most cases, they are not consumed by man. The use of these feedstuffs is very common in the rural areas of Sub-Saharan Africa among those with low income and are actively engaged in fish farming (Gabriel *et al*., 2007). Non-conventional dietary animal protein sources have been experimented as

substitutes for FM in diets for many fish species (Goda *et al*., 2007; Chor *et al*., 2013; Taufek *et al*., 2016)

1.2 Statement of the Research Problem

Due to high protein requirements of fish, fish meal (FM) and soya bean meal (SBM) have become the primary protein sources. However, in the last decade, fish meal and soya bean meal have increased in price along with the other protein sources used in feed due to an increase in the global protein demand. This demand has increased because protein sources are commodities used in feeds for livestock, companion animals and farmed fish (Hardy, 2010), which are designated to meet the global demands of human population growth (Tilman *et al*., 2011).

1.3 Justification for the Study

Fish farming has grown by more than 200% in recent decades as a result of increase in demand (FAO, 2017). The aquafeed industry relies heavily on fish meal in meeting the protein requirements of farmed species. About 20.2 percent of the total global fish catch is currently used for the production of fish meal and fish oil and around 60.8 percent of total fish meal production is consumed by the aquafeed industry (FAO, 2012). Fish meal and fish oil production from marine capture fisheries decreased at annual average rates of 1.7 percent and 2.6 percent, respectively (FAO, 2012). The future growth of aquaculture may be constrained by the availability of fish meal. The limited availability of fish meal has led to increased efforts to identify fish meal replacements, and these efforts include analyses of all possible physiological or metabolic consequences (Kaushik and Seiliez, 2010). Plant meals have been the most common replacement for FM, but plant meals can result in health problems caused by protein and amino acid (AA) deficiencies that impair immune function and increase the susceptibility of animals to infectious diseases (Oliva-Teles, 2012). The contents of starch and anti-nutritional compounds in plant meals also

cause health problems in farmed fish (Francis *et al*., 2001). The different plant meals used in fish nutrition, such as SBM, copra meal, sunflower meal, cotton meal and pea meal, are not parts of natural fish diets, and therefore, their protein contents and AA profiles are not well balanced for fish.

1.4 Aim and Objectives

The research was aimed at investigating some chemical and mineral composition of non-conventional feedstuffs of animal origin namely; crayfish, skin hides (*ponmo or ganda*), hydrolysed feather meal, blood meal, bone meal, chicken *viscera* and grasshopper.

The specific objectives of the research are to;

i. determine the proximate composition of selected non-conventional feedstuff of animal origin namely; blood meal, bone meal, hydrolysed feather meal, skin hides (*ponmo or ganda*), crayfish, chicken viscera and grasshopper meal.
ii. evaluate some mineral composition of selected non-conventional feedstuff of animal origin namely; blood meal, bone meal, Hydrolysed feather meal, skin hides (*ponmo or ganda*), crayfish, chicken viscera and grasshopper meal.
iii. evaluate some chemical composition of the oil extract of selected non-conventional feedstuff of animal origin namely; blood meal, bone meal, hydrolysed feather meal, skin hides (*ponmo or ganda*), crayfish, chicken viscera and grasshopper meal.

1.5 Null Hypothesis (H_O):

i. There is no significant difference (P>0.05) in the proximate composition of selected non-conventional feedstuff of animal origin namely; blood meal, bone meal, hydrolysed feather meal, skin hides (*ponmo or ganda*), crayfish, chicken viscera and grasshopper meal.

ii. There is no significant difference (P>0.05) in the mineral composition of selected non-conventional feedstuff of animal origin namely; blood meal, bone meal, hydrolysed feather meal, skin hides (*ponmo or ganda*), crayfish, chicken viscera and grasshopper meal.

iii. There is no significant difference (P>0.05) in the chemical composition of the oil extract of selected non-conventional feedstuff of animal origin namely; blood meal, bone meal, hydrolysed feather meal, skin hides (*ponmo or ganda*), crayfish, chicken viscera and grasshopper meal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fish Nutrition in Aquaculture

The essential nutrients for fish are amino acids, fatty acids, vitamins, minerals and energy-yielding macro nutrients (protein, lipid and carbohydrate). Diets for fish must supply all essential nutrients and energy required to meet the physiological needs of growing animals (NRC, 2011).

Protein is required in the diet to obtain amino acids, which are utilized to synthesize new proteins or maintain existing proteins in tissues while excess protein is converted to energy (NRC, 2011). Also, lipids supply essential fatty acids and energy in the diet NRC (2011). The requirement of essential fatty acids can only be met by supplying Long Chain (LC) Polyunsaturated Fatty Acids (PUFA) in the diet, specifically α-Linolenic Acid (LNA, 18:3ω3) and Linoleic Acid (LA, 18:2ω6), with varying requirements for eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) depending on species (NRC, 2011).

Dietary carbohydrates can be a source of energy for fish; however, their ability to utilize dietary carbohydrate for energy varies depending on the species and their natural diet (NRC, 2011).

Therefore, depending on species, protein and lipid are the main source of energy for fish. Feeds in aquaculture are formulated with a balance of nutrients in order to meet specific nutrient requirements for different species, life stages and other purposes. The digestibility of nutrients in the feed can affect aquaculture production efficiency and

impact the environment. The bioavailability or digestibility of the diet is the proportion of nutrients in the feed that is digested and absorbed by the fish. Data on the digestibility and available digestible energy of feed ingredients in fish diets are essential or optimization of feed formulations (NRC, 2011).

2.2 Nutrient Requirement of Fish

The three major nutrients required by fish in aquafeeds are proteins, lipids and carbohydrates. Protein consists of large molecules called amino acids, which are the required metabolic compounds used as either a major energy source or for protein synthesis. Lipids are digested in the gut, releasing their fundamental fatty acids that are absorbed and then resynthesized back into lipid and circulated within the blood stream. When required by the organism the lipid is again catabolised into its constituent fatty acids, which can then be used for the synthesis of membranes or further broken down for energy purposes. Carbohydrates are complex molecules and the most common forms are either starch or cellulose. Starch is catabolised into glucose, which is then further burned to give energy purposes energy, whereas cellulose cannot be digested by fish (De Silva and Anderson, 1995).

2.2.1 Energy

Energy requirements for fish are dependent on a range of factors such as fish size, body activity, species, feeding preferences and environmental conditions. Fish have a low energy requirement compared with other terrestrial animals for the following reasons:

(i) fish do not need to maintain internal body temperature (poikilothermic),

(ii) fish live in water and have a swim bladder to adjust body buoyancy so that fish expend less energy to maintain body station in the water column (Goodsell *et al.,* 1996; Trotter *et al.,* 2001), and

(iii) fish have a lower energetic expenditure for the detoxification and removal of ammonia (the end product of protein catabolism) prior to excretion (Brett and Groves, 1979). In general, marine carnivorous fish species have the ability to utilize the energy from dietary protein and lipid more effectively than dietary carbohydrate (CHO) in comparison with omnivorous species (Shimeno *et al.*, 1996). The reduced ability to utilize CHO has been related to lower amylase activity, CHO metabolism and insulin response in some marine species (Vegara and Jauncey, 1993; van Barneveld *et al.*, 1997). However, the inclusion of increasing non-protein energy sources (CHO and lipid) into diets is desirable as they have the ability to spare dietary protein (Shiau and Lan, 1996) by reducing the catabolism of protein for energy which improves protein retention and ultimately growth (Lupatsch *et al.*, 2001; Halver and Hardy, 2002).

2.2.2 Protein

Protein constitutes 65–75% of the dry matter in fish tissues, therefore out of the three major nutrients it is the more essential nutrient in fish nutrition (Wilson, 2002). Most cultured fish species require 30–55% crude protein (CP) in the diet, which provides a suite of essential and unessential amino acids required for cell maintenance, growth, development and health of fish (Hepher, 1988; NRC, 2011). Fish meal (FM) was traditionally the main protein ingredient in fish feeds (NRC, 2011). This is due to its excellent palatability, balanced amino acid profile and good digestibility (Alexis and Nengas, 2001). Fish meal is usually sourced from small, bony fish species that are not generally used for human consumption, including herring, menhaden, capelin, anchovy, pilchard, sardines and mackerel (Halver and Hardy, 2002). Catches of these fish species specifically for reduction into fish meal (FM) and fish oil (FO) have been declining during

recent years, but global fish meal production has remained static since the late 1980s apart from El Nino years (FAO, 2010). One of the reasons is due to increased production of offal from the fish processing industry into FM (Jackson, 2010). The increase in global aquaculture production has escalated pressures on the demand for FM in aquaculture feeds within the past decade (De Silva and Anderson, 1995; Barlow, 2000; Watanabe, 2002). This demand has increased the price of FM to levels that are too expensive to keep using it as the main protein ingredient in aquaculture feeds as the cost of growing juveniles to market size for many commercial species represents approximately 50% of the operating costs (Kissil *et al.*, 1997). Currently, aquaculture uses around 60-70% of the world's annual FM supply, which is 5.7 MMT (FAO, 2010; Jackson, 2010).

