GROWTH RESPONSE OF NILE TILAPIA *OREOCHROMIS NILOTICUS*(L) FINGERLINGS FED *PARKIA BIGLOBOSA*(JACQ BENTH) PULP MEAL AS A PARTIAL REPLACEMENT OF MAIZE MEAL

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AUGUST, 2015.

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.SC) DEGREE IN FISHERIES.

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AUGUST, 2015.

DECLARATION

I declare that the work in this thesis entitled: "Growth Response of Nile Tilapia *Oreochromis niloticus* (L) Fingerlings Fed *Parkia biglobosa* (JACQ Benth) PULP Meal as a Partial Replacement Of Maize Meal" has been performed by me in the Department of Biological Sciences. The information derived from the literatures has been duly acknowledged in the text and a list of reference provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

_____	_____	_____
DANLADI AWAN	SIGNATURE	DATE

CERTIFICATION PAGE

This thesis entitled: "GROWTH RESPONSE OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* (L) FINGERLINGS FED *PARKIA BIGLOBOSA* (JACQ BENTH) PULP MEAL AS A PARTIAL REPLACEMENT OF MAIZE MEAL" by DANLADI AWAN meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Almighty God, my family and lovers of knowledge.

ACKNOWLEDGMENTS

My sincere gratitude goes to God Almighty for His protection and grace given me throughout the research work. My appreciation also go to my Supervisors, Prof. S.A. Abdullahi and Prof. J.A. Adakole for their critical scrutiny of the thesis and for their moral support and advice. My appreciation goes to the entire staff of Biological Sciences especially the Head of Department, Prof. A.K. Adamu, the Post Graduate Coordinator Dr. M. A. Adelanwa, and the Postgraduate Seminar Coordinator, Dr. W.S. Japhet for their various advice and suggestions in the successful completion of the research work. Also worthy of appreciation are Prof. J. Auta, Dr. (Mrs.) B. Suleiman and Dr. T. T. Gbem for their tremendous concern toward this research work. Also worthy of note are Mr. Luka and Mr. Stephen of Fisheries Laboratory, Mallam Magaji of Hydrobiology Laboratory, and Mr. Kwanoh of the Department of Animal Science and faculty of Agriculture for their assistance in he course of the research work.

I am indebted to Air Commandore. J.B. Kwasu and Dr. Yashim Andrew Nuhu of Nigerian Airforce, Kaduna and Department of Haemathology, National Hospital, Abuja, respectively, for their financial and moral assistance during the research work.I also want to express my appreciation to my fellow Postgraduate Students for their wonderful and meaningful contributions during the course of the programme.

Finally, I will not forget to mention my wife and children for their patience with me during the research work.

ABSTRACT

This study was conducted in the Fisheries Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria to investigate the growth response of Nile tilapia, *Oreochromis niloticus*(L) fingerlings. There were five (5) treatment groups with three (3) replicates each. Fifteen (15) white rectangular plastic tanks were used for a period of 13 weeks.0One hundred and fifty (150) fingerlings of *O. niloticus* of mixed sexes and sizes of Initial Mean Weight (4.13 ± 3.04g) were stocked in fifteen (15) white rectangular plastic tanks of water holding capacity of 30 litres (49 x 33.5 x 33.5) with ten (10) fingerlings each per tank and water maintained at 20 litres level for a period of thirteen weeks (13). *Parkia* Pulp Meal (PPM) was used to replace Yellow Maize (YM) at inclusion levels of 0%, 25%, 50%, 75% and 100% for Diet 1 to 5 respectively. Crude protein of 45% was used for the feeds. Proximate analysis of nutrients composition of *Parkia* Pulp recorded Dry Matter (94.48 ± 0.69), Nitrogen Free Extracts (77.30 ± 1.34g). The proximate levels of anti-nutritional factors of *Parkia* Pulp was recorded as Saponin(17.45 ± 0.041mg/100g). The results obtained showed D_4 (75%) *Parkia* Pulp Inclusion being highest in Mean Weight Gain (12.62± 1.44), CrudeProtein(47.39 ± 0.70), Nitrogen Free Extract(17.20±0.28),SpecificGrowthRate(0.635±0.06)and Energy Value (1845.42 ± 18.16), except Protein Intake(277.8 ± 1.27) was found to be highest in D_4 (75%) *Parkia*Pulp Inclusion. The Initial Weight (IW) and Final Weight (FW), Initial Standard Length (ISL), Final Standard Length (FSL), Initial Total Length(ITL) and Final Total Length(FTL), Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR) showed no significant difference (P > 0.05). Also, pH, Temperature (T°C), Dissolved Oxygen (DO), Total Dissolved Solids (TDS) and Electrical Conductivity (EC) showed no significant difference. (P > 0.05). Likewise, Economic Conversion Ratio (ECR), Incidence of Cost (IC) and Profit Index (*PI). It can be concluded that *Parkia* Pulp Meal (PPM) inclusion in the diet of fish of up to 75% can be effectively utilised by the *O. niloticus* fingerlings for optimal growth performance and corresponding economic profitability, hence serve as a good replacement for maize meal in the fish diet.

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LIST OF ABBREVIATIONS

%	Percentage
AC	Ash Content
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
CF	Crude Fibre
CP	Crude Protein
DC	Degree Celsius
DM	Dry Matter
DO	Dissolved Oxygen
ECR	Economic Conversion Ratio
et al	and others
FAO	Food and Agricultural Organization
FCR	Feed Conversion Ratio
FM	Fish Meal
g	Gramme
HCN	Hydrogen Cyanide
IAR	Institute for Agricultural Research
IC	Incidence of Cost
IGF-I-	Immunoglobulin F – I
IITA	International Institute of Tropical Agriculture
M	Mortality
MC	Moisture Content

Mg/L	Milligram Per Litre
MWG	Mean Weight Gain
NaCl	Sodium Chloride
NFE	Nitrogen Free Extracts
NNR	Net Nitrogen Retention
NRC	National Research Council
^{o}N	Degree North
PER	Protein Efficiency Ratio
pH	Hydrogen Ion Concentration
*PI	Profit Index
PI	Protein Intake
PP	*Parkia* Pulp
PPM	*Parkia* Pulp Meal
PV	Profitability Value
SB	Soya Bean
SD	Standard Deviation
SGR	Specific Growth Rate
SPSS	Statistical Package for Social Sciences
SR	Survival Rate
μS / cm	MilliSiemens Per Centimeter
W/V	Weight Per Volume
WHO	World Health Organization
YM	Yellow Maize

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Nutrition comprises all the activities associated with securing the materials necessary for an organism's survival(Falayi,2009). Carbohydrates are many in nature and are highly important in animal feeds. They form about 75% of all the dry matter in plants and the chief source of energy and heat in the feeds of animals (Falayi, 2009).Starch is the major storage carbohydrate in plants. It has found immense industrial use in the manufacture of products such as food, textile, paper, adhesive and pharmaceuticals, reported (Ihegwuagu *et al*., 2009). Carbohydrates are the major source of dietary energy for terrestrial animals; however the ability of fish to utilize carbohydrates varies among fish species (NRC, 1993).

1.1.1 Maize

Maize is a cereal crop consumed by man and livestocks.The increasing prohibitive cost of this maize has necessitated the need to search for an alternative source of energy. Recently, FAO (2006), reported shortages in the production of cereals as a serious issue in many countries including Nigeria. The use of cereal products, especially unjustified in economic terms (Tewe, 2004), because of the ever increasing cost. There is therefore, the need to explore cheaper energy sources to replace expensive cereals in fish feed formulation. To relieve the food feed competition between man and animal and for profit maximization, *Parkia* Pulpis very appropriate for this purpose.

Developments in nutritional physiology, aquaculture technology and economical constraint have triggered the use of cheaper feed ingredients with higher carbohydrate contents (Aderolu *et al.,* 2010). Maize is a cereal grain commonly used as energy/carbohydrate in fish diets; however competition for its use in human foods and animal feeds limits its availability and hence increase its price in the market (Olorin *et al*., 2006,Aderolu *et al.*,2009, Aderolu and Sogbesan, 2010).

The need to solve the problems of feeding in aquaculture has been demonstrated through various researches in the utilization of vegetable sources and agricultural wastes such as plantain peel meal (Fasakin, 2008). Water hyacinth meal (Sotolu, 2008), cassava leaves (Bichi and Ahmadu, 2010), indomie waste (Eniolorunda *et al*., 2008; Aderolu and Sogbesan, 2010), cassava peels, rice brans, cocoa pod husk (Osineye *et al*., 2008) and yam peel (Fagbenro, 1996). Omoregie *et al* (1991)in a study to investigate the growth and feed utilization of *O.niloticus* fish diets containing cassava peels and mango seeds, found that *O.niloticus* can contend with limited amount of cassava peels and mango seeds. Utilization of non conventional carbohydrate had been emphasized in the formulation of least – cost fish feed towards ensuring profitable fish business.

According to Olurin *et al.* (2006), maize is the major source of metabolizable energy in most of the compounded diet of catfish species. This is because it is readily available and digestible. However, the increasing prohibitive cost of this commodity has necessitated the need to search for ingredients that will serve as replacements. In aquaculture, fish feed has been acknowledged generally to constitute the major cost incurred during the production cycle (Eyo, 2003; Jamiu and Ayinla 2003; Akinrotimi *et al*.,2007). The establishment of economically viable fish culture ventures requires the utilization of agro – industrial waste or

by – products. This maize substitutable ingredient had been shown to be highly efficient in replacing maize in the diet of monogastric animals. Falaye(1988) reported that large quantities of these crop residues and agro – industrial wastes that can be utilized as diet components, are either wasted on less significant purposes or denatured.

Locust bean tree (*Parkia biglobosa)* is a perennial leguminous tree that belongs to the Fabaceae family. It grows in the savanna region of West Africa up to the southern edge of the Sahel Zone (Ihegwuagu *et al*., 2009). The tree is not normally cultivated, but can be seen in the wild in the savannah region of Nigeria (Ihegwuagu *et al*., 2009). The seed is commonly called 'Kaluwa' and the fruit "dorowa" or "dazim" around the Northern region of Nigeria. *Parkia biglobosa* tree plays vital economic role in recycling nutrients from the soil. It is a good source of timber and is useful in making pestles, mortars, bows and hoe handles,(Ihegwuagu *et al*., 2009). The seeds serve as a source of useful ingredients for consumption. It has been reported that the husk and the floury pulp can be made into drinks rich in vitamin C and sugar(Obizoba. 1998; Ihegwuagu *et al*., 2009).

