UTILIZATION OF FERMENTED *MUCUNA PRURIENS* LEAF MEAL AS A REPLACEMENT FOR SOYABEAN MEAL IN *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS DIETS

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NIGERIA.

MARCH, 2017

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MARCH, 2017

DECLARATION

I declare that the work in this dissertation entitled "UTILIZATION OF FERMENTED *MUCUNA PRURIENS* LEAF MEAL AS A REPLACEMENT FOR SOYABEAN MEAL IN *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS DIETS" has been carried out by me in the Department of Biology, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and in a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any Institution.

RASAQ IBRAHIM _____ _____

Signature Date

CERTIFICATION

This dissertation entitled "UTILIZATION OF FERMENTED *MUCUNA PRURIENS* LEAF MEAL AS A REPLACEMENT FOR SOYABEAN MEAL IN *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS DIETS" by RASAQ IBRAHIM meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my late Mother Alhaja Ibrahim Rihanat Biala. May Allah grant her Aljanat Firdaus (Amin).

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ABSTRACT

Feeding trial was conducted in 18 plastic tanks (60 × 45× 30 cm) to assess the performance of *Clarias gariepinus* fingerlings fed diets containing fermented *Mucuna pruriens* leaf meal as an alternative protein source to soya bean meal. Five iso-nitrogenous (crude protein- 40%) diets were formulated containing Fermented *Mucuna pruriens* leaf meal at $A_{(100\%F\ MLM),}$ $B_{(75\%\ FMLM)}$, $C_{(50\%\ FMLM),}$ $D_{(25\%\ FMLM)}$ and $E_{(0\%\ FMLM)}$ (control) were fed at 5% to triplicate groups of 10 fingerlings (mean weight ranged from 6.20±1.96g – 6.80±1.97g) of *C. gariepinus* for a period of twelve (12) weeks. The *Mucuna pruriens* leaves were collected and processed by Air-dried Mucuna Leaf Meal (ADMLM), Soaked Mucuna Leaf Meal in cold water (SCMLM), Soaked Mucuna Leaf Meal in hot water (SHMLM) and Fermented Mucuna Leaf Meal (FMLM). Proximate analysis showed that FMLM had highest value (25.94±0.94%) of crude protein. Analysis of anti-nutritional factors showed that hydrocyanic acid, oxalate, phytate, saponin, and tannin were reduced significantly ($P<0.05$) after processing the Mucuna leaf meal. The raw and fermented *Mucuna pruriens* leaf meal recorded revealed there was no significant difference ($P\geq0.05$) in values of mineral composition (calcium, magnesium, phosphorus, sodium, potassium, manganese, copper, zinc and iron). Growth performance and nutrient utilization parameters indicate that $E_{(0\%\ MLM)}$ gave the highest weight gain (168.87±3.97g) but did not significantly ($P\geq0.05$) differ from $E_{(25\%\ MLM)}$, (161.30±6.12g) and the lowest weight gain (89.70±1.96g) was recorded in $B_{(100\%\ MLM)}$. Similarly, the highest specific growth rate (3.79g) was recorded in $F_{(0\%\ MLM)}$ (control) followed by $E_{(25\%\ MLM)}$ which had (3.79±0.03g) and the least value (3.10±0.02g) was obtained in $B_{(100\%\ MLM)}$. However, feed conversion ratio (FCR), protein efficiency ratio (PER), and gross feed conversion efficiency (GFCE) were not significantly different ($P\geq0.05$) among the dietary treatments. The highest FCR (1.72±0.12) and least (1.50±0.02) were obtained in $A_{(100\%\ MLM)}$ and $E_{(0\%FMLM)}$ respectively. The highest (66.54±0.98) and least (58.67±4.47) were recorded in $E_{(0\%\ FMLM)}$ and $A_{(100\%\ MLM)}$, for GFCE respectively. The highest PER (1.64±0.03) and least (1.47±0.11) were obtained in $E_{(0\%FMLM)}$ and $A_{(100\%\ FMLM)}$, respectively. Apparent protein digestibility recorded highest value (61.40±0.60) in diet $E_{(0\%\ FMLM)}$ and least was recorded in diet $A_{(100\%\ MLM)}$ (55.87±±0.90).Cost benefit analysis showed that $D_{(25\%FMLM)}$ had cost per kilogram of diet (₦350±17.32) and profit index (2.60±0.11) while $A_{(100\%\ MLM)}$ recorded least cost per kilogram of feed (₦230±5.77) with correspondingly

lowest profit index (1.69±0.06). The result from this study indicated that Fermented *Mucuna pruriens* leaf meal diet can replace soya bean meal up to 25% in the diets for *C. gariepinus* without compromising growth performance, nutrient utilization apparent digestibility coefficient and cost of production.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The intensive production of farmed fish with compound feeds, has been largely increased mainly due to the growth of aquaculture production, and also because it is the most efficient way of production to meet the fish demand (Olsen and Hasan, 2012). Although, capture fishery production has been relatively stable at about 90 million tonnes since the 1990s, the rising demand for fishery products has not been met by a fast-growing aquaculture industry, which set an all-time high record at 67 million tonnes in 2012, providing 50% of the fish used for human consumption (FAO, 2014). In fish farming, nutrition is critical because fish feed represents about 60% of the total production costs (Sogbesan *et al*., 2006). Protein component represents about 50% of feed cost in intensive culture (El-Sayed, 2005). Therefore, the selection of proper quantity and quality of dietary protein is a necessary tool for successful fish culture practices.

Soya bean meal (SBM) is one of the most nutritious of all plant protein sources (Batal, 2000). Due to its high protein content, high digestibility and relatively well balanced amino acid profiles, it is widely used as feed ingredient for many aquaculture species (Storebakken *et al*., 2000). It is currently the most commonly used plant protein source in fish feed. Lim and Akiyama (1992) reported that soya bean products have been used to replace a significant portion of fish meal in fish feed with nutritional, environmental and economic benefits. However, wider utilization and availability of this conventional source for fish feed is limited by increasing demand for human consumption and by other animal feed industries

(Siddhuraju and Becker, 2001), hence the need to focus on using less expensive and readily available plant protein sources to replace soya bean meal without reducing the nutritional quality of the feed becomes imperative.

The *Mucuna pruriens* belongs to the family Fabaceae and has been described as a multipurpose plant which is used extensively both for its nutritional and medicinal properties (Adepoju and Odubena, 2009). It is twinning and tropical legume known as velvet bean and of common names such as: cow-itch, cowhage, Bengal beans, itchy bean, buffalo bean and velvet bean (English), while it's called Agbara (Igbo), Yerepe (Yoruba) and Karara (Hausa) (Manyham *et al*., 2004). The roots are bitter, stimulants, purgative, aphrodisiac and diuretic. The leaves of *Mucuna pruriens* are used as remedy for various diseases such as diabetes, arthritis, dysentery, and cardiovascular diseases (Barrows *et al*., 2008). *Mucuna pruriens* has been shown to increase testosterone levels (Amin *et al.*,1996), leading to deposition of protein in the muscles and increased muscle mass and strength (Bhasin *et al.,* 1996), and some medicinal properties attributed to the plant include that the roots are thermogenic, antihelminthic, and also used to relieve constipation, neuropathy and ulcer (Warrier *et al.*,1996). The seeds have been found to have anti-depressant properties when consumed, and it has also shown to be neuro-protective (Manyham *et al.,* 2004).

Some of the most popular cultured fish species in Nigeria and other parts of Africa are the *Clarias gariepenus* and *Heterobranchus bidorsalis* which are members of the catfish family called *Clariidae* (Vanden and Bernacsek, 1990; Ojutiku, 2008). The Clariid exhibit many qualities which make them suitable for culture; these include fast growth rate, high resistance to disease, tolerance to adverse environmental conditions, ability to feed on wide range of feed and capacity to withstand low pH and oxygen (Fagbenro *et al*., 1992). It also has high

feed efficiency and utilization (Adebayo and Olanrewaju, 2000). There is, however, no information on the utilization of *Mucuna pruriens* leaf meal in the diets of *Clarias gariepinus* fingerlings.

1.2 Statement of the Research Problem

The major challenge facing aquaculture nutritionists is the development of cost effective feeds using locally available, cheap and unconventional resources. Non-animal proteins derived from legume and/or oil seeds or cereal gluten are now introduced in fish diets (Médale *et al*., 2013), but plant sources have limitations, such as palatability issues, presence of anti-nutritional substances, low concentrations of sulfur amino acids, and high proportions of fiber and non-starch polysaccharides (Sanchez-Muros *et al*., 2014).

Jamu and Ayinla (2003) reported that the low quality of fish feed and its attendant high cost are the major factor limiting the development of aquaculture sector in Africa and therefore, research in fish nutrition that will utilize locally available ingredients without reducing the quality of the feed is urgent and crucial to the overall success of aquaculture development, growth and expansion in the continent.

Soyabeans, however, is very expensive due to human consumption needs and its use in other animal feeds. It has therefore become vital to search for alternative that are not in direct competition with human and other animals, hence an evaluation into the effect of dietary inclusion of fermented *Mucuna pruriens* leaf meal on growth performance and nutrient utilization of *Clarias gariepinus* fingerlings becomes imperative.

1.3 Justification

Feed is a significant factor in increasing the productivity and profitability of aquaculture. Feed determine the viability of fish farming as it account between 60 and 62% of the total cost of production (Sogbesan *et al*., 2006 and Bolorunduro, 2016). Soya bean is widely used in conventional intensive animal feeding systems because of its known high protein content (38-42%), good amino acid balance and digestibility (Baker and Stein, 2009).

The increasing demand, price, competitions with human needs and its use in other animal feeds of soyabean has emphasized the need for alternative protein sources in aqua feeds.

To ensure a sustainable development in aquaculture, there is an urgent need to reduce the dependence on soyabean as protein source through the introduction of alternative raw materials that will constitute the major protein and lipid sources in fish diets.

The itching bean *Mucuna pruriens* is an underutilized legume species grown predominantly in Africa, Asia and in parts of America (Vadivel and Janardhanan, 2000); the seeds have been found to be rich in minerals such as potassium, calcium, magnesium and phosphorus which are essential for growth performances.

1.4 Aim of the Study

To evaluate the utilization of fermented *Mucuna pruriens* leaf meal as a replacement for soyabean meal in *Clarias gariepinus* fingerlings diets.

1.5 Objectives

The specific objectives of the study were:

I. To determine the proximate composition and anti-nutritional compounds of raw and processed *Mucuna pruriens* leaf meal.

II. To determine the macro element, and amino acid profile of raw and fermented *Mucuna pruriens* leaf meal.

III. To determine the growth performance and feed utilization of *Clarias gariepinus* fed in the various inclusion levels of fermented *Mucuna pruriens* leaf meal diets.

IV. To assess the cost benefit of replacing soyabean meal with graded levels of fermented *Mucuna pruriens* leaf meal in the diets.

1.6 Hypotheses

I. There is no significant difference in the proximate composition and anti-nutrient compounds of raw and processed *Mucuna pruriens* leaf meal

II. There is no significant difference in the amino acids profile and minerals content of raw and fermented *Mucuna pruriens* leaf meal.

III. Growth performance and feed utilization of *Clarias gariepinus* fed various inclusion levels of fermented *Mucuna pruriens* leaf meal diets does not differ significantly.

IV. There is no significant difference in the cost of replacing soya bean meal with graded levels of fermented *Mucuna pruriens* leaf meal in the diets.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology and Culture of *Clarias gariepinus*

With more than 100 different species of the genus *Clarias* described in Africa, a recent systematic revision based on morphological, anatomical and biographical studies has been carried out by Teugels (1986), recognizing only 32 valid species. The large African species which are of interest for aquaculture belong to the subgenus *Clarias* (FAO, 1996). *Clarias gariepinus* which is generally considered to be one of the most important tropical catfish species for aquaculture, has an almost Pan-African distribution, ranging from the Nile to West Africa and from Algeria to Southern Africa. They also occur in Asia Minor (Israel, Syria and South of Turkey) (FAO, 1996).The species is euryphagous and generally regarded as an opportunistic, omnivorous predator (FAO, 2010).

