

**GROWTH RESPONSE OF AFRICAN CATFISH *CLARIAS*
GARIEPINUS(BURCHELL, 1822) JUVENILES FED GRADED LEVELS OF
BOILED *SENNA OBTUSIFOLIA* L. SEED MEAL AS A REPLACEMENT FOR
SOYBEAN MEAL**

BY

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NOVEMBER, 2014

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BY

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(MSc/SCIE/00066/09-10)**

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
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DEGREE IN FISHERIES**

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

NOVEMBER, 2014

DECLARATION

I declare that the work in the thesis entitled “GROWTH RESPONSE OF *CLARIAS GARIEPINUS*(BURCHELL, 1822)JUVENILES FED GRADED LEVELS OF BOILED *SENNA OBTUSIFOLIA* L. SEED MEAL AS A REPLACEMENT FOR SOYBEAN MEAL” was carried out by me in the Department of Biological Sciences Under the supervision of Prof. P.I. Bolorunduro and Prof. J.K. Balogun. 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 in any University.

Abdullateef YUSUF

Signature

Date

CERTIFICATION

This thesis entitled “GROWTH RESPONSE OF *CLARIAS GARIEPINUS*(BURCHELL, 1822) JUVENILES FED GRADED LEVELS OF BOILED *SENNA OBTUSIFOLIA* L. SEED MEAL AS A REPLACEMENT FOR SOYBEAN MEAL” by Abdullateef YUSUF meets the regulations governing the award of the degree of Master of Science (M. Sc.) fisheries of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to my parents whose constant guidance has made this work possible.

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Firstly, I thank the Almighty God for giving me good health throughout my studies. I wish to express my gratitude and appreciation to my supervisory team Prof. P.I. Bolorunduro and Prof. J.K. Balogun for their persistent encouragement and quick response each time I needed attention which has made this work successful. My profound gratitude goes to the Department of Biological Sciences, Ahmadu Bello University, Zaria that provided the training and facilities in making this work a success. I also want to thank all entire staffs of the Department.

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ABSTRACT

The growth response of *C.gariepinus* juveniles fed graded levels of boiled *S. obtusifolia* seed meal was studied for 84 days in a flow through system. The proximate analysis of *Senna obtusifolia* seed had 20.63, 6.81, 16.20, 4.52, 5.89 and 46.22% representing value for crude protein, ether extract, crude fibre, ash, moisture and nitrogen free extract respectively while anti-nutrient in seed were hydrocyanic acid, oxalate, phytate, saponnin and tannin with various concentration of 1.42, 2.64, 4.16, 0.96 and 2.44 mg/100g respectively. Six diet were formulated; which were Diet D₁ (Control: with 0% inclusion level boiled *S. obtusifolia* seed meal (100% soybean meal), D₂ (10%), D₃ (20%), D₄ (40%), D₅ (60%) and D₆ with 100% inclusion level (total replacement of soya bean meal). The water quality parameters were monitored weekly and weight was taken every fortnight. The result showed that the best diet with boiled *S. obtusifolia* seed was D₃ as it gave the best MWG (13.83g), SGR (1.31), Protein Efficiency Ratio PER (1.34), profit index (2.28) and the lowest FCR (1.39) when compared with D₂ (10%), D₄ (40%), D₅ (60%) and D₆ (100%). The lowest growth and feed utilization parameters were observed in diet D₆ with total replacement level. The condition factor (K) revealed that fish were in good condition throughout the period of study. The result of carcass composition showed all fish fed with experimental diet had an increase in carcass protein which ranged between (55.12-68.65%). The cost of feed production decreased with increase in inclusion level of *S. obtusifolia* seeds in the diets. The weight gain of fish also decreased with increase in *S. obtusifolia* seed replacement level above 20%. The use of *S. obtusifolia* seed at 20% inclusion level was the best because there was no significant difference between control diet D₁ and D₃ ($P \geq 0.05$). In conclusion 20% is the best

replacement level for soybean meal with no deleterious effect on the growth of *C.gariepinus* juveniles. The use of *S. obtusifolia* seed at 20% replacement level for soybean is highly recommended in the diet of *C. gariepinus* juvenile.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Aquaculture is the rearing of aquatic animals like fish, oysters, prawn, etc in an enclosed natural or artificial water bodies (Suleiman, 2006). It is aimed at improving the nutritional as well as economic status of man through boosting the provision of high quality protein as well as an opportunity to generate income (Otubusin, 1998). In the Nigerian experience, aquaculture practices are still in the extensive and semi-intensive levels of intensification (Adikwu, 1999).

Aquaculture is more than a science, in its infancy; it is now recognised as a viable and profitable enterprise worldwide. As aquaculture technology has evolved, the push toward higher yield and fast growth has involved the enhancement or replacement of natural foods with prepared diets. In many aquaculture operation today, feed account for more than half of the variable operating cost. Therefore, knowledge on nutrition and practical feeding of fish is essential to successful aquaculture.

Aquaculture nutrition to a great extent relies on pond water productivity and the use of various feed supplements (Suleiman, 2006). Fish food consists of natural food and artificial (supplementary) feeds. When natural foods are not available in sufficient quantities to provide adequate nutrition for fish growth feed that are manufactured may be fed at regular interval (weekly, monthly, etc)

Generally, in fish culture, the major factors affecting fish growth are stocking rate, stocking density, the water temperature, the quantity (adequacy) and quality of feeds and feeding method and frequency (Bolorunduro, 2002).

As at now nutritional data are available to allow the nutritionist to formulate catfish feed on a least cost basis. The primary constraint limiting the use of least cost programmes for formulating catfish feeds is that relatively few feed stuffs are available that are suitable for use in catfish feed (Robinson,1991).

Fish, like all other animal, require the provision of essential nutrient and energy for their growth, development and physiological processes (Ochang, 2008). Aquaculture can play a complimentary role to terrestrial animal production in ensuring food security to the teeming Nigerian population. This is even more critical at a time such as this when there is a global food crisis. Feed cost is known to account for over two third of the variable cost of an intensive aquaculture system (Aderolu and Sogbesan, 2010). The cost of fish feeds can be significantly reduced if some locally available ingredients are incorporated in feed formulation. While Soybean and maize remain best choices as plant protein and energy sources respectively. Other less popular legumes can be used as substitutes, especially as partial replacers for these nutrient sources that have high food value for human population.

To mitigate the effect of higher feed cost in fish culture, aqua culturists have always sought to replace fish meal and fish oil components of the diet. Fish meal, known to be the most expensive component of the diet, is usually replaced at least partially with other cheaper animal protein source. Other plant protein sources which have not been traditionally used in

feeds are being assessed for the suitability in fish feed formulation. When suitable ingredients are found that have little value as nutrient sources for food, their cost will be less and this will enhance the profitability of the aquaculture enterprises (Ochang, 2008).

Protein comprise about 70% of the dry weight of fish muscle (Robinson, 1991), a continual supply of protein is needed throughout life for maintenance and growth. Usually, the most economical source of these molecules is a mixture of protein in feed stuffs. Most of the studies on protein requirements of fish have been based on weight gain and feed efficiency. Data from those studies indicate that the dietary protein requirement for fish ranges from about 25% to 50%. In a practical feed, amino acid requirements are best met by feeding a mixture of feed stuffs supplemented with amino acid.

Soaring food prices have triggered an increase in hunger worldwide, the competition between human and livestock for the consumption of soybean and the increasing role of soybean in the world as a biodiesel (Cotula, 2008) have increased its cost and demand and heightened the competition between human and beast for soybeans, this is also the case of other popular plant protein sources. One of such legumes that can be evaluated as alternative and replacement for soybean could be *S. obtusifolia* (sickle pod seed). This study is therefore designed to evaluate the nutritional value of *S. obtusifolia* plant growing in the tropics in order to generate data for the development of this plant as an alternative plant protein in the developing countries of the tropics. The scope for improving the aquaculture nutritional practices in Nigeria is very wide with new developments of integrated cultures arising and paving way for better aquaculture

nutritional practices with a resulting increase in production for food security and income generation for fish farmers.

1.2 Statement of the Research Problem

The principal constraint in fish culture is feed. High competition between animal and human population for crops as conventional plant protein, increasing economic uses and export potentials found for the conventional plant protein source soybean, has led to increased value of the crop, scarcity and resultant high cost.

Furthermore, one of the greatest challenges is the increase in human population without corresponding increase in the annual fish production because it is a known fact that fish serves as a cheap source of animal protein to the poor.

1.3 Justification

Aduku (1992) reported that the key to abundant animal production is availability of cheap and balanced feed. Fish feed account for about 60% recurrent costs of aquaculture operation (Eyo, 1995). The high cost of formulated commercial fish feed is a major constraint to the expansion and growth of aquaculture sector in Nigeria and therefore, concerted effort is needed to seek suitable alternative feed ingredient.

The demand for fish is very high, and in Nigeria, fish culture is getting popular both in homestead and commercial farms. Some species of fish cultured include catfish, highly relished in Nigeria because of its fast growth and high taste value (Oladosun *et al.*, 1994). It is necessary to reduce the dependence on soybean and groundnut by partial replacement with

less popular wild legume seeds, using wild legume seeds to substitute feed ingredients as source of high food value for man.

Consequently, the search for a novel, high quality, cheap and readily available source of plant protein to replace popular costlier ones is now a major concern in aquaculture industry. One of such legumes with great prospect as alternative and replacement of soybean is *Senna obtusifolia* (sickle pod). Ingweye *et al.*(2010), worked on the nutritional evaluation of wild sickle pod seeds. They evaluated the Proximate composition of the seed, results showed high dry matter (92.50%), crude protein (29.54%) and crude fibre (10.18%), which can be a good source of plant protein for catfish.

1.4 Objectives of the Study

The general aim of the study is to evaluate the growth response of *C. gariepinus* fed graded levels of boiled *S. obtusifolia* seed meal.

The specific objectives are:-

1. To determine the proximate composition, anti-nutritional factors, macro element, vitamins and amino acid profile of raw and boiled *S. obtusifolia* seed.
2. To substitute soybean meal with boiled *S. obtusifolia* seed meal and determine the growth response and feed utilization of *C. gariepinus* juveniles fed varying dietary protein levels of substituted meal.
3. To determine the cost effectiveness of replacing soybean cake with graded levels of boiled *S. obtusifolia* seed meal.

1.5

Hypotheses

Hypotheses postulated are:-

1. There is no significant difference in the proximate composition, anti-nutritional factors, macro element, vitamins and amino acid profile of raw and boiled *S. obtusifolia* seed.
2. There is no significant difference in growth response and feed utilization of *C. gariepinus* fed graded levels of boiled *S. obtusifolia* seed meal.
3. There is no significant difference in the cost effectiveness of replacing soybean meal with graded levels of boiled *S. obtusifolia* seed meal.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Feeding Habits of *Clarias gariepinus*

Catfish species are euryphagous and generally regarded as an opportunistic, omnivorous predator. Normally catfish are 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 via individual foraging, individual shoveling, surface feeding and formation feeding (FAO, 2010). *C. gariepinus* is an omnivorous with predatory tendency, feeding mainly on fish, shrimps, zooplankton and higher plants (Ugwumba and Ugwumba, 2007).

2.2 Nutritional Requirement of *C. gariepinus*

The nutrition of catfish is similar to that of other animal species i.e catfish require the same nutrients as other animals for normal metabolic function (Robinson, 1991). However the specified amount of a particular nutrient needed by catfish may differ from that of other animals. Robinson, (1991) reported that requirements for catfish have generally been based on studies with small fish conducted under condition presumed to be near optimum; the requirement being based primary on weight gain and food efficiency.

2.2.1 Carbohydrate (energy requirement)

Carbohydrates are compounds of carbon, hydrogen and oxygen that include sugars, starch, cellulose, gums and other closely related compounds. One of the striking differences in nutrition between fish and farm animals is the amount of energy required for protein synthesis is much less for fish than for warm blooded animals (Ayinla, and Bekibele, 1992). Animal's tissue contains small amounts of stored carbohydrate. Glucose in the blood of animals is relatively constant at 0.05% to 0.1%. Generally, glycogen stores in the liver are small, representing only about 3% to 7% of liver weight in most animals.

Carbohydrate are important dietary components as an inexpensive source of energy as precursors for various metabolic intermediates, as an aid in pelleting practical catfish feeds, and they reduce the amount of protein used for energy thereby sparing protein for growth. It would appear that the average permissible carbohydrate level is around 27 percent (FAO, 2010) and Ali (2011) suggested that *C. gariepinus* cannot utilize dietary carbohydrate levels above 35 percent. On the other hand Pantazis (2005) discovered that dietary carbohydrate levels of between 26 and 32 percent had a significant protein sparing effect, advocating the greater use of carbohydrates in catfish diet formulation.