Nile tilapia,*Oreochromis niloticus* is a cichlid fish of African origin. It belongs to the order Perciformes and a member of the Cilchlidae family. It is reported to be a plankton feeder, an omnivore and also feeds on higher plants to the extent that, it may be used in control of aquatic weeds. It is a species of highly economic value and is widely introduced outside its natural range after *O. mossambicus*. It is the most cultured cichlid. In recent research in Kenya, it has been suggested that the fish can feed on mosquito larvae, hence it can be used in the fight against malaria in Africa. Tilapias are considered suitable for aquaculture because of their high tolerance to adverse environmental conditions, their relatively fast growth and the ease with which they can breed, good utilization of artificial diet, resistance to diseases,

excellent quality of their firmly textured flesh, moreso, they are appetizing to consumers. *Oreochromis niloticus*is one of the most important tilapia species farmed in aquaculture in Africa, especially Nigeria (Sekoni *et al*., 2008).

1.2 Statement of Research Problem

Fish feed accounts for at least 60% of the total cost of production(Fagbenro and Adeparusi, 2003; Gabriel *et al*.,2007).Expensive feeds could negatively affect the profitability of fish farming. Thus, incapacitating the expansion of farms to increase production and consequently, low yield in terms of quality and quantity of fish.

Maize has been a traditional energy source in formulated feeds, but competition between human beings and livestocks for the grain has led to the rising cost of maize and its accompanying scarcity is making it increasingly uneconomical to feed the grain to livestock including fish (Sotolu and Byanyiko, 2010),

*Parkia biglobosa*pulp is a predominant waste from the processing of locust bean, in Northern Nigeria (Sotolu and Byanyiko, 2010). Its abundance could serve as a source of a water pollution in nearby streams, rivers and even fish ponds, leading to eutrophication and a resultant depletion of dissolved oxygen thereby affecting aquatic lives (Falayi, 2009).

1.3 Justification

The use of local, cheap and unsuitable feedstuffs for direct human consumption as alternative energy feed could reduce the cost of production without compromising fish quality. (Sogbesan and Ugwumba 2008; Iluyemi *et al*., 2010), thus the need for this study.

It is equally important that more researches should be focused on finding alternative suitable energy sources to maize in fish diet, considering the rising cost and accompanying scarcity of maize (Sotolu and Byanyiko, 2010). This could reduce the competition for the grain between humans and livestocks.

The abundance of *P.biglobosa* pulp could serve as alternative energy source therefore constitute huge benefit for the sustainability of the aquaculture industry, if properly harnessed. Also a lot of research had been conducted on *P.biglobosa* seeds for protein,leaves andbarks for medicinal purposes, but little has been done on *P.biglobosa* yellow pulp as an energy source, thus the need for this research work.

1.4 Aim

To evaluate the growth response of Nile Tilapia, *O. niloticus* fingerlings fed *P. Biglobosa*pulp meal in percental inclusion levels, replacing maize meal.

1.5 Objectives

To determine the:-

1. Levels of anti-nutritional factors of *P.biglobosa* pulp.
2. Proximate nutrients composition of *P. biglobosa* pulp
3. Growth performance of Nile Tilapia*O. niloticus* fingerlings fed *P.biglobosa* pulp meal at different percental inclusions.
4. Economic profitability of*O. niloticus* production using*P.biglobosa* pulp meal.

1.6 Hypotheses

1. There is no significant difference in the levels of anti-nutritional factors in *P. biglobosa* pulp.
2. *Parkia biglobosa* pulpmeal has no significant difference with respect to its nutrient composition.
3. There is no significant growth difference among*O. niloticus* fingerlings fed *P. biglobosa* pulp mealat different percental inclusion levels.
4. There is no significant difference in the economic profitability of *O. niloticus* production using *P. biglobosa* pulp meal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Food Usage of African Locust Bean Tree

The trees of the *Parkia* species are usually and carefully preserved by the inhabitants of the area where they grow because they are valuable sources of reliable food, especially the seeds which serve as source of useful ingredients for consumption (Alabi *et al.*,2005). It has been reported that the husks and pods are good food for livestock (Obizoba, 1998). Earlier investigators have mentioned the food and nutritive values of *P. biglobosa* (Alabi, 1993; Alabi *et al.,* 2004). Ayelaagbe *et al.* (1996) and Alabi *et al.* (2004) reported that oil derived from the cotyledons of the seeds have significantly high saponfication value 160.60mg/100g, which makes*P. biglobosa* seed oil very usueful for soap making. In many communities in South West Nigeria, edible fruits from wild plants are often taken as food or added to food as condiments to supplement important minerals and vitamins in human diets Olujobi (2012).

Some wild fruits have higher nutritional values in term of protein compared with the levels found in some cultivated fruits (Eromosele *et al.*,1991; Alabi *et al.*,2005). African locust bean (*P. biglobosa)* seed has been found to be very useful especially in its fermented form and it is widely eaten among all the tribes across the country (Olujobi, 2012). However, the yellow dry powdery fruit pulp has not attracted much attention (Olujobi, 2012). The fruit pulp is used in rural communities in Africa during emergencies, when the grain stores are empty (Edem and Mranda, 2011). The pulp is also used as an ingredient in the preparation of various stews and soups (Odebunmi *et al.,* 2010). According to Uwaegbute (1996) the powdery fruit pulp

contains more carbohydrate than the seeds, the carhobydrates being primarily reducing sugars (19.00%), non-reducing sugars (9.00%) and other complex carbohydrates (36.00%).

2.2 Medicinal Importance of Africa Locust Bean Tree

Plants are known to have the ability to produce and store a wide range of chemical substances (Udobi and Onaolapo, 2012). A lot of them are responsible for plant flavours, while others give plants their characteristic odour and yet, others are responsible for the kind of pigments seen in plants (Cowan, 1999). The anti-microbial properties of plants are traceable to these substances divided into phenolic groups and hydroxyl groups. Cowan (1999) observed that increased hydroxylation of pigment and extract from locust beans sourcesleads to higher toxicity to microorganisms.*Parkia biglobosa* has so much medicinal use especially in West Africa. It is used against bronchitis, pneumonia and diarrhoea. A decoction of the stem bark is used as a mouth wash (Ajaiyeoba, 2002).

The bark is also used with lemon for wounds and ulcers. In Cote d' Ivoire and Nigeria, a bark infusion is used as a tonic for diarrhoea (Duker-Eshun *et al.*,2001). The leaves are also used for burns and toothache as well as for sore eyes in Gambia (Banwo *et al.*,2004). Recently, the attention of researchers has been drawn to the great potentials in *P. biglobosa* as a source of an antibacterial agent. Ajaiyeoba (2002), Banwo *et al.*(2004) and Millogo-Kone*et al*. (2006), have all reportedthe presence of plant secondary metabolites which are known to exhibit anti-bacterial activity against a wide range of organisms. *Parkia biglobosa* was largely prescribed in traditional medicine for its multiple medicinal values. It was reported that its stem bark was successfully used for the treatment of many infectious diseases, dentalcaries, severe cough, diarrhoea, wounds, otitis, dermatosis, amoebiasis, bilharziosis, Leprosis, ankylosis, tracheitis and conjunctivitis (Nacoulme, 1996; Arbonnier, 2002).

2.3 Roots and Tuber Sources of Carbohydrates

Like most roots and tubers, cassava peels and roots are much richer in crude protein, ether extract and ash than edible portion (Solomon et al., 2007). Omoregie *et al.* (1991) in a study to investigate the growth and feed utilization of *Oreochromis niloticus* fed diets containing cassava peelings and mango seed.

The calculated nutritive value of cassava has been reported to be higher than that of Sorghum (Solomon *et al.,* 2007). The root and leaves of cassava plant are the two nutritionally valuable parts which offer potential as a food or feed source. Four diets prepared to contain 0% (control), 33.3%, 66.7% and 100% cassava leaves to replaced equal weight of maizein a study by Bichi and Ahmadu. (2010) the study suggests that 66.7% cassava leaves replacement was considered as the optimum requirement for catfish (*Clarias gariepinus)* growth performance. Cassava is one of the most important energy sources in the diet of people in the tropics(Abu *et al.,*2010). Recent estimates suggest that its storage roots provide 8% or more of the minimum calorie requirement of more than 750 million people (Abu *et al.,*2010). Its starchy root produces more calories per unit of land than any other crop in the world (Presston, 2004). According to Pedrosa (2002), cassava roots are generally rich in calcium and ascorbic acid and contain significant amounts of thiamine, riboflavin and niacin. Salami (2002), reported its starchy, thickened, tuberous roots are a valuable source of cheap calories and its use in animal feed is increasing because of its high energy content and low price. In recent times, the use of cassava as a substitute for cereals in livestock and fish feeds has been under investigation. Inclusion of cassava in the diet of white Fulani herds in Nigeria has been reported to increasemilk production by 22% (IITA, 1990). Oke (2007), reported that cassava products are good energy feed ingredients for both monogastric and ruminant animals. Talthawan *et al.* (2002), reported that starch in cassava is highly digestible when compared to that of maize

due to the high content of amylopectin. Cassava can then be used as a source of energy in fish feed, but attention should be paid to the protein, metabolisable energy and Hydrogen Cyanide (HCN) contents in cassava products.

Studies on the use of cassava meal in animal feed (Oresegun and Alegbeleye, 2001; Faturoti*et al.*, 2002; Eyo, 2003) indicate a great potential for cassava to replace the conventional energy food ingredients such as maize, broken rice and sorghum, which are commonly used in animal diet in most parts of Africa (Akinfala and Tewe, 2001). Inclusion of whole Cassava root meal in the diet of hybrid catfish enhanced growth and survival of the fish (Abu *et al.*,2010). Carbohydrate either of cereal or tuber in fish feed acts as both structural and energy component, which have some influence on the rate of growth of fish provided all other physiological requirements are satisfied (Carter *et al.*, 2003). Olurin *et al* (2006) reported a replacement level of 50% Cassava meal for maize without a depressing growth in*Clariasgariepinus.* Cardoso *et al* (2005) observed that good processing of cassava enhances survival and healthy state of fish at all stages of their life.

2.4 Grain Sources ofCarbohydrate

Maize, wheat, rice, sorghum and millet are cereal grains with almost equal caloric and nitrogenous composition (Solomon *et al.*,2007). The grains are generally 68-72% starch, with 10-12% protein (Halver, 1989). However, due to genetic environmental interactions some could have protein as low as 8%. The relative utilization of dietary carbohydrates by fish varies and appears to be related to their complexity. In general, the complex carbohydrate, such as starch and dextrin are utilized by most fishes better than simple sugar like glucose (Shiau and Lin, 1993). Al-Asgah and Alli(1994), observed that maize grain at 25% in tilapia

diet showed better growth performance and nutrient utilization in comparison with other carbohydrate sources such as corn starch, dextrin, sucrose and glucose.

