REPLACEMENT OF *GLYCINE MAX* (L.) Merr. WITH *TAMARINDUS INDICA* L. SEED IN THE DIET OF *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) FINGERLINGS

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE DEGREE IN FISHERIES

DEPARTMENT OF BIOLOGY

FACULTY OF LIFE SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

NOVEMBER, 2017

DECLARATION

I declare that the work in this dissertation entitled **"REPLACEMENT OF *GLYCINE MAX* (L.) Merr. WITH *TAMARINDUS INDICA* L. SEED IN THE DIET OF *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) FINGERLINGS"** has been carried out by me in the Department of Biology, Ahmadu Bello University, Zaria. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

BASHIR, Abdulmalik Yusuf _____ _____

P14SCBS8028 Signature Date

CERTIFICATION

This dissertation entitled **"REPLACEMENT OF *GLYCINE MAX* (L.) Merr. WITH *TAMARINDUS INDICA* L. SEED IN THE DIET OF *OREOCHROMIS NILOTICUS* (LINNAEUS, 1758) FINGERLINGS"** by **BASHIR ABDULMALIK YUSUF** meets the regulations governing the award of the degree of Master of Science (M. Sc.) in Fisheries of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

Effect of processing on *T. indica* seed nut and growth response of *O. niloticus* fingerlings fed graded levels of fermented *T. indica* seed meal was investigated for 84 days. Fermented and roasted *T. indica* seed had 94.27 and 99.21; 21.44 and 26.86; 3.99 and 4.96; 6.73 and 7.03; 3.73 and 1.73; and 64.12 and 58.62% dry matter, crude protein, crude fibre, lipid, ash and nitrogen free extracts, respectively while alkaloid, saponin, tannin and phytate were in concentrations 1.50 and 6.8; 1.80 and 4.1; 4.22 and 2.38; and 2.68 and 7.71 mg/g^{-1}, respectively. Fermentation and roasting significantly increased the crude protein and lipid content of the seeds by 10.17 and 38.03; and 46.62 and 53.16%, respectively while alkaloid, saponin and phytate content of the fermented seeds were reduced by 59.46, 21.74 and 60.30%, respectively. Five diets were formulated at 30% crude protein; which were D_1 (control: 0% inclusion of fermented *T. indica* seed meal), D_2 (25% inclusion of fermented *T. indica* seed meal), D_3 (50% inclusion of fermented *T. indica* seed meal), D_4 (75% inclusion of fermented *T. indica* seed meal) and D_5 (total replacement of soya beans seed meal with fermented *T. indica* seed meal). The results revealed that the best diet apart from the control diet was D_4 as it gave the best mean weight gain (8.58g), standard length gain (2.54cm), total length gain (3.12cm), specific growth rate (0.85%days), percentage weight gain (1.01), nitrogen metabolism (194.60) and net profit (₦72.37) compared with the other diets. D_4 also had the highest value of fish (₦98.70) apart from that of the control diet. The lowest growth and feed utilization were observed in diet D_5 (total replacement). The condition factor (K) showed that the fish were kept in good condition throughout the period of study (K>1). Carcass composition showed that all fish fed with experimental diet had an increase in carcass crude protein and a decrease in nitrogen free extract. The crude protein of the fish carcass ranged between 49.99-54.02% compared to the initial of 43.91%. Cost

of feed production decreased with increase in inclusion level of fermented *T. indica* seed meal in diets. The use of *T. indica* seed at 75% inclusion level was the best apart from the control diet compared to the other diets. Thus the use of *T. indica* seed at 75% replacement level of *Glycine max* meal is recommended in the diet of *O. niloticus* fingerlings.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Fish is an important source of both food and income to many people in developing countries. The exponential growth of the aquaculture sector during the past two decades is a result of the progressive intensification of production systems and use of quality feeds, which meet the nutritional requirements of cultured fish (FAO, 2006). Stimulated by higher global demand for fish, world fisheries and aquaculture production reached 157million tons in 2012 and is projected to reach about 172 million tons in 2021, with most of the growth coming from aquaculture (FAO, 2013). This increase of aquaculture production must be supported by a corresponding increase in the production of designed diets for the cultured aquatic animals (Rahman *et al*., 2013).

In culturing fish in captivity, nothing is more important than sound nutrition and adequate feeding and feeding takes up 50 to 60 percent of cost of production. If the feed is not consumed by the fish or if the fish are unable to utilize the feed because of some nutrient deficiency, then there will be no growth. An undernourished animal cannot maintain its health and be productive, regardless of the quality of its environment. The production of nutritionally balanced diets for fish requires efforts in research, quality control, and biological evaluation. Faulty nutrition impairs fish productivity and results in a deterioration of health until recognisable diseases ensues.

Tilapia is the common name given to three genera of fish in the family Cichlidae namely *Oreochromis*, *Sarotherodon* and *Tilapia* (Santiago and Laron, 2002). However, Tilapia is

not the most cultured fish in Nigeria but regionally, tilapia is the most preferred cultured fish in East Africa and are the second most important cultured fish in the world after carps (El-Sayed, 2006). Tilapia growth is attributed to high resistance to diseases, ability to survive at low oxygen tensions and ability to feed on wide range of foods including feed with anti-nutrients contents. Nile tilapias are relatively inexpensive fish to feed unlike other carnivorous finfish thanks to their low trophic feeding level and herbivorous mode of feeding.

Tamarind or *Tamarindus indica* L. of the Fabaceae family, subfamily Caesalpinioideae, is an important food in the tropics. It is a multipurpose tree of which almost every part finds at least some use (Kumar and Bhattacharya, 2008), either nutritional or medicinal. *T. indica* is indigenous to tropical Africa but it has been introduced and naturalized worldwide in over 50 countries. It grows well over a wide range of soil and climatic conditions, occurring in low-altitude woodland, savannah and bush, often associated with termite mounds. It prefers semi-arid areas and wooded grassland, and can also be found growing along stream and riverbanks. It does not penetrate into the rainforest. Its extensive root system contributes to its resistance to drought and wind. It also tolerates fog and saline air in coastal districts, and even monsoon climates, where it has proved its value for plantations. A long, well-marked dry season is necessary for fruiting. It grows in most soils but prefers well-drained deep alluvial soil.

The seed is a by-product of the *T. indica* pulp industry. The presence of tannins and other dyeing matter in the testa make the whole seed unsuitable for direct consumption (Rao and Srivastava (1974) cited in Kumar and Bhattacharya, 2008).

In the past, the great bulk of seeds available as a by-product of processing tamarinds, has gone to waste. In 1942, two Indian scientists, T. P. Ghose and S. Krishna, announced that the decorticated kernels contained 46 to 48% of a gel-forming substance. It was then patented as a process for the production of a purified product, called "jellose", "polyose", or "pectin", which has been found superior to fruit pectin in the manufacture of jellies, jams, and marmalades. In Northern Nigeria, there is usually no use for the seed after the pulp have been removed for making kunun tsamiya, usually the seeds are discard. The major industrial product of *T. indica* seed is the tamarind kernel powder (TKP) which is an important sizing material used in the textile, paper, and jute industries (Kumar and Bhattacharya, 2008). *T. indica* seed is also the raw material used in the manufacture of polysaccharide (jellose), adhesive and tannin.

In view of the overall nutrient and chemical composition, tamarind seeds may be adopted as an inexpensive alternative protein source to alleviate protein malnutrition among traditional people living in developing countries (Siddhuraju *et al.*, 1995), but the main question is can it be used as protein source in aquaculture?

1.2 Statement of Research Problem

Nutrition is the most expensive component in the intensive aquaculture industry, where it represents over 50% of operating costs (El-Sayed, 2004). Moreover, protein itself represents about 50% of feed cost in intensive culture (El-Sayed, 2004). Therefore, the selection of proper quantity and quality of dietary protein is a necessary tool for successful tilapia culture practices. The high cost of commercial fish feed is one of the major limiting factor in the growth and development of aquaculture sub-sector in Nigeria.

In Nigeria, there is a recent surge in commercial aquaculture production. However, feeds remain the major challenge to the growth of the industry (Bob-Manuel and Erondu, 2010). This is because most of these farms use imported feeds from Europe, thereby resulting in high production costs. Although, cost is not the most important consideration for feeding a diet to fish or in feed formulation (Sogbesan *et al*., 2004). It is rather important that a practical or alternate diet contains all the essential amino acids, fatty acids, vitamins and minerals required by a fish for rapid growth, sound health and economic profitability.

The production of farmed tilapia is among the fastest expanding food sectors in the world (Fagbenro *et al*., 2010). Tilapia are produced most economically in tropical and sub-tropical countries which have favourable temperatures for their growth (Mensah *et al*., 2013). However, only a few studies have considered both economic and biological evaluation of dietary protein sources for tilapia.

Soybean meal is one of the most nutritious of all plant protein sources. Of the total world production of soybeans, more than 90% is directly used for animal consumption (van Eys, 2012). The overwhelming majority is used in animal feed in the form of various types of soybean meals or specialty soy products. Due to its high protein content, high digestibility and relatively well balanced amino acid profile, it is widely used as feed ingredient for many aquaculture species (Storebakken *et al.,* 2000). The cost attached to this ingredient has a lot to do with its rare availability, consumption by man and competition from other livestock industry (Sogbesan *et al*., 2004). This has led to its scarcity and increase in the crop value and its cost. The aim of fisheries nutritionist is to find and develop new unconventional feed that will be able to replace soybean meal at a reduced cost.

There is therefore, the need for a search for alternative protein sources for Nigerian aquafeeds. Such alternatives must satisfy the nutritional needs of the fish species, and as well minimize production cost so as to optimitize the production process.

1.3 Justification

Capture fishery could not satisfy the ever growing demand for fish in Nigeria. This led to the adoption of fish culture in homestead and commercial farms which has been able to reduce the supply deficit. Nile tilapia (*O. niloticus*) is the most cultured freshwater species among the farmed tilapia and contributes about 71% of the world total tilapia production (FAO, 2002).

Tilapias are suitable for various aquaculture systems due to their ease of propagation, tolerance to handling, fast growth on both natural and manufactured feeds, tolerance of a wide range of environmental conditions, and high palatability, marketability and nutrient content (Teichert-Coddington *et al*., 1997). They are especially well-suited for culture in developing countries due to their short generation time, resistance to stress and disease, ability to reproduce in captivity, and their acceptance of artificial feeds right after yolk-sac absorption (EL-Sayeed, 2006). Considerable attention has been paid to tilapia culture during the past decade, consequently, tilapia culture is currently practiced in more than a 100 countries all around globe (El-Sayeed, 2006).

Utilization of some of the unconventional protein sources in fish feeds may result in a slight reduction in fish performance but can still be more cost effective than standard, expensive protein such as soya bean (Bob-Manuel and Erondu, 2010). *T. indica* is underutilized and waste product of Tamarind pulp industry and local kunun-tsamiya

vendors. Therefore, economic evaluation of such protein sources like *T. indica* seeds which has crude protein content of 13.00 to 26.93% (De Caluwe *et al*., 2010) for fish species like tilapia is necessary.

Adoption of replacement of conventional feed ingredients with non-conventional feed ingredients is one of the major breakthrough in fish culture, hence the need to focus on using less expensive and readily available plant protein sources to replace soybean meal without reducing the nutritional quality of fish feeds (Barros *et al.*, 2002). Researchers have mostly focused on the under-utilized or unutilized plant protein sources in fish diet.

