GROWTH PERFORMANCE OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FINGERLINGS FED PROCESSED SOYBEAN MEAL-BASED DIET, SUPPLEMENTED WITH PHYTASE.

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A PROJECT REPORT SUBMITTED IN PATIAL FULFILMENT OF THE AWARD OF MASTER OF TECHNOLOGY (M. TECH.) IN FISHERIES

TO

THE DEPARTMENT OF FISHERIES AND WILDLIFE,
SCHOOL OF AGRICULTURE AND AGRICULTURAL TECHNOLOGY,
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AKURE, ONDO STATE, NIGERIA.

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CERTIFICATION

I certify that this study was carried out by ADEYO ADESINA O. PIUS, a Master of Technology (M.Tech.) student of the Department of Fisheries and Wildlife, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure , Ondo State.

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DEDICATION

This project is dedicated to Almighty God who spared my life and gave me strength and ability to complete this project.

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Abstract

A 77-day feeding trial was conducted to evaluate the growth performance of African catfish (*Clarias gariepinus*) fingerlings (7.0±1.09) fed variously processed Soybean meal-based diets, supplemented with 8000 units phytase per kg diet. Six experimental diets were formulated using raw soybean meal without phytase supplement as control (diet1), raw soybean meal with phytase supplement as the reference diet(diet 2), oven dried soybean meal plus phytase supplement(diet 3), cooked soybean plus phytase(diet 4), toasted soybean plus phytase(diet 5) and soaked soybean plus phytase(diet6) Evaluation of diets was carried out on the basis of fish growth response, protein utilization, feed conversion and protein digestibility. Fish treated with diets 5 and 4 differed significantly ($P<0.05$) from fish fed other diets in their mean weight gain, feed conversion ratio, protein efficiency ratio, digestibility, protein productive value and specific growth rate (SGR). Fish fed diet 1 (control diet) and 2 (reference diet) showed no significant differences ($P>0.05$) in mean weight gain, specific growth rate and protein efficiency ratio, but significant differences ($P<0.05$) in feed intake, feed conversion ratio, protein utilization and digestibility. Fish fed diets 2 and 6 exhibited no significant differences ($P>0.05$) in feed conversion ratio, protein productive value and protein digestibility of the fish, but differed significantly ($P<0.05$) in mean weight gain, feed intake and protein

efficiency ratio .In conclusion, the diet which contained toasted soybean meal supplemented with 8000 units per kg phytase is most economically and environmentally suitable for raising *Clarias gariepnus* fingerlings.

1.0 INTRODUCTION

Feed is the most expensive input in intensive fish productions. Determination of effects of supplementary feeding is very crucial to the economic production of *Clarias gariepinus* because feed represents 65% of the total cost of fish production to fish farmers in Nigeria (Helfrich 2002).

Adequate supply of suitable feedstuffs at economic prices is a major constraint to aquaculture production in Nigeria. This is because fish meal, an essential component of fish feed, is scarce and expensive, because there is increasing competition with other livestock industries for the available supply (Felix and Selvaraj 2004). High cost of complete feeds reduces profit margin and discourages aquaculture investment in Nigeria. Hence for aquaculture to expand with benefit, alternative feedstuff to fishmeal has to be harnessed and utilized in fish feed formulation.

Plant protein feedstuffs such as cotton seed, soybean, groundnut and sunflower cakes have bean incorporated into fish diets to replace fish meal component (Balogun and Ologhobo 1989; Boomyaratpalin 1998; Nyirenda 2000) in order to reduce feed cost, and has yielded good result.

Aquaculture is fast growing industry. Successful and sustainable aquaculture depends on economically viable and environmental friendly feed. The major feed ingredient, fishmeal, is expensive and there is increasing competition with other livestock industries for the available supply. Hence, the need to focus on alternative to fishmeal. One alternative

is to substitute fishmeal with soybean. Various researchers have evaluated soybean meal as substitute for fishmeal (Olivia-Teles et al 1994)

Soybean meal is the most widely used primary plant protein source in many fish diets (Lovell, 1988) because it contains good amino acid profile, similar to that of fishmeal.

Processing of soybean meal contributes greatly to its effectiveness in diets, which invariably improves the growth performance of the fish. Heat treatments are often applied to destroy the anti-nutritional factors present in soybean meal and to make it more digestible (Eyo 1999).

Phytase provide additional tools that can destroy anti-nutritional factors and enhance the nutritional value of plant-based protein feeds. They provide a natural way to transform complex feed components into absorbable nutrients. The addition of phytase in feed can improve nutrient utilization reducing feed cost and the excretion of nutrients into the environment.

Phytate found in legumes is bound with phosphorus and also with calcium and magnesium, trace elements like iron and Zinc, Protein and amino acids. (Felix and Selvaraj 2004). Fishes do not possess their own enzymes to break down the phytate and release the nutrients so they pass through the fish undigested. This is why higher proportion of valuable nutrients from vegetable sources is not utilized by the fish and is wasted as excreta. The enzyme phytase not only releases phosphorus from the phytate

but also release minerals and amino acids that are also bound, paving the way for maximum utilization of nutrients.

Benefits of phytase

Since the phosphorus bound in phytate becomes available as nutrient due to the addition of phytase, the inclusion of inorganic phosphorus in fish feed can be drastically reduced.

Phytase added to the diets improves protein and amino acid digestion in fishes (Felix and Selvaraj 2004). It can improve the metabolisable energy of feed by breaking down the phytate-lipid complex (Felix and Selvaraj 2004).

In addition, phytase reduces the release of nutrients into the environment by making the bound phosphorus available to the fish for growth.

1.2 Justification for the study.

The importance of phosphorus in the growth and development of fishes for a range of functions such as metabolic regulation, macromolecular structure and energy generation cannot be over emphasized. Due to the lack of phytase in fish digestive tract, they cannot utilize dietary phytate that is the major storage form of phosphorous in plant seed (Carla and Elizabeth 2001). The high phytate content in fish feaces results in elevated phosphorous level in water and accompanying environmental concerns. The use of phytase to degrade dietary phytate has potential for reducing the negative environmental impact of fish production. Phytase in the diet also increases the phosphorus available for the growth and development of the fishes (Foster et al 1999).

Soybeans are available in many parts of the world at a lower cost than animal proteins. Among plant proteins, soybeans have the best amino acid profile for meeting the essential amino acid requirement of aquatic species (Lovell 1989). Soybean contains anti-nutritional factors and other toxic substances that adversely affect the growth and well-being of animals. The activity of these anti-nutritional factors can be reduced/removed by appropriate processing and or by addition of phytase.

Therefore, this research proposes to compare the variously processed soybean meal supplemented with phytase in practical diets on the production of African catfish fingerlings.

1.3 Objectives of the Study

The objectives of the research are to determine:

1. the levels of anti-nutrients in the processed soybean meal.
2. the growth response of *Clarias gariepinus* fingerlings fed diets of processed soybean meal supplemented with phytase.
3. the nutrient utilization by *C. gariepinus* fingerlings fed soybean-based diets supplemented with phytase.
4. the digestibility of nutrients by *C. gariepinus* fingerlings fed the experimental diets.
5. the effect of the phytase on the mineral composition of the fish.
6. the effect of phytase on carcass composition of the fingerlings fed experimental diets.

CHAPTER TWO

2.0 Literature Review

2.1 Feeding Habit of Clarias gariepinus

Clarias gariepinus is Omnivores with the propensity for being Carnivorous. (Bardarch et al, 1973) stated that, the food of *Clarias gariepinus* consist of only animal, vegetable matter, fish, insects, debris and rotten vegetable. This is applicable to the fish in the wild. *Clarias gariepinus* under on intensive culture (enclosure) receive compounded ration. Reed et al (1967) has earlier mentioned that *Clarias gariepinus* feed on all food material found in the pond including rotten vegetable and plantain. They also feed on artificial food comprising many domestic and agricultural wastes such as Guinea corn, rice bran and Wheat bran.

2.2 The Importance of Claridae in Aquaculture

Catfishes of genus *Clarias* are the second most important group of farmed catfish in the world (FAO, 1993) In 1991, the world production of *Clarias* catfish amounted to 67,000 MT and increased since 1985 with about 7% per year (FAO 1993) recent estimates suggest even a total production of 125, 000 MT.

This farm output refers principally to *Clarias gariepinus* in Hong Kong. C. macrocephalus in the Philippines and *C. gariepinus* in Africa (mainly in south Africa and Nigeria, Middle East in the North to the Orange

river in the south (Tegel,1984) during the last decade, *Clarias gariepinus* has also been introduced into Europe, Asia and America (Brazil).

2.3 Artificial diets

Prepared or artificial feed may be either complete or supplemental. Complete diets supply all the nutrients (protein carbohydrate fats, vitamins and minerals) necessary for the optimal growth and health of the fish (Helfrich,2002). most fish formers use complete diets containing the required protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (<8.5%) phosphorus (<1.5%) water (<1%) and trace amount of vitamin and mineral (Helfrich, 2002)

2.4 Fish Meal

About one third (1/3) of the world's production of 70 million tones of fish is not used for direct human consumption and may be considered as biomaterial for fishery by-products. (Barlow and Windsor, 1984).

The most important by-products are fishmeal production, more than 90% of which are produced from oily species such as Anchovy, Capeline and Menhaden while less than 10% is being produced from white fish offal such as Cod and Haddock. Fishmeal is the predominant product as 95% of

all raw materials not used for direct human consumption is processed into fishmeal because it is stable.

2.5 Feedstuff Processing

Feedstuff is heat treated in order to destroy the anti –nutritional factors present in them and to make them more digestible (Eyo 1999). Anti-nutritional factors are substances which affect the nutritional value of feedstuff or the health status of a fish. They may be inherent in the feedstuff from other source e.g. toxins (mycotoxin) produce by mould which grow on damp feed during storage.

While the ones inherent in feedstuff are tripsin inhibitors e.g. gossypol (in cotton seed) phytate (in soybean) some of them are destroyed during processing.

