GROWTH RESPONSE AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* JUVENILES FED GRADED LEVELS OF BOILED *PUERARIA PHASEOLOIDES* (TROPICAL KUDZU*)* SEED MEAL

BY

ABDULLAHI, GAMBO TAHIR

DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

APRIL, 2015.

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BY

Abdullahi Tahir GAMBO, B. Sc. (ED) BIOLOGY, (ABU) 2001
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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN EDUCATIONAL BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

APRIL, 2015.

DEDICATION

This work is dedicated to the Almighty Allah, Kano State Government, my parents and my entire family.

DECLARATION

I declare that the work in this thesis entitled 'GROWTH RESPONSE AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* JUVENILES FED GRADED LEVELS OF BOILED *PUERARIA PHASEOLOIDES* (TROPICAL KUDZU*)* SEED MEAL' has been carried out by me in the Department of Biological Sciences under the supervisions of Professor S. J. Oniye and Prof. J. Auta. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Abdullahi Tahir GAMBO _____ _____

Signature Date

CERTIFICATION

This thesis entitled "**GROWTH RESPONSE AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* JUVENILES FED GRADED LEVELS OF BOILED *PUERARIA PHASEOLOIDES* (TROPICAL KUDZU*)* SEED MEAL."** by Abdullahi Gambo TAHIR, 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.

Prof. S. J. Oniye Chairman, Supervisory Committee Department of Biological Sciences, Ahmadu Bello University, Zaria	_____ Signature	_____ Date
Prof. J. Auta Member, Supervisory Committee Department of Biological Sciences, Ahmadu Bello University, Zaria	_____ Signature	_____ Date
Prof. A. K. Adamu Head of Department, Department Biological Sciences, Ahmadu Bello University, Zaria	_____ Signature	_____ Date
Prof. A. Z. Hassan Dean, School of Postgraduate Studies, Ahmadu Bello University, Zaria.	_____ Signature	_____ Date

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ABSTRACT

The effects of feeding varying levels of boiled *Pueraria phaseoloides* seed meal in the diet of *Clarias gariepinus* juveniles for growth performance and feed utilization was investigated in comparison with conventional commercial fish feed. Partial replacement of fish meal with varying levels of *P. phaseoloides* seed meal at 10%, 20%, 25% and 30% were fed to *Clarias gariepinus* juveniles for 56 days. The experiment comprised of six treatments with 12 replicates in the Fisheries laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Data for each parameter were subjected to Analysis of Variance (ANOVA) and the means for various experimental diets were compared for significance differences at 5% level. Fish fed with (diet C) 20% had the best mean weight gain (MWG) of 61.11g, mean length gain (MLG) of 9.57cm, specific growth rate (SGR) of 1.93, feed conversion ratio (FCR) of 0.74, apparent net protein utilization (ANPU) of 2.54, Feed efficiency ratio (FER) of 1.35, and Gross Feed Conversion Efficiency (GFCE) of 11.19. While diet E (30%) boiled Kudzu gave the least growth performance. The fish carcass composition was significantly higher ($P<0.05$) with diet C 20% boiled *Pueraria* seed inculusion this is indicative that protein in the feed was deposited into flesh of the fish.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Some decades ago, fish was mainly the diet of poor and low income people. As a protein source, it is an important component in the building blocks for growth and development in man and other animals. It is nutritionally better than beef in protein, it has high essential minerals, and low in saturated fats (Babbitt, 1990). Fish improve the defense mechanisms in human and assist in life prolongation, thus prevent diseases such as diabetes and cancer, and enhances growth and development in human, especially children (Babbit, 1990).

Imported commercial fish feed have been ranked as the most favoured feed for fish since the inception of fish culture or aquaculture technology in Nigeria, because they support satisfactory growth in fish. However the exhorbitant cost of imported fish feeds has been reported to be as high as 60-70% of production cost (Eyo, 2003). This has made catfish feeding economically unattractive for the small-scale fish farmers.

Pueraria seeds contains 31% Crude protein, 1.13 Methionine, 7.23 Leucine and 4.36 Lysine (Owunsu-Dumfeh, 1979a) when processed and compounded into fish feed, it may reduced the high cost of imported fish feed which remain a major problem to aquaculture development in Nigeria (Madu et *al*., 2003).

Recently, the analysis of nutritional value of wild plant materials has attracted attention and they have shown to contain significant amount of essential nutrients, (proteins, amino

acids, vitamins minerals oils and carbohydrates) that can be used for the formulation of animal feeds. The proximate composition of seeds of some wild plants of Nigerian origin reveals that they could be adequately used in the formulation of animal feeds provided the levels of their toxic substances are reduced or eliminated (Eromosele and Eromosele, 1993). *Moringa oliefera* and *Detarium microcarpum* have been reported to contain an excellent amount of protein (Anhwange *et al.*, 2004). *Amarantus viridis* is an excellent source of protein, its amino acid composition compare favorably with that of World Health Organization (WHO) standard protein (Sena *et al,* 1998). Although fish meal has been recognized to be the best source of animal protein containing an excellent array of amino acids, it is as a result of this, and numerous attempts have been made to replace fish meal with less expensive feedstuff (Eyo, 2003). Marian (2010) replaced fish meal with *Pauletia monandra* seed meal to feed fingerlings of *Clarias gariepinus.* Soybeans (*Glycine max*) and groundnut (*Arachis hypogea*) can equally replace fishmeal in the diet of *Clarias gariepinus* (Fafioye *et al.,* 2005*).* The use of other legumes such as Sword beans (*Camelina sativa*), Yam beans (*Sphenostylis stenocarpa*), Lima beans (*Phaseolus lunatus*), Banbara nut (*Vigna subterranes*), Jack beans (*Canavalia enciformis*), *Delonix regia* seed meal and Locust bean seed have been researched for fish feed formulation (Nielson *et al*., 1996; Balogun *et al*., 2004; Duniya, 2006).

1.2 Statement of Research Problem

The exorbitant cost of fish meal as feed inputs is a major problem to fish culturists in Nigeria (Madu *et al*., 2003). Plant protein sources contain low essential amino acids, due to high fiber contents, and the presence of anti-nutrient factors which hinders its use by forming a ‘shield effect’ on the protein molecules preventing proteases and similar

digestive enzymes from reaching them (Eyo, 2003). Most commercial feeds are formulated with cereals (maize, wheat, guinea corn, soybeans) which are largely utilized in human nutrition; hence the high cost of such feeds (Balogun *et al*., 2004).

1.3 Justification

Aquaculture is fast growing in Nigeria and worldwide since its inception in 1970s. To satisfy the high demand for fish due to high population growth, high production is necessary (Spinelli, 2006). Furthermore, the human population in Nigeria is fast growing and demand for fish is increasing at an alarming rate. Nutritionists have being evaluating alternate protein sources in aquaculture diets in the last seven years than during the previous 50 years (Spinelli, 2006). Several information exist on the utilization of most well known and cultivated legumes as fish feed. Research had been conducted in utilization of *Pueraria phaseoloides* as meals supplement to other ruminant animals, poultry and *Oreochromis niloticus* (Halim, 1992).

Clarias gariepinus is chosen for this research because it is an established aquaculture species in Africa and most common cultured catfish in Nigeria. Faturoti (2003). reported that catfishes have taken the center stage of the aquaculture sub-sector in Nigeria. In addition, they are suitable to low-technology farming systems and have a propensity to consume a variety of supplementary feeds (Fagbenro *et al.*, 2003). This species is recognized for its astonishing tolerance to poor environmental conditions, its disease resistance, good growth rate and wide market acceptability makes it very suitable for marginal economies (Babbit, 1990).

1.4 Aim and Objectives of the Study

The aim of the study was to evaluate the use of *Pueraria* seeds in raw and processed forms in the diet of African Catfish, *Clarias gariepenus*

The specific objectives of the study were to:

i. Evaluate the proximate composition and anti-nutritional factors in raw and processed seeds of *Pueraria phaseoloides*

ii. Determine the growth performance and feed utilization of *Clarias gariepinus* fed diets containing processed *P. phaseoloides*

iii. Determine the carcass composition of *C. gariepinus* fed graded levels of boiled *P. phaseoloIides* seed meal.

1.5 Hypotheses

a. There is no significant difference in the proximate composition of raw and processed *P. phaseoloides* seed meals.

b. There is no significant difference in growth response of *Clarias gariepinus* fed diets containing different concentrations of *P. phaseoloides* seed meal.

c. There is no significant difference in the Carcass composition of the *C. gariepinus* fed with different concentration of *P. phaseoloide* seed meals.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Scientific Classification of *Pueraria phaseoloides*

Pueraria phaseoloides belong to the family: Fabaceae, subfamily: Faboideae, tribe: phaseoleae, subtribe: glycinae, *genus: Pueraria*, species *phaseoloides.*

2.1.1 Common names

Puero (Australia), tropical kudzu (most of the tropics). Centro grande,feuille, Waken gayan- gayan (Hausa - Kano), Waken gizo (Hausa-Zaria).

2.2 Description and Distribution of *P. phaseoloides*

Vigorous twining and climbing, slightly woody, hairy perennial legume, deep-rooting and rather slender. Its main stems are about 0.6 cm in diameter and may extend for 5 to 6 meters. They may root at the nodes and from the nodes a number of lateral or secondary branches are formed. These inter twine and may result in a tangled mass of vegetation 60 to 75 cm deep within eight to nine months of sowing. The young shoots are densely covered with brown hairs. The leaves are large and trifoliate, borne on petioles 5 to 10 cm long covered with ascending hairs. Leaflets are thin, triangular-ovate and very shallowly lobed. Small deep purple flowers are borne in scattered pairs in axillary racemes about 15 to 30 cm long on peduncles about 12.5 cm long. The pod is straight, or slightly curved, linear, cylindrical, 7.5 to 8.5 cm long, thinly clothed with stiff and pressed hairs, black when mature and containing 10 to 20 (usually about 16) seeds, oblong to squarish with rounded corners, brown to brownish black, about 3 mm (Halim, 1992). It is native to

south-east Asia Malaysia and Indonesia and is now widespread throughout the wet tropics.