As a result, this is done by substituting the costly feed ingredient with cheap, readily available and equally high quality ones. One of such feed ingredients is *T. indica* seed. Akajiaku *et al*. (2014), worked on the proximate and mineral composition of processed Tamarind seed nut and found its protein content to range from 22.16 – 22.57%, which means it has high level of protein, comparable with that of other common legume grains.

1.4 Aim

To evaluate the growth performance of *O. niloticus* fingerlings fed with varying inclusion levels of fermented *T. indica* seed meal.

1.5 Objectives

The objectives of this study were to determine:

i. the effect of fermentation on proximate and anti-nutrient composition of *T. indica* seed nut.

ii. the growth performance and feed utilization of *O. niloticus* fingerlings fed varying inclusion levels of fermented *T. indica* seed meal.

iii. the carcass composition of *O. niloticus* fingerlings fed varying inclusion levels of fermented *T. indica* seed meal.

iv. the cost effectiveness of replacing soybean meal with varying inclusion levels of fermented *T. indica* seed meal.

1.6 Hypotheses

i. Fermentation does not significantly affect the nutrient and anti-nutrients composition of *T. indica* seeds.

ii. Fermenting *T. indica* seed meal does not significantly enhance the growth performance and feed utilization of *O. niloticus* fingerlings fed varying inclusion levels of the feed.

iii. Fermented *Tamarindus indica* seed meal does not significantly affect the carcass composition of *Oreochromis niloticus* fed varying inclusion levels of the feed.

iv. Fermented *T. indica* seed meal does not have significantly better cost benefit value compared to soya bean meal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Feeding Habits of *Oreochromis niloticus*

Early juveniles and young fish are omnivorous, feeding mainly on zooplanktons and zoobenthos but also ingest detritus and feed on aufwuchs and phytoplankton (FAO, 2015). At around 6cm total length, Tilapia becomes almost entirely herbivorous feeding mainly on phytoplankton, using the mucus trap mechanism and its pharyngeal teeth (Moriarty *et al*., 1973 in FAO, 2015). The digestive tract of Nile Tilapia is at least six times the total length of the fish, providing abundant surface area for digestion and absorption of nutrients from its mainly plant based food sources (Opuszynski and Shireman, 1995 in FAO, 2015).

2.2 Nutritional Requirement of *Oreochromis niloticus*

Even though the information on the exact quantitative nutritional requirement for other life stages of Nile tilapia is lacking, it can be expected that early juvenile fish (0.02 – 10.00g) would require a diet higher in protein, lipid, vitamin and minerals and lower in carbohydrates. El-Sayed (2006) have made comprehensive reviews of Nile tilapia nutrition.

2.2.1 Protein Requirements of *Oreochromis niloticus*

O. niloticus requires ten essential amino acids as other fin fishes. Dietary proteins are used continuously by fish for maintenance, growth and reproduction functions. Protein requirement for Nile tilapia decreases with age and size (Mjoun *et al*., 2010). FAO (2015) reported that first feed larvae requires 45 - 50% protein, fry and fingerlings (0.02 – 10.00g) requires 35 – 40%, juveniles (10.0 – 25.0g) requires 30 – 35% and on-growing (>25.0g) requires 28 – 30% protein. The quality of protein is dependent on its amino acid

composition. For optimum growth and development, a protein ingredient has to contain the ten amino acids (Histidine, Arginine, Isoleucine, Leucine, Lysine, Methionine, Phenylalnine, Threonine, Tryptophan and Valine). Therefore, when formulating fish feeds to meet amino acid requirement, all ten essential amino acids must be available for optimum growth and development.

2.2.2 Carbohydrate Requirement of *Oreochromis niloticus*

Carbohydrates are included in fish feeds to provide a cheap source of energy and for improving pellet binding properties. Carbohydrate utilization by Tilapia is affected by a number of factors, including carbohydrate source, other dietary ingredients, fish species and size and feeding frequency (El-Sayed, 2006). Nile tilapia is capable of utilizing high levels of various carbohydrate of between 30–70% of the diet.

Abdel-Hakim *et al*. (2008) reported that the energy digestibility coefficients of Nile tilapia ranges from 67.37–68.06% and Craig and Helfrich (2002) also reported that up to about 20% of dietary carbohydrate can be used by fish.

2.2.3 Vitamins Requirement of *Oreochromis niloticus*

Vitamins are generally necessary for optimum growth and health of Tilapia in intensive culture systems where limited natural food is available (FAO, 2015). Several vitamins requirements of Tilapia are known to be affected by other dietary factors and they must be taken into consideration in diet formulations. For example, the Vitamin E requirement is influenced by dietary lipid level with Nile tilapia requiring 50–100mg/kg when fed diets with 5% lipid. It was reported that Nile tilapia requires 4mg/kg of Thiamine (Lim *et al*., 2000 in Mjoun *et al*., 2010), 5mg/kg of Riboflavin (Lim *et al*., 1993 in Mjoun *et al*., 2010),

50mg/kg of Ascorbic acid (Abdelghany, 1996 in Mjoun *et al*., 2010) and 5000mg/kg of Retinol (Saleh *et al*., 1995 in Mjoun *et al*., 2010).

2.2.4 Lipid Requirement of *Oreochromis niloticus*

Dietary lipids provide a major source of energy, facilitate the absorption of fat soluble vitamins, play an important role in membrane structure and function and serve as metabolizable source of essential fatty acids.

The minimum requirement of dietary lipid in Tilapia diet is 5% but improved growth and protein utilization efficiency has been reported for diets with 10-15% lipids (FAO, 2015). Both n-3 and n-6 polyunsaturated fatty acids (PUFA) have been shown to be essential for maximal growth of Hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*). Nile tilapia's quantitative requirement for n-6 PUFA is around 0.5–1.0% (FAO, 2015). Jauncey (2000) suggested that to maximize protein utilization, dietary fat concentration should be between 8–12% for Tilapia up to 25g, and 6–8% for larger fish.

2.2.5 Mineral Requirement of *Oreochromis niloticus*

Like other aquatic animals, Tilapias are able to absorb minerals from the culture water which makes the quantitative determination of these elements difficult to carry out. For example, when Nile tilapia reared in fertilized ponds were fed with diets either containing complete mineral mixes or one deficient in Calcium, Phosphorus, Magnesium, Sodium, Potassium, Iron, Zinc, Manganese or Iodine and it was found that only the addition of phosphorus significantly affected weight gain, food conversion ratio and protein efficiency ratio (Stickney, 1997). Despite its ability to absorb minerals from the culture water and the presence minerals in feed ingredients, Tilapia feeds should contain supplement mineral

premixes. This is to ensure that sufficient levels are available to protect against mineral deficiencies caused by reduced bioavailability such as when plant phosphorus sources are used in Tilapia feeds (FAO, 2015).

2.3 Non-conventional Feedstuffs of Plant Origin Used in Fish Feed Formulation

The utilization of nonconventional feedstuffs of plant origin had been limited as a result of the presence of Alkaloids, Oxalic acids, Glycosides, Phytates, Protease inhibitors, Haematoglutinin, Saponegin. Mimosine, Cyanoglycosides, Limamarin to mention a few despite their nutrient values and low cost implications. These anti-nutritional factors limit growth and other physiological activities at higher concentrations.

Non-conventional Feed Resources (NCFRs) are feeds that are not usually common in the market and are not the traditional ingredients used for commercial fish feed production (Madu *et al*., 2003). NCFRs are credited for being noncompetitive in terms of human consumption, very cheap to purchase, byproducts or waste products from agriculture, farm made feeds and processing industries and are able to serve as a form of waste management in enhancing good sanitation (Abowei and Ekubo, 2011). These include all types of feedstuffs from plant waste; cottonseed meal, jack bean, *Cajanus caja*, khaya, duckweed, maize bran, rice bran, palm kernel cake, brewers waste, canola seed etc.

Obasa *et al*. (2003) substituted soybean meal with pressure-cooked and sun-dried pigeon meal (25% crude protein) at 60% dietary level in the diet of Nile tilapia and it had significant better growth response and nutrient utilization.

Soltan (2005) reported that soaking canola meal in 0.1% citric acid solution followed by heating treatment improved its nutritional value and could substitute up to 50% of fishmeal

in Tilapia diets and that it reduced feed cost by 22.47% without significant effect on growth performance. The treatment was believed to have reduced their anti-nutritional factors and improved its nutritional value.

El-Sayed and Kwanna (2008) found that cottonseed meal used as the only feed input for Nile tilapia reared in earthen ponds fertilized with cattle manure for 100 days, resulted in a sharp increase in fish weight. Cottonseed meal can be used as protein source in commercial pelleted feeds for Tilapia and also used as a source of fertilizer in semi-intensive Tilapia culture to increase natural food production.

Bichi and Ahmad (2010) fed *Clarias gariepinus* varying dietary levels of processed cassava leaves and suggested that 66.7% cassava leaves replacement of maize meal is optimum for the growth of the fish.

Agbo *et al.* (2011) worked on the replacement of fishmeal with cotton seed meal in the diets of *O. niloticus* and found that replacement of 50% fishmeal protein with cottonseed meal was successful without adversely affecting the growth and feed utilization of the fish.

Olaniyi and Oladunjoye (2012) substituted 25% fishmeal with duckweed meal in the diet of *O. niloticus* which promoted growth with better feed utilization than feeding with only fishmeal which can lead to reduction in cost of feeding fish and profit margin. However, exceeding the limit (25%) resulted in depressed weight gain.

Jimoh *et al*. (2013) also replaced soybean meal in the diet of *C. gariepinus* fingerlings with cooked *Luffa cylindrica* seed meal, there were no significant variations in growth and

nutrient utilization parameters of fish fed the control diet and those fed diet containing 15% *Luffa cylindrica.*

Khan *et al*. (2013) replaced fishmeal with rice polish (up to 20%) and mustard oil cake (up to 22%) in the diet of Nile tilapia which was economically efficient by reducing about 24% of the feed formulation cost without changing the nutritional quality.

Adewumi (2014) replaced fishmeal in the diet of *C. gariepinus* with 10% Moringa lam meal, and it gave good growth, and nutrient utilization comparable with the fishmeal meal diet.

2.4 Description and Habitat of *Tamarindus indica*

Tamarind is a medium to large evergreen tree reaching 40 to 60 feet tall. It has a moderate growth rate and is long lived. Young trees have an airy canopy. When grown unencumbered, older trees develop a densely round canopy usually on a short trunk (Brown and Marshall, 2013).

Leaves are alternate, compound, with 10-18 pairs of opposite leaflets; leaflets narrowly oblong, 12-32mm x 3-11mm, petiole and rachis finely haired, midrib and net veining more or less conspicuous on both surfaces; apex rounded to almost square, slightly notched; base rounded, asymmetric, with a tuft of yellow hairs; margin entire, fringed with fine hairs. Stipules present, falling very early.

Flowers are attractive pale yellow or pinkish, in small, lax spikes about 2.5cmin width. Flower buds completely enclosed by 2 bracteoles, which fall very early; sepals 4, petals 5, the upper 3 well developed, the lower 2 minute**.**

Fruit is a pod, indehiscent, subcylindrical, 10- 18 x 4cm, straight or curved, velvety, rusty-brown; the shell of the pod is brittle and the seeds are embedded in a sticky edible pulp. Seeds 3-10, approximately 1.6cm long, irregularly shaped, testa is hard, shiny and smooth.