2.2.3 Lipid

Lipids are a source of essential fatty acids, energy, eicosanoids, components of the cell membrane (phospholipids) and assist in the uptake of lipid soluble nutrients(Storebakken, 2002). Currently, aquaculture uses around 80–90% of the global FO supply,which is 1.3 MMT (FAO, 2010; Jackson, 2010). Changes in temperature can lead to manyphysiological changes in fish. Research has shown that fish can exploit the structural diversity of lipids within their membranes to adapt to changes in ambient temperature (Miller *et al.*, 2006). The omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFA),eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) function as structural and physiological components of the cell membrane in most fish tissues (Sargent *et al.*, 1993). At low temperatures the chemical structure of n-3 LC-PUFA permits a greater degree of unsaturation compared with saturated fatty acids (SFA) (Hazel, 1984). This unsaturation is necessary to maintain flexibility and permeability in the phospholipid bi-layer (Lovell, 1998). This means that cold and temperate water species require a certain level of n-3 LC-PUFA to maintain cell membrane fluidity and

digestion at low temperatures Not only is the tissue fatty acid composition highly influenced by water temperature, but it can also change the dietary fatty acid composition (Glencross, 2009), digestibility (Torstensen *et al.,* 2000), transport and uptake, elongation and desaturation processes (Bell *et al.,* 2001; 2002), and b-oxidation of fatty acids (Froyland *et al.,* 2000), which can affect the membrane and depositing lipid composition (Torstensen and Tocher, 2011). The ability of fish to digest and absorb lipids is based on the fatty acid composition and degree of unsaturation and chain length of the lipid ingredient. These factors are responsible in determining the lipid ingredients melting point, which is a good indicator of lipid digestibility (Turchini *et al.,* 2009). There is a wide variety of vegetable oil and animal fat ingredients produced globally that are now commonly used in aquaculture feeds (reviewed by Turchini *et al.,* 2009).

2.2.3.1 *Chemical characteristics of lipid*s

a) Saponification numbers of lipids

Saponification is the process of breaking down or degrading a neutral fat into glycerol and fatty acids by treating the fat with alkali. The saponification number (value) is defined as the milligrams of potassium hydroxide (KOH) required to saponify 1g of fat. It is an index of average molecular weight of the triacylglycerols in the sample. The molecular weight of the triacylglycerols may be divided by three (3) to give an approximate average molecular weight of the fatty acid present. Kirk and Sawyer, (1991) reported that high saponification values of fats and oils are due to the predominantly high proportion of shorter carbon chain lengths of the fatty acids. This assertion was confirmed by Nielson, 1994, that the smaller the saponification values the longer the average fatty acid chain. If the fatty acids present in the glycerides are low molecular weight (short-chain acids), there will be more glycerides molecules per gram of fat than if the acids are high in

molecular weight (long-chain acids). Thus, since each glyceride molecule requires three potassium hydroxide molecules for saponification, fats containing glycerides of low molecular weight correspondingly have higher saponification values (Aurand *et al.,* 1987). Saponification values have been reported to be inversely related to the average molecular weight of the fatty acids in the oil fractions (Abayeh *et al.,* 1998).

b) Iodine value

The iodine value is an identity characteristics nature of oil. The iodine value of an oil or fat is defined as the grams of iodine absorbed by 100g sample. The iodine value or iodine number is the generally accepted parameter expressing the degree of unsaturation, the number of carbon-carbon double bonds in fats or oils (Pomeranz and Meloan, 1987). This value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of oil to oxidation. As the iodine is a measure for unsaturation of the fatty acids in the fat, the number of double bonds of a pure substance is:

$$\text{Double bonds} = IV \times \frac{\text{molecular weight of substance}}{253.84 \times 100}$$

(Cocks and Rede Van, 1966).

The determination of the iodine value is also important in classifying oils and fats (Kapila *et al.,* 2005). Classification of oils and fats as drying, semi drying and non-drying is given as follows: Drying oils: IV 200-130, Semi drying: IV 130-100 and Non-drying: IV lower than 100 (Duel, 1951). The IV of free fatty acids is higher than that of glycerides. For each %FFA the IV increases by 0.00045×IV (Cocks and Rede Van, 1966). The higher the amount of unsaturation, the more iodine is absorbed: therefore, the higher the iodine value the greater the degree of unsaturation (Nelson, 1994).

c. Peroxide value (PV)

Peroxide value (PV) is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. PV is one of the most widely used tests for the measurement of oxidative rancidity and or deterioration of oils and fats. It has been showed that off-flavours, nutritional losses and other deteriorative changes in oil arise by reaction with atmospheric oxygen, i.e., oxidative rancidity, or by hydrolytic reactions catalysed by lipases from food or from microorganisms (Shahina *et al.*, 2004). The peroxide value (PV), anisidine value (AV) and free fatty acids (FFA) are good guides to the quality of oil. Good quality oil should have a PV less than 10 units before off-flavours are encountered (Rossell, 1994).

d. Acid value or free fatty acids (FFA):

Acid value of an oil or fat is defined as the number of mg potassium hydroxide required to neutralize the free acid in g of the sample. The result is often expressed as the percentage of free acidity. The acid value measures the extent to which the glycerides in the oil have been decomposed by lipase action. the decomposition is accelerated by heat and light.

As rancidity is usually accompanied by free fatty acid formation, the determination is often used as a general indication of the condition and edibility of oils.

The FFA figure is usually calculated as oleic acid (1 ml 0.1M Sodium hydroxide 0.0282g oleic acid), in which case the acid value 2 x FFA

e. Thiobarbituric acid number or value (Tba)

The detectable rise in red pigment produced when thiobarbituric acid (TBA) reacts with oxidized lipids as oxidative rancidity shoots up have been used in assessing fatty foods.

Sinnhuber *et al.* (1958) working with rancid Salmon oil, have shown that Malonadehyde (an end product of oxidative decomposition) is involved in the reaction. Indeed, what makes TBA test interesting is that it determines the deterioration in both extractable and non-extractable lipids and therefore has often been used in compound fatty foods especially flesh foods rather than pure oils and fats.

2.2.4 Minerals requirement

All aquatic animals require minerals for their vital physiological and biochemical functions and to maintain their normal life processes. Fish live in a wide range of salinity levels (0-35 0/00) in freshwater (FW), seawater (SW) and brackish water (BW) environments, and, unlike other vertebrates, absorb minerals from the diet as well as the surrounding water. Most of the essential minerals required for animals and other vertebrates (Suttle, 2010) have been detected in fish tissues. The essentiality of macro minerals (calcium, phosphorus, magnesium, sodium, potassium and chloride) and certain trace elements (cobalt, copper, iodine, iron, manganese selenium and zinc) have been confirmed in fish (NRC, 1993; 2011).

2.3 Non-Conventional Feedstuff

Unconventional dietary animal protein sources have been experimented as substitutes for FM in diets for many fish species (Goda *et al.*, 2007; Chor *et al.*, 2013; Taufek *et al.*, 2016). Non-Conventional Feed Ingredients are locally available feedstuffs that are not standardized and the usage is not widely-spread. In most cases, they are not consumed by man. The use of these feedstuffs is very common in the rural areas of Sub-Saharan Africa

among those with low income and is actively engaged in fish farming (Gabriel *et al.,* 2007). Non-conventional feedstuffs usually come from 3 sources:

2.3.1 Kitchen wastes**:**

These are remnants of household wastes are used to feed the fish especially in backyard fish farming. They are used in an indiscriminate manner without any standard and are mostly utilized at household level of aquaculture. Remnants of bread, cooked rice and yam are commonly used in fish culture (Gabriel *et al.,* 2007).

The advantage of kitchen wastes is that they are readily available while the disadvantage is that some nutrients may be absent in such feeds. Some common examples of kitchen wastes are yam peels, cassava peels, remnants of bread, cooked rice, cooked yam.

2.3.2 Animal sources:

These feedstuffs are of high-quality feed ingredients and could compare to some extent with the conventional types. The advantage of non-conventional feedstuff of animal origin is that they are cheaper by virtue of the fact that there is no competition for human consumption but the disadvantage with the feedstuff is their unavailability in large commercial quantities for the sustenance of aquaculture industry as they are available in small quantities in most parts of Africa. Their production is not consistent and sporadic in nature.

2.3.3 Plant sources:

Generally, they are known as non-conventional plant feedstuff (NCPF) and are abundant in almost every locality in Africa. The levels at which they are being included in aquafeed varies and largely depends on their availability, nutrient level, processing technique,

species of fish and the pattern of cultural farming that is prevalent in the locality (Gabriel *et al.,* 2007). The advantage is that some of these feedstuffs like maize bran is palatable and free from anti-nutritional factors but the disadvantage is that the energy contents tend to be high hence limits the use in fish feed. Some examples include maize bran, rice bran, palm kernel cake, jack bean, cotton seed meal, soybean meal, cajanus, ground nut cake, duckweed, brewers waste.