2.3 Proximate Composition and Anti-nutritional Factors of *Pueraria phaseoloides* Seeds

The proximate composition of *Pueraria* seeds contains 31% crude protein, 1.13% Methionine, 7.23% Luecine and 4.36% Lysine (Owunsu-Dumfeh, 1979b). According to their study, raw seeds had the highest crude protein value of 31.14% followed by roasted seeds 30.90% and the least was toasted seeds which had 23.08%. Toasted seeds had the lowest fat content of (2.82%) followed by roasted seeds (3.99%) and raw seeds had the highest fat content of (4.93%). Hoffman *et al*. (1997), found that *Pueraria* contained 8.4% fibre and 3.65% protein with 22.59% dry matter. In the Kimberley district, Adugna (2007) reported 11.6% protein and Adamneh *et al.* (2007) reported 19.5% protein.

2.4 Anti-Nutritional Compounds in *Pueraria phaseoloides* Seed

Leguminous seeds are commonly used as food for humans. Before leguminous seeds are used in feed formulation such as castor oil seed, the seed need to be subjected to supplementary processing such as boiling, toasting and fermentation to remove the toxic contents (Lewington, 1990).

Pueraria phaseoloides seeds, like other legumes, contain the anti-nutritional factors such as trypsin inhibitor, lectins, phytates, tannin and saponin, which negatively affect the nutritive value of *Pueraria* through direct and indirect reactions such as induce phatological changes in the intestine and liver tissue thus affecting metabolism (Bressani, 1993). These effects limit the use of the raw seed meal, although various processing

techniques like cooking and heating (toasting) tend to reduce the anti nutritional compounds. According to Emenalom and Udebibie (2005), soaking the seed in water prior to cooking was more effective in improving the nutritional value of the seed, and that soaking prior to cooking may have opened up more surface area for heat penetration. The raw seed proved toxic to rat (Ezeagu *et al.*, 2001).

2.5 Utilization of *Pueraria phaseoloides* as Meal Supplement to Animals.

Pueraria phaseoloides is primarily, grown for pasture, hay, and silage. It is palatable to all types of livestock. *Pueraria phaseoloides* is nearly equal to alfalfa in nutritive value (John *et al*., 1999). Leaves, shoots and roots are eaten by some humans. Useful fiber is obtained from stems, and starch is obtained from the tuberous root (roots up to 35 kg each). In China and Japan Ko-fen flour, made from the roots, is used in soups. Said to be cultivated for its tuber in the uplands of New Guinea and New Caledonia (John *et al*., 1999). *Pueraria phaseoloides* seed were used to feed *Oreochromis niloticus* from the related literature. *Pueraria* flower is used in traditional Chinese medicine to reduce reaction to alcohol consumption and it is used as cover crop mixtures or as a component of grass-legume pasture in the humid-tropics (Halim, 1992).

2.6 Utilization of Plant Proteins in Fish Nutrition

Proper nutrition is one of the essential factors that influence the ability of fish to attain genetic potential, growth, reproduction and longetivity (Lall, 1991). Knowledge of the carcass composition of fish is a good measure for assessing the growth and quality of protein and its utilization, whether the diet promoted growth or simply accumulated only as fat. Studies have been carried out on the carcass composition of some indigenous

fishes. Abdullahi *et al.* (2001) reported that the carcass of *C. gariepinus* is composed of moisture (75.00%), ash (10.50%), carbohydrate (10.20%), crude lipid (27.10) and crude protein (52.20%). The amino acid composition (mg/100g) include lysine (10.13), histidine (2.84), arginine (4.40), aspartic acid (8.30), threonine (2.89), serine (2.07), glutamic acid (14.85), proline (2.54), glycine (8.86), alanine (4.90,) cystine (2.62), vasline (3.01), methionine (1.97), isoleucine (5.64), trysine (1.10), and phnylamine (2.0). The mineral content (mg/100g) was reported as calcium (39mg/g), potassium 6.3mg/g, iron 1.71mg/g, magnesium 3.1mg/g, sodium 2.6mg/g, copper 2.0mg/g, phosphorus 1.6mg/g and zinc 19%. Mineral are important in the metabolic and physiological activities and subsequent growth and development of any organism. Eyo (2001), reported that all feed stuff such as maize, corn, palm kernel cake, blood meal, soybeans meal and fish meal contain a fair amount of calcium, potassium, sodium, phosphorus, magnesium and manganese. Soybean has been identified as possible alternative to fish meal in fish feeds due to its abundant protein. It contains all the essential amino acids and can be used in combination with other plant proteins to improve their nutritive value. The low methionine level in soybean meal can be augmented by addition of synthetic methionine in the diet (Eyo and Olatunde, 1998).

Groundnut cake is another conventional feed of plant origin used in the diet of fish. Eyo (1994), substituted soybean meal with different level of groundnut cake in the diet of *Clarias angularis* and the growth performance and feed utilization was observed at 50% inclusion level.

Banyigl *et al.* (2001), reported that diets containing bambara nut meals fed to *Oreochromis niloticus* and *Clarias gariepinus* was highly digested by the fish, with highest value obtained in the fish diet that was traditionally toasted. This indicated that the bambara nut has the potential of being used in the fish feed formulation if appropriately processed.

2.7 Proximate Composition of Some Conventional Fish Feeds

In order to enhance aquaculture production and to improve food security, and reduce the level of poverty in developing countries, a search for inexpensive and locally available feedstuffs is required. Fish feed play a major role in aquaculture viability and profitability because it accounts for at least 40 – 60% of the total cost of fish production (Shang, 1992; Jamu and Ayinla, 2003). In order to meet the increasing demand for fish, a low cost – effective and high quality fish feed is necessary (Gabriel *et al*., 2007). Locally produced feed reduces the cost of production and hence, cheaper means of meeting the protein requirement, improved food security and reduce the level of poverty in developing countries, thus inexpensive and locally available feedstuffs are to be identified, and further more the search for alternative proteins sources should be focused on by – products and materials which are not suitable for direct human consumption (Hoffman *et al,*. 1997).

Several agricultural and agro – industrial by – products available in the tropical region around the world have been evaluated for their production potential in poultry and livestock feed (Beker, 1985; Lema, 1992; Adugna, 2007; Negesse 2009 and Ajebu,

2010). However, only few data are available which cover the suitability of this resource for fish feed (Adamneh *et al*, 2007).

Supplying energy from suitable sources in order to satisfy the energy requirements of fish that will save dietary protein for growth (Jauncey 1998, Sang-Main and Tae-Jun 2005). High dietary fiber concentration can lead to growth depression, due to various factors, such as faster gastric emptying, reduced feed intake, digestibility and nutrient utilization (NRC 1993). It is reported that at least 24% of crude protein was found to be essential for a satisfactory growth of *O. niloticus* under typical East African aquaculture conditions (Liti *et al.*, 2005).

Recently, the analysis of nutritional value of wild plant materials attracted attention, they have shown to contain significant amount of essential nutrients, (proteins, amino acids, vitamins minerals oils and carbohydrates) that can be used for the formulation of fish feeds.

2.8 An overview of Conventional Feeds in Fish Nutrition.

Abowei and Ekubo (2011) reported that essential or indispensable amino acids (EAAs) cannot be synthesized by fish and often remain inadequate but are needed for growth and tissue development. Generally oil seed cakes and legume seeds such as soybean meal, groundnut cake, cotton seed cake, sunflower cake, palm kernel cake, rape seed meal, jack bean, pigeon pea meal, castor seed meal are considered suitable as alternative dietary protein and energy sources for fish feed and are available in the tropical Africa and worldwide on large scale (Fagbenro *et al*., 2003). Among the ingredients being investigated as alternative to fish meal, soybean cake is the most promising (Lim *et al*.,

1998, Hardy 1999, Storebakken *et al.*, 2000, Swick, 2000) because of the security of supply, price as well as its protein and essential amino acid composition. The plant sources of fish diets however include, leaf protein, leaf meal, aquatic macrophytes, cultivable pulses such as mucuna bean, yam beans, bread beans, winged beans or any legume ornamental that can yield pods with seeds. Leaf proteins are abundant in the tropics growing freely without cultivation. All contain diverse levels of protein, which can produce an inexhaustible and inexpensive source of nutrient for fish. Examples of plants with nutritionally valued leaves are cassava (*Manihot esculenta*), pawpaw (*Carica papaya*), pineapple (*Ananas Comosus*), Groundnut (*Arachis hypogea*), soya bean (*Glycine max*) and plantain (*Musa paradisica*) (Abowei and Ekubu 2011; Egwui *et al*., 2013).

2.9 Anti-Nutritional Factors

The utilization of conventional feedstuffs of plant origin had been limited as a result of the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponegin, momosine, cyanoglycosides and linamarin despite their nutrient values and low cost implications (Sogbesan *et al*., 2006).These anti-nutritional factors negate growth and other physiological activities at higher inclusion levels (Oresegun and Alegbeleye, 2001). The presence of anti-nutritional factors in most of the legumes and oil seeds limit its utilization (Olili *and* Krogahal, 1994). Anti-nutritional factors reduces the growth rate of young monogastric animals (Van Damme *et al.*, 1997), and requires removal or inactivation through processing prior to usage within aqua-feed (Tacon, 1995). High level of oxalic and phytic acids in Sesame may reduce the palatability of feed (Narasinga, 1985).

The presence of protease inhibitors, phyto - haemagglutin and Saponin in lentil hulls, which could reduce apparent digestibility of protein and lipid, and inhibit absorption of vitamin and cholesterol metabolism may limit the use of lentil hull and other legumes in fish feed (Berg-lea *et al.*, 1989, Ashild *et al.*, 2010). The presence of toxic substance in Jackbean meal (JBM) had been reported, for example the L-Cananine which limits its use as feed ingredient for livestock, especially monogastric animals, including fish (Udedibe, 1997).