T. indica grows well over a wide range of soil and climatic conditions, occurring in low-altitude woodland, savannah and bush, often associated with termite mounds. It prefers semi-arid areas and wooded grassland, and can also be found growing along stream and riverbanks. It does not penetrate into the rainforest. Its extensive root system contributes to its resistance to drought and wind. It also tolerates fog and saline air in coastal districts, and even monsoon climates, where it has proved its value for plantations. Young trees are killed by the slightest frost, but older trees seem more cold resistant than mango, avocado or lime. A long, well-marked dry season is necessary for fruiting (Orwa *et al*., 2009)**.**

2.5 Origin and Geographical Distribution of *Tamarindus indica*

The natural distribution of tamarind individuals was limited to the Sudanian and the Sudano-Guinean regions and their density declined with increasing moisture, being highest (2 trees per km^2) in the Sudanian region and lowest in the Guineo-Congolian region (scarce) (Fandohan *et al.*, 2011).

T. indica is native to Burkina Faso, Central African Republic, Chad, Eritrea, Ethiopia, Gambia, Guinea, Guinea-Bissau, Kenya, Madagascar, Mali, Mozambique, Niger, Nigeria, Senegal, Sudan, Tanzania, Uganda and Zimbabwe. And are exotic species in Afghanistan, Australia, Bangladesh, Brazil, Brunei, Cambodia, China, Colombia, Cote d'Ivoire, Cuba, Dominican Republic, Egypt, Ghana, Guatemala, Haiti, Honduras, India, Indonesia, Iran, Jamaica, Laos, Liberia, Malaysia, Mauritania, Mexico, Myanmar, Nepal, Nicaragua,

Pakistan, Panama, Papua New Guinea, Philippines, Puerto Rico, Sri Lanka, Thailand, Togo, United States of America, Vietnam and Zambia

2.6 Phytochemical Composition of *Tamarindus indica*

The pulp is composed of tartaric acid, citric and malic acids, potassium bitartarate, pectin, gum, water and parenchymatous fiber (Nyadoi and Abdullahi, 2004). Abubakar *et al*. (2008) carried out the phytochemical screening and antibacterial activity of *T. indica* pulp extract and found them to include saponins (2.2%), alkaloids (4.32%) and glycosides (1.59%).

From the Tamarind pericarp, polyphenolic compounds were isolated by semi preparative HPLC. After Soxhleth extraction with methanol the total yields were 6.54g/kg in the seed sand 2.82g/kg in the pericarp. The polyphenolics in the pericarp are dominated by proanthocyanidins (73.4%) in form of catechin (2.0%), epicatechin (9.4%), procyanindin B2 (8.2%), procyanidin tetramers till procyanidinhexamers and the flavonoids taxifolin, apigenin, eriodictyol, luteolin, and naringenin.

The tamarind seeds comprised only procyanidins, represented by their tri - till hexamers, with lower amounts of procyanidin B2 (5.5%) and -/- epicatechin (4.8%).

Here one must assume that a part of the polyphenolic substances can be originated by the acid hydrolysis during the extraction. The antioxidant capacities of the Soxhleth methanolic extracts were determined. Authors believe them to be an important source of cancer preventive natural products in tropical regions (Sudjaroen *et al.*, 2005). In the leaves of the plant two triterpenes, lupanone and lupeol were found (Iman *et al*., 2007).

Phytate is one of the most powerful anti-nutritional factors in plant feedstuffs. The antinutritional activity of phytate can be eliminated by the addition of enzymes such as phytase. Phytate in cereals, legume grains and oil seeds are bound with phosphorus, calcium and magnesium, trace elements such as iron and zinc, and protein and amino acids. The phytate content can be lowered by processing (Esenwah and Ikenebomeh, 2008) and has been considered as an anti-nutritional component in cereals, seeds and beans. However, recent research has shown that phytate has many health benefits such as antioxidant, anticancer, hypocholesterolemic and hypolipidemic effects (Banupriya and Vijayakumar, 2016).

2.7 Economic Importance of *Tamarindus indica*

Tamarind plant parts products have been used as fodder (leaves and cooked seed), apiculture (flowers are reportedly a good source for honey production), fuel (tree trunk and branches provides good firewood with calorific value of 4,850 kcal/kg, it also produces an excellent charcoal (Orwa *et al*., 2009)), tannin or dyestuff (both leaves and bark are rich in tannin; the bark tannins can be used in ink or for fixing dyes), timber (the tree trunk is used for general carpentry, sugar mills, wheels, hubs, wooden utensils, agricultural tools, mortars, boat planks, toys, panels and furniture).

2.7.1 Feed Usage

2.7.1.1 Leaves and Flowers

Tamarind leaves and flowers can be eaten as vegetables and are prepared in a variety of dishes (ICRAF, 2007). They are used to make curries, salads, stews and soups in many countries, especially in times of scarcity (Benthall (1933) cited in El-Siddig *et al.*, 2006).

Before consumption, leaves are sometimes boiled in water and prepared as tamarind fruits (Nordeide *et al.,* 1996).

2.7.1.2 Fruit

Tamarind fruit pulp is used for seasoning, as a food component, to flavour confections, curries and sauces, and is a main component in juices and certain beverages (De Caluwé *et al*., 2010). Tamarind fruit pulp is eaten fresh and often made into a juice, infusion or brine (El-Siddig *et al.,* 2006), and can also be processed into jam and sweets. The refreshing drinks are popular in many countries around the world, though there are many different recipes. In some African countries, the juice obtained from the fruit pulp is mixed with wood ash to neutralize the sour taste of the tartaric acid (De Caluwé *et al*., 2010). However, the most common method is to add sugar to make a pleasantly acid drink. In Ghana, the pulp is mixed with sugar and honey to make a sweet drink (De Caluwé *et al*., 2010). Most of the producing countries manufacture drinks commercially. Sometimes pulp is fermented into an alcoholic beverage (FAO (1988) cited in El-Siddig *et al.,* 2006).

2.7.1.3 Seeds

Tamarind seed is a by-product of the tamarind pulp industry. The presence of tannins (dyeing matter) in the testa makes the whole seed unsuitable for direct consumption (Rao & Srivastava (1974) cited in Kumar & Bhattacharya, 2008). However, the seeds become edible after soaking and boiling in water, which removes the seed coat (El-Siddig *et al.*, 2006). In the past, and even today, seeds have been wasted (El-Siddig *et al.*, 2006) even though they could be ground to make a palatable livestock feed (NAS (1979) cited in El-Siddig *et al.,* 2006). The major industrial product of tamarind seed is the tamarind kernel powder (TKP) which is an important sizing material used in the textile, paper, and jute

industries (Kumar & Bhattacharya, 2008). Tamarind seed is also the raw material used in the manufacture of polysaccharide (jellose), adhesive and tannin. In 1942, two Indian scientists announced that decorticated kernels contained 46-48% of a gel-forming substance. This polysaccharide (pectin) with carbohydrate character and jelly forming properties, named 'jellose' (Rao (1948 and 1956) cited in El-Siddig *et al.*, 2006), has been recommended for use as a stabilizer in ice cream, mayonnaise and cheese, and as an ingredient or agent in a number of pharmaceutical products (El-Siddig *et al.*, 2006). Flour from the seed may be made into cake and bread. Roasted seeds are claimed to be superior to groundnuts in flavour (ICRAF, 2007).

2.7.2 Medicinal Usage

The bark is astringent and tonic and its ash may be given internally as a digestive. Incorporated into lotions or poultices, the bark may be used to relives sores, ulcers, boils and rashes. It may also be administered as a decoction against asthma and amenorrhea and as a febrifuge. Leaf extracts exhibit anti-oxidant activity in the liver and are common ingredient in cardiac and blood sugar reducing medicines. Young leaves may be used in fomentation for rheumatism, applied to sores and wounds, or administered as a poultice for inflammation of joints to reduce swelling and relieve pain. A sweetened decoction of the leaves is good against throat infection, cough, fever, and even intestinal worms. Filtered hot juice of young leaves and a poultice of the flowers are used for conjunctivitis. The pulp may be used as a massage is used to treat rheumatism, as an acid refrigerant, a mild laxative and also to treat scurvy. Powdered seeds may be given to cure dysentery and diarrhea (Orwa *et al*., 2009).

In Kaduna state of Nigeria, a general inquiry was made about the indigenous knowledge for treating Trypanosomiasis in domestic animals. Questionnaires and interviews were conducted with about 200 livestock farmers and traders. Between 14 of them used plants, *T. indica* was included (Atawodi *et al*., 2002).

The bark and leaves of *T. indica* have been used to treat jaundice and for fortification as blood tonic, with decoction of fresh plant parts with potash in Nigeria (Doughari (2006) cited in Havinga *et al.*, 2010).

T. indica has been used for treatment of cuts, wounds and abscesses. Fandohan (2007) reported the use of a leaf decoction to wash circumcision wounds in Benin.

2.8 Description of *Glycine max*

Glycine max (L.) Merr. (soya bean) is an erect leguminous plant, up to 1m high. It is a fast growing herbaceous annual with tap-root and can extend to 2m deep in good soil conditions. It is native to Asia but currently grown worldwide. Roots bear nodules, leaves are trifoliate and leaflets are oval to lanceolate. The flowers are white, pink, purple or bluish (Giller *et al*., 2007). Fruits are two or three-seeded pods containing yellow, rounded seeds with a hilum colour ranging from yellow to black (Koivisto, 2006).

2.9 Origin and Geographical Distribution of *Glycine max*

Soya bean is native to Asia. It was domesticated in North China 3000 years ago, and is now produced in almost all continents between 53°N to 53°S, and from sea level up to an altitude of 2000m (Heuze and Tran, 2016). The main producing countries are the USA, Brazil, Argentina, China and India.

Optimal growth conditions are average day-temperatures around 30°C, 850mm annual rainfall, and not less than 500mm water during the growing season, and soil pH ranging from 5.5 to 7.5 with good drainage. Soya is sensitive to soil acidity and aluminium toxicity. It can withstand short periods of waterlogging and short droughts.

2.10 Phytochemicals Compositions of *Glycine max*

Soya bean seeds contain several anti-nutrients. The most important ones are trypsin inhibitors, hemagglutinins, lectins and saponins. Soya bean was indeed used to demonstrate the existence and the role of trypsin (Rackis *et al*., 1986).

Raw soya beans contain trypsin inhibitors that bind with trypsin (a digestive enzyme) in the small intestine and form an inactive complex that prevents trypsin from degrading feed proteins (van Eys *et al*., 2010). Trypsin inhibitors also induce pancreatic enlargement, increased trypsin secretion and, therefore, lower nitrogen retention, growth and feed conversion (van Eys *et al*., 2010). Leaves and stems do not contain trypsin inhibitors and there is no problem in using soya beans as roughage or silage (Koivisto, 2006). Due to these anti-nutrients, raw soya beans are unable to support growth requirements in monogastrics. Ruminants are not as sensitive because trypsin inhibitors are deactivated and degraded in the rumen (Hoffmann *et al*., 2003). However, trypsin inhibitors are heat labile and largely destroyed by heating (Rackis *et al*., 1986) so heat treatment is required before feeding soya bean products to animals, particularly to monogastrics.

Lectins are glycoproteins that bind with intestinal villi and reduce nutrient uptake. They cause damage to the lining of the intestine and then interfere with absorption of nutrients.

Saponins are glycosides that diminish uptake of other nutrients. Whole soya beans contain from 0.17 to 6.16% saponins. They are common in a large number of plants and plant products that are important in human and animal nutrition (Francis *et al*., 2002). Saponins have long been known to cause lysis of the erthrocytes when given in vitro. The hemolytic activity of saponins, coupled with this cholesterol inhibition effect, has been extensively used as a means of detecting and quantifying saponins in plant material (Dourado *et al*., 2011).