2.4 Feed Ingredients in Aquaculture

2.4.1 Animal sources

2.4.1.1 Fish meal as an ingredient in fish diet

Feed manufacturing industry heavily depends on the fish meal as a dietary protein ingredient. Fish meal is an ideal resource to meet the essential amino acid requirement of the fish. The fish meal also has high protein content and good availability of micronutrients. On the other hand, fish oil contains some highly unsaturated fatty acids. Reduced usage of other meat and bone meals due to the Bovine spongiform encephalopathy/mad cow disease is another impetus for fish meal usage in diets all over the world (Trushenski *et al.,* 2006). Fish meal contains unique nutrients such as Taurine and other components that are not identified yet (VKM, 2009). Full replacement of fish meal could deprive these nutrients and could have a negative effect on fish health and growth response (Hardy, 2010). Despite these available positives, fish meal and fish oil production are highly dependent upon the marine fishery productions and bycatch. Global fish meal production statistics revealed that production rates are declining an average of 1.7 per year (Tacon, 2011; SKRETTING, 2012). Extreme pressure upon the marine capture fishery productions and declining stocks also influenced the global fish meal and fish oil productions. On a global scale, a greater part of the fish meal is used in feed for

terrestrial animals such as swine and poultry (Trushenski *et al.,* 2006). Consequently, increasing terrestrial animal production is competing for fish meal as a high-quality feed ingredient. Increasing competition and limited availability of fish meal and fish oil have motivated the aquaculture industry to find out new alternative feed ingredients.

2.4.1.2 Insects as potential feed ingredient

Use of insect as fish feed ingredients is quite novel to the aquaculture sector. However, a wide range of insect species is currently used in aquaculture practices. This includes rat tail maggots (*Musca domestica*), black soldier flies (*Hermetia illucens*), silkworm pupae (*Bombyx mori),* Grasshoppers, termites and mealworms (*Tenebrio molitor*). Broader scope in availability (availability over seven taxa), rich in protein content and favourable lipid profiles make insects as ideal candidates for replacing fish meal. Replacement of fish meal with insect diet primarily depends upon the nutritional profile of insects. The protein contents in insects diets vary from 50-82% (dry matter basis) which is in the same range as fish meal (Henry *et al.,* 2015). Insect meal is rich with EAA (lysine and methionine) although some minor variations are visible depending upon the taxon. Insect meal also contains compounds such as taurine and hydroxyproline which are deficient in plant diets ((Henry *et al.,* 2015). Lipid composition. of the insects contain a higher amount of polyunsaturated fatty acids (PUFA) n-6 than fish meal (Alegbeleye *et al.,* 2012). Various studies have reported positive results when fish were fed with insect meal in the diet. Increased growth rate and higher protein efficiency ratio have been recorded in fish fed with *Z. variegatus* (Alegbeleye *et al.,* 2012). Increased antioxidant activity (Taufek *et al.,* 2016). and recovery from lesions by improving the haematological parameters (red blood cells, white blood cells) also recorded when fish were fed with insect diets (Johri *et al.,* 2010). However, results may vary with fish/insect species, inclusion rate, and processing methods. In production perspective, insect meal can readily be produced in

farm facilities. It requires no land/water environment for productions. Most of the insect larvae can grow on poultry and livestock waste. Insects growing on these substrates could convert farm waste into organic manure. Insects also have an ability to reduce the pathogenic load in farm waste. In an environmental perspective, low emission of carbon can be achieved by biodegradation activity by insects.

Challenges of insects as fish meal substitute are that the exoskeleton of the insects is made of polysaccharide chitin. Chitin in insects particularly remains indigestible in most of the fishes despite the availability of chitinase enzyme. Bioaccumulation of pesticides through insects in fish has been also reported (Ogunji *et al.*, 2007). In terms of fatty acids, low amount of PUFA in terrestrial insects reduces its suitability as marine fish feed. Mass production of insects for aquaculture practices is still in developing stage. Therefore, future studies should be more focused on technological improvements to enhance insect productions and understand the effect of insect meal on fish health.

2.4.1.3 Blood meal

Clean and fresh animal blood is used in the production of blood meal. Due to the strict regulations and safety concerns, only non-ruminant blood is currently used in aquaculture industry (especially in Europe) (NRC, 2011).

Crude protein level and crude fat level of blood meal usually reach approximately 85% and 0.5%-3% respectively (NRC, 2011). Blood meal has favourable content of lysine and histidine. Inclusion rates of blood meal vary according to the life stage of the species of concern. However, the inclusion of blood meal at the rate of 5-10% may gain optimal results (Hertramp and Piedad-Pascual, 2000).

Previous research has shown that blood meal can be incorporated up to a level of 6% to 10 % in diets of the grouper, *Epinephelus coioides* (Martins and Guzman, 1994),

tambaqui, Colossoma macropomum (Martínez-Llorens *et al.,* 2008), and gilthead sea bream, *Sparus aurata* (Ribeiro *et al.,* 2011), and 20% in diets of juvenile trout, *Oncorhynchus mykiss* (Luzier *et al.,* 1995). Moreover, Agbebi *et al.* (2009) reported that fish meal can be replaced completely (100%) by blood meal with no adverse effect on growth, survival and feed conversion in cat fish, *Clarias gariepinus,* juveniles. However, the level of fish meal replacement was species-specific and varied according to fish size and feeding habits (Barnes *et al.,* 2012; Dedeke *et al.,* 2013). The challenge of blood meal in aqua-diets is that it processing method has a significant effect on the digestibility. An Inverse relationship between heat application and lysine availability of blood meal was also reported (Batterham *et al.,* 1986). Increased heat application usually deteriorates haemoglobin and cause low palatability. Blood meal produced by spray drying has shown higher digestibility than other methods (Nogueira *et al.,* 2012).

2.4.1.4 Hydrolysed feather meal (HFM)

Hydrolysed feather meal (HFM) is a rich protein source - 74 to 91% crude protein (CP; Baker *et al.,* 1981; Bureau *et al.,* 1999; 2000; Grazziotin *et al.,* 2008; Klemesrud *et al.,* 2000), rich in cystine (4 -5% CP), but deficient in lysine (2%) and methionine (1% CP; Baker *et al.,* 1981; Klemesrud *et al.,* 2000). HF is a good source of proteases, polyunsaturated fatty acids and antioxidant peptides, and has a low content of ash and phosphorus, allowing low water pollution formulations (Lasekan *et al.,* 2013). When obtained from bacterial hydrolysis, HF also shows antihypertensive activities (Fontoura *et al.,* 2014). The nutrient availability of HF can vary according to the rendering process applied to the feathers. Previous studies reported that protein apparent digestibility coefficient (ADC) of HF is high in Australian silver perch (*Bidyanus bidyanus*, 93%), a freshwater species (Allan *et al.,* 2000), but reduced in salmonids such as Coho salmon (*Oncorhynchus kisutch*, 80%) and rainbow trout (77 – 86%; Bureau *et al.,* 1999; Cheng

et al., 2004; Sugiura *et al.,* 1998), and in marine species such as gilthead seabream (*Sparus aurata*, 22%), turbot (*Psetta maxima*, 47%), Atlantic cod (*Gadus morhua*, 62%),European seabass (*Dicentrarchus labrax*, 67%) and rockfish (*Sebastes schlegeli*, 79%; Davies *et al.,* 2009; Lee, 2002). The use of HF as fish meal replacement was shown to bepossible up to 25% in malabar grouper (*Epinephelus malabaricus*; Li *et al.,* 2009). Above these replacement rates for each species, reduced feed intake, weight gain and whole- body protein content were observed, which could be due to the amino acid imbalance andlow palatability and digestibility of HF, as reviewed by Yu (2008).

Higher inclusion rates of feather meal in fish diets are depending upon production technology (Bureau *et al.,* 1999; Shapawi *et al.,* 2007). However, supplementation of these essential amino acids along with feather meal could enhance the growth of fish. In contrast to that, the presence of antimicrobial residues in feather meal could accumulate through feeding practices (love *et al.,* 2012).

2.4.1.5 Chicken viscera

Poultry offal meal (POM) is an animal by-product, generally consisting of parts of the animal considered ill-favoured for human consumption. High in protein, with an essential amino acid profile that more closely resembles fish meal in comparison to terrestrial plant derived protein sources (Riche, 2015), POM is low in ash and contains little to no anti-nutritional factors found in plant-derived protein sources (e.g., proteolytic enzyme inhibitors) (Francis *et al.,* 2001). Due to its aforementioned advantages, global use of POM-based aquafeed has steadily increased (Badillo *et al.,* 2014; Riche, 2015). The suitability of POM inclusion has been evaluated for many commercially important aquaculture species, including *Oncorhynchus mykiss* (rainbow trout) (Badillo *et al.,*

2014), *Lates calcarifer* (barramundi) (Lewis *et al.,* 2019), and *Salmo salar* (Atlantic salmon) (Hatlen *et al.*, 2015).

