REPLACEMENT VALUE OF NEEM (*AZADIRACHTA INDICA*) SEED CAKE FOR SOYA BEAN MEAL IN THE DIET OF AFRICAN CATFISH *CLARIAS GARIEPINUS* FINGERLINGS

BY

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**THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
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DECLARATION

I declare that the thesis entitled "REPLACEMENT VALUE OF NEEM (*AZADIRACHTA INDICA*) SEED CAKE FOR SOYA BEAN MEAL IN THE DIET OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FINGERLING**s**" has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for any other degree or diploma at this or any other Institution.

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Name Of student		

CERTIFICATION

This thesis entitled "REPLACEMENT VALUE OF NEEM (*AZADIRACHTA INDICA*) SEED CAKE FOR SOYA BEAN MEAL IN THE DIET OF AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FINGERLINGS" by Mohammed HASSAN meets the requirements governing the awards of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my late grand Mother Ya Dattah. May her soul rest in peace.

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ABSTRACT

One of the critical problems facing aquaculture development in Nigeria is high cost of protein rich fish feeds. The present study investigated the nutritional suitability and cost effectiveness of *Azadirachta indica* seed cake as alternative protein source to soya bean meal in the diet of African catfish (*Clarias gariepinus)*.The neem seed cake was processed using water washed method to reduce the bitterness and toxicity of the cake. Diets, containing the neem seed cake meals at T1 (0%), T2 (9.62%), T3 (17.35%), T4(28.58%) and T5 (commercial feed) were formulated to be iso-nitrogenous (40%Cp)) and fed to triplicate groups of 10 fingerlings (averaged initial weight 6.5g) of *C. gariepinus* for a period of eight (8) weeks. Proximate analysis showed that raw and processed neem seed cake had 25.38% and 31.87% crude protein, respectively. Analysis of anti nutritional factors showed that tannin, phytate, saponin and oxalate were significantly ($P<0.05$) reduced by 50.8%, 51.4%, 56.25% and 88%, respectively, after processing of the cake. Growth performance and nutrient utilization parameters indicate that treatment1 (control) gave the best weight gain (23.24g) but did not significantly ($p>0.05$) differ from treatment2 (9.62%WNSC) 20.80g and the lowest weight gain (16.63g) was recorded in treatment4(28.58% WNSC). Similarly, the highest specific growth rate (0.024g/d) was recorded in T1(control) followed by T2 (9.62%WNSC) which had 0.023g/d) and the lowest value (0.021g/d) was obtained in T4(28.58WNSC). Nutrient utilization indicates that highest feed intake by fish (30.72g) was recorded in T1 and significantly ($p<0.05$) reduced (25.66g) at T4(28.58%WNSC). However, protein efficiency ratio (PER), net protein utilization (NPU) and feed conversion ratio (FCR) were not significantly different ($p>0.05$) among the dietary treatments. The highest (1.88) and the lowest (1.6) were recorded in T1 (control) and T4 (28.58%WNSC), respectively. Best net

protein utilization (100%) and the lowest (87%) were obtained in T5 and T3, respectively. The highest feed conversion ratio (1.53) and the lowest (1.29) were obtained in T4 and T1 respectively. Cost benefit analysis showed that T4 had the lowest cost per kilogram of diet (199.99) and highest profit index (4.13) while T5 recorded the highest cost per kilogram of feed (450) with correspondingly lowest profit index (1.16). The result recommended that soya bean protein can be replaced by WNSC from 9.62% to 28.58% levels in diet of the African catfish (*Clarias gariepinus)* without compromising fish growth performance and cost of production. This will reduce the high cost of catfish production.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

With global population expansion, the demand for high quality animal protein is rising dramatically (Yisa *et al*., 2006). Increased aquaculture production is clearly needed to meet this demand, because capture fisheries are showing serious declines due to over fishing, aquatic habitat destruction and pollution (FAO, 2004). World capture fisheries have been in a stable state throughout much of the past decades, and it has been estimated that maximum capture fisheries potential from global waters has been reached (in 2007 as many as 50 percent of stocks were labeled as fully exploited (FAO, 2008).

Fish farming is increasing in Nigeria today, if this development is sustained, then aquaculture will create a great impact on the economy and provide proteinous food for its population (Alatise *et al.,* 2006). Catfish is one of the major aquaculture fish in Nigeria and commands high commercial value (Ogwuba, 2003). It is very hardy and has accessory breathing organs which enable it to tolerate low dissolved oxygen and other adverse aquatic environmental conditions where most culturable species cannot survive. It has been advocated that among the Clarias species, *Clarias gariepinus* contributes substantially to the total fish produced under culture system in Nigeria (Aniebo et al., 2009).

Feed is one of the major inputs in aquaculture production and fish feed technology is one of the least developed sectors of aquaculture particularly in Africa and other developing countries of the world (Gabriel *et al*., 2007). In Nigeria the bulk of feed used in fish production, especially for catfish are imported and this has led to high production cost of farmed fish (Aniebo *et al*., 2009). Cost of feed has been observed as

one of the problems hampering aquaculture development in Nigeria (Gabriel, 2007). Expensive feeds marginalize or even minify the profitability of fish farming thereby incapacitating the expansion of farms to increase production and consequently low yield in terms of quality and quantity, resulting in the scarcity of the commodity (fish) and eventually high cost of the few available ones to the disadvantage of the populace (Adikwu, 1992).