Thiaminase, an enzyme that destroys thiamine (Vitamin B1) one of the essential water soluble vitamins, is mostly found in fresh water fish and is destroyed by heat (i.e.) cooking) (Juli – Anne et al 2003).

2.6 Standard Diet

Yanong, (1999) opined one simple formulation, which is used traditionally to feed ornamental fish in ponds, this consists of a mixture of 30% ground and processed wheat, and 50% of fish meal, 2-3% of fish oil, and a 0.3% vitamin and a 1% mineral premix are added to the mixture. This

mixture is blended with water and can be formed into dough balls of different sizes.

2.6.1 Binding Agent

Binding agent is purposely in fish diet to provide stability to the pellets and reduce leaching of nutrient into the water. Starch, cellulose and various other polysaccharides, such as extracts or derivatives from animals (gelatin), plants (gum Arabic) locus bean and sea weeds (agar) are also popular binding agent.

2.6.2 Mineral Requirement Of Catfish.

Minerals are inorganic elements necessary in the diet for normal body function. They can be divided into two groups (macro–minerals and micro–minerals) based on the quantity required in the diet and the amount present in fish. Common macro–mineral are sodium chloride, potassium and phosphorus. These minerals regulate osmotic balance and aid in bone formation.

The skin of fish contains much of the total calcium about 40% (Andrew et al 1973).

Channel catfish can absorb calcium from the water to meet their requirement (Robinson et-al 1986). However calcium deficiency has been induced when channel catfish were reared in calcium free water (Robinson

et al 1986). Lovell (1984) reported that mineral deficiencies in practical catfish culture have not been identified as a problem but when diets containing low quantity of animal by product with no mineral supplement are fed intensively to catfish mineral deficiency could possible occur. The phosphorus required for catfish was reported to be approximately 0.45% of the diets (Lovell 1978) phosphorus deficiency signs in catfish include poor weight gain feed conversion and bone mineralization (Lovell 1978).

Selenium is normally supplemented in the Vitamin premix at a level to provide 0.1mg/kg of feed.

2.6.3 Protein Requirement Of Catfish.

Protein is the most expensive part of fish feed therefore it is important to accurately determine the protein requirement for each species fish cultured. Protein is formed by linkage of individual amino acids.

Protein levels in aquaculture feed generally average 18-20%for marine shrimp, 28-32% for tilapia, 32-38 %for catfish and 38-42% for hybrid striped bass (N R C 1993). According to (N R C 1993), fishmeal, soybean meal, fish hydrosylate, skin mill powder, legumes, and wheat gluten are excellent source of protein. Machiels and Henken, (1985) reported that the best growth rate and protein gain in *Clarias gariepinus* were found in fish fed a high C. P diet (40%) rather than a low C P diet 25,

30, 35%. It was also observed that the plant protein source (soybean meal) and animal protein source (fish meal) are very essential in the diet for *Clarias gariepinus*.

2.6.4 Lipid Requirement Of Catfish.

Lipid (fats) are high energy nutrients that can be utilized to partially spare protein in aquaculture feed, lipids supply about twice the energy as protein and carbohydrates. Lipids typically comprise about 15% of fish diets (Helfrich et al 2002). According to Helfrich et al (2002), a recent trend in fish feeds is to use higher level of lipid in the diets. Although increasing dietary lipids can help reduce the high costs of diets by partially sparing protein in the feed, problem such as excessive fat deposition in the liver can decrease the health and market quality of fish. It was reported that Catfish fed diets containing animal fat or fish oil showed better growth than fish fed diets containing vegetable oil (Stickney and Andrew, 1972).Catfish are able to synthesize most of their required fatty acid, thus, there is no optimum level of lipid to feed them (Stikney 1979), reported that Catfish fed diet of 10% lipids performed very well but no significant benefit can be attributed to that level of lipid, he then recommended less than 5% lipid in diets for catfish

2.6.5 Energy Requirement Of Catfish.

One of the remarkable differences between fish and farm animal is that the amount of energy required for metabolism is much less for fish than for warm-blooded animals (Lovell, 1979).Cowey, (1979), stated that fish

requires lower energy/protein ratio in their diets, which they are physiologically equipped to utilize and excrete.

Lovell (1989), reported that for maximum growth rate, 1-3g. Channel catfish (*Ictalurus punctatus*) fingerling fed ad-libitum required 16.8kcal of digestible energy (D.E)/100g weight daily per gram of diet, the juvenile fish in the weight range of 10-20g requires 11.4 kcal/100g weight; while larger fish in the weight range of 100 – 250 g requires 9kcal/100g weight.

According to Wilson (1989) energy requirement for maintenance and maximum growth of fish were 62.8 and 72.4 kj/kg body weight per day respectively.

2.6.6 Mineral Supplements

Livestock needs a regular intake of certain amount of minerals for good health and maximum production. If there food does not contain enough minerals they should be given mineral as supplement (Komolafe et al, 1980).

Although some minerals are found in the body skeleton, soft tissue is another area of the body where mineral is very important for digestion, absorption and in the transformation of food to release energy, as well as excretion (Komolafe et al (1980).

Some elements (such as Sodium, Potassium, Calcium, Phosphorus, Magnesium, Chlorine, Iron and Sulphur) are known as major minerals

because they are required in large quantities than the minor or trace minerals (which include iodine, cobalt, copper, zinc, manganese) by the fish (Helfrick 2002).

Calcium and phosphorus together form over 90% of the total mineral matter of the animal body. A deficiency of either or both elements can lead to a characteristics malformation of bones of young animals (Komolafe et al 1980).

2.6.7 Vitamin Supplements

Vitamins are organic compounds necessary in the diets for normal fish growth and health. They are often not synthesized by fish, and must be supplied in the diet.

According to NRC (1993), the minimum vitamin A requirement to prevent deficiency symptoms in Catfish ranges between 1000 – 2000 I.U.

Lovell (1978) reported that 50% of the supplementary Ascorbic acid is destroyed during manufacturing process, due to its vulnerability therefore, excess Ascorbic acid is routinely added to commercial formulation to ensure that an adequate level of the vitamin is retained in the final product.

National research Council, (1993) recommended 500-1000 I.U 30mg/kg, 60 mg/kg, 1.0 mg/kg, 3.0 mg/kg, 10-20 mg/kg and 14 mg/kg as a

minimum level of vitamins D, E, C. Thiamin, riboflavin, pyridoxine, panthrothemic acid and nicotinic acid respectively.

According to Robinson (1990) the vitamin deficiency symptoms of vitamin D is reduced bone ash, Vitamin E are mortality, fatty liver, oxidative diathesis, de-pigmatation and anaemia, Vitamin C are Lordosis, scoliosis (bent bone disease) impaired collagen formation, increased susceptibility to disease, thiamine are dark colouration and mortality, pyridoxine are lethany, , nervous disorder mortality and green-blue colouration pantothenic acid are clubbed gill, eroded lower jaw, fin and barbells anaemia and nicotinic acid are exophthalmia lesion and haemorrhage on skin and fins.

2.70 Feed Conversion and Efficiency Calculations

Feed conversion ratio (FCR) or feed efficiency (FE) is important calculation for growth because feed is expensive. They are necessary to avoid feed wastage.

According to Holihan et al (2001), (FCR) feed conversion ratio is calculated as weight of the feed fed to the fish divided by the weight of fish growth (FE) feed efficiency is simply the reciprocal of FCR, (1/FCR).

Fish are not completely efficient (Helfrich et al 2002), i.e. when fed 50kg of feed, could not exhibit 50g of growth because they must use some

of the energy in feed for metabolic heat, digestive processing, respiration, name impulses, salt balance, swimming and other living activities. Food conversion ratios will vary among species, sizes and activity levels of fish, environmental parameters and the culture system used.

2.7.1 Evaluation of Proteins

Quantity of protein obtained from a diet depends on the amount of the food and on the protein content of food. Food at low protein content may provide useful amount at protein if consumed in sufficient quantity.

In order to test changes in amino acid availability, the use of biological test using the animal is necessary (Church and Pond, 1988).

2.7.2 Protein and Amino acid requirement

Protein makes up approximately 70% of the dry weight of catfish muscle (Robinson 1989). Optimum lysine requirement for *Clarias gariepinus* fingerlings and juvenile are 1.91% (5.25%) of protein) and 1.06%(3.12% of protein) respectively (Balogun, 1992).

About 40 quality nutrients have been identified as necessary for the normal metabolic function of catfish with the quality requirement value available for about 30 nutrients including amino acids, fatty acid, mineral and vitamin C (Wilson and Morean, 1996). Degani et.al (1989)

recommended dietary crude protein of 40% for the rearing of *Clarias gariepinus*

Generally the nutrient requirement of fish change as fish grow and mature. Plant protein material commonly used in fish feeds is soybean meal groundnut cake and cotton seed cake (Eyo 1999).

2.8 Proximate Composition

The terms that one needs to understand to formulate fish diets are: Crude Protein level, energy level, either expressed as metabolizable energy (M.E) or as digestible energy (DE); Specific amino acid level; crude fibre level and ash level.

The fish spawning season affects the proximate composition of the fishmeals. Similarly, many plant feedstuffs vary in proximate composition with their stage of maturity at harvest, location grown, and other environment condition, such as weather (Hardy 2003).

2.9 Water Parameters in Catfish Culture

Many fish feeding experiments have been carried out in a confinement to prevent interaction of environmental effects such as natural food organism, temperature and water quality, with the nutrient variable being studied. Studies are conducted in tanks placed indoor to allow accurate collection and analysis of data in the hatcheries. Anyanwu (1988),

recommended that on daily basis, dissolved oxygen, ammonium nitrate, carbon dioxide, pH and suspended solids should be checked.

(i) pH

Acid and alkaline death points are 4 and 11 respectively Anyanwu (1988). pH of 6.5 – 9.0 was reported by Akinyemi (1988) and Boyd, (1986) respectively as optimum for fish growth, while Viveen et al (1985) reported 6.5 – 8.0 as the optimum pH range for fish growth.