On the other hand, in poultry processing, viscera are mostly considered as a waste product and disposal of these by-products is becoming a major problem for industries causing wastage of precious proteins source and environmental contamination (Patil and Nag, 2011). Poultry by products such as chicken viscera have significant potential as feed ingredients in fish feed (Alofa *et al.,* 2016; Oké *et al.,* 2016; Rossi and Davis, 2012). Chicken viscera has high protein content, balanced amino acids profile, total digestible dry matter and total energy (Giri *et al.,* 2010) It's less expensive animal protein source as compared to fish meal (Abdel-Warith *et al*., 2001).

Previous research has shown that blood meal can be incorporated up to a level of 6% to 10 % in diets of the grouper, *Epinephelus coioides* (Martins and Guzman, 1994), *tambaqui, Colossoma macropomum* (Martínez-Llorens *et al.,* 2008), and gilthead sea bream, *Sparus aurata* (Ribeiro *et al.,* 2011), and 20% in diets of juvenile trout, *Oncorhynchus mykiss* (Luzier *et al.,* 1995). Moreover0) reported that fish meal can be replaced completely (100%) by blood meal with no adverse effect on growth, survival and feed conversion in cat fish, *Clarias gariepinus,* juveniles. However, the level of fish meal replacement was species-specific and varied according to fish size and feeding habits (Barnes *et al.,* 2012; Dedeke *et al.,* 2013).

2.4.1.6 Grasshopper meal

Edible Grasshoppers and locusts which include *Nomadacris septemfasciata, Kraussaria sp., Katantop sp., Anacridium sp., Cataloipus sp., Hieroglyphycus sp., Gelestorhinus sp.,* and Locusta sp. are found to invade most of the North-eastern and Central States of Nigeria at a particular season of the year causing great consequences on crops Sharah

(Richardson *et al.,* 1985). These grasshoppers also serve as a delicacy to nation of North Eastern Nigeria during these invasions. These grasshoppers are as rich as the fish meal in terms of its amino acid profile. Olaleye (2015) reported that grasshopper have crude protein level of 64.51. Encouraged by the similarity in the quality of the amino acid profile of fish and grasshopper meal, this research decided to replace fish meal with grasshopper meal to ascertain if these qualities of the grasshopper can compare favourable in growth production of *Clarias gariepinus* as that obtained or fish meal in the same species (Olaleye, 2015).

2.4.2 Plant as an alternative feed ingredient

Incorporation of plant ingredients in the fish diet is widely used practice driven by higher abundance and lower price compared to the fish meal. A range of plant ingredients is used in aquaculture industry including grains (wheat and corn), oilseeds (soybean, sunflower, rapeseeds and cottonseed), and pulses (beans, lupins, and peas) this is according to Tharindu, (2018).

Challenges of plant ingredients in aquaculture are Imbalanced amino acid profile, lower protein content, and presence of anti-nutritional factors are several challenges associated with plant ingredients. “Anti-nutrients have been defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals” (Makkar, 1993).

All plants have phytochemicals for protection against predators. Therefore, use of plant ingredients in fish feed without proper treatments may cause significant challenges in fish health. Anti-nutritional factors have a different mode of actions but may have an adverse effect on feed intake and nutrient digestibility in fish species. An extensive review of major anti-nutrient factors has been presented by Francis *et al.,* 2001.

However, more recent studies have addressed the effect of plant oil on the gut morphology of fish (Moldal *et al.*, 2014, Sørensen *et al.*, 2011). Transcriptomic approach to assess the fish health after fed with plant ingredients has shown that full replacement of fish meal by plant diets usually alter the lipid metabolism, nitrogen metabolism and cause overexpression of hepatic genes (Panserat *et al.*, 2009). Imbalance in the nutrient composition is another drawback in plant ingredients. This limitation is appearing in amino acid profile and the fatty acid profile in plant ingredients. Amino acid profile of plant ingredients is not totally compensated EAA (Essential Amino Acid) requirement of fish (e.g., soybean; higher in lysine but deficient in methionine, cysteine; corn gluten: low in lysine). This leads to the combined use of one or more plant ingredients to correct balance of the AA profile of fish. One major example is a mixture of corn gluten (high in methionine but low in arginine and lysine) and soybean (high in arginine and lysine, low in methionine) meal to compensate the deficient AA requirement. However, these kinds of combinations could be challenged by the interaction of different anti-nutritional factors in plant ingredients. Fatty acids in plant ingredients are devoid of HUFA (Highly Unsaturated Fatty Acid), especially in DHA and EPA. Full replacement of fish oil with plant oil may cause a deficiency in essential fatty acid profile in fish. In contrast to that, plant oils are rich in medium chain tryglycerols (MCT) that increase the performance of fish (VKM, 2009). But higher inclusions usually increase the fish mortality (VKM, 2009). Ingredients processing techniques have a significant impact on the availability of EAA and other major nutrients in plant proteins. Most of the plant protein ingredients are by-products of residual from the industrial manufacturing process of vegetable oil and starch (Refstie and Storebakken, 2001).

Other challenges in the plant ingredients also include the presence of components such as mycotoxins and environmental pollutants. Mycotoxins are prevalent in plant diets

including grains (maize, cottonseed and other grains). Frequent monitoring programs, proper storage of ingredients and risk assessments are needed to avoid contaminations of plant ingredients with these external toxins. Although there are several undesirable characteristics, the value of plant ingredients in aquaculture practices is innumerable. Major obstacles in plant ingredients can be achieved by application of the various level of technology. For an example, higher protein content in soy protein concentrates (e.g., soybean, corn gluten meal) is achieved by removing the carbohydrate fraction. Treatment of both heat labile and heat stable anti-nutritional compounds of plant ingredients can be achieved by extrusion, heat treatment and fractionating of the crops respectively (Drew *et al.,* 2007).

Plant oils are widely used in aquaculture since last decade due to its lower cost and higher availability. Soybean, palm, rapeseed and sunflower oil are the major ingredients that use for production of plant oils (Sorensen *et al.,* 2011).

Wide-scale cultivation of these crops can be done in semi-arid/ arid regions with minimum water requirement. In addition to that increased biofuel/ethanol production from plants also produce different co-products. Those co-products can be used in aquaculture practices with further processing. Some of these co-products (e.g., dried distilleries grain) is not included anti-nutritional factors and contain moderate protein level. In an environmental perspective, low phosphorus level of these dried distilleries-grains leaves less ecological footprint (FAO, 2012).

2.4.3. Microbial ingredients for fish meal replacement

Over the past decade, use of microbial feed ingredients in aquaculture practices has gained wider attention. Microbial ingredients primarily include bacteria, microalgae, andyeast. Most of these microbial ingredients can be produced by waste treatment or obtain

as a by-product of refinery processes. Methanotrophic bacteria can be grown in a larger amount with minimum dependence on soil, water, and climatic conditions (Øverland *et al.,* 2010).

Amino acid profile of bacterial meal is high in tryptophan but lower in lysine compared to the fish meal. Lipid profile of bacterial meal consisted of phospholipids. The fatty acid profile is dominated by C 16:0 and C 16:1 n-7 (Sørensen *et al.,* 2011; Øverland *et al.,* 2010). The Vitamin content is dominated by vitamin B (Øverland *et al.,* 2010). Recent studies have shown promising results when methanotrophic bacteria used as a fish meal replacement. These include increased growth efficiency, feed conversation rate, and improved gut health in Atlantic salmon and rainbow trout (Øverland *et al.,* 2010). In terms of gut health, the bacterial meal can be helpful in recovering soy meal-induced enteritis in salmon. Moreover, the bacterial meal has proven capability to reduce expression of genes associated with inflammatory response in distal intestine of fish (Øverland *et al.,* 2010). Yeast is a single cell organism, widely used in various industries including brewery and bakery. Dietary yeast can enhance the gut health of fish species as a probiotic and it also bears a number of nutritional properties. The crude protein content of yeast ranges between 42-55% and has shown higher digestibility in farmed fish (Tacon, 2014). Potential benefits of the microalgal products have been recorded over various fish species. These include, effect on the growth of fish (depends upon inclusion level), the effect on skin coloration, improved carcass quality, diseases and stress tolerance (Kiron *et al.,* 2012; Kiron *et al.,* 2016; Roy and Pal, 2015). Challenges of microbial feed ingredients are numerous. Most of the microalgal products contain rigid cell walls that limit nutrient bioavailability. Rigid cell walls are also characteristic in bacterial ingredients and yeast. Although a number of processing methods are available to solve this issue, some of the

processing methods (e.g., pressure application) are incapable of improving the bioavailability of the nutrients (Øverland *et al.,* 2010).