Catfish are predominantly bottom feeders, but their feeding habits are adaptable and they occasionally filter feed in groups at the water surface. There are four recognized feeding modes, viz. individual foraging, individual shoveling, surface feeding and formation feeding (FAO, 2010).The species is equipped to feed on a variety of food organisms ranging from phytoplankton to fish. Predation is more efficient on invertebrate prey. Most feeding takes place at night on active benthic organisms, but they may also feed during the day and at the water surface. Individual bottom foraging is the normal mode of feeding, although catfish may also feed in groups at the water surface (Bruton, 2010).

They are not specific in their food requirements. They are known to feed on insects, plankton, snails, crabs, shrimp, and other invertebrates. They are also capable of eating dead animals,

birds, reptiles, amphibians, small mammals, other fishes, eggs, and plant matter such as fruit and seeds (Bruton, 2010).

2.2 Nutritional requirements of *Clarias gariepinus*

Although the species is euryphagic, it feeds predominantly on fish (Bruton, 1979). Its propensity toward a carnivorous feeding habit suggests that *C. gariepinus* has a relatively high dietary protein requirement, in the order of 40–50 percent of crude protein on a dry weight basis(FAO, 2010). The fact that the animal also feeds on plant material reflects its ability to digest plant proteins and utilize carbohydrates as an energy source (Van Weerd, 1995). From a farming perspective, euryphagy holds the benefit that a wide variety of feed ingredients of animal and plant origin may be considered in formulating feeds that will satisfy the fish's dietary requirements (FAO, 2010).

2.2.1 Carbohydrate requirements

Carbohydrate-containing feed stuffs are available in great quantities at low prices. Carbohydrates (starches and sugars) are the most economical and inexpensive sources of energy for fish diets. Carbohydrates are used in fish diets primarily as energy sources and for their binding properties (Krogdahl *et al*., 2005). Starches, pectins and hemicelluloses have pellet-binding characteristics of great importance to feed manufacturers (Krogdahl *et al*., 2005). Therefore, carbohydrates may be added to the feed in excess of the amounts that can be efficiently utilized for energy by the fish. Cooking starch during the extrusion process makes it more biologically available to fish. In fish, carbohydrates are stored as glycogen that can be mobilized to satisfy energy demands (Steven, 2009). Fish species differ greatly in their ability to digest carbohydrates, and this variability reflects anatomical and functional

deference of the gastrointestinal tract and associated organs. Digestive functions capable of hydrolyzing a greater variety of carbohydrate-containing feed stuffs have developed in herbivorous and omnivorous fish in contrast to carnivorous fish (Steven, 2009). Digestive organs of fish vary from short and simple to complex ruminant-type, reflecting the variation in nutrient sources (Krogdahl *et al*., 2005). Robinson (1991) reported that utilization of carbohydrate by catfish appears different, depending on the complexity of the carbohydrate starch or dextrin (partially hydrolysed starch) which are used more efficiently by catfish than sugars such as glucose and sucrose.

2.2.2 Protein and amino acids requirements

Proteins are composed of carbon (50%), nitrogen (16%), oxygen (21.5%), and hydrogen (6.5%)(CAN, 1993). Fish are capable of using a high protein diet, and these proteins are formed by linkages of individual amino acids. Although over 200 amino acids occur in nature, only about 20 amino acids are common (CAN, 1993). Of these, 10 are essential (indispensable) amino acids that cannot be synthesized by fish. The 10 essential amino acids that must be supplied by the diet are: methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine. Of these, lysine and methionine are often the first limiting amino acids (Eyo, 2003). Protein levels in aquaculture feeds generally average 18-20% for marine shrimp, 28 – 32% for tilapia, 32 – 38% for catfish, 38 – 42% for hybrid striped bass (Craig and Helfrich, 2004). Protein requirements usually are lower for herbivorous fish and omnivorous fish than they are for carnivorous fish, and are higher for fish reared in high density (re-circulating aquaculture) than low density (pond aquaculture) systems (CAN, 1993).Protein requirements generally are higher for smaller fish, as fish grow larger; their protein requirements usually decrease. Protein requirements also vary with

rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish. Protein is used for fish growth if adequate levels of fats and carbohydrates are present in the diet. If not, protein maybe used for energy and life support rather than growth (CAN, 1993).

2.2.3 Vitamins requirements

Vitamins are highly diverse in chemical structure and physiological function (Robinson, 1993). They are generally defined as organic compounds that are required in small amounts in the diet for normal growth, health and reproduction of animal. They often are not synthesized by fish, and must be supplied in the diet (FAO, 1988).

The two groups of vitamins are water-soluble and fat-soluble. Water-soluble vitamins include: the B vitamins, choline, inositol, folic acid, pantothenic acid, biotin and ascorbic acid (vitamin C). Of these, vitamin C probably is the most important because it is a powerful antioxidant and helps the immune system in fish. The fat-soluble vitamins include vitamin A, retinols (responsible for vision); the D vitamins, cholecaciferols (bone integrity); E vitamins, the tocopherols (antioxidants); and K vitamins such as menadione (blood clotting, skin integrity) (FAO, 1988). Of these, vitamin E receives the most attention for its important role as an antioxidant. Deficiency of each vitamin has certain specific symptoms, but reduced growth is the most common symptom of any vitamin deficiency (FAO, 1988; King and Burgess, 1993).Scoliosis (bent backbone symptom) and dark coloration may result from deficiencies of ascorbic acid and folic acid vitamins, respectively (King and Burgess, 1993). Catfish feeds are generally supplemented with a vitamin premix that contains all essential

vitamins in sufficient quantities to meet dietary requirements including losses due to feed processing (FAO, 1988).

2.2.4 Lipid and fatty acid requirements

Lipids are the generic names assigned to a group of fat soluble compounds found in the tissues of plants and animals: and are broadly classified as: fats, phospholipids, sphingomyelins, waxes, and sterols.

Lipids (fats and oils) are a highly digestible source of concentrated energy; it contains about 2.25 times as much energy as does an equivalent amount of carbohydrates. Lipids play several important roles in an animal's metabolism, such as supplying essential fatty acids, serving as a vehicle for absorption of fat-soluble vitamins, and serving as precursors for steroid hormones and other compounds (Robert, 1979). The use of lipids in fish feeds may increase feed palatability, as body lipid stores affect the flavour of fish, as well as help maintain neutral buoyancy. The type and amount of lipid used in catfish diets is based on essential fatty acid requirements, economics, constraints of feed manufacture, and quality of fish flesh desired (Robert, 1979).

Essential fatty acids (EFA) are ones that cannot be synthesized in the animal's body; thus, they must be provided in the diet. The EFAs are classified based on their chemical structure and are designated as either omega-3 (n-3) or omega-6 (n-6) fatty acids. In general, fish appear to require n-3 fatty acids, while land animals appear to require n-6 fatty acids. However, this generalization does not always hold true. Certain fish (including some species of tilapia and carp) apparently require both n-3 and n-6 fatty acids (Robert, 1979).

The EFA requirements for catfish and most other warm water fish have not been precisely defined, but catfish apparently require a small amount of n-3 fatty acids. It appears that 1–2% dietary linolenic acid (18:3 n-3) is as good as 0.5–0.75% highly unsaturated fatty acids for normal growth, because catfish apparently elongate and desaturate linolenic acid to synthesize highly unsaturated fatty acids (Robert, 1979). The EFA requirement can be supplied by marine fish oil such as menhaden oil. Natural food organisms, such as zooplankton, found in the pond are also a good source of EFA. Catfish appear to have the ability to synthesize most of their fatty acids; thus, nutritionally there may be no "best" level of dietary lipid except that needed to provide EFA (Robert, 1979). Generally, weight gain and feed efficiency are depressed when fish are fed diets containing 15% or more lipids (Robert, 1979; Robinson *et al*., 2001).

Catfish have been fed diets containing up to 16% lipid without conclusive evidence as to which level is best for optimum growth (Robinson *et al*., 2001). Even so, there is likely an optimum level of lipid to be used in catfish feeds with respect to protein sparing, product quality, and constraints of feed manufacture. Since lipid is a concentrated source of energy and can spare the more expensive protein, some lipid should be included in catfish diets. However, too much dietary lipid may result in excessive fat deposition in the body cavity and tissues that may adversely affect processing yield, product quality, and storage of processed products (Robinson *et al*., 2001). Also, high-lipid feeds are more difficult to pellet, but if needed, supplemental lipid can be sprayed onto the finished feed pellets. Lipid levels in commercial feeds for food-sized catfish rarely exceed 5–6% (Robinson, 1991). About 3–4% of the lipid is inherent in the feed ingredients, with the remaining 1–2% being sprayed onto the finished pellets. Spraying feed pellets with lipid increases dietary energy and aids

in the reduction of feed dust ("fines"). A mixture of vegetable and animal lipids has been used in commercial catfish feeds (Robinson, 1991).

In addition, there is evidence that dietary menhaden oil levels of 2% or more reduced survival of catfish exposed to the bacterial pathogen *Edwardsiella ictaluri* (Fracalossi and Lovell, 1994; Li *et al*., 1994). The negative effects of menhaden oil on bacterial resistance are likely caused by the immuno-suppressive effect of highly unsaturated n-3 fatty acids. Catfish feeds manufactured in Mississippi are generally sprayed with catfish oil, which is a local product extracted from catfish offal. In some cases, menhaden oil or a mixture of catfish oil and menhaden oil is used.

2.2.5 Minerals requirements

Minerals are inorganic elements necessary in the diet for normal body functions. Mineral requirements of fish are similar to those of terrestrial animals (Helfrich and Craig, 2002). They can be divided into two groups (macro-minerals and micro-minerals) based on the quantity required in the diet and the amount present in fish. Common macro-minerals are sodium, chloride, potassium and phosphorous. These minerals regulate osmotic balance and aid in bone formation and integrity. Micro-minerals (trace minerals) are required in small amounts as components in enzyme and hormone systems. Common trace minerals are copper, chromium, iodine, zinc and selenium. Fish can absorb many minerals directly from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet (Hepher, 1990).

Fourteen minerals are considered essential for catfish. Among macro-minerals, phosphorus is particularly important in fish feeds because fish require a relatively large quantity of the

mineral in the diet (Hepher, 1990). Feedstuffs, especially those of plant origin are poor sources of biologically available phosphorus, and fish do not obtain significant amounts of phosphorus from pond water. Therefore, catfish feeds are usually supplemented with phosphorus to provide the 0.3-0.4% biologically available phosphorus that is required.

2.3 Non-conventional Feedstuffs of Plant Origin in Catfish Feed Formulation

Commercial fish feeds are usually expensive because the traditional or conventional protein source ingredient such as fish meal, soya bean meal, groundnut cake, maize, millet, guinea corn, and vegetable oil are competing for human and livestock consumption (Madu *et al*., 2003). Unconventional fish feeds are potential feed ingredients, which have not been fully used in fish feed production for some reasons:

i. They are not well known or understood.
ii. No effective study of the method of production with a view to commercializing them.
iii. They are not readily available and,
iv. They can be toxic or poisonous (Moel and Harwart, 2005).