Soya beans contain a high level of phytate, which has many effects including acting as an antioxidant and a chelating agent. The beneficial claims for phytate include reducing cancer (Vucenik and Shamsuddin, 2003), minimizing diabetes, and reducing inflammation (Sudheer *et al*., 2004). However, phytate is also criticized for reducing vital minerals absorption due to its chelating effect, especially for diets already low in minerals.

Alkaloids are oxidized in the liver resulting in metabolites, such as dehydrosparteine, which are responsible for the observed toxicity. The level of toxicity is influenced by the structure of the alkaloids. There is a high degree of variation in the ability of different animal species to deal with these compounds. The toxic effects of alkaloids and their metabolites are primarily mediated through the nervous system, although they are also stimulate copper uptake by liver cells thus leading to copper toxicity (Dublecz *et al*., 2011).

Others anti-nutrients of soya bean are antivitamins, urease and plant sterols that may interfere with the absorption of carotenoids and vitamin E (Brown *et al*., 2008).

2.11 Economic Importance of *Glycine max*

Soya bean is primarily an oilseed containing about 20% oil. The extraction of oil results in a high-protein cake that can be further processed into a variety of products for feed and food uses. One of these products, soya bean meal, is one of the major feed commodities and is the main protein source in many animal diets (Heuze and Tran, 2016). While soya bean used to be grown primarily for its oil, the expansion of the crop is now driven by the demand for soya bean meal and feed use accounted for about two-thirds of the value of soya beans in recent years (FAO, 2006).

Soya bean is used as food in tropical Africa and Asia. It may be roasted and eaten as a snack, or fermented to make soya bean cake 'Awara'. Immature soya beans are also eaten as a vegetable, as well as bean sprouts (Giller *et al*., 2007).

Soya bean leaves and stems can be grazed, ensiled or dried to make hay. The foliage is very palatable to cattle, has a high nutritive value and is highly digestible (Koivisto, 2006). Average world soya bean yields are 2.25 t/ha while average African soya bean yields are 0.5 t/ha (Giller *et al*., 2007).

The major soya bean products for feed use are soya bean oil, soya bean meal (the by-product of oil extraction) and whole soya beans (usually called "full-fat"), the latter being usually heat processed in order to destroy the anti-nutrients and improve their feed value. These products can be ground, pelleted, flaked or powdered.

Soya beans are also used in industrial products, including oils, soap, cosmetics, resins, plastics, inks, crayons, solvents, and clothing. Soya bean oil is the primary source of biodiesel in the United States, accounting for 80% of domestic biodiesel production.

Soya beans have also been used since 2001 as fermenting stock in the manufacture of a brand of vodka. Other products include soya bean flour, soya bean protein concentrates and isolates, soya bean solubles and molasses, soya bean mill feed, and soya bean mill run (Heuze and Tran, 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of *Oreochromis niloticus* Fingerlings

The *O. niloticus* fingerlings used for this research were purchased from Songhai Nigeria Partnership Initiative LTD/GTE Funtua Branch, Katsina State.

3.2 Source of *Tamarindus indica* Seeds

Tamarindus indica seeds were obtained from the wild around Zaria and identified at Herbarium Unit of Department of Botany, Ahmadu Bello University, Zaria.

3.3 Sample Preparation

T. indica seeds were prepared to remove dirt and bad seeds (Plate I), the seeds were weighed and processed. Processing was by fermentation and roasting.

Fermentation was done according to the method of Shlini and Siddalinga Murthy (2015). This was done by soaking the seeds for 72 hours and then dehulled mechanically with mortar and pistil (Plate II). Then the dehulled seeds were fermented by putting them into an airtight container for 72 hours (Plate III and IV). The fermented seeds were then sun dried, milled into flour and were oven dried at a temperature of 60˚C and further dried under the sun. The flour was then packaged in an air tight container until when it was used.

Roasting was carried out according to the recommended methods of Akajiaku *et al*. (2014). The seeds were roasted in an open pan at a temperature of 100°C for 15 minutes and mechanically dehulled. The roasted seeds were milled into flour and oven dried at a

temperature of 60°C and further dried under the sun at 36°C. The samples were sieved to obtain fine flour and packaged in an air tight plastic container until when it was used.

Glycine max seeds were also prepared by roasting. The seeds were roasted in an open pan at a temperature of 100°C for 15 minutes and mechanically dehulled. The roasted seeds were milled into flour and oven dried at a temperature of 60°C and further dried under the sun at 36°C. The samples were sieved to obtain fine flour and packaged in an air tight plastic container until when it was used.



Plate I: *Tamarindus indica* seed after sorting



Plate II: Dehulled *Tamarindus indica* before fermentation



Plate III: Plastic container made airtight by covering with polythene bag



Plate IV: Container covered with a lid and left to ferment for 72 hours

3.4 Proximate Analysis of Feedstuff

The proximate analysis was determined using recommended methods of the Association of Official Analytical Chemist (AOAC, 1990) procedures for moisture, ash, crude fibre, crude protein and lipid content. Carbohydrate content (NFE) was determined by difference; thus the sum of measured moisture (% M.C); ash (% ash); protein (% N); crude fibre (% C.F) and lipid (%EE) deducted from 100 (i.e. 100 - % M.C + % Ash + % N + % C.F + % EE = NFE).

3.4.1 Determination of Moisture Content

Moisture content was determined using the method of AOAC (1990). A clean crucible was dried to a constant weight in an oven at 150°C, cooled in a desiccator and weighed (W_1). Two grams of the sample flour was weighed into the crucible (W_2) and dried in the oven at 105°C. The crucible and its content were then cooled to room temperature in a desiccator and weighed. The procedure was continued until a constant weight was obtained (W_3).

The moisture content was calculated as follows:

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

3.4.2 Determination of Ash Content

Ash was determined using the method of AOAC (1990). Two grams of the finely ground sample was weighed (W_2) into a previously weighed, clean and empty crucible (W_1). The sample was then ignited and cooled in a desiccator before taking to the furnace. After maintaining the sample at 550°C in a muffle furnace for eight hours, the crucible and its

residual ash were removed from the furnace and then allowed to cool to room temperature in a desiccator before it was weighed (W_3).

The ash content was calculated as follows:

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

3.4.3 Determination of Crude Fibre Content

Crude fibre was determined using the method of AOAC (1990). Two grams of the finely ground sample was put into a round bottom flask. Then 100mL of a 0.023M Sulphuric acid solution was added and the mixture boiled under reflux for 30minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. This was then quantitatively transferred into the flask and 100mL of 0.312M Sodium Hydroxide solution was added and the mixture boiled again under reflux for 30minutes before it was quickly filtered under suction. The insoluble residue was washed until it was base free, then dried to constant weight in an oven set at 100°C, cooled in a desiccator and weighed (C_2). This residue was then incinerated in a muffle furnace at 550°C for two hours, cooled in desiccators and reweighed (C_3).

The crude fibre content was calculated as follows:

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

Where $C_2 - C_3$ = loss in weight on ashing (incineration)

W = weight of original sample

3.4.4 Determination of Crude Protein (Kjeldahl method) Content

Crude protein was determined using the method of AOAC (1990). Two grams of the sample was weighed into 100mL Kjeldahl digestion flask. Then 1g of catalyst mixture (Na_2SO_4 + anhydrous $CuSO_4$, ratio 1:10) was added to speed up the reaction. Exactly 25mL of concentrated Sulphuric acid (Specific gravity 1.84) was also added into the flask. The content in the Kjeldahl digestion flask was then heated slowly at first in a Kjeldahl digestion heating unit until fretting subsides and then more vigorously (with occasional rotation of the flask to ensure even digestion and prevent overheating of the content) until the green digest is obtained. The solution was transferred into a 100mL volumetric flask and diluted to the mark with distilled water. Then 10mL aliquot of the diluted solution (or digest) was pipetted into a Kjeldahl distillation flask and 10mL of 40% Sodium Hydroxide solution was added. The solution was steam distilled and liberated ammonia was trapped in a 250ml conical flask containing 10mL of 4% Boric acid and a drop of mixed indicator (methyl red and methyl blue, ratio 2:1). Distillation was continued until the pink colour of the indicator turns greenish. The content of the conical flask was titrated with 0.1M HCl and endpoint indicated by a change from greenish to pink colour. The volume of the acid used for each distillate (as well as the blank) were recorded.

% Total N per sample was calculated from the formula:

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

Where V_1 = Volume of HCl required for 10ml sample solution

V_0 = Volume of HCl required for the blank

M = Concentration of HCl (0.1M)

14 = Atomic weight of nitrogen

1000 = Total volume of digest (mL)

10 = Volume of digest distilled (mL)

2 = Weight of sample (gram)

1000 = Conversion factor to dm

The crude protein was calculated as follows:

Crude protein = 6.25 × %N

3.4.5 Determination of Lipid (Ether Extract)

Ether extract was determined using method of AOAC (1990). Exactly 200cm^3 of petroleum ether (40-60°C) was transferred into a clean dry 250mL round bottom flask fitted with Soxhlet extraction unit after some boiling chips was added. Fat free extraction thimble containing 20g of the sample flour was then fixed into the Soxhlet extraction unit with forceps and cold water circulation was put on. The heating mantle was switched on and heating rate was adjusted to a temperature between 40 and 60°C until the solvent starts refluxing at a steady rate. Extraction was carried out for six hours after which the heating mantle was switched off. By means of a rotary evaporator, the solvent was recovered and the oil left behind was quantified.

The extractable lipid of the sample was calculated as follows:

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

3.4.6 Determination of Nitrogen Free Extracts Content

The AOAC (1990) method was used. Carbohydrate as nitrogen free extract (NFE) was calculated by difference:

NFE = 100 – (Crude protein + Ether extract + Ash + Moisture + Crude fibre)

3. 5 Anti-Nutrients in *Tamarindus indica* Seed Nut

Alkaloid, Saponin, Tannin and Phytate were determined using recommended methods of the Association of Official Analytical Chemist procedures (AOAC, 1980).

3. 5.1 Determination of Alkaloids

Exactly 5g of the sample was weighed into 250mL beaker with 200mL of 20% acetic acid in ethanol added, covered and allowed to stand for 4h at 25°C. This was filtered with paper No. 42 and filtrate was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was collected and washed with dilute NH_4OH (1% Ammonia solution) and then filtered with pre-weighed filter paper. The residue on the filter paper is the alkaloid which was dried in the oven (precision Electro thermal model BMP9052 England) at 80oC. Alkaloid content was calculated and expressed as percentage of the sample analysed.

Calculation:

$$\%wt\ of\ alkaloid = \frac{wt\ of\ filter\ paper\ with\ residue - wt\ of\ filter\ paper}{wt\ of\ sample\ analysed} \times \frac{100}{1}$$

3.5.2 Determination of Saponin

Exactly 5g ground was weighed into a thimble and transferred into the Soxhlet extractor chamber filled with a condenser. Some quantity of acetone reflux was poured into the flask. The sample was exhaustively extracted, at its lipid for 3 hours and the solvent distilled off. This was first extraction.

The second extraction, a pre-weighed flask was fitted unto the Soxhlet apparatus (bearing the sample containing thimble) and methonal poured into the flask. The methanol caused a reflux. The saponin was then exhaustively extracted for 3 hours. The flask was reweighed. The difference in weight represents the weight of Saponin extracted.