2.10 Protein Requirement of Catfish

Protein is the major constituent of fish diet. Knowledge of the protein requirement of fish is essential for the formulation of a well balance artificial diet for an economical fish feeding. Brown (1977) stated that channel catfish (*Ictalur spunctatus*) could be raised in still water. Brown (1977) recommended cheap complete catfish feed for various weight categories. Better feed efficiency may be obtained from a well-balanced diet containing 24% protein than from a poorly balanced diet containing 36% protein. Fed free choice and balanced in amino acids and energy, 25-30% protein is adequate for larger fish; Fingerlings respond to higher protein levels of 30-36%. Fish meal, soybean meal, fish hydrosylate, skim milk powder, legumes, and wheat gluten are excellent sources of protein. Additionally, the building blocks of proteins (free amino acids) such as lysine and methionine are commercially available to supplement the diet. According to Caesar (2000) unlike domesticated farm animals, many fish species currently being cultured have high dietary protein requirement (30%-50%), which vary for each species and with each particular life stage. For example, fish will require less protein at lower temperature and pH and higher protein content at higher temperature and pH levels. Also fish at a fry

state will require a protein level of 40% and above, fingerlings will require 40% and adult will require the protein of about 35% (Eyo and Olatunde, 2001).NRC (1993), gave the following amount of amino acid requirement for warm water catfish. Lysine 1.43%, leusine 0.98%, arginine 1.2%; methionine 0.64%, valine 0.84%, histidine 0.42%, tryptophan 0.14%, isoleusine 0.72%, phenylalanine 1.40% and threonine 0.65% .

CHAPTER THREE

3.0 Materials And Methods

3.1 Seed Collection

Dry pods of *Pueraria phasoloides* (Kudzu) were collected from plant in different areas in Gwarzo, Zango, S/Gida, Unguwar Tudu, Karkari and Getso areas of Kano State where they are grown naturally as weed plants. They were threshed and later transferred to a motar and pestle and lightly crushed for complete separation of seeds from the pods, and then winnowed to get clear seeds (plate 1).

3.1.1 Seed Processing

Kudzu seeds were divided into four (4) and processed differently. The seeds were Raw (T_1), Toasted (T_2), Boiled (T_3), Roasted (T_4).

The toasting was done with a frying pan at a temperature of 100^oC for 30 minutes by constant stirring the seeds to permits evenly distribution of heat until it turned brown. It was then collected in a tray and air – cooled outside the laboratory and stored.

The boiling was done with alluminium pot using one part of the raw whole seeds to 10 part of clean water on an electric stove for one hour. Roasting was done in an electric oven at 100^oC for 45minutes.

The boiled seeds were sun – dried while the toasted seeds were exposed to room temperature for cooling. The processed seeds were subsequently milled to obtain a

Plate 1: *Pueraria phaseoloides* seeds

homogeneous powder and stored in an air – tight stopper glassware before analysis was carried out.

3.2 Proximate Analysis

The proximate composition (moisture, ash, crude carbohydrates, crude proteins and crude fats) of the raw, fried, boiled and toasted seeds; experimental fish before and after the experiment were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2005). All chemical analysis was carried out in duplicate.

3.2.1 Moisture determination

Principle: This was done based on the difference between the wet weight and the weight after drying to a constant at 100^{o}C for 24 hours.

Procedure: Crucibles were washed and dried to a constant weight in an oven at 100^{o}C, they were later removed and cooled in a desiccators and weight (W_1). 2 grams of the grounded sample were placed in the crucible (W_2) and kept in an oven at 100^{o}C for 24 hours. It was reweighed after about 3 hours to ensure a constant weight (W_3). The moisture content was calculated as:

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where: W_1 = weight of an empty Crucible.

W_2 = weight of a known amount of sample fresh + crucible

W_3 = weight of oven dried sample

3.2.2 Determination of lipid content AOAC(2005)

Principle: This is the continuous extraction of fat content from the sample using suitable solvent e.g. petroleum ether (40 – 60^{o}C) in a Soxhlet extractor.

Procedure: Two round bottom flask were clean and few anti bump granules were added to prevent bumping. 300mls of petroleum ether (40 – 60^{o}C) boiling point were poured into the flask. This were fitted into the Soxhlet extraction units. Extraction thimble was weighed and twenty milliliters of the sample was placed into it and weighed (W_1), the thimble was fixed into the Soxhlet extraction unit with forceps and cold water in circulation. The heating mantle was switched on and solvent refluxing was adjusted at a steady rate. Extraction was carried out for eight hours. The thimble was removed and dried to constant weight in an oven at 70^{o}C and was weighed (W_2). The extractible lipid was calculated as:

$$\% \text{ Lipid} = \frac{\text{Wt of lipid extracted}}{\text{Wt of dried sample}} \times \frac{100}{1}$$

Where the weight of lipid extracted is given by the loss in weight between $w_1 - w_2$ of thimble content after extraction.

3.2.3 Determination of nitrogen and crude protein

Principle: These are the major compounds containing nitrogen (minor nitrogenous ingredients of food includes Amino acids, Purines, Ammonium salts and Vitamin B_1). So nitrogen was used as an index of protein termed 'crude protein' as distinct from true protein.

Procedure: Proteins determination was carried out in three stages, as follows

A. Digestion: two grammes of sample was weighed and placed into a 50ml digestion – flask and the Kjeldahl mixture which acts as a digestion catalyst was added. The flask containing the sample mixture was heated gently at an inclined angle in a Kjeldahl digestion rack until frothing subsided. It was then boiled until the solution became colorless. Heating of the mixture released the nitrogen in the various samples which was then converted to ammonia with the concentrated Sulphuric acid. It was later allowed to cool. The sample was transferred to a 100ml volumetric flask and diluted with distilled water to the mark. It was then mixed thoroughly. The mixture was further allowed to cool before distillation. A blank containing only the Sulphuric acid and catalyst was also heated

$$\text{Conc. } H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

(N – free)

$$C \xrightarrow{\text{Oxidation}} CO_2$$

A. Distillation: A known aliquot (10ml) was transferred to the sample addition funnel of the distillation apparatus and then introduced to the sample chamber 10ml of 40% sodium hydroxide was added to the sample addition tunnel and released to the sample chamber at a slow rate. The ammonia was entrapped in a receiving solution containing 10ml 2% boric acid solution into which 4 drops of bromocresol green/2 drops of methyl red indicator had been put. Distillation was continued until the pink colour turned greenish.

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2Na_2SO_4 + 2H_2O + 2NH_3$$

$$NH_3 + H_2O \longrightarrow NH_4OH$$

$$H_3BO_3 + 3NH_4OH \longrightarrow (NH_4)_3BO_3 + H_2O$$

(A) **Titration**: The back titration method was employed i.e. the ammonia reacts with the Boric acid in the receiving flask and the amount of excess acid was determined by titration with HCl.

$(NH_4)_3BO_3 + 3HCl \longrightarrow NH_4Cl + H_3BO_3$

The percentage total nitrogen was calculated and crude protein was estimated by multiplying the percentage nitrogen with standard conversion factor 6.25. (i.e. % crude protein (cp) = 6.25 x N

$$\% N = \frac{V_1 - V_0 \times M \times 14 \times 100 \times 100}{0.2 \times 1000 \times 10 \times 1}$$

V_o = Vol. of HCl require for blank

V_1 = Vol. of the HCl required for 10ml sample solution

M = Molarity of acid (0.1M)

14 = atomic weight of N_2

100 = total volume of digest

100 = % conversion

10 = Volume of distillate

0.2 = amount of sample taken in gram

Note: protein contains 16% N_2. This makes the general conversion factor to be 6.25.

3.2.4 Determination of crude fiber (AOAC, 2006)

Principle: The bulk of roughage in foods is referred to as Fiber. This is the non – digestible portion of the carbohydrate contained in the sample and was estimated as crude fiber.

Procedure: Two grams of the grounded *Phaseoloides* seeds were placed in a round bottom flask. 100ml of 0.25M H_2SO_4 was added and the mixture boiled under reflux for 30 minutes. The insoluble water was washed several times with hot water until it was acid free. Thereafter, it was transferred into a flask containing 100ml of hot 0.312M NaOH solution. The mixture was boiled again under reflux for 30 minutes and filtered under section, the insoluble residues was washed with hot water until it was base free. It was dried to constant weight in an oven at 100^{o}C, cooled in a desiccator and weighed (C_2). The weighed sample was incinerated in furnace at 550^{o}C for 2 hours. It was put off and allows to cool down. It was removed cooled. It was removed cooled in a desiccator and weighed (C_3). The crude fiber was calculated as the loss in weight on ashing:

Weight of the original sample = W

$$\% \text{ Crude fibre} = \frac{C_2 - C_3}{W} \times \frac{100}{1}$$

3.2.5 Determination of carbohydrate (Pearson, 1976**)**

Principle: The total of protein, moisture content, ash content and lipid content subtracted from 100 gives the carbohydrate, and this is referred to as estimation by difference.

3.2.6 Determination of ash content (AOAC 2005)

The ash content was determined from the loss in weight that occurs during igniting at 550^{o}C in muffle furnace which was enough to allow all organic matter to burn off without permitting any appreciable decomposition of the ash constituent.

Procedure: Crucibles were cleaned and dried in the oven. After drying they were corked in the desiccator and weighed (W_1). 2g of the grounded sample was placed in the crucible

and weighed (W_2) they were transferred into furnace and set to 550^{o}C. The crucible containing the ash was removed and cooled in the desiccator and weighted (W_3). The weight of the residue in the crucible corresponds to the organic matter content.

% Ash and organic matter was calculated as:

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times \frac{100}{1}$$

$$= \frac{w_3 - w_1}{w_2 - w_1} \times \frac{100}{1}$$

$$\% \text{ organic matter} = \frac{\text{loss of weight}}{\text{weight of sample}} \times \frac{100}{1}$$

$$= \frac{w_3 - w_1}{w_2 - w_1} \times \frac{100}{1}$$

3.3 Anti-Nutritional Factors

The anti-nutrients that were determined were Oxalate, Tannin, Saponin, Phytic acid (phytate) and Trypsine. Using the Standard Methods of Association of Officials Analytical Chemist (AOAC, 1980).

