EFFECTS OF REPLACEMENT OF SOYA BEAN (*GLYCINE MAX*. LINN*)* MEAL WITH SOYA BEAN OFFAL MEAL IN THE DIET OF NILE TILAPIA *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) FINGERLINGS

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES

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DEPARTMENT OF BIOLOGY,

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ZARIA, NIGERIA

MARCH, 2020

DECLARATION

I declare that the work in this dissertation entitled "**Effects of Replacement of Soya Bean (*Glycine max*. Linn*)* Meal with Soya Bean offal Meal in the Diet of Nile Tilapia *Oreochromis niloticus* (Linnaeus,1758) Fingerlings** . " has been carried out by me in the Department of Biology, Ahmadu Bello University, Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and in a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any Institution.

SULEIMAN MUSTAPHA _____ _____

Signature **Date**

CERTIFICATION

This dissertation entitled "**EFFECTS OF REPLACEMENT OF SOYA BEAN (*GLYCINE MAX*. LINN*)* MEAL WITH SOYA BEAN OFFAL MEAL IN THE DIET OF NILE TILAPIA *OREOCHROMIS NILOTICUS* (LINNAEUS,1758) FINGERLINGS**" meets the regulations governing the award of the degree of Master of Science in Fisheries of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to Almighty Allah for seeing me throughout my studies and also to my family.

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ABSTRACT

The study was conducted to investigate the effect of replacing soya bean meal with soya bean offal meal in the diet of Nile tilapia *O. niloticus* fingerlings and to determine its growth performance. Fifteen (15) plastic rearing tanks (30cm x 45cm x 35cm depth) were used for the experiment. Plastic rearing tanks were randomly assigned in three replicates to five treatments. Three hundreds (300) fingerlings of *O. niloticus* of mixed sexes and sizes of initial mean weight of (6.0 ± 2.04^{a} g) were stocked in fifteen (15) white rectangular plastic tanks of water holding capacity of 50 litres (30cm x 45cm and 35cm depth) with twenty (20) fingerlings per tank and water maintained at 40 litres level for a period of twelve (12) weeks. Soya bean Offal meal (SBOFFM) was used to replace Soya bean meal (SBM) at inclusion levels of 0%, 25%, 50%, 75% and 100% for Diet 1 to 5 respectively. T1- 24.82 ± 0.72^{b} CP, T2- 25.34 ± 1.93^{a} CP, T3- 24.75 ± 1.01^{b} CP, T4- 24.21 ± 0.43^{b} CP and T5- 25.12 ± 0.70^{a} CP. Total growing period was 97 days (14 days of acclimatization and 83 days of feeding). The results obtained showed T2 (25.34 ± 1.93^{a}) (25%) inclusion Soya bean Offal had the highest Mean Weight Gain (MWG) of (21.97 ± 4.29^{a} g), Mean Length Gain (MLG) of (1.76 ± 0.23^{a} cm), Protein Efficiency Ratio (PER) of 0.867 ± 0.5^{a} g, Net Protein Utilization (NPU) of 150.35 ± 0.2^{a} %, Feed Efficiency Ratio (FER) of 1.89 ± 0.4^{a}, Gross Feed Conversion Efficiency (GFCE) of 200.00 ± 0.4^{a} and crude protein of *O. niltocus* carcass (38.1 ± 0.20^{a} g/100g DM). Feed Conversion Ratio (FCR) and Specific Growth Rate showed no significant difference ($P\geq0.05$). Also, pH (1-12), Temperature (T°C), Dissolved Oxygen (DO) (mg/l), Electrical Conductivity (µ/S), and Total Dissolve Solute (TDS) (mg/l) showed no significant difference ($P \geq0.05$). The study results demonstrate that soybean offal meal could replace the soya bean meal in the diet of Nile tilapia without negative effects on

growth, or on total production and even leading to high net economic returns as shown in the diets with 25% CP crude protein from plant source.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

As aquaculture production becomes more and more intensive in Nigeria, fish feed could be a significant factor in increasing the productivity and profitability of aquaculture as it accounts for at least 60 percent of the cost of fish production (Jamiu and Ayinla, 2003).The need to intensify the culture of the fish, so as to meet the ever increasing demand for fish has made it essential to develop suitable diets either in supplementary forms for ponds or as complete feed in tanks (Olukunle, 2006). For the purpose of nutritional and economic benefits, previous researchers have made attempts at increasing the use of non-conventional plant and animal materials to replace conventional feed ingredients like maize and fish meal in fish feed ration. Nigeria is the second largest producer of farm-raised tilapia in Africa, after Egypt (Fagbenro *et al.*, 2010), however, her contribution to global output is just a meager 28,950 metric tons. One of the greatest advantage of tilapia for aquaculture is that they feed on a low trophic level. The members of the genus *Oreochromis* are all herbivorous, feeding on algae, aquatic plants, and other, detrital material (Agbo *et al.*, 2015).

Supplemental diets are usually much less expensive than complete diets and usually high in carbohydrates as well as increasing productivity (Abu *et.al.*, 2010). Tilapia exhibit their best growth rates when they are fed a balanced diet that provides a proper mixture of protein, carbohydrates, lipids, vitamins, mineral and fibre. Krome *et.al.* (2016) provided excellent reviews that examined the details of tilapia nutrition. The nutritional requirements are slightly different for each species and more importantly vary with life stages. Fry and fingerlings require a diet higher in protein, lipids, vitamins and minerals and lower in carbohydrates as they are

developing muscle, internal organs and bone with rapid growth. Sub-adult fish need more calories from fat and carbohydrates for basal metabolism and a smaller percentage of protein for growth. Adult fish need even less protein, however the amino acids that make up that protein need to be available (Agbo *et al*., 2015). Corn, wheat, rice and a number of agricultural products are typical carbohydrate sources (Agbo *et al*., 2015). The ratio of energy, lipids and carbohydrates to the proteins available in the diet are often critical measures. Ng and Romano (2013) provided a comprehensive review of carbohydrate and fiber utilization in tilapia. Vitamins and minerals are critical to proper nutrition in tilapia and considerable research has been conducted to determine these requirements (Agbo *et al.,* 2015). Specific nutritional needs vary with species, age of fish, production system, and salinity. According to FAO (2006), fish supplies from capture fisheries will therefore, not be able to meet the growing global demand for aquatic food. There is need for a viable alternative fish production system that can sufficiently meet this demand, and aquaculture fits exactly into this role (Abu *et al*., 2010). Precocious breeding and high cost of feed are among the militating factors to tilapia production. Although, there has been breakthrough in checking the precocious breeding of tilapia through genetically modified parent stock and administration of Methyl-testosterone (MT) feed, availability of cost effective and highly potent feed still remains a major constraint to tilapia culture.

1.2 Statement of the Research Problem

Fish feed account for about 60% of recurrent cost of aquaculture operation (Eyo, 2013). Expensive feeds could negatively affect the profitability of fish farming through incapacitating the expansion of farms to increase production and consequently, low yield in term of quality and quantity of fishes (Gabriel *et al*., 2007). Aquaculture has traditionally relied on products from industrial fisheries; namely fish meal and oil which are most expensive global resource. The

global fish meal price has increased more than two fold in recent years (FAO, 2013). In Asia alone, the production of fish feed increased from 40% in 2000 to 60% in 2008 while the fish meal consumption for tilapia increased from 0.8 million tons to 1.7 million tons during same period (Tacon and Metian, 2015). Despite the increase in fish feed prices, the farm gate prices of aquaculture products have remained static, literally impinging on the economic viability of thousands of small-scale producers that form the backbone of the aquaculture sector (Rana, 2009). Indeed, the increasing price of feed ingredients (fish meal, fish oil and cereal), energy and transportation costs have complicated the availability of aqua-feeds to many fish farmers worldwide. This global phenomenon could influence small-scale producers to change businesses and/or result to poverty, vulnerability and loss of livelihood especially in developing countries (Rana, 2009). Fish feeds account for the highest operational costs in aquaculture with protein being the most expensive diet (Munguti *et al*., 2012). Fish Meal (FM) is the most expensive protein source in aquaculture feeds (Amaya *et al*., 2007). Fish require high proportion of protein in their diet because they metabolize protein as energy source (Aladetohun and Sogbesan, 2013).The development of commercial aqua-feeds has been traditionally based on fish meal as the main protein source with high protein content and balanced Essential Amino Acid (EAA) profile (Agbo *et al.*,2015).

1.3 Justification

The need to increase food production for the ever-increasing population of people in the world including Nigeria cannot be over emphasized. To solve these problems there is the need to exploit any available food resources. The most important food content that promotes growth and good health is protein. Sources of protein readily available apart from poultry are fish and plant proteins resources. Since fish feed account for 60% of recurrent cost of aquaculture operation,

therefore this research may reduce the economic burden to the aquaculturist through finding °cheap and available protein supplement (Omeru and Solomon, 2016). The efficiency of production and growth of fish in the cultural system depends on feeding complete feed at levels not exceeding the dietary requirement of the fish (Krome *et al.* 2014) .The choice of *O .niloticus* among other fish species was due to availability of the species in almost all freshwater bodies in Nigeria and also it is among the commonest accepted fish in our local markets. Nile tilapia is highly culturable of a high fecundity, which make it breed even in captivity (FAO, 2013). *Oreochromis niloticus* is highly palatable. Nile tilapia is among few fish species that are cultured by many aqua-culturists, this is because it is disease resistant, could be cultured in small water bodies and is a good converter of feeds (FAO, 2013).

1.4 Aim

To replace the soya bean meal with soya bean offal meal in the diet of *Oreochromis niloticus.*

1.5 Objectives

i. To determine the proximate and anti-nutritional composition of soya bean offal meal.

ii. To assess the growth performance of *Oreochromis niloticus* fed soya bean offal meal at different inclusion levels.

iii. To determine the cost effectiveness of replacing soya bean meal with soya bean offal meal at different inclusion levels.

1.6 Hypotheses

I. There is no significant difference in the proximate and anti-nutritional composition of soya bean meal and soya bean offal meal.

II. The growth response and feed utilization of *Oreochromis niloticus* fed at different inclusion levels of soya bean offal meal do not differ significantly.

III. There is no significant difference in the cost effectiveness of replacing soya bean meal with soya bean offal meal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Non- conventional Feed Ingredients used in Tilapia Diets

The primary source of high quality proteins of plant origin are soya beans, cowpea, groundnut, Bambara nut, pigeon pea, beans, quality proteins maize and other plant source (Augustine and Derner, 2012). There are also nine known legumes in the south western Nigeria that include; *Mallotus subulatus* (white variety), *Cassia hirsutta*, *Canavalia ensiformis*, *Vignea subterranean* (checked variety), *Vigna racemosa*, *Mallotus subulatus* (red variety), *Vignea subterranean* (cream variety), *Sphenostylis sterocapa* and *Cajanus cajan* (Solomon *et al*., 2007).

Like most roots and tubers, cassava peels and roots are much richer in crude protein, ether extract and ash than edible portion (Solomon *et al*., 2007). Solomon and Ezigbo (2010) in a study to investigate the growth and feed utilization of *O.niloticus* fed diets containing cassava peelings and mango seed. The calculated nutritive value of cassava has been reported to be higher than that of Sorghum (Solomon *et al*., 2007). The root and leaves of cassava plant are the two nutritionally valuable parts which offer potential as a food or feed source. Four diets prepared to contain 0% (control), 33.3%, 66.7% and 100% cassava leaves to replace equal weight of maize in a study by Bichi and Ahmadu (2010) which suggested that 66.7% cassava leaves replacement was considered as the requirement for optimum growth performance catfish *C. gariepinus*. Cassava is one of the most important energy sources in the diet of people in the tropics (Abu *et al*., 2010). Recent estimates suggest that its storage roots provide 8% or more of the minimum calorie requirement of more than 750 million people (Abu *et al*., 2010). Its starchy root produces more calories per unit of land than any other crop in the world (Presston, 2004).

According to Pedrosa (2002), cassava roots are generally rich in calcium and ascorbic acid and contain significant amounts of thiamine, riboflavin and niacin. Salami (2002) reported its starchy, thickened, tuberous roots are a valuable source of cheap calories and its use in animal feed is increasing because of its high energy content and low price. In recent times, the use of cassava as a substitute for cereals in livestock and fish feeds has been under investigation. Inclusion of cassava in the diet of white Fulani herds in Nigeria has been reported to increase milk production by 22% (IITA, 2013). Oke (2007) reported that cassava products are good energy feed ingredients for both monogastric and ruminant animals.