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

3.5.3 Determination of Tannin

One gram of 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 filter paper was washed with distilled water and extract diluted to volume (this is the extract volume). Exactly 50mL of distilled water and 10mL of dilute extract (aliquot volume) was pipetted into a 100mL conical flask, followed by 5mL Folin-Denis reagent and 10mL of saturated Na_2CO_3 solution. It was then diluted to volume with distilled water. After mixing, the solution was allowed to stand for 30minutes in a water bath (25°C). Optical density was measured at 700nm and the absorbance compared on a standard tannic acid (tannin) curve. The tannic acid standard curve was prepared by dissolving 0.2g of tannic acid in distilled to 200mL (1mg/mL). Varying concentrations (0.1 – 1.0mg/mL) of standard tannic acid solutions was

pipetted into 10 different 100mL conical flask, then 5mL Folin-Denis reagent and 10mL saturated Na_2CO_3 solution was pipetted into the test tubes and made up to 100mL mark with distilled water. The solution was left to stand for 30minutes and optical density was measured at 700nm. a plot of optical, density of tannic acid concentration was made; with the line passing through the origin. % Tannin content was calculated as follows:

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

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

3.5.4 Determination of Phytate Content

Exactly one gram of the defatted sample was extracted for one hour with 100mL of 3% TCA with occasional swirling by hand. The suspension was centrifuged and 10mL of the supernatant was precipitated with 4mL of ferric chloride solution (containing 2mg of ferric ion ml"l in 3% TCA). The precipitate of ferric phytate was converted to ferric hydroxide with 4mL 1.5M NaOH. The ferric hydroxide was dissolved in hot 40mL 3.2M HNO_3 and transferred to 100mL volumetric flask. The volume was made up with distilled water.

Exactly 5ml of the aliquot was transferred to another 200ml volumetric flask and diluted to approximately 70 ml to the contents, 20ml of 1.5M KSCN was added and volume made up with water. The ion was determined colorimetrically 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 ($FeCl_3$) in distilled water and volume made up to 1 litre. This stock solution contained 3.4 × 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:

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

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

Therefore $C_{unknown} = C_{iron} = C_{phytate\text{-}P}$

$$Phytate = C_{phytate\text{-}P} - 28\%\ C_{phytate\text{-}P}$$

3.6 Experimental Diet

The seeds were milled and added to replace soybean meal ingredient using various graded levels. The experimental feed for *O. niloticus* was formulated using conventional feed ingredients applying the Pearson's square method. The ingredients were mixed together in each case (each protein inclusion level) and water was added and mixed thoroughly.

A hand pelletizer was used in pelleting the feed (Plate V), and followed by sun drying. Each experimental feed concentrate was packed in a separate container and kept in a dried and cool condition to prevent fungi attack; the feeds were collected and kept separately in polythene bags based on various inclusion levels. Five diets including the control diet at varying inclusion levels (0%, 25%, 50%, 75% and 100%) were formulated.

Table 3.1: Composition of Experimental Diets Used for Feeding Trial

Ingredients	Inclusion Levels of *T. indica* Fermented Seed (%)				
	0	25	50	75	100
Soya bean meal	40.90	30.68	20.45	10.23	0.00
T. indica meal	0.00	10.23	20.45	30.68	40.90
Fish meal	20.45	20.45	20.45	20.45	20.45
Yellow maize	28.64	28.64	28.64	28.64	28.64
Bone meal	1.00	1.00	1.00	1.00	1.00
Palm oil	3.50	3.50	3.50	3.50	3.50
Salt	0.80	0.80	0.80	0.80	0.80
Vitamin Premix	0.70	0.70	0.70	0.70	0.70
Methionine	2.00	2.00	2.00	2.00	2.00
Lysine	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100



Plate V: Pelletized feed

3.7 Experimental Fish

One hundred (100) *O. niloticus* fingerlings were collected and acclimatized within the laboratory for two weeks (14 days).

3.8 Experimental Setup

The feeding and growth experiment were conducted in the Fisheries Laboratory of Department of Biology, Ahmadu Bello University, Zaria. Ten plastic aquaria with dimension of 50 × 45 × 35cm were used (the experiment was replicated twice with two aquaria/experimental diet) in a static culture system, each containing dechlorinated water (Plate VI). Ten fingerlings of *O. niloticus* were stocked randomly in each aquarium.



Plate VI: Experimental set-up

3.9 Weighing and Measuring of Fish

The mean weight of fish was determined using an electronic weighing balance. This was done after acclimatization period. Mean weight per aquarium was recorded after taking the bulk weight of fish. Mean weight per aquarium was taken every fourteenth night throughout the twelve (12) weeks period of experiment. Both standard length (SL) and total length (TL) of the fish were recorded using a measuring board. Growth performance and feed utilization parameters such as weight gain (WG), percentage live weight gain (PWG), specific growth rate (SGR), feed conversion ratio (FCR), gross feed conversion efficiency (GFCE), protein efficiency ratio (PER), net protein utilization (NPU) and nitrogen metabolism (Nm) were calculated.

3.10 Feed Administration

Feeding frequency recommended by Marinmuthu *et al*. (2010) was adopted. Feed were administered four times daily. Experimental fish were fed at 5% body weight.

3.11 Growth and Feed Utilization Computation

3.11.1 Weight Gain (WG)

Weight gained from the initial weight to the end of monitoring exercise. This was computed as the difference between the final weight and the initial weight of the fish that being cultured as follows:

$$\textit{Weight gain (WG)} = w_t - w_0$$

Where: w_t = weight at the time of observation

w_0 = initial weight

3.11.2 Percentage Live Weight Gain (PWG)

Percentage live weight gain (PWG) was computed as difference between initial and final fish weight divided by the initial weight expressed as percentage (Falayi, 2009) as follows:

$$\% \text{ Live Weight Gain (PWG)} = \frac{w_t - w_0}{w_0} \times 100$$

Where: w_t = weight at the time of observation

w_0 = initial weight

3.11.3 Specific Growth Rate (SGR)

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

$$SGR = \frac{\log_e w_t - \log_e w_0}{t - t_0} \qquad (\%days)$$

where: w_t = weight at the time of observation

w_0 = initial weight

t-t_0= duration of the experiment

e = the base of natural logarithm (10)

3.11.4 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) was calculated using the formula:

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

3.11.5 Gross Feed Conversion Efficiency (GFCE)

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

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

3.11.6 Protein Efficiency Ratio (PER)

Protein efficiency ratio was computed as ratio of fish weight gain to protein intake of fish (Feed intake crude protein of feed) as described by Aderolu and Sogbesan (2010).

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

3.11.7 Net Protein Utilization (NPU)

Method described by Balogun *et al.* (2004) was adopted to calculate NPU as follows:

$$NPU = \frac{Fish\ protein\ gain}{Protein\ fed} \times 100 \quad (\%)$$

3.11.8 Nitrogen Metabolism (Nm)

The values of nitrogen metabolism (Nm) was calculated using the formula of Dabrowski (1977) as follows:

$$\%\ Nitrogen = \frac{(0.54)(b\text{-}a)h}{2}$$

Where: a = initial weight of fish (g)

b = final weight of fish (g)

h = Experimental period in days

3.11.9 Condition Factor (K)

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

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

Where: K = Condition factor

L = Length (cm)

W = Body weight of fish (g)

3.11.10 Length-Weight Relationship

Length-weight relationship was estimated by the equation:

$W=a\,L^b$ (Le Cren, 1951)

Which is further expressed logarithmically as:

$Log\ W=Log\ a\ +b\ Log\ L$

Where: W = Weight of the fish

L = Total length of the fish

a = Constant and

b = Exponent

3.12 Cost Benefit Analyses

The recommended methods of Konyeme *et al.* (2005) were used to calculate the cost effectiveness of replacing soybean meal with varying inclusion levels of fermented *T. indica* seed meal as follows:

Estimated investment cost analysis = cost of feeding (₦) + cost of fingerlings stocked (₦)

Net Profit = Total cost of fish cropped (₦) – Total expenditure (₦)

Profit Index = Value of fish (₦) / Cost of feed (₦)

Benefit Cost Ratio = Cost of feed (₦) / Value of fish (₦).

3.13 Data Analysis

Microsoft Office Excel (2013) statistical software package was used to run all statistical analysis. Descriptive statistics was used to summarize data obtained. Student's t-test was used to determine if there was significant difference between the proximate and anti-nutritional content of the raw and fermented seed. One-way ANOVA was adopted to determine if there was significant difference ($P \leq 0.05$) between the growth performance, feed utilization, cost benefit and carcass composition of *O. niloticus* fed varying inclusion levels of fermented *T. indica* seed meal. Linear regression was used to calculate 'a', 'b' and 'r' (value of correlation).

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical Analyses of Feedstuff

4.1.1 Proximate Composition of *Tamarindus indica* Seeds

The proximate composition of *T. indica* seed is presented in Table 4.1. The roasted seed had the highest value for dry matter (99.21%), crude protein (26.86%) and lipid (7.03%). Fermentation increased nitrogen free extract (64.12%) the most. While the raw seed had higher crude fibre (5.09%) and ash contents (6.84%) than the rest. There was significant difference ($P<0.05$) in all proximate contents of the three seed samples. Fermentation and roasting significantly increased crude protein by 10.17 and 38.03% and lipid by 46.62 and 53.16%, respectively.

4.1.2 Anti-nutrient Levels of *Tamarindus indica* Seeds

The results of anti-nutrient composition of *T. indica* seed is presented in Table 4.1. The values for alkaloid content of fermented and roasted samples were 1.50mg/g^{-1} and 6.80 mg/g^{-1}, respectively. The alkaloid content differed significantly ($P<0.05$). The values of the saponin content of fermented and roasted samples (1.80mg/g^{-1} and 4.10mg/g^{-1}, respectively) differed significantly ($P<0.05$). Phytate content for the roasted sample (7.71mg/g^{-1}) was significantly higher ($P<0.05$) than that of the fermented seeds (2.68mg/g^{-1}). Fermentation also significantly reduced the alkaloid, saponin and phytate content of the seeds by 59.46, 21.74 and 60.30%, respectively.

4.2 Proximate Composition of Experimental Diets Fed to *Oreochromis niloticus*

The proximate composition of experimental diets fed to *O. niloticus* is presented in Table 4.2. Experimental diet with 75% inclusion level of fermented *T. indica* seed meal, had the highest dry matter (93.17%), followed by those with 50%, 100%, 25% and 0% inclusion levels of fermented *T. indica* seed meal, respectively. It also had the highest crude fibre (3.33%) followed by 25%, 50%, 100% and 75% inclusion level of fermented *T. indica* seed meal, respectively. However, 25% inclusion level of fermented *T. indica* seed meal had the highest crude protein compared to the rest. Experimental diet D_4 had the least crude protein (29.63%). Ash content was higher in 25% inclusion level of fermented *T. indica* seed meal (11.66%), lipid (5.44%) and nitrogen free extract (55.08%) were higher in 75% inclusion level of fermented *T. indica* seed meal. There was significant difference ($P<0.05$) in all proximate contents except for dry matter and crude protein.