The determination was done as follows:

3.3.1 Determination of oxalate

Oxalate was determined by using the method of Oke (1969). Two grammes (2g) of the sample was placed in a 250ml volumetric flask, 190ml of distilled water and 10ml of 6MHcl were added. The mixture was warmed in a water bath at 90^{o}C for four hours and the digested sample was centrifuged at a speed of a 2,000rpm for 5 minutes. The supernatant was then diluted to 250ml. three 50ml aliquots of the supernatant was evaporated to 25ml, the brown precipitate was filtered off and washed. The combined

solution and washings was titrated with concentrated ammonia solution in drops until salmon pink color of methyl orange changed to faint yellow.

The solution was heated in a water bath to 90^{o}C and the oxalate was precipitated with 10ml of 50% calcium chloride ($CaCl_2$) solution. The solution was allowed to stand overnight and then was centrifuged. The precipitate was washed into a beaker with hot 25% H_2SO_4, diluted to 125ml with distilled water and after warming to 90^{o}C, it was against 0.05m $KMnO_4$.

Calculation

1ml 0.05M $KMnO_4$ = 2.2mg oxalate.

3.3.2 Determination of tannin

Tannin was determined using the standard method described by AOAC (2005). Two grammes of the dried sample was boiled with 300ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non – obsorbent cotton wool. A volume of 25ml of the infusion was measured into a 2 liter porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution changed green; then few drops of 0.1N potassium permanganate was added. The titre was multiplied by 0.006235 to obtain the amount of tannin in the sample.

The equation is below:

0.1N oxalic acid = 0.0066235g tannin

3.3.3 Determination of saponin

The standard method of AOAC (2005) was used to determine saponin in the samples. A gravimetric method employing the use of Soxhlet extractor and two different organic solvent were used. The first solvent extract lipids and interfering pigments while the second solvent extracts the Saponin proper.

A known weight of the dried grounded sample was weighed and fitted unto the soxhlet apparatus (bearing the sample containing thimble) and methanol poured into the flask. The methanol should be enough to cause a reflux. The saponin was there exhaustively extract for 3hours. The flask was re-weighed. The difference in weight represents the weight of saponin extracted.

$$\%\ \text{saponnin} = \frac{\text{wt ofsaponin}}{\text{wt ofsample}} \times \frac{100}{1}$$

3.3.4 Phytic acid (Phytate) determination

Phytic phosphorus was determined by the method of Wheeler and Ferrel (1971). A known weight of each ground sample was soaked into 100m/of 2% HCl for into 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 color persisted for 5 minutes. The concentration of the $FeCl_3$ was 1.04% w/v.

Calculation:

Mole ratio of Fe to Phytate = 1:1

$$\text{Conc. of phytate phosporus} = \frac{\text{Titre values}}{1000} \times \text{weight ofsample}$$

3.3.5 Trypsin determination

1.0g of ground sample was weighed and mixture dispersed into 50mls 0.05M NaCl solution. The mixture was stirred for 30 minutes at room temperature and centrifuged. Filtered the supernatant through Whatman No. 41 filter paper. The filtrate (extract volume) was used for the assay.

Preparation of standard Trypsin and substrate 1mg/ml of trypsin in 0.1M HCl was prepared 2% casein substrate in 0.1M phosphate buffer pH 7.6 was prepared.

Procedure: To 2.0ml of standard trypsin solution add 1ml of trypsin inhibitor extract and then 10ml of the substrate in a test tube. Prepare a blank of 10ml of the sample substrate in a test tube to stand for at least 5 minutes and then measures spectrophotometrically at 410mm.

The trypsin inhibitor activity was expressed as the number of trypsin unit inhibitor (TUI) per unit weight at the sample analyzed.

Calculation:

$$TUI/_{mg} = \frac{b - a}{0.01} \times F$$

Where b = absorbance of test sample solution

a = absorbance of the blank

$$F = {}^{1}/_{W} \times {}^{V_1}/_{V_a} \times D$$

Where W = weight of sample

V_1 = total volume extract

V_a = volume of extract used in the assay

D = Dilution factor (if any)

3.4 Experimental Diets

3.4.1 Diet formulation and composition

The crude protein values of the *Pueraria* seeds derived from the proximate analysis were used to formulate feed at a crude protein level of 40% using Pearson Square method as shown in Table 3.1 below: The experimental diets comprised of *Pueraria* meal, fishmeal (clupeid), yellow maize, bone meal/blood meal, palm oil, vitamin premix, salt and wheat flour. From the analysis carried out on the different processed sample of *Pueraria*, the boiled seeds were chosen for feeding trial for the *Clarias gariepinus*. Diet 1 – served as "positive control diet´. Diet 2, 3 , 4, 5 and 6 contains the boiled seed meal with 10%, 20% , 25%,30 and 0% respectively which serve as negative control. And it was also observed the boiled seed samples had lowered anti – nutritional factors when compared with the fried and toasted seed samples. All diets were isoproteic (40% protein).

3.4.2 Diet preparation

Five experimental diets were prepared through mixing of various ingredient based on the percentages of crude protein required. The proportion of the ingredients was weighted separately. Each of the mixture was first mixed dry and later with just enough hot water to obtain homogenous hard dough. The mixture was then moulded, pelleted using a local pelleting machine. The local pelleter measured about 0.2cm in diameter and 2cm in length. The finished products were sun dried and stored in labeled polyethylene bags. The cassava served as a suitable binder. Sample of each diet was analysed in the laboratory for proximate composition of the fish feed pelleted following standard method of analysis (AOAC, 2005).

Table 3.1: Gross composition of the experimental diets fed to *Clarias gariepinus* juveniles

Percentage of *Pueraria* diet (Boiled kudzu)						
Ingredients	**Control Diet A Commercial feed**	**Diet O 0% Kudzu**	**Diet B 10% Kudzu**	**Diet C 20% Kudzu**	**Diet D 25% Kudzu**	**Diet E 30% Kudzu**
Pueraria seed meal		-	6.63	13.28	16.59	19.92
Fish Meal		64.38	57.74	52.11	47.79	44.47
Bone meal		2.00	2.00	2.00	2.00	2.00
Yellowmaize		6.17	6.17	6.17	6.17	6.17
Wheat flour		9.66	9.66	9.66	9.66	9.66
Palm oil		2.19	2.19	2.19	2.19	2.19
Vitamin premix		4.00	4.00	4.00	4.00	4.00
Fat		6.8	6.8	6.8	6.8	6.8
Salt		0.5	0.5	0.5	0.5	0.5
Lysine		3.0	3.0	3.0	3.0	3.0
Methionine		2.0	2.0	2.0	2.0	2.0
Total		100.0	100.0	100.0	100.0	100.0

This was to ensure that the final product contained all the nutritional and energy requirement needed for optimal fish growth and physiological functions.

3.5 Source of Experimental Fish

Clarias gariepinus was obtained from Aqua consult fish/hatchery farm, Kaduna, opposite National Teachers Institutes, along Kaduna-Zaria road, Kaduna state, Nigeria. The fish were transported in two 50L plastic jerry cans to the Fisheries Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria. On arrival, the fish were transferred into two large containers for a period of two weeks for acclimatization. During the acclimatization period, the fish were fed with commercial feed at five percent (5%) total body weight. Water parameters were monitored as described in 3.9.

3.6 Experimental Design

Twelve aquaria with dimensions of 60 x 30 x 30cm were used, each was thoroughly washed, cleaned and disinfected with dettol containing thirty five (35) liters of dechlorinated water, which provide a water environment for the fish. Ten (10) *Clarias gariepinus* were stocked randomly in each of the six treatments with two replicates each, giving a total of 120 fish. The set – up was covered with mesh on the top to avoid fish from jumping out. (Plate II).

3.7 Feeding Rate and Practices

Feeding of fish was done twice daily at 8:00am in the morning and 6:00pm in the evening (i.e. 2.5% in the morning and 2.5% in the evening of their body weight). Fish were weighed after every two weeks and amount of feed to be given was adjusted or increased to reflect the new body weight of fish. Feeding trail lasted for 8 weeks.