(Robinson, 1991), also indicated that the utilization of carbohydrate by catfish appears to differ depending on the complexity of the carbohydrate starch or dextrin (partially hydrolyzed starch) are used more efficiently by catfish than are sugars such as glucose and sucrose.

A typical commercial catfish feed contain 25% or more soluble (digestible) carbohydrate, additional 3% to 6% carbohydrate is generally present as crude fibre. Fibre is considered to be indigestible by catfish; thus it is not desirable in catfish feed because indigestible material may pollute the water. However there is always some fibre inherent in practical feed ingredients.

2.2.2 Protein and amino acid requirement

Protein comprises about 70% of dry weight of fish muscle (Robinson, 1991). A continual supply of protein is needed throughout life for maintenance and growth.

Ayinla (1988) reported 31-34%, 31-34% and 40% crude protein requirement for *C. gariepinus* fingerlings, juvenile and adult respectively. Madu (1992) recommended 40%

crude protein for *C. anguillar* and Fagbenro *et.al.*(1992) recommended 40-42.5% for *Heterobranchus bidorsalis*.

The protein requirement of fish changes with size of the fish, water temperature and the balance of individual components in a given ratio (Ayinla and Bekibele, 1992). Robinson (1991) reported that it is difficult to set a level of protein that is optimum for all situations because of the factors that affect the dietary protein requirement of catfish, these include water temperature, feed allowance, fish size amount of non-protein energy in the diet, protein quality, natural food availability and management practices. The quality of protein is principally influenced by its amino acid composition, and feeding stuffs deficient in any of the ten essential amino acids (Arginine, Histidine, Isoleucine, Leucine, Lysine. Methionine, Phenylalanine, Threonine, Tryptophan and Valine) will cause depressed appetite and growth rate of fish. Thus in formulating fish feeds to meet amino acids requirements, the total essential amino acid content of the feed ingredient must be corrected for availability (digestibility) to allow the optimum amount of amino acids in the diet.

2.2.3Vitaminrequirements

Vitamins are highly diverse in chemical structure and physiological function (Robinson 1991). Auta (2001) reported that vitamins are organic compounds required in the diet in relatively small quantities for health and function in animals.

They are generally defined as organic compounds that are required in small amounts in the diet for normal growth, health and reproduction animal species.

Vitamins are classified as water and fat soluble. 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.

Robinson (1991) reported that dietary levels of fat soluble vitamin; vitamin A, D and E required to prevent sign of deficiency in a catfish are 450-900IU, 110-220IU, and 23IU respectively, vitamin K is also required in a reasonable quantity. Also water soluble Vitamins such as Thiamin, Riboflavin, Pyridoxine, Pantothenic and Niacin requirement for catfish are 1.0ppm, 9.0ppm, 3.0ppm, 15ppm and 14ppm respectively.

2.2.4Lipid and fatty acid requirement

The use of lipids (Fat and oils) in catfish feeds is desirable because lipids are a highly digestible source of concentrated energy (containing about 2.25 times as much energy as does an equivalent amount of carbohydrate), supply essential fatty acids, serve as vehicle for absorption of fat-soluble vitamins, increase feed palatability and serve as precursors for steroid hormones and other compounds (Robinson,1991).

The types and amount of lipid used in catfish diet is based on essential fatty acid requirements, economics, constraints of feed manufacture and quality of fish flesh desired. Essential fatty acid (EFA_s) are classified based on their chemical structure and are designated as either omega-3 (n3) or omega (n6) fatty amino acid.

Catacutan (1991) revealed that homoeothermic animal have requirement for essential fatty acid. Gatlin (2010) recommended that they oil should not be present at excessive in the diet of fish, fish feed are generally known to contain low amount of oils (Umar, 2006).

Okoye and Falaye (2001) reported that addition of oil to Tilapia diet would significantly improve growth. However, too much dietary lipid may result in excessive fat deposition in the visceral activity and tissues that adversely affect yield, products. Also high lipid feeds are difficult to pellet. Robinson (1991) reported that EFA requirement for catfish and most other warm water fish have not been precisely defined, but it appears that catfish require a small amount (about 0.50-0.75%) of n3 fatty acids. He further stated that lipid levels in commercial catfish grow out feeds rarely exceed 5% to 6%.

2.2.5 Mineral requirements

Mineral requirements of fish are similar to those of terrestrial animal (Umar, 2006). The same minerals required for metabolism and skeletal structure of other animals are apparently required by catfish. Minerals may be classified as macro minerals or micro minerals, depending on the amount required in the diet, macro minerals required in relationally large quantities and micro minerals are required in trace quantities.

Aduku (1993) reported that blood meal, fish meal, lime stone etc. are natural sources of macro and micro minerals used in feed formulation for animals. Fish require large amount of calcium and phosphorus (Eyo, 2003). Ayinla and Bekibele (1992) stated that information on minerals requirement of cultivable fish species is very scanty, this is probably because of general problem in mineral nutrition such as formulating mineral free diets and over-coming tissue stores of minerals, fish absorb dissolved minerals from water.

However, Robinson (1991) stated that dietary levels required to prevent deficiency signs in catfish for macro-minerals were Calcium (< 0.1%, 0.45%), Phosphorus (0.45%), Magnesium

(0.04%) Sodium, Potassium, Chloride and Sulphur (Not determined). He also reported micro minerals cobalt, iodine, (not determined), zinc (20ppm), selenium (0.25 ppm), manganese (2.4ppm), Iron (20ppm) and Copper (4.8ppm).

Lawrence *et al.* (2009) studied the phosphorus requirements of African catfish, *C. gariepinus*, based on broke line regression analysis methods, and stated that phosphorus is an essential and critical nutrient for animal production. The authors further suggested that phosphorus (p) of 6.70 – 8.20g/kg diet as the requirement for the growth of African catfish of 10.2g weight.

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

Commercial fish feeds are usually expensive because the traditional or conventional protein source ingredient such as fish meal, soybean meal, groundnut cake, maize, millet, guinea corn, vegetable oil etc. are competing for human and livestock consumption (Madu *et al.*, 2003). There have been concerted effort by aqua culturist to mitigate the cost of feed in aquaculture, this have led to a lot researches conducted to investigate non-conventional feed stuff for formulation of feed (for both protein and energy from plant source).

Banyigyi *et al.* (2001) reported the use of bambara groundnut in Nile tilapia (*Oreochromis niloticus*) diets and they also evaluated it use as feed for catfish juvenile (*C. gariepinus*) and concluded that 50% use of Bambara groundnut had the best growth performance. *Cassia fistuala* meal has been evaluated as a replacement for soybean meal in practical diets for the Nile tilapia, *Oreochromis niloticus* fingerlings (Adebayo *et al.*, 2004) in which they recommended 40% replacement level without adverse effect. However, *C. fistuala* meal

substitution for soybean meal of up to 250kg practical diet does not appear to adversely affect growth performance and nutrient utilization (Adebayo *et al.*, 2004).

Bekibele (2005) used *Mucuna* beans to replace soya bean meal to evaluate its effect on growth performance of *C. gariepinus* and concluded that mucuna bean protein can replace commercial soya bean meal protein up to 50% level without adverse effect on the growth of catfish; rather it enhances the quality of the fish carcass by reducing body fat content.

Nweke and Ugwumba (2005) in their study on the effect of replacement of fish meal with Duck weed (*Lemna paucicostata*) meal on the growth of *C. gariepinus*, reported that for adequate growth and nutrient utilizations, duck weed can replace fish meal up to 50% level of inclusion. Auta *et al.* (2007) reported that castors seed (*Ricinus communis*) meal show high digestibility of 71.4% in fish feed with diet having processed castor seed (boiling at 100⁰C for 50 min) and also fish carcass protein in all dietary treatments was higher than the initial carcass protein, thus indicating the potential of incorporating castor seed in fish feed formulation for *C. gariepinus*.

Tiamiyu *et al.* (2001) replaced maize with cooked Bread fruit (*Artocarpus altilis*) as carbohydrate source in the diet of *C. gariepinus* fingerling. They suggested a net advantage of replacing dietary maize with cooked bread fruit seed meal for *C.gariepinus* fingerling at 50% and above levels of inclusion under the condition of the experiment (using outdoor Hapas system).

Umar (2006) used parboiled *S. occidentalis* seed meal to replace fishmeal in the diet of *Oreochromis niloticus* and concluded that fish fed with 30% replacement level had the best

performance. Ademola and Olubodun (2009) investigated the dietary effect of coconut oil and peanut oil in improving biochemical characteristics of *C. gariepinus* juvenile. Fish were fed five isocaloric and isonitrogenous diets containing 5% and 10% graded levels of coconut oil and peanut oil, respectively over a period of 6 weeks. Results showed that fish fed diets containing graded levels of oil had significantly higher weight gain, Feed Efficiency Ratio and Specific Growth Rate when compared with control diet that had no oil. They reported that inferences from this study conclusively support the inclusion of graded level of coconut and peanut oil in the diet of *C. gariepinus* for better growth and economic value in the sustenance of aquaculture.

Aderolu and Sogbesan (2010) evaluated the potential of cocoyam as carbohydrate source in cat fish diet and recommended 25% replacement of cocoyam for maize for growth and economic benefit for sustainable aquaculture. Aminu (2011) used cottonseed meal as an ingredient in the diet of *C. gariepinus* fingerlings. He recommended 20% cottonseed meal inclusion level in catfish diet for good growth and development. Agbugui *et al.* (2011) fed processed *Pauletia monandra* seed meal to *C. gariepinus* and recommended the use of *P. monandra* seed in combination with imported commercial feed in catfish production.

2.4 Non-conventional Feedstuffs of Animal Origin Used for Catfish Feed Formulation

Ayinla and Akande (1988) reported that *C. gariepinus* fed with fish silage at 11%, 33.5% and 41% inclusion levels demonstrated feed acceptability without any symptom of nutritional deficiency although they attested to the superiority of fish meal over fish silage as a protein source.

Fagbenro and Jauncey (1993) used fermented fish-silage from whole tilapia and concluded that fermented silage stored for 15 days is beneficial as protein supplement in aqua feeds in culturing catfish. Erturk and Sevgili, (2003) replaced fish meal with poultry by-product meal on apparent digestibility, body composition and protein efficiency ratio in a practical diets 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.

Nwanna (2003) evaluated nutritional value and digestibility of fermented shrimp head waste meal by African catfish *C. gariepinus*. Nwanna, (2003) stated that 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. Ekelemu and Ogba, (2005) used rations of maggot meal as replacement for fish meal and stated that maggot can be used as a replacement for fish meal at 50% replacement level to reduce cost of producing fish feed, still obtain optimal production while maximizing profit.

Keremah and Green (2005) evaluated the effect of replacing fish meal with graded level of fish offal on growth and survival of Hybrid catfish fingerlings. Fish diet of 0, 25, 50, 75 and 100% level of fish offal substitution were formulated to contain 40% protein and similar energy levels. Keremah and Green (2005) concluded that diets with fish meal replaced by 25% and 50% fish offal could be fed to hybrid catfish without adverse effect on growth and survival.

Agbabiaka *et al.* (2012) fed enzyme supplemented dried rumen digesta to catfish, revealed that enzyme supplemented dried rumen digesta can be tolerated by *C.gariepinus* fingerlings and support growth at 40% with little effect on Specific Growth Rate and Food Conversion Ratio. They also reported that there was no mortality encountered during the experimental period.

2.5 Description and Habitat of *S. obtusifolia*

The plant *S. obtusifolia* is a legume belonging to the family Fabaceae, subfamily Caesalpinoideae, commonly called sickle pod plant and locally called “tsafasa” in Hausa language. Okezie and Agyakwa (1998) and Queensland Government (2006) gave a description of the plant as an erect, branched bushy annual or perennial up to 90cm high, that reproduces from seed. The stem is robust cylindrical, hairless or sparingly hair. The leaves are alternate, pinnate, 2.5-7.5cm long with petioles that 2.5cm long. The rachis always has one to two glands between the lowest pair of leaflets. The leaflets consist of three pairs. Each leaflet is obvate 1.5-5cm long and 1-3cm wide, mucronate at the apex, sessile and smooth. The inflorescence consists of one or two flower in the axils of the upper leaves. The flowers are yellow 2-3cm across and borne on pedicels that are 1.5-3.5cm long. The fruits are cylindrical pod that are 10-20cm long and 5-6mm wide they are curved and long-beaked, one are usually two in an axil. The seed are about 5mm long and 2mm wide somewhat rhomboid, brown and smooth. A pan-tropic weed of road sides, grass lands and cultivated fields, common near settlements wide spread in West Africa (Okezie and Agyakwa, 1998). It grows on well- drained fertile soil (Ismaila *et al.*, 2011). *Senna obtusifolia* is found along river and on lake shores, as well as on cultivated land, up to 1700m altitude (Irwin and Barneby, 1982).