2.4.4 Genetically modified ingredients

Statistics have shown that production of Genetically Modified (GM) crops is increasing by 4% in each year as reported by James, (2014). Inevitably, this increment has urged the feed manufacturing industry to use GM plant ingredients in feed manufacturing process. GM plant ingredients have more favourable characteristics such as disease/ pest resistance, higher nutritional quality, longer shelf life, and free from anti-nutritional factors. Recent advances in recombinant DNA technology have increased the EAA profiles in several crops. Increased lysine profile in GM maize was achieved by incorporating *Corynobacterium glutamicum* genes (Lucas *et al.,* 2007).

GM plant ingredients in world market include GM-soybean (RRS®), RR canola, GM-maze (MON 810) and GM-lupins. A number of studies have been conducted to understand the effects of GM ingredients on immunological changes, histopathological changes, digestibility and expression of transgenic DNA in fish. The basic concern of GM ingredients is an expression of the modified gene in targeted species. However, there is no solid evidence on horizontal gene transfer from GM plant ingredients to the fish or gut-associated microflora (Sissener *et al.,* 2011).

Challenges of the use of GM plant ingredients in aquaculture industry is in its earliest stage. The inclusion of the GM plant ingredients is also strictly regulated by the certain parts of the world, especially in Europe. According to EU legislation (1829/2003 and 1830/2003), any feed material which is exceeded 9g/kg of GM material should be labeled

(Sanden *et al.,* 2004). Although the nutritional composition of plant ingredients has been improved by gene modification, negative effects on nutrient balance and growth rate are also visible in fish fed with GM diets (Sissener *et al.,* 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The experiment was carried out at the Laboratory of the Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology Minna, Gidan Kwano campus, Niger State. It is located within latitude 09^0 30´ and 09^0 45´ and longitude 06^0 30´and 06^0 045´E with an altitude of 1475m above sea level. The vegetation type mainly of southern guinea savannah grassland. The mean annual rainfall is 1200mm-1300mm (*www.nigisservices.com*)

3.2 Preparation of Experimental Feed.

Eight (8) feed ingredients were purchased from Minna, Central Market, Niger State which includes one (1) kg each of fish meal, crayfish, dry edible hides, grasshopper meal, blood meal, bone meal, hydrolysed feather meal and poultry viscera. The poultry viscera were washed properly then placed in an electric oven to dry. Each of the feed ingredients were grounded into powder form and subjected to proximate, minerals and chemical analysis according to AOAC, (2005).

3.3 Proximate Composition

Determination of moisture, crude protein, lipid and ash content was determined using standard procedures (AOAC, 2005).

This is an analysis that is used to determine the nutritional content of food, feed, plants etc. this analysis entails crude protein, crude fibre, crude fat, ash, carbohydrate and moisture.

3.3.1 Crude protein determination

This test was carried out to know the percentage of crude protein in the sample.

a. Materials**.**

i. Complete digestion block set.

ii. Sulphuric acid(H_2SO_4)

iii. Weighing balance.

iv. Hydrochloric acid.

v. Boric acid.

vi. Sodium hydro-oxide (NaOH).

vii. Burette.

viii. Pipette.

ix. Pipette filler.

x. Conical flask.

xi. Makahmps apparatus.

xii. Indicator (Bromocresol green and Methyl red).

xiii. Selenium tablet,

b. Procedures

i. Digestion stage:

Method: 0.5g of sample was taken and added into the digestion tube where also 20ml of concentrated sulphuric acid was added. One selenium tablet was added as catalyst. The

content in the tube was heated at a temperature of 350^{o}c for 6 hours until a clear digest was achieved that is a clear solution. This solution was poured into a standard flask and made up to 100ml.

ii. Distillation stage:

Method: 10ml of 2% boric acid was taken into a 100ml conical flask and added with three drops of mixed indicator (Bromocresol green and Methyl red) and the colour changes to pink which was then placed under the collecting spot. 10ml of the digested sample was pipetted into the open chamber of the makhamps apparatus then followed by 10ml of 40%NaOH. The mixture was force to boil by the steam produced by the boiling water in the flat bottom flask. As the mixtures boil, a gas (ammonia) was evolved and condense by the condenser of the apparatus which was collected inform of liquid into the boric acid. As the ammonia is collected in the boric acid, the solution turns blue.

iii. Titration stage:

The distillate collected was titrated using 0.1M Hcl until an end point is reached by the colour of the distillate changing to pink colour which is the initial colour of the boric acid and the mixed indicator.

Crude protein is calculated as

TVx0.014xMAxdfx100/wt of S

TV=titre value.

0.014=nitrogen standard.

MA=molarity of acid.

Wt of S=weight of sample.

3.4.2 Determination of crude fibre.

This test was carried out to know the amount of fibre in sample.

a Materials**:**

i. Hot plate
ii. 250ml cornical flask.
iii. Sodium hydro-oxide (NaOH).
iv. Hydrogen tetraoxosulphate (VI) acid.
v. Filter paper.
vi. Funnel.
vii. Hydrochloric acid.
viii. Ethanol.
ix. Measuring cylinder.
x. Furnance

b. Method**:**

2g of defatted sample was weighed into a 250ml conical flask, and added with 200ml of1.25% of H_2SO_4 using a measuring cylinder and boil for 30min on a hot plate, after which it was filtered and the residue poured back into the same conical flask. 200ml of 1.25% of NaOH was added and boil for another 30min and filter. The residue was washed with 10% HCL and later ethanol. The residue was poured into a crucible and dried in the

oven and weighed. After the weighing, the crucible and the content were further ash using the furnace at 550^{o}c for 30min.

Crude fibre was calculated as

A-Bx100/wt of S

A=weight of dry sample.

B=weight of ashed sample.

Wt of S=weight of sample boiled.

3.3.2 Determination of ash

This test is carried out `to know the percentage of inorganic component of the sample.

a. Materials:

i. Crucible.

ii. Muffle furnace.

iii. Spatula.

iv. Weighing balance**.**

b. Method:

2g of sample was weighed into a crucible and ash in a muffle furnace at about 550^{0}c for three hours. The crucible with its content was removed cooled and weighed to get the ash weight.

Ash was calculated as

A/Bx100

A=weight of ash

B=weight of initial sample.

3.3.3 Determination of crude fat

This analysis is carried to know the amount of fat in a given sample.

a. Materials:

i. Soxhlet apparatus.

ii. Thimble.

iii. Spatula.

iv. Petroluem ether.

v. Oven.

vi. Weighing balance.

b. Method**:**

5g of the sample was taken and introduce into the thimble which it was then corked with cotton wool. 200ml of petroleum ether was poured in the round bottom flask of the Soxhlet apparatus and corked with Soxhlet chamber. The corked sample in the thimble was introduce into the Soxhlet and heated at 40-600^{0}c for three hours after which the extracted sample removed and weighed.

Fat is calculated as

A-B/wt Sx100.

A=weight of fresh sample.

B=weight of extracted sample.

Wt of S=weight of initial sample.

3.3.4 Determination of moisture content

This analysis is carried out to ascertain the amount of moisture in a giving sample.

a. Materials**.**

i. Oven.

ii. Weighing balance.

iii. Spatula.

iv. Petri dish.

b. Method:

10g of sample was weighed into a dry petri dish using a spatula. The weighed sample was loaded into the oven and dried at 105^0c until a constant weight is achieved. The sample was removed, cooled and weighed.

Moisture content was calculated as.

A-B/Ax100.

A=initial weight f sample.

B=weight of oven dry sample.

3.4 Determination of Chemical Composition of Oil Extract

The peroxide value and FFA/ acid value were determined by the AOCS 2004, the saponification value (SV) was determined by AOAC (2000) and the iodine value was determined using AOCS Method Cd 1-25 (1993), also known as the wajis methods.

3.4.1 Acid value or free fatty acids (FFA):

Procedure:

i. Mix 25ml diethyl ether with 25ml alcohol and 1ml phenolphthalein solution (1%) and carefully neutralize with 0.1M NaOH.

ii. Dissolve 1-10g of the oil or melted fat in the mixed neutral solvent and titrate with aqueous 0.1M NaOH shaking constantly until a pink colour which persists for 15 seconds is obtained.

Calculation:

$$\text{Acid value} = \frac{\text{Titre (m)} \times 5.61}{\text{Weight of sample used}}$$

The FFA figure is usually calculated as oleic acid (1 ml 0.1M Sodium hydroxide 0.0282g oleic acid), in which case the acid value 2 x FFA.

For most oils acidity begins to be noticeable to the palate when the FFA calculated as oleic acid is about 0.5-1.5%.

For palm oil as palmitic (I ml o.1M NaOH = 0.0256g). for palm kernel, coconut and similar lauric acid (Iml 0. 1M NaOH= 0.0200g)

Procedure

i. a 10% W/V suspension of sample was diluted in water
ii. the mixture was mixed thoroughly in a warring blender, then the pH was measured with a pH meter.