Talthawan *et al*. (2002) reported that starch in cassava is highly digestible when compared to that of maize due to the high content of amylopectin. Cassava can then be used as a source of energy in fish feed, but attention should be paid to the protein, metabolisable energy and Hydrogen Cyanide (HCN) contents in cassava products. Studies on the use of cassava meal in animal feed (Oresegun and Alegbeleye, 2001; Faturoti *et al*., 2002) indicate a great potential for cassava to replace the conventional energy food ingredients such as maize, broken rice and sorghum, which are commonly used in animal diet in most parts of Africa (Akinfala and Tewe, 2004). Inclusion of whole Cassava root meal in the diet of hybrid catfish enhanced growth and survival of the fish (Abu *et al*.,2010). Carbohydrate either of cereal or tuber in fish feed acts as both structural and energy component, which have some influence on the rate of growth of fish provided all other physiological requirements are satisfied (Carter *et al*., 2003). Olurin *et al.* (2006) reported a replacement level of 50% Cassava meal for maize without a depressing growth in *Clarias gariepinus*. Cardoso *et al.* (2005) observed that good processing of cassava enhances survival and healthy state of fish at all stages of their life. Maize, wheat, rice, sorghum and millet are cereal grains with almost equal caloric and nitrogenous composition (Solomon *et al*., 2007). The grains

are generally 68-72% starch, with 10-12% protein (Solomon *et al*., 2007). However, due to genetic environmental interactions some could have protein as low as 8%. The relative utilization of dietary carbohydrates by fish varies and appears to be related to their complexity. In general, the complex carbohydrate, such as starch and dextrin are utilized by most fishes better than simple sugar like glucose (Ng and Romano, 2013). Kamalam *et al*. (2017) observed that maize grain at 25% in tilapia diet showed better growth performance and nutrient utilization in comparison with other carbohydrate sources such as corn starch, dextrin, sucrose and glucose. However, the performance of *O. niloticus* has been reported to decrease with the increasing level (25-43%) of maize grain in the diet (Solomon *et al*., 2007).

Solomon *et al*. (2007) reported that wheat, maize and rice increased the growth performance of common carp more than barley and sorghum when fed fish meal-soya bean meal diets with cereal grains and grain offals. Solomon *et al*. (2007) who fed different grain sources to tilapia reported that the best growth performance was obtained for sorghum based diet whereas barley based diet gave the poorest growth performance. Bergot (1993) reported that wheat starch appeared to be the most digestible form out of a series of seven untreated carbohydrate sources. However, the digestibility of starch is affected not only by the source and nature of carbohydrate, but also by the level of its incorporation (Solomon *et al*., 2007). The establishment of economically viable fish culture ventures requires the utilization of agro-industrial cassava peels, rice bran, cocoa pod husk (Thomson, 2013), Kola nut husk (Osineye *et al*., 2008). Jamiu and Ayinla (2003) expressed that feed management determines the viability of aquaculture as it accounts for at least 60% of the cost of fish production.

2.2 Products of Plant Origin

A lot of work has been done by aquaculturists on the replacement efficiency of animal sources of protein with their plant counterparts in the diet of fish because of high cost of the animal proteins. Soybean has been identified as possible alternative to fishmeal in fish feeds due to its abundant protein. Solomon and Ezigbo (2010) worked on the seed and found it to contain all the essential amino acids and can be used in combination with other plant proteins to improve their nutritive value. They concluded that the low methionine levels in soya bean meal could be augmented by the addition of synthetic methionine in the diet.

2.3 Anti-nutritional Factors Present in the Soya bean Seed

Anti-nutritional factors are growth-inhibitors that are found in virtually all available ingredients of plant origin. Eyo (2013) reported the presence of trypsin inhibitor in raw soybean seed, which is destroyed during toasting the seed in an electric oven at 100°C for one hour. An effective means of reducing phytic acid content in soya bean seed. WHO reported a substantial decrease in phytic acid content after bacterial fermentation with *Lactobacillus acidophilus*. Fermentation with lactic acid bacteria L. acidophilus also reduces the amount of tannin in the seed from 20 to l0g kg1 (Atama *et al*., 2013). Inclusion of skinned dry toad meals as a good replacement for compounded feed since fish fed this diet have better growth response than those fed either whole dry toad meal or compounded feed. With the emergence of large scale poultry industries in Nigeria, there is the availability of enormous poultry waste that can be used in fish feed formulation. Chicken feather and offal were tried and the gross profit and profit index were best at 75% and 100% inclusions of chicken offal in the diet fed to *Clarias gariepinus*. Live maggot has a crude protein of 43.8, crude lipid 1.9, and 14.3 crude fibre (Madu and Ufodike, 2003), and it has also been used in the diets of *Clarias gariepinus* fingerlings. The result indicated that live

maggot utilization *Clarias gariepinus* was of better economic value as compared to the costly compounded fish feed. *Clarias gariepinus* fingerlings were able to convert the protein present in the maggot more efficiently than the compounded feed (Sogbesan, 2006).

2.4 Feeding Habits of Nile Tilapia

Tilapia is herbivorous and as in all herbivores, relatively large quantities of material have to be eaten because a large proportion of food is indigestible (Salzburger, 2009). He also added that *S.mossambicus* is omnivorous and feed especially on plankton and vegetation, the young fish feed equally on plankton but also seeds filamentous algae, notably chlorophyceae, higher plants and decomposing vegetable matter. When vegetable feeds are scarce, worms, crustaceans, fish larvae and detritus are eaten. Pond fish readily takes artificial feeds like rice bran, oil cakes, chopped leaves and kitchen feed refusal. Salzburger (2009) indicated that overcrowding of Tilapia forces the fish to canibalism and it engulfs tadpoles or frogs as well. In general tilapia of the genus *Sarotherodon* are primarily Omnivores taking phytoplankton , periphyton or detritus while members of the genus Tilapia tend to take coarser food including macrophytase and are consequently used to control weed growth in irrigation channels, ponds and dams (Salzburger, 2009).Tilapia measuring more than 4-5 cm readily takes artificial feeds (Salzburger, 2009) but smaller fish have been noted to be fed by the parent who chews up the food into fine mash and sprays this into a school of fry. In the development and management of an aquaculture enterprise, fish feed plays a vital role in its growth and expansion (Gabriel *et al*., 2007). The high cost of feed has affected the development and expansion of aquaculture enterprise in most African countries contributing in no small way, to the low protein intake in many developing countries (Abu *et al*., 2010). Non-conventional feed resources are credited for being non-competitive in terms of human consumption, very cheap by-products or waste products from

agriculture (Iluyemi *et al*., 2010). The main sources of carbohydrate in many farm based fish diets in Ghana and other West African countries are wheat bran and maize (Abarike *et al*., 2013). A fair proportion of industries in Ghana, Nigeria and other West African countries which are agriculture – based and produce a range of by-products which could be rich ingredients for the formulation of fish feed as they already contribute to livestock and poultry feed (Abarike *et al*., 2013). Abarike *et al*. (2013) used pito mash, a by-products obtained from sorghum used in the production of local drink known as "Pito", rice bran and groundnut bran to feed *O. niloticus* fingerlings.

The palatability and acceptability of the diets containing pito mash, rice bran and wheat bran, indicated the feasibility of using these agro-industrial by-products in formulating feed for fingerlings of *O. niloticus* (Abarike *et al*. 2013) . Similar results have been reported for *O. niloticus* by Liti *et al*. (2006) and Attipoe *et al*. (2009), and for Florida Red Tilapia (*O. niloticus* crossed with *O. mosambicus*) by El-Dakar *et al*. (2008). Availability of these agro-industrial by-products is a significant step towards more efficient utilisation of plant by-products (Abarike *et al*., 2013). The ability of *O. niloticus* to utilize various diets could be attributed to wide spectrum of preference for foods (Abarike *et al*., 2013). This is in agreement with Gonzalez and Allan (2007) and Audu *et al*. (2008) who reported that, *O. niloticus* readily adapts to eating a wide variety of feeds and that they have very long intestines necessary to digest plant materials. Solomon *et al*. (2007) used different grain sources (maize, wheat, rice, sorghum and millet) to assess the growth of tilapia in out-door hapas, reported that the best mean weight gain (M.W.G) for fish fed with maize grain > wheat grain > sorghum grain > millet grain > rice grain in this order. Gonzalez and Allan (2007) and El-Dakar *et al*. (2008) reported that high fibre levels in the diet of tilapia resulted in poor weight gain and nutrient utilisation. Chatzifotis *et al*. (2010)

demonstrated that growth can be reduced and Protein Efficiency Ratio lowered if fishes were fed with excessive dietary lipid levels. This is because excessive lipids not only spare proteins, but can also result in higher fat deposits and impaired growth performance.

Manjappa *et al*. (2002) reported that, growth of fish fed with diets which have higher lipid level (above 12%) is poorer, reflecting a negative impact of dietary fat beyond the optimum level. El-Dakar *et al*. (2008) reported an incidence cost of 2.26 for diets containing up to 50% fig jam by-products replacing wheat bran in the diet of red tilapia and recommended it as an economically efficient diet for red tilapia fry. Liti *et al*. (2006) reported that wheat bran based diet gave higher returns than the other test brans on the economic performance of *O. niloticus*. The use of carbohydrate-rich diets has been considered economical as fish would utilise the inexpensive carbohydrate as a source of energy (Liti *et al*., 2006; Mohanta *et al*., 2007). Amisah *et al.* (2009) indicated that in developing countries like Ghana, Nigeria and other West African countries, most feeds have to be imported using the limited foreign exchange of the state. (According to FAO 2006), fish supplies from capture fisheries will therefore, not be able to meet the growing global demand for aquatic food. There is need for a viable alternative fish production system that can sufficiently meet this demand, and aquaculture fits exactly into this role.

For the purpose of nutritional and economic benefits, previous researchers have made attempts at increasing the use of non-conventional plant and animal materials to replace conventional feed ingredients like maize and fish meal in fish feed ration (Eyo, 2004). Olurin *et al.* (2006) reported a replacement level of 50% cassava meal for maize without a depressing growth in *Clarias gariepinus*.

Abu *et al.* (2010) reported a growth performance and nutrient utilisation of fish feed 66% level of whole cassava root meal, which implies that high inclusion levels of whole cassava root meal

in the diet of hybrid catfish favours enhanced growth rate. Fakunmoju *et al*. (2014) reported that cassava peels could be used to replace maize in the diet of *O. niloticus* with considerable economic gain. The relative utilisation of dietary carbohydrate by fish varies and appears to be related to the level, source and complexity of the carbohydrate (Aderolu and Sogbesan, 2010). Nwanna *et al* (2009) observed that very high inclusion levels of unconventional dietary carbohydrate sources often result in poor performance of the fish. This was also confirmed in the work of Jesu *et al*. (2008) when potato peel was fed to *O. niloticus*. Orire and Ricketts (2013) reported that *O. niloticus* digested the protein in melon shell meal based diet as high as 93.2%. Orire and Ricketts (2013) reported that a complete replacement of maize at 100% appears to be unsuitable in the diet of *O. niloticus*, but inclusion rate up to 75% was found to be appropriate in the diets of *O. niloticus* fingerlings.

A number of animal and plant source proteins have been evaluated for fish meal replacement in diets for a number of different species. In freshwater omnivores, such as tilapia *Oreochromis* sp. and channel catfish *Ictalurus punctatus*, fish meal can be completely replaced by plant protein sources such as soybean meal Al-kenawy *et al*., 2008. Because of the limited supplies and the high price of fish meal, other alternative sources of protein must be considered. Plant proteins are generally cheaper per unit of nutrient than animal protein. Relatively, few plant protein sources have been used in fish feeds, because fish require high levels of dietary protein. Commercial aquaculture feeds for grow out contain 25 to 45% crude protein. Thus, only high protein content plant feedstuffs, such as oilseed residues, are used in fish feed. The most commonly utilized by feed manufacturers are soybean meal, peanut meal, and cottonseed meal. Soy protein is considered the best plant protein source for meeting the essential amino acid requirements of tilapia and other fish species commercially grown. It is highly digestible by fish and the digestion

coefficients are comparable or higher than fish meal protein (Alceste and Jory, 2000). Over the past few years, the American Soybean Association (ASA) has conducted several tilapia feeding studies with the objective of replacing fish meal with soybean meal and examining potential benefits of extrusion over pelleting (Swick, 2002). ASA feeding studies have demonstrated that replacement of fishmeal with soybean meal has no impact on fish performance but can improve net income (Swick, 2002). Tilapia generally has been shown to respond favorably to the dietary replacement of fish meal with soybean meal. Davis (2004) conducted a 4 x 4 factorial experiment in aquaria with blue tilapia to evaluate four different combinations of soybean meal and fish meal (each constituting 0, 33, 67 or 100% of dietary protein) at each of four dietary protein levels (15, 22, 29 and 36%). Diets containing all protein combinations, except the 36% protein diet with 100% fish meal were supplemented with DL-methionine. Fish fed diets containing 36% protein experienced the greatest weight gain and feed efficiency.