(ii) Dissolved Oxygen

Oxygen is one of the most critical factors for survival of fish and for a successful fish production. Oxygen is a gas present in air but fish breathes oxygen in water through its gills as dissolved oxygen, i.e. oxygen in-solution. Fishes use the oxygen to oxidize food to produce energy.

Oxygen demand of fish increases as the food intake increases. Viveen et al (1985) reported that at eggs, larvae to fry, fingerlings and adult stages, oxygen concentration closed to saturation is desirable for *Clarias gariepinus* but catfish with full grown arborescent organ can survive low dissolved oxygen content as low as 0.33 ppm (Viveen et al. 1985). Dissolved oxygen of 3-8mg/l and 5mg/l were reported by Anyanwu, (1988) and Boyd, (1979) respectively.

(iii) Temperature

Temperature is the most important environmental factors affecting the equilibrium and rates of chemical reactions, growth rates, nutritional requirements, and the enzymatic and chemical composition of cells.

Temperature has been found to affect the growth of *Clarias gariepinus* (Veveen et al, 1985).

Temperature is by far the most pervasive of all environmental stresses disturbing the maintenance of homeostasis in a living system. Poikilotherms e.g. fish cannot adapt their internal temperature to the environmental temperature. A number of fishes are eurythermal, capable of being tolerant to wide fluctuation in ambient temperature. Temperature has an over ruling effect in all metabolic rates including development (Boyd, 1986).

Viveen et al (1985) reported 25-27°C as optimum for catfishes.

2.10 Anti-Nutritional factors

The anti-nutritional factors (ANFs) may be defined as those substances generated in natural feed stuffs by the normal metabolism of species and by different mechanism (e.g. Inactivation of some nutrients, diminutions of the digestive process or metabolic utilization of feed which exert effects contrary to optimum nutrition.

2.11 Enzymes Additives

Enzymes are protein molecules that catalyze specific chemical reactions. Enzymes are specific in their function. Digestive enzymes are essential to animals because complex feed are not readily absorbed by the digestive tract unless degraded to more simple molecules.

Several digestive enzymes have been studied for use as additives to enhance animal performance with success in monograstrics including poultry and swine diets (Limin 2004).

Morgavi et al (2000) reported that spraying fibrolytic enzymes on to feed prior to in vitro fermentation improves digestion. Spraying enzymes onto feed offer exciting possibilities for using enzymes to improve nutrient digestion, utilization, and productivity and at the same time reduce feacal material and pollution (Limin, 2004). Limin (2004) also reported that a growing body of evidence exists that supports improvement in animal productivity when feeds are treated with enzymes prior feeding.

Phytase added to the diets improves protein and amino acid digestion in fishes (Felix et al 2004). Felix et al 2004, also reported that phytase can improve the metabolic energy of feed by breaking down the phytate and lipid complex.

Table:1 Major enzyme complexes their substrates and primary end products.

Enzymes complex	Substrates	Simplers End products
Proteases/Deaminases	Proteins, Peptids	Amino acids
Amylase	Starch	Glucose
Cellulase	Cellulose	Glucose
Hemi Cellulase (xylanase)	Hemicellulose	Xylose

2.12 Apparent digestibility of ingredients

Fagbenro (1998) working on the apparent digestibility of crude protein and gross energy in some plant and animal based feed stuffs by *Clarias isheriensis* observed that shrimp/fish meal dry matter digestibility was high, because of its attribute as a feed attractant.

According to Fagbenro and Jáuncey (1994) *Clarias gariepinus* and *Oreochromis niloticus* set fed 50% fishmeal diets gave the best digestibility.

2.13 Growth Parameter/Feeding frequency

Farmers can calculate optimum feeding frequency based on the average size in length or weight and the number of fish in the tank, raceway or pond (Robinson et al 1998).Farmed fish typically are fed 1 – 4% of their body weight per day (Lovell T. 1989). Fish growth rate is in a straight line related to ration level. Huisman, (1987) opined that smaller fish ate more food and more frequency in relation to their size than larger fish.Eyo, A.A.

1999, in his own report opined that fish should be fed at 3-5% of their body weight to minimize wastage.

2.14 Managing Fish wastes

The most important rule in fish nutrition is to avoid overfeeding. Overfeeding is a waste of expensive feed. It also results in water pollution, low dissolved oxygen levels, increased biological oxygen demand, and increased bacterial loads. (Nwanna 2004). Usually fish should be fed only the amount of feed that they can consume quickly (less than 25 minutes) (Helfrich et al 2002).

Even with careful management, some feed ends up as waste. (Helfrich et al 2002), for example, out of 100 units of feed fed to fish, typically about 10 units of solid and 30 units of liquid waste (50% total waste) are produced in fish. Of the remaining feed about 25% is used for growth and another 25% is used for metabolism (heat energy for life processing). These numbers may vary greatly with species, sizes, activity, water temperature, and other environmental condition.

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials: Soybeans (Glycine max) used in this study was obtained from a commercial feed store in Akure, Ondo State, Nigeria. The beans were screened and distributed into six batches. Two batches were left raw; while the remaining four batches were either oven dried, cooked, toasted or soaked. The other ingredients that were combined with soybean meal to formulate the diets include, yellow maize, fishmeal, groundnut oil, vitamin and mineral premix and starch, they were all purchased from the same source. Phytase was used as enzyme supplements.

3.2 EXPERIMENTAL PROCEDURE:

3.2.1 Preparation of soybean meals:

Raw: 500g of raw soybean meal was prepared by grinding the beans in a milling machine, put in a polythene bag and stored at room temperature as raw soybean meal (RSB).

Oven-drying: 500g of milled soybean was mixed with 0.5 litres of water and made into a paste and oven dried at 120^0C for 4 hours, it was ground to form oven-dried Soybean meal (OSB).

Cooking: 500g of soybeans were cooked with 3 litres of boiling water in an aluminum pot for 30 minutes. The water was drained off and the cooked beans was sun-dried at ambient temperature and ground to form meal (CSB).

Toasting: 500g of soybeans was put in an aluminium plate and toasted in an oven LCON53Cf at 120^0C for 4 hours. The toasted beans were ground to yield the toasted soybean meal (TSB).

Soaking: 500g of soybeans were soaked inside 3 litres of water at room temperature for 24 hours. After the soaking, the soybeans were sun-dried at ambient temperature and ground to form soaked soybean meal (SSB).

3.2.2 Analysis of anti-nutrients of the raw and pretreated soybean meal.

3.2.2(i) Phytate/Phytin phosphorous:

Eight grams each of the finely ground raw and processed samples were soaked in 200ml of 20% hydrochloric acid and allowed standing for three hours. The extract was thereafter filtered through two layers of hardened filter paper. Fifty milliliters of the filtrate was pipetted in duplicate into 400 ml capacity beakers before the addition of 10ml 0.3% ammonium thiocyanate solution as an indicator, and 107ml of distilled water to obtain the proper acidity (pH 4.5). The solution was then titrated with a standard

iron chloride ($FeCl_3$) solution containing 0.00195 gm Fe/ml until a brownish yellow colour persists for 5 min.

Phytin-phosphorus was determined and phytin content was calculated by multiplying the value of phytin-phosphorus by 3.55 (Young and Greaves, 1940). Each milligram of iron is equivalent to 1.19 mg of phytin-phosphorus.

Phytate = X x 564.11 mg/100g

Where X = titre value (ml).

3.2.2.(ii) Tannin:

200mg of finely ground raw and processed samples were weighed into a 20ml capacity beaker. 10ml of 70% acetone was added to the beaker, the beaker was placed in an ice water bath coupled with a shaker for 12 mins. The sample was allowed to cool for 3 mins. The content or sample (4^0C) was centrifuged for 20 mins at about 3,000xg. The supernatant was collected into a glass tube and kept on ice.

Total tannin was analyzed by using suitable aliquot by taking 0.2 in test tubes, these values were made to 1.0ml with distilled water, than 0.5ml of folin reagent (freshly prepared) and 2.5ml of 20% sodium carbonate were added to each sample, left for 40 mins and then

some solution were transferred into 10mm part length glass cuvette and the absorbance was read in "spectrophotometer" at 725nm. 1 ml of distilled water was also pipetted into another test tube, 0.5ml folin reagent (freshly prepared) and 2.5ml of 20% sodium carbonate were added. It was left for 40 mins to serve as blank.

From the prepared standard, 0.6, 0.02, 0.03, 0.04, 0.05 and 0.06ml tannic acid solution was pipetted into test tubes and made up to 1 ml with distilled water. 0.5 ml of folin reagent and 2.5 ml of 20% sodium carbonate were added subsequently into each test tube containing the standard solution. They were left for 40 mins in a water bath coupled with shaker. They were then transferred subsequently into 10mm part length glass curvette and the absorbance were read at 725nm using "spectrophotometer 20". The amount of tannic acid was calculated from standard curve.

Finally total tannin content was expressed on a dry matter basses (X%).

3.2.2(iii) Lignin:

200 mg of ground sample was weighed accurately into 100ml centrifuge tube, 2ml of 75% w/w H_2So_4 was added and the sample was stirred and dispersed thoroughly with a glass rod. The tube and the content

were then incubated in a water bath at 30^{0}C for one hour after which 50ml of distilled water was added. This was autoclaved at 121^{0}C, 15psi for one hour. The sample was removed from autoclave and the lignin filtered off with a glass fibre filters. The content was rinsed carefully into a crucible and then dried at 105^{0}C in an oven overnight, cooled and weighed to constant weight.

The lignin content was calculated as:

$$\% \text{ lignin} = \frac{Wa}{Wb} \times 100$$

Wa = Weight of lignin

Wb = Weight of sample.