However, the performance of *O. niloticus* has been reported to decrease with the increasing level (25-43%) of maize grain in the diet (Al-Ogaily *et al.*,1994). Shalaby *et al.* (1989) reported that wheat, maize and rice increased the growth performance of common carp more than barley and sorghum when fed fish meal-soyabean meal diets with cereal grains and grain by-products. Al-Ogaily *et al.*(1996) who fed different grain sources to tilapia reported that the best growth performance was obtained for sorghum based diet whereas barley based diet gave the poorest growth performance. Bergot (1993) reported that wheat starch appeared to be the most digestible form out of a series of seven untreated carbohydrate sources. However, the digestibility of starch is affected not only by the source and nature of carbohydrate, but also by the level of its incorporation (Al-Ogaily *et al.*,1996).

2.5 Agro-Industrial Waste Sources of Carbohydrates

The establishment of economically viable fish culture ventures requires the utilization of agro-industrial cassava peels, rice bran, cocoa pod husk (Osei *et al.*,1990), Kola nut husk (Osineye *et al.,* 2008). Jamiu and Ayinla (2003) expressed that feed management determines the viability of aquaculture as it accounts for atleast 60% of the cost of fish production.

2.6 Growth and Nutrient Utilisation

According to De Silva (2001) feed conversion ratio is between 1.2 to 1.8 for fish fed optimal prepared-diets. Davis (2004) observed that protein efficiency ratio is a measure of how well the protein sources in a diet could provide the essential amino acid requirement of the fish fed.

Abu *et al.* (2010) reported a highest level of whole cassava root meal inclusion value of 66%.Abu *et al.* (2010) reported the highest value of 1.85±0.04,while the lowest value was recorded in diet D66% with a value of 1.71± 0.04,for Feed Conversion Ratio (FCR) of cassava root meal inclusion. Aderolu and Sogbesan (2010) reported the mean weight Gain was highest at the 25% Cocoyam inclusion level replacing maize in *Clarias gariepinus* diet. Also, specific growth rate recorded highest at the 25% cocoyam inclusion level. Aderoluand Sogbesan, (2010) also reported the least feed conversion ratio of 0.86 and the highest protein efficiency ratio of 2.94 in the diet with25% Cocoyam inclusion level replacing maize.

2.7 Proximate Composition of Nutrients and Anti-nutrients of *Parkia biglobosa*

Germah *et al.* (2007) reported a moisture content of 8.41%, protein 6.56%and Carbohydrate of 67.30% for *Pakia* fruit pulp. According to Uwaegbute. (1996) the powdery fruit-pulp contains more carbohydrate than the seeds, the carbohydrates being primarily reducing sugar (19.00%), non-reducing sugars (9.00%) and other complex carbohydrates (36.00%). The fruit-pulp of the African Locust bean is sweet to the taste which indicates presence of natural sugar and thus a potential energy source, Germal *et al* (2007). The attractive yellow colour indicates the presence of photo-nutrients, possibly carotenoids, an important precursor of Retinol (Vitamin A). It has a sour taste which indicates the presence of Ascorbic acid (Vitamin C).

The fruit pulp is used in rural Africa during emergencies, when the grain stores are empty, which is an indication of its edibility and non-toxicity(Akoma *et al.*,2001). It may also be used as an ingredient in the preparation of various stews, soups and sauces for the consumption of cereals; pressed into cakes and preserved for later use or used in the preparation of some indigenous drinks and is more than adequate to meet the FAO/WHO recommended daily allowance of protein of 0.59g/kg body weight for an average healthy

individual and 0.88g/kg body weight for children aged 1 to 10 years (Shakuntala and Shadaksharaswamy, 1987).

Uwaegbute (1996) reported acarbohydrate content of 67.32% higher than the 49.49% in the seeds,showed that the fruit pulp contains more carbohydrates than the seeds. It is also higher than most legume seeds with only Lentils and Bambara groundnuts coming close with a value of 65.0% (Muller, 1988). Though proteins and fats also provide energy, carbohydrates are much cheaper and more easily digested and absorbed (Fox and Cameron, 1989).

Crude fibre wasfound to be 11.15%, reported Germah *et al.* (2007). Though crude fibre does not contribute nutrients or energy, it is a source of dietary fibre which is essential for good bowel movement and help in preventing obesity, diabetes, cancer of the colon and other ailments of the gastro-intestinal tract of man, crude fat was found to be 1.80% (Germah *et al.*, 2007). Germah *et al*. 2007, reported an ash content of 4.19%, which is within the range for most legumes such as 2.00% in peas and 5.00% in soya beans.

Akegbejo-Samson *et al.* (2004) reported presence of essential amino acids. Shiau and Lin(1993) reported that complex carbohydrates are more easily utilized for growth in fish such as tilapia than simple sugars. Maize Kernel consist up to 73% starch, while *Parkia* Pulp (PP) has up to 32.14% starch (Akegbejo-Samson *et al*., 2004). Phytic acid content was found to be 60.00mg/100g (Germah *et al.*, 2007). Germah *et al.* (2007) reported a Saponin content of 17.80mg/100g. This is much lower than in other everyday food stuffs like Lima beans (24.50mg/100g) and Millet (19.47mg/100g) as reported by Osagie (1998) and therefore, considered to be safe. Germah *et al.*(2007) reported tannin content of 81.00mg/100g in

African Locust bean pulp, which is much lower than values in some legumes like Lima beans (140.00mg/100g) and Pigeon Pea (100.00mg/100g) as reported by Osagie(1998).

2.8 Feeding of *O. niloticus*

In the development and management of an aquaculture enterprise, fish feed plays a vital role in its growth and expansion (Gabriel *et al*., 2007). The high cost of feeding has affected the development and expansion of aquaculture enterprise in most African countries contributing in no small way, to the low protein intake in many developing countries (Abu *et al*., 2010). Non conventional feed resources are credited for being non competitive in terms of human consumption, very cheap by-products or waste products from agriculture (Sogbesan and Ugwumba, 2008; Iluyemi *et al*., 2010). The main sources of carbohydrate in many farm based fish diets in Ghana and other West African countries are wheat bran and maize (Abarike *et al*., 2013). A fair proportion of industries in Ghana, Nigeria and other West African countries which are agriculture – based and produce a range of by-products which could be rich ingredients for the formulation of fish feed as they already contribute to livestock and poultry feed (Abarike *et al*., 2013). Abarike *et al.* (2013) uses pito mash, a by-product obtained from sorghum used in the production of local drink known as "Pito", rice bran and groundnut bran to feed *O. niloticus* fingerlings. The palatability and acceptability of the diets containing pito mash, rice bran and wheat bran, Abarike *et al.*(2013) indicates the feasibility of using these agro-industrial by-products in formulating feed for fingerlings of *O. niloticus.* Similar results have been reported for *O. niloticus* by Liti *et al.*(2006) and Attipoe *et al.* (2009), and for Florida Red Tilapia (*O. niloticus* crossed with *O. mosambicus*) by El-Dakar *et al.* (2008).

Availability of these agro-industrial by-products is a significant step towards more efficient utilisation of plant by-products (Abarike *et al*., 2013). The ability of *O. niloticus* to utilise

various diets could be attributed to wide spectrum of preference for foods (Abarike *et al*., 2013). This is in agreement with Gonzalez and Allan (2007) and Audu*et al.* (2008) who reported that, *O. niloticus* readily adapts to eating a wide variety of feeds and that they have very long intestines necessary to digest plant materials. Solomon *et al.* (2007) used different grain sources (maize, wheat, rice, sorghum and millet) to assess the growth of tilapia in out-door hapas, reported that the best mean Weight Gain for fish fed with maize grain > wheat grain > sorghum grain > millet grain > rice grain in this order. Gonzalez and Allan (2007) and El-Dakar *et al.*(2008) reported that high fibre levels in the diet of tilapia resulted in poor weight gain and nutrient utilisation. Chatzifotis *et al.*(2010) demonstrated that growth can be reduced and Protein Efficiency Ratio lowered if fishes were fed with excessive dietary lipid levels. This is because excessive lipids not only spare proteins, but can also result in higher fat deposits and impaired growth performance. Manjappa *et al.* (2002) reported that, growth of fish fed with diets which have higher lipid level (above 12%) is poorer, reflecting a negative impact of dietary fat beyond the optimum level. El-Dakar *et al.* (2008) reported an incidence cost of 2.26 for diets containing up to 50% fig jam by-products replacing wheat bran in the diet of red tilapia and recommended it as an economically efficient diet for red tilapia fry. Liti *et al.*(2006) reported that wheat bran based diet gave higher returns than the other test brans on the economic performance of *O. niloticus.* The use of carbohydrate-rich diets has been considered economical as fish would utilise the inexpensive carbohydrate as a source of energy (Liti *et al*., 2006; Mohanta *et al*., 2007). Amisah *et al* (2009) indicated that in developing countries like Ghana, Nigeria and other West African countries, most feeds have to be imported using the limited foreign exchange of the state. According to FAO (2006), fish supplies from capture fisheries will therefore, not be able to meet the growing global demand for aquatic food. There is need for a viable alternative fish production system that can sufficiently meet this demand, and aquaculture fits exactly into this role (Abu *et al.*,2010). As

aquaculture production becomes more and more intensive in Nigeria, fish feed could be a significant factor in increasing the productivity and profitability of aquaculture as it accounts for at least 60 percent of the cost of fish production (Jamiu and Ayinla, 2003).The need to intensify the culture of the fish, so as to meet the ever increasing demand for fish has made it essential to develop suitable diets either in supplementary forms for ponds or as complete feed in tanks (Olukunle, 2006). For the purpose of nutritional and economic benefits, previous researchers have made attempts at increasing the use of non-conventional plant and animal materials to replace conventional feed ingredients like maize and fish meal in fish feed ration (Baruah *et al*., 2003; Eyo, 2004). Olurin *et al* (2006) reported a replacement level of 50% cassava meal for maize without a depressing growth in *Clarias gariepinus*. Abu *et al* (2010) reported a growth performance and nutrient utilisation of fish feed 66% level of whole cassava root meal, which implies that high inclusion levels of whole cassava root meal in the diet of hybrid catfish favours enhanced growth rate. Fakunmoju *et al.* (2014) reported that cassava peels could be used to replace maize in the diet of Nile Tilapia (*O. niloticus*) with considerable economic gain.