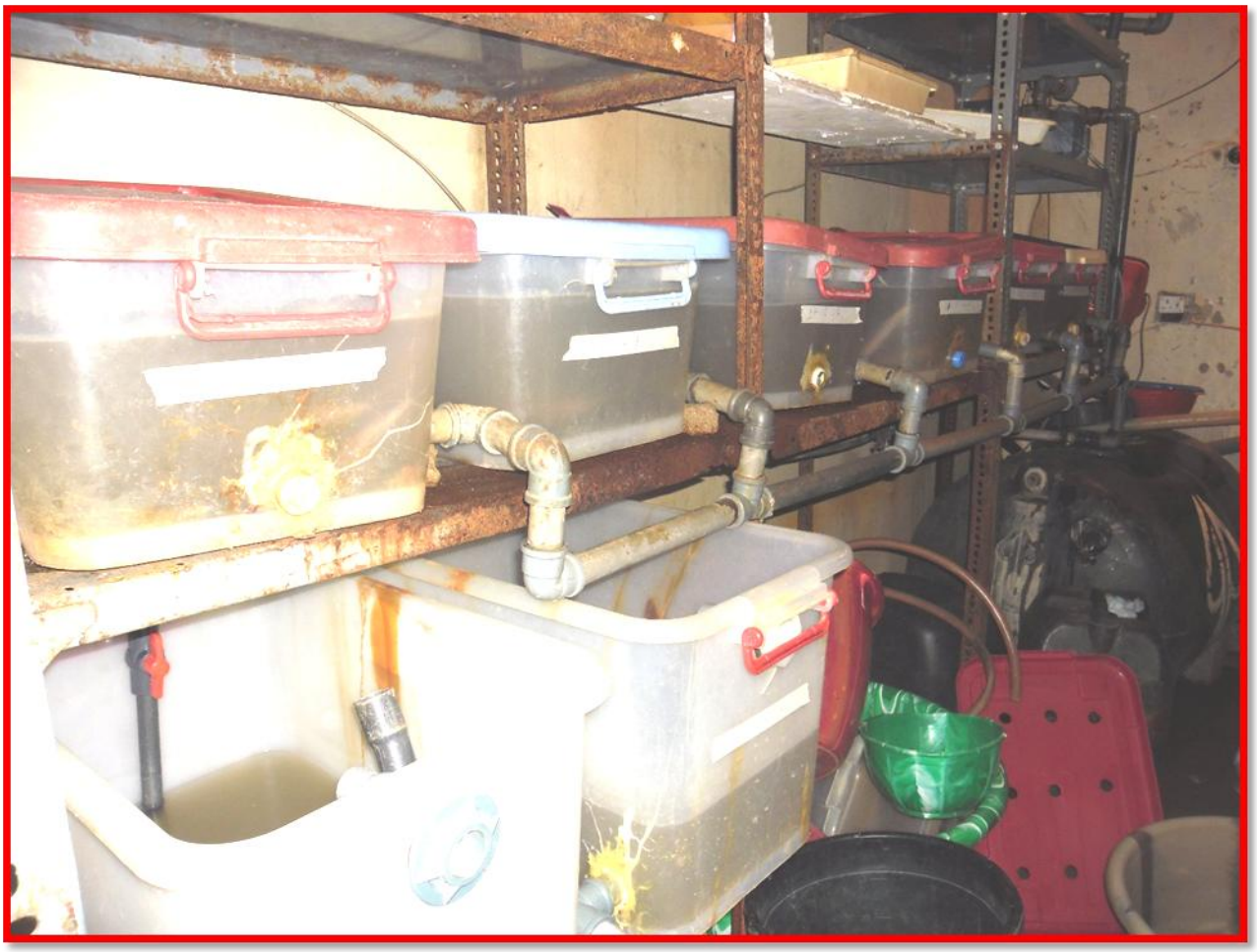

Plate II: Experimental setup for feeding trials

3.8 Growth Performance and Feed Utilization Parameters

3.8.1 Mean weight gain (WG)

The fish fresh weight gain (WG) was calculated as the difference between the mean final weight of fish at the end of the experiment and the mean initial weight in grams.

3.8.2 Percentage live weight gain (PLWG%)

The percentage LWG was computed as the difference between the mean initial and mean final fish weight divided by the initial weight expressed as percentage (Wannigama *et al*, 1985).

$$PLWG = \frac{wt_1 - wt_0}{wt_0} X\ 100$$

Where:

Wt=mean final weight

Wt_0=mean initial weight

3.8.3 Specific growth rate (SGR)

The specific growth rate was calculated as described by Hepper (1988) as follows:

$$SGR = \frac{\log_e wt - \log_e wo}{t - t_o} (\%\ days)$$

Where: wt = weight at the time of observation

wo = initial weight

$t - t_o$ = the period under study in days, and e = the base of natural logarithm (10)

3.8.4 Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated as the dry weight of feed consumed divided by the wet weight gain of fish (Stickney, 1979 and Hepper, 1988) this was expressed as:

$$\text{FCR} = \frac{\text{Weight feed consumed (g)}}{\text{Weight gained}}$$

3.8.5 Gross food conversion efficiency (GFCE)

The GFCE is the reciprocal of the FCR expressed as a percentage (Stickney, 1979), it was expressed as:

$$\text{GFCE} = \frac{1}{\text{FCR}} \times 100$$

3.8.6 Protein efficiency ratio (PER)

This is the efficiency with which the fish utilizes dietary protein and is defined by the equation given by Osborne *et al.* (1919).

$$\text{PER} = \frac{\text{Wet weight gained by fish (g)}}{\text{weight of crude protein fed (g)}}$$

3.8.7 Apparent Net protein utilization (ANPU)

$$\text{ANPU} = \frac{\text{Fish Protein Gain(g)}}{\text{Protein Fed (g)}} \times 100$$

Where: protein gain = final body protein – initial body protein

Protein consumed = total dietary protein fed. (Dabrowski and Kozak, 1979)

3.9 Determination of Water Parameters

Water parameters were determined throughout the 8 weeks experimental period. The parameters include: water pH, water temperature and Dissolved Oxygen (D.O).

3.9.1 Water pH

Water pH was determined using Hanna instrument model. The meter was standardized with the use of the buffer solution at the pH of 4.0, 7.0 and 9.0. The pH meter was usually lowered into the water collected in each aquarium from water bath for both 5 minutes before reading was taken. The reading was taken every week.

3.9.2 Water temperature

Water temperature was taken with the use of Hanna instrument model HI 98129. The reading was taken weekly. The thermometer was lowered in to each water – filled aquarium and remain deep for about 5 minutes before reading and recording.

3.9.3 Dissolved oxygen (D.O)

Hanna instrument was used to measure the dissolved oxygen. The set up was held in position for about 5 minutes. Reading and recording was done.

3.9.4 Condition factor (K)

$$K = \frac{100W}{L^3}$$

(Madu and Akilo, 2001)

Where;

W= Body weight (g)

L=Standard length (cm)

3.9.5 Carcass analysis

Before the feeding trial commenced, four fish were selected from each tank and dried. At the end of the feeding trial, four fish were selected from each tank and dried. The tissues from these representative samples of fish were grounded separately using an electric milling machine and subjected to proximate analysis in accordance to AOAC methods (AOAC, 2005).

3.9.6 Statistical analysis

Analysis of variance (ANOVA) was conducted to find out if there was significant difference ($P< 0.05$) between the means. Duncan multiple range test (DMRT) (Duncan, 1955) was used to separate treatment means. Analysis was conducted using SPSS statistical package version 20.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Raw and Processed *Pueraria phaseoloides* Seed Meals

Table 4.1 shows the result for the proximate analysis of the raw and processed *P. phaseoloides* seed meals. Raw seeds had the highest crude protein (31.14%) followed by roasted seed (30.90%) and the least was toasted seeds with (23.08%). Toasted seeds had the lowest fat content (2.82%), followed by roasted seeds (3.99%) and raw seeds had the highest fat content of (4.93%). The fiber content values ranged from 8.80-5.40%. Toasted seed recorded the highest value of 8.80% while the boiled seed recorded 5.40%. The ash content value ranged from 3.26-2.14%. Boiled seed has the lowest ash content of 2.14 while the toasted seed recorded the highest value of 3.26%.

4.2 Anti-Nutritional Factors

Boiling was very effective in reducing the anti-nutritional factors of *P. phaseoloides* In both the raw and processed seeds, the lowest value of Trypsin (0.81%), Saponin (1.33%), Phytic (0.13%) and Oxalate (0.16%) were recorded in seeds that were boiled. The raw seeds had the highest value of Trypsin (1.48), Saponin (4.98), Phytic (0.25),Tannin (0.16) and Oxalate (2.66) compared to others (Table 4.2).

4.3 Physico-Chemical Parameters of Water

The Weekly temperature, hydrogen ion concentration (pH) and dissolved oxygen (DO) during the eight weeks feeding period of *Clarias gariepinus* is presented in Table 4.3.

Table 4.1 Proximate analysis of *P. phaseoloides* seed meal

Sample	**% Moisture**	**% Fat**	**% Ash**	**% Crude Protein**	**% Crude Fiber**	**% CHO**
Raw *P. phaseoloides*	7.35	4.93	3.01	31.14	6.55	57.02
P. phaseoloides (Toasted)	1.43	2.82	3.26	23.08	8.80	69.30
P. phaseoloides (Boiled)	6.67	4.08	2.14	29.17	5.40	65.94
P. phaseoloides (Roasted)	1.36	3.99	3.03	30.90	7.00	60.72

Table 4.2 Anti-nutritional factors of raw and treated *P. phaseoloides* seeds.

Sample	**% Trypsin**	**% Saponin**	**% Phytic**	**% Tannin**	**% Oxalate**
Raw *P. phaseoloides*	1.48	4.98	0.25	0.16	2.66
P. phaseoloides (Toasted)	0.84	2.44	O.25	0.10	0.36
% Reduction	72.97	51.00	0.00	75.5	86.47
P. phaseoloides (Boiled)	0.81	1.33	0.13	0.13	0.16
% Reduction	45.27	73.29	48.0	18.75	93.98
P. phaseoloides (Roasted)	0.82	2.04	0.23	0.12	1.60
% Reduction	44.59	53.03	8.0	25.0	39.85

TABLE 4.3: Physico-chemical parameters of water in the experimental tanks

Parameters	Treatment A (Com. feed)	Treatment B (10%)	Treatment C (20%)	Treatment D (25%)	Treatment E (30%)	Treatment F (Control 0%)
Temperature	26.35±2.50[a]	25.05±1.00[a]	25.00±1.00[a]	25.00±1.00[a]	24.93±1.00[a]	25.23±1.00[a]
pH	6.96±1.00[a]	6.42±1.00[a]	6.34±1.00[a]	6.38±1.00[a]	6.35±1.00[a]	6.41±1.00[a]
DO	4.05±1.00[a]	5.15±1.00[a]	3.85±1.00[a]	5.60±1.00[a]	5.73±1.00[a]	4.53±1.00[a]
Dissolved Solid	135.25±1.00[b]	120.25±1.00[c]	141.75±1.00[a]	103.75±1.00[d]	100.25±1.00[e]	119.25±1.00[c]
Electrical Conductivity	198.83±1.00[e]	240.00±1.00[b]	286.00±1.00[a]	207.00±1.00[c]	203.50±1.00[d]	240.00±1.00[b]

Values with the same superscription on the same rows are not significantly different (P>0.05).

The water temperature in the experiment ranged from (24.93) to (26.35°C). The highest temperature was recorded in tank A while the lowest was recorded in tank E. There was no significant difference (P>0.05) within the range of temperature during the experimental period. pH ranged from (6.34) to (6.96) with highest value of 6.96 in tank A. The dissolved oxygen was within the range of (3.85) to 5.73mg/l. Tank E recorded the highest value while tank C had the least value of 3.85. There was no significant difference (P>0.05) between the pH values and Dissolve oxygen values throughout the experimental period.

4.4 Proximate Composition of Experimental Diets Fed *Clarias gariepinus*

Table 4.4 presents the proximate composition of the experimental diets fed to the fish

4.4.1 Dry matter (DM)

Dry matter values ranged from 95.98% - 97.70%. Diets 5, 4 and 1 had the highest values of 97.70, 96.89, and 96.30% while Diets 3, 2 and 6 had the following values (96.09, 96.00 and 95.98%). There was no significant difference (P>0.05) among all the treatments in terms of dry matter.