These feeds are generally referred to as unconventional feed ingredients, and they could contain high quality feed nutrients that can compare favourably with conventional feed types. They are expected to be cheaper by virtue of less or no competition for human consumption and these feedstuffs can be of animal or plant source (Jamu and Ayinla, 2003).They are locally available feed stuffs that are not standardized, whose usage is not widely-spread and they are not consumed by man in most cases. Their utilization in aqua feed is very common especially in the rural area of sub-saharan Africa, among low income group that are actively

engaged in fish farming (Omoregie, 2001; Baraigi *et al*., 2004; Wingkeong, 2002; Bekibele, 2005).

Okomoda *et al*. (2015) reported the use of *Leucaena leucocephala* leaf in the diet of the *Clarias gariepinus* fingerlings and concluded that fish fed up to 20% Leucaena leaf meal had the best performance in terms of weight gain, specific growth rate (SGR) and feed conversion ratio

Omoregie (2001) reported the use of Palm kernel meal in juveniles of *Labeo senegalensis* diets, and concluded that 10% inclusion level use of Palm kernel meal had the best growth performance. Also Wingkeong (2002) also reported no significant difference in the growth performance of hybrid Asian catfish fed up to 20% Palm kernel meal. Banyigi *et al*. (2004) reported that diets containing Bambara groundnut meals fed to Nile tilapia (*Oreochromis niloticus*) and *Clarias gariepinus* had the best growth performance and feed utilization at 50% inclusion level. Bekibele (2005) studies the effect of Mucuna beans to replace soyabean meal and to evaluate its effects on growth performance of *Clarias gariepinus* and concluded that Mucuna bean protein can replace soya bean meal protein up to 50% inclusion level without adverse effect on the growth performance; rather it enhances the quality of the carcass by reducing body fat content.

2.4 Non-conventional Feedstuffs of Animal Origin in Catfish Feed Formulation

The non conventional feed stuff of animal origin are high quality feed ingredients that could compare to some extent with the conventional types (Okoye and Sule, 2001). These are cheaper by virtue of the fact that there is no competition for human consumption. However, the only problem with these feed stuffs is their unavailability in large commercial quantities

for the sustenance of aquaculture industry (Roberts, 1989)). In most parts of Africa, these are available in small quantities and their production is inconsistent and sporadic in nature.

Idowu and Afolayan (2008) used ration of Maggot meal as replacement for fish meal and stated that the best growth performance and feed utilization would be realized by replacing fish meal with 50% of maggot meal in the diet of *Clarias gariepinus*. Keremah and Green (2005) evaluated the effect of replacing fish meal with graded level of fish offal on growth and survival of hybrid catfish fingerings and concluded that the diets with fish meal replaced by 25% and 50% fish offal could be fed to hybrid catfish without adverse effect on growth and survival rate. Nwanna (2003) evaluated nutritional value and digestibility of fermented shrimp head waste meal by African catfish *Clarias gariepinus* and results findings showed no significant variation in apparent digestibility coefficient (ADC) of nutrients $ADC_{protein}$ and ADC_{energy} , protein efficiency ratio (PER), food protein energy conversion ratio (FCR) and hepasomatic index of the fishes fed all the diets, but comparative costs analyses indicated that the best profit margin would be realized by replacing fish meal with 30% FSHM in the diet of the fish.

Madu and Ufodike (2003) reported that live maggot has a crude protein of 43.8,crude lipid 1.9 and 14.3 crude fibre, and it has also been used in the diets of *Clarias gariepinus* fingerlings. The result indicated that live maggot utilization by *C. gariepinus* is of better economic value as compared to the costly compounded fish feed. The *Clarias gariepinus* fingerlings were able to convert the protein present in the maggot more efficiently than the compounded feed. Chicken feather and offal were tried by Faturoti (2000), it was reported that the gross profit and profit index were best at 75% and 100% inclusions of chicken offal meal in the diet fed to *Clarias gariepinus.* With the emergence of large scale poultry

industries in Nigeria, there is the availability of enormous poultry waste that can be used in fish feed formulation.

Erturk and Sevgili (2003) replaced fish meal with poultry by-product meal on apparent digestibility, body composition and protein efficiency ratio in a practical diet for Rainbow Trout, *Onchorynchus mykiss*. They concluded that poultry by-product meal in a proportion of 20% may replace about 40% of fish meal in Rainbow trout diet without significant impairment of growth.

2.5 Ecology of *Mucuna pruriens*

The *Mucuna pruriens*, belongs to the family Fabaceae and the genus *Mucuna* includes approximately 150 species of annual and perennial legumes and is among the various under-utilized wild legumes, the velvet bean *Mucuna pruriens* is widespread in tropical and sub-tropical regions of the world (Lampariello *et al*., 2012). It is a twinning and tropical legume known as velvet bean and of common names such as: cow-itch, cowhage, Bengal beans, itchy bean, buffalo bean and velvet bean (English), while it's called Agbara (Igbo), Yerepe (Yoruba) and Karara (Hausa).

The plant *Mucuna pruriens*, widely known as "velvet bean," is a vigorous annual climbing legume originally from southern China and eastern India, where it was at one time widely cultivated as a green vegetable crop (Duke, 1981). It is one of the most popular green crops currently known in the tropics; velvet beans have great potential as both food and feed as suggested by experiences worldwide (Lampariello *et al*., 2012). The velvet bean has been traditionally used as a food source by certain ethnic groups in a number of countries. For instance, it is cultivated in Asia, America, Africa, and the Pacific Islands, where its pods are

used as a vegetable for human consumption, and its young leaves are used as animal fodder (Lampariello *et al*., 2012).

According to Lampariello *et al*. (2012) the plant has long, slender branches; alternate, lanceolate leaves; and white flowers with a bluish-purple, butterfly-shaped corolla. The pods or legumes are hairy, thick, and leathery; averaging 4 inches long; are shaped like violin sound holes; and contain four to six seeds. They are of a rich dark brown color, and thickly covered with stiff hairs. In India, the mature seeds of *Mucuna* bean are traditionally consumed by a South Indian hill tribe, the Kanikkars, after repeated boiling to remove anti-nutritional factors.

Most *Mucuna* species exhibit reasonable tolerance to a number of abiotic stresses; including drought, low soil fertility, and high soil acidity, although they are sensitive to frost and grow poorly in cold, wet soils (Duke, 1981). The genus thrives best under warm, moist conditions, below 1500 m above sea level, and in areas with plentiful rainfall. Like most legumes, the velvet bean has the potential to fix atmospheric nitrogen via a symbiotic relationship with soil microorganisms (Lampariello *et al*., 2012).

2.6 Nutritive Value of *Mucuna pruriens*

It is considered a viable source of dietary proteins (Pugalenthi *et al*., 2005) due to its high protein concentration (23–35%) in addition its digestibility, which is comparable to that of other pulses such as soya bean, rice bean, and lima bean (Gurumoorthi *et al*., 2003). It is therefore regarded a good source of food. The velvet bean has been traditionally used as a food source by certain ethnic groups in a number of countries. Tavares *et al*.(2015) investigated the nutritional composition, phytocemicals and microbiological quality of the

legume, *Mucuna pruriens* and concluded that both flour and extract of *Mucuna pruriens* are good sources of carbohydrates, fiber and protein and also exhibit considerable amounts of iron, potassium and phosphorus. Also, Lampariello *et al.* (2012) stated that *Mucuna pruriens* is an exceptional plant and it is a good source of food, as it is rich in crude protein, essential fatty acids, starch content, and certain essential amino acids.

2.7 Medicinal usage of *Mucuna pruriens*

All parts of the Mucuna plant possess medicinal properties (Sathiyanarayanan and Arulmozhi, 2007). *M. pruriens* is a popular Indian medicinal plant, which has long been used in traditional Ayurvedic Indian medicine, for diseases including Parkinsonism (Sathiyanarayanan and Arulmozhi, 2007). This plant is widely used in Ayurveda, which is an ancient traditional medical science that has been practiced in India since the Vedic times (1500–1000 BC). *Mucuna pruriens* is reported to contain L-dopa as one of its constituents (Chaudhri, 1996). The beans have also been employed as a powerful aphrodisiac in Ayurveda (Amin *et al*., 1996) and have been used to treat nervous disorders and arthritis (Jeyaweera, 1981). The bean, if applied as a paste on scorpion stings, is thought to absorb the poison (Jeyaweera, 1981).

The non-protein amino acid-derived L-dopa(3,4-dihydroxy phenylalanine) found in this underutilized legume seed resists attack from insects, and thus controls biological infestation during storage. According to D'Mello (1995), all anti-nutritional compounds confer insect and disease resistance to plants. Further, L-dopa has been extracted from the seeds to provid e commercial drugs for the treatment of Parkinson's disease. L-Dopa is a potent neurotransmitter precursor that is believed, in part, to be responsible for the toxicity of the

Mucuna seeds (Lorenzetti *et al*., 1998). Anti epileptic and anti-neoplastic activity of methanol extract of *M. pruriens* has been reported (Gupta *et al*., 1997). A methanol extract of *M. pruriens* seeds has demonstrated significant in vitro anti-oxidant activity, and there are also indications that methanol extracts of *M. pruriens* may be a potential source of natural anti-oxidants and anti-microbial agents (Rajeshwar *et al*., 2005).Its anti-venom activities have been investigated by Guerranti *et al*. (2002) and its anti-helminthic activity has been demonstrated by Jalalpure(2007). The *M. pruriens* has also been shown to be neuro-protective (Misra and Wagner, 2007), and has demonstrated analgesic and anti-inflammatory activity (Hishika *et al*., 1981).The root is used as diuretic, tonic and stimulant. It is recommended for the nervous system disorder, facial paralysis, hemiplegia, delirium in fevers, and dropsy, in decoction. The infusion of the pods is also good for dropsy. The hairs of the pods are used for threadworms. The seeds are considered astringent, aphrodisiac, tonic, nervine and nutritive. They are given in powder or in decoction in cases of leukorrhea, spermatorrhea, and menstrual disorders (Voogelbreinder, 2009).

2.8 Phytochemical Composition of *Mucuna pruriens*

Ifemeje (2016) investigated chemical and phytochemical compositions of *Mucuna pruriens* leaves. He found out that the crude proteins, 34.16 had high percentage of the nutritional composition of the *M. pruriens* and crude fibre, 32.50 with relatively high quantity of ash, 5.80 and crude fat, 2.30. *M. pruriens* contained 8.10 mg/100g of iron and 5.10 mg/100g of zinc. Among the three anti-oxidant vitamins analysed (vitamin A, C and E), vitamin C concentration was highest (82.50 mg/100g) with concentration of vitamin A as the least (0.50 mg/100g). The phytochemical analysis revealed a relatively high amount of oxalates, 5.00 mg/100g. Saponins, tannins, alkaloids, flavonoids, phytates and cyanogenic glycoside were

respectively found to be 3.50mg/100g, 3.25 mg/100g, 2.67 mg/100g, 2.86 mg/100g, 1.00 mg/100g and 0.02 mg/100g.He concluded that *M. pruriens* is a very good source of phytochemical, mineral and vitamins.