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

2.9 Physico – Chemical Parameters

Abu *et al.*(2010) reported the results of the water quality variables, indicated that mean values of pH ranged from 6.60 to 8.55, while concentration of Dissolved Oxygen (DO) ranged from 4.99 to 7.10mg/L^{-1}. Water temperature ranged from 27.11 to 29.14^{o}C, nitrite ranged 0.0010 to 0.0054mg/L^{-1},ammonia 0.30 to 0.46mg/L^{-1} and total hardness 46.05 to 80.08. Aderolu and Sogbesan. (2010) reported water parameter ranges were 27.5 to 29.5^{o}C, 4.5 to 4.8mg/L^{-1} and 7.3 to 8.0 for temperature, dissolved oxygen and pH, respectively, for the treatments of *clariasgariepinus*juvaniles. Tropical fish species generally prefer temperatures above 20^{o}C are unsuitable. Temperature on digestion is hinged with metabolic rate, which varies with the activity of enzymes. A narrow or wide range of suitable temperature for digestion depends on the species of fish (Ugwumba and Ugwumba,2007). In tilapias such as *O. niloticus* and *Sarotherodon melanotheron* as well as in the African bony-tongue, *Heterotis niloticus,* intestinal amylase activity is maximum between pH 7.0-8.5 being optimal at the upper level (Ugwumba and Abumoye. (1998).



Plate I:*Parkia biglobosa* tree
a – Trunk



Plate II: ***Parkia biglobosa flower and leaves***

b - Flower
c - Leaves



Plate III:*Parkia biglobosa* fruit pods

d – Unripe fruit pods

CHAPTER THREE

3. 0 MATERIALS AND METHODS

3.1 Experimental Site

The research was conducted in the Fisheries Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

3.1.1. Experimental design

There were five (5) treatment groups with three (3) replicates each. Fifteen (15) white rectangular plastic tanks were used for a period of 13 weeks. Each tank has a water holding capacity of 30 litres (49x33.5x33.5cm); but water was maintained at the 20 litres level throughout the study and ten (10) fingerlings of *Oreochromis niloticus* were stocked per tank according to Eyo (1994). The tanks were covered with net meshes to prevent fish from jumping out. A partial flow-through system with fresh dechlorinated water was used throughout the experiment. Solid wastes in tanks were siphoned forthnightly during which data on body weight were taken to determine fish growth performance.

The fish were starved for 24 hours in order to empty their stomach and prepare their appetite for the new feed. Fish were fed twice daily morning (9:00am) and evening (4:00pm) with the experimental diets at the rate of 3%body weight. An electric meter Zurich (Swiss made) top-loading balance model Cp8201 was used to weigh fish forthnightly. Standard and total lengths were also determined using a meter rule.

3.1.2. Experinmental Set-up

The fiften (15) white rectangular plastic tanks were supplied with dechlorinated water source from the tank/Reserviour shown by a horizontal arrow labbeled (A). The taps labelled with

arrows vertically (B) supplied each plastic tank, which are inlets to the plastic ponds, while the arrows labelled vertically (C) are outlets for removal of waste and polluted water while washing the tanks. (Plate IV).

3.2 Collection and Processing of Materials

3.2.1. Fingerlings collection

One hundred and fifty (150) fingerlings of *O.niloticus* of mixed sexes and sizes were procured from Bagauda Fisheries Farm, Kano state, Nigeria. They were conveyed to the Fisheries laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, in oxygenated polythene bags.

The fingerlings were acclimatized for two (2) weeks, during which they were fed at the rate of 3% body weight with commercial (control diet) pelleted feed. They were treated with antibiotic (Ampiclox) and vitamin C to relief them from bacterial infections and stress, respectively.

3.2.2. Collection of feedstuffs

Yellow Maize (YM), *Parkia* Pulp (PP), Fish Meal (FM) and Soya Bean (SB) 0.41Kg, 0.409Kg, 1.52Kg, 2Kg, were purchased.*Parkia* Pulp was purchased at a market in Jagindi, Jema'a Local Government Area of Kaduna State, Nigeria. Yellow maize and soya bean were purchased at a market in Sabon-Tasha, Kaduna State, Nigeria. Fish meal was purchased at Sabon – Gari market, Zaria, Kaduna State, Nigeria.Vitamin premix lysine, Methionine, bone meal, and sodium chloride (common salt) of 4.5g, 1.8g, 0.9g, 2.7g and 0.9g resepectively,were purchased at Rebson Agric. Enterprise Ltd, Samaru, Zaria, Nigeria.



Plate IV: Cross Section of Experimental Set-up

Note:

a - Inlet from tank
b - Inlet to plastic ponds
c - Outlet

3.3 Processing of Ingredients

The *Parkia*pulpwere extracted after opening the pods. The yellow pulp which embedded the seeds were sundried and the seeds were separated from the yellow pulp using a threshing machine. The yellow pulp were further sun dried, ground into fine powder, sieved using a small mesh size (0.5mm) to separate the powder from the chaff. The pulp were transported in polythene bags and stored in a dry place till further use. (Sotolu and Byanyiko, 2010).

Soya Bean (SB) was toasted in an electric oven at 100°C for 15 – 30 minutes(Eyo, 1999). The toasted soya bean was ground in a Hammer mill machine to fine texture and sieved with (0.5mm) mesh size openings. The powder was transported in polythene bags and stored in air dried places for further use, Sotolu *et al* (2010).

The yellow maize was toasted in an electric oven at 100°Cfor 15-30minutes (Eyo, 1999). The toasted yellow maize was ground into fine powder with a hammer mill machine and sieved with a small mesh size (0.5mm) to separate the powder from the chaff. The powder wasstored in polythene bags and kept in a dry place till further use(Sotolu *et al.*,2010).

Fish meal was prepared by sun-drying of the fish. The dried fish were ground into powder using a Hammer mill machine, sieved and stored in polythene bags tillfurther use(Sotolu *et al.*2010).

3.3.1. Feed formulation

Feedstuffs were prepared and formulated into feeds, using the Pearson square method to determine the proper dietary proportion of feedstuffs bearing the protein requirement of the fish being 45% crude protein.Five (5) experimental feeds were formulated at varying percental inclusions of *P. biglobosa* pulp as (D1=0%, D2= 25%, D3=50%, D4=75%, D5=

100%) replacing yellow maize (YM) and D0= control diet was a commercial feed. Equal proportions of vitamin premix, bone meal methionine, lysine and NaCl (common salt) were added to the formulated diets. All other feed ingredients except *P.biglobosa* pulp and yellow maize were included at equal proportions in all the formulated diets (Table 3.1).

3.3.2. Dry and Wet Mixing of ingridents

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

3.3.3. Procedures for Pelleting of diets

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

Table 3.1: Feed Composition of Experimental Diets

S/N	Ingredients	D_1 (0%)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
1	Fish meal	42.6	42.6	42.6	42.6	42.6
2	Soya Bean	30.3	30.3	30.3	30.3	30.3
3	Yellow Maize	16.4	12.3	8.2	4.1	-
4	Parkia Pulp	-	4.1	8.2	12.2	16.4
5	Vit. Premix	4.5	4.5	4.5	4.5	4.5
6	Lysine	1.8	1.8	1.8	1.8	1.8
7	Methionine	0.9	0.9	0.9	0.9	0.9
8	Bone meal	2.7	2.7	2.7	2.7	2.7
9	NaCl	0.9	0.9	0.9	0.9	0.9
	Total	100.1%	100.1%	100.1%	100%	100.1%

Where: D_1(0%) = Diet 1

D_2(25%) = Diet 2

D_3(50%) = Diet 3

D_4(75%) = Diet 4

D_5(100%) = Diet 5

3.4 Proximate Analysis

Proximate analysis was conducted in the Food Science Laboratory, Institute for Agricultural Research, Zaria, Nigeria. Proximate analysis of *Parkia* pulp and anti nutritional compositions were determined. Experimental diets, initial and final fish samples (carcasses) were also analysed for Crude Protein (CP), Dry Matter (DM), Crude Fibre (CF), Lipid Content, Free Nitrogen Extracts, Ash Content and Moisture Content, using AOAC (1990) methods.

3.4.1 Determination of dry matter

Dry Matter (DM) was determined by drying two grams of each representative sample in a (Gallen Kamp UK) at 105°C for 24 hours.Ash content was determine by the incineration of two grams of each sample in Muffle furnace (Lenton furnace, England) at 600°Cfor 2 hours using AOAC (1990) methods.Nitrogen content of the samples were estimation using Micro-Kjeldahl method crude protein contents were estimated by manipulating their respective percentage nitrogen values by the factor 6.5(AOAC 1990).

3.4.2 Determination of moisture content

Moisture content was determined using the method of AOAC (1990). A clean crucible was dried to a content weight in an oven at 105^{o}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^{o}C. The crucible and its contents were cooled to room temperature in a desiccator and weighed. The procedure was continued until a constant weight was obtained (W_3).

The moisture content was calculated thus:

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

3.4.3 Determine of ash content

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

The ash content was calculated thus:

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

3.4.4 Determination of ether extract

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

The extractable lipid of the sample was calculated as:

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

3.4.5 Determination of crude fibre content

Crude fibre was determined using the method of AOAC (1990). Two grams of the finely ground sample was put into a round bottom flask. Then 100 ml of a 0.023 M Sulphuric Acid solution was added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. This was then quantitatively transferred into the flask and 100 ml of 0.312 m Sodium Hydroxide solution was added and the mixture boiled again under reflux for 30 minutes before quickly filtered under suction. The insoluble residue was washed until it was base free, dried to constant weight in an oven set at 100^{O}C, cooled in a desiccator and weighed (C2). This residue was then incinerated in a muffle furnace at 550 ^{o}C for two hours, cooled in desiccators and reweighed (C3).

The crude fibre content was calculated as:

$$\% \text{ Crude fibre} = \frac{C2-C3}{W} \text{x } 100$$

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

Weight of original sample = W

3.4.6 Determination of crude Protein (Kjeldahl method) content

Crude protein was determined using the method of AOAC (1990). Two grams (2g) of the sample was weighed into 100 ml Kjeldahl digestion flask. Then 1g of catalyst mixture (Na2SO4 + anhydrous CuSO4, ratio 1:10) was added to speed up the reaction. Exactly 25 ml of concentrated Sulphuric Acid (Specific gravity 1.84) was also added into the flask. The content in the Kjeldahl digestion flask was then heated slowly at first in a Kjeldahl digestion heating unit until fretting subsided and then more vigorously (with occasional rotation of the flask to ensure even digestion and to prevent overheating of the content) until the green digest was obtained. The solution was transferred into a 100 ml volumetric flask and diluted to the

mark with distilled water. Then ten ml aliquot of the diluted solution (or digest) was pipetted into a Kjeldahl distillation flask and ten ml of 40% Sodium hydroxide solution was added. The solution was steam distilled and the liberated ammonia was trapped in a 250 ml conical flask containing 10 ml of 4% Boric Acid and a drop of mixed indicator (methyl red and methyl blue ratio 2:1). Distillation was continued until the pink colour of the indicator turned greenish. The content of the conical flask was titrated with 0.1 M HCI and endpoint was indicated by a change from greenish to pink colour. The volume of the acid used for each distillate (as well as the blank) was noted.