4.4.2 Ash content

The ash values ranges from (5.57% -6.21%). Diet 6 had the highest value of (6.21%), followed by diets 2, 5, 1, 3 and 4 which recorded the following values (5.96, 5.95, 5.89, 5.70 and 5.57%) respectively. There was no significant difference (P>0.05) among them.

4.4.3 Crude fiber (CF)

The values of the crude fiber in the diets ranged from 8.89% -8.27%. Diets 3, 6, and 4 had 8.89, 8.70, and 8.52% values respectively. Diets 1, 5 and 2 recorded the following

TABLE 4.4: Proximate composition of experimental diet fed to *Clarias gariepinus:*

Composition	**Diet 1Com.feed**	**Diet 2 0%**	**Diet 3 10%**	**Diet 4 20%**	**Diet 5 25%**	**Diet 6 30%**
Crude protein	40.98±0.50[a]	40.80±1.00 [a]	40.20±0.80 [a]	40.72±2.28[a]	40.57±0.43 [a]	40.60±0.80 [a]
Crude fiber	8.49±1.00 [a]	8.70±1.00 [a]	8.27±1.00 [a]	8.89±1.00 [a]	8.52±1.00 [a]	8.40±1.00 [a]
Crude Fat	6.25±1.00 [a]	6.15±1.00 [a]	8.31±1.00 [a]	9.26±1.00 [a]	7.22±1.00 [a]	8.25±1.00 [a]
Ash	5.89±1.00 [a]	6.21±1.00 [a]	5.96±1.00 [a]	5.70±1.00 [a]	5.57±1.00 [a]	5.95±1.00 [a]
NFE	21.41±1.00 [a]	20.97±1.00 [a]	21.38±1.00 [a]	20.98±1.00 [a]	19.96±1.00 [a]	21.37±1.00 [a]
Moisture	3.40±0.10a[b]	2.00±0.00 [e]	3.22±0.03 [cd]	3.52±0.02 [a]	3.12±0.02 [d]	3.30±0.01[bc]
Organic Matter	90.72±0.02 [a]	90.25±0.05 [a]	90.05±2.00 [a]	90.40±0.90 [a]	91.30±0.90 [a]	91.50±1.02 [a]
Dry Matter	96.30±0.90 [a]	95.98±1.50 [a]	96.00±2.00 [a]	96.09±2.00 [a]	96.89±2.04 [a]	97.70±1.20 [a]

Values with the same superscription on the same rows are not significantly different (P<0.05).

(8.49, 8.40 and 8.27%) values respectively. There was no significant difference (P>0.05) among the treatments.

4.4.4 Nitrogen free extracts (NFE)

The nitrogen free extract values were as follows (21.41, 21.38, 20.98, 19.96, 21.37 and 20.97%) for diets 1, 2, 3, 4, 5 and 6 respectively. Statistically there was no significant difference (P>0.05) among the diets.

4.4.5 Crude protein (CP)

The crude protein values are presented as follows (40.98, 40.20, 40.72, 40.57, 40.60, and 40.80%) for Diet 1, 2, 3, 4, 5 and 6 respectively. There was no significant difference (p>0.05) between them.

4.4.6 Crude fat

The crude fat values ranged between (6.15 - 9.24%). Diet 1, 2, 3, 4, 5 and 6 had recorded the following values of crude fat as (6.25, 8.31, 9.26, 7.22, 8.25 and 6.15%) respectively. There was no significant difference (P>0.05) among the diets.

4.4.7 Moisture content

The moisture content values were as follows 3.40, 3.22, 3.52, 3.12, 3.30 and 2.00% representing treatment 1, 2, 3, 4, 5 and 6 respectively. There was significant difference between them (P<0.05).

4.4.8 Organic matter

The Organic matter content in treatments 1, 2, 3, 4, 5 and 6 had the following values (90.72, 90.05, 90.40, 91.30, 91.50, and 90.25%) respectively. The difference was not significant (P>0.05).

4.5 Growth Performance Of *Clarias gariepinus* fed experimental diets

4.5.1 Mean weight gain (MWG)

Table 4.5 shows growth assessment and feed utilization of *Clarias gariepinus* fed the processed *P. phaseoloides* seed meal. The mean weight gain of diet A (Commercial feed) was 63.70g, representing (268.2%) percentage live weight gain (PLWG) and it was the highest followed by F (62.77g), C (61.11g), B (59.49g), D (56.88g), and E (54.61g) representing, (264.96, 239.50, 236.62, 262.26, and 213.73%) percentage live weight gain respectively. Diet C which was the 20% kudzu inclusion recorded 61.11g MWG indicated that 20% inclusion was the best or highest. The MWG of the experimental diets were significantly different (P<0.05).

4.5.2 Mean length gain (MLG)

The values of the mean length gain (MLG) indicated that sample A (11.12cm) had the highest value representing 70.60% percentage length gain (PLG) while sample B(10.53cm), F (10.14), C (9.59cm), D (9.43cm) and E (9.16cm) representing 65.36%, 63.93%, 60.47%, 61.47% and 56.37% Percentage length gains (PLG). There was no significant difference (P>0.05) between the values.

4.5.3 Specific growth rates (SGR)

The specific growth rates values shows no significant difference (P>0.05) among treatments. That fish fed Diet A (Commercial feed), had the highest SGR of 1.96 while fish fed with diets B, E and D had similar values of 1.89, 1.87 and 1.82, respectively. Diet C recorded 1.93 indicating highest value of *P. phaseoloides* seed inclusion. The control diet F had 1.92 SGR. However, results showed no significant difference (P> 0.05) between the treatments.

4.5.4 Feed conversion ratio (FCR**)**

The feed conversion ratio (FCR) of diet C (20%) with 0.74 was the lowest, followed by diet A (30%) with 0.98.The highest FCR was obtained in diets B (I.61). Diets E, D, and F had 1.44, 1.22, and 1.21 respectively. There was significant differences (P<0.05) between the values of FCR obtained for the treatments.

4.5.5 Protein efficiency ratio (FER)

The Feed Efficiency Ratio (FER) values indicated that diet E had the lowest value FER (0.13). The highest PER value was obtained from diet A (1.02). Diet F, D and B had (0.83), (0.82) and (0.48) respectively. The difference among the values were not significant (P>0.05).

4.5.6 Apparent net protein utilization (ANPU).

The ANPU values ranged between 0.22 and 3.57. The ANPU value of Diet A (Commercial diet) had 3.57 which was highest followed by diet C with 2.54. Diets F, D and E recorded 1.89, 1.76, and 1.40 respectively. There was significant difference (P<0.05) between the ANPU values.

Table 4.5: Growth Assessment and Feed Utilization of *Clarias gariepinus* Fed With Experimental Diets

Parameters **Diets**	**Commercial Feed A**	**10% boiled seed B**	**20% Boiled Seed C**	**25% Boiled Seed D**	**30% Boiled Seed E**	**0% F (Control)**
Mean initial body weight (g)	22.01±1.42[a]	25.15±50.45[a]	25.51±1.17[a]	21.57±1.22[a]	25.55±0.36[a]	23.69±0.15[a]
Mean final body weight (g)	85.72±1.60[b]	84.66±9.28[ab]	86.62±4.37[a]	78.14±7.72[ab]	80.16±10.96[ab]	86.46±1.57[a]
Mean weight gain(g)	63.70±3.22[a]	59.51±9.72[a]	61.10±3.19[a]	56.57±8.93[a]	54.61±10.60[a]	62.78±1.42[a]
Percentage live weight gain (%)	268.2±1.00[a]	236.62±1.00[b]	239.50±1.00[b]	262.26±1.00[a]	214.23±0.50[c]	264.96±1.00[a]
Mean initial standard length(cm)	15.75±1.00[a]	16.11±1.00[a]	15.84±1.00[a]	15.34±1.00[a]	16.25±1.00[a]	15.86±1.00[a]
Mean Final standard length (cm)	23.88±1.00[a]	26.64±1.00[a]	25.41±1.00[a]	24.77±1.00[a]	25.41±1.00[a]	26.00±1.00[a]
Mean body weight gain (MWG) (g)	11.12±1.00[a]	10.53±1.00[a]	9.58±1.00[a]	9.43±1.00[a]	9.16±1.00[a]	10.14±1.00[a]
Specific growth rate(%)	1.96±1.00[a]	1.89±1.00[a]	1.93±1.00[a]	1.82±1.00[a]	1.87±1.00[a]	1.92±1.00[a]
Feed conversion ratio	0.98±1.00[a]	1.61±1.00[a]	0.74±1.00[a]	1.22±1.00[a]	1.44±1.00[a]	1.21±1.00[a]
Apparent net protein utilization	3.57±1.00[a]	0.22±0.01[a]	2.54±1.00[a]	1.76±1.00[a]	1.40±1.00[a]	1.89±1.00[a]
Protein efficiency ratio	1.02±1.00[a]	0.48±0.01[a]	1.35±1.00[a]	0.82±0.01[a]	0.13±1.00[a]	0.83±0.01[a]
Gross feed conversion efficiency	9.34±1.00[b]	11.09±1.00[b]	11.19±1.00[b]	12.25±1.00[b]	21.73±1.00[a]	10.82±1.00[b]
Percentage Survival Rate (%)	100.00±1.00[a]	70.00±1.00[e]	95.00±1.00[b]	90.00±1.00[c]	60.00±1.00[f]	76.00±1.00[d]
Condition factor	0.64±0.01[a]	0.45±0.03[a]	0.53±0.02[a]	0.51±0.06[a]	0.49±0.04[a]	0.49±0.05[a]

Values of the same superscription on the same rows are not significantly different (P>0.05)

4.5.7 Gross feed conversion efficiency (GFCE) %

The diet with lowest GFCE value was diet A (9.34), while diet E had the highest GFCE value (21.73). Diets D, C, B and F had the following values 12.25, 11.19, 11.09 and 10.82 respectively. There were significant differences ($P<0.05$) among them.