2.9 Anti-nutrients Present in *Mucuna pruriens*

Many food stuffs that are commonly used in preparing diets for animals contain anti-nutritional factors. These factors interfere with the utilization of dietary nutrients in a variety of ways including reducing protein digestibility, binding to various nutrients or damaging the gut wall and thereby reducing digestive efficiency (Karoly, 2011). Lampariello *et al.* (2012) stated that in addition to the low levels of sulfur-containing amino acids in *M. pruriens* seeds, the presence of anti physiological and toxic factors may contribute to a decrease in their overall nutritional quality. These factors include polyphenols, trypsin inhibitors, phytate, cyanogenic glycosides, oligosaccharides, saponins, lectins, and alkaloids. Polyphenols (or tannins) are able to bind to proteins, thus lowering their digestibility. Phenolic compounds inhibit the activity of digestive as well as hydrolytic enzymes such as amylase, trypsin, chymotrypsin, and lipase. Trypsin inhibitors belong to the group of proteinase inhibitors that include polypeptides or proteins that inhibit trypsin activity. Tannins exhibit weak interactions with trypsin, and thus also inhibit trypsin activity. Phytic acid [myoinositol-1,2,3,4,5,6-hexa(Dihydrogen phosphate)] is a major component of all plant seeds, which can reduce the bioavailability of certain minerals such as zinc, calcium, magnesium, iron, and phosphorus, as well as trace minerals, via the formation of insoluble complexes at intestinal pH. Phytate-protein complexes may also result in the reduced solubility of proteins, which can affect the functional properties of proteins (Lampariello *et al.,* 2012). Cyanogenic glycosides are plant toxins that upon hydrolysis liberate hydrogen

cyanide. The toxic effects of the free cyanide are well documented and affect a wide spectrum of organisms since their mode of action is inhibition of the cytochromes of the electron transport system (Laurena *et al*., 1994).

2.10 Methods Used in Anti-Nutritional Factors Removal from Feed stuffs

Santosh and Richard (2002) reported that physical and chemical methods employed to reduce or remove anti-nutritional factors including soaking, cooking, germination, fermentation, selective extraction, irradiation n and enzymic treatment. They also stated industrial processes; including canning, toasting, fractionation and isolation of protein concentrate have also been shown to be effective in reducing or removing anti-nutritional factors. However, it should be noted that processing can also introduce undesirable compounds for example volatile aldehydes, ketones and peroxides as a direct result of lipid oxidation, or reduce levels of desirable compounds for example protein and essential minerals. They further stated that application of a single technique is frequently insufficient for effective treatment and so combinations are commonly employed, thus the most effective methods for reducing saponin contents have been reported to be soaking and cooking.

An effective means of reducing phytic acid content in sesame seeds was suggested by Mukhopadyay and Ray (1999) who reported a substantial decrease in phytic acids content after bacterial fermentation with *Lactobacillus acidophilus* and also reduces amount of tannin in the seed from 20 to10 gkg^{-1}.

2.11 Cost benefit of using plant protein sources

Studies have been conducted on the use of non conventional feedstuffs such as leaves and tubers, and plant by products which can probably reduce feed cost and ultimately the

production cost in fish farming in particular. For the purpose of nutritional and economic benefits, previous researchers have made attempts at increasing the use of non conventional plant and animal materials such as maize cobs (Sogbesan *et al*., 2012). Uchechukwu *et al*. (2016) studies effect of feed protein: Lipid Ratio on Growth Parameters of African Catfish *Clarias gariepinus* after fish meal substitution in the diet with bambaranut (*Voandzeia subterranea*) meal and soya bean (*Glycine max*) meal and concluded that bambaranut and soya bean meal could favourably replace over 60% fish meal in the diets of Africa catfish (*Clarias gariepinus*) with better economic conversion ratio than 60% fish meal diets and non-significant reduction in growth rate which is compensated by cheaper production cost.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and preparation of *Mucuna pruriens* leaf meal

Mucuna pruriens leaves were obtained from Hanwa Area of Zaria and authentication was carried out in the Department of Botany, Ahmadu Bello University Zaria by the Herbarium

curator. Then the leaves were washed using tap water to remove the dust and different methods of processing were employed to prepare the leaf meal and thereafter the proximate analysis and anti-nutrients of the samples was determined. The samples were subjected to the following processing techniques:

i. The *Mucuna pruriens* leaf was air-dried (ADMLM) at room temperature for a week,
ii. The *Mucuna pruriens* leaf weighing 1 kilogram was soaked in cold water at 1 kilogram leaf per 5 litre of water (SCMLM) for 36 hours. The leaf were then collected by losing the water and then air-dried at room temperature for a week. The leaf was later milled for further use
iii. The *Mucuna pruriens* leaf weighing 1 kilogram was soaked in 60°C hot water (SHMLM) and was allowed to cool down for 24 hours. The leaf were then collected by losing the water and air-dried at room temperature for a week. The leaf meal was later milled for further use.
iv. The *Mucuna pruriens* leaf weighing 1 kilogram was fermented with yeast (*Saccharomyces cerevisiae*) of 2g in airtight container for 72 hours at room temperature (Padmavathy and Shobha, 1987).

3.2 Feed Formulation

The feed ingredients used in the experiment composed of fish meal (Clupeid), Soya bean meal, fermented *Mucuna pruriens* leaf meal, yellow maize, bone meal, palm oil, salt, vitamin premix, methionine and lysine.

3.3 Experimental Site

The experiment was conducted in the Fisheries Research Laboratory of the Department of Biology, Ahmadu Bello University Zaria, Nigeria.

3.4 Experimental Fish

A total number of 180 fingerlings of *Clarias gariepinus* of mean weight (6.20±1.96g – 6.80±1.97g) and length 7.8±0.24cm were procured from reputable fish farm in Zaria, Nigeria. The fish were transported in 50L plastic container, where they were acclimatized and fed commercial feed for two weeks.

3.5 Experimental Feed Ingredients

All the dietary ingredients were separately processed and milled to fine particle size. The soya beans were thoroughly sorted to remove all extraneous materials. The clean beans were toasted for about 15 minutes and then de-hulled, followed by winnowing, milling and sieving (Ademulegun and Koleosho, 2012). The dry ingredients were then weighed out according the formulation (Table 3.1), following Pearson Square Method. The ingredients were mixed until uniformly blended. Water was added slowly to the mixture with continuous stirring to form dough.

3.6 Proximate Analysis

Proximate analysis of major dietary ingredients, experimental diets, fish whole body and faecal matter samples were carried out according to AOAC (1990) procedures. Whole body proximate analysis was used to determine body composition of fish. The proximate analysis

followed methods described by the AOAC (1990). Components such as moisture, crude protein, crude lipid and ash were analyzed.

3.6.1 Crude protein

The micro-kjeldahl method according to AOAC (1990) was used for the crude protein determination in triplicate as follows; 200g of sample was digested in concentrated sulphuric acid and ammonia from the digest is released which reacts with 40% sodium hydroxide and distilled water, trapped in 2% boric acid and quantified by titration against 0.2M hydrochloric acid. Crude protein was calculated by multiplying the nitrogen by a factor 6.25 based on the assumption that most plant protein contains 16% nitrogen. Thus, 1mg nitrogen = 6.25 protein.

3.6.2 Moisture

Moisture determinations are used to convert all other nutrients to a dry matter basis. Sample containing 2g dry material was dried to constant weight at 95 – 100 C under pressure <100 mmHg. Loss in weight was reported as moisture. This was achieved by weighing the sample before and after drying. Moisture content was calculated using the formula below (AOAC, 1990).

$$\text{Moisture} = \frac{\text{initialmass} - \text{finalmass}}{\text{initialmass}} \times 100$$ (Sullivan, 2008)

Table 3.1: Gross Compositions of Experimental Diets

	Treatments

Ingredients	$A_{(100\% \text{ FMLM})}$	$B_{(75\% \text{ FMLM})}$	$C_{(50\% \text{ FMLM})}$	$D_{(25\% \text{ FMLM})}$	$E_{(0\% \textbf{ FMLM})}$
Fermented Mucuna leaf meal	**24.72**	**18.54**	**12.36**	**6.18**	**0**
Fish meal	**49.44**	**49.44**	**49.44**	**49.44**	**49.44**
Soya bean meal	**0**	**6.18**	**12.36**	**18.54**	**24.72**
Maize	**15.84**	**15.84**	**15.84**	**15.84**	**15.84**
Bone meal	**1**	**1**	**1**	**1**	**1**
Palm oil	**3.5**	**3.5**	**3.5**	**3.5**	**3.5**
Salt	**0.8**	**0.8**	**0.8**	**0.8**	**0.8**
Vit. Premix	**0.7**	**0.7**	**0.7**	**0.7**	**0.7**
Methionine	**2**	**2**	**2**	**2**	**2**
Lysine	**2**	**2**	**2**	**2**	**2**
Total	**100**	**100**	**100**	**100**	**100**

3.6.3 Ash content

To determine the ash content, samples were weighed before and after being placed in a muffle furnace for approximately 6hrs or until powdery white. Then cooled, and then place in desiccators for further cooling to room temperature before the final mass was taken. The formula below was used to calculate the ash content.

$$\text{ASH} = \frac{\text{initial mass} - \text{final mass}}{\text{initial mass}} \times 100$$

(Sullivan, 2008)

3.6.4 Lipid

To determine the crude lipid content, the Soxhlet method (AOAC, 1990) was used to extract the lipid from the samples. Approximately 1 g of sample was placed in the cellulose thimble and extracted using 150 ml of acetone solvent. The system was heated in a water bath for approximately 10 h after which time the solvent was evaporated using a rotary evaporator. The flask was placed in a drying oven for 1 h to remove water. After cooling, the flask was weighed and the lipid content calculated using the following formula:

$$\text{Lipid} = \frac{\text{Initial lipid mass} - \text{Final lipid mass}}{\text{initial lipid mass}} \times 100$$

(Sullivan, 2008)

3.7 Determination of Anti-nutritional Compounds

Triplicate samples of the raw and processed *Mucuna pruriens* leaf were taken to the Department Animals Science of the Ahmadu Bello University Zaria for analysis of an-nutritional factors according to method described by AOAC (1990).

3.7.1 Tannin

Two grams (2g) of the ground sample was defatted for 2hours using soxhlet extraction apparatus. The residue was placed in an oven for 24hours, retrieved and boiled at 100°C with300ml of distilled water, diluted to 500ml in a standard volumetric and filtered through nonabsorbent cotton wool. A volume of 25ml of the infusion was measured in to 2litre porcelain dish and titrated with 0.1N oxalic acid until blue solution changed to green, then few drops of 0.1potassium permanganates was added. The difference between the two titration was multiplied by 0.006235 to obtain the amount of tannin in sample, since 0.1N oxalic acid=0.006235g tannin (AOAC, 1990).

3.7.2 Oxalate

Two gram (2g) of aliquot of the ground *Mucuna pruriens* leaf was weighed to a 250 ml flask; 190ml distilled water and 10ml of 6m hydrochloric acid were added. The mixture was digested for 1hour on boiling water bath, then cooled, transferred in to a 250ml volumetric flask, diluted to volume and filtered. Four drops of methyl indicator were added followed by concentrated ammonia until the solution turn to faint yellow. It was then heated to 100°C, allowed to cool and filtered. The filtrate was boiled with 10ml of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through whatman No. 40 filter paper. The precipitate was rinsed several times with distilled water, transferred to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80°Cthen cooled and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. Blank test was also run for the test sample. From the amount potassium permanganate the oxalate was calculated according to methods described by AOAC (1990). Thus;1ml of potassium permanganate =2.24mg oxalate.

3.7.3 Determination of Saponin

The standard method of A.O.A.C (1990) was used to determine Saponin in the samples. A gravimetric method employing the use of Soxhlet extractor and two different organic solvent was used. The first solvent extracted lipids and interfering pigments while the second solvent extracted the Saponin proper. A known weight of the dried ground sample was weighed and fitted unto the soxhlet apparatus (bearing the sample containing thimble) and methanol poured into the flask. The methanol was enough to cause a reflux. The saponin was then exhaustively extracted for3hours. The flask was re-weighed. The difference in weight represents the weight of saponin extracted.