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

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

Where,

V0 = Volume of HCI required for the blank

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

M= Concentration of HC1 (0.1M)

14 = Atomic weight of nitrogen

100 = Total volume of digest (ml)

10 = Volume of digest distilled (ml)

2 = Weight of sample taken in (gram)

1000 = Conversion factor to dm

The crude protein was calculated as:

Crude Protein = 6.25 x %N

3.4.7 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).

3.4.8 Energy Values

Energy values for *Parkia* Pulp, experimental diets, initial and final fish samples (Carcasses) were estimated in Kcal/100g/dm, by multiplying the percentages of Crude Protein, Crude Lipids and Nitrogen Free Extracts by the factors 17.7, 37.7 respectively and summoning up the values. All determinations were carried out in triplicates and reported as mean ± standard deviation following standard recommendations of the AOAC (1990).

3.5 Determination of anti-nutritional factors

3.5.1 Determination of Oxalate

Oxalate was determined by the method of Oke (1969). One gram of the sample was placed a 200ml volumetric flask 150ml of distilled water and 10ml of 6m HCI were added. The mixture was warmed on a water bath at 70^{o}C for 4 minutes. The supernatant was then diluted to 200ml. There 50ml aliquots of the supernatants were evaporated 25ml; the brown precipitate was filtered off and washed. The combined solution and washings was titrated with concentrated ammonia solution in drops until salmon pink colour of methyl orange changed to faint yellow.

The solution was heated on water bath to 90^{o}C and the oxalate precipitated with 10ml of 5% calcium chloride (CaC12) solution. The solution was allowed to stand overnight and then was centrifuged. The precipitate was washed into a beaker with hot 25% H2SO4 dilute to 125mls with distilled water and after warming to 90^{o}C, it was titrated against 0.05m KMnO4.

Calculation: 1ml of 0.05mnO4 = 2.2mg oxalate,

3.5.2 Determination of phytic acid / phytate

Phytate phosphorous was determined by the method of AOAC (1980). Exactly one gram of the defatted sample was extracted for 1 hour with 100ml of 3% TCA with occasional swirling by hand. The suspension was centrifuged and a definite volume (10ml) of the supernatant was precipitated with 4ml of ferric chloride solution (containing 2mg of ferric ion ml" 1 in 3% TCA). The precipitate of ferric Phytate was converted to ferric hydroxide with 4ml 1.5M NaOH. The ferric hydroxide was dissolved in hot 40ml 3.2M HNO3 and transferred 10 100ml volumetric flask. The volume was made up with distil water.

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

Standard Iron: This was prepared by dissolving 0.5g of iron III chloride (FeC13) in distilled water and the volume made up to 1 litre. This stock solution contained 3.4 x 105 ppm of iron.

Working Standard: 1ml of the stock solution was pipetted into 100ml volumetric flask and diluted to mark with, distilled water, there by obtaining the concentration of 3ppm. Absorbance was measured at 480nm with 1.5M KSCN.

Calculation

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

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

Therefore C unknown = C iron = C phytate-p

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

3.5.3 Determination of tannin

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

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

The tannic acid standard curve was prepared by dissolving 0.2g of tannic acid in distilled to 200ml (1mg/ml)., Varying concentration (0.1-1.0mg/ml) of standard tannic acid solutions are pipetted into 10 different 100ml conical flask. 5ml Folin Denis reagent and 10 ml saturated Na2CO3 solution was pipetted into the test tubes. Then make up to the 100ml mark with distilled water. The solution is left to stand for 30mins and the optical density was measured at 700mm. A plot of optical, density tannic acid concentration was made; with the line passing through the origin. % Tannin content was calculated thus:

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

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

3.5.4 Determination of saponin

The method of AOAC (1980) was used, 5.0g ground was weighed into a thimble and transferred into the soxhlet extractor chamber filled with a condenser. Some quantity of

acetone reflux was poured into the flask. The sample was exhaustively extracted, at its lipid for 3 hours and the solvent distilled off. This is the first extraction.

The second extraction, a pre-weighed flask was fitted unto the soxhle apparatus (bearing the sample containing thimble) and methanol poured into the flask. The methanol should be enough to cause a reflux. The Saponin was there exhaustively extracted for 3 hours. The flask was re-weighed. The difference in weight represents the weight of Saponin extracted.

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

Nitrogen Determination:

A small amount (200mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjelcihal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na2SO4), copper sulphate (CuSO4) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

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

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

$$\text{Percentage Nitrogen} = \frac{(a-b)\ x\ 0.01x14xvx100}{wxc}$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100ml)

w. = Weight of dried sample (mg)

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

14. = Nitrogen constant in mg.

3.6 Weighing and Measuring of Fish

The mean weight of fish was determined using an electric Mettler Zurich (Swiss made) top-loading balance model CP8201. This was done after acclimatization period. Mean weight per aquarium was recorded after taken the bulk weight of fish. Mean weight per aquarium was taken every fortnight throughout the thirteen (13) weeks period of experiment. Both standard length (SL) and the total length (TL) of fish used for the experiment were recorded using a measuring board, a meter rule was used to take both total and standard length of fish. Growth performance parameters such as specific growth rate (SGR), feed conversion ratio (FCR), etc were calculated.

3.7 Growth Performance and Computation

Data collected were processed to determine fish growth performance and nutrients utilization as follows:-

3.7.1. Mean weight gain

This is the difference between the Final Weight and the Initial Weight of the Fish that is being cultured (Falayi, 2009).The difference between the final weight and initial weight was determined as follows:-

Mean weight gain (MWG) = W2 –W1

Where W1 = Initial Weight

W2 = Final Weight.

3.7.2. Specific growth rate (SGR %)

It is an index showing the best growth in a set of growths. This was determined to observe the best growth in a set of growth. This was obtained using the method of Brown, (1957) thus:

$$SGR\% = \frac{\log \text{ of } W2 - \log \text{ of } W1}{T2\ T1} \times 100$$

Where W1= Initial weight
W2= Finial weight
T1= Initial time
T2= Final time

3.7.3. Protein efficiency ratio (PER)

It is described as live Weight Gain per gram of protein fed. This is estimated from the relationship between the increment in weight and the protein consumed by the fish.

Protein Efficiency Ratio is based on the Weight Gain of a test subject divided by its Intake of a particular food protein during the test period. This was estimated from the relationship between the increment in weight (i.e Weight gain) and the protein consumed by the fish.

$$PER = \frac{\text{Weight (g) (wet)}}{\text{Protein intake (g)}}$$

3.7.4. Protein Intake (PI)

This was determined from the proportation of Protein Content in the total feed.

$$PI = \frac{\text{Total Feed Consumed x \% Protein in Fee}}{100}$$

3.7.5. Feed conversion ratio (FCR)

It is a numerical value used to measure the gross utilization of food for growth in fish and other animals. It is assumes that Weight Gain in fish and other animals is due to an increase in body weight. A lower FCR therefore implies efficient food utilization by the animal. This was measured as the gross utilization of food for growth in the fish (Falayi, 2009).

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

A lower level of feed conversion ratio therefore implies efficient food utilization by the fish.

3.7.6. Net nitrogen retention (NNR)

This is expressed as a percentage thus:

$$NNR = \frac{\text{Initial body protein}}{\text{Final body protein}} \times 100$$

3.7.7. Mortality:

This was calculated as follows

$$M = \frac{\text{numbers of fish dead at the end of the experiment}}{\text{Initial numbers of fish stocked}} \times 100$$

Falayi (2009)

3.7.8 Condition Factor (K)

This was calculated according to (Amisah et al; 2009):

$$K = \frac{100 \times W}{L3}$$

Where K = Condition Factor
L = Length (cm)
W = Body Weight of Fish (g)

3.8 Determination of Water Quality Parameters

Water parameters were determined throughout the 13 weeks experimental period (readings were taken bi-weekly). The parameters include: water pH, water temperature and Dissolved oxygen (DO), Total dissolved solid (TDS) and Electrical conductivity (EC). Hanna pH/EC/TDS/Temp instrument H19830 was used to determine Electrical conductivity (EC), Total dissolve solid (TDS), water pH and water temperature. Dissolved oxygen was determined using D_O meter (Hanna model D1946) after it has been calibrated and the protective cap has been removed. The tip of the probe was immersed into the water samples, allowed for 2 minutes and reading was recorded in mg/l. pH, EC, TDS and Temperature were obtained using Hanna pH/EC/ TDS/ Temperature instrument after it protective cap has been removed, the instrument was held while the tip of probe was immersed into water sample and allowed to read until the parameters value became static which correspond to the appearance of a clock symbol on the screen of the instrument.

3.9 Economic Analyses of Experimental Diets

Konyeme *et al.* (2005) method was adopted. The economic analysis was computed to estimate the cost of feed required to raise a kilogram of fish using various experimental diets. Estimated Investment cost analysis= Cost of feeding (₦) + Cost of fingerlings stocked. Profit Index = Value of fish (₦) / cost of feed (₦).

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



Plate V: Pelleted Fishmeal Formulated with *Parkia biglobosa* pulp



Plate VI: Lateral view of *Oreochromis niloticus* fingerling

3.9.1 Economic conversion ratio (ECR)

This is also known as feed conversion efficiency. It is one of the most important principles for a fish farmer to understand. The cost of fish feed claims over half of the total budget for most fish farms. It is very important for a fish farmer to get the optimum performing feed for the most economical price. Feed conversion efficiency or ratio is a fairly simple calculation to perform.

ECR = Feed Intake x Feed Conversion Ratio

3.9.2 Incidence of cost (IC)

$$IC = \frac{\text{Cost of feed consumed(₦)}}{\text{Mean weight gained by the fish (g)}}$$

$$PI = \frac{\text{Value of fish produced(₦)}}{\text{Cost of feed consumed(₦)}}$$

3.10 Data Analysis

Data were analyzed using one way analysis of variance (ANOVA) to evaluate significant differencesin means. Duncan's Multiple Range Test was used to rank and separate means where ANOVA showed significance. SPSS V.20 (2011) was adopted (statistical package) to show mean deviation and standard errors. Statistical signficance was set at $P \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 *Parkia biglobosa* Pulp

This study conducted with the purpose of evaluating the growth response of Nile tilapia, *O. niloticus* fingerlings fed *P. biglobosa* pulp meal in percental inclusion, replacing maize meal came up with the following proximate composition of *P. biglobosa* pulphad a dry matter of 94.48±0.69g, Moisture Content of 5.32±0.54g, Lipid Content of 2.42±0.82g, Ash Content of 6.47±0.67g, Crude Protein of 5.53±0.89g, Crude Fibre of 8.46±0.79g, Nitrogen Free Extracts of 77.30±1.34g and Energy Value of 1553.66±0.77kcal/100g/dry matter as presented in Table 4.1.