2.6 Origin and Geographical Distribution of *S. obtusifolia*

S. obtusifolia plant is found throughout tropical Africa with the exception of Madagascar (Irwin and Barneby, 1982). It is considered on early introduction into Africa from America when it shows for more variation. In Africa the fruits are broad as in specimens from the Caribbean and Southern United States which suggest a Caribbean origin of the African plants. In Asia plants with broad fruits are widespread, but in the Philippine only plant with needle like fruit occur. *Senna obtusifolia* is considered a weed worldwide.

2.7 Phytochemical Composition of *S obtusifolia*

Ismaila *et al.* (2011) investigated on nutritional and phyto-chemical constituent of *Senna obtusifolia* indigenous to Mubi, Nigeria. The phytochemical constituents present in the seeds and leaves observed were tannins, flavonoids, steroids and terpenoids, and alkaloids were found only in the plant leaves. Saponins were only found in the seeds. Flavonoids were observed to be more abundant in seeds extract (99%) than in leaves extract (66%), Alkaloids absent in seeds and only 33% in leaves. Both leaves and seed showed sufficient element, Ca, N, Cu and Na with good nutritive value and rich in carbohydrates and proteins but low in fat.

Ingweye *et al.* (2010), evaluated the nutritional potentials of wild sickle pod seeds from Obanliku South-Eastern Nigeria, reported that the concentration of anti-nutrients in the legume seeds recorded high value (260, 185, 388, 50 and 83.25 mg / 100g) for alkaloid Saponin, tannin and oxalate respectively, while phytate, hydrocyanic acid and phytohaematoglutinin levels were low.

Also, the seed were abundant in Calcium 960mg/100g), Potassium 1,200mg/100g), Phosphorus (810mg/100g), Sodium (600mg / 100g) Magnesium (6.40mg / 100g), Iron (234.60mg /100g) Zinc (53.12mg/100g) and Copper (10.48mg/100g) but low in Molybdenum, Cobalt Chromium, Selenium, Sulphur and Flourine. The amino acid profile revealed a high concentration of Leucine (17.60g/100g of protein) Histidine (2.11g/100g protein) Prolin (2.33g/100g protein), and Glycine (4.11g/100g protein) while the rest of amino acids were of low concentration in the raw seed. Fresh leaves of *Senna obtusifolia* contain per 100g edible portion water 79.7g, energy 251kg (60kcal), protein 5.6g, fat 0.2g, carbohydrate 12.5g, fibre 2.3g, Ca 589mg, P 96mg, Riboflavin 0.71mg, Niacin 1.5mg, ascorbic acid 113mg (Irwin and Barneby, 1982).

2.8 Economic Importance of *S. obtusifolia*

Sicklepod (*Senna obtusifolia*) is a leguminous weed species that has become a severe problem in soya bean production areas throughout the Southern United State (Abbott, 1998). Sickle pod is an annual plant considered serious weed in many places. It is a competitive wood shrub that grows vigorously across the tropic (Ingweye *et al.*, 2010).

2.8.1 Food usage of *S. obtusifolia*

Irwin and Barneby (1982) reported that the young tender leaves of *Senna obtusifolia* are occasionally used as a vegetable throughout Africa and elsewhere and the plant is cultivated in home gardens for this purpose in several countries including Senegal, Ghana, Cameroun, and Ethiopia. Older leaves, if eaten frequently or in large quantities will cause diarrhea. Cattle sheep, goat, and ostriches browse the plants, but the growth stage of the plants may influence

acceptability. In both Africa and United States, mycotoxicosis is an often fatal disease of cattle grazing *Senna obtusifolia* and other *Senna* species.

In Uganda the seed are occasionally dried and ground into powdered which is cooked and eaten as a staple food in moderate amount. The seeds have been eaten in times of famine in the Sahel region as well. As the seeds are reputedly poisonous, cooking or roasting is deemed necessary to make the safe to eat.

2.8.2 Medicinal usage of *S. obtusifolia*

Senna obtusifolia though eaten as a vegetable has also been reported to be useful in the treatment of many diseases. The leaves are used as a laxative and a poultice to treat skin infections, sores, ulcers and insect bites. The leaves are further used as an anthelmintic and against vomiting and stomach ache. A decoction of the leaves is used to treat eye complaints in Senegal and Zanzibar (Irwin and Barneby, 1982).

Roasted seed have been used as a leaves for a tea-like infusion. In Sudan the powered and fermented leaves are used as condiment. Manandhar (2002) reported that the leaves' and seed are anti-cholesterolemic, anti-spasmodic, carminative, emollient, hepatic, ophthalmic and purgative, according to him the powered leaves are used in the treatment of indigestion and stomach pains.

The seeds have also been reported to contain anthraquinones which makes it very useful in Pharmaceutical industries. It is anti-helminthic, anti-bacterial, anti-fungal and hepatic, also the seed are used in Korea to treat constipation, oedema, glaucoma, myctalopia and to protect the liver (WHO, 1998).

2.9 Anti-nutrients Present in Feed Stuffs

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients (Oxford Dictionary of Biochemistry and Molecular Biology, 2008). The terms anti-nutrient or natural toxicant have been widely employed to describe plant defense metabolites in the food and nutrition literature (Santosh and Richard, 2002). The anti – nutritional factors are substances, which affect the nutritional value of the feed stuffs and the same time affect the health of fish (Bolorunduro, 2002).

Many food stuffs that are commonly used in preparing diets for animals contain anti-nutritional factors (ANFs) 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 (Károly, 2011). The main ANFs that interfere with nutrient digestion and absorption are lectins, protease inhibitors, tannins, antigenic proteins, phytic acid, glucosinolates and gossypol.

2.10 Methods Used in ANFs Removal from Feed Stuffs

Santosh and Richard (2002) reported that physical and chemical methods employed to reduce or remove anti-nutritional factors include soaking, cooking, germination, fermentation, selective extraction, irradiation 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 borne in mind 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 e.g. 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.

Khatab *et al.* (2009) in their work on Nutritional quality of legume seed as affected by some physical treatments, using raw and treated cowpea, pea and kidney bean seed, were investigated for their content of anti-nutritional factors, applying water soaking, boiling, roasting, microwave cooking, autoclaving, fermentation and micronisation. They revealed that boiling caused significant decrease in tannins, phytic acid trypsin inhibitors and oligosaccharides followed by autoclaving and microwave cooking. Food processing methods such as boiling reduces the amount of these phytochemicals in plant product (Piorrock *et al.*, 1984).

Ikemefuna *et al.* (1991) reported the effects of soaking, sprouting, cooking and fermentation on some nutrient composition and anti – nutrients factor of sorghum (*Guinesia*) seeds. They concluded that soaking, sprouting, cooking and fermentation appeared to have beneficial effects as methods of processing.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source and Collection of *S. obtusifolia* Seed

The collection of *S. obtusifolia* seed was made from Zaria; from the wild (from abandoned farm lands, road side and refuse dump areas) in Zaria. Zaria is located between Latitude 11° 11' N and Longitude 07° 38' E, at an altitude of 686 metres above sea level. It lies within the Guinea Savanna zone, and has 3 distinct seasons including harmattan (Nov- Feb), hot (Mar - May) and rainy (Jun- Oct) (Ayo *et al.*, 1998). Its annual rainfall, average temperature and relative humidity are 1055 millimetres, 24.55° C and 43.6% respectively (IAR, 2009). Samples of plant collected were taken to the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria, for proper identification as seen in (plate I).

3.2 Processing of *S. obtusifolia* Seed

S. obtusifolia seeds were manually threshed from its pods (Plate II). The seeds were then washed thoroughly and sundried to remove all impurities after which the seed were packaged in a polythene bags. *S. obtusifolia* seeds were boiled at 100°C for 40 mins. Boiled seeds were sundried and taken for proximate and anti-nutrient analysis at Department of Animal Science, Ahmadu Bello University, Zaria.



(a)



(b)

**Plate I: *S. obtusifolia* Plant Growing on the Field in Zaria (a) Fresh plant
(b) Dried Plant**



(a)



(b)

Plate II: *S. obtusifolia* (a) Pods (b) Seeds

3.3

Chemical Analyses of Feedstuff

3.3.1 Determination of moisture content

Moisture content was determined using the method of AOAC (1990). A clean crucible was dried to a constant weight in an oven at 105°C, cooled in a desiccator and weighed (W_1). Two grams of the sample flour was weighed into the crucible (W_2) and dried in the oven at 105°C. The crucible and its contents were cooled to room temperature in a desiccator and weighed. The procedure was continued until a constant weight was obtained (W_3).

The moisture content was calculated thus:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

3.3.2 Determination of ash content

Ash was determined using the method of AOAC (1990). Two grams of the finely ground sample was weighed (W_2) into a previously weighed, clean and empty crucible (W_1). The sample was then ignited and cooled in a desiccator before being taken to the furnace. After maintaining the sample at 550°C in a muffle furnace for eight hours, the crucible and its residual ash were removed from the furnace and then allowed to cool to room temperature in a desiccator before being weighed (W_3).

The ash content was calculated thus:

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

3.3.3 Determination of ether extract

Ether extract was determined using the method of AOAC (1990). Exactly 200 cm³ of petroleum ether (40-60°C) was transferred into a clean dry 250 ml round bottom flask fitted with soxhlet extraction unit after some boiling chips were added. Fat free extraction thimble containing 20 g of the sample flour was then fixed into the soxhlet extraction unit with forceps and cold water circulation was put on. The heating mantle was switched on and heating rate adjusted to a temperature between 40 and 60°C until the solvent was refluxing at a steady rate. Extraction was carried out for six hours after which the heating mantle was switched off. By means of a rotary evaporator, the solvent was recovered and the oil left behind was quantified.

The extractable lipid of the sample was calculated as:

$$\% \text{ Lipid} = \frac{\text{Weight of lipid extracted}}{\text{Weight of direct sample}} \times 100$$

3.3.4 Determination of crude fibre content

Crude fibre was determined using the method of AOAC (1990). Two grams of the finely ground sample was put into a round bottom flask. Then 100 ml of a 0.023 M Sulphuric Acid solution was added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. This was then quantitatively transferred into the flask and 100 ml of 0.312 M Sodium Hydroxide solution was added and the mixture boiled again under reflux for 30 minutes before being quickly filtered under suction. The insoluble residue was washed until it was base free, dried to constant weight in an oven set at 100°C, cooled in a desiccator

and weighed (C₂). This residue was then incinerated in a muffle furnace at 550 °C for two hours, cooled in desiccator and reweighed (C₃).

The crude fibre content was calculated as:

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

The loss in weight on ashing (incineration) = C₂ – C₃

Weight of original sample = W

3.3.5 Determination of crude protein (Kjeldahl method) content

Crude protein was determined using the method of AOAC (1990). Two grams (2g) of the sample was weighed into 100 ml Kjeldahl digestion flask. Then 1g of catalyst mixture (Na₂SO₄ + anhydrous CuSO₄, ratio 1:10) was added to speed up the reaction. Exactly 25 ml of concentrated Sulphuric Acid (Specific gravity 1.84) was also added into the flask. The content in the Kjeldahl digestion flask was then heated slowly at first in a Kjeldahl digestion heating unit until fretting subsided and then more vigorously (with occasional rotation of the flask to ensure even digestion and to prevent overheating of the content) until the green digest was obtained. The solution was transferred into a 100 ml volumetric flask and diluted to the mark with distilled water. Then ten ml aliquot of the diluted solution (or digest) was pipetted into a Kjeldahl distillation flask and ten ml of 40% Sodium Hydroxide solution was added. The solution was steam distilled and the liberated ammonia was trapped in a 250 ml conical flask containing 10 ml of 4% Boric Acid and a drop of mixed indicator (methyl red and methyl blue ratio 2:1). Distillation was continued until the pink colour of the indicator turned greenish. The content of the conical flask was titrated with 0.1 M HCl and endpoint was

indicated by a change from greenish to pink colour. The volume of the acid used for each distillate (as well as the blank) was noted.