3.2.3 Diet Preparation

Six isoproteic (40%CP) diets were formulated using each of the differently prepared soybean meals mixing with other ingredients(Table 2). The control diet (Diet 1) contained raw soybean meal (RSb_1) without phytase supplement. The reference diet (Diet 2) contained raw soybean meal (RSb_2) with 8000 units of phytase (Ronozyme P) kg per diet.

The remaining diets contained either oven dried, cooked, toasted or soaked soybean meal supplemented with 8000 units of phytase

(Ronozyme P) per kg diet. All other ingredients including vitamin and mineral premix, fishmeal, maize meal, groundnut oil were added and mixed together before corn starch were added. The ingredients were mixed thoroughly. The dough formed was made into pellets using the Hobart A – 200T pelleting machine to obtain a homogeneous mass and passed through a mincer to produce 0.8mm pellets, which was immediately oven dried (40-45^{0}C). The diets were broken up into fine particles and stored at room temperature before use.

Table 2: Gross composition of experimental diets (g/kg DM).

INGREDIENTS	DIETS					
	1	2	3	4	5	6
Fishmeal	36.46	36.46	36.46	36.46	36.46	36.46
Raw SB	31.33	31.33	-	-	-	-
Oven dried OSB	-	-	31.33	-	-	-
Cooked SB	-	-	-	31.33	-	-
Toasted SB	-	-	-	-	31.33	-
Soaked SB	-	-	-	-	-	31.33
Yellow Maize	25.13	25.13	25.13	25.13	25.13	25.13
Groundnut Oil	4.28	4.28	4.28	4.28	4.28	4.28
Vit/Min Premix*	1.75	1.75	1.75	1.75	1.75	1.75
Corn Starch (binder)	1.05	1.05	1.05	1.05	1.05	1.05
Phytase (8000 units/kg diet)	-	+	+	+	+	+

*Composition according to formulation provided by Hoffman La Roche Balse Switzerland. (Vitamin A, B, D, E, K, Riboflavin, Folic acid, Selenium, Phosphorus, Nicotinic acid, Calcium, Iodine, Copper, Manganese, Zinc, Iron, Anti oxidant, Anti-caking agent and Terramycin.

SB = Soybean meal.

3.2.4 Procurement of Experimental Fish

Clarias gariepinus fingerlings (7-8g) were procured from Ondo State Agricultural Development Project, Akure, located at G.R.A Algbaka Akure.

3.2.5 Acclimation of experimental Fishes .

The fish were acclimated to experimental conditions for 2 weeks in glass tanks (75x30x30) cm^3 containing 22.5 litres of water at the rate of 10 fingerlings per tank, during which they were fed with commercial diet of 40% CP.

3.2.6 Fish stocking and feeding

Ten fingerlings were stocked into each tank with three replicates per treatment. Water was supplied to the tank from a borehole after aeration for 24 hours. A blower and airstones provided continuous aeration. The fish were fed at 5% body weight/day in two equal portions at 8.00 – 9.00 and 15.00 – 17.00 hours for 77 days. The left over feed was siphoned out two hours after every feeding to prevent fouling of tank water.

3.27 Water Quality Monitoring

The water in the tanks was replaced at two days interval with freshly oxygenated water . The dissolved Oxygen (DO_2), Water temperature and

pH in all tanks were measured weekly between 8.00 – 9.00 hours using oxygen meter (Model 9071,Jenway) and pH meter(Model 320, Mettlar Toledo) respectively.

3.2.8 Fish Weight Measurement

Weight measurement of the fish in each experimental tank was done weekly using an electronic weighing balance (Mettlar Toledo). Feed adjustment was based on the respective weekly weights measured. The values of the fish weekly weight changes were used to assess the growth performance of the fish as described by Olvera – Novoa et al (1990) as follows:

- The weight gain was being determined at the end of the experiment by the weight difference. (Final weight – initial weight).
- Specific growth rate: - Specific growth rate is represented as:

$$\frac{\text{Log}_e \text{ final weight} - \text{log}_e \text{ initial weight}}{\text{Culture period (days)}} \times 100$$

- Protein efficiency ratio:

This is represented as :

$$\text{PER} = \frac{\text{Weight gain}}{\text{Protein consumed.}}$$

Feed conversion ratio: = Feed fed

Weight gain.

3.2.9 Digestibility Measurement

Protein digestibility Coefficient

Feacal samples were collected seven hours after each feeding and oven-dried. Samples from the same tank were pooled together, packed in cellophane bags and stored in a freezer.

Acid insoluble ash (AIA) analyses were carried out on the diets and feaces according to Halver et al (1993) methods.

The percentage AIA was determined as:

% AIA = weight of ash – weight of AIA x 100

weight of ash .

Therefore, the apparent digestibility coefficient was determined thus:

ADC= 100-100[%AIA in feed x % nutrient in feaces]

[%AIA in feaces % nutrient in feed]

3.3.0 MOISTURE CONTENT:

This was determined after oven-drying a known weights of the fish and diet samples separately at 105°C for three hours. Samples were cooled

in dessicator before weighing and the moisture calculated by difference in weight of samples.

3.3.1 Crude Protein:

The crude protein was determined using the micro-kjeldahl distillation procedures as described by AOAC. (1990) using Gallenkamp equipment. Thes percentage crude protein was calculated by multiplying the total nitrogen by a factor of 6.25.

$$\text{Total Nitrogen (N)} = \frac{\text{Vol. of acid x molarity x 0.014 x diluting factor}}{\text{Weight of sample used}}$$

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

3.3.2 Lipid:

The fat content was determined by subjecting the sample to a continuous extraction with petroleum ether using Gallenkamp soxhlet equipment as described by AOAC (1990). The ether residue was the residue obtained from evaporation of the solvent.

3.3.3 Crude Fibre:

Crude fibre was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH.

$$\% \text{ Crude fibre} = \frac{\text{Wt of Residues} - \text{Wt of Ash}}{\text{Wt of sample}} \times 100$$

3.3.4 Ash Content:

This was determined by subjecting the already oven dried samples with known weight to ignition in a muffle furnace (Gallenkamp) at 550^{0}C for 5 hours after which samples were cooled in a dessicator and weighed.

$$\% \text{ Ash} = \frac{\text{Wt of Ash}}{\text{Wt of sample}} \times 100$$

3.3.5 Nitrogen Free Extract (NFE):

This is a measure of carbohydrate available in the diet and fish respectively. It was determined by subtraction method.

% NFE = 100 - (% crude protein + % crude lipid + % crude fibre + % Ash + water).

3.3.6 Determination of Minerals.

The mineral analysis was carried out using an Atomic Absorption / emission spectrophotometer Buck scientific model 200-A. About 2g of sample was ashed (dry ashing) at 550°C for 6hours in an electric muffle furnace, the

ashed sample was digested in 5ml of 10% Hcl. This was filtered and made to the mark in 50ml volumetric flask using distilled water, the solution was measured using AAS. The formula below was used to determine the value of the minerals present in the flesh and feacal matter of the experimental fish in part per million (ppm)

$$\frac{Rx\ V}{W} = ppm$$

Where,

R=Value of each mineral

W=Weight of sample

V = Volume of digested sample used.

But R was calculated using the formula: $R = \frac{10ppm \times \text{sample read.}}{\text{Standard.}}$

3.3.7 Determination of Phosphorus

About 2g of sample was ashed (dry ashing) at 550°C for 6 hours in an electric Muffle furnace, the ashed sample was digested in 5ml of 10% Hcl. This was filtered and made to the mark in 50ml volumetric flask; the filtrate was used in phosphorus analysis using Vanado-molybdate reagent and standard solution.

10ml of the vanadium molybdate was pipetted into a 50ml standard volumetric flask and 10ml of the sample was added , shaken and enough

distilled water was added till it get to meniscus. The solution was allowed to stand for about 15mins. Serial stands(0,2,4,6,8,10ppm) and blank(0ppm) solution were prepared using 100ppm stock .

The absorbance was read at 450nm of standard. The absorbencies of samples were read as in the standards.

Therefore, Phosphorus was calculated using the formula:

$$P\ (ppm) = A \times \frac{FV \times D}{Wt}$$

Where A= conc. of sample as obtained from graph.

FV = Final volume of sample digest.

Wt = weight of sample used.

D = dilution factor.

3.3.8 Statistical Analysis

One way analysis of variance (ANOVA) was used to find the treatment means of the data measured. The differences among the means were separated using Duncan's multiple range test (Duncan 1955).

CHAPTER FOUR

4.0 Results

4.1 Proximate Composition of the raw and processed soybean meals:

Table 3 shows the proximate composition of the raw and pretreated soybean meal. The value of crude protein in treatments 1and 2 were the same. While that of treatment 3 is slightly differed from 1 and 2, and significantly differs from 4 and 5 that were higher. Treatment 4 and 5 were closely related. Treatment 6 differed from all others. The values of ash and ether extract were closely related indicating no significant effect of processing method in all the treatment. The value of fibre in all the treatments was closely related. The NFE values of treatment1, 2,3 and 6 were closely related with those of treatments 4 and 5.

Table 3a: PROXIMATE COMPOSITION OF THE RAW AND PROCESSED SOYBEAN MEALS(%DM).

	Ether extract	Ash	Crude protein	Fibre	NFE
T1(RSB$^-$)	7.71 ±0.3	5.87 ±0.3	42.61±0.1	5.56 ±0.4	42.15 ±1.0
T2(RSB$^+$)	7.39±0.1	5.34 ±0.3	42.20 ±0.1	5.27 ±0.6	43.68 ±0.8
T3(OSB)	7.67±0.2	5.33 ±0.1	41.82 ±0.2	4.23 ±0.6	40.94 ±1.1
T4CSB)	7.73±0.2	5.81 ±0.1	47.06 ±0.2	5.23 ±0.1	38.53 ±1.0
T5(TSB)	7.55±0.3	5.03 ±0.4	46.94 ±0.3	4.12 ±0.3	36.36 ±0.7
T6(SSB)	7.56±0.5	5.10 ±0.2	46.42 ±0.5	4.92 ±0.4	40.34 ±0.8

Values are means ± SD from three replicates.