These responses were not influenced significantly by any combination of soybean meal to fish meal. However, at the lower dietary protein levels, a general improvement in growth and feed efficiency was seen with increasing amounts of fish meal in the diet. Francis *et al*. (2001) suggested that up to 67% of fish meal in milk fish feed could be replaced by commercial hexane-extracted soybean meal and a methionine supplement without any adverse effect on milk fish growth and feed conversion rate. Hybrid tilapia (*O.niloticus x O. aureus*) fed a diet in which 30% of the fish meal in the control diet was replaced with SBM had similar weight gain, feed efficiency, protein efficiency ratio and protein digestibility, compared to fish fed the control diet (Al-kenawy *et al*., 2008). In recent studies, some attempts were carried out to replace the high cost animal protein source by plant source proteins (El-Saidy and Gaber, 2002; Olvera-Novoa *et al*., 2002; Borgeson *et al*. 2006; Sanz *et al*., 2009). El-Saidy and Gaber, (2002) compared a

commercial tilapia diet containing 20% fish meal and 30% soybean meal to diets with all of the protein coming from soybean meal with graded levels of L-lysine supplementation formulated for Nile tilapia fingerlings. After feeding for ten weeks, the diet containing 55% soybean meal and 0.5% L-lysine was significantly ($P<0.05$) better than the commercial tilapia diet with respect to final weight, weight gain, feed conversion, protein efficiency ratio and feed intake. This diet had the highest digestibility coefficients for protein, fat and energy. These researchers suggested that a diet with 55% soybean meal supplemented with 0.05% L-lysine can totally replace fish meal in a diet for Nile tilapia fingerlings without adverse effect on fish performance .

2.5 Economic Importance of Soya bean (*Glycine max*. Linn.)

The International Institute of Tropical Agriculture (IITA, 2013) in one of its reports says leading infant food manufacturers in the country use soya beans because of its high nutritional value. Soya bean is also processed into flour and its oil is used in local paint, cosmetics, and soap making industries (Ademola *et al*., 2013). Direct human consumption of soya beans and its usage as animal feeds are also very significant in Nigeria, especially among rural low-income groups that cannot really afford animal protein sources such as meat, fish and eggs (Ademola *et al*., 2013). There is an all year round demand for soya bean in Nigeria, "after sorghum, soya bean is the most profitable grains to invest in, whether the investor wants to grow it or trade in it (Gbadamosi *et al.,* 2000) . Processing or reprocessing it for the industrial market is also highly profitable. In fact, the profit from processed soya beans is higher. Soya bean was compatible with the farmers available resources and needs (Gbadamosi *et al.,* 2000). Soya bean farmers had low awareness and perception of soya beans benefits, but had high levels of utilization of soya beans with low levels of constraints to its uses (Amusat, 2013).Soya beans serve as an essential sources of fatty acid, calcium, magnesium, lecithin, riboflavin, thiamin, fibre, folate and iron, (Agbede, 2000).The role of

leguminous seeds in animal and man nutrition in developing countries is well documented (Agbede, 2000). They are rich in nutrients such as digestible protein with good array of amino acid (Agbede, 2000). The percent crude proteins of most legumes vary from 20-50% (Adebowale *et al*., 2003; Aletor, 2007) and have been judged a good source of minerals (Aletor, 2007). Leguminous seed have been reported to be excellent sources of energy (Soetan and Oyewole, 2009) in animal and human diets. Since the beginning of 1970, there has been a great breakthrough in the popularization of soybean-based food in Nigeria and in many parts of the developing world, especially for use in the prevention of kwashiorkor. Since 1975, soybean has become a main source of daily dietary protein in many parts of Nigeria as a result of the successful incorporation of soybean products into almost all traditional Nigerian foods. This is a review of previous work in Nigeria on eliminating the beany flavour, bitter taste, and flatus factors in soybean milk and cooked soybean paste preparations (Soetan and Oyewole, 2009).

2.6 Growth and Nutrient Utilisation of Nile Tilapia

According to De Silva (2001) feed conversion ratio is between 1.2 to 1.8 for fish fed optimal prepared-diets. Davis (2004) observed that protein efficiency ratio is a measure of how well the protein sources in a diet could provide the essential amino acid requirement of the fish fed. Abu *et al*. (2010) reported a highest level of whole cassava root meal inclusion value of 66%.Abu *et al*. (2010) reported the highest value of 1.85±0.04, while the lowest value was recorded in diet D66% with a value of 1.71± 0.04, for Feed Conversion Ratio (FCR) of cassava root meal inclusion. Aderolu and Sogbesan (2010) reported the mean weight gain was highest at the 25% Cocoyam inclusion level replacing maize in *Clarias gariepinus* diet. Also, specific growth rate recorded highest at the 25% cocoyam inclusion level. Aderolu and Sogbesan, (2010) also

reported the least feed conversion ratio of 0.86 and the highest protein efficiency ratio of 2.94 in the diet with25% Cocoyam inclusion level replacing maize.

2.7 Distribution of Nile Tilapia

Nile tilapia as an important food fish in developing countries is widely used in commercial farming systems for intensive aquaculture. Tilapia family Cichlidae have being an important source of food for man at least since recorded history began (Odhiambo *et al*., 2011). However, Abdel- Hakim *et al*. (2008) reported that the family Cichlidae is very diverse and widely distributed in Africa, most of south America and in parts of India. Tilapia is however, mainly indigenous to Africa where some 100 species can be found. indicated that the relative ease for culture of Tilapia and its rapid growth rate under tropical climates has led to its widespread distribution such that today, with the probable exception of the common carp *(Cyprinus carpio)* no other species is more widely cultured. However, the spread of Tilapia around the world was so great that it was feared that the true identity of transplanted species would be lost (Abdel-Hakim *et al*., 2008). The same authors reported that the spread of Tilapia in the Far East began soon after the Second World War, but the distribution of introduced species for Africa is still incompletely documented and therefore is not included (Abdel- Hakim *et al*., 2008).

2.8 The Biology and Ecology of Nile Tilapia

Odhiambo *et al*. (2011) reported that the male of Nile tilapia has two orifices situated just forward of the anal fin .One is the anus, the other a urogenital aperture which usually forms into a small papilla in some species for example *Sarotherodon macrochir* the papilla is less prominent (Salzburger, 2009) However, female of Nile tilapia has three orifices, namely the anus, a transversal genital opening and a microscopic urinary orifice which is scarcely visible to

the naked eye. The genital opening is fairly easy to recognize in *S.mossambicus* and *S.macrochir* but more difficult in *Tilapia pendalli* because of the belly (Salzburger, 2009). Seasonal differences in food availability, reproductive activity and change in environmental factors such as light and temperature undoubtedly contribute to observed changes in growth rates, but effects of individual variables have rarely been studied and investigation of the existence of long- term endogenous rhythms (Abdel- Hakim *et al*., 2008).

Tilapia have a distinct preference for the higher water temperature. Hence temperature is one of the most important factors to be considered when selecting a particular species for pond culture (Odhiambo *et al*., 2011). Salzburger (2009) showed that the number of eggs per clutch increases with body weight so that weight of eggs is proportional to the weight of the female and represent the reproductive drain on the female in ponds, or any confined. Relative to other sparking fish Tilapia feed less materials during spawning, e.g., in perch 27% of total weight is made up by the ovaries, while in *S. variables* the ovaries only make up 5% of body weight, but an equivalent amount is lost as they breed more frequently. Losses at spawning are also greatest in smaller fish as is demonstrated in the case of *T.zillii*. Also, generally under natural condition matured in 2-3 years. *Oreochromis niloticus* mature at 17 cm length. In ponds Tilapia matured much sooner in less than six months, and this is now a well-recognized phenomenon (Salzburger, 2009). Odhiambo *et al*. (2011) found that temperature plays an important role in triggering of reproduction. Generally Tilapia spawns between 20-23°C and above 20°C secondary sexual characteristics become prominent. The observation reported by Salzburger (2009) showed that the maintenance of Tilapia at temperatures below 20°C prevent reproduction. The reduction in quantitative production was more or less compensated by an improvement in quality of water. The light plays a major role in courtship behavior of Tilapia courtship in aquaria could be

triggered off by a long photoperiod. Salzburger (2009) reported that Tilapia maturation to be accelerated by exposure to full light and related this to sexual precocity in ponds and swamps. It would therefore appear that both photoperiod and light intensity influence early maturation. Characteristics of all fish, growth of Tilapia is indeterminate continually slowing down but apparently does not seem to stop and continuous after sexual maturity is achieved. In this respect fish differ from mammals where growth virtually ceases at maturity. It is not a general rule amongst Tilapia that growth rate declines after maturity, e.g *S. galilaeus* (Odhiambo *et al*., 2011).

2.9 Physico – Chemical Parameters within the Plastic Rearing Tanks

Abu *et al*.(2010) reported the results of the water quality variables, indicated that mean values of pH ranged from 6.60 to 8.55, while concentration of Dissolved Oxygen (D.O) ranged from 4.99 to 7.10mg/l. Water temperature ranged from 27.11 to 29.14°C, nitrite ranged 0.0010 to 0.0054mg/l, ammonia 0.30 to 0.46mg/l and total hardness 46.05 to 80.08. Aderolu and Sogbesan (2010) reported water parameter ranges were 27.5 to 29.5°C, 4.5 to 4.8mg/l and 7.3 to 8.0 for temperature, dissolved oxygen and pH, respectively, for the treatments of *Clarias gariepinus* juvanile and *O. niloticus*. Tropical fish species generally prefer temperatures above 20°C are unsuitable. Temperature on digestion is hinged with metabolic rate, which varies with the activity of enzymes. A narrow or wide range of suitable temperature for digestion depends on the species of fish (Ugwumba and Ugwumba, 2007). In tilapias such as *O. niloticus* and *Sarotherodon melanotheron* as well as in the African bony-tongue, *Heterotis niloticus*, intestinal amylase activity is maximum between pH 7.0-8.5 being optimal at the upper level (Ugwumba and Ugwumba, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Proximate Analysis of the Feed

Proximate analysis was conducted in the Food Science Laboratory of the Institute for Agricultural Research (I.A.R) Zaria, Nigeria and National Animal Production Research Institute (NAPRI) Shika for comparism. Anti- nutritional factors determination of soya bean offal meal was determined at National Research Institute for Chemical Technology (NARICT) Zaria. . Experimental diets, initial and final fish samples (carcasses) were also analysed for Crude Protein (CP), Crude Fibre (CF), Lipid Content, Ash Content and Moisture Content, using AOAC (1990) methods.

3.1.1 Determination of the Moisture Content

The method described by AOAC (2005) was adopted. A clean crucible was dried to a constant weight in an air oven at 110° C, cooled in a dessicator and weighed (W1). 2g of finely ground sample was accurately weighed into the previously labeled crucible and re- weighed (W2).The crucible containing the sample was dried in an oven to a constant weight (W3). The percentage moisture calculated as follows:

$$\% \text{ Moisture content} = \frac{W2-W3}{W2-W1} \times 100$$

Where:

W1= weight of dried crucible

W2= weight of crucible containing 2g of sample

W3= weight of crucible with sample and dried in the oven

3.1.2 Determination of Ash Content

The AOAC (2005) method was used. The porcelain crucible was dried in an oven at 100°C for ten minutes cooled in a desiccator and weighed (W1). 2g of the finely ground sample was placed into the previously weighed porcelain crucible and reweighed (W2). It first ignited and then transferred in to a furnace, which was set at 550°C. The sample was left in the furnace for 8 hours to ensure proper ashing. The crucible containing the ash was removed cooled in a desiccator and weighed (W3). The percentage ash content was calculated as

$$\% \text{ Ash content} = \frac{W3-W1 \times 100}{W2-W1}$$

where: WI = dried crucible

W2 = crucible containing 2g of the sample

W3 = crucible that was Removed from the muffle furnace

3.1.3 Determination of Crude Lipid Content

The lipid content was determined using AOAC (2005) method. A clean dried 500 ml round bottom flask containing few anti-bumping granules was weighed (W1) and 300ml of petroleum either (40-60^{o}C) for the extraction was poured into the flask fitted with soxhlet extraction unit. The extractor thimble containing 20g was fixed into the soxhlet extraction unit. The round bottom flask and a condenser was connected to the soxhlet extractor, cooled water circulation was put on.