4.2 Anti-nutritional Factors in *Parkia biglobosa* Pulp

The proximate evaluation of the anti-nutritional factors in *P. biglobosa* pulp. This study revealed and recorded a saponin concentration of 17.45±0.041mg/100g/dry matter, phytic acid concentration 325±0.51mg/100g/dry matter and an oxalate concentration of 88±0.72mg/100g/dry matter as shown in Table 4.2.

4.3 Proximate Composition of Experimental Diets

In this study, dry matter of the experimental diets showed no significant difference ($p \geq 0.05$) between D_1(%) and D_4(75%)of *P. biglobosa* pulpinclusion with values of 98.14±0.06g and 98.04±0.25g, respectively. There is a significant difference ($p \leq 0.05$) between the dry matter of experimental diet of D_1 (0%), D_4 (75%) and those of other treatments. The dry matter ranged from 96.26±0.76g to 98.14±0.06g between D_3 (50%) and D_1 (0%) of *P. biglobosa* inclusions. This indicates that D_1 (0%) recorded the highest dry matter, while D_3 (50%) recorded the lowest dry matter in the different treatments.

Table 4.1: Proximate Composition of *Parkia* Pulp

S.No	Proximate Contents (%)	Beans Pulp (Means±SD)
1.	Dry Matter (g)	94.48±0.69
2.	Moisture (g)	5.32±0.54
3.	Fat (g)	2.42±0.82
4.	Ash (g)	6.47±0.67
5.	Crude Protein (g)	5.53±0.89
6.	Crude Fibres (g)	8.46±0.79
7.	Nitrogen Free Extracts (g)	77.30±1.34
8.	Energy values (kcal)	1553.66±0.77

Note: Values are expressed as means ±SD (Standard Deviation).

Table 4.2: Levels of Anti – Nutritional Factors in*Parkia* Pulp

S.No	Anti – nutritional Contents (mg)	Beans pulp (Means±SD	NRC, Tolerable limit of anti – nutrients
1.	Saponin	17.45±0.041	1.0g/kg
2.	Tannins	27.0±0.41	15mg/kg
3.	Phytic Acid	325.0±0.51	5g/kg
4.	Oxalate	88.0±0.72	10-20ppm/kg

Where ppm – parts per millions

Note: Values are expressed as means±SD (Standard Deviation).

Source: National Research Council (NRC), 1993.

The Moisture Content of the experimental diet was highest at D_3 (50%) and lowest at D_1 (0%) with values of 3.74±0.76g, and 1.86±0.06g respectively. The Moisture Content of D_3 (50%) of value 3.74±0.76g showed significant difference ($p \leq 0.05$) with other treatments. Moisture Content in the experimental diet ranged from 1.86±0.06g to 3.74±0.76g for D_1 (0%) and D_3 (50%), respectively.

Lipid Content of the experimental diets was highest at D_5 (100%) and lowest at D_1 (0%) with values of 18.66±0.72g and 13.34±1.38g, respectively. The Lipid Content ranged from 23.34±1.38g to 18.66±0.72g between D_1 (0%) and D_5 (100%).*Parkia biglobosa* inclusion showed significant difference ($p \leq 0.05$) with other experimental diets in the other treatments.

Ash Content in the experimental diets showed no significant difference ($p \leq 0.05$) with other experimental diets in the other treatments. Ash Content in the experimental diets showed no significant difference ($p > 0.05$) between D_2 (25%) and D_4 (75%), but D_2 (25%) and D_4 (75%) showed a significant difference ($p \leq 0.05$) with the Ash Content of other treatments. Ash Content ranged from 10.67±0.49g to 14.53±0.71g between D_1 (0%) and D_4 (75%) of *P. biglobosa* pulp inclusion. Crude Protein of the experimental diets showed significant difference ($p \leq 0.05$) between D_5 (100%) with the Crude Protein of the other treatments. Crude Protein of the experimental diets ranged from 38.98±1.93g to 47.39±0.70g between D_2 (25%) and D_5 (100%) of *P. biglobosa* pulp inclusion. D_5 (100%) recorded the highest Crude Protein in the experimental diet, while D_2 (25%) recorded the least with values of 47.39±0.70 and 38.98±1.93 respectively. Crude Protein of the experimental diets in D_4 (75%) showed no significant difference ($P > 0.05$) with D_1 (0%).

The highest Crude Fibre (6.63±0.85g) in the experimental diets was recorded in D_1 (0%), while the lowest (2.67±1.28g) was recorded in D_2 (25%). The Crude Fibre of the experimental diet ranged from 2.67±1.25g to 4.63±0.85g between D_2 (25%) and D_1 (0%) of *P. biglobosa* pulpinclusion. Crude Fibre in D_1 (0%) significantly differ ($p \leq 0.05$) from Crude Fibre of the experimental diets in other treatments.The experimental diets showed that D_1 (0%) and D_2 (25%) showed no significant difference ($p > 0.05$) with respect to Nitrogen Free Extracts, with values of 26.77±0.96g and 27.14±0.75g, respectively. There was a significant difference ($p \leq 0.05$) between D_1 (0%), D_2 (25%) with other treatments. Nitrogen Free Extracts had a range from 11.48±1.18g to 27.14±0.75g between D_4 (75%) and D_2 (25%) respectively. In other words, the D_2 (25%) has the highest while D_4 (75%) has the lowest levels of Nitrogen Free Extracts.

The Energy values of the experimental dies recorded highest in D_5 (100%), while D_1 (0%) recorded the lowest value of 1848.42 ± 18.16kj/100g/dry matter and 1762.83 ± 32.16kcal/100 g/dry matter. There was a significant difference ($p \leq 0.05$) between the energy value of D_5 (100%) and other treatments in the experimental diets. Apart from D_5 (100%) of *P. biglobosa* pulp inclusion, other treatments showed no significant difference ($p > 0.05$) among each other in regards to Energy value in the experimental diet (Table 4.3).

Table 4.3: Proximate Composition of Experimental Diet in means/SD.

DIET	D_1(0%)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
Dry matter (g)	98.14 ± 0.06^{a}	97.50 ± 0.44^{ab}	96.26 ± 0.76^{c}	98.04 ± 0.25^{a}	97.12 ± 0.13^{b}
Moisture (g)	1.86 ± 0.06^{c}	$2.50\pm0.44b^{c}$	3.74 ± 0.76^{a}	1.96 ± 0.25^{c}	2.88 ± 0.13^{b}
Lipids (g)	13.34 ± 1.38^{c}	$16.12\pm0.44b^{c}$	16.52 ± 0.66^{b}	16.40 ± 0.66^{b}	18.66 ± 0.72^{a}
Ash content (g)	10.67 ± 0.49^{c}	14.43 ± 0.39^{a}	13.34 ± 1.25^{ab}	14.53 ± 0.71^{a}	12.44 ± 0.61^{b}
Crude protein (g)	44.41 ± 0.72^{c}	38.98 ± 1.93^{e}	41.51 ± 1.09^{d}	44.68 ± 0.43^{c}	47.39 ± 0.70^{a}
Crude fibre (g)	4.63 ± 0.85^{a}	2.67 ± 1.28^{b}	3.49 ± 0.64^{ab}	2.76 ± 0.98^{b}	3.24 ± 0.12^{ab}
Nitrogen Free Extracts (g)	26.77 ± 0.96^{a}	27.14 ± 0.75^{a}	23.12 ± 0.87^{b}	11.48 ± 1.18^{d}	17.20 ± 0.28^{c}
Energy value in (kcal)	1762.83 ± 32.16^{b}	1778.04 ± 10.06^{b}	1764.88 ± 11.07^{b}	1789.44 ± 2.56^{b}	1845.42 ± 18.16^{a}

Note: values are expressed as mean ± SD (standard deviation)

Mean with different superscripted alphabets across rows are significantly different ($p\leq0.05$).

Table 4.4: GrowthRate of *O. niloticus*.

Fish	D_1 (0%)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
I W (g)	4.50 ± 3.32^{a}	4.30 ± 3.43^{a}	3.99 ± 3.04^{a}	3.98 ± 2.79^{a}	3.60 ± 1.64^{a}
FW (g)	15.86 ± 11.46^{a}	14.01 ± 9.63^{a}	14.87 ± 9.53^{a}	16.60 ± 0.67^{a}	15.13 ± 9.86^{a}
WG (g)	11.36 ± 2.19^{abc}	9.71 ± 1.67^{bc}	10.88 ± 2.48^{bc}	12.62 ± 1.44^{a}	11.53 ± 2.08^{ab}
SGR (%)	0.559 ± 0.04^{a}	0.525 ± 0.09^{a}	0.581 ± 0.07^{a}	0.635 ± 0.07^{a}	0.632 ± 0.06^{a}
ISL (cm)	5.20 ± 1.26^{a}	5.13 ± 1.34^{a}	5.00 ± 1.31^{a}	5.00 ± 1.15^{a}	4.93 ± 0.83^{a}
FSL (cm)	8.19 ± 2.16^{a}	8.07 ± 2.22^{a}	8.35 ± 1.85^{a}	8.49 ± 2.15^{a}	8.36 ± 1.97^{a}
ITL (cm)	6.28 ± 1.59^{a}	6.14 ± 1.56^{a}	5.93 ± 1.47^{a}	5.85 ± 1.44^{a}	5.89 ± 0.91^{a}
FTL (cm)	9.42 ± 2.16^{a}	9.31 ± 2.27^{a}	9.94 ± 2.22^{a}	9.84 ± 2.33^{a}	9.70 ± 2.14^{a}
CF	1.77 ± 0.83^{a}	1.57 ± 0.24^{ab}	1.35 ± 0.24^{b}	1.56 ± 0.36^{ab}	1.49 ± 0.34^{ab}
PI (g)	225.70 ± 2.12^{c}	198.13 ± 5.68^{e}	210.97 ± 3.20^{d}	277.8 ± 1.27^{a}	240.87 ± 2.07^{b}
FCR	0.67 ± 0.02^{a}	0.72 ± 0.06^{a}	0.67 ± 0.00^{a}	0.69 ± 0.02^{a}	0.72 ± 0.04^{a}
PER	0.82 ± 0.01^{c}	1.07 ± 0.06^{a}	0.94 ± 0.03^{b}	0.54 ± 0.01^{e}	0.73 ± 0.00^{d}
M(%)	20.00 ± 5.77^{a}	15.00 ± 5.00^{a}	15.00 ± 5.00^{a}	23.33 ± 8.82^{a}	26.67 ± 8.82^{a}
NNR (%)	87.82 ± 0.57^{a}	87.87 ± 0.97^{a}	86.59 ± 0.60^{a}	86.84 ± 0.41^{a}	85.91 ± 0.45^{a}

Note: Values are expressed as mean ±SD (standard deviation).

Means with different superscripted alphabets across rows are significantly different at $p\leq0.05$.