4.2 Anti-nutrients and the Phosphorous content of the raw and processed soybean meals.

Table 3b shows the anti-nutrients and the phosphorus content of the raw and variously processed soybean meals used in formulating the diets. The table showed no significant differences($p>0.05$) in the phytate, Tannin, Lignin and total Phosphorous content of both untreated and treated soybean meals. However, the phytate of the untreated soybean meal was highest, while the sample treated by cooking had the least value. Similarly, the sample treated by toasting had numerically lower phytate level than the sample treated by either oven drying or soaking. The soybean meal treated by cooking had the highest total Phosphorous followed by the sample treated by toasting.

Table 3b Ant-nutrients and phosphorous content of the raw and processed soybean meals

	Phytate mg/100g	Phytin phosphorous g/100g	Total phosphorous (mg/g)	Tannin mg/100g	Lignin %
T1	2021.3±187	4.70 ±0.03	4.87±0.12	1.50 ±0.02	0.20 ±0.00
T2	1927.3±183	4.46 ±0.06	4.72±0.6	1.20 ±0.01	0.25 ±0.01
T3	1937 ±65	4.17 ±0.01	4.69±0.11	2.30 ±0.02	0.05 ±0.00
T4	1654 ±65	3.57 ±0.12	4.95±0.5	1.75 ±0.00	ND
T5	1824 ±33	3.81 ±0.21	4.91±0.10	1.75 ±0.20	0.35 ±0.02
T6	1918 ±169	4.40 ±0.34	4.89±0.10	2.05 ±0.01	0.05 ±0.01

NOTE: Value obtained from the mean of three analyses on one preparation.

4.3 Water Quality Parameters:

Table 4 shows the result of water parameters monitored during the experiment. The temperature ranged between 23.33± 0.91 and 23.50± 0.27, while the dissolved oxygen (DO) ranged between 4.24± 0.21 and 4.57± 0.13 and the pH values ranged between 6.32± 0.50 and 6.45± 0.14.The table showed that values of the water parameters recorded were closely related indicating that the tanks were subjected to the same experimental condition.

Table 4: Mean water quality parameters recorded during the experimental period:

Tanks	Temperature °C	DO	pH
1	23.33±0.91	4.48±0.46	6.32±0.50
2	23.47±0.26	4.52±0.29	6.42±0.12
3	23.50±0.27	4.57±0.13	6.38±0.02
4	22.38±0.13	4.24±0.21	6.45±0.02
5	22.46±0.92	4.50±0.11	6.45±0.14
6	23.48±0.24	4.55±0.21	6.38±0.13

4.4 Proximate composition of the experimental diets:

Table 5 shows the proximate composition of the experimental diet, the values of the proximate composition of the crude protein (CP), either extract, ash, crude fibre, nitrogen free extract(NFE); and the gross energy were closely related, indicating no clear effect as a result of the different treatments of the SBM.

Table 5: PROXIMATE COMPOSITION (g/100g) OF EXPERIMENTAL DIETS.

Parameters	T1	T2	T3	T4	T5	T6
Crude protein	40.22 ±0.5	40.30 ±0.1	40.42 ±0.2	40.47 ±0.2	40.43 ±0.3	40.38 ±0.4
Ether extract	14.25 ±0.3	15.56 ±0.1	14.02 ±0.4	14.53 ±0.2	14.28 ±0.2	13.98 ±0.1
Ash	15.93 ±0.2	14.11 ±0.3	14.72 ±0.2	13.52 ±0.3	13.30 ±0.3	14.08 ±0.4
NFE	23.04 ±0.1	23.74 ±0.1	25.51 ±0.4	25.15 ±0.1	26.81 ±0.1	25.62 ±0.0
Crude fibre	6.56 ±0.1	6.29 ±0.1	5.33 ±0.2	6.33 ±0.1	5.18 ±0.0	5.94 ±0.0
Energy (kcal/100g)	435.51 ±2.2	435.9 ±3.1	435.48 ±2.3	435.65±3.4	435.5 ±3.5	435.05 ±3.1

Values are means ± SD from three replicates.

4.5 Growth Response:

The growth response of *C.gariepinus* fingerlings fed experimental diets is summarized in Table 6.

The growth response of fish fed with diet 5 (i.e. toasted soybean meal supplemented with phytase) and diet 4 (cooked soybean meal supplemented with phytase) were superior to the fish fed with other diets, buts showed no significant difference ($P>0.05$) between each other. However, fish fed with diet 5 and 4 have Mean weight gain 18.39± 0.99g and 18.18± 1.22g respectively. These were significantly different ($P<0.05$) from those fed with diet 1 (raw soybean meal without phytase supplement, which serve as control diet) and diet 2 (reference diet, which is raw soybean meal with phytase inclusion).

Fish fed with diets 1 and 2 were not significantly different ($P>0.05$) in their growth response. However their mean weight gain are 8.36± 1.04 and 9.55± 1.24 respectively. The fish fed with diets 1 and 6 also exhibited similar growth responses as 8.36± 1.04 and 7.03± 0.24 respectively.

Fish fed with diet 3 (oven dried soybean meal supplemented with phytase) with mean weight gain of 12.86± 0.15 showed significant difference ($P<0.05$) from fish fed with other diets.

There was significant difference ($P<0.05$) in the values recorded for specific growth rate (SGR) of fish within the treatments. However no

significant different (P>0.05) between fish fed with diets1 and 2, between diets 3 and 6, and between diets 4 and 5 in their specific growth rate values.

Table 6: **GROWTH RESPONSE, PROTEIN UTILIZATION AND FEED CONVERSION OF THE FINGERLINGS FED VARIOUSLY PROCESSED SOYBEAN MEAL-BASED DIETS, SUPPLEMENTED WITH PHYTASE FOR 77DAYS.**

Parameters	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet6**
Initial Weight(g)	7.15± 6.05	7.27 ±0.04	7.23±0.04	7.30 ±0.02	7.24±0.04	7.22 ± 0.02
Final weight(g)	15.5± 1.01	16.82± 1.21	20.09± 6.11	25.48± 1.23	25.63± 0.96	14.25± 0.26
Mean weight(g)	8.36 ± 1.04ad	9.55 ±1.24a	12.86±0.15c	18.18 ±1.22b	18..39±0.99b	7.03 ± 0.24d
Weight gain %	117.06± 15.2ad	131.5 ±17.7a	178.01 ± 2.87c	249.18 ±16.15b	254.17±14.92b	97.36 ±3.05d
SGR%/day	0.45± 0.23a	0.31 ±0.03a	0.96 ± 0.58c	1.03 ±0.03b	1.02± 0.03b	0.57±.07ac
Feed fed(g)	41.70± 2.70a	46.40 ±0.20e	50.33 ± 1.47c	62.11 ±2.63d	60.60 ±0.46b	41.30 ±0.60a
FCR	2.97±0.46a	2. 12±0.27bc	1.9 ±0.25cd	1.4 ±0.19e	1.5 ±0.16de	2.4 ±0.21b
PER	0.46±0.08bc	0.51±0.07b	0.63±0.01d	0.72±0.02a	0.75±0.05a	0.42±0.01c
PPV%	10.59±0.70c	13.16±0.06b	14.62±0.43d	15.94±0.70a	16.22±0.12a	13.07±0.19b
ADC protein	75.30d	81.47c	83.49b	85. 35a	85.69a	80.92c

Means within the same row followed by the same subscripts are not significantly different (P> 0.05)

4.6 Feed Conversion Ratio (FCR):

Table 6 shows the calculated values for feed conversion ratio for the diets. The result shows significant difference ($P<0.05$) in the FCR values with diets 4 and 5 being superior over other diets. Fish fed diet 4 (cooked soybean meal with enzyme supplement) has the best FRC of 1.4±0.19, followed by fish fed diet 5 (i.e. Toasted soybean meal with enzyme supplement) with FCR of 1.5±0.16.

Fish fed diets 1 and 2 also showed significant difference ($P<0.05$ in their FRC values. Those fed diet 1 (raw soybean meal without enzyme) has 2.97±0.46, while those of diet 2 (reference diet) (raw with enzyme supplement) showed better feed conversion ratio over diet 1 by having 2.12±0.27 FCR value. FCR values of fish fed diets 2 and 3 were not significantly different ($P>0.05$). Also fish fed diets 2 and 6 were not significantly different ($P>0.05$) in their FCR values, while fish fed diets 3 and 5 also exhibited no significant difference ($P>0.05$) in their FCR values.

4.7 Protein Utilization:

Protein Efficiency Ration (PER) result is showed in Table 6. Fish fed diets 4 and 5 were superior in their PER with 0.72±0.02 and 0.75±0.05 respectively. There was no significant difference (P>0.05) in PER between fish fed diets 1 and 6 with 0.46±0.08 and 0.42±0.01 respectively. Fish fed diets 3 and 2 differed significantly (P<0.05) from each other and from those fed other diets with 0.63±0.01 and 0.51±0.07, respectively.

4.8 Digestibility

Table 7 shows the digestibility result. The diets exhibited significant difference (P<0.05) in their values with diets 4 and 5 being superior with values 85.35% and 85.69% respectively.

Diets 2 and 6 showed no significant difference (P>0.05) with 81.47 and 80.92 %respectively. Diet 1 and 3 showed significant variation from each other and from the remaining diets with values 75.30% and 83.49% respectively.

Table 7: Digestibility Result (AIA)

	T1	T2	T3	T4	T5	T6
Nutrient(CP) in feed	40.22	40.30	40.42	40.47	40.43	40.38
Nutrient in feaces	14.08±1.2	12.55±0.6	11.81±0.01	9.77±0.70	10.04±1.18	11.71±0.99
%AIA in feed	0.67±0.02	0.56±0.00	0.52±0.01	0.51±0.04	0.49±0.01	0.81±0.02
% AIA in feaces	0.95±0.01	0.94±0.04	0.9±0.01	0.84±0.02	0.85±0.01	1.18±0.01
Digestibility	75.30d	81.47c	83.49b	85.35a	85.69a	80.92c

Values are means ± SD from three replicates.