The % total N per sample was calculated from the formula below:

$$\% N = \frac{V_1 - V_0 \times M \times 14 \times 100}{2 \times 1000 \times 10 \times 1} \times 100$$

Where,

V₀ = Volume of HCl required for the blank

V₁ = Volume of HCl required for 10 ml sample solution,

M = Concentration of HCl (0.1 M)

14 = Atomic weight of nitrogen

100 = Total volume of digest (ml)

10 = Volume of digest distilled (ml)

2 = Weight of sample taken in (gram)

1000 = Conversion factor to dm

The crude protein was calculated as:

$$\text{Crude Protein} = 6.25 \times \%N$$

3.3.6 Determination of nitrogen free extracts content

The AOAC (1990) method was used. Carbohydrate as nitrogen free extract (NFE) was calculated by difference: $NFE = 100 - (\text{Crude protein} + \text{ether extract} + \text{Ash} + \text{Moisture} + \text{Crude fibre})$.

3.3.7 Determination of anti-nutritional factors

3.3.7.1 Determination of Oxalate

Oxalate was determined by the method of Oke (1969). One gram of the sample was placed a 200ml volumetric flask 150ml of distilled water and 10ml of 6m HCl were added. The mixture was warmed on a water bath at 70°C for 4 minutes. The supernatant was then diluted to 200ml. Three 50ml aliquots of the supernatants were evaporated 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 colour of methyl orange changed to faint yellow.

The solution was heated on water bath to 90°C and the oxalate was precipitated with 10ml of 5% calcium chloride (CaCl₂) solution. The solution was allowed to stand overnight and then was centrifuged. The precipitate was washed into a beaker with hot 25% H₂SO₄ dilute to 125mls with distilled water and after warming to 90°C, it was titrated against 0.05m KMnO₄.

Calculation: 1ml of 0.05m KMnO₄ = 2.2mg oxalate,

3.3.7.2 Determination of phytic acid / phytate

Phytate phosphorus was determined by the method of AOAC (1980). Exactly one gram of the defatted sample was extracted for 1 hour with 100ml of 3% TCA with occasional swirling by

hand. The suspension was centrifuged and a definite volume (10ml) of the supernatant was precipitated with 4ml of ferric chloride solution (containing 2mg of ferric ion ml⁻¹ in 3% TCA). The precipitate of ferric Phytate was converted to ferric hydroxide with 4ml 1.5M NaOH. The ferric hydroxide was dissolved in hot 40ml 3.2M HNO₃ and transferred to 100ml volumetric flask. The volume was made up with distilled water.

Five milliliters of the aliquot was transferred to another 200ml volumetric flask and diluted to approximately 70mL to the contents, 20ml of 1.5M KSCN was added and volume made up with water. The iron was determined calorimetrically using 1.5M KSCN. The absorbance was read at 480nm against 1.5M KSCN as reagent blank.

Standard Iron: This was prepared by dissolving 0.5g of iron III chloride (FeCl₃) in distilled water and the volume made up to 1 litre. This stock solution contained 3.4 x 10⁵ ppm of iron.

Working Standard: 1ml of the stock solution was pipetted into 100ml volumetric flask and diluted to mark with, distilled water, thereby obtaining the concentration of 3ppm.

Absorbance was measured at 480nm with 1.5M KSCN.

Calculation

$$C_{unknown} = \frac{A_{unknown} \times C_{standard}}{A_{standard}}$$

Phytic acid content was calculated on the assumption that it contained 28.20% phosphorus by weight. The mole of iron to Phytate phosphorus is 1:1.

Therefore C unknown = C iron = C phytate-p

Phytic acid = C phytate-p – 28% C phytate-p

3.3.7.3 Determination of tannin

The method of AOAC (1990) was used 1.0g ground sample was weighed into a conical flask and 100ml of distilled water added. This was boiled gently on a hot plate for one hour and filtrated through a NO.44Whatman filter paper into a 10ml volumetric flask. The paper was washed with distilled water and extract diluted to volume (this is the extract volume) Pipette 50ml of distilled water and 10ml of dilute extract(aliquot volume) into a 100ml conical flask followed by, 5ml

Folin-Denis reagent and 10ml of saturated Na_2CO_3 solution. Dilute to volume with distilled water. After mixing the solution is allowed to standard for 30 minutes in a water bath (25°C). Optical density is measured at 700nm and the absorbance compared on a standard tannic acid (tannin) curve.

The tannic acid standard curve was prepared by dissolving 0.2g of tannic acid in distilled to 200ml (1mg/ml)., Varying concentration (0.1-1.0mg/ml) of standard tannic acid solutions are pipetted into 10 .different 100ml conical flask. 5ml Folin Denis reagent and 10 ml saturated Na_2CO_3 solution was pipetted into the test tubes. Then make up to the 100ml mark with distilled water. The solution is left to stand for 30mins and the optical density was measured at 700nm. A plot of optical, density tannic acid concentration was made; with the line passing through the origin. % Tannin content was calculated thus:

$$\text{Tannin content } C \text{ (mg/100g)} = \frac{C \text{ (mg)} \times \text{extract volume} \times 100}{\text{Aliquot volume} \times \text{Wt of sample, (g)}}$$

Where C (mg) = concentration of Tannic acid read off the graph

3.3.7.4*Determination of saponin*

The method of AOAC (1980) was used, 5.0g ground was weighed into a thimble and transferred into the soxhlet extractor chamber filled with a condenser. Some quantity of acetone reflux was poured into the flask. The sample was exhaustively extracted, at its lipid for 3 hours and the solvent distilled off. This is the first extraction.

The second extraction, a pre-weighed flask was fitted unto the soxhle 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 extracted for 3 hours. The flask was re-weighed. The difference in weight represents the weight of Saponin extracted.

$$\% \text{ Saponin} = \frac{(\text{wt of saponin})}{(\text{Wt of sample})} \times 100$$

3.3.7.5*Determination of hydrocyanic acid*

The hydrocyanic acid determination was done following the method of AOAC (1990). 1.0g of ground sample was weighed into a 250ml flask; 200ml of distilled H₂O was added and allowed to stand for 2 hours. Full distillation was then carried out and 150-170ml of distillate (extractive volume- was collected in a 250ml conical flask containing 20ml of 2.5% NaOH. To 100ml of the distillate (aling of volume) containing cyanogenic glycoside, 8ml of 6N Ammonium hydroxide and 2ml 5% KI was added, nixed and titrated with 0.02N AgNO₃ against a background. Permanent turbidity end loint. Cyanogenic glycoside content (mg/ml).

3.3.8 Minerals determination

AOAC, (1990) procedure was adopted. Working solution for digestion was prepared using Nitric acid 600ml, perchloric acid 80ml and sulphoric acid 20ml.

Apparatus: 100ml beaker, crucible tongue, Digestion system (temperature at 450°C), measuring cylinder and grinding or milling machine.

0.3g of ground sample was weighed into 100ml beaker and 30ml of the working solution was added. Sample was digested in a digesting system (at 450°C), until the fuming become pure white and no carbon or particles is longer seen, then sample made up to 50ml distill water. Atomic Absorption spectrometer (AAS) was used to determine the mineral composition of the sample in part per million (ppm).

$$\frac{Conc (ppm) \times 50}{0.3}$$

3.3.9 Spectrophotometric determination of vitamins

This was carried out in the National Research Institute of Chemical Technology (NARICT), Zaria, using Shimadzu UV-VIS 2550 model of UV-VIS Spectrophotometer to determine the vitamins present in the seed. Retinol (25 mg) was dissolved in isopropanol and taken as stock solution. An aliquot of the stock solution was diluted and its absorbance measured at typical wave length of 325nm against isopropanol and calculated. Alpha-Tocopherol was dissolved in isopropanol, an aliquot of this stock solution diluted its absorbance measured against isopropanol at 292nm and calculated. Standard solutions were prepared by appropriate dilution of the measured stock solution.

Extraction of the sample

20ml of isopropyl alcohol was added to 5g-10g of the sample and allowed to stand in a test tube for about 45minutes at room temperature. The mixture was gently swirled and filtered using what man filter paper. The collected filtrate was measured at different wave length for various vitamins for example 325nm and 292nm for vitamin A and E respectively.

3.3.10 Amino acid profile determination

The Amino Acid profile in the known sample was determined using methods described by Benitez (1989). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

Defatting Sample:

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 4g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

Nitrogen Determination:

A small amount (200mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate (CuSO_4) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times v \times 100}{w \times c}$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10ml)

14. = Nitrogen constant in mg.

Hydrolysis of the sample

A known weight of the defeated sample was weighed into glass ampoule. 7ml of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible

oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $1050\text{C} \pm 50\text{C}$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humans. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis.

The filtrate was then evaporated to dryness at 400C under vacuum in a rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into TSM analyzer

The amount loaded was between 5 to 10 microlitre. This was dispended into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an Analysis lasted for 76 minutes.

Method of Calculating Amino Acid Values from the Chromatogram Peaks

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids. Alternatively, the net height of each peak produced by the chart recorder of TSM (each representing an Amino) was measured. The half-height of the peak on the chart was found and width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula.

$$NE = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{std} = NE_{std} \times \text{Molecular weight} \times \mu\text{MAA}_{std}$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

Concentration (g/100g protein) = $NH \times W @ NH/2 \times S_{std} \times C$

$$\text{Where } C = \frac{\text{Dilution} \times 16}{\text{Sample Wt (g)} \times N\% \times 10 \times \text{Vol.loaded}} \div NH \times W(nleu)$$

Where: NH = Net height

W = Width @ half height

nleu = Norleucine

3.4 Experimental Diet

The seeds were milled and added to replace soybean meal ingredient using various graded levels. The experimental feed for *C.gariepinus* were formulated using conventional feed ingredients applying computer feed formulation package. The ingredients were mixed together in each case (each protein inclusion level) and water was added and mixed thoroughly.

A pelleting machine was used in pelleting the feed as seen in (Plate III), and followed by sun drying. Each experimental feed concentrate was packed in a separate container and kept in a dried and cool condition to prevent fungal attack; the feed was collected and kept separately in polythene bags based on various inclusion levels. Six diets including the control diet at varying inclusion levels were formulated. Diet D₁ control contained 0% inclusion level of processed *Senna* seed, D₂ (10% inclusion level), D₃ (20% inclusion level), D₄ (40% inclusion level), D₅ (60% inclusion level) and D₆ (100% inclusion level), (Table 3.1).

3.5 Experimental Fish

One hundred and eighty (180) *C. gariepinus* (catfish) juveniles with average of 5.74g were obtained from Bangiwa farm in Funtua, Katsina state. The fish were transported in a fifty litres (50) half-filled plastic Jerican with water very early in the morning between 7.00am-8.00am to the Fisheries Laboratory at the Department of Biological Sciences, Ahmadu Bello University, Zaria, this was done to take advantage of the early morning low temperature and were immediately transferred into two large water baths with dimension of 150x50x40cm for a period of two weeks (acclimatization period). Fish was fed during acclimatization period with control diet (commercial feed) at five percent (5%) total body weight.