3.7.4 Phytic acid (phytate)

Phytic phosphorus was determined by the method of AOAC (1990). A known weight of each ground sample was soaked into 100mlof 2% HCl in a conical flask, 50$cm^3$of 0.3% potassium thiocynate solution was added. The mixture was titrated in a standard solution of $FeCl_3$ until a brownish-yellow colour persisted for 5 minutes. The concentration of the $FeCl_3$ was 1.04% w/v.Mole ratio of Fe to Phytate = 1:1

$$\text{Conc. of phytate phosporus} = \frac{\text{Titrevalues}}{1000} \times \text{weight of sample}$$

3.7.5 Hydrogen Cyanide

The alkaline titration method was used to determine the cyanide content of the cake. 5g of sample was placed in a 2000ml conical flask and 50ml of distilled water was added. The content of the flask was stirred and allowed to stand for 4hrs. The filtrate was collected in to a beaker using a glass wool which was placed in a funnel and steamed, distilled in to 20ml of 2.5% sodium hydroxide. About 75ml of the distilled water was collected. This was titrated with0.02N silver nitrate after the addition of 8ml of 6N ammonium hydroxide and 2ml of 2% potassium iodide.

3.8 Feed Formulation and Compounding

Five (5) iso-nitrogenous diets were formulated according to the nutritional requirements of the experimental fish (*Clarias gariepinus*) at 40% crude protein. The protein level was chosen on the basis of some previous studies (Fagbenro, 1998), where 40% protein in formulated feed demonstrated better growth of Catfish. Feed ingredients; soya bean, yellow maize, fish meal and the fermented *Mucuna pruriens* leaf meal were separately milled, screened to fine particle size (<250µm).The dry ingredients were then weighed out according the formulation (Table 3.1)following Pearson Square Method. The ingredients were mixed until uniformly blended. Water was added slowly to the mixture with continuous stirring until dough was formed. This was followed by pelleting using a die size of 2mm. The pelleted feeds were sun dried and packaged in two layers of plastic bags measuring two kilogram (3kg) each and stored in a well ventilated room under ambient temperatures.

3.9 Experimental Design

The experimental fish were acclimated for two weeks, during which they were fed commercial feed (Coppens) at a daily rate of 5% of biomass with half of the daily ration fed in the morning(8:00am) and the other half in the evening (5:00pm). Thereafter, batch weighing and length measurement of fish was done to ascertain their initial mean weight in grams and initial mean length in centimetres, using top-load weighing balance (Meter Tolardo 567) and fish measuring board, respectively, before the commencement of the experiment and subsequently after every two weeks. The fingerlings were randomly grouped into six treatments of ten fish per fifty litre (50L) plastic tanks. The treatments were allocated as, $A_{(100\%FMLM)}$, $B_{(75\%FMLM)}$, $C_{(50\%FMLM)}$, $D_{(25\%FMLM)}$, and $E_{(0\%FMLM)}$respectively. Each treatment was in triplicate. The experiment lasted for 12 weeks.

3.9.1 Feeding procedures

The fish were fed at five percent (5%) of their body weight twice daily during the week. To monitor the amount of feed administered, each tank had its own labelled container.

3.9.2 Determination of water quality parameters

Water quality parameters such as Temperature, Dissolved Oxygen and pH were monitored daily using mercury -in- glass Thermometer and automatic DO/pH Analyzer (model jpb 607)respectively.

3.10 Growth Measurements

Body weighing (g) and body length (cm) of individual fish of each experimental tank were recorded every 2 weeks (14 days) during the experimental period using weighing balance and fish measuring board respectively. Mortalities were recorded as they occurred. Parameters used in evaluating growth performance in this study were, weight gain by fish

and specific growth rate (SGR). All these parameters were measured for all the treatments and their replicates including the control diets.

a) Weight gain (WG): - Is the difference between the final body weight and the initial bodyweight offish over a period of time.

$$WG = W2 - W1$$

Percentage weight gain was calculated using the formula

$$\mathbf{WG} = \frac{\mathrm{FBW} - \mathrm{IBW}}{\mathrm{FBW}} \times 100$$

Where, FBW is final mean body weight (g), IBW is initial mean body weight (g).

b) Specific Growth Rate: - Is the instantaneous change in weight of fish expressed as the percentage increase in body weight per day over any given time interval. It is calculated by taking natural logarithms of body weight, and expresses growth as %.day-1 (Ricker, 1979).

$$\mathbf{SGR} = \frac{\ln\mathrm{FBW} - \ln\mathrm{IBW}}{\mathrm{D}}$$

Where D is the number of days of the experimental period.

3.11 Nutrient Utilization Parameters

A. Feed Conversion Ratio (FCR)

Conversion of feed stuffs into high quality protein by fish for human consumption at a profit for the farmer is the main objective of fish culture (Balogun *et al*., 2005) FCR is defined as the amount of dry feed fed per unit live weight gain (Nelson, 2005). It often serves as a measure of efficiency of the diet. The more suitable the diet for growth, the less food is required to produce a unit weight gain, i.e. a lower FCR (De Silva and Anderson, 1995). It is calculated as;

$$\mathbf{FCR} = \frac{\text{live weight gain}}{\text{feed fed}}$$

B. Protein Efficiency Ratio

Protein efficiency ratio (PER) is defined as the ratio between the weight gain of fish and the amount of protein fed (De Silva and Anderson, 1995):

$$\mathbf{PER} = \frac{\text{weight gain (g)}}{\text{crude protein fed(g)}}$$

C. Productive Protein Value (PPV)

PPV sometimes also called efficiency of protein utilization' (Gerking, 1971), evaluates the protein in the diet by the ratio between the protein retained in fish tissues and the dietary protein fed. PPV is determined by carcass analyses of samples of fish taken before and after feeding with the evaluated protein, and generally expressed as a percentage of the protein fed.

$$\mathbf{PPV} = \frac{\text{retained protein in tissues}}{\text{Dietary protein consummed}} \times 100$$

PPV is a more refined criterion for the evaluation of dietary protein compared to PER since it takes into account the transformation of the dietary protein into body protein rather than the overall increase in body weight (Hepher, 1988).

Due to practical constraints in experiments with fish, it is not possible to ensure that all food presented is ingested nor is it possible to collect uneaten food from the experimental tanks. Therefore for calculation of FCR, PER and PPV (ANPU – Apparent Net Protein Utilization) the amount of feed fed (instead of feed consumed/intake) will be used without correction being made for any wastage. This could actually lead to overestimation of feed and underestimation of the ratios (Smita *et al.,* 2005).

c) Carcass Composition of Experimental Fish

Whole body proximate analysis and hepatosomatic index (HSI) were used to determine carcass composition of fish. The proximate analysis will follow methods described by the AOAC (1990). Components such as moisture, crude protein, crude lipid and ash will be analysed and expressed as percentage of fresh weight. At the end of each experiment 18 fishes were randomly selected from each treatment, including the control.

Survival Rates of Fish;

$$\mathbf{R} = \frac{\text{Initial number stocked}}{\text{number survived}} \times 100\%$$

Adedeyi, (2004)

a) Apparent Digestibility Coefficient

The direct determinations of digestibility were used in this study by relating the total quantity of nutrients (protein, carbohydrates, lipids as well as calorific values) in a known quantity of feed consumed.

The apparent digestibility coefficients (ADC) for the nutrients of the diets were calculated as follows (Bureau *et al*., 1999; Forster, 1999):

$$\mathrm{ADC}(\%) = \frac{\text{nutrient intake} - \text{nutrient in faeces}}{\text{nutrient intake}} \times 100$$

3.12 Cost and Benefit Analysis of Diets

Economic Analysis of Diets

A simple economic analysis was conducted to assess the cost effectiveness of the experimental diets. Only the cost of feed was used in the calculations with the assumption that all other operating costs remained constant. Costs of the feeds were calculated using market prices of ingredients as at the time of the experiment. Economic evaluation of the experimental diets was calculated by evaluating the feed cost in Nigerian naira (FC) needed to produce 1 kg of live weight gain of each experimental fish group (Eleamer and Sharon, 1984).

Feed cost (₦) = (feed cost/kg) x (food consumption)

Price of one kg gain in weight (₦) (₦/gain "kg") = (feed cost/kg) X FCR

The profit Index was used to evaluate the profitability of the diets (Miller, 1976).

$$\textbf{Profit index} = \frac{\text{cost of feeding}}{\text{value of fish cropped}}$$

3.13 Data Analyses

The data was subjected to t-test (paired T-test) and analysis of variance (ANOVA) to test the significance among treatment means. Where there was significant difference, Duncan multiple range test was applied to rank treatment means ($P< 0.05$). All statistical analyses were computed using SPSS (IBM) Statistical package Version 20 for Windows.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Raw and Processed *Mucuna pruriens* leaf

The proximate Composition of Raw and Processed *Mucuna pruriens* leaf are presented in Table 4.1.The dry matter percentages ranged from 93.23±0.22 to 94.33±0.33 while the crude protein percentages ranged from 23.00±0.00 to 25.94±0.94. The highest crude protein a value (25.94%) was recorded in fermented mucuna leaf meal and the lowest values (23.00%) was recorded in soaked mucuna leaf meal in fresh water. The highest value of ether extract was obtained in raw mucuna leaf meal (20.17±0.17) while the least (14.31±0.31) was obtained in air-dried mucuna leaf meal. The ash content percentages ranged from 21.26±0.26 to 24.89±0.89 while the crude fibre percentages ranged from 21.26±0.26 to 24.89±0.89.

4.2Anti-nutritional Compounds of Raw and Processed *Mucuna pruriens* leaf

Anti-nutritional compounds such as hydrocyanic acid, oxalate, phytate, saponin and tannin as presented in Table 4.2 indicates that all the components determined were greatly reduced after processing methods of the leaf. In raw leaf, hydrocyanic acids had the highest value (15.1±0.10) and oxalate had the lowest value (0.34±0.00). In the raw and processed *Mucuna pruriens* leaf meal the values of phytate ranged from 1.63±0.63 to 3.15±0.15 while the saponin ranged from 4.30±0.30 to 8.46±0.46.

4.1 Proximate Composition of Raw and Processed *Mucuna Pruriens* Leaf Meal

	Leaf Meal				
Parameters (%)	RMLM	ADMLM	SCMLM	SHMLM	FMLM
Dry matter	94.33±0.33^{a}	94.09±0.09^{a}	93.84±0.84^{a}	93.23±0.23^{a}	93.31±0.31^{a}
Crude protein	24.88±0.88^{ab}	24.38±0.38^{ab}	23.00±0.00^{b}	23.94±0.94^{ab}	25.94±0.94^{a}
Ether extract	20.17±0.17^{a}	14.31±0.31^{c}	17.06±0.06^{b}	17.67±0.67^{b}	17.00±0.00^{b}
Ash content	10.55±0.55^{a}	7.64±0.64^{bc}	9.33±0.33^{ab}	7.33±0.33^{bc}	5.88±0.88^{c}
Crude fibre	24.89±0.89^{a}	23.78±0.78^{ab}	23.94±0.94^{ab}	22.99±0.99^{ab}	21.26±0.26^{b}
Nitrogen free extract	19.51±0.51^{c}	29.89±0.89^{a}	26.67±0.67^{b}	28.07±0.07^{ab}	29.92±0.92^{a}

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

RMLM = Raw Mucuna Leaf Meal

ADMLM = Air-dried Mucuna Leaf Meal

SCMLM = Soaked Mucuna Leaf Meal in cold water

SHMLM = Soaked Mucuna Leaf Meal in hot water

FMLM = Fermented Mucuna Leaf Meal

Table4.2 Anti-nutritional Compounds of Raw and Processed *Mucuna pruriens* leaf meal

	Leaf Meal				
Parameter	RMLM	ADMLM	SCMLM	SHMLM	FMLM
Phytate	3.15±0.15[a]	1.79±0.79[a]	2.44±0.44[a]	2.06±0.06[a]	1.63±0.63[a]
Oxalate	0.34±0.00[a]	0.33±0.00[a]	0.16±0.01[a]	0.16±0.00[a]	0.11±0.01[a]
Tannin	2.38±0.38[a]	2.01±0.01[a]	0.22±0.00[b]	0.38±0.00[b]	0.38±0.01[b]
Saponin	8.46±0.46[a]	8.35±0.04[a]	4.30±0.30[c]	6.40±0.40[b]	5.60±0.60[bc]
Hydrocyanic acid	15.1±0.10[a]	7.60±0.60[b]	3.20±0.20[d]	5.40±0.04[c]	5.40±0.00[c]

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

RMLM = Raw Mucuna Leaf Meal

ADMLM = Air-dried Mucuna Leaf Meal

SCMLM = Soaked Mucuna Leaf Meal in cold water

SHMLM = Soaked Mucuna Leaf Meal in hot water

FMLM = Fermented Mucuna Leaf Meal

4.3 Proximate composition of major ingredients in experimental diets

The proximate composition of major ingredients is presented in Table 4.3, revealed that the highest crude protein was recorded in fish meal (65.00±0.00%) and least recorded in yellow maize (8.90±0.90%). the nitrogen free extract ranged from 17.10±0.10% - 82.00±0.00% while ether extract ranged from 4.00±0.00% - 17.00±0.88%. The yellow maize had the least value of ash content (1.30±0.30%) and fish meal had highest value (7.22±0.22%).