4.5.8 Percentage survival rate (PSR) of experimental fish

There was a high survival rate in all the experimental treatments with Diet A having the highest value of 100% survival (Commercial feed), Diet C ,D, F, and B had 95%, 90%, 76% and 70% survival rate values respectively, while diet E (60%) had the least survival rate. The difference were significant ($P<0.05$) among them.

4.6 Carcass Composition of Experimental Fish Before And After Feeding Period

Table 4.6 shows the proximate composition of the fish (*Clarias gariepinus)* fed experimental diets.

4.6.1 Dry matter

The initial dry matter (DM) of fish were 95.84, 93.54, 94.06, 92.65, 93.46, and 93.67% for the commercial diet A and B, C, D, E and F respectively. The percentage dry matter for all treatments was not significantly different ($P > 0.05$) of fish at the beginning of the experiment.

Table 4.6: Carcass Composition of Experimental Fish (*Clarias gariepinus*) fed experimental diets.

Composition	**Initial**	**Com. Feed A**	**10% B**	**20% C**	**25% D**	**30% E**	**0% F**
Dry Matter	95.84±1.00^{a}	93.67±1.00 ^{a}	94.06±1.00 ^{a}	92.65±1.00^{a}	93.45±1.00 ^{a}	94.46±1.00 ^{a}	93.54±1.00 ^{a}
Crude Protein	46.75±1.00^{d}	60.86±1.00 ^{a}	47.93±1.00^{cd}	60.64±1.00^{a}	54.92±1.00^{b}	50.38±1.00^{c}	57.52±1.00^{ab}
Crude Fiber	0.46±0.04 ^{a}	0.06±0.01^{b}	0.03±0.01^{bc}	0.05±0.01^{bc}	0.09±0.01^{b}	0.08±0.01^{bc}	0.02±0.01^{c}
Crude Fat/Oil	14.24±1.00 ^{a}	7.68±1.00^{b}	8.06±1.00^{b}	7.78±1.00^{b}	7.83±1.0b	7.68±1.00^{b}	8.03±1.00^{b}
Ash	11.06±1.00 ^{a}	7.62±1.00 ^{a}	8.58±1.00 ^{a}	8.38±1.00^{a}	7.70±1.00 ^{a}	10.19±1.00 ^{a}	10.30±1.00 ^{a}
Carbohydrates	22.63±1.00^{c}	23.79±1.00^{c}	34.42±1.00 ^{a}	23.20±1.00^{c}	30.43±1.00^{b}	30.75±1.00^{b}	24.29±1.00^{c}

Values with the same superscription on the same rows are not significantly different ($P<0.05$)

4.6.2 Crude protein

The fish fed diet 1 (A) had the highest percentage of 60.86% followed by diet 3 (20% inclusion) which was diet C. while fish fed on 10% (B) had the least protein content of 47.52%. Fish fed diets D, E and F had 54.92%, 50.38 and 50.28% respectively. The crude protein content of diet A was significantly higher (P > 0.05) than others (Table 4.6).

4.6.3 Crude fiber

The crude fiber values of initial and other treatment were 0.46g/100g, 0.02g/100g, 0.03g/100g, 0.05g/100g, 0.09g/100g, 0.08g/100g and 0.06g/100g for fish fed diets A, B, C, D and E respectively. This showed significant difference between the treatments (P> 0.05). The crude fiber content before the feeding trial was significantly higher (P< 0.05) than those of the fish after the experiment (Table 4.6).

4.6.4 Crude ether extract

The crude fat/oil of the carcass has the following values of 14.24%, 8.03%, 8.06%, 7.78%, 7.83%, 7.68%, and 7.68% for commercial feed diets A, B, C, D and E respectively. Fish fed commercial diet has the highest values of 14.24g and those fed diet D recorded the lowest value of 7.68.

Statistically fish fed commercial diet were significantly higher in fat (p < 0.05) than those in the other treatments.

4.6. 5 Ash content

The crude ash content values were as follows A (11.06g / 100g), E (10.19g/100g) and D (7.7g/100g) recorded the lowest value. Statistically, there were significant differences between the treatments ($P< 0.05$).

4.6.6 Carbohydrates

Nitrogen free extract of fish at the beginning was 22.63g/100g, after the feeding trials the NFE were 24.29g/100g, 34.42g/100g, 23.2g/100g, 30.43/100gg and 23.79g/100g for fish fed diet E, B, C, D, and F respectively. Diet B (10%) recorded the highest value, while fish fed diet C(20%) recorded the lowest values

CHAPTER FIVE

5.0 DISCUSSION

The proximate analysis of the seeds of *P. phaseoloides* showed that crude fibre values ranged from 5.40 to 8.80%. The toasted seed had the highest value while the boiled seed had the lowest value. The high fibre content of seeds of *P. phaseoloides* is attributed to the inclusion of seed coat during milling. The value of Nitrogen free extract (NFE) content of seed ranged from 57 to 69% with highest value obtained in toasted seeds followed by boiled seeds while the lowest value was recorded in raw seed. The NFE content of the seeds is adequate for providing energy and could be compared favourably to those of bambara nut (66%), mucuna beans (53%) and cotton seed cake (42.93%) (NRC, 1993). The lipid content of the seeds ranged from 2.82 to 4.93%. The highest value was obtained in the raw seed followed by boiled seed while the lowest value was recorded in toasted seeds. This is an indication that, the onset of rancidity in the seeds would be low, particularly if stored for a long time, and that it is not an oil seed, because oil seeds are grouped as those legumes with oil content ranging from 18% as in soybeans, to 45% in groundnut (Eyo, 2003). The protein content ranged from 23.08 to 31.14% with highest value obtained in raw seeds, followed by the roasted seed and the boiled seed. The toasted seeds recorded the lowest crude protein content. The protein content in the raw seeds of *P. phaseoloides* is higher than the values obtained in boiled, roasted and toasted seeds, which is associated with denaturing of protein molecules due to high temperature during heating (Abbey and Berezi, 1988).

The anti-nutritional factors present in *P. phaseoloides* seeds were reduced after boiling, an indication that boiling process reduced to a large extent the levels of anti-nutrients present in the seed. The trypsin values reduced drastically in treated seeds compared to the trypsin value in the raw seed which had high value, while the boiled seeds recorded the least value of trypsin. The saponin values of the seed ranged from 1.33 to 4.98% with least value obtained in the boiled seed and the highest saponin was obtained in the raw seed. The phytic acids values ranged from 0.13 to 0.25% with highest values obtained in raw seeds and toasted seeds, followed by roasted seed. While the least value of phytic was recorded in boiled seeds. The raw seeds of *P. phaseoloides* recorded the highest value of tannin, followed by the boiled seeds, while the least value of tannin was obtained in toasted seeds. The values of oxalate ranged from 0.16 to 2.66% and the highest value was obtained in the raw seeds while the least value was recorded in boiled seeds. Similarly, the boiling of seeds of *Paulina monandra* reduced the levels of the saponin, tannin, oxalate and phytates (Marian, 2010). These anti-nutritional factors are growth inhibitors and occur in almost all plant materials. In most cases they form a shield in the protein molecule of the ingredient, thus preventing the proteases (digestive enzyme) from getting to the protein molecule, making them unavailable for digestion, absorption and hence impair the growth of fish. This result in wastage as the crude proteins is passed out along with feaces (Eyo, 2003). Therefore, the decrease in the level of anti- nutritional factors in the seeds portends to make the seeds more digestible, absorbable and provide the nutritional element of the seed to the fish.

Claris gariepinus (catfish) is a fresh water fish. It requires certain water parameters to grow best under culture conditions. The range of temperature, pH and dissolve oxygen

obtained in the experiment fall within the recommended range. Ayoola and Fredrick (2012) reported that pH of 6.5-9.0 and temperature of 22-27^{o}C gives the best growth by cultured tropical fishes. Ayoola and Fredrick (2012) reported that for tropical fish, an average temperature of 28^{o}C, D.O of 6.9 and pH 7.3 are optimal for normal growth. Auta (1993) reported that temperatures of 25^{o}C and 30^{o}C, pH 6.7 9.0 would be adequate for fresh water fish culture.

5.1 Growth Performances

The increase in body weight and length of the experimental fish confirms that the fish responded positively to the diets, which may be associated to the reduction of anti – nutrients as a result of processing. Similar finding were reported by Duniya (2006) where higher growth values were attained for fish fed processed locust bean seeds. Balogun *et al.* (2004) reported a similar finding in fingerlings of *Clarias gariepinus* fed processed *Delonix regia* seed meal. The weight increase of fish fed processed soybean was higher than fish fed raw soybean (Fafioye *et al.,* 2005), the higher weight gain was attributed to adequate consumption and utilization of the feed by the fish.

The fish showed good appetite to all the treatment diets; this is attested to by the increase in body weight, total length and standard length.. However the greatest weight gain by the experimental fish fed experimental diet was (61.10g) achieved with the treatment C containing 20% inclusion and the least weight gain (54.61g) was recorded with the treatment E containing 30% *P. phaseoloides* seeds inclusion. The greatest mean daily weight gain (MWG) (11.12g) was achieved by the treatment A, and the least mean weight gain was recorded by the treatment E (9.16g) containing 30% *P. phaseoloides*

seeds inclusion. This is similar to the values obtained for *Oreochromis niloticus* by Faturoti and Akibote (1986). The greatest specific growth rate, feed conversion efficiency and protein efficiency ratio were achieved in the treatment C containing 20% *P. phaseoloides* seeds inclusion and the least specific growth rate, feed conversion efficiency and protein efficiency were recorded by the treatment E containing 30% inclusion. This observation is similar to that reported by Oresegun and Alegbeleye (2001), for *Oreochromis niloticus* fed cassava peels. Fish in all the treatment diets indicated that growth due to increased in *P. phaseoloides* seeds meal were not significant ($P<0.05$). The best growth response was achieve in the fish fed 20% *P. phaseoloides* seed meal inclusion and the least was recorded by the fish fed 30% *P. phaseoloides* seed meal inclusion. The lower growth response by fish fed 30% *P. phaseoloides* seed inclusion was probably caused by reduced palatability of the diet which causes reduced in feed intake.The lower weight gain attained in diets D and E might be associated with low utilization of the experimental feed by the fish compared to other rations; thus may have contributed to poor utilization of essential nutrients for growth and development. It indicates that the inclusion of *P. phaseoloides* seed meal above 30% in the diets of *C. gariepinus* may not assist desirable growth.