3.6 Experimental Setup

This Research was conducted in the Fishery Laboratory of the Department of Biological Sciences, Ahmadu Bello University Zaria. Eighteen plastic aquaria with dimension of 50x45x35cm was used (three aquaria/ experimental diet), each containing dechlorinated water provided suitable environment for the fish (Plate IV). Ten (10) juveniles of *C. gariepinus* were stocked randomly in each aquarium. The set up was wire mesh to avoid fish



Plate III: Pelletized Feed Formulated Using Graded Levels of Boiled *S. obtusifolia* Seed

3.1: Composition of Experimental Diets Used for Feeding Trial

Inclusion Levels of Boiled <i>S. obtusifolia</i> Seed Meal, (%)						
Ingredient	0 (c)	10	20	40	60	100
Senna seed	0.00	2.46	4.93	9.86	14.78	24.64
Maize	3.55	3.55	3.55	3.55	3.55	3.55
G. corn	15.77	15.77	15.77	15.77	15.77	15.77
G/nut cake	23.68	23.68	23.68	23.68	23.68	23.68
Soybean meal	24.64	22.18	19.71	14.78	9.86	0.00
Beniseed cake	9.86	9.86	9.86	9.86	9.86	9.86
Fish meal	19.71	19.71	19.71	19.71	19.71	19.71
Oil sludge	0.99	0.99	0.99	0.99	0.99	0.99
Lime stone	0.24	0.24	0.24	0.24	0.24	0.24
Bone meal	0.49	0.49	0.49	0.49	0.49	0.49
Salt	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Premix*	0.49	0.49	0.49	0.49	0.49	0.49
L-lysine	0.049	0.049	0.049	0.049	0.049	0.049
Enzyme (phytase)	0.0099	0.0099	0.0099	0.0099	0.0099	0.0099
Vitamin-C	0.030	0.030	0.030	0.030	0.030	0.030
Klino feed mycotoxin inhibitor	0.049	0.049	0.049	0.049	0.041	0.049
Pellet Binder	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100

Bio-organics Vitamin/ Mineral Premix* for Broiler Starter per Kg; Vitamin A: 10,000,000IU, Vitamin D3: 2,000,000IU, Vitamin K3: 2,000mg, Vitamin E: 23,000mg, Vitamin B1: 1,800mg, Vitamin B2: 5,500mg, Vitamin B6: 3,000mg, Vitamin B12: 15mg, Niacin: 27,500mg, Pantothenic: 7,500mg, Folic acid: 7,500mg, Biotin H2: 60mg, choline chloride: 300,000mg, Cobalt: 200mg, Copper: 3,000mg, Iodine: 1,000mg, Manganese: 40,000mg, Iron: 20,000mg, Selenium: 200mg, Zinc: 30,000mg, antioxidant: 1,250mg and Calcium Sulphate Guar gum (pellet binder).

Note: (c) = control diet



Plate IV: Experimental setup Used During the Feeding Trial

from jumping out. Water replacement was done every three (3) days using the flow through system. This eliminated faecal matter and unutilized feed by the fish.

3.7 Weighing and Measuring of Fish

The mean weight of fish was determined using an electric Mettler Zurich (Swiss made) top-loading balance model CP8201. This was done after acclimatization period. Mean weight per aquarium was recorded after taken the bulk weight of fish. Mean weight per aquarium was taken every fortnight throughout the twelve (12) weeks period of experiment. Both standard length (SL) and the total length (TL) of fish used for the experiment were recorded using a measuring board. Growth performance parameters such as specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), etc were calculated.

3.8 Feed Administration

Feeding frequency recommended by Marinmuthu *et al.* (2010) was adopted. Feed was administered twice daily; early morning, and in the evening. Experimental fish was fed 5% body weight.

3.9 Growth and Feed Utilization Computation

3.9.1 Weight gain (WG)

The weight gained from the initial weight at the end of monitoring exercise. This is computed as the difference between the final weight and the initial weight of the fish that is been cultured.

3.9.2 Percentage live weight gain (PWG)

The percentage live weight gain (PWG) computed as the difference between the initial and final fish weight divided by the initial weight expressed as percentage (Falayi, 2009).

3.9.3 Specific growth rate (SGR)

The specific growth rate was calculated as described by Aderolu and Sogbesan (2010) as follows:

$$SGR = \frac{\log e w_t - \log e w_0}{t - t_0} \quad (\% \text{days})$$

Where: W_t = weight at the time of observation

W_0 = initial weight

$t - t_0$ = the period under study in days, and

e = the base of Natural Logarithm (10)

3.9.4 Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated using the formular

$$FCR = \frac{\text{Total weight of diet fed (g)}}{\text{Total weight gain of fish (g)}}$$

3.9.5 Gross feed conversion efficiency (GFCE)

The GFCE is the reciprocal of the FCR expressed as a percentage, it is expressed as:

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

3.9.6 Protein efficiency ratio (PER)

Protein efficiency ratio was computed as a ratio of fish weight gain to protein intake of fish.

$$PER = \frac{\text{Weight gain (g)}}{\text{Protein intake of fish (g crude protein fed)}}$$

3.9.7 Net protein utilization (NPU)

$$NPU = \frac{\text{Fish protein gain}}{\text{protein fed}} \times 100$$

Where; protein gain = final body protein - Initial body Protein.

Protein Consumed = Total dietary- protein fed. (Falayi, 2009)

3.9.8 Nitrogen metabolism (Nm)

The values of nitrogen metabolism (Nm) were calculated using the formula of Dabrowski (1977). It is as follows

$$Nm = \frac{(0.54)(b-a)h}{2}$$

Where: a = Initial weight of fish (g)

b =Final weight of fish (g)

h = Experimental period in days.

3.9.9 Condition factor (K)

This was calculated according to (Amisha *et al.*, 2009):

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

Where: K= condition factor; L= length (cm); W= body weight of fish (g)

3.10 Determination of Water Quality Parameters

Water parameters were determined throughout the 12 weeks experimental period (readings were taken bi-weekly). The parameters include: water pH, water temperature and Dissolved oxygen (DO), Total dissolved solid (TDS) and Electrical conductivity (EC). Hanna pH /EC/TDS/Temp instrument H19830 was used to determine Electrical conductivity (EC), Total dissolve solid (TDS), water pH and water temperature.

DO was determined using DO meter (Hanna model D1946) after it has been calibrated and the protective cap has been removed. The tip of the probe was immersed into the water samples, allowed for 2 minutes and reading was recorded in mg/l. pH, EC, TDS and Temperature were obtained using Hanna pH/EC/ TDS/ Temperature instrument after it protective cap has been removed, the instrument was held while the tip of probe was immersed into water sample and allowed to read until the parameters value became static which correspond to the appearance of a clock symbol on the screen of the instrument.

3.11 Economics Analyses of Experimental Diets and Fish Cropped

Konyeme *et al.* (2005) method was adopted. The economic analysis was computed to estimate the cost of feed required to raise a kilogram of fish using the various experimental diets.

Estimated Investment cost analysis= Cost of feeding (₦) + Cost of fingerlings stocked.

Profit Index= Value of fish (₦) / cost of feed (₦).

Incident of Cost=Cost of feed (₦) / mean weight gain of fish produced (g).

Net Profit = Total Cost of Fish cropped (₦)-Total Expenditure (₦)

3.12 Data Analyses

SPSS Version 20 (2011) statistical software package was adopted to run all statistical analyses. Descriptive statistics was used to summarize data obtained. Paired sample t-test was used to determine if there was significant difference between the proximate and anti-nutritional content of the raw and boiled seed. The level of significance was set at $p < 0.05$. One way ANOVA was adopted to determine if there was any significant difference between the treatments (varying dietary inclusion levels) and growth performance of fish. Turkey HSD was used to rank the means that showed significant difference.

CHAPTER FOUR

4.0

RESULTS

4.1

Chemical analysis of feedstuff

This includes the proximate, anti-nutritional factors, macro-minerals, vitamins and amino acid profile of *S.obtusifolia* seed and the proximate composition of diets fed to *C. gariepinus*.

4.1.1 Proximate composition and anti-nutritional content of *S. obtusifolia* seed

The proximate composition of *S. obtusifolia* seeds as presented below (Table 4.1), the boiled seeds had higher value in dry matter, crude fibre, ether extract and nitrogen free extract while boiled seeds had higher values in moisture content, crude protein and ash content.

Anti-nutritional factors such as hydrocyanic acid, oxalate, phytate, saponin and tannin were detected in the raw seeds. In raw seeds, phytate had the highest value and saponin had the lowest value (Table 4.1), that anti-nutritional factors detected in boiled seeds were lower than that of raw seeds, tannin had the highest percentage reduction (91.83%) after boiling and the lowest percentage reduction (54.17%) was observed in saponin.

4.1.2 Macro-mineral and vitamin compositions of raw and boiled *S. obtusifolia* seeds

The macro-minerals determined in the raw and boiled seed were Calcium (Ca), Iron (Fe), Magnesium (Mg), Phosphorus (P), Potassium (K), Sodium (Na), Copper (Cu) and Zinc (Zn), (K) had the highest value followed by (Na), (Ca), (P), (Mg), Iron (Fe), (Zn) and (Cu) had the lowest value. Raw seeds had higher concentration of macro-elements compared to boiled seeds in (Table 4.2).

Table4.1: Proximate Composition and anti-nutritional contents of Raw and Boiled *S. obtusifolia* Seed

Proximate content	Seed, (%)		
	Raw	Boiled	
Moisture content	5.89	8.12	
Dry matter	94.11	91.88	
Crude protein	20.36	20.87	
Crude fibre	16.20	15.04	
Ether extract	6.81	6.03	
Ash content	4.52	4.66	
Nitrogen free extract	46.22	45.28	
S.E.M	±12.33	±11.97	
Anti-nutritional content	Concentration (mg/100g)	Percentage Reduction (%)	
Hydrocyanic acid	1.42 ^a	0.14 ^b	90.14
Oxalate	2.64 ^a	0.26 ^b	90.15
Phytate	4.16 ^a	0.96 ^b	76.92
Saponin	0.96 ^a	0.44 ^b	54.17
Tannin	2.44 ^a	0.20 ^b	91.83
S.E.M	±.56	±.15	

No significant difference between proximate composition of raw and boiled seed. ($P \geq 0.05$), Statistical analysis showed significant differences in anti-nutritional contents between raw and boiled seeds ($p < 0.05$)

Table 4.2: Macro-minerals Composition of Raw and Boiled *S. obtusifolia* Seed

Minerals	Raw <i>S. obtusifolia</i> Seed (%)	Boiled <i>S. obtusifolia</i> Seed (%)	Soybean (SBM) Meal (%)	Fish Meal (FM) (%)
Calcium (Ca)	0.78	0.70	0.30	3.74
Phosphorus (P)	0.70	0.62	0.69	2.48
Potassium (K)	1.80	1.32	2.10	0.72
Magnesium (Mg)	0.25	0.23	0.29	0.25
Sodium (Na)	0.80	0.71	0.04	0.88
Iron (Fe)	0.045	0.0059	0.01	0.021
Zinc (Zn)	0.005	0.004	ND	0.01
Copper (Cu)	0.0005	0.0003	0.0018	0.0009
S.E.M	±0.07	±0.02		

No significant difference in macro-mineral composition between raw and boiled *S. obtusifolia* seeds, ($p \geq 0.05$)

Bold= NRC (2001) standard for macro-minerals composition of soybean meal and fish meal, ND= Not Determined

Vitamin composition of seeds as presented (Table 4.3), *S. obtusifolia* raw seeds had higher concentration of vitamins compared to the boiled seeds. In the raw seed vitamin B₂ had the highest concentration and the lowest concentration was observed in vitamin C, vitamin B₁ had the highest values in boiled seeds and vitamin C was equally the lowest.

4.1.3 Amino acid profile of raw and boiled *S. obtusifolia* seed

The amino acid profile as presented in (Table 4.4), amino acids profile of raw *S. obtusifolia* was higher in abundance compared to the boiled seed. Glutamic acid had the highest amino acid profile and the lowest was obtained for cysteine as observed in both raw and boiled seeds.

4.1.4 Proximate Composition of Experimental Diets Fed to *C. gariepinus* Juveniles

Moisture content of Experimental diets of 100% inclusion level had the highest value followed by 60, 40, 20, 10 and the lowest in 0% (control). The control diet with 0% inclusion level had the highest value of dry matter followed by 10, 20, 40, 60 and least was diet with 100% inclusion level (D₆).

Diet with 0% inclusion level of boiled *S. obtusifolia* seed meal had the highest value of crude protein and diet with 100% inclusion level had the lowest, Diet with 100% inclusion level had the highest percentage crude fibre, and next to it was 60, 40, 20, 10 and diet with 0% boiled *Senna* seed meal had the lowest in that order.