Table 4.3: Proximate composition of major ingredients in experimental diets

	Composition (%)					
Ingredients	Dry Matter	Crude Protein	Crude Fibre	Ether Extract	Ash Content	Nitrogen Free Extract
Fish meal	88.70±0.30	65.00±0.00	0.00±0.00	10.56±0.44	7.22±0.22	17.10±0.10
Soya bean	93.19±0.19	40.00±0.00	21.26±0.26	8.88±0.12	5.32±0.32	39.63±0.63
Yellow maize	90.73±0.73	8.90±0.90	3.80±0.80	4.00±0.00	1.30±0.30	82.00±0.00
FMLM	93.31±0.31	25.94±0.94	21.26±0.00	17.00±0.88	5.88±0.26	29.92±0.92

Statistical analysis showed mean values and standard error

FMLM = Fermented Mucuna Leaf meal

4.4Macro-mineralof Raw and Fermented *Mucuna pruriens* leaf

The macro-minerals determined in the raw and fermented leaf were Calcium (Ca), Magnesium (Mg), Phosphorus (P), Sodium (Na), Potassium (K), Manganese (Mn), Copper (Cu), Zinc (Zn) and Iron (Fe). The result indicates an insignificant variations ($P \geq 0.05$) between the raw and fermented mucuna leaf meal.

4.4 Amino acid profile of Raw and Fermented *Mucuna pruriens* leaf

The amino acid profile is presented in (Table 4.4). Amino acids profile of raw *Mucuna pruriens* were higher in abundance compared to the fermented leaf. Glutamic acids had the highest amino acids profile and the lowest obtained for cysteine as observed in both raw and fermented leaves.

4.5 Proximate Composition of Experimental Diets

The proximate composition of the experimental diets fed to *C. gariepinus*(Table 4.5). The diets were iso nitrogenous as there was no significant difference ($P \geq 0.05$) in the protein composition of the diets at 40% crude protein and they all met the set targets specification for the experiment. The crude protein ranged from 40.00±0.19 to 40.47±0.27. The Ether Extract in the feeds ranged from 10.57±0.17 to 11.99±0.52 while the Nitrogen Free Extract ranged from 20.31±0.19 to 25.37±0.32.

Table4.4: Minerals Composition of Raw and Fermented *Mucuna pruriens* leaf meal

	Leaf Meal		
Mineral	RMLM (%)	FMLM (%)	Soya Bean Meal (mg/100g)(Udo and Umoren, 2011)
Sodium (Na)	0.20	0.20	14.99
Potassium (K)	0.80	0.50	15.00
Calcium (Ca)	0.72	0.69	135.48
Magnesium (Mg)	0.41	0.38	80.19
Phosphorus (P)	1.10	0.70	286.61
Zinc (Zn)	0.0104	0.0099	3.99
Copper (Cu)	0.00019	0.0021	0.46
Manganese (Mn)	0.012	0.013	4.53
Iron (Fe)	0.022	0.026	2.54
S.E.M.	±0.14	±0.10	

No significant difference ($P \geq 0.05$) in macro-mineral composition between raw and fermented *Mucuna pruriens* leaf meal.

RMLM = Raw *Mucuna pruriens* Leaf Meal,

FMLM = Fermented *Mucuna pruriens* Leaf Meal

Table 4.4: Amino Acid Profile of Raw and Fermented *Mucuna pruriens* leaf meal

	Leaf Meal		
Amino Acids(g/100g)	RMLM	FMLM	Soya Bean Meal (Ari *et al*., 2012)
Leucine	7.00	6.69	6.00
Lysine	3.42	3.18	3.60
Isoleucine	3.17	2.68	2.32
Phenylalanine	4.96	4.25	3.06
Valine	4.59	3.94	2.85
Methionine	1.39	1.23	0.88

Proline	3.14	2.43	3.08
Arginine	3.18	2.58	4.48
Tyrosine	3.95	3.27	2.63
Histidine	1.88	1.72	3.00
Cysteine	0.60	0.36	0.70
Alanine	3.49	3.03	3.04
Glutamic acid	10.29	9.25	14.94
Glycine	2.99	2.09	3.35
Threonine	3.60	3.11	2.80
Serine	3.99	3.29	1.90
Aspartic acid	6.60	5.70	10.49
S.E.M.	±0.55	±0.45	

No significant difference ($P \geq 0.05$) in amino acids profile between raw and fermented *Mucuna pruriens* leaf meal.

Table 4.5: Proximate Composition of Experimental Diets Fed

	Treatments				
Parameters	A	B	C	D	E

	(100% FMLM)	(75% FMLM)	(50% FMLM)	(25% FMLM)	(0% FMLM)
Dry Matter	93.40±0.29^{b}	93.06±0.35^{b}	93.20±0.17^{b}	94.10±0.46^{ab}	94.20±0.17^{ab}
Crude Protein	40.00±0.19^{a}	40.00±0.19^{a}	40.00±0.06^{a}	40.20±0.12^{a}	40.47±0.27^{a}
Ether Extract	11.99±0.52^{a}	11.27±0.35^{ab}	10.57±0.17^{b}	11.06±0.52^{ab}	10.27±0.15^{b}
Ash Content	10.85±0.52^{a}	10.42±0.24^{a}	9.89±0.51^{a}	9.92±0.40^{a}	9.64±0.40^{a}
Moisture Content	9.43±0.28^{a}	8.99±0.05^{a}	8.91±0.35^{a}	9.21±0.46^{a}	8.87±0.09^{a}
Crude Fibre	7.42±0.24^{a}	7.23±0.13^{a}	6.23±0.13^{b}	5.89±0.08^{b}	5.28±0.16^{c}
Nitrogen Free Extract	20.31±0.19^{c}	22.09±0.76^{b}	24.40±0.69^{a}	23.72±0.14^{ab}	25.37±0.32^{a}

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

% FMLM = Fermented Mucuna Leaf Meal with respective percentage levels of inclusion

4.6 Growth performance and Nutrient Utilization of *Clarias gariepinus* fingerlings

The result of the statistical analysis as presented in Table 4.6. Fish fed control diet $E_{(0\% FMLM)}$had the highest weight gain (g) per fish (168.87±3.97), followed by fish fed with, $D_{(25\% FMLM)}$ (161.30±6.32), $C_{(50\% FMLM)}$,(115.77±5.08), $B_{(75\% FMLM)}$ (104.00±4.26), while the fish fed diet $A_{(100\% FMLM)}$ had the lowest weight gain per fish (89.70±1.96). The total length (cm) ranged from 11.13±0.48 to 16.40±0.23. Specific Growth Rate (SGR) with the highest value was observed in fish fed with diet $E_{(0\% FMLM)}$ (3.79±0.03), next to it $D_{(25\% FMLM)}$ (3.74±0.04), $C_{(50\% FMLM)}$ (3.37±0.06), $B_{(75\% FMLM)}$ (3.26±0.05), and the fish fed diet $A_{(100\% FMLM)}$ (3.10±0.02) had the lowest growth rate. Percentage live Weight Gain (PWG) fish with the highest value was observed in the fish fed with diet $E_{(0\% FMLM)}$(96.29±0.08), followed by $D_{(25\% FMLM)}$(96.11±0.14), $C_{(50\% FMLM)}$(94.67±0.24), $B_{(75\% FMLM)}$(94.09±0.24), and the fish fed diet $A_{(100\% FMLM)}$(93.24±0.12)had the lowest PWG. Percentage Survival Rate (PSR) for fish fed different experimental diets ranged between 90.00±0.00 – 96.67±3.33. The value for Feed Conversion Ratio (FCR) ranged from 1.50±0.02 to 1.72±0.12 and no significant variation ($P \geq 0.05$) was observed among the treatments. Gross Feed Conversion Efficiency (GFCE) obtained showed that fish fed with diet $E_{(0\% FMLM)}$had the highest (66.54±0.98) and the least was recorded from fish fed with diet $A_{(100\% FMLM)}$(58.67±4.47). The Protein Efficiency Ratio (PER) ranged from 1.47±0.11 to 1.65±0.05.

Figure 1 revealed growth response of *Clarias gariepinus* fingerlings fed experimental diets. There was a uniform pattern of growth for all diets for the first week. Fish fed diets $E_{(0\% FMLM)}$

and $D_{(25\% FMLM)}$ increased in weight above the other treatments after week one and continued this trend until week twelve. This was followed by fish fed diets $C_{(50\% FMLM)}$ and $B_{(75\% FMLM)}$. Fish fed diet $A_{(100\% FMLM)}$ lagged behind those of diets $C_{(50\% FMLM)}$ and $B_{(75\% FMLM)}$.after the eight week. Therefore, growth response of $E_{(0\% FMLM)}$ and $D_{(25\% FMLM)}$ where far better than the others.