3.4.2 Saponification value

i. 2g of the oil or fat was weigh into a conical flask and exactly 25ml of alcoholic potassium hydroxide solution was added.
ii. a reflux condenser was Attach and the flask was heated in a boiling water for 1hr
iii. add 1ml of phenolphthalein (1%) solution and titrate hot the excess alkali with 0.5M hydrochloric acid (titration =aml

iv. a blank was carried out at the same time (titration= bml)

calculation

saponification value = $\frac{(b-a) \times 28.05}{\text{wt (g) of sample}}$

3.4.4 Iodine value

Determination of iodine value:

i. Pour the oil into a small beaker, add a small rod and weigh Out a suitable quantity of the sample by difference into a dry glass-stoppered bottle of about 250ml capacity. The approximate weight in 8 of the oil to be taken can be calculated by dividing 20 by the highest expected iodine value.

ii. Add 1Oml of carbon tetrachloride to the oil or melted fat and dissolve.

iii. Add 20ml of Wijis' solution, insert the stopper (previously moistened with potassium 1odine solution) and allow to stand in the dark for 30 minutes.

iv. Add 15ml of potassium 10dide solution (10%) and 100ml water, mix and titrate with 0.IM thiosulphate solution using starch as indicator Just berore the end-point (titration= aml).

v. Carry out a blank at the same time commencing with 10ml of carbon tetrachloride (titration = bml).

Iodine Value = $\frac{(b-a) \times 1.2\ 69}{\text{Wt (g) of sample}}$

Note: If (b-a) is greater than b/2 the test must be repeated using a smaller amount of the sample.

it should be noted also that the less unsaturated fats with low iodine values are solid at room temperature, or conversely, oils that are more highly unsaturated are liquid

(Showing there is a relationship between melting points and the iodine value).

Preparation of Wijis' Solution**:**

i. Dissolve 8g iodine trichloride in 200ml glacial acetic acid.

ii. Dissolve 9g iodine in 300ml carbon tetrachloride.

iii. Mix the two solutions and dilute to 1000ml with glacial acetic acid.

3.4.5 Peroxide value:

Procedure:

i. Weight out 1g of oil or fat into a clean dry boiling tube and while still liquid add 1g powdered potassium iodide and 20ml of solvent mixture (2 vol glacial acetic acid + 1vol chloroform).

ii. Place the tube in boiling water so that the liquid boils within 30 seconds and allow to boil vigorously for not more than 30 scconds.

iii. Pour the contents quickly into a flask containing 20ml of potassium iodide solution (5%), wash out the tube twice with 25ml water and titrate with 0.002M sodium thiosulphate solution using starch.

iv. Perform a blank at the same time

The peroxide value is often reported as the number of ml of 0.002

N (M) sodium thiosulphate per g of sample.

If the value so obtained is multiplied by 2, the figure then equals milliequivalents

of peroxide oxygen per kg of sample (ml M per kg), which has greater international recognition.

3.5 Mineral Composition

3.5.1 Determination of phosphorus (Molybdate method, Fiske and Subbarrow, 1925)

Phosphorous in a sample is determined by molybdate method using hydroquinone as a reducing agent. To 0.5m1 of the mineral digest are added 1.0mI of ammonium molybdate, 1.0ml sodium sulphate and 1.0ml of hydroquinone. The mixture is agitated and allowed to stand for 30 minutes. The blue colour that develops is quantitated using a colorimeter at 660nm against a standard curve.

a. Preparation of sample

i. To a 16x125mm test-tube, 0.5ml of the mineral digest and 9.5ml of 10% trichloroacetic acid are added

ii. The Mixture is agitated to mix, centrifuged for 5 minutes and then filtered through 7 cm filter paper.

iii. 5ml of the filtrate is measured into 19mm covet.

iv. 5ml of the filtered trichloroacetic acid and 5ml of the Working standard are measured into two covets to serve as a blank and standard respectively. These are treated the same way as the sample filtrate.

v. 0.5ml of molybdate reagent is added to each tube and mixed.

vi. 0.2ml of sulphuric acid reagent is added.

vii. The contents are stoppered, mixed and allowed to stand for 10 minutes.

viii. The absorbance of the test and standard are read in a spectrophotometry at 660nm with the blank set at Zero.

Calculations:

$$\frac{\text{Absorbance of test x conc. of std. (5mg/d1)}}{\text{Absorbance of std.}} = \text{P (mgldl)}$$

Phosphorus concentration can also be obtained from the calibration

curve of standards.

b. Preparation of standard solution

Phosphorus standard is prepared from dibasic potassium phosphate K_2HPO_4, (Mol. Wt. = 174). A standard containing 200mg of phosphorus per litre is prepared from the dibasic potassium phosphate and calculated as follows:

Molecular weight of K_2HPO_4= 174.

One mole of the salt contains I mole of 31g of Phosphorus.

In order to get 200mg of phosphorus,

(174/3 1 x 200) = 1.1224g of K_2HPO_4, is required.

Phosphorus stock standard 20mg/d1:

1.1224g of K_2HPO_4 is dissolved in 500m1 of water and transferred to one-litre volumetric flask. 8ml of conc. HCl is added and diluted to 1 litre with water. This is stored in a refrigerator.

3.5.2 Determination of calcium and magnesium by atomic absorption spectrophotometer

About 1.0g of the sample is first digested with 20ml of acid mixture (650ml conc HNO^3; 80m1 Perchloric acid (PCA); 20ml conc H_2SO_4) and aliquots of the diluted clear digest used for atomic absorption spectrophotometry using filters that match the different elements.

a. Sample Preparation

i. Weigh out lg of the dried sample into a digestion flask and add 20mnl of the acid mixture (650ml conc HNO_3; 8Oml PCA; 20ml conc H_2SO_4).

ii. Heat the flask until a clear digest is obtained.

iii. Dilute the digest with distilled water to the 500ml mark.

iv. Appropriate dilutions are then made for each element.

v. For the determination of calcium and magnesium add enough SrCl2, solution containing 10,000mg/ml to yield a 1,500mg/ml of Sr^{2+} in the final solution.

vi. Calibration curves are prepared for each element using standard solution. The appropriate lamps and correct wavelength for each element are usually specified on the instruction manual of the instrument.

The concentrations of these elements mentioned above are determined using the calibration curve

b. Preparation of standard solutions:

i. Calcium

Dissolve 2.497g of oven-dried CaCO3 and dilute to 100m1 with de-ionized water. This solution contains 100Omg Ca^{2+} ions. From this stock solution, prepare calcium standard solutions with the following concentrations 0.0, 3.0, 6.0, 9.0.

ii. Magnesium

1.0g of Magnesium ribbon is accurately weighed and dissolved into 10ml conc. HCl. The solution is then boiled and evaporated almost to dryness on a water bath. De-ionized water is added and the solution transferred into a 100ml volumetric flask. The solution is made to mark with de-ionized water. From this stock solution which contains 1000mg/ml of Mg ions, four standard solutions of concentrations 0.0, 0.5, 1.0 and 1.5 ppm are prepared. To each of the calcium and Magnesium solutions, strontium chloride solution is added such that there is 1500/mg of strontium ions in the final solution.

3.5.3 Determination of sodium and potassium by flame photometry (A.0.A.C.,2010)

Above 1.0g of the sample is digested with 20ml of acid mixture (650m1 of concentrated HNO_3; 80ml PCA; 20ml conc H_2SO_4) and aliquots of the diluted clear digest taken for photometry using Flame analyser.

a. Sample preparation

The sample is prepared as earlier described for atomic absorption spectrophotometry and suitable dilutions made from the perchloric acid digest and ran in the instrument according to the instructions in the manual.

Absorbance for Na is read at 767nm while that for K is read at 589nm. Sodium and potassium concentrations are obtained from the calibration curves obtained from the standards.

b. Preparation of standard solutions

i. Prepare the Sodium stock solution of 1000 ppm Na by dissolving 2.542g of dry NaCl in water and diluting to one litre.

ii. Prepare the potassium stock solution of 1000 ppm K by dissolving 1.907g of KCl in water and diluting to one litre.

iii. Prepare the standard solutions from the stock solutions by obtaining dilutions s of the following concentrations, 10pm, 8ppm and 2ppm.

iv. The concentrations of these elements mentioned above are determined using the calibration curve.