Table 4.1: Proximate and Antinutrients Composition of *Tamarindus indica* Seed

Parameters (g/100g)	Raw *T. indica* Seed	Fermented *T. indica* Seed	Roasted *T. indica* Seed
Dry Matter	94.18 ± 0.00^{b}	94.27 ± 0.00^{b}	99.21 ± 0.03^{a}
Crude Protein	19.46 ± 0.07^{c}	21.44 ± 1.01^{b}	26.86 ± 0.04^{a}
Crude Fibre	5.09 ± 0.27^{a}	3.99 ± 2.17^{b}	4.96 ± 0.06^{a}
Lipid	4.59 ± 1.39^{c}	6.73 ± 1.07^{b}	7.03 ± 0.02^{a}
Ash	6.84 ± 0.62^{a}	3.73 ± 0.65^{b}	1.73 ± 0.00^{c}
Nitrogen Free Extract	64.02 ± 1.11^{a}	64.12 ± 2.88^{a}	58.62 ± 0.01^{b}
Anti-nutrients (mg/g^{-1})			
	Raw *T. indica* Seed	Fermented *T. indica* Seed	Roasted *T. indica* Seed
Alkaloid	3.70 ± 0.10^{b}	1.50 ± 0.05^{c}	6.8 ± 0.00^{a}
Saponin	2.30 ± 0.10^{b}	1.80 ± 0.12^{c}	4.1 ± 0.10^{a}
Tannin	2.37 ± 0.02^{b}	4.22 ± 0.16^{a}	2.38 ± 0.02^{b}
Phytate	6.75 ± 0.15^{a}	2.68 ± 0.09^{b}	7.71 ± 0.01^{a}

Means with the same superscript along rows do not vary significantly ($P>0.05$)

Table 4.2: Proximate Composition of Experimental Diets Fed to *Oreochromis niloticus*

Parameters	Inclusion Levels				
	0%	25%	50%	75%	100%
Dry Matter	90.15 ± 0.00^{a}	91.72 ± 0.00^{a}	92.88 ± 0.00^{a}	93.17 ± 0.00^{a}	91.84 ± 0.00^{a}
Crude Protein	30.50 ± 0.64^{a}	31.04 ± 0.00^{a}	30.25 ± 0.33^{a}	29.63 ± 1.39^{a}	30.88 ± 0.44^{a}
Crude Fibre	3.33 ± 0.23^{a}	2.50 ± 0.21^{b}	2.50 ± 0.12^{b}	1.67 ± 0.03^{c}	2.50 ± 0.08^{b}
Lipid	4.34 ± 0.10^{d}	4.63 ± 0.09^{cd}	5.09 ± 0.11^{b}	5.44 ± 0.40^{a}	4.90 ± 0.10^{bc}
Ash	10.53 ± 0.52^{ab}	11.66 ± 0.18^{a}	9.32 ± 0.09^{bc}	8.18 ± 0.07^{c}	9.83 ± 0.58^{b}
NFE	51.31 ± 1.03^{ab}	50.17 ± 0.06^{b}	52.84 ± 0.92^{ab}	55.08 ± 1.53^{a}	51.89 ± 0.84^{ab}

Means with the same superscripts along rows do not vary significantly ($P>0.05$).

4.3 Growth Performance of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Growth performance of *O. niloticus* fed varying inclusion levels of fermented *T. indica* seed meal is presented in Table 4.3. The highest final weight (21.78g) in this study was observed in fish fed 0% inclusion level of fermented *T. indica* seed meal. However, fish fed 75% inclusion level of fermented *T. indica* seed meal was higher (17.54g) than that of 25%, 50% and 100% inclusion levels of fermented *T. indica* seed meal. The final weight for all experimental diets varied significantly ($P<0.05$).

Diet D_1 recorded highest value for weight gain (12.40g) while experimental diet with 100% inclusion level of fermented *T. indica* seed meal had the least weight gain (2.25g). And all varied significantly ($P<0.05$).

The highest final standard length (8.93cm), standard length gain (2.81cm), final total length (11.20cm) and total length gain (3.58cm) were observed in fish fed 0% inclusion level of fermented *T. indica* seed meal. Whereas, the lowest final standard length (7.52cm), standard length gain (0.70cm), final total length (9.40cm) and total length gain (0.72cm) were observed in those fed 25% inclusion level of fermented *T. indica* seed meal. All growth parameters varied significantly ($P<0.05$).

The highest value for specific growth rate (1.05%days) was observed in fish fed 0% inclusion level of fermented *T. indica* seed meal, followed by 75% (0.85%days), 25% (0.55%days), 50% (0.37%days) and 100% (0.25) inclusion levels of fermented *T. indica* seed meal, respectively.

There was significant difference (P<0.05) in the percentage live weight gain (PWG) of fish fed varying inclusion levels of fermented *T. indica* seed meal, with 100% inclusion levels of fermented *T. indica* seed meal having the least (0.23) and 0% inclusion levels of fermented *T. indica* seed meal having the highest (1.42).

4.3.1 Growth Pattern of Fish Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

The growth pattern of *O. niloticus* fingerlings fed varying inclusion levels of fermented *T. indica* seed meal for 12 weeks is presented in Figure 4.1. The control (0%), 25% and 75% inclusion levels all had increase in growth of above 30% in the first two weeks. Diets D_3 and D_5 both had a poor increase in growth of less than 20% in the first two weeks. Diets D_1 and D_4 both gave the best growth performance of more than 40%, which were 59.30% and 48.92%, respectively.

4.4 Length-Weight Relationship of *Oreochromis niloticus* Fingerlings Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

The length-weight relationship of *O. niloticus* fingerlings fed varying inclusion levels of fermented *T. indica* seed meal is presented in Table 4.4. The values for constant (a), exponent (b) and correlation coefficient (r) ranged between 0.42-0.59, 2.30-3.93 and 0.8045-0.9861, respectively. Fish fed 0% inclusion level of fermented *T. indica* seed meal had the highest ‘a’ and ‘b’, while 100% inclusion level of fermented *T. indica* seed meal had the lowest.

The condition factor obtained in this study ranged between 1.28-1.60 (Table 4.4). The values for condition factor did not vary significantly (P>0.05).

4.5 Feed Utilization *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Feed utilization parameters for this study is presented in Table 4.5. The feed conversion ratio (FCR) and gross feed conversion efficiency (GFCE) value did not vary significantly ($P>0.05$) for all inclusion levels of fermented *T. indica* seed meal.

Protein efficiency ratio (PER) obtained in this study varied significantly ($P<0.05$). 0% inclusion levels of fermented *T. indica* seed meal had the highest (0.14), while the least (0.03) was recorded in 100% inclusion levels of fermented *T. indica* seed meal. Net protein utilization values for this study ranged between 0.09-0.22% and did not vary significantly ($P>0.05$).

There was great significant difference ($P<0.05$) in the nitrogen metabolism (Nm) obtained in this study, which ranged between 281.24-50.92. 0% inclusion levels of fermented *T. indica* seed meal had the highest (281.24) while 100% inclusion levels of fermented *T. indica* seed meal had the lowest (50.92).

4.6 Carcass composition of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Table 4.6 show the carcass composition of *O. niloticus* fed the experimental diets. Fish fed the diet with 0% inclusion level of fermented *T. indica* seed meal had the highest carcass crude protein (54.02%) followed by the diet with 75% inclusion level of fermented *T. indica* seed meal (53.07%). 100% inclusion level of fermented *T. indica* seed meal gave the least carcass crude protein (49.99%). However, all feeds formulated significantly ($P<0.05$) increased the crude protein content of *O. niloticus*. The feed significantly ($P<0.05$) increased ash content (13.03% for initial and 13.64% for the least of those fed the

experimental diets) and decreased the nitrogen free extract content (34.51% for the initial and 26.09% for the highest of those fed the diets).

The dry matter content recorded for fish in this study ranged between 87.72-91.00%. Dry matter, crude fibre and lipid contents did not vary significantly (P>0.05) for both initial fish and fish fed varying inclusion levels of fermented *T. indica* seed meal.

Table 4.3*:* Growth Performance of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Parameters	Inclusion Levels				
	0%	25%	50%	75%	100%
IW (g/fish)	9.38 ± 2.36^{a}	8.39 ± 0.37^{a}	9.39 ± 1.44^{a}	8.96 ± 2.44^{a}	9.63 ± 2.90^{a}
FW (g/fish)	21.78 ± 2.03^{a}	13.15 ± 0.58^{b}	12.58 ± 0.11^{b}	17.54 ± 1.76^{ab}	11.88 ± 3.68^{b}
WG (g/fish)	12.40 ± 0.33^{a}	4.77 ± 0.92^{c}	3.19 ± 1.54^{c}	8.58 ± 0.68^{b}	2.25 ± 0.78^{c}
ISL (cm/fish)	6.12 ± 1.49^{a}	6.82 ± 0.09^{a}	6.80 ± 0.13^{a}	5.61 ± 0.39^{a}	6.38 ± 0.53^{a}
FSL (cm/fish)	8.93 ± 0.28^{a}	7.52 ± 0.12^{b}	8.0 ± 0.60^{ab}	8.15 ± 0.15^{ab}	7.61 ± 0.31^{b}
SLG (cm/fish)	2.81 ± 1.76^{a}	0.70 ± 0.03^{c}	1.21 ± 0.48^{b}	2.54 ± 0.24^{a}	1.24 ± 0.22^{b}
ITL (cm/fish)	7.62 ± 0.10^{a}	8.68 ± 0.10^{a}	8.23 ± 0.65^{a}	7.18 ± 0.44^{a}	8.15 ± 0.58^{a}
FTL (cm/fish)	11.20 ± 0.20^{a}	9.40 ± 0.10^{b}	10.11 ± 0.90^{ab}	10.30 ± 0.30^{ab}	9.51 ± 0.30^{ab}
TLG (cm/fish)	3.58 ± 1.80^{a}	0.72 ± 0.00^{c}	1.88 ± 0.24^{b}	3.12 ± 0.14^{a}	1.37 ± 0.32^{b}
SGR (%days)	1.05 ± 0.20^{a}	0.55 ± 0.12^{ab}	0.37 ± 0.20^{b}	0.85 ± 0.22^{ab}	0.25 ± 0.01^{b}
PWG	1.42 ± 0.39^{a}	0.58 ± 0.14^{ab}	0.37 ± 0.22^{ab}	1.01 ± 0.41^{ab}	0.23 ± 0.01^{b}

Means with the same superscripts along rows do not vary significantly ($p>0.05$)

Initial Weight (IW), Final Weight (FW), Weight Gain (WG), Initial Standard Length (ISL), Final Standard Length (FSL), Standard Length Gain (SLG), Initial Total Length (ITL), Final Total Length (FTL), Total Length Gain (TLG), Specific Growth Rate (SGR) and Percentage Weight Gain (PWG).

Table 4.4: Length-Weight Relationship of *Oreochromis niloticus* Fingerlings Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Inclusion Level (%)	a	b	r^2	Log W	K
0	0.59	3.93	0.8409	3.89	1.55
25	0.51	2.37	0.9861	2.01	1.59
50	0.47	2.37	0.8991	2.05	1.28
75	0.56	3.25	0.8045	3.04	1.60
100	0.42	2.30	0.8421	1.87	1.36

Constant/Intercept (a), Exponent (b), correlation coefficient (r^2), Weight (W) and Condition factor (K).