The heating mantle was switched on and the heating rate was adjusted until the solvent was refluxing at a ready rate. Extraction was carried out for 6 hours. The solvent was recovered and the oil was dried in the oven at 70° C for one hour. The round bottom flask and oil was cooled and then weighed (W2). The lipid content was calculated as follows;

$$\% \text{ crude lipid content} = \frac{W2 - W1}{\text{Wt of sample}} \times 100$$

Where: W1 = empty filter paper
W2 = filter paper containing 2g of the sample
W3 = residue removed from oven.

3.1.4 Determination of Crude Protein

The method of AOAC (2005) was used. Exactly 1.5 gramme (1.5g) of the ground defatted sample in ash-less filter paper was dropped into 300 ml Kjeldahl flask. Twenty five 25 milliliters concentration H_2SO_4 and 3g of digesting mixed catalyst (weighed separately into an ashless filter) was dropped into the kjeldahl flask. The flask was then transferred to the Kjehldahjl digestion apparatus. The sample was digested until a clear green colour was obtained. The digest was transferred into 500ml kjehldahl flask containing anti-bumping chips. Two hundred and fifty milliliters distilled waters was added (rinsing the 300ml flask into the 500ml flask with part of the 250ml water). About 70-120ml of 40% NaOH was slowly added the side of the flask and then followed 3 drops of 1% phenolphthalein indicator. A 500ml conical flask containing a mixture of 125ml 4% boric acid and 4 drops of mixed indicator was used to trap the ammonia being liberated. The Kjehldahl and the conical flask was then placed on Kjehldahl distillation apparatus, with the tubes inserted into the conical flask and Kjehldahl flask. The distillation was carried out until 125ml of the distilled was trapped in the boric acid solution to make a total volume of 250ml. The distillate was titrated with 0.5ml H_2SO_4. The content of the conical flask was titrated with 0.1 M HCI 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.

Calculation:

$$\% N_2 = \frac{V_1 - V_0 \times M \times 14 \times 100}{2 \times 1000 \times 10 \times 1} \times 100$$

Where,

V0 = Volume of HCI required for the blank

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

M= Concentration of HC1 (0.1M)

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:

Crude Protein = 6.25 x %N

3.1.5 Determination of Carbohydrates

The total carbohydrates content was determined by difference. The sum of percentage Moisture, Ash, Crude Lipid, Crude Protein, and Crude Fibre is subtracted from 100 (Francis *et al*., 2016)

CHO = 100- (% Mosture + % Ash + % Protein + % Fats)

3.1.6 Determination of Crude Fibre Content

The crude fibre content was determined, using the methods of AOAC (2005). Two grams (2g) of the sample was refluxed in 100ml digestion mixture including 450 glacial acetic acid, 20g trichloro acetic acid, 50ml nitric acid and 50ml distilled water. The extracted mixture was filtered through ash less filter paper, washed with 100ml boiling water followed 50ml ethanol and 50ml

petroleum ether and dried to a constant weight. The residue was ashed incineration at 750° C for 6 hours. The crude fibre was calculated at the differences in weight between the residue and ash.

$$\% \text{ Fibre} = \frac{C2-C3}{W}$$

Where: C2= Oven dried residue

C3 = The residue removed from the muffle furnace

W = Weight of the sample

3.2 Anti-nutritional analysis of the experimental feed

3.2.1 Determination of oxalate

Oxalate was determined by the method of Umaru *et al*. (2007). One gram of the sample was placed a 200ml volumetric flask 150ml of distilled water and 10ml of 6m HCI were added. The mixture was warmed on a water bath at 70°C for 4 minutes. The supernatant was then diluted to 200ml. There 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 precipitated with 10ml of 5% calcium chloride ($CaC1_2$) solution. The solution was allowed to stand overnight and then was centrifuged. The precipitate was washed into a beaker with hot 25% H_2SO4 dilute to 125mls with distilled water and after warming to 90°C, it was titrated against 0.05m $KMnO_4$.

Calculation: 1ml of $0.05MnO_4$ = 2.2mg oxalate,

$$\% \text{ Oxalate} = \frac{\text{Wt of oxalate}}{\text{Wt of the sample}} \times 100$$

3.2.2 Determination of Tannin

The method of AOAC (2005) was used.l.0g ground sample was weighed into a conical flask and 100ml of distilled water was added. This was boiled gently on a hot plate for one hour and filtered through a No. 44 Whatman 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 dilutee 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 was allowed to stand for 30mins in water bath (25°C). Optical density was measured at 700mm and the absorbance was compared on a standard tannic acid (tannin) curve. The tannic acid standard curve was prepared dissolving 0.2g of tannic acid in distilled water and diluted to 200ml mark (lmg/ml). Varying concentration (0.1- l.0mg/ml) of standard tannic acid solutions was pipetted into 10 different 100ml conical flask. 5ml Folin Denis reagent and 10ml saturated Na_2CO_3 solution was pipetted into the test tubes. Then make up to the 100ml mark with distilled water. The solution was left to stand for 30mins and the optical density was measured at 700mm. A plot of optical density tannic acid concentration was made; with the line passing through the origin.

Tannin content (mg/100g) was calculated thus:

$$\frac{C(mg) \times \text{Extract volume}}{\text{Aliquot Volume} \times \text{Wt of sample (g)}} \times 100$$

Where C(mg) = Concentration of tannic acid read off the graph

3.2.3 Determination of Saponin

The methods of AOAC (2005) were used. 5.0g ground sample was weighed into a thimble and transferred into the soxhlet extractor chamber filled with a condenser and a round/flat bottomed flask. Some quantity of acetone, enough to cause a reflux was poured into the flask. The sample was exhaustively extracted at its lipid for 3 hours and the solvent distilled off. This was the first extraction. The second extraction, a pre-weigh flask was fitted unto the soxhlet apparatus (bearing the sample containing thimble) and methanol poured into the flask. The methanol should be enough to cause a reflux. The saponin was exhaustively extracted for 3 hours. The flask was re-weighed. The difference in weight represents the weight of saponin extracted.

$$\% \text{ saponin} = \frac{\text{wt of saponin}}{\text{wt of sample}} \times 100$$

3.2.4 Determination of phytate

Phytate phosphorous was determined by the method of AOAC (2005). 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% CA). 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 HNO_3 and transferred 10 100ml volumetric flask. The volume was made up with distil water. Five milliters 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 make 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 (FeC13) 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, there by obtaining the concentration of 3ppm. Absorbance was measured at 480nm with 1.5M KSCN. Calculation:

$$C\ (unknown) = \frac{A\ (unknown)\ X\ C(standard)}{A\ (standard)}$$

Phytate 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

Phytate = C phytate-P – 28% C phytate-p

3.2.5 Determination of Trypsin Inhibitor (Ti)

Extraction of Sample:

Three 50ml to 2.0ml of standard trypsin solution, l.0 g of ground sample was weighed and mixture was dispersed into 50mls 0.05M NaCI solution. The mixture was stirred for 30mins at room temperature and centrifuged. Filter the supernatant through whatman No. 41 filter paper. The filtrate (extract volume) was used for the assay. Preparation of Standard Trypsin and Substrate Img/ml of trypsin in 0.1M HCI was prepared 2% casein substrate in 0.1M phosphate buffer pH 7.6 was prepared. Procedure :for 3 hours. The flask was re-weighed.1ml of Trypsin inhibitor extract and then 10ml of the substrate in a test tube was added. Prepare a blank of 10ml of the sample substrate in a test tube but with no trypsin inhibitor extract was added. The content of test tubes was allowed to stand for at least 5mins and then measures spectophotometrically at

410mm.The trypsin inhibitor activity was expressed as the number of trypsin unit inhibited (TUI) per unit weight of the sample analyzed. Calculation:

TUI/mg = b - a × **F**

Where b = absorbance of test sample solution

a= absorbance of the blank

F = $1 / w \times V/V_a \times D$

Where w = weight of sample

V, = Total volume of extract

V_a= Volume of extract used in the assay

D = dilution factor (if any)

3.2.6 Determination of Cyanogenic Glycosides

The method of AOAC (2005) was used.l.0g ground sample was weighed into a 250ml flask. 200ml of distilled H_2O is added and allowed to stand for 2 hours. Full distillation was 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 (align of volume) containing cyanogenic glucoside, 8ml of 6N Ammonium hydroxide and 2ml 5% KI were added, mixed and titrated with 0.02N $AgNO_3$ against a black background. Permanent turbidity indicates end point. Cyanogenic glucoside content (mg/l00g)

$$\frac{\text{Titre volume x 1 .08(G) extract volume}}{\text{Aliquot volume xsample weight (g)}} \times 100$$

Calculation:

$\% N_2 = \frac{0.014 \times M \times V \times 100}{\text{weight of test sample}}$

% crude protein = % nitrogen (N2) x 6.25

Where M = actual morality of acid

V = Volume of H_2S0_4. Used

3.3 Determination of Growth Performance Parameters

3.3.1 Fish Weight Gain

The weight gain was calculated as the difference between the final weight of the fish at the end of the experiment and the initial weight at the beginning of the experiment.

W.G = FW – Wo

Where: FW= final weght

Wo = initial weight

3.3.2 Percentage Live Weight Gain (LWG%)

This was computed as the difference between the initial and final fish weight divided the initial weight expressed as percentage (Mbahinzireki *et al*., 2001).

$$LGW = \frac{W_t - W_0}{W_0} \times 100$$

Where W_0 = initial fish weight (g)

W_t = Weight at time t days (g)

3.3.3 Specific Growth Rate (SGR)

This was described by Fafioye *et al*. (2005) as

$$SGR = \frac{\text{Log } W - \text{Log } W_0}{t\text{-to}}$$

Where W, = Weight at the time of observation W_0 = initial weight

t-to "the period under study in days.

3.3.4 Feed Conversion Ratio (FCR)

This was calculated as the dry weight of feed offered divided by the weight

Gain of fish (Mbahinzireki *et al*., 2001). This was expressed as

$$FCR = \frac{\text{Feed applied (x)}}{\text{Net fish produced (g)}}$$

3.3.5 Gross Food Conversion Efficiency (GFCE)

This is the reciprocal of the FCR expressed as a percentage (Mbahinzireki *et al*., 2001):

$$\text{Thus: GFCE} = \frac{1}{\text{FCR}} \quad \text{x l 0 0}$$

3.3.6 Feed Efficiency (FER)

It was computed as a ratio that expressed the fish weight gain to the quantity of Feed

$$FE = \frac{\text{Weight gain (g)}}{\text{Feed fed (g)}}$$

3.3.7 Protein Efficiency Ratio (PER)

This was computed as a ratio of fish weight gain to the crude protein consumed, as determined Saha and Ray (2011) as

$$PER = \frac{\text{Fish weight gain (g)}}{\text{Crude protein fed (g)}}$$

3.3.8 Apparent Net Protein Utilization (A_{P1}>NPU)

Saha and Ray (2011) was computed as

$$\text{App NPU} = \frac{\text{Fish Protein Gain}}{\text{Protein fed}} \times 100$$

3.3.9 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. (Santigosa *et al*., 2008)

3.3.10 Productive Protein Value (PPV)

This was calculated as a ratio between the protein retained in fish tissue and the Dietary protein consumed (Harpaz *et al*., 2001). That is:

$$PPU = \frac{\text{Protein retained in tissues}}{\text{Dietary protein consumed}}$$

3.3.11 Nitrogen Metabolism (Nm)

The values of nitrogen metabolism (Nm) was calculated using the Formula of (Lin *et al.*, 2007). It is as follows:

$$Nm = (0.\frac{549Kb-a)\ h}{2}$$

Where: a = Initial weight of fish (g) b = Final weight of fish (g)

h = Experimental period in days.