Table 4.3: Vitamins Composition of Raw and Boiled *S. obtusifolia* Seed

Vitamins	Raw Seed	Boiled Seed	Soybean Meal (SBM)	Fish Meal (FM)
Vitamin A (mg/l)	15.418 ^a	6.270 ^b	ND	ND
Vitamin B ₁ (mg/l)	477.449 ^a	25.865 ^b	7.3	2.9
Vitamin B ₂ (mg/l)	963.049 ^a	12.296 ^b	5.98	0.52
Vitamin B ₆ (mg/l)	518.378 ^a	36.348 ^b	6.00	4.71
Vitamin B ₁₂ (mg/l)	106.561 ^a	2.279 ^b	ND	14.47
Vitamin C (mg/ml)	4.871 ^a	0.478 ^b	ND	ND
Vitamin E (mg/l)	363.175 ^a	20.046 ^b	2.4	3.7
S.E.M	±130.10	±5.01		

Means with different superscript are significantly different ($p < 0.05$)

Bold= NRC (2001) standard for vitamin composition of soybean meal and fish meal, ND= Not Determined

Table 4.4: Amino Acid Profile of Raw and Boiled *S. obtusifolia* Seed

Amino Acid	Raw Seed(g/100g)	Boiled Seed (g/100g)
Lysine	3.19	3.49
Histidine	2.19	1.82
Argenine	5.25	4.94
Aspartic acid	7.54	7.35
Threonine	2.11	3.05
Serine	3.21	2.27
Glutamic acid	8.94	9.16
Proline	2.23	2.18
Glycine	3.50	3.31
Alanine	4.02	3.71
Cystine	0.53	0.40
Valine	3.66	3.52
Methionine	1.02	0.81
Isoleucine	2.20	2.51
Leucine	6.95	6.75
Tyrosine	2.25	2.25
Phenylalanine	3.89	3.38
S.E.M	±.55	±.60

No significant difference amino acid profile between raw and boiled *S.obtusifolia* seeds, ($p \geq 0.05$)

Diets with 0% inclusion level had the highest value of ether extract followed by 10, 20, 40, 50 and the lowest was observed in diet with 100% inclusion level. Diet with 0% inclusion level had the highest Ash content (14.94%) while the lowest was observed in D₅ which was had 60% inclusion level of boiled *Senna* seed meal (10.06%). NFE for Diet with 100% inclusion level had the highest value (36.22%) followed by 60, 40, 10, 20 and diet with 0% inclusion level had the lowest value (32.47%) as seen in (Table 4.5)

4.2 Fish Growth and Feed Utilization of *C. gariepinus* Fed Graded Levels of Boiled *S. obtusifolia* Seed Meal

4.2.1 Growth response of fish fed graded levels of boiled *S. obtusifolia* seed meal

The initial weight per fish fed diet with 0% inclusion level, 10%, 20%, 40%, 60% and 100% ranged between (5.41-6.05g). The highest final weight (g / fish) in this study came from fish fed 0% boiled *Senna* seed meal that is 100% soybean meal (control), 20%, 10%, 40%, 60% and fish fed with 100% inclusion level had the lowest.

Fish fed control diet had the highest weight gain per fish (16.65g) followed by fish fed with 20% inclusion level of boiled seed, 10%, 40%, 60% and fish fed diet 100% inclusion level had the lowest weight gain per fish (4.81g). The initial standard length per fish of *C. gariepinus* used for this study ranged between (8.18 – 8.85cm). The highest final standard lengths per fish of fish was recorded for diet with 0% inclusion level and fish fed with 100% had the lowest. The length gain per fish for *C. gariepinus* in this study ranged between (4.98-3.49cm).

Table 4.5: Proximate Composition of Experimental Diets Fed to *C. gariepinus* Juveniles

Proximate Content	Inclusion Levels of Boiled <i>S. obtusifolia</i> Seed Meal, (%)						S.E.M
	0	10	20	40	60	100	
Dry matter	96.73 ^a	96.68 ^a	96.40 ^a	96.09 ^b	95.06 ^b	94.90 ^b	±0.22
Crude protein	35.99 ^a	34.30 ^a	35.93 ^a	33.62 ^a	30.22 ^b	29.52 ^b	±2.58
Crude Fibre	7.11 ^d	8.33 ^c	8.46 ^c	10.03 ^b	13.18 ^a	13.46 ^a	±0.73
Ether extract	6.20 ^a	5.82 ^b	5.62 ^b	4.75 ^c	4.71 ^c	4.66 ^c	±0.10
Ash Content	14.96 ^a	14.24 ^b	14.18 ^b	11.82 ^c	10.76 ^d	11.04 ^d	±0.51
Moisture	3.28 ^c	3.32 ^c	3.60 ^b	3.92 ^b	4.94 ^a	5.10 ^a	±0.22
NFE	32.47 ^c	33.99 ^b	32.71 ^b	35.87 ^a	36.19 ^a	36.22 ^a	±0.48

Values with the same superscript along row were not significantly different ($p \geq 0.05$)

The initial total length per fish ranged between (9.43 – 9.93cm).The highest final total length per fish of fish was observed in *C. gariepinus* fed diet (D₁) with 0% inclusion level followed by fish fed 10%, 20%, 40%, 60% and fish fed 100% (D₆) had the least.

The initial total length per fish recorded used in this study ranged between (3.82-5.46cm). Condition factor (K) obtained in this study ranged between (0.98-0.61), (Table 4.6).

4.2.2 Carcass Composition of *C. gariepinus* Fed Graded Levels of Boiled *S. obtusifolia* Seed Meal

The highest carcass crude protein of fish was recorded in fish fed with diet with 0% inclusion level (D₁) next to it was fish fed 20% inclusion level (D₃), 10% (D₂), 40% (D₄), 60% (D₅) and fish fed diet with 100% inclusion level(D₆)had the lowest carcass crude protein. Ether extract ranged between (10.33-8.30%). Fish fed 100% inclusion level had the highest carcass ash value,next to it were fish fed 60%, 40%, 20%, 10% and the lowest was observed in fish fed diet with 0% inclusion level.

The moisture content recorded for fish in this study ranged between (8.76-7.45%). NFE content of fish fed diet 100% inclusion level (D₆) had the highest value followed by 60%, 40%, 10%, 20% and fish fed with 0% inclusion level(D₁;control diet) had the lowest (table 4.7).

4.2.3 Feed utilization efficiency parameters of *C. gariepinus* fed graded levels of boiled *S. obtusifolia* seeds meal

Feed utilization efficiency parameters as presented (Table 4.8), specific growth rate (SGR) of fish with the highest value was seen in fish fed diet that had 0% inclusion level,

Table 4.6: *Clarias gariepinus* Growth Parameters Fed Graded Levels of Boiled *S. obtusifolia* Seed Meal

Parameters	Inclusion Levels of Boiled <i>S. obtusifolia</i> Seed Meal, (%)						S.E.M
	0	10	20	40	60	100	
Initial Weight(g/ fish)	5.58 ^a	5.88 ^a	6.05 ^a	5.78 ^a	5.72 ^a	5.41 ^a	±0.06
Final Weight(g/ fish)	22.24 ^a	16.42 ^a	19.86 ^a	14.00 ^b	10.98 ^c	10.22 ^c	±1.07
Weight Gain(g/ fish)	16.66 ^a	10.54 ^b	13.81 ^a	8.22 ^b	5.26 ^c	4.81 ^c	±1.05
Initial Standard Length (cm / fish)	8.18 ^a	8.33 ^a	8.36 ^a	8.32 ^a	8.55 ^a	8.43 ^a	±0.02
Final Standard Length (cm / fish)	13.16 ^a	12.54 ^b	12.98 ^b	12.43 ^b	12.21 ^c	11.92 ^c	±0.10
Length Gain (cm/ fish)	4.98 ^a	4.21 ^b	4.62 ^a	4.11 ^b	3.66 ^c	3.49 ^c	±0.12
Initial Total Length (cm / fish)	9.43 ^a	9.60 ^a	9.67 ^a	9.78 ^a	9.93 ^a	9.77 ^a	±0.03
Final Total Length (cm / fish)	14.98 ^a	14.31 ^a	14.73 ^a	13.98 ^{ab}	13.84 ^{ab}	13.59 ^b	±0.49
Total Length Gain (cm / fish)	5.46 ^a	4.69 ^b	5.07 ^a	4.34 ^c	3.91 ^c	3.82 ^c	±1.45
Condition factor (k)	0.98 ^a	0.83 ^b	0.90 ^{ab}	0.72 ^c	0.61 ^c	0.60 ^c	±0.04

All values represent mean of three replicates, values along row with same superscript were not significantly different ($p \geq 0.05$)

Table 4.7: Carcass Composition on Dry Matter Basis of *Clarias gariepinus* before and After Feeding Trail

Parameter	Inclusion Levels of Boiled <i>S. obtusifolia</i> Seed Meal, (%)							S.E.M
	Initial	0	10	20	40	60	100	
Crude protein	51.16 ^e	68.65 ^a	65.23 ^b	67.56 ^a	62.63 ^c	55.46 ^d	55.12 ^d	±1.42
Ether extract	13.99 ^a	10.33 ^b	9.43 ^c	9.33 ^c	8.44 ^d	8.32 ^d	8.30 ^d	±0.42
Ash content	15.37 ^a	7.74 ^d	8.14 ^d	8.11 ^d	10.82 ^c	11.21 ^{bc}	11.44 ^b	±0.56
Moisture content	10.23 ^a	8.76 ^b	8.16 ^b	8.38 ^b	7.66 ^c	7.52 ^c	7.45 ^c	±0.20
Nitrogen free extract (NFE)	9.25 ^b	4.54 ^d	8.97 ^c	6.63 ^d	10.45 ^b	17.42 ^a	17.70 ^a	±1.05

All values represent mean of three replicates, values with same superscript along row were not significantly different ($p \geq 0.05$)

Table 4.8: Feed Utilization Efficiency Parameters of *C. gariepinus* Fed with Graded Levels of Boiled *S. obtusifolia* Seed Meal

Inclusion Levels of Boiled <i>S. obtusifolia</i> Seed Meal, (%)							
Parameters	0	10	20	40	60	100	S.E.M
SGR	1.62 ^a	1.10 ^b	1.31 ^b	0.77 ^c	0.61 ^c	0.54 ^c	±0.10
FCR	1.29 ^a	1.57 ^{ab}	1.39 ^a	2.02 ^b	2.32 ^c	2.55 ^c	±0.11
GFCE	77.45 ^a	63.70 ^a	71.77 ^a	49.60 ^b	43.15 ^c	39.18 ^c	±3.51
PWG	284.49 ^a	150.03 ^c	195.63 ^b	94.13 ^d	66.54 ^{de}	56.91 ^e	±19.58
PER	1.59 ^a	1.12 ^a	1.34 ^a	0.72 ^b	0.57 ^c	0.48 ^c	±0.10
_{App} NPU	48.59 ^a	41.02 ^c	46.27 ^b	34.11 ^d	14.23 ^e	13.40 ^e	±3.46
PSR	96.70 ^a	90.00 ^b	90.00 ^b	86.70 ^c	86.70 ^c	83.30 ^d	±1.46
PMR	3.30 ^d	10.00 ^c	10.00 ^c	13.30 ^b	13.30 ^b	16.70 ^a	±1.59
Nm	3673.91 ^a	2030.64 ^a	2726.22 ^a	1467.26 ^b	871.59 ^c	710.19 ^c	±254.97

All values represent mean of three replicates, values with same superscript 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, _{App}NPU: Apparent Net Protein Utilization, PSR: Percentage Survival Rate, PMR: Percentage Mortality Rate, Nm: Nitrogen Metabolism

next to it were fish fed 20%, 10%, 40%, 60% and fish fed 100% inclusion level had the lowest growth rate. Feed conversion ratio (FCR) of fish fed diet containing 100% inclusion level had the highest FCR and the lowest recorded for fish fed diet that had 0% inclusion level (D₁).

Gross feed conversion efficiency (GFCE) obtained shows that 0% inclusion level diet had the highest (77.45%) and the least GFCE was recorded from fish fed with diet with 100% inclusion level(39.18%). Percentage live weight gain (PWG) of fish fed various diets in descending order 0%, 20%, 10%, 40%, 60% and fish fed diet 100% inclusion level had the lowest PWG.

Apparent Net Protein Utilization (_{App} NPU) fish fed diet with 0% inclusion level(D₁) had the highest _{App} NPU value followed by 20%, 10%, 40%, 60% and the lowest was recorded for fish fed 100% inclusion level. Percentage survival rate for fish fed with different inclusion level of boiled *S.obtusifolia* seed meal ranged between (96.70-83.30%). Percentage mortality rate of fish fed with diet D₁ (control) had the lowest mortality of (3.30%) while the highest mortality was recorded for fish fed with diet D₆(16.70%).Nitrogen metabolism computed for fish fed different dietary level of feed ranged between (3673.91-710.19).

Fish fed with D₁ (0% inclusion level which was the control feed recorded the highest growth response across the 12 weeks period followed by D₃ (20% inclusion level),

D₂ (10% inclusion level), D₄ (40% inclusion level), D₅ (60% inclusion level) and the lowest was observed in *C.gariepinus* fed with 100% inclusion level boiled *Senna* seed meal D₆ (Figure 1).