Table4.6: Growth performance and Nutrient Utilization of *Clarias gariepinus* fingerlings

Parameters	Treatments				
	A (100% FMLM)	B (75% FMLM)	C (50% FMLM)	D (25% FMLM)	E (0% FMLM)
Mean weight gain (g)	89.70±1.96^{c}	104.00±4.26^{cb}	115.77±5.08^{b}	161.30±6.12^{a}	168.87±3.97^{a}
Total length gain (cm)	11.13±0.48^{c}	13.87±0.37^{b}	13.10±0.55^{b}	16.20±0.10^{a}	16.40±0.23^{a}
SGR	3.10±0.02^{c}	3.26±0.05^{b}	3.37±0.06^{b}	3.74±0.04^{a}	3.79±0.03^{a}
PWG	93.24±0.12^{d}	94.09±0.24^{c}	94.67±0.24^{b}	96.11±0.14^{a}	96.29±0.08^{a}
PSR	96.67±3.33^{a}	93.33±3.33^{a}	93.33±3.33^{a}	90.00±0.00^{a}	90.00±0.00^{a}
FCR	1.72±0.12^{a}	1.63±0.07^{a}	1.54±0.10^{a}	1.50±0.04^{a}	1.50±0.02^{a}
GFCE	58.67±4.47^{a}	61.47±2.83^{a}	65.50±4.25^{a}	66.61±1.73^{a}	66.54±0.98^{a}
PER	1.47±0.11^{a}	1.54±0.07^{a}	1.64±0.11^{a}	1.65±0.05^{a}	1.64±0.03^{a}

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

SGR = Specific Growth Rate,

FCR = Feed Conversion Ratio,

GFCE = Gross Feed Conversion Efficiency,

PWG = Percentage Live Weight Gained,

PER = Protein Efficiency Ratio,

PSR= Percentage Survival Rate,

% FMLM = Fermented Mucuna Leaf Meal with respective percentage levels of inclusion

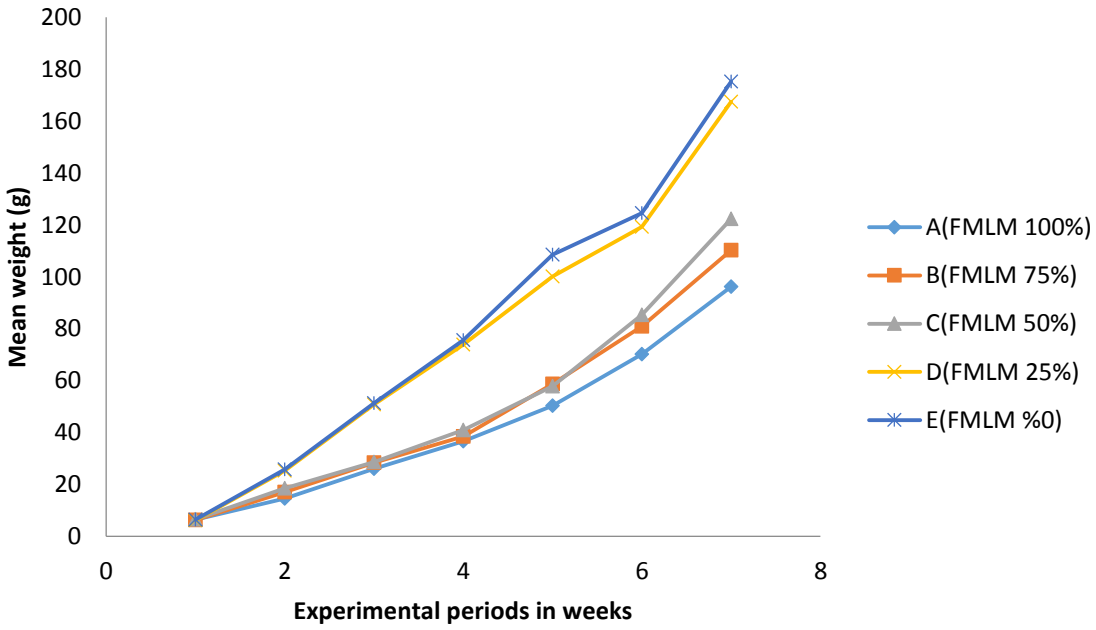


Figure 4.1: Growth Response of *Clarias gariepinus* fingerlings fed experimental diets

%FMLM = Fermented Mucuna Leaf Meal with respective percentage levels of inclusion

4.7 Carcass Composition of fish fed experimental diets

The result of carcass composition as presented in Table 4.7, revealed that the highest carcass crude protein of fish was recorded in fish fed with diet $E_{(0\% \text{ FMLM})}$ (64.23±0.34),followed by diet $D_{(25\% \text{ FMLM})}$ (61.62±0.43), $C_{(50\% \text{ FMLM})}$ (55.36±0.35), $B_{(75\% \text{ FMLM})}$ (55.16±2.86), $B_{(100\% \text{ FMLM})}$ (51.12±0.00) while the initial value (50.16±0.14) before feeding trial was the lowest carcass crude protein. Ether extract ranged between 8.30±0.06 – 14.92±0.73. The ash content also ranged between 8.42±0.08 - 16.37±0.59. The highest moisture content value ranged between (7.63±0.13 – 11.04±0.31). NFE content of fish fed ranged between 8.73±0.41 – 17.54±0.38

4.8 Apparent digestibility of the feed fed to *Clarias gariepinus* Fingerlings

Apparent digestibility parameter is presented in Table 4.8. Fish fed with diet $E_{(0\% \text{ FMLM})}$,had the highest of APD value (61.40±0.06) followed by $D_{(25\% \text{ FMLM})}$ (60.67±0.76), $C_{(50\% \text{ FMLM})}$(58.34±3.35), $A_{(0\% \text{ FMLM})}$ (55.87±0.90), and the fish fed diet $B_{(0\% \text{ FMLM})}$had the lowest APD value (50.38±2.21). Apparent lipid digestibility (ALD) of fish with the highest value (35.27±2.90) was seen in fish fed diet $A_{(100\% \text{ FMLM})}$ and the lowest in fish fed with diet $D_{(25\% \text{ FMLM})}$ (19.37±0.58). Apparent ash digestibility (AAD) obtained showed that fish fed with diet $A_{(100\% \text{ FMLM})}$ had the highest value (26.76±0.39) and the lowest in fish fed with diet $D_{(25\%}$

FMLM)(10.52±0.53). Apparent carbohydrate digestibility (ACD) ranged from 38.47±1.80 to 54.61±2.54.

Table 4.7: Carcass Composition of fish fed experimental diets

Parameters	Treatments					
	Initial Value	A (100% FMLM)	B (75% FMLM)	C (50% FMLM)	D (25% FMLM)	E (0% FMLM)
Crude Protein	50.16±0.14^{c}	51.12±0.00^{c}	55.16±2.86^{b}	55.36±0.35^{b}	61.62±0.43^{a}	64.23±0.34^{a}
Ether Extract	14.29±0.73^{a}	13.92±0.51^{a}	8.30±0.06^{b}	8.31±0.18^{b}	8.44±0.36^{b}	9.42±0.51^{b}
Ash Content	15.32±0.50^{a}	16.37±0.59^{a}	11.24±0.41^{b}	11.23±0.50^{b}	11.21±0.38^{b}	8.42±0.08^{c}
Moisture Content	11.04±0.31^{a}	10.22±0.06^{b}	7.76±0.52^{d}	7.82±0.37^{d}	7.63±0.13^{d}	9.20±0.12^{c}
Nitrogen Free Extract	10.00±0.34^{b}	8.37±0.21^{c}	17.54±0.38^{a}	17.28±0.39^{a}	11.10±0.57^{b}	8.73±0.41^{c}

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

% FMLM = Fermented Mucuna Leaf Meal with respective percentage levels of inclusion.

Table 4.8: Apparent Digestibility of the Feed Fed to *Clarias gariepinus* Fingerlings

Parameters	Treatments				
	A (100% FMLM)	B (75% FMLM)	C (50% FMLM)	D (25% FMLM)	E (0% FMLM)
APD	55.87±0.90^{ab}	50.38±2.21^{b}	58.34±3.35^{a}	60.67±0.76^{a}	61.40±0.60^{a}
ALD	35.27±2.90^{ab}	28.47±1.12^{c}	39.57±0.01^{a}	19.37±0.58^{d}	31.60±0.42^{bc}
AAD	26.76±0.39^{a}	20.30±1.07^{b}	16.81±1.82^{b}	10.52±0.53^{c}	17.33±1.34^{b}
ACD	38.47±1.80^{c}	46.29±3.50^{b}	50.29±0.55^{ab}	48.96±1.24^{ab}	54.61±2.54^{a}

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

APD = Apparent Protein Digestibility,

ALD = Apparent Lipid Digestibility,

AAD = Apparent Ash Digestibility,

ACD = Apparent Carbohydrate Digestibility,

% FMLM = Fermented Mucuna Leaf Meal with respective percentage levels of inclusion.

4.9 Physico-chemical Parameters of Water

Physicochemical parameter of water determined is presented in Table 4.6. The hydrogen ion concentration pH ranged between 7.56 - 7.86 in all the treatments. The dissolved oxygen ranged between 6.54 – 6.75, and temperature range was 27.94 – 28.13.The result indicates an insignificant variations ($P \geq 0.05$) among the experimental tanks.

4.10 Cost -Benefit Analyses of Experimental Diets

The cost analysis of experimental diets and fish cropped is presented in Table 4.9. The fish fed with diet $E_{(0\% \text{ FMLM})}$ had the highest value (6309.6±143.5) of fish cropped followed by, $D_{(25\% \text{ FMLM})}$ (6030±224.5), $C_{(50\% \text{ FMLM})}$ (4569.07±288.0), $B_{(75\% \text{ FMLM})}$ (4121.33±248.9), and the fish fed diet $A_{(100\% \text{ FMLM})}$had the lowest cropping value (3720±164.7). Total expenses ranged from ₦2196.37 – ₦2356.23 with the highest value observed in fish fed with diet $E_{(0\% \text{ FMLM})}$, and the lowest in fish fed with diet $A_{(100\% \text{ FMLM}).}$ Net profit value for fish fed with diet $E_{(0\% \text{ FMLM})}$had the highest value (₦3953.37) while the lowest observed in fish fed with diet $A_{(100\% \text{ FMLM})}$(₦1523.63). Incidence cost ranged from 0.63 – 0.83. Profit index ranged from1.69 to

2.68, in which fish fed with diet $A_{(100\% FMLM)}$was the lowest while the fish fed with diet $E_{(0\% FMLM)}$ recorded the highest profit index. The benefit cost ratio ranged from 0.69 – 1.68.

Table 9: Physicochemical Parameters Recorded during Experimental Period

	Physico-chemical parameters				
Treatments	pH ±S.E.M	Temp(^{0}C) ±S.E.M	DO(mg/l) ±S.E.M	Electrical Conductivity (µs) ±S.E.M	TDS (ppt) ±S.E.M
$A_{(100\% FMLM)}$	7.74±0.02	28.23±0.09	6.70±0.05	205.00±0.01	101±0.00
$B_{(75\% FMLM)}$	7.68±0.03	28.10±0.07	6.64±0.05	205.00±0.01	101±0.00
$C_{(50\% FMLM)}$	7.74±0.03	28.18±0.07	6.63±0.07	205.00±0.00	101±0.01
$D_{(25\% FMLM)}$	7.56±0.04	27.94±0.07	6.65±0.09	205.00±0.00	102±0.00
$E_{(0\% FMLM)}$	7.68±0.04	28.13±0.09	6.54±0.07	205.00±0.00	101±0.01

Statistical analysis showed no significant difference ($P \geq 0.05$) among the experimental tanks

Temp. = Temperature,

pH = Hydrogen ion concentration,

DO = Dissolved oxygen,

TDS = Total Dissolved Solid,

% FMLM = Fermented Mucuna Leaf Meal with respective percentage levels of inclusion

Table 4.10: Cost – Benefit Analyses of Experimental Diets

Parameters	Treatments				
	A (100% FMLM)	B (75% FMLM)	C (50% FMLM)	D (25% FMLM)	E (0% FMLM)
Cost of Fingerlings (₦30)	300±0.00[a]	300±0.00[a]	300±0.00[a]	300±0.00[a]	300±0.00[a]
Value of Fish Cropped(₦)	3720±165[c]	4121±249[cb]	4569.1±288.0 b	6030±224.5[a] b	6309.6±143.5 a

Total Expenses (₦)	2196±28.2^{d}	2241±19.5^{dc}	2277±31.7^{cb}	2326±13.5^{a}	2356±8.1^{a}
Net Profit (₦)	1524±141.4^{c}	1880±249.9^{bc}	2292±273.3^{b}	3704±238.0^{a}	3953±150.9^{a}
Cost of Producing a kg Feed (₦)	230±5.77^{d}	270±5.77^{c}	310±5.77^{b}	350±17.32^{a}	370±17.32^{a}
Incidence of Cost	0.83±0.06^{a}	0.80±0.61^{ab}	0.78±0.62^{ab}	0.63±0.38^{b}	0.63±0.23^{b}
Benefit Cost Ratio	0.69±0.06^{b}	0.84±0.11^{b}	1.01±0.12^{b}	1.60±0.11^{a}	1.68±0.07^{a}
Profit Index	1.69±0.06^{b}	1.86±0.11^{b}	2.01±0.12^{b}	2.60±0.11^{a}	2.68±0.07^{a}