3.3.12 Standard Length Gain

The length gain was calculated as the difference between the final length of the fish and the initial length at the beginning of the experiment.

$SLG = FL - L_0$

Where: FL = final length

L_0 = initial length

3.3.13 Standard Length Gain Percentage

This was computed as the difference between the initial and final standard length divided by the initial length expressed as percentage.

$$SLG\% = \frac{FL - L_0}{L_0}$$

Where: FL = final length

L_0 = initial length

3.4 Experimental site

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

3.5 Experimental Design

Completely Randomised Design (C.R.D) was used in the experiment. Using five treatments and two replication to each treatment, T3R2, T2R2, TIR3, T5R1, T1R2, T3R3, T5R2, T4R1, T1R1, T2R1, T4R2, T3R1, T4R3, T5R3 and T2R3.

3.5 Experimental Fish

Three hundreds (300) *Oreochromis niloticus* fingerlings of initial mean weight of (6.0±2.04g) were used in the experiment and were obtained from Fagam fish farm, Mariri Ward, Kumbotso Local Government Area, Kano State. The *O. niloticus* fingerlings were transported very early in the morning between 7.00am-8.00am to the Fisheries Laboratory, Department of Biology, Ahmadu Bello University Zaria. Nigeria. The Nile Tilapia fingerlings were placed in to fifteen plastic rearing tanks that contained dechlorinated water for a period of two weeks (acclimatization period), during this period they were fed with a commercial feed at five percent

(5%) total body weight. Chemical analysis of fish samples was carried out before and after the feeding trials following the AOAC (2005) methods. After period of acclimatization the Nile tilapia fingerlings were weighed using the sensitive weighing scale and recorded as Initial weight of (6.0±2.04g). The total and standard lengths were measured and recorded using a measuring board. The weighing and length measurements of the fish were taken bi-weekly throughout the experimental period. Total growing period was 97 days (14 days of acclimatization and 83 days of feeding).

3.6 Experimental feedstuffs

Feedstuffs used in the experiment were fish meal, soya bean meal, soya bean offal meal as shown in Appendices I, II and III as protein concentrate and yellow maize as source of carbohydrate and binder. Additives used include methionine, vitamin premixes, bone meal, sodium chloride, lysine, and palm- oil. The proximate analysis and anti-nutritional composition of soya bean offal meal were determined following the AOAC (2005) methods.

3.7 Processing of Feed Ingredients

Wet soya bean offal was obtained directly from women making the soya bean cake and soya milk in Makurdi village. Wet Soya bean offal obtained was sun dried and grounded to fine particles. Soya Bean (SB) was toasted in an electric oven at 100℃ for 15 – 30 minutes (Omeru and Solomon, 2016). The toasted soya bean was ground in a Hammer mill machine to fine texture and sieved with (0.5mm) mesh size openings. The powder was transported in polythene bags and stored in air dried places for further use (Sotolu and Byanyiko, 2010). The yellow maize was toasted in an electric oven at 100℃ for 15-30minutes (Omeru and Solomon, 2016). The toasted yellow maize was ground into fine powder with a hammer mill machine and sieved with a small mesh size (0.5mm) to separate the powder from the chaff. The powder was stored in

polythene bags and kept in a dry place till further use (Sotolu and Byanyiko, 2010). Fish meal was prepared by sun-drying of the fish. The dried fish were ground into powder using a Hammer mill machine, sieved and stored in polythene bags till further use (Sotolu and Byanyiko, 2010). The rice bran was steamed to remove all the oil before it was grounded into powder and stored in a polythene bags and kept till further use (Omeru and Solomon, 2016).

3.8 Feed Formulation

Five diets of varying proteins replacement were formulated at 100% soya bean meal (31.7g), 0% soya bean offal meal,75% soya bean meal (23.77g), 25% soya bean offal (7.93g), 50% soya bean meal (15.85g) , 50% soya bean offal meal(13.55g), 25% soya bean meal (7.93g),75% soya bean offal meal (20.33g) , 0% soya bean meal, 100% soya bean offal meal (27.1g) as shown in table 3.1. The percentage inclusion of each ingredient was calculated using the Pearson square methods. Each ingredient was weighed using a sensitive weighing scale separately.

3.8.1 Dry and Wet mixing of ingredients

All ingredients that were weighed in different containers according to formulation were properly mixed and agitated to obtain homogenous quality of sample called dry mixing. After thorough mixing of the dry mash, warm water was introduced at a ratio of 4:1(4 kilograms of mixture to 1 litres of warm water).The soya bean offal also served as a binding agent (Falayi, 2009).

Table 3. 1: Composition of Experimental Diets used for the feeding Trial

Ingredients	Inclusions Levels of Soya bean Offal Meal (%)				
	0	25	50	75	100
Soya bean Meal	31.7	23.77	15.85	7.93	00
Soya bean Offal Meal	00	7.93	13.55	20.33	27.1
Fish Meal	31.7	31.7	31.7	31.7	27.1
Yellow Maize	41.2	41.2	41.2	41.2	41.2
Bone Meal	1.2	1.2	1.2	1.2	1.2
Palm Oil	4.2	4.2	4.2	4.2	4.2
Salt	0.96	0.96	0.96	0.96	0.96
Vitamin Premix	0.84	0.84	0.84	0.84	0.84
Methionine	2.4	2.4	2.4	2.4	2.4
Lysine	2.4	2.4	2.4	2.4	2.4
Total	100	100	100	100	100

3.8.2 Procedures for pelleting of diets

The hopper received the dough into an inner drum flanked by propelled shaft or conveyor, across into a waiting disk adjusted to 3mm size. The drum with flanges was propelled by a motorized pulley with belt which conveyed the dough directly into the waiting disk with holes adjusted at 3mm size. The wet doughs were collected in flat trays and spread in oven to dry at controlled temperatures (Falayi, 2009).The five (5) prepared and formulated experimental diets were moulded into 3mm sized pellets and were labelled separately as diet 1-5 for easy identification in air tight polythene bags as shown in Appendix IV. The experimental diets were stored in a dry place during the experimental duration to avoid nutrients deterioration due to fungal growth (Bolorunduro, 2002).

3.9 Feeding the Fingerlings

The fingerlings were starved for 24 hours in order to empty their gastro intestinal tract (G.I.T) and prepare their appetite for the new feed. The experimental fishes were fed at 5% body weight daily with their respective experimental diets. The feed was divided into three portions so that 33% of the feed for a day was given in the morning between 7.00am-8.00am, 33% was given in the evening between 4.00pm-6.00pm and the remaining 33% was given in the mid-night 12:00pm. The quantity of daily ration was adjusted after every two weeks based on fish weight gain for 98 days.

3.10 Experimental Set-up

Fifteen plastic rearing tanks of 30cm x 45cm x 35cm depth were used for the experiment set horizontally with each having an outlet separately as shown in Plate V. Five treatment diets were used in the experiment with the first treatment as a control. Each treatment has two replicates,

twenty (20) fingerlings of *O.niloticus* were stocked per tank according to Ofor (2007). The tanks were covered with net meshes to prevent fish from jmping out. A partial flow-through system with fresh dechlorinated water was used throughout the experiment. Solid wastes in tanks were siphoned forthnightly during which data on body weight were taken to determine fish growth performance. The fish were starved for 24 hours in order to empty their stomach and prepare their appetite for the new feed. Fish were fed four times daily morning (7:00am), afternoon (1:00pm), evening (6:00pm) and mid-night (12:00pm) with the experimental diets at the rate of 5% body weight. A sensitive Electronic Compact Scale Kerro (German made) top- loading balance model BL2001 was used to weigh fish forthnightly. Standard and total lengths were also determined using a measuring board.

3.11 Economic Analyses of Experimental Diets

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 various experimental diets.

I. Estimated Investment cost = Cost of feeding + Cost of fingerlings stocked.

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

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

IV. Economic Conversion Ratio (ECR) = Feed Intake x Feed Conversion Ratio

3.12 Monitoring of Physico-Chemical Parameters in the Tanks

The temperature was recorded on bi- weekly basis, using Hanna (HI98129). It was dipped in to the water and left for about three minutes before the reading was taken. The pH was determined with Hanna (HI98129) meter. The water used to be standardized with buffer solutions at pH 4.0, 7.0, and 9.0 After it has been calibrated and the protective cap has been removed The pH meter

was dipped into water samples collected inside it for about 3-5 minutes before taking the reading. Electrical Conductivity and Total Dissolved Solute EC and TDS were determined using Hanna instrument. 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. The physico-chemical parameters reading were taken on bi-weekly basis. The dissolved oxygen was measured on a bi-weekly basis through the Wrinkler's method described by APHA (2005).

3.13 Data Analysis

The student T-test was used to determine the proximate and anti-nutritional compositions of soya bean and soyabean offal meal. The data collected at the end of the experiment was subjected to a one way Analysis of Variance (ANOVA) using the statistical package (SPSS) software version 20.0. The mean was further separated using Duncan's multiple range test at the 95% confidence limit.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Soya bean offal and Soya bean Meal

The proximate composition of soya bean offal meal and soya bean meal are shown in Table 4.1. There was significant difference ($p \leq 0.05$) in moisture contents of the samples where by it was higher in the soya bean meal with 8.93g/100g DM than soya bean offal meal with the value of 2.10 g/100g DM, The ash content was significantly higher ($p \leq 0.05$) in soya bean meal (4.15 g/100g DM), than the soya bean offal meal (3.32 g/100g DM). There was significant different ($p \leq 0.05$) in lipid content between soya bean meal and soya bean offal meal, it was significantly higher in the soya bean meal (18.38 g/100g DM) ($p \leq 0.05$) than the soya bean offal meal (11.98 g/100g DM). Protein value of the soya bean meal (44.00 g/100g DM) was significantly higher ($p \leq 0.05$) than protein content of soya bean offal meal (24.17 g/100g DM). There was significant difference in the crude fibre and carbohydrate (NFE) values between the soya bean meal and soya bean offal meal, where by crude fibre 12.31 ± 0.05^{a} and carbohydrate 58.48 ± 0.01^{a} content were significantly higher ($p \leq 0.05$) in soya bean offal meal than the soya bean meal 5.12 ± 0.4^{b} and 40.81 ± 0.2^{b}.

4.2 Anti-nutritional Factors in Soyabean offal and soya bean meal

The result obtained for Anti-nutritional Factors ANFs in soyabean offal and soyabean meal is presented In Table 4.2. The concentration of oxalate was significantly higher in soya bean meal (3.5mg/g) than the soya bean offal meal (2.50mg/g) ($p \leq 0.05$), so also the percentage concentration of Phytate 6.3 ± 0.06^{a}, Tannin 21.5 ± 1.3^{a}, Saponin 6.2 ± 0.91^{a} and Cyanogen 8.1 ± 0.3^{a} were significantly higher ($p \leq 0.05$) in the soya bean meal than the soya bean offal meal 4.30 ± 0.067^{b}, 1.55 ± 0.1^{b}, 1.26 ± 0.2^{b} and 5.30 ± 0.2^{b} respectively. But the concentration of flavonoid 14.20 ± 1.4^{a} and

Trypsin Inhibitor13.20±0.3[a] were significantly higher ($p \leq 0.05$) in the soya bean offal meal than the soya bean meal 2.1±0.2[b], and 5.9±0.01[b] respectively.