Table 4.5: Feed Utilization of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Parameters	Inclusion Levels				
	0%	25%	50%	75%	100%
FCR	1.82 ± 0.32^{a}	2.29 ± 0.27^{a}	2.73 ± 0.56^{a}	1.92 ± 0.17^{a}	2.78 ± 0.08^{a}
GFCE	56.70 ± 9.97^{a}	44.36 ± 5.14^{a}	36.63 ± 5.28^{a}	52.08 ± 2.65^{a}	36.01 ± 1.04^{a}
PER	0.14 ± 0.14^{a}	0.07 ± 0.02^{b}	0.06 ± 0.01^{b}	0.12 ± 0.02^{a}	0.03 ± 0.00^{b}
NPU	0.12 ± 0.01^{a}	0.12 ± 0.01^{a}	0.22 ± 0.13^{a}	0.13 ± 0.03^{a}	0.09 ± 0.03^{a}
Nm	281.24 ± 7.5^{a}	108.07 ± 20.8^{c}	72.35 ± 34.9^{c}	194.60 ± 15.43^{b}	50.92 ± 17.58^{c}

Means with the same superscript along rows do not vary significantly ($P>0.05$)

Feed Conversion Ratio (FCR), Gross Feed Conversion Efficiency (GFCE), Protein Efficiency Ratio (PER), Net Protein Utilization (NPU) and Nitrogen Metabolism (Nm).

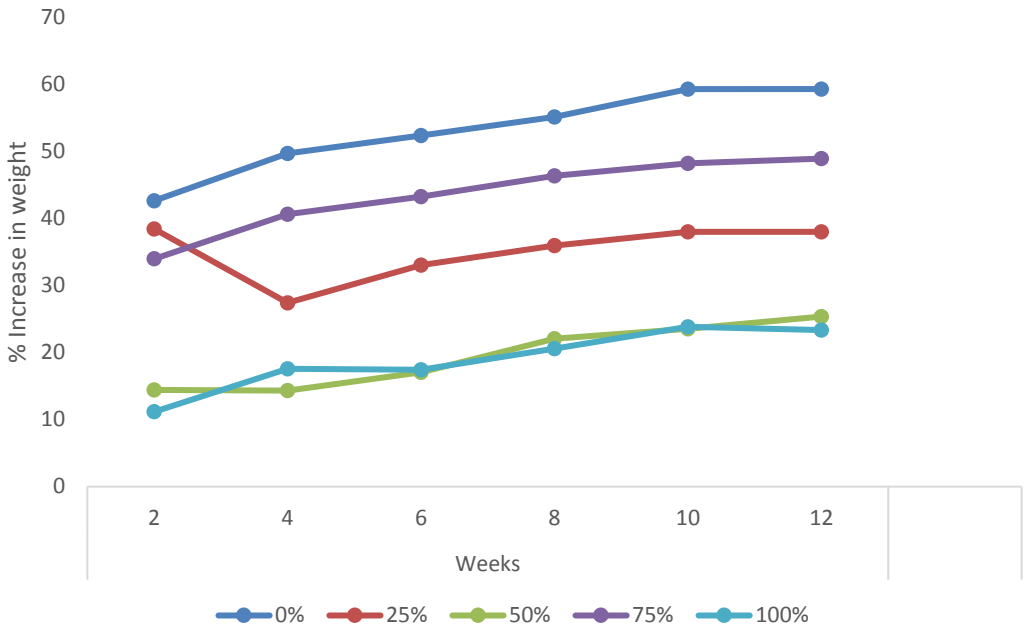


Figure 4.1: Growth Pattern of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal for 12 Weeks

Table 4.6: Carcass Composition (g/100g) of *Oreochromis niloticus* Fingerlings Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

P	Initial	Inclusion levels after feeding trial				
		0%	25%	50%	75%	100%
DM	89.84 ± 0.00[a]	91.00 ± 0.00[a]	89.91 ± 0.00[a]	90.05 ± 0.00[a]	87.72 ± 0.00[a]	89.20 ± 0.00[a]
CP	43.91 ± 0.29[d]	54.02 ± 0.08[a]	52.61 ± 0.30[b]	52.77 ± 0.27[b]	53.07 ± 0.13[b]	49.99 ± 0.01[c]
CF	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
EE	8.56 ± 0.17[a]	9.91 ± 0.48[a]	8.78 ± 0.78[a]	9.79 ± 0.27[a]	10.15 ± 0.90[a]	9.95 ± 0.33[a]
Ash	13.03 ± 0.05[c]	14.31 ± 0.25[ab]	13.81 ± 0.07[ab]	13.64 ± 0.36[ab]	14.76 ± 0.37[a]	13.98 ± 0.13[ab]
NFE	34.51 ± 0.07[a]	21.77 ± 0.81[c]	24.81 ± 1.14[bc]	24.69 ± 1.52[bc]	22.04 ± 1.39[c]	26.09 ± 0.45[b]

Means with the same superscripts along rows do not vary significantly (P>0.05)

Parameters (P), Dry Matter (DM), Crude Protein (CP), Crude Fibre (CF), Lipid (EE) and Nitrogen Free Extract (NFE).

4.7 Cost Benefit Analyses of Experimental Diets and Fish Fed Varying Inclusion Levels of Fermented *Tamarindus Indica* Seed Meal

Cost benefit analyses of experimental diets and fish cropped is presented in Table 4.7. The highest value (₦114) was gotten from fish fed 0% inclusion level followed by 75% (₦98.70), 25% (₦78.82), 50% (₦71.76) and 100% (₦66.49) inclusion levels of fermented *T. indica* seed meal, respectively.

The highest expenditure value (₦111.15) was recorded in diet 0% inclusion level of fermented *T. indica* seed meal, then 75%, 100%, and 25% and the lowest was recorded in diet 50% inclusion level of fermented *T. indica* seed meal.

The highest net profit was obtained from diet 0% inclusion level of fermented *T. indica* seed meal while the lowest was diet 50% inclusion level. The cost of producing a Kg of feed for 0% inclusion level was the highest (₦99.97) and diet 100% inclusion level cost the least (₦80.64). The highest cost of feeding (₦95.46) was recorded in diet 0% inclusion level of fermented *T. indica* seed meal, while 50% inclusion level of fermented *T. indica* seed meal had the least (₦52.55) cost of feeding.

The profit index for 75% inclusion level was the highest (1.63). The profit index of fish fed 100% inclusion level was the lowest (1.12). The benefit cost ratio of the cropped fish ranged between (1.23-1.53). There was significant difference ($P<0.05$) for all cost analyses except for cost of fingerlings and benefit cost ratio.

Table 4.7: Cost Benefit Analyses of Experimental Diets and Fish Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Parameters	Inclusion Levels				
	0%	25%	50%	75%	100%
E (₦)	111.15 ± 10.79^{a}	74.09 ± 9.52^{b}	73.59 ± 20.81^{b}	75.89 ± 14.56^{b}	75.26 ± 15.02^{b}
CF (₦)	95.46 ± 12.16^{a}	59.54 ± 7.28^{b}	52.55 ± 19.42^{b}	59.67 ± 11.30^{b}	57.58 ± 11.39^{b}
CFg (₦)	192.60 ± 0.00^{a}	192.60 ± 0.00^{a}	192.60 ± 0.00^{a}	192.60 ± 0.00^{a}	192.60 ± 0.00^{a}
VF (₦)	114 ± 25.47^{a}	78.82 ± 9.67^{b}	71.76 ± 27.31^{c}	98.70 ± 23.93^{b}	66.49 ± 12.24^{c}
CFd (₦)/Kg	99.97 ± 0.00^{a}	95.47 ± 0.00^{b}	90.66 ± 0.00^{c}	85.58 ± 0.00^{d}	80.64 ± 0.00^{e}
NP (₦)	164.84 ± 30.73^{a}	70.59 ± 12.67^{b}	45.69 ± 11.22^{c}	72.37 ± 11.09^{b}	50.69 ± 3.81^{c}
PI	1.30 ± 0.42^{b}	1.20 ± 0.33^{b}	1.13 ± 1.07^{c}	1.63 ± 0.21^{a}	1.12 ± 0.38^{c}
BCR	1.52 ± 0.26^{a}	1.41 ± 0.31^{a}	1.31 ± 0.58^{a}	1.53 ± 0.15^{a}	1.23 ± 0.22^{a}

Means with the same superscripts along rows do not vary significantly (P>0.05)

Expenditure (E), Cost of Feeding (CF), Cost of Fingerlings (CFg), Value of Fish (VF), Cost of Feed (CFd), Net Profit (NP), Profit Index (PI) and Benefit Cost Ratio (BCR).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical Analyses of Feedstuff

5.1.1 Proximate Composition of *Tamarindus indica* Seeds

Fermentation and roasting significantly increased ($P<0.05$) the lipid content of the seed by 46.62 and 53.16%, respectively. Many animals cannot synthesize one or more of the fatty acids and must ingest them in foods (Chicago: Encyclopædia Britannica, 2014a). Ouoba *et al*. (2003a) reported significant lipolysis of legumes by fermentation which yielded predominantly oleic, linoleic and linolenic acids which are essential fatty acids. Nwanna *et al*. (2004) reported lipid content value of 0.35% for raw *T. indica* seed nut in Ondo State, which is lower than that observed in this study. Crude fibre content were all lower than that reported by Akajiaku *et al*. (2014) in Imo State, which were 6.15% for raw sample and 6.30% for roasted sample of the same seed. High fibre in the diet of animals leads to higher cellulose activities and results in high number of microorganisms in their caecum. However, low fibre induces amylase secretion for fermentation of more digestible carbohydrate residue in the caecum, which is indigestible in the small intestine (Hsu *et al*., 2000).

The values for crude protein were increased by processing which is of great importance in fish feed formulation. The higher protein value obtained for the roasted sample is due to the increased activity of proteolytic enzymes, which hydrolysed inherent proteins to their constituent amino acids and peptides (Olanipekun *et al*., 2015). In most fermented high-protein products, the extent of protein hydrolysis is one of the most important factors in the changes in texture and flavour (Achi, 2005). Soluble low molecular weight peptides and

amino acids that contribute to flavour are produced through the enzymatic breakdown of proteins (Ouoba *et al*., 2003b) as a result of fermentation.

Roasting reduced the level of carbohydrate in the seed and can be attributed to the high temperature that have been reported to reduce the oligosaccharide content of legumes (Hedley, 2000). Most legumes contain large amounts of non-digestible carbohydrates, which may include arabinogalactan, stachyose, sucrose and raffinose (Achi, 2005). These carbohydrates are associated with abdominal distention and flatulence in animals (Naczk *et al*., 1997; Chicago: Encyclopædia Britannica, 2014b). Fermentation reduced total flatus factors from 2.0 to 16.5 mg/g in soybeans (Winarno and Reddy, 1986), and from 0.16% to <0.1% in groundnut (Fardiaz and Markakis, 1981). The value for both samples are higher than that of the carbohydrate value of roasted and soaked *T. indica* seed nut (58.08% and 56.24%) reported by Akajiaku *et al*. (2015). The values of the proximate composition obtained from this research are higher than those reported by Heuze and Tran (2015) in India except for ash and fibre contents, which could be as a result of difference in geographical locations in which the seeds were obtained.

5.1.2 Anti-nutrients of *Tamarindus indica* Seeds

Alkaloid containing grain legumes, such as lupins (*Lupinus albinus*), are otherwise ideally suited as feedstuff in aquafeeds because of high digestible protein content (Kumar *et al*., 2012). The low level of alkaloid in the fermented sample could be due to the extended hours spent during soaking of the seed sample to remove the seed coat and fermentation process, since aqueous extraction and treatment are known to remove alkaloids from seed materials (Kumar *et al*., 2012).