5.2 Nutrient Utilization

The general feed acceptance in all the experimental tanks is appreciable. This may be due to feed palatability and good heat processing method involved to detoxify the anti-nutrients to minimum level. Agbabiaka *et al.*, (2013) reported that palatability can be determined by the rate of ingestion of feed by the fish during the feeding trial. The nutrients utilization of *Clarias gariepinus* fed diets containing different inclusion levels

of *Pueraria phaseoloides* seed meals show no significant differences (P>0.05) in feed conversion ratio (FCR) and Net protein utilization among the experimental diets compared to the control. This appreciable performance may be due to the good composition of essential and non essential amino acids in *Pueraria phaseoloides* seed. The good performance also attributed to the processing method and good experimental management during the feeding trial (Eyo, 2003). The protein efficiency ratio of all the diets were not significantly different from control (P>0.05). The apparent net protein utilization (ANPU) were not significantly different (P>0.05). Dietary levels of *P. phaseoloides* up to 30% inclusion level therefore had nutritional attributes as feedstuff in the diet of *Clarias gariepinus* Juvenile (Anyanwu *et al*., 2012).

5.3 Carcass Composition of the Fish

Fish fed the experimental diets showed that, the crude protein content in all the fish was significantly higher (p<0.05) at the end of the experiment than that of the fish at the beginning. This indicates reasonable protein accumulation due to optimal protein utilization for growth (Eyo, 2003; Alegbeleye *et al*., 2004; Balogun *et al*., 2004).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Pueraria phaseoloides seeds have potentials of competing with other conventional sources of plant protein in the diet of *Clarias gariepinus.*

It is concluded that all the Null (Ho) hypotheses are rejected because:

i. There were significant differences between the proximate composition of raw and boiled seeds of *Pueraria phaseoloides;* and there was significant difference in the anti-nutritional factors in the raw and boiled seeds of *Pueraria phaseoloides;*

ii. There was significant difference in the growth performance and feed utilization of *C. gariepinus* fed diets containing boiled *P. phaseoloides* seeds meal when compared with the control diet (raw).

iii. There were significant differences in the carcass composition of the experimental fish.

6.2 Recommendations

It is recommended that:

i. Fish farmers are adviced to use boiled *P. phaseoloides* seed meal at 20% inclusion level.

ii. The study using *P. phaseoloides* requires further studies on best processing methods that would eradicate the anti-nutritional compounds.

iii. Other culturable fresh water fish species such as *Hetrobranchus* and *Tilapia* species should be experimentally fed with *Pueraria phaseoloides* seeds meal.

iv. The study using *P. phaseoloides* (Tropical kudzu) as feed should be carried out in other cultural system such as concrete tanks and earthen ponds.

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APPENDICES

Appendix 1: Bi-weekly Sampling of the experimental fish

CONC	REP	Length				
		INITIAL	1st sampling	2nd sampling	3rd sampling	4th sampling
0	1	15.73	19.08	19.77	22.53	26.75
0	2	16.00	18.54	20.16	21.70	25.25
10	1	16.37	17.67	20.49	21.68	25.83
10	2	15.86	19.31	20.66	23.10	27.46
20	1	15.99	19.37	18.91	21.50	26.80
20	2	15.68	19.24	18.46	22.29	27.47
25	1	15.60	17.67	18.47	18.59	23.50
25	2	15.07	18.24	18.55	19.63	26.03
30	1	16.33	19.18	18.79	21.07	27.23
30	2	16.18	19.01	15.80	19.40	23.60
CC	1	15.97	18.69	20.70	20.91	23.78
CC	2	15.54	18.56	17.05	20.52	23.97

Appendix 2: Bi-weekly Sampling (Weight) of the experimental fish

CONC	REP	INITIAL	1st sampling	2nd sampling	3rd sampling	4th sampling
0	1	23.84	34.46	35.86	79.95	88.03
0	2	23.54	33.08	33.13	72.68	84.90
10	1	25.59	41.68	44.40	69.50	75.38
10	2	24.74	40.23	44.44	85.30	93.94
20	1	26.68	41.06	33.05	71.24	90.98
20	2	24.34	74.96	40.45	79.35	82.25
25	1	22.19	30.30	36.20	46.13	70.45
25	2	20.35	33.56	33.29	56.45	85.85
30	1	25.91	32.72	37.81	73.74	91.11
30	2	25.19	34.75	33.20	47.10	69.20
CC	1	23.83	36.97	36.86	57.32	62.32
CC	2	23.83	35.47	36.47	55.76	59.12

Appendix 3: Percentage Composition of the Experimental Diet (grammes)

Ingredients	**Percentage of *Pueraria* diet (Boiled kudzu)**					
	Diet A Commercial feed)	Diet B 10% Kudzu	Diet C 20% Kudzu	Diet D 25% Kudzu	Diet E 30% Kudzu	Diet O Kudzu 0% Control
***Pueraria* seed meal**	203.4g	406.8g	508.5g	610.2g	-	
Fish meal	1830.6g	1627.2g	1525.5g	1423.8g	2034.0g	
Bone meal	63g	63g	63g	63g	63g	
Yellow maize	189g	189g	189g	189g	189g	
Wheat flour	296g	296g	296g	296g	296g	
Palm oil	126g	126g	126g	126g	126g	
Vitamin premix	157.5g	157.5g	157.5g	157.5g	157.5g	
Fat	500.0g	500.0g	500.0g	500.0g	500.0g	
Salt	15.75g	15.75g	15.75g	15.75g	15.75g	
Lysine	94.5g	94.5g	94.5g	94.5g	94.5g	
Methionine	63g	63g	63g	63g	63g	
Total	3063.95g	3063.95g	3063.95g	3063.95g	3063.95g	

Bone Meal -2% represents 63g per replicate Vitamin Premix -2% represents 63g per replicate Palm oil -4% represents 126g per replicate Nacl (Salt) – 0.3% represents 9.45g per replicate
Lysine – 3% represents 94.5g per replicate Methionine -2% represents 63g per replicate.

Plate III: THE YOUNG *PUERARIA PHASEOLOIDES*

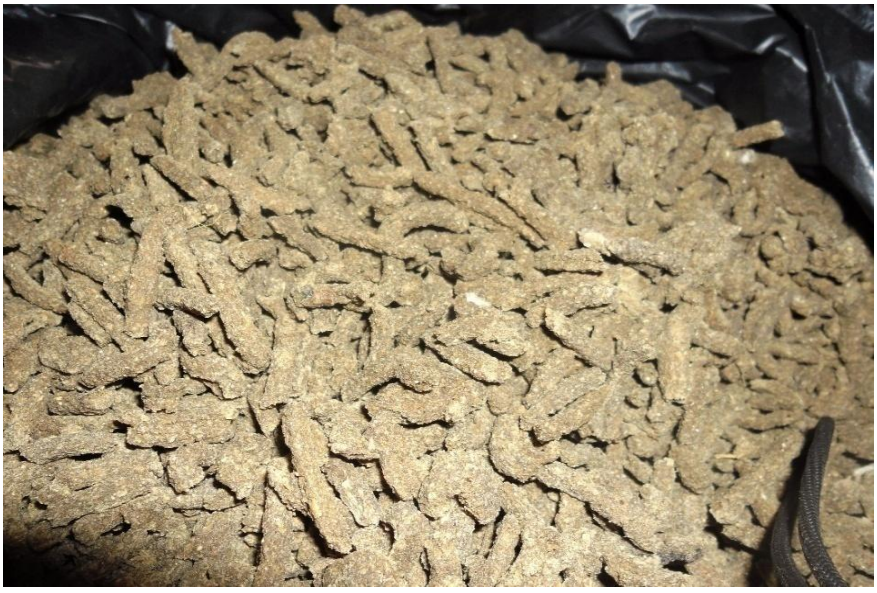

Plate IV : Formulated diet at 10% inclusion

Plate V: Formulated diet at 20% inclusion

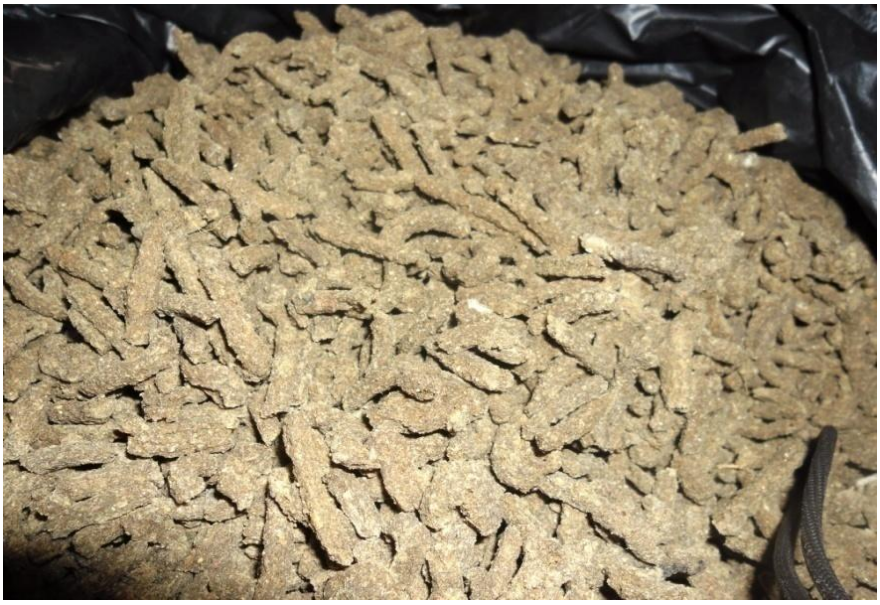

Plate VI: Formulated diet at 25% inclusion

Plate VII: Formulated diet at 30% inclusion

Plate VIII: Formulated diet at 0% inclusion