4.3 Physicochemical parameters of water recorded during this study

Physicochemical parameters of water determined as presented (Table 4.9), the hydrogen concentration ion pH ranged between 6.77-6.87 for all plastic aquaria. The temperature range was 25.65-25.76°C, electrical conductivity of water was the same (0.20µs-1) which shows that the total dissolve solid (TDS) was relatively the same (0.10ppt). The dissolved oxygen ranged between 6.98-7.02mg/l.

4.4 Cost Analyses Estimation of Experimental Diets and Fish Fed Graded Levels of Boiled *S. obtusifolia* Seed Meal

The cost analysis of experimental diets and fish cropped as presented (Table 4.10), fish fed 0% inclusion level had the highest value following it was fish fed with diet 10%, 30%, 40%, 60% and fish fed 100% inclusion level had the lowest cropping value. Expenditure value showed that 20% inclusion level had the lowest value, followed by 0%, 10%, 40%, 60% and the highest value was recorded for 100%.

Net profit value for 100% inclusion level (N926.28) had the lowest value and the highest was recorded for 0% (N2385.72). Cost of producing a kg feed for diet with 0% inclusion level had the highest value next to it was 10%, 20%, 40%, 60% and the lowest value was obtained for 100% inclusion level in that order. Cost of feeding a fish to a kg for 60% inclusion level had highest value (N341.04) and 0% had the lowest value (N212.25).

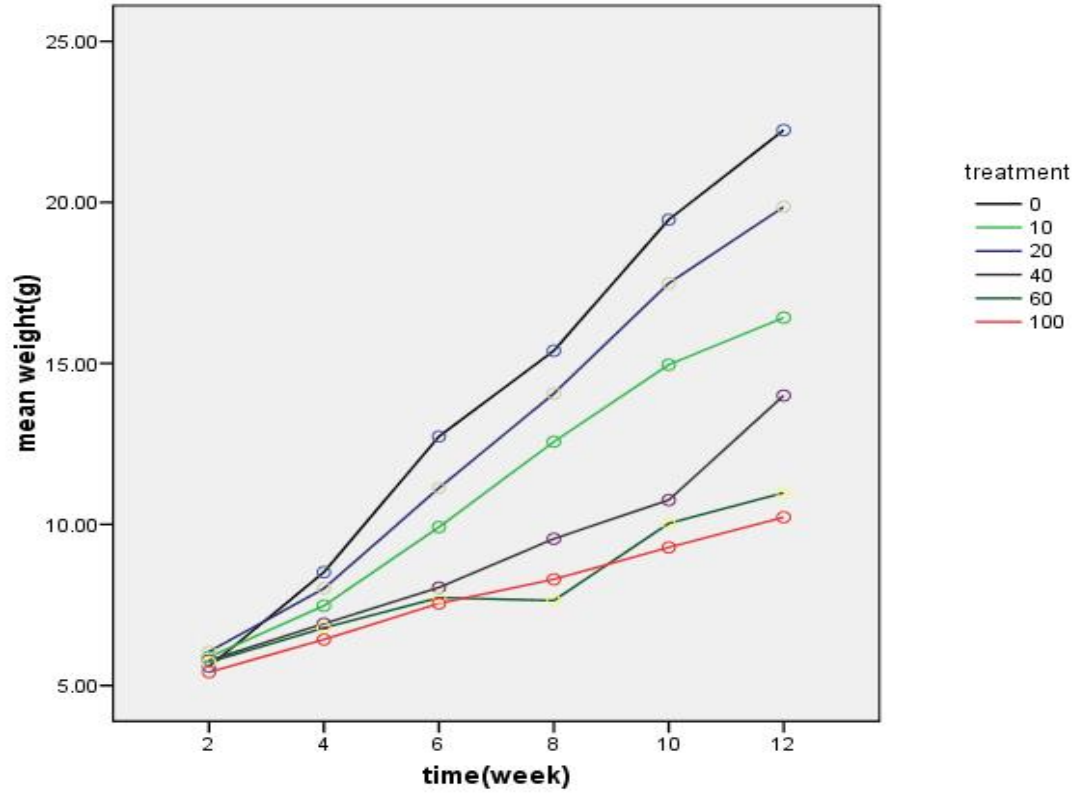


Figure 1: Growth Response of *C. gariepinus* Fed Graded Levels of Boiled *S. obtusifolia* Seed Meal for Twelve Weeks

Table 4.9: Physicochemical Parameters Recorded within the Period of Feeding Trial and FEPA Range for Aquaculture Practice

Tank/inclusion level	pH \pm SE	Temp($^{\circ}$ C) \pm SE	Electrical conductivity (μ s) \pm SE	TDS (ppt) \pm SE	DO (mg/l) \pm SE
Tank 1 (10%)	6.87 \pm 0.02	25.73 \pm 0.02	0.20 \pm 0.00	0.10 \pm 0.00	6.99 \pm 0.30
Tank 2 (20%)	6.84 \pm 0.01	25.73 \pm 0.02	0.20 \pm 0.00	0.10 \pm 0.00	7.00 \pm 0.10
Tank 3 (40%)	6.80 \pm 0.03	25.66 \pm 0.01	0.20 \pm 0.00	0.10 \pm 0.00	6.98 \pm 0.10
Tank 4 (60%)	6.81 \pm 0.01	25.65 \pm 0.02	0.20 \pm 0.00	0.10 \pm 0.00	7.02 \pm 0.20
Tank 5 (100%)	6.79 \pm 0.02	25.71 \pm 0.02	0.20 \pm 0.01	0.10 \pm 0.00	6.98 \pm 0.10
Tank 6 (0%)	6.77 \pm 0.02	25.76 \pm 0.02	0.20 \pm 0.01	0.10 \pm 0.01	6.99 \pm 0.10
FEPA range	5.00-9.00	20.00-35.00	20.00-1500.0	10-750ppm	>1.00

All values represent mean of three replicates, statistical analysis showed no significant difference of the parameters at varying inclusion levels ($p \geq 0.05$)

FEPA= Federal Environmental Protection Agency, DO = Dissolved oxygen, TDS = Total Dissolved Solid

Table 4.10: Cost Analysis Estimation of Experimental Diets and Fish Fed Graded Levels of Boiled *S. obtusifolia* Seed Meal

Cost Analyses Parameter	Inclusion Levels of Boiled <i>S. obtusifolia</i> Seed Meal, (%)						S.E.M
	0	10	20	40	60	100	
Cost of Fish Cropped (₦) EIA (Expenditure)	4833.33 ^a	4666.67 ^a	4500.00 ^a	4333.33 ^a	4333.33 ^a	4166.67 ^a	±75.32
(₦) Net Profit	2447.61 ^c	2759.37 ^b	2363.77 ^c	3053.88 ^{ab}	3353.21 ^a	3240.38 ^a	±95.00
(₦) Cost of Producing a kg Feed	2385.72 ^a	1907.14 ^b	2136.23 ^{ab}	1279.45 ^c	980.13 ^c	926.28 ^c	±141.33
(₦) Cost of feeding a fish to a Kg	164.00 ^a	160.95 ^a	157.91 ^b	151.18 ^c	145.70 ^c	133.50 ^d	±2.50
(₦) Cost of juveniles (₦40/juvenile)	212.25 ^d	252.91 ^c	219.20 ^d	306.32 ^b	341.04 ^a	340.78 ^a	±13.00
Incidence of cost	400.00 ^a	400.00 ^a	400.00 ^a	400.00 ^a	400.00 ^a	400.00 ^a	±0.00
Profit Index	12.77 ^a	24.29 ^b	15.87 ^a	37.25 ^c	63.75 ^d	71.54 ^e	±5.57
Benefit Cost Ratio	2.05 ^a	1.98 ^a	2.28 ^a	1.63 ^b	1.47 ^c	1.47 ^c	±0.07
	1.97 ^a	1.69 ^a	1.90 ^a	1.42 ^b	1.29 ^c	1.28 ^c	±0.07

All values represent mean of three replicates, statistical analysis showed significant difference of the parameters at varying inclusion levels ($p < 0.05$)

Cost of juvenile was the same for all groups each juvenile cost N40/juvenile. Incidence of Cost for 100% inclusion level had highest incident of cost(71.54) and the lowest was obtained for fish fed diet with 0% inclusion level (12.77).

The highest profit index was observed in 20% level (2.28), the profit index of fish fed 100% and 60% had the lowest value. The benefit cost ratio computed in this study ranged between (1.28-1.97).

CHAPTER FIVE

5.0

DISCUSSION

The proximate composition of raw *S. obtusifolia* seed obtained from this work did not differ to that reported by Ingweye *et al.* (2010) where they nutritionally evaluated raw seeds of *S. obtusifolia* plant obtained from Obanliku, south eastern Nigeria. The proximate composition of raw sickle pod seed obtained from Ingweye *et al.* (2010) study recorded 92.50, 29.54, 10.18, 3.70 and 46.77% for dry matter, crude protein, crude fibre, ash and NFE respectively. The dry matter, crude fibre and ash obtained in this study was higher than result obtained by Ingweye *et al.* (2010), also comparing the crude protein, this work recorded a lower value, while NFE did not differ. Ismaila *et al.* (2011) did a similar work on nutritional and phytochemical screening of raw seed, showed that crude fat (lipid) crude fibre, moisture, ash, crude protein and carbohydrate had 3%, 0.20, 4.00, 6.00, 18.46 and 68.54% respectively, there was however slight variation in result obtained by Ismaila *et al.* (2011) when compared to values obtained in this work, their findings showed higher values for carbohydrate and ash while crude protein, moisture and ether extract were lower than value obtained from this work. The differences observed in the proximate composition of raw seeds from these studies are probably as a result of factors, such as; geographical location of the plant, soil and climatic conditions of a place (FAO, 2004). Difference was also observed in Umar (2006) using *Senna occidentalis*. He obtained crude protein, crude fibre, Ash, ether extract, NFE and moisture to be 14.40, 16.10, 8.40, 4.20, 52.90 and 4.00% respectively.

Senna occidentalis showed higher value for carbohydrate and ash, while crude protein, moisture and crude lipid were lower when compared p the proximate composition result

obtained from this work. The crude fibre reported by Umar (2006) and that of this study did not differ significantly. The difference in proximate composition observed from both studies is probably due to differences seen at inter-species level. Irwin and Barneby (1982) also gave variation at inter species levels.

The moisture content in raw and boiled seeds were higher than the values reported by Umar (2006) who reported 4.00% (raw) and 3.41% (parboiled) using seed of *Senna occidentalis*, Suleiman, (2006) reported that this differences could be due to the processing method used. Boiling increased the moisture content, crude protein and ash while it reduced crude fibre, carbohydrate, crude lipid and dry matter. This trend was in conformity with the work of Umar (2006).

There was no significant difference ($p \geq 0.05$) in the proximate values obtained from raw and boiled seed. The high dry matter content was similar to the value obtained from most raw seeds of legumes like *Milletia purpureus*, *Phaseolus aurens* and *Vigna sinensis* (Umoren *et al.*, 2005; Osman,2007; Mubarak, 2005; Khattab *et al.*, 2009), this will ensure long term storage (Ingyewe *et al.*, 2010).

The crude protein content was higher than the lower limits range and slightly the same with the upper limit range (15.52 - 20.74%) reported for *Cassia hirsuta* (vadivel and Janardhana, 2000), *Senna occidentalis* (Umar, 2006) and *Azelia africana* (Obun and Ayanwale, 2008).

Differences observed could be due to the variety at inter-specie level(Ingyewe *et. al*, 2010). The high crude protein value will enhance it replacement value for soybeans in aqua feeds. The anti-nutritional content in raw were higher than that of boiled meaning that boiling

significantly reduced anti-nutritional contents in raw seed ($P < 0.05$). Phytate was higher in raw seeds 4.16mg/100g and the least was saponin 0.96mg/100g. All anti-nutrients present were lower than those reported by Ingyewe *et al.* (2010), this could be suggestive that environment could be the determining factor of presence or absence of anti-nutrients in *Senna obtusifolia* plant as a result of plants absorbing substances from their environment such as soil and atmosphere (Ismaila *et al.*, 2011).

Boiling reduce the anti-nutrient greatly, tannin had the highest percentage reduction level 91.80% while saponin had the lowest reduction level 54.16%. Boiling might have leached out a large percentage of tannin because it is mainly found in seed coat (Umar, 2006). Dakare (2005) reported that boiling of *Parkia* seed reduces the anti – nutritional content of the seed.

The hydrocyanic acid content was lower than 2.60mg/100g (i.e 26mg/kg) and 1.79–3.47mg/100g (i.e. 17.9 – 34.0mg/kgDM) reported for *M. obanensis* (Umoren *et al.*, 2005) and *L. purpureus* bean (Abeke *et al.*, 2008), respectively.