Table 4. 1 : Proximate Composition of Soya bean offal Meal and Soya bean Meal

Samples (g/100g DM)	SBOFFM	SBM
Moisture	2.10 ± 0.05^{b}	8.93 ± 0.1^{a}
Ash	3.32 ± 0.06^{b}	4.15 ± 0.02^{a}
Crude Lipid	11.98 ± 0.01^{b}	18.38 ± 1.2^{a}
Crude Protein	24.17 ± 0.05^{b}	44.00 ± 0.2^{a}
Crude Fibre	12.31 ± 0.05^{a}	5.12 ± 0.4^{b}
N.F.E	58.48 ± 0.01^{a}	40.81 ± 0.2^{b}

Means with different superscript letter (s) across the rows are significantly different ($p\leq0.05$)

Key:
SBOFFM: Soya bean offal meal
SBM: Soya bean meal

Table 4. 2 : Anti-nutritional Factors (mg/g) of Soya bean Offal and Soya bean Meal

Anti-Nutrients	SBOFFM	SBM
Oxalate	2.50 ± 0.08^{b}	3.50 ± 0.07^{a}
Phytate	4.30 ± 0.067^{b}	6.3 ± 0.06^{a}
Tannin	1.55 ± 0.1^{b}	21.5 ± 1.3^{a}
Trypsin Inhibitor	13.20 ± 0.3^{a}	5.9 ± 0.01^{b}
Saponin	1.26 ± 0.2^{b}	6.2 ± 0.91^{a}
Flavonoid	14.20 ± 1.4^{a}	2.1 ± 0.2^{b}
Alkaloid	12.30 ± 0.7^{a}	10.20 ± 0.8^{b}
Cyanogen	5.30 ± 0.2^{b}	8.1 ± 0.3^{a}

Means with different superscript letter(s) across the rows are significantly different ($p\leq0.05$)

Key:
SBOFFM: Soya bean offal meal
SBM: Soya bean meal

4.3 Proximate composition of the Experimental diets (g/100g DM)

There was no significant difference in the moisture content, Dry matter, Ash and crude fibre in all the inclusion levels (Table 4.3). The Lipid content was significantly higher ($p \leq 0.05$) at 0% inclusion level (22.60 ± 0.72^{a}), 25% ($22.53 \pm 0.44^{a)}$ and 50% (22.47 ± 0.66^{a}) inclusion levels than 75% (21.40 ± 0.66^{b}) and 100% (19.34 ± 1.38^{c}) inclusion levels. Crude Protein was significantly higher ($p \leq 0.05$) at 25% (25.34 ± 1.93^{a}) and 100% (25.12 ± 0.70^{a}) inclusion levels than 0% (24.82 ± 0.72^{b}), 50% (24.75 ± 1.01^{b}) and 75% (24.21 ± 0.43^{b}) inclusion levels. Nitrogen Free Extract was higher (NFE) ($p \leq 0.05$) at 100% inclusion level (34.39 ± 0.9^{a}) than 0% (33.3 ± 0.4^{b}), 25% (31.41 ± 0.7^{c}), 50% (32.66 ± 1.4^{c}) and 75% (31.67 ± 1.8^{c}) inclusion levels.

4.4 Growth rate and feed utilization of *O. niloticus* fed at different inclusion levels.

The growth parameters as shown in Table 4.4 observed in this growth study revealed that the Mean Weight Gain (MWG) was apparently highest ($p \leq 0.05$) in T2 (25%) 21.97 ± 4.29^{a} followed by T1 (0%) 18.42 ± 8.17^{b} and T4 (75%) 17.33 ± 3.95^{b} and least in T3 (50%) 13.75 ± 2.01^{c} and T5 (100%) 12.65 ± 2.93^{c}. The mean length gain was also higher ($p \geq 0.05$) in T2 (25%) 1.76 ± 0.23^{a} than T5 (100%) 1.11 ± 0.12^{a}. The specific growth rate (SGR) was apparently higher ($p \geq 0.05$) in T2 (25%) 0.008 ± 0.3^{a} and lower in T5 (100%) 0.005 ± 0.4^{a} all the treatments showed no significant difference ($p \geq 0.05$) in specific growth rate (SGR). There was no significant difference observed in the feed conversion ratio (FCR) of T1 (0%), T2 (25%), T3 (50%), T4 (75%) and T5 (100%) ($p \geq 0.05$). The gross feed conversion efficiency (GFCE) was significantly higher ($p \leq 0.05$) in T2 (25%) 200.00 ± 0.4^{a} than in T1 (0%) 166.66 ± 0.1^{b} and T4 (75%) respectively and least in T3 (50%) 142.85 ± 1.2^{c} and T5 (100%) respectively. There was significant difference in the feed efficiency ratio (FER) among the treatments it was significantly higher ($p \leq 0.05$) in T2 (25%) 1.89 ± 0.4^{a} and least in T3 (50%) 1.26 ± 0.1^{c} and T5 (100%) 1.27 ± 0.13^{c} . There was

significant difference in the net protein utilization (NPU), it was significantly higher ($p \geq 0.05$) in T2 (25%) 150.35 ± 0.2^{a} followed by T1 (0%) 146.53 ± 0.1^{b} and T3 (50%) 145.17 ± 0.5^{b} and least in T4 (75%) 134.65 ± 1.2^{c} and T5 (100%) 115.72 ± 1.4^{d} . There was also significant difference in the protein efficiency ratio (PER) it was highest ($p \geq 0.05$) in T2 (25%) 0.867 ± 0.5^{a} followed by T1 (0%) 0.742 ± 0.3^{b} and T4 (75%) 0.715 ± 0.1^{b} and least in T3 (50%) 0.555 ± 0.1^{c} and T5 (100%) 0.503 ± 0.5^{c} respectively.

Table 4. 3 : Proximate Composition of Experimental Diets Fed to *Oreochromis niloticus* (g/100g DM)

Parameters	Inclusion Levels				
	0%	25%	50%	75%	100%
Dry Matter	101.78±0.00[a]	101.02±0.00[a]	101.85±0.00[a]	99.53±0.00[a]	100.39±0.00[a]
Moisture	8.40±0.76[a]	8.71±0.44[a]	8.87±0.13[a]	8.96±0.25[a]	8.86±0.06[a]
Lipid	22.60±0.72[a]	22.53 ±0.44[a]	22.47±0.66[a]	21.40±0.66[b]	19.34±1.38[c]
Ash	7.23±0.49[a]	7.69±0.39[a]	7.64±1.25[a]	7.53±0.71[a]	7.44±0.61[a]
Crude Protein	24.82±0.72[b]	25.34±1.93[a]	24.75±1.01[b]	24.21±0.43[b]	25.12±0.70[a]
Crude Fibre	5.43±0.64[a]	5.34±1.28[a]	5.46±0.85[a]	5.76±0.98[a]	5.24±0.12[a]
N.F.E	33.3±0.4[b]	31.41±0.7[c]	32.66±1.4[c]	31.67±1.8[c]	34.39±0.9[a]

Means with different superscript letter(s) across the rows are significantly different ($P \leq 0.05$)

Table 4. 4 : Growth Rate and Feed Utilization of *O. niloticus* Fed at Different Inclusion Levels

Growth Parameters	T1	T2	T3	T4	T5
MIW (g)	6.27±1.19[a]	5.27±1.37[b]	5.20±1.20[b]	6.67±1.33[a]	6.67±1.33[a]
MFW (g)	24.69±7.04[b]	27.23±4.22[a]	18.95±0.92[c]	24.00±2.63[b]	19.31±1.81[c]
MWG(g)	18.42±8.17[b]	21.97±4.29[a]	13.75±2.01[c]	17.33±3.95[b]	12.65±2.93[c]
MLG (cm)	1.39±0.19[a]	1.76±0.23[a]	1.36±0.13[a]	1.17±0.09[a]	1.11±0.12[a]
SGR (g)	0.007±0.1[a]	0.008±0.3[a]	0.006±0.2[a]	0.006±0.1[a]	0.005±0.4[a]
FCR (g)	0.61±0.01[a]	0.52±0.02[a]	0.78±0.01[a]	0.62±0.01[a]	0.78±0.04[a]
GFCE (%)	166.66±0.1[b]	200.00±0.4[a]	142.85±1.2[c]	166.66±1.3[b]	142.85±1.3[c]
FER (g)	1.64±0.1[b]	1.89±0.4[a]	1.26±0.1[c]	1.59±0.2[b]	1.27±0.13[c]
NPU (%)	146.53±0.1[b]	150.35±0.2[a]	145.17±0.5b[b]	134.65±1.2[c]	115.72±1.4[d]
PER (g)	0.742±0.3[b]	0.867±0.5[a]	0.555±0.1[c]	0.715±0.1[b]	0.503±0.5[c]

Means with different superscript letter(s) across the rows are significantly different ($p \leq 0.05$)

Key:
MIW = Mean Initial weight
MFW = Mean Final weight
MWG = Mean weight gain
PER = Protein efficiency ratio
MLG = Mean length gain
NPU = Net protein utilization
SGR = Specific growth rate
FER = Feed efficiency ratio
FCR = Feed conversion ratio
GFCR = Gross feed conversion ratio

4.5 Carcass Composition of *O. niloticus* (g/100g DM)

The proximate composition of *O. niloticus* carcass before and after the experiment is shown in Table 4.5. There was significant different ($p \leq 0.05$) in Crude protein, Crude lipid, Ash and Nitrogen Free Extracts in the fish samples before and after the experiment. Crude fibre concentration differ significantly ($p \leq 0.05$). Similar observation was made in ash (before = 10.54 ± 0.00^{d} g/100g DM ; after = 16.72 ± 0.00^{a} g/100g DM) and protein value (before = 28.95 ± 0.02^{d} g/100g DM; after = 38.10 ± 0.20^{a} g/100g DM). The concentration of lipid (before = 27.41 ± 0.61^{d} g/100g DM; after = 35.41 ± 0.14^{a} g/100g DM) and NFE (before = 19.35 ± 0.45^{a} g/100g DM ; after = 10.04 ± 1.04^{b} g/100g DM) Nitrogen free extracts concentration was higher in the fish samples before the experiment than after the experiment.

4.6 Physico-chemical Parameters of water Monitored in the experimental tanks

Table 4.6 shows the physicochemical properties of water during the experimental period. There was no significant difference ($p \geq 0.05$) in DO concentration T5 (2.15 ± 0.04^{a} mg/l) had slightly higher value, than T1 (2.08 ± 0.04^{a} mg/l) which had the least concentration. Similarly, there was no significant difference ($p \geq 0.05$) in temperature, pH, TDS and EC of the water. Nonetheless, T3 (23.26 ± 0.31^{a} °C) had the highest temperature, while T4 (23.06 ± 0.34^{a} °C) had the least; for pH it was also higher in T3 (8.32 ± 0.11^{a}) and lowest in T4 (8.17 ± 0.08^{a}); Total Dissolved Solutes of water, was highest in T1 (287.44 ± 8.20^{a} mg/l) and lowest in T2 (269.59 ± 16.13^{a} mg/l); Electrical conductivity, was highest and lowest in the control T1 ($575.17 \pm 16.16.28^{a}$ µ/S) and T3 (523.32 ± 39.61^{a} µ/S) respectively.

Table 4. 5 : Carcass Composition of *O. niloticus* after the experiment (g/100g DM)

		Inclusion Levels of Soya Bean Offal Meal				
Parameters	Initial	0%	25%	50%	75%	100%
Crude Protein	28.95±0.02^{d}	36.37±0.02^{b}	38.1±0.20^{a}	35.93±0.05^{b}	32.60±0.79^{c}	29.07±0.45^{d}
Crude Fibre	0.00±0.00^{a}	0.00±0.00^{a}	0.00±00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Crude Lipid	27.41±0.61^{d}	35.41±0.14^{a}	31.30±0.23^{b}	32.27±0.06^{b}	33.34±0.08^{b}	29.35±0.14^{c}
Ash	10.54±0.00^{d}	12.45±0.00^{c}	11.22±0.00^{d}	14.27±0.00^{b}	14.88±0.03^{b}	16.72±0.00^{a}
Moisture	11.24±0.05^{c}	12.23±0.21^{c}	9.33±0.00^{d}	13.41±0.01^{b}	14.57±0.02^{a}	15.76±0.04^{a}
Nitrogen Free Extracts	19.35±0.45^{a}	3.53±1.05^{d}	10.04±1.04^{b}	4.11±1.06^{c}	4.6±0.06c	9.10±1.05^{b}

Means with different superscript letter(s) across the rows are significantly different ($p \leq 0.05$)

Table 4.6: Monitored Physicochemical Parameters

	Physicochemical parameters				
Treatment	Temperature (°c)	pH (1-12)	EC (µ/S)	TDS (mg/l)	DO (mg/l)
T1	23.16±0.32[a]	8.37±0.10[a]	575.17±16.28[a]	287.44±8.20[a]	2.08±0.04[a]
T2	23.07±0.34[a]	8.20±0.11[a]	540.00±32.10[a]	269.59±16.13[a]	2.12±0.05[a]
T3	23.26±0.31[a]	8.32±0.11[a]	523.32±39.61[a]	276.94±12.70[a]	2.09±0.08[a]
T4	23.06±0.34[a]	8.17±0.08[a]	564.61±21.36[a]	280.92±10.86[a]	2.11±0.06[a]
T5	23.21±0.30[a]	8.21±0.09[a]	538.47±25.72[a]	271.75±12.96[a]	2.15±0.04[a]

Means with different superscript letter (s) across the column are significantly different ($p \leq 0.05$)

Key:
DO = dissolved oxygen
pH = Hydrogen ions concentration
T1-T5 = Treatments 1-5

4.7 Cost and quantity of feed ingredients used in formulating diets

The cost implication of feed formulation for *O. niloticus* is shown in Figure 4.1. Yellow maize meal (1.475 g) had the highest quantity formulated, followed by soybean and soybean offal meal with 630g each, followed by Fish meal with (420.1g). The quantity of Palm oil (21g), Methionine and lysine 12g each, Bone meal (6g), Sodium chloride (4.8 g) and Vitamin Premix (4.2 g) were the least used for the formulation of feed. The cost implication of Yellow maize meal (₦ 532.6) was the highest, followed by Fish meal (₦ 420.1), Palm oil (₦ 210), Soya bean meal (₦ 73.09), Soya bean offal meal (₦ 25) and Methionine (₦ 19.2). The following product cost less in this order: Lysine (₦9.6), Vitamin Premixes (₦2.1) Sodium chloride (₦0.48) and Bone meal (₦ 0.42).