4.9 Carcass Composition experimental fish.

The carcass composition (Table 8) showed close relationship between the values of crude protein, ether extract , ash , crude fibre and nitrogen free extract of the fish under the different treatments. This is also an indication that treatments of the SBM and or addition of phytase had no clear effect on the values of the carcass yield. However, the CP of the fish showed slight increase over the CP of the fish before the experiment, while the crude fibre of the fish before the experiment was slightly higher than that after the experiment.

TABLE 8 : Carcass composition of the experimental fish.

Sample	Ash	Crude protein	Lipid	NFE
T1	11.48±0.1	66.91 ±1.2	4.55 ±0.1	17.06 ±0.2
T2	12.84 ±0.0	67.60 ±1.4	4.01 ±0.0	15.55 ±0.1
T3	12.39 ±0.2	68.11 ±1.3	3.25 ±0.0	16.25 ±0.4
T4	12.04 ±0.1	69.14 ±1.0	3.22 ±0.1	15.60 ±0.8
T5	11.99 ±0.0	69.11 ±0.8	3.51 ±0.1	16.43 ±0.5
T6	12.13 ±0.2	67.32 ±0.5	4.12 ±0.0	17.27 ±0.3
Initial	14.14 ±0.0	65.14 ±0.4	3.45 ±0.0	5.46 ±0.0

NFE=Nitrogen free extract

Values are means ± SD from three replicates.

4.10 Mineral contents of the fish after the experiment.

The carcass (whole body) mineral concentration of the fish is presented in Table 9 . The groups of. fish fed diets2,3 and6had higher ($p<0.05$) Ca deposition than the composition in fish fed other diets. Fish fed with diet 5 also had higher ($p<.05$) Ca than the fish fed diets l and 4, which had similar but lowest Ca concentration. The pattern of Ca distribution indicated that fish fed soaked, oven-dried or untreated SBM supplemented with phytase produced higher ($P<0.05$) Ca in the body of the fish. Mg content of the fish fed diets 1,2,3,4 and 5 was the same ($p>0.05$) while the Mg contents of the fish fed diets 1,2,4 and 5 were higher ($p<0.05$) than the Mg content of the fish fed diet 6. P deposition was similar ($p>0.05$) in the fish fed diets 4 and 5, but higher ($p<0.05$) than the concentration in the fish fed diet 2 and 3.

Fish fed with diets 1and 6 had similar ($p>0.05$), but poorest P composition. Zn concentration in the fish fed all the diets was statistically the same. Mn composition in the fish fed diets 2,3,4 and 6 was the same ($P>0.05$), while the composition in fish fed diets 2,3 and 6 was higher ($p<0.05$) than the composition in the fish fed diets 1 and 5. The trend in the mineral distribution indicated that generally, and, except in Mg, fish fed diets with phytase had higher mineral concentration than the fish fed diets without phytase, while fish fed diet with untreated SBN plus phytase, contained similar Ca with fish fed diets 3and 6; similar Mg with fish fed diets 2,3,4 and 5; similar P with the fish fed diet 3 and the same Mg with fish fed diet 3 and 6. These results showed that treated or untreated SBM plus phytase can produce similar mineral deposition, unlike in growth parameters where treatment of SBM significantly improved the nutrient utilization and growth rates of the fish.

TABLE 9 : Mineral content of the fish carcass.

Trt	T1	T2	T3	T4	T5	T6
Mn (µg/g)	19.59±1.15a	25.00±0.01b	24.23±0.15bc	21.49±1.31ac	20.54±1.45a	23.39±3.10bc
Zn (µg/g)	25.78±2.10a	30.14±1.31a	30.11±0.3a	26.5±0.30a	29.30±3.33a	31.18±11.19a
Mg (mg/g)	230.4±10.57a	219.40±9.15a	211.98±3.73ac	230.24±10.01a	232.97±10.97ab	198.78±18.18c
Fe (mg/g)	26.01±7.49a	34.38±4.90a	32.58±3.91a	29.14±0.03a	30.88±0.04a	33.31±1.6a
Ca (mg/g)	19.08±10.6a	22.97±6.74bc	22.87±4.01cbe	19.36±2.01a	20.47±0.89d	22.45±0.91e
P (mg/g)	22.54±6.40a	24.98±7.0b	25.18±7.78c	26.65±6.80d	26.44±8.70e	23.26±3.70f

Means within the same row followed by the same subscripts were not significantly different ($p>0.05$)

4.11 Mineral content of the faeces from the experimental fish.

The faecal minerals (Table 10) describe that except in Ca discharges, other minerals were generally lower than the mineral discharged by the fish fed diet without phytase. Also faecal Ca and P discharged by the fish fed untreated SBM plus phytase were generally higher than the discharges from the fish fed treated SBM plus phytase. Faecal Ca discharges was significantly lowest in fish fed diet 6, followed by discharges from the fish fed diets 5,4,3,2,1 in that order.Fish fed diets1,3, 4and 5 discharged sthe same ($P>0.05$) level of Mg while the Mg discharged by the fish fed diets 2 and 6 was lower ($p<0.05$). The faecal P discharged by the fish fed diet 1 was significantly highest, followed by the discharges from the fish fed diets 2,6,3,4 and 5 in that order. Zn discharges was highest ($p<0.05$) from the fish fed diet1 compared to the discharges from the fish fed diets 2,3,4 and 6. Zn discharged by the fish fed diet 2 was l lowest in comparison with discharges from other fish.

However, Zn discharged by fish fed diets 2 and 6 were the same ($p>0.05$), but lower ($p>0.05$) than the discharges from fish fed diets 3,4 and 5. Fish fed diet 1 discharged the highest concentration of Mg into the culture system. The discharges from the fish fed diet 3 was higher ($p<0.05$) than the discharges from the fish fed diets 2,4,5 and 6 were similar but significantly higher than the discharges from the fish fed diet 4. It can be summarized

that most minerals discharged by the fish fed diets with phytase was significantly less than the discharges from the fish fed diet without phytase.

TABLE 10: Mineral content of the feacal matter of the experimental fish.

Trt	T1	T2	T3	T4	T5	T6
Mn (µg/g)	69.34±4.10a	44.33±2.9bef	49.81±1.10c	35.55±0.01d	41.51±0.02ef	44.64±0.02f
Zn (µg/g)	104.61±4.51a	59.16±10.0bc	86.01±3.01cd	83.03±2.01d	96.02±3.10ac	66.50±2.04e
Mg (mg/g)	54.02±11.56a	49.12±13.01b	53.29±13.40a	51.84±15.10ac	52.93±15.30a	50.38±17.05cb
Fe (mg/g)	10.96±13.40a	12.74±10.5ac	8.38±6.70b	10.63±10.51c	12.54±13.01a	9.47±5.01dbc
Ca (mg/g)	55.00±17.51a	57.83±13.51b	50.49±15.5c	47.40±11.5d	43.91±13.6e	40.18±14.51f
P (mg/g)	78.79±9.40a	53.61±13.80b	45.35±10.40c	40.50±7.30d	32.29±17.65e	46.46±45.39f

Means within the same row followed by the same subscripts were not significantly different ($p>0.05$)

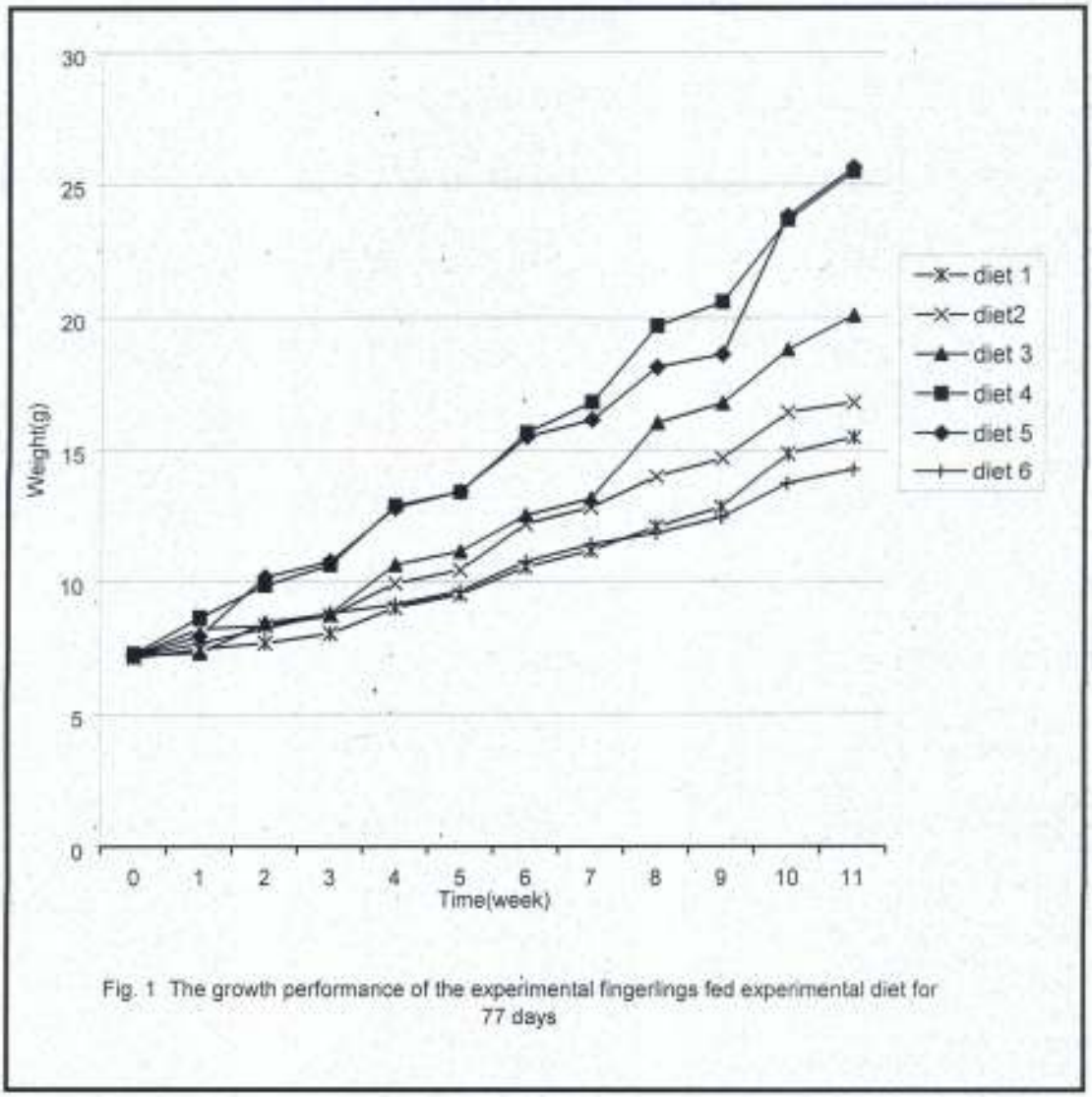

Fig. 1 The growth performance of the experimental fingerlings fed experimental diet for 77 days