High concentration of hydrocyanic acid acts by inhibiting the action of porhyrin enzymes (cytochrome oxidase) in tissues to cause suffocation (Umar, 2006). The oxalate value was low compared to 37.5mg/100g in *M. obanensis* (Umoren *et al.*, 2005). High oxalate content in seed has the potential to cause metabolic calcium deficiency by inhibiting its absorption from the intestine (McDonald *et al.*, 1995).

The phytic acid content in seeds was lowered by boiling but had the lowest reduction levels. Abu (2005) reported fermentation reduces phytate by 17.77%. Kumar *et al.* (1998) reported that cooking decreased both water and acid extractable phytate phosphorous in

legumes. Phytic acid can bind with essential minerals in the body such as zinc, calcium and aluminum and limit their bioavailability (Umar, 2006).

This work recorded same mineral element reported by Ingweye *et al.* (2010), the most abundant was potassium (K), Ingweye *et al.* (2010) recorded K to be the most abundant mineral. This most abundance level of K is not surprising as members of the bean family are rich in K (Vasadevan and Sreekumari, 2007). Potassium is necessary for nerve transmission, maintenance of osmotic pressure and acid base equilibrium, activation of certain enzymes, and uptake of certain amino acids as well as carbohydrate and protein metabolism (Banerjee, 2004).

The calcium value recorded in this work was also low compared to 13.9, 47.4, 585.00 and 84mg/100g reported for raw *Lathyrus maritimus* (beach, pea) *M.obanensis* (Odudu Nigeria cowpea *Vigna Spp*) and mung bean (*P. aurens*) seeds respectively (Shahudi *et al.*, 1999; Umoren *et al.*, 2008). Calcium is necessary for teeth and bone formation, blood clotting, the working of muscles, reputation of heart beat and maintenance of acid base equilibrium in the body of animals and it is said to be abundant in legumes (Banarjee, 2004).

The presence of sodium (Na) in *S. obtusifolia* seed shows that feeding livestock feed that is rich in *S. obtusifolia* has the potential to maintain body fluid pH support nerve transmission and muscular contraction in livestock (McDonald *et. al*, 1995). The Mg value was also low compared to 128.148, 92.3, 183 and 55.6mg/100g reported for Nigeria cowpea, *M. obanensis* and mung beans (Chinma *et al.*, 2008; Umeron *et al.*, 2005; Shahidi *et al.*, 1995). Mg in the body sustains lower irritability of neuromuscular system and activates

enzymes like phosphate which require ATP (Banerjee, 2004). Phosphorus (P) in the form of phosphate ion is needed for formation of bone and teeth, production of high energy compound e.g. ATP, DNA and RNA formation and activation of enzymes (Ingweye *et al.*, 2010). The P content was lower than that reported by Ingweye *et al.* (2010) in *Sennaobtusifolia* obtained from Obanliku Southeastern part of the country. Iron content was low compared to 11.5 and mg/100g for soybean and Mung beans (Vasudevan and Sreekumari, 2007).

The Vitamins obtained in this study were the same as that reported by Ingweye *et al.* (2010), Vitamin C recorded the lowest value and this is because Vitamin C is more abundant in fruit than in seed (Ingweye *et al.*, 2010). Gatlin (2010) gave specific function of various vitamin in fish; Vitamin A (retinol); epithelial tissue maintenance and vision. Vitamin E (tocopherol); biological antioxidant, B₁ (thiamin); Carbohydrate metabolism, B₂ (riboflavin); hydrogen transfer, B₆ (pyridoxine); protein metabolism, B₁₂ (cyanocobalamin); red blood cell formation and C (ascorbic acid); blood clotting and collagen synthesis. The presence of these Vitamins in *S. obtusifolia* seed shows that it has potential of performing these functions in fish. There was a significant reduction of vitamins between the raw and boiled seed this is probably as a result of heat during boiling and this was taken into consideration because vitamin premix was added to the formulated diets. The vitamins obtained were higher than NRC standard compositions of soybean meal and fish meal.

There was no significant difference between the amino acid profiles of raw and boiledseeds, this show that processing method used did not significantly affect the amino acids in the seed.

The dry matter of experimental diets ranged between 94.60 – 96.73% this is in conformity with Umar (2006) and Aminu (2011), high dry matters means that experimental feed will have longer shelf life and can easily be stored and preserved without any difficulties. The dry matter decreased as inclusion level increased but this showed no significant differences.

The highest diet crude protein was obtained from 0% inclusion level(35.99%) and the lowest was 100% level(29.52%). 0% (D₁) and 20% (D₃)inclusion level crude protein were in conformity with value recommended by Ayinla (1988), Robinson (1991), Bolorunduro (2002), Bekibele (2005), and Gatlin (2010) as crude protein requirement for *C. gariepinus* juvenile. There was significant difference in crude protein of experimental diets ($P < 0.05$), this can be attributed to level of inclusion Aderolu and Sogbesan (2010). Also soybean meal is higher in crude protein compared to boiled *S. obtusifolia* seed. The crude fibre, carbohydrate and moisture content increase with increase in inclusion level while the reverse was the case for crude lipid of experimental diet which decreased with increase in inclusion level and this showed high significant differences ($P < 0.05$). The fish carcass protein for all dietary treatment was higher than the initial carcass protein, indicating that there was synthesis and increased tissue protein production and growth of fish was not due to increase in weight only (Fuller, 1969). The highest carcass protein was recorded in fish fed with 0% inclusion level while the least was observed in 100% inclusion level. Carcass protein of fish decreased with increased in inclusion level, this can be attributed to the reduction in crude protein and increase in crude fibre content of experimental diet carcass protein was in conformity with Auta *et al.* (2007) where they used boiled castor seed meal to feed fingerling of *C. gariepinus*. Gatlin (2010) reported that the amount of crude fibre in

fish feed is usually less than 7 percent of the diet to limit the amount of undigested material entering the culture system. Carcass crude lipid of fish at the end of the experiment was lower than the initial, this shows that increase in weight was not due to the accumulation of fat fish (Bekibele, 2003).

Carcass crude lipid of fish decreased with the increase in inclusion level of *S. obtusifolia* seed in experimental diets. Carcass Ash, Carbohydrate increased with increase in inclusion level. The protein sparing effect was greater in fish fed with D₁ followed by D₃ while the least protein sparing effect was observed in fish fed with diet D₆, this can be attributed to the carbohydrate level of each experimental diet. Pantazis (2005) found that dietary carbohydrate level of between 26 and 32 percent had a significant protein sparing effect in *C. gariepinus*. Also, Ali (2011) suggested that, *C. gariepinus* could not utilize dietary carbohydrate level above 35 percent.

The mean weight gained showed that fish fed D₁ recorded the highest weight gained 16.66g and the least was obtained in fish fed with D₆ 4.81g. These differences in mean weight gained can be attributed to differences in crude protein, crude fibre and carbohydrate 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. The weight gained decreased with increase inclusion level above 20% of *S. obtusifolia* seed. There was significant differences in mean weight gained among fish fed with different inclusion level of *S. obtusifolia* seed ($P < 0.05$).

The condition factor (K) of fish revealed that they were in good condition throughout the study. This is probably due to reduction in the anti-nutrients content in seed through

boiling (Auta *et al.*, 2007) the condition factor was within the range (0.55 – 1.72) reported by Agbugiu *et al.* (2011). The seeming slow growth rate that was apparent both in control and sickle pod seed replacement level may have resulted from the high inclusion of soya bean meal in formulating diets and low level of fish meal in the diet (Cai and Burtle 1996; Bolton and Blair 1977; FAO, 1983 and Bekibele 2005).

FCR recorded in this study revealed that as the inclusion level in diet increases the FCR also increases, by increase in crude fibre which can translate to increase in indigestibility of diet (Gatlin, 2010; Aderolu and Sogbesan 2010). The highest FCR was recorded in D₆ (2.55) and lowest in D₁ (1.29). Falayi, (2010) reported a lower FCR which therefore implies efficient food utilization by that animal. Irwin and Barneby, (1982) advised the used of *S. obtusifolia* in a very low amount because feeding on *S. obtusifolia* in high amount can cause indigestibility and diarrhea in animal. GFCE, PWG, PER NPU, NM and percentage fish survival decreased as inclusion level of boiled sickle pod seed increases above 20% replacement level. Percentage mortality rate increases as the level of *S. obtusifolia* seed increases.

The water quality parameters recorded in this work were within the range recommended for the culture of *C. gariepinus* defined for warm fish species. Boyd (1981) reported temperature range of 22 – 27°C, pH range from 6.5 – 9.0 and dissolved oxygen of 6.3mg/l and 9.6 mg/l gives the best growth in cultured tropical fishes. Mazid *et al.* (1972) also reported a similar value for tropical fish, an average temperature of 28°C, dissolved oxygen of 6.9mg/l and pH 7.3 are optimal for normal growth. Auta (1993) reported temperature between 25°C and 30°C and pH (6.7 and 9.0) which were adequate for fresh water fish culture.

Balogun, *et al.* (2004) also found that a temperature ranged of 23°C to 27°C, pH of 6.3 to 7.8 and dissolved oxygen of 6.3 to 9.6mg/l were adequate for an optimal growth and development of Nile Tilapia (*Oreochromis niloticus*) cultured under aquaria conditions. There was no significant difference in water quality parameters recorded in this work for various plastic aquaria ($P \geq 0.05$) this probably due to the fact that all plastic aquaria were placed under the same environmental conditions (in the same laboratory for the twelve weeks period of this work), the physicochemical parameters of water used during the feed trials were within FEPA range for aquaculture practice, therefore it was concluded that formulated diets should be the only varying factor for fish growth in the experimental setup.

Economic analysis of feed showed that estimated cost of fish cropped showed no significant difference ($P \geq 0.05$) but the higher the inclusion level above 20% the longer the duration for culturing the fish to marketable size. There is no significant difference in estimated investment analysis between diet D₁ and D₃ (20% inclusion level of *S. obtusifolia*) N2385.75 and N2136.23 respectively. Increasing the inclusion level of boiled *S. obtusifolia* seed in diet will correspond increased the expenditure; this can probably be due to growth performance of fish.

The fish feed with higher level of boiled *S. obtusifolia* seed above 20% showed lower crude protein in diet reducing growth rate which means it will take the fish longer time to attain marketable sizes, this was in conformity with result of Aderolu and Sogbesan (2010) and Bekibele (2005). Net profit of fish show that D₁ and D₃ recorded the highest value N2, 385.72 and N2, 136.23 respectively and the least was recorded in D₆ N926.28.

This shows that increase in boiled *S. obtusifolia* seed above 20% will lead to decrease in Net profit. The cost of production of feed reduced as inclusion level increased. This was in conformity with report of Aderolu and Sogbesan (2010) and Konyeme *et al.* (2005). The profit index and benefit cost ratio showed that Diet D₁ and D₃ performed best compared to other fish fed with diet having above 20% inclusion level.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

There is a significant difference ($p < 0.05$) in nutritional value at graded levels of boiled *S. obtusifolia* seed meal in the growth performance of *C. gariepinus* juveniles.

There is a significant difference ($p < 0.05$) in feed utilization between various diets of sickle pod seed meal at 10%, 40% and above, and the control diet fed to *C. gariepinus*.

In order to meet the demand for fish, the development of cheap, balanced and nutritionally adequate diets are required, *S. obtusifolia* seed is cheap to get, it has considerable protein, minerals and vitamins, amino acid and its anti-nutritional factors can easily be removed by boiling.

Also the growth rate parameters and economic analyses of diet fed to *C. gariepinus* using *S. obtusifolia* seeds have proven to be nutritive source of protein and a good replacement for soybean meal at 20% inclusion level in formulating aqua feed for *C. gariepinus* juveniles without deleterious effect.

6.2 Recommendations

Catfish feed formulators should maintain 20% replacement level of soybean meal for an effective utilization of *S. obtusifolia* seed in aqua feed for *C. gariepinus*.

The replacement level of *S. obtusifolia* seed for soybean meal should not be much for an effective growth performance, It is important to formulate aqua feed for *C. gariepinus* such that the fish meal (animal protein) should be greater in composition compared to plant

protein because they are more of carnivores than omnivores for the fish to record a high specific growth rate.

Other heat treatment methods for removal of ANFs should be conducted to evaluate the most effective method for best use in wild legumes seeds.

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