4.8 Cost effectiveness of formulated feed

There was no significant difference ($p \geq 0.05$) in the Economic convertion ratio (ECR) it was significantly higher in diet 3 ($588.924^{a)}$ and diet (4588.924^{a}) and least in diet 1 (574.56^{a}),diet 2 (574.56^{a}) and diet 5 (574.56^{a}) respectively. Incidence cost (IC) was significantly ($p \leq 0.05$) higher in diet 4 (21.38^{a})and least in diet 2 (14.74^{d}). There was significant difference ($p \leq 0.05$) in the Profit index it was higher in diet 2 (0.081^{a}) and least in diet 4 (0.056^{d}) .

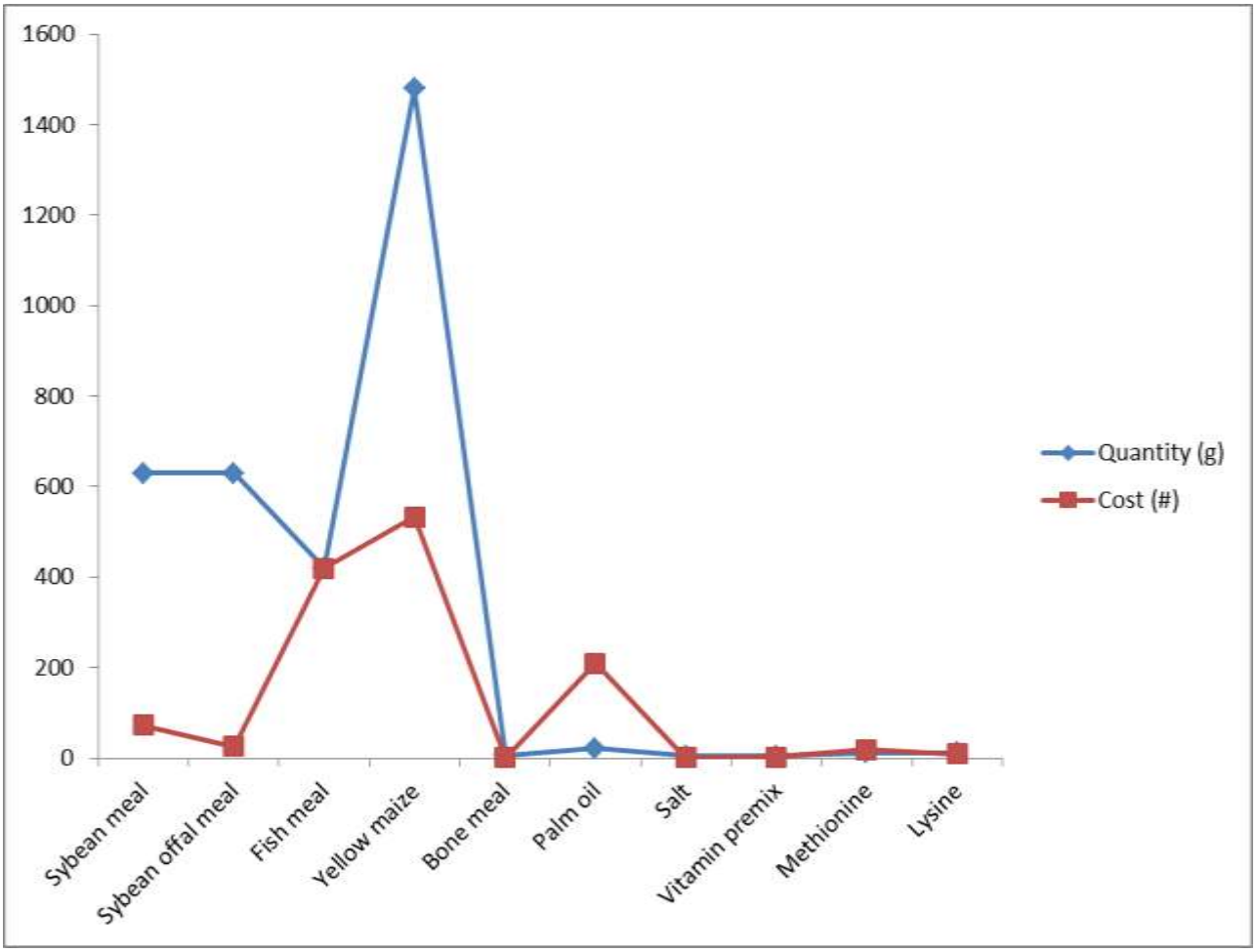


Figure 4. 1 : Cost and quantity of feed ingredients used in formulating of diets for *O.niloticus*

Table 4. 6 : Cost effectiveness of feed formulation

Economics Factors ₦	D1	D2	D3	D4	D5
ECR	574.56[a]	574.56[a]	588.924[a]	588.924[a]	574.56[a]
IC	16.96[c]	14.74[d]	19.54[b]	21.38[a]	17.73[c]
PI	0.070[b]	0.081[a]	0.061[c]	0.056[d]	0.067[c]

Means with different superscript letter (s) across the rows are significantly different ($p \leq 0.05$)

Key:
ECR = Economic conversion ratio
IC = Incidence cost
PI = Profit index

CHAPTER FIVE

5.0 DISCUSSION

5.1 The Proximate and Anti-nutritional Composition of Soya bean and Soya bean offal Meal

The proximate composition results obtained from this study showed that the concentration of moisture for soya bean offal meal was significantly lower ($p<0.05$) than that of soya bean meal, this makes the soya bean offal to have more storage life. Likewise the concentration of Ash, Lipids and Crude protein were higher in the soya bean meal ($p<0.05$) than the soya bean offal meal because traditional processing significantly improved the nutritional quality of soybean and reduced the anti- nutritional properties of soybean (El-Saidy and Gaber 2002). The crude Fibre and Carbohydrates were significantly higher ($p<0.05$) in the soya bean offal meal than the soya bean meal. *O.niloticus* has a preference for plant protein as it possesses morphological and physiological adaptations for diets high in fibre content (El-Saidy and Gaber 2002).

The anti-nutritional composition results obtained from this study revealed that the concentration of Oxalate, Phytate, Tannin and Saponin were significantly higher in the soya bean meal ($p<0.05$) than the soya bean offal meal but the concentration of Flavonoid, Alkaloid and Trypsin inhibitor were higher ($p<0.05$) in soya bean offal meal than the soya bean meal, traditional processing methods of toasting and soaking significantly improved the nutritional quality of soya bean and reduced the anti- nutritional properties of soya bean (El-Saidy and Gaber, 2002).The determined levels of anti-nutritional factors showed a Saponin concentration of 1.26mg/100g/dry matter which disagreed with the report of Danladi (2015) who found saponin concentration from Parkia pulp of 17.45 ± 0.41mg/100g/dry matter, and the report of Germah *et al*. (2007) who reported a Saponin concentration of 17.80mg/100g/dry matter for the amount was

found to be much lower than some other everyday consumed legumes like Lima Beans 24.50mg/100g and Millet 19.47mg/100g as reported by Gernah *et al*. (2007). The tannin concentration of 1.55mg/100g/dry matter was recorded which disagreed with report of Danladi (2015) who recorded tannin levels of 27.0 ± 0.41mg/100g/dry matter from Parkia pulp meal and the findings of Germah *et al*. (2007) who reported a tannin level of 81.00mg/100g from Perkia pulp meal. Tannin reduces protein solubility by combining with it to form a complex there by reducing its bioavailability. The phytate content of 4.30% was recorded which disagreed with the report of Umar (2006) who reported a phytate of raw cowpea senna of 112.40 mg/ml reduced to 100.20 mg/ml in parboiled cowpea senna. because heating reduces both water and phytate acid extractible from legumes, This was in accordance with Lv *et al*. (2017) who reported that low dietary protein observed in castor seed resulted from heat denaturation and destruction. The proximate composition of the experimental diets revealed that diet 1 (0%), diet 2 (25%) and diet 5 (100%) inclusion levels were higher ($p \leq 0.05$) than diet 3 (50%) inclusion level. The crude lipid content of 11.98 ± 0.01^{b} was recorded which agreed with the report by Lv *et al*. (2017) on replacement of dietary saturated fats with other types of fats and carbohydrates.

5.2 Growth Performance and Nutrients Utilization of *O. niloticus*

The study was conducted with the aim to evaluate the growth response of *O. niloticus* fingerlings fed soya bean offal meal in various inclusion levels, replacing soya bean meal. The growth performance of the treatments showed that T2 25% inclusion level had the highest Mean Weight Gain (MWG), (SGR) and crude proteins of *O. niloticus* carcass. The highest Mean Weight Gain (MWG) observed in T2 (25%) agreed with partial soya bean meal inclusion diets by Al-Kenawy *et al*. (2008), Borgeson *et al*. (2006), Furuya *et al*. (2004) and

El-Saidy and Gaber (2003). The differences were significant ($P<0.05$). The Mean Length Gain (MLG) was significantly highest in T2, this result disagreed with the results of Umar (2006) who found the Mean Length Gain (MLG) was highest in Diet D (25.41%) of Parboiled senna that had the highest Mean Length Gain MLG value of 1.184 and percentage length gain of 15.260%. The least MLG value came from diet C 0.5% of Raw senna. The differences among the values were significant ($p\leq0.05\%$). Specific Growth Rate (SGR) was significantly higher in T2 and least in and T5. The study revealed that the Feed Efficiency Ratio (FER) was highest in T2 and also the Net Protein Utilization (NPU) was significantly highest in T2 (25%) (25CP). The Feed Conversion Ratio (FCR) and Gross Feed Conversion Ratio (GFCR) were significantly higher in T2 (25) the differences were significant ($P\leq0.05$). This was documented by Siddiqui and Khan (2009) who said that the higher the Specific Growth Rate and the smaller the Feed Conversation Ratio. Toasting the seeds (soya bean) at higher temperatures than the recommended values of 121^0C-130^0C denatures the essential amino acids especially lysine, hence rendering the proteins in soya bean useless at high temperature.

However at lower temperature, the anti- nutrients were still active. The significant difference observed in the growth parameters was due to nutrients, such as amino acids and minerals, presence of toxic substance or anti-nutrients factors, or decrease of palatability and pallet water stability value in fish diets in some cases reduced growth and poor feed efficiency. Eyo (2001) reported that the use of inferior feedstuffs affect the digestibility and nutritive value of the compounded feeds. Eyo (2001) also was of the opinion that apart from nutrition, other factors such as anti-nutrients could be responsible for low values in growth indices. The growth parameters results obtained in this study agreed with the report of growth and nutrient utilization that were significantly high ($p<0.05$) in the partially soya bean meal (PSM) and only

soya bean meal plus methionine groups(OSM+M) , in contrast to no soya bean meal (NSM)and only soya bean meal group (OSM). This indicates that partial replacement of fish meal with soybean meal did not compromise growth.