Where: IW - Initial Weight FW - Final Weight WG - Weight Gain SGR - Specific Growth Rate ISL - Initial Standard Length

FSL-Final Standard Length ITL - Initial Total Length FTL - Final Total Length CF - Condition Factor PI - Protein Intake

FCR - Feed Conversion Ratio PER - Protein Efficiency Ratio M - Mortality NNR -Net Nitrogen Retention

4.4 Growth Rate of *O. niloticus*

The growth parameters as shown in (Table 4.4) observed in this growth study revealed that the Initial Weight (IW) was apparently highest in D_1(0%)and least in D_5(100%) with values of 4.50±3.32g and 3.60±1.64g respectively, that is Initial Weight (IW) ranged from 3.60±1.64g to 4.50± for treatments D_5 (100%) and D_1(0%) which is the control, respectively. Initial Weight (IW) showed no significant difference ($p>0.05$) for all the different treatments.

At the end of this study, the Final Weight (FW) was found to be apparently highest in D_4 (75%) and least in the D_2 (25%), with no significance with other treatments. In other word, the Final Weight ranged from 14.01±9.63g to 16.60± 0.67g in the D_2 (25%) and D_4 (75%) respectively. The lowest Initial Weight (IW) and Final Weight (FW) were recorded in D_5 (100%) and D_2(25%)with values of 3.60±1.64g and 14.01±9.63g respectively.

The result of growth parameters in this study showed a mean Weight Gain (WG) ranged from 9.71±1.67g to 12.62±1.44g for D_2 (25%) and D_4 (75%) of *P. biglobosa* pulp inclusion. Mean weight Gain (WG) in D_4 (75%) showed an apparent difference from those of all the other treatments. The Specific Growth Rate (SGR) was apparently highest in D_4 (100%) and lowest in D_2 (25%) with values of 0.635±0.06 and 0.525±0.09, respectively. All the treatments showed no significant difference ($p>0.05$) among the treatments for Specific Growth Rate (SGR).

The Initial Standard Length (ISL) was apparently lowest in D_5 (100%) and highest in D_1 (0%) with values of 4.93±0.83cm and 5.20±1.26cm respectively. The Initial Standard Length (ISL) showed no significant difference ($p>0.05$) among Initial Standard Length (ISL) of all the treatments. The Final Standard Length (FSL) ranged from 8.07±2.22cm to 8.49±2.15cm for D_2 (25%) and D_4 (75%), respectively. Also, just like in the case of Initial Standard Length, the

Final Standard Length showed no significant differences ($p>0.05$) among all the treatments. The apparently least Initial and Final Standard Length were recorded in D_4 (75%) and D_2 (25%), with values of 5.00±1.15cm, and 8.07±2.22, while the apparently highest Initial and Final Standard Length were recorded in D_1 (0%) and D_4 (75%) with values of 5.20±1.26cm and 8.49±2.15cm, respectively.

This study revealed that D_4 (75%), has the initial total length of 5.85±1.44cm, while D_1 (0%) recorded 6.28±1.59cm. The Final Total Length (FTL) was found to be lowest in D_2(25%), while D_3 (50%) recorded the highest Final Total Length (FTL) with values of 9.31±2.27cm and 9.94±2.22cm respectively. The Initial and Final Total Length were least in D_4 (75%) and D_2(25%) with values of 9.31±2.27cm and 9.31±2.27cm respectively. Also, the Initial and Final Standard Length were found to be highest in D_1 (0%) and D_3 (50% with values of 6.28±1.59cm and 9.94±2.22cm respectively. Both Initial and Final Standard Length showed no significant difference ($p>0.05$) among all the treatments. The Condition Factors (CF) showed no significant difference ($p>0.05$) between the treatments, although D_1 (0%) was apparently highest than other treatments with a value of 1.77±0.83. Also, D_2 (25%), D_4 (75%) and D_5 (100%) showed no significant difference ($p>0.05$) with values of 1.57±0.24, 1.56±0.36 and 1.49±0.34 respectively. The (D_1) recorded the highest condition factors, while the lowest was recorded in D_3 (50%) with values of 1.77±0.83 and 1.35±0.24, respectively.

The Protein Intake (PI) was observed to be highest in D_4 (75%), while D_2 (25%) recorded the least Protein Intake with values of 277.8±1.27g and 198.13±5.68g respectively. Protein Intake was significantly different ($p\leq0.05$) between the treatments, with exception ofD_1(0%). There was no significant difference ($p>0.05$) among the treatments with respect to feed conversion ratio, although D_2 (25%) was apparently highest with a value of 0.72±0.06.

The Protein Efficiency Ratio (PER) in D_4 (75%) and D_2 (25%), (25%) ranged from 0.54±0.01 to 1.07±0.06 respectively. This showed that the least was in D_4 (75%) and highest in D_2 (25%). Significant difference ($p \leq 0.05$) in PER was observed across most of the treatments. Mortality showed no significant difference ($p > 0.05$) among the different treatments, though it ranged from 15.00±5.00% to 26.67±8.82% for D_2, D_3 and D_5 respectively.

The Net Nitrogen Ratio (NNR) showed no significant difference ($P > 0.05$) among treatments. The net Nitrogen retention ranged from 85.91±0.45% to 87.87±0.97% in D_5 (100%) and D_2 (25%), respectively.

4.5 Initial / Carcass Composition of *Oreochromis niloticus*

This study showed a dry matter highest in D_1(0%), with values of 23.70±0.15g, while D_2(25%), D_3 (50%) and D_4(75%) had the least values of 21.88±0.18g, 21.79±0.25g and 21.80±0.14grespectively. The initial composiiton of dry matter shows apparent difefrence with carcass with a value of 24.54±0.29g.

The Moisture Content in the fish in this study, showed no significant difference ($p \leq 0.05$) among some of the treatments. The highest values were recorded for D_2 (25%), D_3 (50%) and D_4 (75%) with values of 78.12±0.18g, 78.21±0,25g and 78.20±14g, respectively. While D_1 (0%) of 76.30±0.15g recorded the least Moisture Content. There was also no significant difference between initial moisture content and that of the carcasses.

The Crude Protein Content in the fish was observed to show no significant difference ($p \leq 0.05$) for all the tretament, but there was a significant difference (≤ 0.05) between the crude protein of initial and that of the carcasses. The study recorded a Crude Fibre of 0.00±0.00 in all the treatments, including the initial crude fibre. With respect to the Lipid Content of the fish in

this study, D_1 (0%) was no significantly lower ($p \leq 0.05$) with values of 4.37±0.59g, compared to other treatments, although the initial lipid content composition was significantly higher than that of the carcasses with a value of 12.07±0.90g.

The Ash Content of the fish in this study recorded on significantly difference among the carcasses, but was significantly different on ($p \leq 0.05$) with the initial ash content composition of 16.16±0.68g. The Nitrogen Free Extracts in the Carcass showed no significant difference ($p \geq 0.05$) among the different treatments, but the initial nitrogen free extracts showed significant difference ($p \leq 0.05$) with a value of 17.13±0.77g. The energy values of the Carcass showed no significant difference, but the carcasses were significantly different from the initial energy value of 1061±9.58 kcal (Table 4.5).

4.6 Physico-Chemical Quality of Culture Water

The Physico-chemical Parameters of culture water shown in Table 4.6 revealed that the pH ranges from 6.48±0.13 to 6.96±0.10 between D_5 (100%) and D_3 (50%)respectively, although the pH of the culture water in the different treatments showed no significant difference ($p \geq 0.05$).

The temperature of the culture water gave a range of 23.77±0.16^{o}C to 24.06±020^{o}C between D_5 (100%) and D_3 (50%) of *P. biglobosa* pulp inclusion respectively. Dissolved Oxygen (DO) of the culture water during the study has a range of 4.16±0.36mg/L to 4.54±0.57mg/L between D_4 (75%) and D_3 (50%) of *P. biglobosa* pulp inclusion, respectively. Total Dissolved Solids (TDS) of the culture water also ranged from 133.71±13.45mg/L to 153.57±16.73mg/L between D_4 (75%) and D_3 (50%) of *P. biglobosa* pulpinclusion respectively. The Electrical conductivity of the Culture Water indicates a range of 68.71±5.79 µs/cm to 80.86±9.04 µs/cm

to 80.86±9.04µs/cm between D_4 (75%) and D_3 (50%) of *P. biglobosa* pulp inclusion respectively. In this study, the physico-chemical parameters of pH, temperature, Dissolved Oxygen, total dissolved solids and electrical conductivity showed no significant difference ($p>0.05$) in all the treatments.

Table 4.5: Initial / Carcass composition and Energy Values of *0. niloticus* in means/SD.

	Initial	Carcass				
		D_1(0%)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
Dry matter (g)	24.54±0.29	23.70±0.15^a	21.88±0.18^c	21.79±0.25^c	21.80±0.14^c	22.64±0.27^b
MC (g)	75.46±0.29	76.30±0.15^c	78.12±0.18^a	78.21±0.25^a	78.20±0.14^a	77.36±0.27^b
CP (g)	54.65±0.57	62.22±0.71^a	62.20±1.18^a	63.11±0.77^a	62.92±0.52^a	63.60±0.58^a
Crude fibre (g)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Lipids (g)	12.07±0.90	4.37±0.59^b	5.61±0.59^a	6.02±0.38^a	5.94±0.06^a	6.32±0.59^a
Ash content (g)	16.16±0.68	23.77±0.09^a	21.45±0.8^b	21.29±1.16^b	21.73±0.25^b	22.12±0.35^b
NFE (g)	17.13±0.77	9.64±1.20^a	10.74±2.62^a	9.57±0.06^a	9.41±0.58^a	7.96±1.18^a
EV (kcal)	1061±9.58	1436.66±13.23^b	1502.59±5.29^a	1513.50±28.05^a	1503.51±4.87^a	1504.81±14.68^a

Note: values are expressed as mean ± SD (standard deviation).
Means with different superscripted alphabets across rows are significantly different at $p \leq 0.05$.

Where: MC - Moisture Content, CP - Crude Protein , NFE - Nitrogen Free Extracts, EV - Energy Value

Table 4.6: Physico-Chemical Quality of culture Water in means±SD.

Water Quality Parameter	D_1 (0%)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
pH	6.89±0.06[a]	6.59±0.11[a]	6.96±0.10[a]	6.73±0.14[a]	6.48±0.13[a]
Temperature (^oC)	23.96±0.11[a]	23.90±0.11[a]	24.06±0.20[a]	23.97±0.10[a]	23.77±0.16[a]
DO (mg/L)	4.26±0.25[a]	4.43±0.25[a]	4.54±0.57[a]	4.16±0.36[a]	4.53±0.26[a]
TDS (mg/L	149.43±20.53[a]	152.86±14.69[a]	153.57±16.73[a]	133.71±13.45[a]	137.14±11.31[a]
EC (µs/cm)	73.29±9.63[a]	73.71±4.78[a]	80.86±9.04[a]	68.71±5.79[a]	71.00±6.03[a]

Note: Values are expressed as mean ± SD (Standard Deviation).