CHAPTER FIVE

Discussion

This research demonstrates the necessity of heat in increasing nutritional quality of soybean meal. The presence of anti-nutritional factors such as phytate, tannin and lignin in the meals confirm the findings of Balogun and Ologbobo (1989) who reported the presence of anti-nutrient in the plant based ingredients. Also. Felix and Selvaraji (2004) reported phytate (phytic acid) as the most powerful anti-nutritional factors in plant base ingredient.

Several studies have reported successful use of enzymes to combat anti-nutritional factors in plant protein for fish feeds. A feeding trial conducted with tilapia *Oreochromis niloticus* fingerlings in Brazil showed the significance of phytase in plant protein based diets (Felix and selvaraj 2004).

The observation of the present study compares favourably with the earlier report by Felix and selvaraj (2004) on Tilapia *Oreochromis niloticus* fingerlings that fishes fed diet supplemented with enzyme (Natuphos) showed higher weight gain and better feed conversion ratio.

The present study compared favourably with the report of Adeparusi and Jimoh (2002) that toasting shows the best digestibility coefficient. The higher temperature with a short processing time improves digestion of

protein; this could be due to destruction of cell walls, thereby making the contacts more available to enzymatic action, or the carbohydrate fraction becoming more susceptible to hydrolysis(Olivia-Teles et al, (1994).

The similarity in mean weight gain (MWG) of fish treated on diets 1and2 is an indication that addition of phytase to untreated SBM was not enough to produce a significant differece in growth compare to the fish fed untreated SBM without phytase (diet1). Also, the fish fed diet6 had the same MWG with those fed diet1, emphasizing thatsoaking was not adequate to reduce the anti-nutrient in the SBMso as to significantly improve upon the growth more than in the fish fed untreated SBM without phytase.

Growth response of fish fed diets that containd oven dried, cooked or toasted SBM supplemented with phytase were significantly higher than fish fed either diet of untreated SBM or untreated SBM plus phytase as shown in Table 6. This could be that anti-nutritional factors such as phytate, tannin and lignin in diets 1, 2 and 6 depressed the growth of the fish. This agrees with the work of Aletor and Aladetimi (1989), which reported, that the presence of anti-nutrients in the plant protein has inhibitory effect on the growth performance of poultry.Findings from the study showed that heat treatment of the SBM enhanced the activities of the phytase, indicating that phytase alone may not be able to degrade all the anti-nutrients to the extent of producing significant difference in the growth of the fish. The increase in growth performance of fish fed diets 5and 4 followed by

diet 3 could be attributed to the heat processing effects which improved the nutrient quality of the diets.

The increase in growth performance in the fish agrees with the work of Jaffe (1973), who reported that heat has positive effects on protein quality. Heat decreases and eliminates activities of anti-nutrients and improves growth performance, Balogun and Ologbobo (1989) reported cooking as the best processing method for soybean meal, the present study showed toasting as the best processing method. However, there was no significant difference ($p>0.05$) between the effects of cooked meals based diets and toasted meal based diets.

This research demonstrates that phytase improves the performance of feed when fed to catfish (*Clarias gariepinus*) and this could be attributed to its most important function of improvements in phosphorus libration, utilization and stimulation of appetite which increased feed intake and consequently the growth rate.

Significant differences in feed conversion ratio (FCR) and protein efficiency ratio (PER) as shown in Table 6, may be due to the presence of anti-nutritional factors in raw soybean meal based diet (diet1) with values 2.97 and 0.46 for feed conversion ratio and protein efficiency ratio respectively.

Although diet 2 (reference diet) was raw soybean meal based, the enzyme supplement could have contributed to its improved protein

efficiency ratio with value 0.51, and reduction in the value of feed conversion ratio (2.12) compared to diet 1 (2.97). Steven and Helfrich (2002) reported that feed conversion ratio of 1.5-2.0 are considered best for most fish species. This agrees with the result from the present study where fish fed diet 3,4 and 5 had FCR of 1.94 , 1.40 and 1.50 respectively. However, the variation in the values of diets 3,4,and 5, which were processed, soybean meal and phytase and diet 2 which was raw plus phytase could be attributed to the necessity of heat in improvement of nutrient utilization of soybean. The reduction of protein efficiency in fish fed diet 6 could be due to leaching of the nutrient and the inability of soaking method to remove the anti-nutrients of the soybean.

Also, the significant difference in digestibility observed between diets 2 (reference), 3,4 and 5 could be ascribed to the processing effect on the diets. Heat decreases and eliminates activities of anti-nutrients and improves gross energy and protein digestibility. This agrees with the work of Adeparusi and Jimoh (2002), who reported that digestibility coefficient of protein, was highest in fish fed with toasted lima bean diet and lowest in fish fed the raw lima bean diets.

Apparent digestibility coefficient of diets is shown in Table 6. Protein digestibility of diet 1 (control) was 75.30 and was lower than the values obtained from the diet2 (reference diet), which was 81.47. This could be

connected with the effect of enzyme supplement in the reference diet (diet 2), which was not in 1 (control).

This research agrees with the work of FRDC (1993) which reported that phytase has significant effects on digestibility of major nutrient and on growth and when added to an extruded diet containing soybean (33%protein) the growth response was greater than that on the control diet. The protein digestibility of diet 3, 4 and 5 (processed diets) ranged between 83 and 85%, which agrees with the work of Robinson, and Meng (2003) that protein digestibility of cottonseed meal-based diet to catfish is about 84%.

The mineral composition of the carcass and feacal matters of the experimental fish revealed the various degree of amount required by the fish in terms of macro and micro minerals. Tables 9 and 10 show the mineral analysis of the flesh and feacal matter respectively. The observation from this present study agrees with Steven and Helfrich (2002), who reported that mineral such as Fe, Zn, and Mn are needed in trace form as component of enzyme and hormone system.

The minerals are regarded as macro minerals because of their large amount in the flesh of the fish. They are needed for osmotic balance and aid in bone formation. This study reveals significant differences in the carcass minerals composition except the Iron (Fe) and Zinc (Zn) composition, which showed no significant differences.

The feacal mineral composition of all the treatment was significantly different. The phosphorus was highest in diet 1(control) which could be attributed to the lack of enzyme. The lowest values recorded in diets 5 and 4 were due to the absorption and utilization of the dietary phosphorus. This observation agrees with those of Forster et al (1999) who reported that the addition of phytase reduces the release of nutrients into the environment by making the bound phosphorus available to the fish for growth –so it is incorporated into the fish body instead.

Similarly the present study supports the work of Felix and Selvaraj(2004) that addition of phytase in diets of rainbow trout reduced phosphorus load in their feacal matter. All the fish fed treated SBM plus phytase released significantly lower phosphorous than the fish fed diet with untreated SBM plus phytase . This means that a combination of any of the treatment methods with phytase would greatly reduce phosphorous discharges into the environment.

CHAPTER SIX

Conclusions and Recommendations.

The results of this study showed that soybean meal is best used as plant protein source for catfish diet, when toasted and supplemented with phytase. The anti-nutrients in soybean is deactivated when it is toasted. Phytase improves growth as well as phosphorous and protein utilization.

The research has identified potentials of soybean meal and enzyme additives in future commercial catfish feeds. It is recommended that further investigation be done to analyze the interaction between the phytase and water. This can assist in determining the role of phytase in the water body.

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APPENDICES

Analysis of Variance Table on Cumulative Weight Gain

Source	DF	SS	Ms	F	Fc
TRT	5	368.33	73.67	85.79	3.11
Error	12	10.304	0.859		
Total	17	378.64			

$P<0.05$

Analysis of Variance Table on Percentage Weight Gain

Source	DF	SS	Ms	F	Fcal
TRT	5	68918	13784	79.09	3.11
Error	12	2091	174		
Total	17	71009			

P<0.05

Analysis of Variance Table on Specific Growth Rate

Source	DF	SS	Ms	F	Fcal
TRT	5	1.528	0.306	4.68	3.11
Error	12	0.784	0.065		
Total	17	2.311			

$p<0.05$

Analysis of Variance Table on Cumulative Weight Gain

Source	DF	SS	Ms	F	Fcal
TRT	5	368.33	73.67	85.79	3.11
Error	12	10.304	0.859		
Total	17	378.64			

$p<0.05$

Layout of ANOVIA Table for feed fed.