Similar result was reported with partial replacement of up to two third of fishmeal with soybean meal in the diet of tilapia (El-Saidy and Gaber, 2002). Similar results were obtained with partial soya bean meal inclusion diets by Al-Kenawy *et al*. (2008), Borgeson *et al*. (2006), Furuya *et al*. (2004) and El-Saidy and Gaber (2003). However, total replacement caused a reduction in growth and feed utilization as exhibited by the only Soya bean Meal (OSM) groups. Soya bean was reported to contain approximately 30% carbohydrate of which oligosaccharides make up 10% and Non Starch Polysaccharides (NSP) make up the remaining 20% (Storebakken *et al*. 2000). The carbohydrates are not digestible by monogastric animals, with some components having antinutritional effects. The low growth and nutrient utilization in fish fed diet with complete replacement of fishmeal with soya bean meal could be attributed to an increase in the carbohydrate component of the diet. Also, Koumi *et al*. (2008) observed lower growth in *O. niloticus* when fishmeal was totally replaced by soya bean meal. Davis *et al*. (2005) reported a decrease in growth response of juvenile red snapper, *Lutjanus campechanus* when soya bean meal was totally used as a replacement for fishmeal. Methionine supplement improved growth and nutrient utilization parameters in *O. niloticus*.

This result is in agreement with Francis *et al*. (2001) who reported improved growth when diets of tilapia were supplemented with crystalline methionine. In contrast, El-Saidy and Gaber, (2002) reported that feeding blue tilapia and Nile tilapia, respectively with 100% SBM, firstly with or without methionine supplementation had no significant effect on growth parameters and feed utilization. Meanwhile, the reduced growth rate observed in the no soya

beans meals NSM group could be due to the fact that *O. niloticus* has a preference for plant protein as it possesses morphological and physiological adaptations for diets high in fibre content. Ayoola, (2010) opined that the protein quality of the feed fed the fish determines whether the feed material accepted by the fish is balanced or not. Proximate analysis of the carcass of partially soya beans meals PSM group revealed high percentage dry matter and crude protein. The highest crude fat and ash was obtained in soya beans meals plus methionine SBM+M group and this may be due to the availability of sufficient energy in the diet. This assertion is supported by Abdelghany (2003), who reported that diets containing soya bean meal for *O. niloticus* increased body crude protein.

5.3 Carcass Composition of *O. niloticus*

The carcass proximate composition of the experimental fish indicated a significant difference ($p<0.05$) in the crude protein content of the carcass fish fed all the various diets as compared to the initial. This suggested a reasonable level of protein retention due to optimal utilization of the protein for growth and development (Alegbeleye *et al.*, 2004). The crude protein was highest in T2 (25%) inclusion level . This indicated that the anti-nutrients present in Diet 2 were low enough to prevent protein utilization by the fish. Apart from the nutritional aspect, other factors that could be responsible for the low values recorded for the growth indices may be attributed to processing, drying or storage methods used.

The physiological status of the fish were also unknown (Eyo, 2001). The aquaria were also siphoned weekly instead of bi-weekly and it was observed that the less the frequency of water replacement, the better the growth performance and survival rate of fish (Eyo, 2001). The Ash contents of the carcass showed that sample A (T1) had 2.9% that was the highest Ash content this sample had low FER, NPU and PER. Decrease in Ash content generally goes with an

increase in crude lipid and protein, was in line with the report of Nwanna (2003) who described that ash and moisture contents of Africa catfish decreased after feeding trials, while the crude protein and lipid contents increased. It was observed that crude lipid/fat of the carcass of sample D (24.39 %) was higher than sample A (21.80%). The lipid content was observed to increase with crude protein of the diets. The differences were significant ($P<0.05$). Generally the carcass crude protein increases appreciably after the feeding trials. The differences were significant ($p<0.05$). This followed the report of Nwanna (2003), who said that crude protein and liquid content increased after feeding trials. The crude fibre (CF) content indicated appreciable increase with crude protein contents of the diets with T5 (100%) having the highest crude fibre content of 0.42%. the least CF came from T3 (0.40%) the differences were significant ($P<0.05$).

The crude fibre contents however were generally below 1% in all the samples. (Eyo 2001) reported that the use of inferior feed stuffs could affect the digestibility and nutritive value of compounded feeds. Therefore the relatively low performance could be associated with anti-nutients present in the experimental diets. The carbohydrate content of T1 (34.55%) was the highest while that of T3 (32.47%) was the least. This was also associated with the quality of the feedstuff and the processing method. The differences were significant ($P<0.05$) among the values. The variation in growth indices might be attributed to fact that the fish used was from an unknown parental stock. Their ages and physiological status were also unknown. Also frequency of water replacement. The quality of pelleted feed might have also contributed in the variation of growth indices. This has been attributed to the fact that the feed is prone to leaching of nutrients in to water due to poor stability and degradation of the feed to the bottom of water at feeding. This has been shown to cause significant losses in Agricultural management (Falayi, 2003). The carcass proximate composition indicate that there was significant differences

(P<0.05) among the samples crude protein, Ash, Crude lipids, Crude fibre and carbohydrate contents.

5.4 Cost effectiveness of feed formulation and Physico-chemical Parameters of water in experimental tanks.

The cost effectiveness of feed formulation for *O. niloticus* showed that, there was significant difference in the Economic conversion ratio(ECR) (P≤0.05) diet 3 and diet 4 were significantly higher than diet 1, diet 2 and diet 5. Incidence cost (IC) was significantly highest in diet 4 (D4) (P≤0.05) and least in diet 2 (D2). There was significant difference in the Profit index it was higher in diet 2 and least in diet 4. Survival rate was comparable among the treatments (P≥0.05). The high survival rate of (87.5%) obtained in this study could be attributed to good management procedures during the study i.e. proper handling and storage of feed to avoid mould growth and contamination by rodents, proper handling of fish during sampling and conducive physico-chemical parameters within the culture system. Cury *et al.* (2011) reported that a temperature range of 22°C – 27°C, pH range of 6.5 – 9.0 and dissolved oxygen range of 6.3mg/l – 9.6mg/l gives the best growth in cultured tropical fishes. Nwanna *et al.* (2004) reported a temperature range of 23°C - 27°C, pH 6.3 – 7.8 and dissolved oxygen of 6.3mg/l – 9.6mg/l for the optimal growth and development of *O. niloticus* cultured under aquaria conditions. However, In the present experiment, it is clear that inclusion levels of soya bean offal meal had no effect on the survival rate of fish which was in agreement with Diaa Al-kanewy *et al.* (2008).

CHAPTER SIX

6.1 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.2 SUMMARY

The study was conducted to investigate the effect of replacing soya bean meal with soya bean offal meal in the diet of Nile tilapia *O. niloticus* fingerlings and to determine its growth performance. Fifteen (15) plastic rearing tanks (30cm x 45cm x 35cm depth) were used for the experiment. Plastic rearing tanks were randomly assigned in three replicates to five treatments. Three hundreds (300) fingerlings of *O. niloticus* of mixed sexes and sizes of initial mean weight of (6.0 ± 2.04^{a} g) were stocked in fifteen (15) white rectangular plastic tanks of water holding capacity of 50 litres (30cm x 45cm and 35cm depth) with twenty (20) fingerlings per tank and water maintained at 40 litres level for a period of twelve (12) weeks. Soya bean Offal meal (SBOFFM) was used to replace Soya bean meal (SBM) at inclusion levels of 0%, 25%, 50%, 75% and 100% for Diet 1 to 5 respectively. T1- 24.82 ± 0.72^{b} CP, T2- 25.34 ± 1.93^{a} CP, T3- 24.75 ± 1.01^{b} CP, T4- 24.21 ± 0.43^{b} CP and T5- 25.12 ± 0.70^{a} CP. Total growing period was 97 days (14 days of acclimatization and 83 days of feeding). The results obtained showed T2 (25.34 ± 1.93^{a}) (25%) inclusion Soya bean Offal had the highest Mean Weight Gain (MWG) of (21.97 ± 4.29^{a} g), Mean Length Gain (MLG) of (1.76 ± 0.23^{a} cm), Protein Efficiency Ratio (PER) of 0.867 ± 0.5^{a} g, Net Protein Utilization (NPU) of 150.35 ± 0.2^{a} %, Gross Feed Conversion Efficiency (GFCE) of 200.00 ± 0.4^{a} , Feed Efficiency Ratio (FER) of 1.89±0.4a , and crude protein of *O. niltocus* carcass (38.1 ± 0.20^{a} g/100g DM). Feed Conversion Ratio (FCR) and Specific Growth Rate showed no significant difference ($P\geq0.05$). Also, pH (1-12), Temperature (T°C), Dissolved Oxygen (DO) (mg/l), Electrical Conductivity (µ/S), and Total Dissolve Solute (TDS) (mg/l) showed no significant difference ($P \geq0.05$). The study results demonstrate that

soybean offal meal could replace the soya bean meal in the diet of Nile tilapia without negative effects on growth, or on total production and even leading to high net economic returns as shown in the diets with 25% CP crude protein from plant source.

6.3 CONCLUSION

There are significant differences ($p \leq 0.05$) in the proximate and anti-nutritional composition between soya bean meal and soya bean offal meal, where by soya bean meal has higher ($p<0.05$) concentration of crude protein (44.00 ± 0.2^{a}) and crude lipid (18.38 ± 1.2^{a}) than soya bean offal meal (24.17 ± 0.05^{b}) and (11.98 ± 0.01^{b}) respectively, but soya bean offal meal has higher ($p<0.05$) concentration of nitrogen free extracts (58.48 ± 0.01^{a}) and crude fibre (12.31 ± 0.05^{a}) than the soya bean meal (40.81 ± 0.2^{b}) and (5.12 ± 0.4^{b}). However the concentration of tannin (21.5 ± 1.3^{a}) and sapon in (6.2 ± 0.9^{a}) were higher ($p<0.05$) in the soya bean meal than the soya bean offal meal (1.55 ± 0.1^{b}) and (1.26 ± 0.2^{b}) accordingly, but soya bean offal meal has higher ($p<0.05$) concentration of trypsin inhibitor (13.20 ± 0.3^{a}), flavonoid (14.20 ± 1.4^{a}) and alkaloid (12.30 ± 0.7^{a}) than the soya bean meal (5.9 ± 0.01^{b}), (2.1 ± 0.2^{b}) and (10.20 ± 0.8^{b}) respectively.

Nile tilapia (*O.niloticus*) growth was better in T2 (25.34CP) (25%) Soya bean offal Inclusion had the highest Mean Weight Gain ((21.97 ± 4.29^{a} g), Mean Lengh Gain (MLG) of (1.7 ± 0.23^{a} cm), Protein Efficiency Ratio (PER) of (0.867 ± 0.5^{a} g), Net Protein Utilization (NPU) of (150.35 ± 0.2^{a} %) Gross feed conversion efficiency (GFCE) of 200.00 ± 0.4^{a} and crude protein of O. *niltocus* carcass (38.1 ± 0.20^{a} g/100g DM).This indicated that T2 25% inclusion (25.34 ± 1.93^{a} CP) is the best diet that support the growth of *O.niloticus* during the experiment.

The study demonstrated that soybean offal meal could replace the soya bean meal in diet for Nile tilapia reared in plastic rearing tanks without negative effects on growth, or on total production and even leading to high net economic returns.

6.4 RECOMMENDATIONS

1- Soya bean offal meal can be used to replace the soya bean meal in the diet of Nile tilapia at 25% replacement.

2- The anti-nutritional composition of soya bean offal that includes Flavonoid (14.20 ± 1.4^{a}) Trypsin Inhibitor (13.20 ± 0.3^{a}) and Alkaloid (12.30 ± 0.7^{a}) should be lowered through the use of other processing methods.

3- The use and effect of Soyabean Offal meal on survival, growth and nutrient utilization of other culturable fresh water fishes should be investigated.

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APPENDICES



Appendix I : Soya bean plant (*Glycine max*,Linn)



Appendix II : Soya bean seed (*Glycine max*, Linn)



Appendix III : Soya bean offal meal (*Glycine max* Linn*).*



Appendix IV : Pelleted Fish feed Formulated with Soya bean offal Meal



Appendix V : Cross section of experimental set-up

Note: a - Inlet from tank

b - Inlet to plastic ponds

c – Outlet



Appendix VI : Lateral view of *O.niloticus* after the experiment