Saponins are steroids or triterpenoid glycosides found in many of the potential, alternate plant-derived feed ingredients for fish, such as various legume seeds (18-41 mg/kg) and defatted roasted soybean flour (67mg/kg) (Kumar *et al*., 2012). And when added to water, are highly toxic to fish, with detergent action causing damage to the respiratory epithelium of gills and inhibits active transport of nutrients (Kumar *et al*., 2012). Saponins increase the digestibility of carbohydrate rich foods. However, simultaneous consumption of saponin and tannin results in the loss of individual toxicity because the formation of tannin-saponin complexes inactivates the separate biological activity of both tannin and saponins. Plants containing saponins are used to heal wounds (Okwu and Josiah, 2006) because saponins have the ability to precipitate and coagulate red blood cells (Sood *et al*., 2012).

Tannins are secondary compounds of various chemical structures widely occurring in plants and are divided into hydrolysable and condensed forms. Anti-nutritional effects of tannins include interference with digestion by binding to proteins or minerals. The higher level of tannin in the fermented sample could be due to the extended hours spent during soaking of the seed sample to remove the seed coat and fermentation process which made the hydrolysable tannin content more available within the seed. However, lower level of tannins in the roasted sample may be due to the removal of condensed form of tannin from the tannin-rich outer layer of seed by roasting and dehulling.

The decrease in phytate content by soaking (fermentation: keeping of the seed for longer hours in water), cooking of pre-soaked bean or germination may be due to leaching out of this compound in water (Osman, 2007).

Pretreatments such as soaking, soaking and boiling, effected significant reduction of the anti-nutrients concentrations and toxicants present in *Mucuna pruriens* (Velvet Beans) seeds (Nwaoguikpe *et al*., 2011).

5.2 Proximate Composition of Experimental Diets Fed to *Oreochromis niloticus* Fingerlings

All crude protein contents were within the range recommended for Nile tilapia by El-Sayeed (2004) who used dose-response procedures. There was no significant difference ($P>0.05$) in crude protein of the experimental diets. All other proximate contents varied significantly ($P<0.05$) except for dry matter contents. The small variations in the proximate content could be due to variations of feed ingredients in inclusion levels of the diets. The higher carbohydrate contents in the 50%, 75% and 100% inclusion levels could be due to higher inclusion of fermented *T. indica* seed meal which contributed more carbohydrate content (64.12%) compared to soya beans meal (31.82%) (FAO, 1990).

5.3 Growth Performance of *Oreochromis niloticus* Fingerlings Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

From this study, it was observed that growth response was significantly influenced by varying inclusion levels of fermented *T. indica* seed meal. Which means weight gain and SGR decreased with increase in inclusion level of fermented *T. indica* seed meal. However, SLG and TLG increased with increase in inclusion level. The 0% inclusion level had the best growth response, followed by 75% inclusion level of fermented *T. indica*. The growth response in respect to weight gain and SGR are in conformity with the findings of Agbo *et al.* (2011) who reported decrease in growth rate with increase in the level of cotton seed meal in the diet of *O. niloticus*. However, SLG and TLG in this study are contrary to their findings. Khan *et al*. (2013) also reported decrease in growth performance of *O. niloticus*

with increase in inclusion level of rice polish. All growth response parameters varied significantly ($P<0.05$).

5.4 Length-Weight Relationship of *Oreochromis niloticus* Fingerlings Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

In this study, the growth performance of fish fed 0% and 75% inclusion levels of fermented *T. indica* seed meal were high since exponential value exhibits a high degree of positive allometric correlation between the length-weight relationships. None of the inclusion levels gave an isometric growth, in that none of them had an exponent value that is equal to 3. However, D_1 and D_4 gave positive allometric growth while D_2, D_3 and D_5 gave a negative allometric growth. The values of 'r' for all inclusion levels are close to 1.0 (0.8045-0.9861), indicating high degree of length-weight relationships. The higher 'b' values for D_1 and D_4 may be indicating the higher feeding proficiencies (Saikia *et al*., 2011), observed for the diets.

The condition factor for all inclusion levels did not vary significantly ($P>0.05$) and were very high (1.28-1.60) indicating that the fish were kept in good condition. The good condition of the culture system could probably be due to reduction in the anti-nutrient content of the seed by fermentation, and falls within the range reported by Ighwela *et al*. (2011) for *O. niloticus* (1.19-1.98). The slow weight gain might be due to the fact that the fish were not able to utilized the feed given to them efficiently which may be due to non-palatability of the feeds.

5.5 Feed Utilization of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

The values recorded for FCR in this study did not vary significantly ($P>0.05$). However, the values were high for 25%, 50% and 100% inclusion levels which suggests indigestibility of the diet and can be attributed to high crude fibre in the diet (Aderolu and Sogbesan, 2010). The low value for FCR obtained from 0% and 75% inclusion levels indicates that the fishes in the diets were able to digest their feed efficiently. A number of studies opined that low level of FCR is an indicator of feed utilization efficiency of formulated feed (Kader *et al*., 2003; Khan *et al*., 2013; Shipton and Hecht, 2013 and RIAS Inc., 2016). High-energy diet produced the lowest FCR and the highest nutrient retention (Zamal *et al*., 2009). Similarly, GFCE was not significantly different ($P>0.05$) for all experimental fish.

Net protein utilization is the ratio of amino acids converted to proteins to the ratio of amino acids supplied. As a value, NPU can range from 1 to 0, with the value of 1 indicating 100% utilization of dietary nitrogen as protein and a value of 0 indicating that none of the nitrogen supplied was converted to protein. The values for NPU from this study reveals that the fishes were able to convert some of the amino acids supplied by the diets. The values for NPU did not vary significantly ($P>0.05$) and ranged between 0.09-0.22.

Nm and PER decreased with increase in inclusion level of fermented *T. indica* seed meal and all varied significantly ($P<0.05$). PER increased with the quantity of protein fed. The high value for Diets D_1 and D_4 can be attributed to their higher cost of feeding.

5.6 Carcass composition of *Oreochromis niloticus* Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

All experimental diets significantly increased the protein and lipid contents of experimental fish. Obaroh *et al.* (2015) reported that protein, lipids and moisture contents are the major constituents when evaluating nutritive value of fish. This result implies that there was synthesis and increased tissue protein production (Yusuf *et al*., 2016). 0% inclusion level of fermented *T. indica* seed meal had the highest carcass protein (54.02%), while the least (49.99%) was recorded for 100% inclusion level of fermented *T. indica* seed meal (D_5). This could be due to the higher proportion of soya bean meal protein that was not included in D_5.

Increase in lipid content of the carcass can be due to accumulation of fat (Bekibele, 2005) which has contributed to increase in weight. However, there was no significant (P>0.05) increase in the lipid content of all fish carcass from this study.

Carbohydrate content increased with increase in inclusion level and must have resulted in D_5 having the least WG, SGR, GFCE, PWG, PER, NPU and Nm. Also, Pantazis (2005) reported that high dietary carbohydrate level has significant protein sparing effect in fish.

5.7 Cost Benefit Analyses of Experimental Diets and Fish Fed Varying Inclusion Levels of Fermented *Tamarindus indica* Seed Meal

Cost benefit analysis of feed revealed that there was no significant difference (P>0.05) in the cost of fish reared across treatments. Expenditure for Diet D_1 (0% inclusion level) was the highest (₦111.15) and varied significantly (P<0.05) with the other diets (25%, 50%, 75% and 100%). Cost of feeding for 0% inclusion was also the highest (₦95.46) and also varied significantly (P<0.05) with the rest. This could be the most probable reason why 0%

inclusion level gave the best growth performance and coupled with the fact that the fish in the 0% inclusion level of fermented *T. indica* seed meal ate of feed than the rest. The high cost of purchasing soya beans used for feed formulation caused the higher cost of feed and feeding for diet D_1. However, this consequently resulted in the better growth performance by fish fed the feed with the 0% inclusion of fermented *T. indica* seed meal.

All other economic analysis varied significantly ($P<0.05$) except for Benefit cost ratio. Net profit is the actual profit after working expenses. Net profit of fish was better for 0% inclusion level followed by 75% inclusion level. However, the lower net profit of D_3 and D_5 can be attributed to the lower weight gain of fishes fed the diets.

Profit index (PI) qualifies the amount of value created per unit of investment and is usually accepted when PI is greater than 1. Profit index was better (1.63) in D_4 which could be due to the fact that D_4 had a lesser expenditure (₦75.89), cost of feeding (₦59.67) and cost of feed (₦85.58) than D_1 and the rest.

Benefit cost ratio (BCR) summarizes the overall value for money of feeding trial. Benefit cost ratio (which ranged from 1.23–1.53) were all greater than 1, which means all inclusion levels are viable. Yakubu *et al*. (2014) stated that benefit cost ratio that is greater than 1 is viable.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

In this study, seeds of *T. indica* were fermented for 72hours. Fermentation increased the protein, lipid and carbohydrate content of *T. indica* seed by 10.17, 46.62 and 0.16%, respectively. Alkaloid, saponin and phytate content of the seed were also reduced by 59.46, 21.74 and 60.30%, respectively. The fermented seed meal was used to compound feed for *O. niloticus* fingerlings reared for 12 weeks under laboratory condition replacing soya bean meal at varying inclusion levels (0%(control), 25%, 50%, 75% and 100%). Of all inclusion levels of fermented *T. indica* seed meal, 75% inclusion level gave the best growth performance and utilization (weight gain: 8.58g, total length gain: 3.12, specific growth rate: 0.85, percentage weight gain: 1.01, feed conversion ratio: 1.92. gross feed conversion efficiency: 52.08, protein efficiency ratio: 0.12 and net protein utilization: 0.12). A high degree of length-weight relationship was observed in all inclusion levels. Diets D_1 and D_4 had a positive allometric growth while D_2, D_3 and D_5 had a negative allometric growth. Carcass composition of *O. niloticus* fingerlings fed varying inclusion levels of fermented *T. indica* seed meal was best for the fish fed 75% inclusion level. Diet D_4 (75% inclusion level) had the best value of fish (₦98.70), net profit (₦72.37), profit index (1.63) and benefit cost ratio (1.53).

6.2 Conclusions

Fermentation and roasting significantly (P<0.05) increased the nutritive content (crude protein by 10.17 and 38.03%, and lipid by 46.62 and 53.16%) of *T. indica* seed nut and

also, fermentation significantly decreased the anti-nutrients of the seed except for tannin content (alkaloid: 59.46%, saponin: 21.74%, tannin: 43.84% and phytate: 60.30%).

The growth performance of *O. niloticus* fed 75% inclusion level of fermented *T. indica* seed meal (WG: 8.58g/fish, TLG: 3.12cm/fish and SGR: 0.85) varied significantly ($P<0.05$) compared to those fed other inclusion levels, and also maximally utilized (FCR: 1.92, PWG: 1.01 and Nm: 194.60) fermented *T. indica* seed meal at 75% inclusion level compared to the rest, and was second best only to the control diet (0% inclusion level).

All inclusion levels significantly ($P<0.05$) increased the carcass composition of *O. niloticus*. 75% inclusion level gave the best crude protein (53.07%) followed by 50, 25 and 100% inclusion levels apart from the control (0% inclusion level).

Diet 4, 75% inclusion level of fermented *T. indica* seed meal (VF: ₦98.70, NP: ₦72.37, PI: 1.63 and BCR: 1.53) was more cost effective than the other inclusion levels. Therefore, soya bean meal can be replaced at 75% inclusion level of fermented *T. indica* seed meal in the diet *O. niloticus*.

6.3 Recommendations

Further work should be carried out using other processing methods like boiling and germination to improve the nutritive value of *T. indica* seeds and also to boost the growth performance and feed utilization of fish.

Also, the performance of *T. indica* seed meal can be augmented by increasing the composition of fish meal, other animal protein sources or other better plant protein sources in addition to *T. indica* seed to attain a better growth response.

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