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

% FMLM = Fermented Mucuna Leaf Meal with respective percentage levels of inclusion

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition and Anti-nutritional Compounds

The proximate composition of raw *Mucuna pruriens* leaf obtained from this work differs to that reported by Ifemeje (2016) where he examined the nutritional and phytochemical composition of *Mucuna pruriens* leaves obtained from Umuoma village in Ihiala Local Government Area of Anambra State, South Eastern Nigeria. Ifemeje (2016) reported the percentage proximate composition as 8.30%, 34.16%, 2.30%, 5.80%, 16.94%, and 32.50% for moisture, crude protein, crude fat, ash content carbohydrate and crude fibre respectively. The carbohydrate, ash and ether extract obtained in this study was higher than that reported by Ifemeje (2016), while the crude protein and crude fibre recorded a lower value. The differences observed in the proximate composition of raw *Mucuna pruriens* leaf from these studies are probably as a result of factors, such as geographical location of the plant, soil and climatic conditions of cultured environment (FAO, 2004), which stated that these factors directly affect the composition of plant physiological and chemical structures. It could be observed that the crude protein of fermented *Mucuna pruriens* leaf meal differed from that of the soaked in both cold and hot water. The observed difference may be attributed to leaching of soluble protein into the water. This suggestion agrees with the observation of Ani (2008) that showed that Mucuna bean seeds soaked in an aqueous solution of potassium bicarbonate at room temperature for 24 hours led to the solubilization and removal of some nitrogenous substance in the bean. The proximate composition of the fermented *Mucuna pruriens* leaf meal indicates a significant increase in the crude protein composition of the leaf (25.94%). The increased level of crude protein is consistent with the findings of Ramachandran *et al*. (2005) who reported that increase in protein value with fermentation

could be attributed to net synthesis of protein by fermenting of the leaf, which might have resulted in the production of some amino acids during protein synthesis. Lipid contents were found to be significantly lower in the fermented *Mucuna pruriens* leaf than in raw leaf. This decrease in lipid contents might be attributed to the increased activities of the lipolytic enzymes during fermentation which hydrolyses fat components in to fatty acid and glycerol (Chinma *et al*., 2009).

The results of the anti-nutritional factors of the raw *Mucuna pruriens* leaf meal obtained in this work show that tannins, saponin, phytate have higher values, while oxalate recorded lower values than those reported by Ifemeje (2016), this could be as a result difference in environment probably being a determining factor of type of anti-nutrients factors in plant and this may be as a result of plants absorbing substances from their environment. The anti-nutritional factors of the processed *Mucuna pruriens* leaf showed significant reduction. This significant reduction of the anti nutritional compounds soaked in cold and hot water and this may be as result of efficacy of water leaching out anti-nutrient in the leaves as reported by Bichi and Ahmad (2010). The anti-nutritional compounds of the fermented *Mucuna pruriens* leaf showed significant reduction. These observations however, agree with the reports of Oseni and Ekperigin (2007) on reduction of phytate by fermentation, when pure strain of *Aspergillus niger* was used to ferment maize cobs, but contradicts the report of Oladele and Oshodi (2008) who observed an increase in phytate and tannin levels by fermentation; it is however possible that the mode of fermentation and the species of organisms involved play crucial roles in the fermentation processes. Fermentation, or any other treatment has been reported to reduce anti nutrient constituents of plant materials (e.g. seeds, leaves, roots), these

offer promise for inclusion of products from plants in animal and fish diets (Makkar and Becker, 1999).

5.2 Minerals and Amino acid profile of raw and fermented *Mucuna pruriens* leaf meal

Minerals are known to play important metabolic and physiological role in the living system (Enechi and Odonwodo, 2003). The values of Iron (Fe) and Zinc (Zn) are 0.022mg/100g and 0.0104 mg/100g respectively in raw *Mucuna pruriens* leaf were found to be lower as compared to the values of Fe and Zn by Ifemeje (2016) (8.10±0.12 and 5.10±0.05mg/100g respectively). Although, there is slight decrease in the mineral composition of fermented *M. pruriens* leaf, but not significantly different from the values obtained for the raw leaf of *M. pruriens*.

There was no significant difference between the amino acid profiles of raw and fermented *Mucuna pruriens* leaf, this implies that processing method used did not significantly affect the amino acids in the leaf.

5.3 Growth Performances and Nutrient Utilization

Growth of fish depends on the nutritive quality of feeds, especially its crude protein. The proximate composition of the experimental diets for crude protein were similar (40%) for $A_{(100\%\ FMLM)}$, $B_{(75\%\ FMLM)}$, $C_{(50\%\ FMLM)}$,and (40.20%) for $D_{(25\%\ FMLM)}$, while (40.47%) for $E_{(0\%\ FMLM)}$,in dietary inclusion levels of *Mucuna pruriens* leaf meal. Comparing the crude protein recorded in this study, they did not differ from the values estimated by Bolorunduro (2002) of 20-25% crude protein for catfish in semi-intensive system and 40-48% for fingerlings production in intensive system. The decreasing trend in growth performance may be due to residual anti nutritional compounds inherent in the leaf meal. The better growth

performances recorded in dietary $E_{(0\% \text{ FMLM})}$ and $D_{(25\% \text{ FMLM})}$ may be due to lower level of inclusion which enhanced its nutritional composition, palatability and bioavailability. The specific growth rate (SGR) also displayed a decreasing trend with increasing levels of *Mucuna pruriens* leaf meal in the diets. The best performance was obtained in fish fed the control diet $E_{(0\% \text{ FMLM})}$ followed by fish fed $D_{(25\% \text{ FMLM})}$,diet while the lowest value was recorded in fish fed with $A_{(100\% \text{ FMLM})}$and $B_{(75\% \text{ FMLM})}$,diet. The trend in SGR of the experimental fish might be an indication of their relative responses to the varied dietary inclusion levels of *Mucuna pruriens* leaf meal. Nutrients seemed to be best converted into flesh by the fish on the $E_{(0\% \text{ FMLM})}$, leaf meal dietary treatment, followed by that of the $D_{(25\% \text{ FMLM})}$, while the ones of $B_{(75\% \text{ FMLM})}$ and $A_{(100\% \text{ FMLM})}$were least converted. Sotolu and Adejumoh, (2008) reported varying nutrient levels to affect growth responses of fish, where they found out that fish fed cassava based diet had inferior growth response to the control of zero cassava peel.

The feed conversion ratio recorded in this experiment ranged from 1.50–1.72 in which the control diet $E_{(0\% \text{ FMLM})}$, had the lowest next to it, $D_{(25\% \text{ FMLM})}$, and $A_{(100\% \text{ FMLM})}$ had the highest value for FCR. Poor feed conversion ratio will lead to poor growth and may be due to high fibre content of the plant-based diets. High fibre content in diets causes dilution of the nutrients, and therefore reduces digestibility, resulting in growth depression, as the diets become inconsistent (Adewumi, 2014).

The GFCE, PWG, PER, and percentage mortality rate decreased as inclusion level of fermented *Mucuna pruriens* leaf meal increases. Percentage Survival rate increases as the level of fermented *Mucuna pruriens* leaf meal increases also. The growth and nutrient utilization by fish decreased as level of Mucuna Leaf Meal inclusion increases in the diets.

These differences in mean weight gained and nutrients utilization can be attributed to the differences in crude fibre and nitrogen free extract of experimental diets because crude protein is known to be very important in optimal growth, crude fibre can affect digestibility and carbohydrate is known to have protein sparing effect (Fagbenro *et al*., 1993; Orire and Sadiku, 2013).

5.4 Carcass Composition

Generally, the carcass crude protein of *Clarias gariepinus* in this experiment increased significantly ($P<0.05$) after the feeding trial. The mean initial carcass crude protein (50.16%) was significantly ($P<0.05$) lower than the values obtained after the feeding trial. However, the carcass crude protein decreases with increase in inclusion level of *Mucuna pruriens* leaf meal. Carcass crude lipid of fish at the end of the experiment was lower than the initial value, this showed that increase in weight was not due to the accumulation of fat by the experimental fish (Bekibele, 2005).

5.5 Apparent Digestibility Coefficient

Apparent digestibility is mainly used to describe how efficiently feeds or feed ingredients are being digested and how much of their nutrient composition can be made available to fish for maintenance and growth (Fagbenro, 2001). Apparent digestibility of crude protein was highest (61.40%) in the fish fed $E_{(0\%\ FMLM)}$ diet next to it was (60.67%) $E_{(25\%\ FMLM)}$, and lowest in $A_{(100\%\ FMLM)}$diet (55.87%), the values were significantly different among all dietary treatments and fell within the recommended range as reported by Fagbenro(2001).

5.6 Physico-chemical Parameters

The water quality parameters obtained in this work were within the range recommended for the culture of *Clarias gariepinus* (Boyd, 2000; Ajani, 2006). Boyd (2000) reported temperature range of 22 - 27^{O}C, pH range from 6.5 – 9.0 and dissolved oxygen of 6.3 – 9.6mg/l, as optimum and best for high growth performance in cultured tropical fishes.

5.7 Cost and Benefit Analyses of Experimental Diets

Cost evaluation indicated that the incorporation of *Mucuna pruriens* leaf meal as a substitute of soya bean meal up to 25% decreased feed costs and increased weight gained and survival thus, giving a high feasibility for aquaculture production. The cost required to produce a kilogram of feed decreased from ₦370 to ₦230 $E_{(0\% \text{ FMLM})}$, and $A_{(100\% \text{ FMLM})}$ respectively.

The profit index and benefit cost ratio showed that diet $F_{(0\% \text{ FMLM})}$, and $E_{(25\% \text{ FMLM})}$, performed best compared to other fish fed with diet having above 25% inclusion level of *Mucuna pruriens* leaf meal. However, the use of fermented mucuna leaf meal in fish feed production may be more economical taken in to consideration it availability and less competition by humans. Amisah *et al*. (2009) stated that when selecting alternative feed ingredients it is important that selected protein source that do not conflict with human food security interest.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The fermentation of *Mucuna pruriens* leaf improved the crude protein 25.89% and reduced the crude fibre 21.26% and the anti-nutritional factors also reduced.

Fish fed 25% inclusion level of fermented *Mucuna pruriens* leaf meal performed better in terms of growth performance, nutrient utilization and apparent digestibility coefficient. Production cost can be considerably reduced and profitability enhanced at 25% fermented Mucuna leaf meal inclusion.

6.2 Recommendations

I. Fermented Mucuna leaf meals at 25% replacement level of soya bean meal for better performance is suggested.
II. The return on investment is higher when Mucuna leaf meal is used to replace soya bean meal at 25% inclusion level in *Clarias gariepinus* fingerlings.

CONTRIBUTION TO KNOWLEDGE

Aquaculture has huge potentials of alleviating poverty, contributes to food security, generates income and improves livelihood. However, the lack of affordable quality fish feed is one of the major constraints to aquaculture development in Nigeria. The high prices of conventional feed ingredients and commercial feeds have made feed costly beyond the reach of the average farmer. In order for aquaculture to continue to grow with use of affordable and available quality feeds there is the need to reduce dependence on soya bean and other conventional proteins. Therefore, this study evaluated the utilization of fermented mucuna leaf meal as a replacement for soya bean meal in *Clarias gariepinus* fingerlings diets.

I. The result on the processing methods will serves as baseline information on the utilization of *Mucuna pruriens* leaf meal for Africa catfish (*Clarias gariepinus*).

II. Fermented *Mucuna pruriens* leaf meal can be used to partially replace the soya bean meal in the diet of *Claria gariepinus* fingerlings up to 25% level of inclusion after it was established that its gave the high growth performance and nutrient utilization of the feed by *Clarias gariepinus.*

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