3.6 Data Analysis

Data were processed into their descriptive statistics and analysis (Zar, 1984). Means analysis was done using One-way Analysis of Variance (ANOVA) and compared using multiple range test (Duncan 1955), by employing Minitab Software Student version 17.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

Table 4.1 depict that all treatments differed significantly ($p<0.05$) in terms of moisture contents. The percentage moisture content ranged from 1.20% to 10.09%, where T3 (grasshopper meal) and T7 (hydrolysed feather meal) recorded the highest and lowest value of 10.09% and 1.20% respectively. There was significant ($p<0.05$) difference in terms of the crude protein level of all the treatments. However, T7(hydrolysed feather meal) and T6 (Bone meal) recorded the highest and lowest value of 84.21% and 4.19% respectively. In terms of the lipid content, all the treatments differed significantly ($p<0.05$). The percentage lipid content ranged from 0.04%-17.46%, where T4 (poultry viscera) and T6(bone meal) recorded the highest and lowest value of 17.46% and 0.04%

respectively. There was significant ($p<0.05$) difference in the crude fibre value of all the treatments. However, T3 (grasshopper meal) recorded the highest value of 15.81% and T5 (blood Meal) and T6 (bone meal) recorded the lowest value of 0%. There was significant ($p<0.05$) difference in terms of the NFE content of all the treatments. However, T4 (poultry viscera) and T6(bone Meal) recorded the highest and lowest value of 7.51% and 0.57% respectively. There was also significant ($p<0.05$) difference in the Ash content of all the treatments. However, T2 (dry edible hides) and T6(bone meal) recorded the lowest and highest value of 1.75% and 91.22% respectively.

Table 4.1: Percentage proximate composition of selected non-conventional feedstuffs of animal origin

PROXIMATE ANALYSIS (%)	T1	T2	T3	T4	T5	T6	T7	T8(FM)	Pooled S.E.M
MOISTURE	3.26±0.02^{b}	4.98±0.01^{d}	10.09±0.02^{g}	7.38±0.02^{f}	5.12±0.16^{d}	3.97±0.01^{c}	1.20±0.02^{a}	5.86±0.01^{e}	0.0625
CRUDE PROTEIN	62.11±0.01^{d}	70.46±0.05^{e}	50.57±0.95^{b}	55.06±0.07^{c}	80.35±0.21^{f}	4.19±0.26^{a}	84.21±0.04^{g}	69.34±0.01^{e}	0.361
LIPID	1.79± 0.01^{b}	13.08±0.02^{g}	12.35±0.00^{f}	17.46±0.0^{h}	9.17±0.01^{d}	0.04±0.00^{a}	4.33±0.02^{c}	10.10±0.01^{e}	0.0158
CRUDE FIBRE	12.31±0.18^{e}	3.34±0.01^{c}	15.81±0.12^{f}	4.8.09±0.32^{d}	0.00±0.00^{a}	0.00±0.00^{a}	2.31±0.04^{b}	0.05±0.01^{a}	0.140
NFE	4.21±0.19^{b}	6.38±0.07^{d}	5.04±0.78^{c}	7.51±0.29^{f}	1.22±0.08^{a}	0.57±0.26^{a}	4.18±0.09^{bc}	4.01±0.01^{b}	0.3236
ASH	16.31±0.02^{c}	1.75±0.00^{a}	6.12±0.02^{b}	4.49±0.01^{b}	4.04±0.01^{b}	91.22±0.01^{d}	3.76±0.01^{b}	14.33±0.01^{c}	0.0150

Mean data on the same row with different superscripts differs significantly ($p<0.05$). KEY: SEM: Standard Error of Mean, T1: Crayfish, T2: Dry edible hides, T3: Grasshopper meal, T4: Poultry viscera, T5: Blood Meal, T6: Bone meal, T7: Hydrolysed Feather Meal, T8: Fish meal (Control), NFE: Nitrogen Free Extract

Table 4.2 showed that there was significant difference ($p<0.05$) in the pH value in all the treatments, T7 (hydrolysed feather meal) had the highest value of 8.19 while T6 (bone meal) had the least value of 0.00 as also seen in other analysis (i.e., FFA, Acid value, peroxide value, iodine value and saponification value). All treatments differed significantly ($p<0.05$) in their FFA content T2 (dry edible hides) having the highest value of 16.39mgKOH/g. In terms of the peroxide value, all treatments which differed significantly ($p<0.05$), however, T8 had the highest value of 20.78meq/kg. There was significant difference ($p<0.05$) in the acid, iodine and saponification value of all the treatments. However, T8 (fish meal) recorded the highest value in all three parameters with the value of 12.25mgKOH/g, 140.52g/100g and 195.77mgKOH/g respectively.

Table 4.2: Chemical composition of oil extract of selected non-conventional feedstuffs of animal origin

Chemical composition	T1	T2	T3	T4	T5	T6	T7	T8(control)	Pooled S.E.M
Ph	5.86±0.02^{d}	4.66±0.05^{c}	6.51±0.04^{ef}	4.22±0.02^{b}	6.72±0.03^{f}	0.00±0.00^{a}	8.19±0.01^{g}	6.25±0.35^{e}	0.1291
FFA (mgKOH/g)	3.37±0.04^{b}	11.71±0.02^{f}	5.86±0.05^{d}	16.39±0.01^{g}	4.16±0.02^{c}	0.00±0.00^{a}	3.56±0.04^{b}	8.05±0.07^{e}	0.0417
Acid value (mgKOH/g)	1.68±0.02^{b}	5.88±0.02^{e}	2.92±0.03^{d}	8.19±0.01^{f}	2.08±0.02^{c}	0.00±0.00^{a}	1.78±0.02^{bc}	12.25±0.35^{g}	0.127
Peroxide value (meq/kg)	1.78±0.04^{b}	7.48±0.02^{e}	3.72±0.02^{d}	12.28±0.02^{f}	3.21±0.01^{c}	0.00±0.00^{a}	2.87±0.01^{c}	20.78±0.02^{g}	0.0275
Iodine value (g/100g)	34.38±0.02^{b}	80.76±0.01^{f}	39.62±0.02^{c}	108.35±0.02^{g}	54.17±0.01^{e}	0.00±0.00^{a}	42.33±0.02^{d}	140.52±0.03^{h}	0.023
Saponification (mgKOH/g)	174.38±0.02^{f}	166.85±0.01^{e}	180.22±0.03^{g}	153.76±0.01^{c}	148.65±0.01^{b}	0.00±0.00^{a}	160.37±0.03^{d}	195.77±0.03^{h}	0.025

Mean data on the same row with different superscripts differs significantly ($p<0.05$). KEY: SEM: Standard Error of Mean, T1: Crayfish, T2: Dry edible hides, T3: Grasshopper meal, T4: Poultry viscera, T5: Blood Meal, T6: Bone meal, T7: Hydrolysed Feather Meal, T8: Fish meal (Control), FFA: Free Fatty Acid.

Table 4.3 showed that all the treatments differ significantly ($p<0.05$) in terms of sodium with T5 (blood meal) and T2 (dry edible hides) having the highest value and lowest value of 266.35mg/100g and 22.37mg/100g respectively. The potassium content differed significantly ($p<0.05$) in all treatments. However, T3 (grasshopper meal) and T8 (fish meal) had the highest and lowest value of 1355.73mg/100g and 137.56mg/100g respectively. As for the calcium content, there was also significant ($p<0.05$) difference in all the treatments, T6 (bone meal) and T1 (crayfish) had the highest and lowest value of 22374.9mg/100g and 94.30mg/100g respectively. In terms of magnesium content, all other treatments differed significantly ($p<0.05$). However, T8 (fish meal) and T2 (dry edible hides) had the highest and lowest value of 1350.49mg/100g and 54.39mg/100g respectively. The phosphorus content of all treatments differed significantly ($p<0.05$) with T5 (blood meal) and T8 (fish meal) having the highest and lowest value of 1350.58mg/100g and 44.51mg/100 respectively.

Table 4.3: Minerals Composition of selected non-conventional feedstuffs of animal origin

Mineral composition (mg/100g)	T1	T2	T3	T4	T5	T6	T7	T8(FM)	S.E.M.
Sodium	78.38±0.02 ^{c}	22.37±0.09 ^{a}	34.78±0.09 ^{b}	85.72±0.03 ^{d}	266.35±0.07^{h}	164.28±0.08^{g}	112.18±0.02^{e}	120.60±0.07 ^{f}	0.069
Potassium	335.86±0.06^{c}	552.60±0.11^{e}	137.56±0.08^{a}	222.28±0.11^{b}	428.14±0.06^{d}	566.37±0.04^{e}	343.27±0.07^{c}	1355.73±0.04^{f}	0.07
Calcium	94.30±0.1 ^{a}	106.20±0.0 ^{a}	984.9±0.0 ^{c}	342.6±0.0 ^{b}	414.3±0.1^{b}	22374.9±0.0^{e}	1988.6±0.1^{d}	1250.3±0.0 ^{c}	0.1
Magnesium	288.69±0.05^{e}	54.39±0.06 ^{a}	224.33±0.06^{d}	98.38±0.11^{b}	188.38±0.03^{c}	72.51±0.06^{a}	616.29±0.01^{f}	1350.49±0.02^{g}	0.06
Phosphorus	246.58±0.04^{e}	84.43±0.04 ^{b}	112.66±0.06^{c}	166.76±0.02^{d}	44.51±0.01^{a}	234.63±0.04^{e}	328.05±0.07^{f}	1350.58±0.11^{g}	0.05

Mean data on the same row with different superscripts differs significantly ($p<0.05$). KEY: SEM: Standard Error of Mean, T1: Crayfish, T2: Dry edible hides, T3: Grasshopper meal, T4: Poultry viscera, T5: Blood Meal, T6: Bone meal, T7: Hydrolysed Feather Meal, T8: Fish meal (Control).