	T1	T2	T3	T4	T5	T6
r1	38.99	60.13	51.80	64.82	46.20	41.93
r2	44.45	61.04	48.86	59.57	46.59	40.74
r3	41.65	60.62	50.33	61.95	46.41	41.23
Total	125.09	181.79	150.99	186.34	139.20	123.90
Mean	41.70	60.60	50.33	62.11	46.40	41.30

$$Cf = \frac{140943.54}{18}$$

$Cf = 7830.20$

SS total = 47015.44- 7830.2
SS total = 39185.24

$$SS\ treatment = \frac{140943.54}{3} - 7830.2$$

$= 46981.18 - 7830.2$
$= 39150.98$

SS error $= 39185.24 - 39150.98$
$= 34.26$

Sv	df	SS	ms	fcal	ftab
Treatment	5	39150.98	7830.20	2737.8	3.11
Error	12	34.26	2.86		
Total	17	39185.24			

Sig.dif $(P<0.05)$
LSD = 3.00

$$LSD = t(0.025,12) \times \sqrt{\frac{2x2.86}{3}}$$

$= 2.18 \times 1.38$
$= 3.00$

T4	186.34[a]	186.34 - 181.71 = 4.55*	181.79 – 150.99 = 30.8*
	181.79[b]	186.34 – 150.99 = 35.35*	181.79 – 137.20 = 42.59*
T2			
T3	150.99[c]	186.34 – 139.20 = 47.14*	181.79 – 125.09 = 56.7*
T5	139.20[d]	186.34 – 125.09 = 61.25*	181.79 – 123.9 = 57.89*
T1	125.09[e]	186.34 – 123.9 = 62.44*	150.99 – 139.20 = 11.79*
T6	123.9[e]		150.99 – 125.09 = 25.90*
			150.99 – 123.9 = 27.09*
			139.20 – 125 .09 = 14.11*
			139.20 – 123 .9 = 15.3*
			125.09 – 123.9 = 1.19 [ns]

Layout of ANOVA Table for feed conversion Ration

$$Cf = \frac{(8.92 + 4.60 + 5.84 + 4.31 + 6.36 + 7.31\ D^2}{18}$$

$$= \frac{247.29}{18} = 13.74$$

SS total = 83.34 – 13. 74
= 69.60

SS treatment = $\frac{247.29}{63}$ = 82.43 – 13. 74
= 68.69

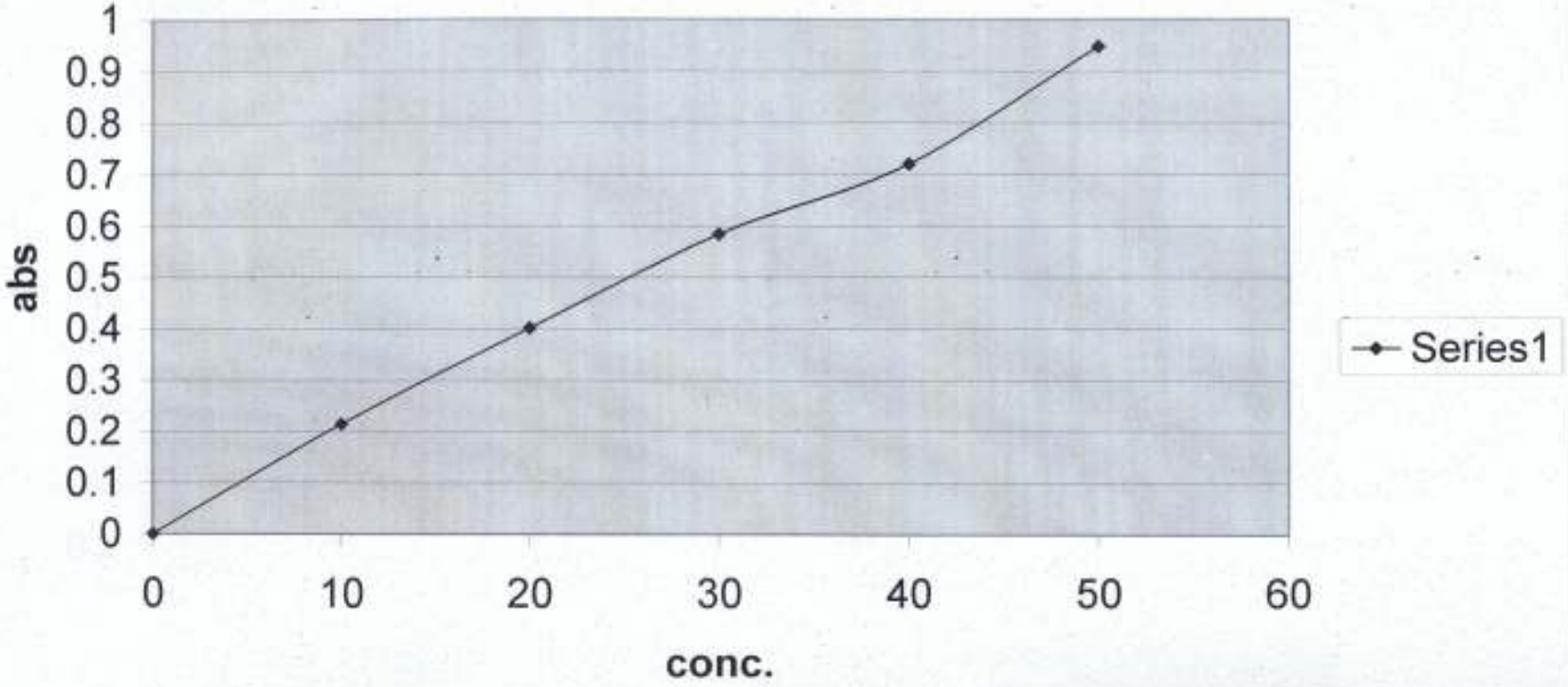
SS error = SS total – SS treat
= 69.60 – 68. 69
= 0.9

Sv	df	SS	ms	fcal	ftab
Treat	5	68.69	13.74	171.75	3.11
Error	12	0.9	0.075		
Total	17	69.60			

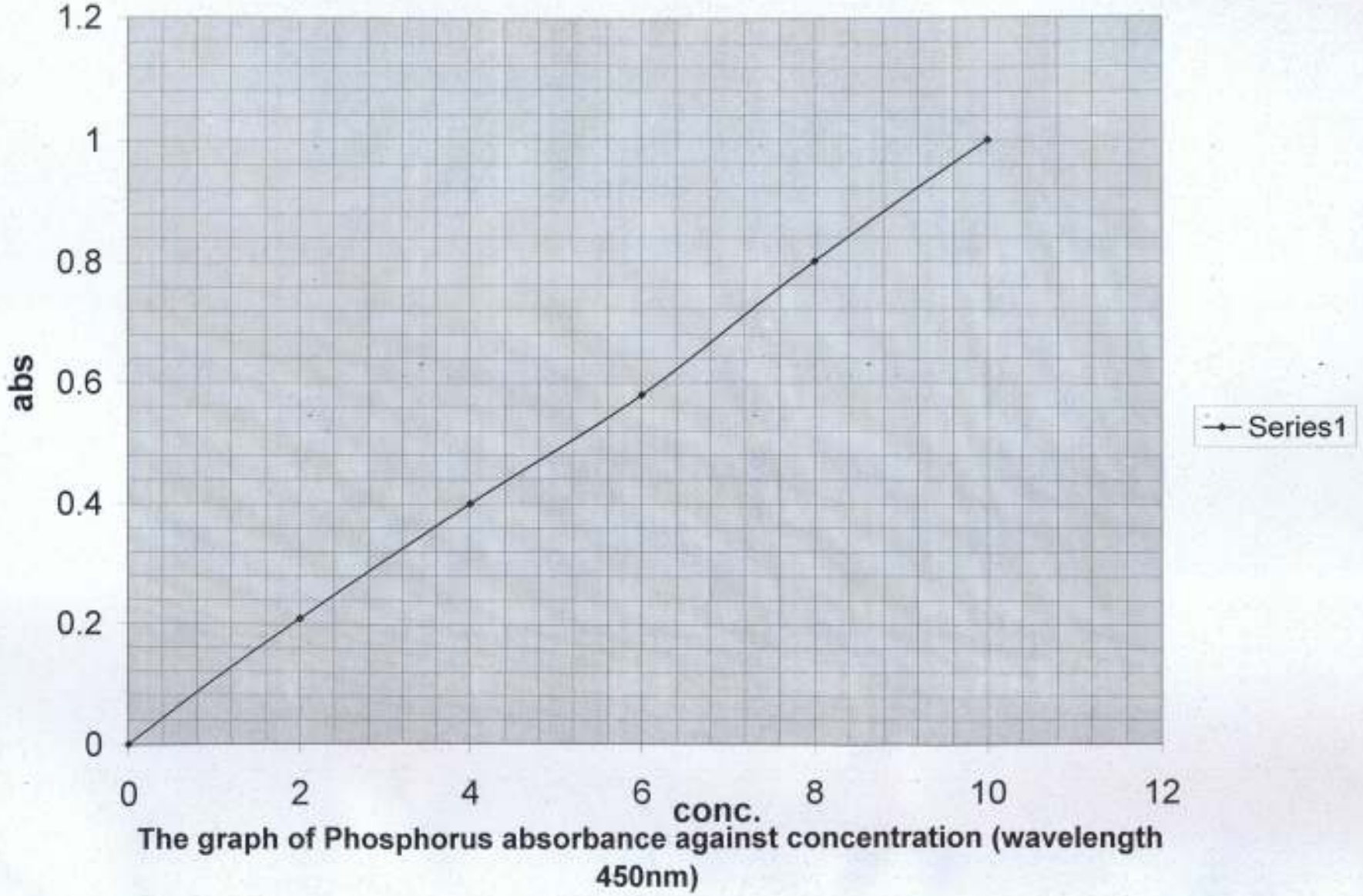
Sig dif (P< 0.05)
LSD Value = 0.5

$$LSD = t\ 0.025, 2 \sqrt{\frac{2 \times 0.08}{3}}$$

= 2.18 x 0.23
= 0.5



Graph of Tannin absorbance against concentration(wavelength 450nm)



The graph of Phosphorus absorbance against concentration (wavelength 450nm)