Mean with different superscripted alphabets across rows are significantly different ($p \leq 0.05$).

Where: pH - Hydrogen ion Concentration, DO - Dissolved Oxygen, TDS - Total Dissolved Solids, EC - Electrical Conductivit

4.7 Economic of Feed Formulation

The economic of feed formulation (Table 4.7) indicates that Economic Conversion Ratio (ECR) showed no significant difference ($p>0.05$) among all the different treatments. Incidence of Cost (IC) ranged from 3.86 ± 0.44 to 4.67 ± 0.71 in D_4 (75%) and D_2 (25%), respectively. The Incidence of Cost showed no significant difference in all the treatments. Profit Index (*PI) ranged from 1.08 ± 0.06 to 1.17 ± 0.04 in D_5 (100%) and D_1 (0%), respectively. The Profit Index showed no significant difference ($p>0.05$) in all the treatments.

Table 4.7: Economic of Feed Formulations in means/SD.

Economic Factors (₦)	D_1(0%)	D_2 (25%)	D_3 (50%)	D_4 (75%)	D_5 (100%)
ECR	1.89±0.06[a]	2.03±0.17[a]	1.91±0.01[a]	1.94±0.05[a]	2.05±0.13[a]
I C	4.26±0.13[a]	4.67±0.71[a]	4.05±0.42[a]	3.86±0.44[a]	4.55±0.62[a]
PI	1.17±0.04[a]	1.11±0.08[a]	1.16±0.00[a]	1.15±0.03[a]	1.08±0.06[a]

Note: Values are expressed as mean ± SD (Standard deviation).

Means with different superscripted alphabets across rows, are significantly different at $p \leq 0.05$

ECR - Ecomonic Conversion Ratio

IC - Incidence of Cost

PI - Profit Index

CHAPTER FIVE

5.0 DISCUSSION

This study was conducted with the aim to evaluate the growth response of Nile tilapia*O. niloticus* fingerlings fed *P. Biglobosa*pulpmeal in various percental inclusions, replacing maize meal. Results obtained from this study showed that the proximate nutrients composition in *P. biglobosa* had Dry Matter (94.48±0.69g), Moisture Content (5.32±0.54g); Lipid Content (2.42 ± 0.82g); Ash Content (6.47 ± 0.69g); Crude Protein (5.53±0.89g); Crude Fibre (8.46 ± 0.79g); Nitrogen Free Extracts (77.30 ± 1.34g) and Energy Value (1553.66 ± 0.77kcal /100g/dry matter). The *Parkia* pulpCrude Protein Content in this study disagrees with the report of Sotolu and Byanyiko*(*2010) who recorded a Crude Protein Content of 9.51g for *Parkia* pulp. This wide difference may be as a result of disparities in methodology, climatic factors or even soil fertility. The Dry Matter (94.48 ± 0.69g) recorded in this study, although slightly higher than when compare agrees with 92.74g of dry matter reported by Olujobi (2012). The *Parkia* pulpNitrogen Free Extracts (77.30 ± 1.34g) in this study revealed that *Parkia* pulpwill serve as a good carbohydrate source for fish and other livestocks. This is comparable to the report of Omoregie *et al.*(1991) who reported a Nitrogen Free Extracts of (74.70g) in Sweet Potato, *Ipomea batatas.*

In this study, the determined levels of anti-nutritional factors showed a Saponin Concentration of (17.45 ± 0.41mg/100g/dry matter). This result agrees with the report of Germah *et al.*(2007) who reported a Saponin Concentration of 17.80mg/100g/dry matter forthe amount was found to be much lower than some other everyday consumed legumes like Lima Beans (24.50mg/100g) and Millet (19.47mg/100g) as reported by Osagie (1998). The tannin concentration of 27.0 ± 0.41mg/100g/dry matter disagrees with the findings of Germah *et al.*

(2007) who reported a tannin level of 81.00mg/100g. All the findings for anti – nutrients fall within the permissable levels of (NRC, 1993), except tannin. The physico-chemical parameters of culture water showed a pH range from 6.48±0.13 to 6.96±0.10. This result falls within the pH range for fish survival and is in agreement with the pH standard. (APHA, 1985) Temperature of the Culture Water ranged from 23.77±0.16^{o}C to 24.06±0.20^{o}C between. This is in agreement with the standard for the physico-chemical parameters for aquatic lives including fish (APHA, 1985). Dissolved Oxygen ranged from 4.16±0.36mg/L to 4.54 ± 0.57mg/L. This agrees with the optimum dissolved oxygen needed for rearingfreshwaterfish with *O. niloticus* inclusive (APHA,1985). The total dissolved solids ranged from133.71 ± 13.45mg/L to 153.57 ± 16.73m g/L. The electrical conductivity of the Culture Water ranged from 68.71 ± 5.79µs/cm to 80.86±9.04µs/cm. The non-significant differences ($p>0.05$) in physico-chemical parameters across the different treatments in this study implies that; the variations seen in this work was not as a result of varyingphysico-chemical indices, but the treatment diets.

The proximate composition of experimental diets in this study revealed a high Moisture Content (3.74±0.76) in D_3 (50%) and a low Moisture Content (1.86±0.076) in D_1 (0%), this implies that the shelf-life or storage span of diet D_3 (50%) will be low, hence high fungal activities, while the storage span will be high in D_1 (0%), hence low fungal activities.

The high Lipid Content recorded in the experimental diet D_5 (100%) suggests the diet to have high source of energy thereby sparing protein to enhance growth response in the fish. This Lipid value of 18.66 ± 0.72g is slightly higher the 16.5g reportedby (Sotolu and Byanyiko, 2010) for the proximate composition of experimental diet presented D_4 (75%) as having the highest ash content. This suggests that the diet D_4 has relatively high amount of mineral salts for the healthy growth of the fish in this study.

The composition of Crude Protein in the experimental diets was found to be highest in D_5 (100%), this suggests that the amount of Crude Protein in the diet will stimulate high growth response in the fish fed this diet. Crude Protein of the experimental diet in D_4 (75%) showed no significant difference ($P>0.05$) with D_1 (0%) which is one of the control in this study. This suggests that D_4 (75%) could serve as the best protein source for good growth performance.
The highest Crude Fibre recorded in D_1 (0%) suggests that maize has high fibre than the *Parkia* pulp. This also suggests that maize may be highly digestible by the fish than the *Parkia* pulp. Nitrogen Free Extracts in the experimental diets presented D_2 (25%) having the highest source of carbohydrates, hence could also be a source of energy.

The energy values of the experimental diet was highest in D_5 (100%) in this study. This corresponded with the Lipid Content and the Crude Protein also found to be highest in D_5 (100%), which showed significant difference ($p\leq0.05$) with the other treatments. Final weight, Final standard length and Final total length showed no significant difference ($p>0.05$) in all the treatments. These observations implied that D_4 (75%) seem to show good growth performance in the fish.

The mean weight gain in this study was observed to be highest in D_4, (75%). The D_4 (75%) *Parkia* pulp inclusion in the diets of *oreochromis niloticus* agrees with Orire and Ricketts (2013) who reported a replacement of maize with melon shell inclusion rate up 75% was found appropriate in the diets of *O. niloticus* fingerlings. The study disagrees with Satolu and Byanyiko (2010) was reported a 40% *Parkia* pulp inclusion rate, replacing yellow maize in the feed of *clarias gariepinus* juveniles; The difference may be as a result of the different feeding habits between the two species. This result disagrees with the report of Solomon *et al.* (2007) that up to 57% inclusion level of cereal grain can be effectively utilized to support

growth of *O. niloticus* without any deleterious effect on their health. The reason of this different may be because of the high carbohydrate content in grains than the *Parkia* pulp.

The specific growth rate was found to be highest in D_4 (75%), although all treatments showed no significant difference ($P>0.05$).The study revealed D_4 (75%) with the highest protein intake, which suggests good growth performance in the fish. The least feed conversion ratio was seen in D_3 (50%), which suggested fish fed D_3 (50%) as the best utilized feed in this study, although feed conversion ratio showed no significant difference ($p>0.05$) among the different treatments. Protein efficiency ratio was observed to be highest in D_2 (25%), which suggested fish fed D_2 (25%) showed great feed efficiency which will equally translates into good growth performance in the fish. Protein efficiency ratio showed a significant difference ($p<0.05$) between D_2 (25%) and other treatments. The highest survival rate and the lowest mortality rate of 90.00 ± 5.77% and 15.00 ± 5.00% showed that the feeding condition and the physico-chemical parameters were conducive for the fish. The carcass composition of Crude Protein and Lipid Content were observed to show no significant differences, the initial crude protein was found to be lower than the crude protein of the carcasses. Lipid content of the initial was significantly different ($p\leq0.05$) with the lipid contents of the carcasses.

The economic of feed formulation for Economic Conversion Ratio (ECR); Incidence of cost and Profit Index showed no significant difference ($P>0.05$) for all the treatments, although these parameters were found to be apparently higher in D_5 (100%), D_2 (25%) and D_1 (0%) for economic conversion ration, incidence of cost and Profit Index, respectively.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

The growth response of *O. niloticus* fingerlings fed *P. Biglobosa*pulpmeal in various percentage inclusion, replacing maize meal were evaluated and had drawn the following findings:-

Parkia pulp (Nitrogen Free Extracts of 77.30±1.34g)is a good source of carbohydrate/energy composition capable of replacing yellow maize in the diet of *O. niloticus.*

Parkia biglobosa pulp anti nutrients in this study were observed to be within the tolerable limits, hence safe for the fish, except tannin.

Highest level of crude protein (47.39 ± 0.70g), lipids (18.66 ± 0.72g) and energy (1845.42 ± 18.16 kcal) values were obtained in D_5 (100%), while mean weight gain of (12.62±1.44g), Final weight of 16.60±0.67, specific growth rate of 0.63±0.07, protein intake of (277.8±1.27g) were found to be highest in *Oreochromis niloticus* fed 75% *Parkia* pulp inclusion.

Parkia pulp can adequately replace yellow maize in formulated diets by fish farmers upto 75% inclusion levels in the culture of *O. niloticus.*

Hence, *Parkia* pulp formulated diets are economically competititve with maize formulated diet.

6.2 Recommendations

The following recommendations are therefore put forward:

1. *Parkia* pulpshould be used to replace yellow maize at 25% up to 75% inclusion levels in formulated diets for *O. niloticus.*

2. Good processing methods of *P. biglobosa* pulp should be used to reduce the tannin content to the bearest minimum to avoid affecting the fish negatively.

3. *Parkia. biglobosa* pulp depletion and deteriorationwas observed, hence proper storage methods should be used.

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