4.2 DISCUSSION

The study on non-conventional feedstuffs of animal originated characterized sevenlocally available feedstuffs with fish meal as a reference feedstuff, viz; crayfish, dry edible hides (*ponmo* or *ganda*) grasshopper, poultry viscera, blood meal, bone meal and hydrolysed feather meal were used. They were labelled as treatments; T1, T2, T3, T4, T5,T6 and T7, respectively while fish meal (T8) served as control. The study showed that T2(70.46%), T5(80.21%), and T7(84.21%) have more crude protein value compared to T8 (60.83%) control. This study is similar to that reported by NRC (2011) stating that crude protein level and crude fat level of blood meal usually reach approximately 85% and 0.5%-3% respectively, Hydrolysed feather meal (HF) is a rich protein source – 74 to91% crude protein (CP; Baker *et al.,* 1981; Bureau *et al.,* 1999; 2000; Grazziotin *et al.,* 2008; Klemesrud *et al.,* 2000), rich in cystine (4 – 5% CP), but deficient in lysine (2%) and methionine (1% CP; Baker *et al.,* 1981; Klemesrud *et al.,* 2000). However, the study differs with that of Sanusi *et al.,* 2016 which reported that crude protein level of dry edible hides is 58.86% and also differs with that of Olaleye, (2015) which shows that grasshopper meal has high crude protein of 64.51% this is a very high value that could completely replace fish meal in fish feed.

Peroxide value (PV) is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. PV is one of the most widely used tests for the measurement of oxidative rancidity and or deterioration of oils and fats. In this study peroxide values of less than 10 were obtained for all lipid sources examined, with exception of fish meal which is the control and poultry viscera which have PV of 20.78meq/kg and 12.28meq/kg respectively. Similar lower peroxide values of less than 10 was obtained in canola oil, corn oil, coconut oil, grape seed oil, olive oil, palm olein, peanut oil, rapeseed oil, rice bran oil, sunflower oil, soybean oil, sesame oil, safflower oil

and walnut oil by Gan *et al*. (2005). However, higher value above this level was obtained in hazelnut oil and extra virgin olive oil by the same author. It has been showed that off-flavours, nutritional losses and other deteriorative changes in oil arise by reaction with atmospheric oxygen, i.e., oxidative rancidity, or by hydrolytic reactions catalysed by lipases from food or from microorganisms (Shahina *et al.,* 2004). The peroxide value (PV), anisidine value (AV) and free fatty acids (FFA) are good guides to the quality of oil. Good quality oil should have a PV less than 10 units before off-flavours are encountered (Rossell, 1994). All the oils used in this study with exception of fish meal which is the control and poultry viscera had acceptable levels of PV which were less than 10 units. The lower peroxide values of the lipids suggest that the oil can be stored for a long period without deterioration. According to Ojeh (1981), oils with high peroxide values are unstable and easily become rancid. Oils become rancid when the peroxide value ranges from 20.0 to 40.0 mg/g oil.

Acid value is used as an indicator for edibility of oil and suitability for use in the paint industry. The total acidity, expressed as acid value of T1, T3, T5 and T7 are similar to the values reported for the pulp and seed of D. edulis (Ajayi and Oderinde, 2002). These values are within the allowable limits for edible oils (Eckey, 1954).

Formation of free fatty acid might be an important measure of rancidity in foods. FFA is formed due to hydrolysis of triglycerides and may be promoted by reaction of oil with moisture (Frega *et al*., 1999). Free fatty acid values of less than 5 obtained for T1, T5 and T7 are within allowable limits for edible oils (Eckey, 1954), also similar to the study of Babalola and Apata (2011) which FFA values of less than 5 was obtained for fish oil, lard, soybean oil, palm oil, sunflower oil, chicken fat and melon seed oil. And also similar to those obtained by Gan *et al*. (2005) suggests that the oils could be a good edible oil that will store for a long time without spoilage via oxidative rancidity. This indicates that these

oils with FFA values above the allowable limits may undergo oxidation and aquafeed produced with such oils may become rancid.

Iodine value is a measure of overall unsaturation and is widely used to characterize oils and fats. It is defined as the number of grams of iodine absorbed by 100 g of fat. The control sample T8 i.e., fish meal obtained the highest value of iodine of 140g/100g followed by T4(poultry viscera) with value of iodine of 108g/100g. This is similar to the study of Babalola and Apata, (2011) which stated that, the high iodine value in FO (109g/100g), SO (120.60g/100g) and SFO (125.17g/100g) shows that these oils are rich source of polyunsaturated fatty acids that possess health benefits, such as regulating blood cholesterol levels and lowering elevated blood pressure. High iodine shows that the oils have the good qualities of edible oil and drying oil purposes (Eromosele *et al.,* 1997). Also similar to the statement of Nielson 1994 who reported that the higher the amount of unsaturation, the more iodine is absorbed; therefore, the higher the iodine value the greater the degree of unsaturation.

According to Codex Alimentarius (2005), the saponification values (SV) range between 250-260 mg KOH/g oil, all treatments did not reach the stipulated range, this could be an indication that the fatty acid chain is long. This assertion was confirmed by Nielson (1994), that the smaller the saponification values the longer the average fatty acid chain.

Ca and P are the primary mineral components of a complete diet. Ca is involved in blood clotting (vertebrates), muscle functions (such as contractions), nerve impulse transmission, osmoregulation, membrane permeability, hormone and enzyme secretions, and acts as a structural component of teeth and bones. In addition, P is a component of nucleotides, skeletal tissues, phospholipids, coenzymes, DNA, RNA, and special enzymes involved in energy production (Takeda *et al*., 2012; Yamauchi *et al.,* 1996).

Moreover, P has a buffering effect and helps an organism maintain a normal pH (Fairweather-Tait and Cashman 2015). Compared to FM, T6(bone meal) and T7(hydrolysed feather meal have higher Ca content. Also, T5 (blood meal) and T6(bone meal) have higher Na content which is an essential macro mineral (NRC 1993; 2011) compare to fish meal. However, fish meal was richer in other mineral elements.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The main objective of this study is to investigate the chemical and mineral composition of some non- conventional feedstuffs of animal origin base on their chemical scoring, focusing more on their protein level, lipid, carbohydrate and minerals composition.

From the result obtained from this study, it is therefore concluded that some non-conventional feedstuffs such as blood meal, hydrolysed feather meal and dry edible hides have the potential to be used in fish feed considering their high crude protein level compare to fish meal.

The peroxide value of all the feedstuffs examined with exception of poultry viscera obtained the required value limit of less than 10 compared to fish meal which exceeded the limit. This shows that the oil extracted from these feedstuffs can be stored for a long time without getting spoilt easily. However, fish meal had high level of iodine value compare to other feedstuffs, this shows that oil extract from fish meal is a rich source of polyunsaturated fatty acids that possess health benefits.

Also, the result obtained from the study indicated that, all the samples fell short in their minerals composition compare to fish meal except in Ca where bone meal and Hydrolysed feather meal had greater value compare to fish meal.

5.2 Recommendations

From the study carried out, it is hereby recommended that;

Blood meal, hydrolysed feather meal and edible skin can be used as protein supplement in aquafeed, the use of these feedstuffs in fish feed will serve as alternative for fish meal. It is also recommended that bone meal and hydrolysed feather meal should be used as source of calcium in aquafeed. Further studies need to be carried out on the amino and fatty acid profile of these feedstuffs. Also, further studies need to be carried out on the appropriate quantity to be utilized in feed and their effects on growth and nutrient utilization of various fish species.

5.3 Contribution to Scientific Knowledge

The evaluation of some chemical and mineral composition of non-conventional feedstuffs of animal origin has contributed in identifying that;

Some non-conventional feedstuffs such as hydrolysed feather meal (84.21%), blood meal (80.35%) and dry edible hides (70.46%) can be used as alternative for fish meal in aquafeed considering their high crude protein level compare to fish meal (69.34%).

The study also identified that oil extract from these feedstuffs can be used as lipid supplement in aquafeed considering their low susceptibility to lipid oxidation, considering their peroxide value ranging from (1.78- 7.48meq/kg) which is within the allowable limit (i.e., less than 10) and also have acid value within the allowable limit of less than 5, ranging from (1.68- 2.92mgKOH/g).

This study also contributed in identifying that some of the feedstuffs used in the experiments such as bone meal (22374.9mg/100g) and feather meal (1988.6mg/100g)

have calcium content which is a primary mineral component of a complete diets higher to than that of fish meal(1250.3mg/100g).

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