Protein is the main constituents of feed for aquaculture animals, and cost of protein rich feed make up a large percentage of the total expenses of aqua feeds. Soya bean meal (SBM) has been the most common source of plant protein as a fish meal replacer in aquaculture feeds, because of its high protein content, relatively well balanced amino acid profiles, reasonable price and steady supply (EI-Sayed, 1999). Lim and Akiyama (1992) reported that soybean products have been used to replace a significant portion of fish meal in fish feed with nutritional, environmental and economic benefits. However, wider utilization and availability of this conventional source for fish feed is limited by increasing demand for human consumption and by other animal feed industries (Siddhuraju and Becker, 2001).

Therefore, the search for alternative plant proteins to replace soybean meal has gained increasing interest in the research on aquaculture feedstuff. Bichi and Ahmad (2010) also stated that this scenario has motivated the research for locally available and cheap ingredients as alternative energy source for animal feed.

Neem *(Azadirachta indica)* seed cake (NSC), a by-product of Neem oil industry, is one of the non-conventional feed ingredients showing great potential for livestock feeding (Gowda *et al.* 1998; Bawa *et al.* 2005). Neem seed cake has been noted as a

rich protein source (34-40%cp) and consists of all the essential amino acid and non essential amino acid except valine and tryptophan. It has good keeping quality and does not spoil on storage or attack by fungi (WNC,2012).

Unfortunately, the use of Neem seed cake as feed stuff has been discouraged because of the presence of bitter and toxic triterpenoids mainly nimbin, nimbidin, azadirachtin and salanin (Paul *et al*. 1996; Nagalakshmi *et al*., 1999)). The bitter taste of Neem Seed is as a result of the presence of sulphur containing compounds, while the tignic acid is the main compound believed to be responsible for its odour, (Gowda *et al.,* 1998).

Attempts have been made over the years to detoxify and reduce the bitterness of the cake for animal use, through extraction, water washing, alkali soaking, urea ammunition and autoclaving (Gowda *et al*., 1998; Uko and Kamalu, 2006; Bawa *et al*., 2007). Consequently, the cake has been treated by various methods such as Urea treatment (Musalia *et al*. 2000), water washing (Rao *et al*., 2002) and solvent treatment

(James *et al*., 2007) all aimed at eliminating the growth depression reported in raw neem seed cake.

It has been reported that out of the several methods tried to detoxify Neem Seed Cake, water washing was found to be the most effective one. Similarly, the regional Research Laboratory in Hyderabad India, has developed a simple extraction process of removing toxic compounds. When Udayashekaraa (1994) employed this

process on Neem seed, the seed was devoid of the bitter taste and its usual foul smell while retaining its nutritional value.

1.2 Research Problem

The current trend in high cost of feed with irregular supply of feedstuffs which pose a great threat to the future of livestock industry is an indication that research attempts have not match up with different challenges of feeding farm animals (Odunsi *et al*., 2009). Moreover, the limited supply of raw materials for the feed industry has resulted in a continuous increase in the cost of production, causing a phenomenal rise in the unit cost of animal products. Thus, these products have become too expensive for the majority of the population (Adesope, 2000; Ebenebe, 2000). In recent times it has been reported that, extensive use of soya bean in animal and human nutrition has made it second expensive source of protein after fish meal (Smita *et al*., 2005). Despite its nutritional value the utilization of Neem seed cake in fish nutrition especially for *Clarias gariepinus* has not been exploited. Smita *et al.* (2005) reported that no information is available on the use of Neem Seed Cake in fish feed.

1.3 Justification of the Study

Soya bean meal is the protein source commonly used to replace fish meal in aquaculture diets, yet it is limited and expensive due to high demand by human and other animal feed industries. Hence, the need to search for other sources of protein with lesser competition and demand by human needs. Neem seed cake which is a bey-product of neem oil extraction, is one of such ingredients showing great potential for inclusion in animal feeds. When properly treated, the cake has high protein contents

similar to that of soya bean. Moreover, increase in the cost of energy and protein sources in Nigeria has been related to their scarcity as a result of the competing demand for these ingredients. The use of these ingredients in animal feed production when human needs have not been met, introduced questions of economic and moral justification (Owens *et al.* 2009). As more and more neem trees (*Azadirachta indica*) are planted in reforestation projects around the world including Nigeria, large quantities of Neem seeds are becoming available (Bawa *et al.,* 2007; Odunsi *et al*. 2009). Therefore, rational use of this nutritive ingredient for fish production can reduce the high price of feedstuffs, the competition and dependence on the conventional protein sources such as soya beans.

1.4 Objectives of the Study

The aim of this research was to develop cost effective diet for the culture of *Clarias gariepinus* fingerlings using Water washed Neem seed cake as alternative protein source to soya bean meal. The specific objectives of the study were to;

1. Determine the proximate composition and anti nutritional factors of raw and treated Neem Seed Cake.
2. Analysis the growth performance and nutrient Utilization of fingerlings fed the neem seed cake based diets.
3. Determine cost and Benefits of feeding the fingerlings using diets containing the neem seed cake..

1.5 Hypotheses

1. The proximate composition and anti-nutritional factors of raw and water washed Neem Seed Cake are the same.

2. There are no significant differences between growth performance and feed utilization of *Clarias gariepinus* fed washed Neem Seed cake diets and those fed soya bean based diets.

3. There are no significant differences between the cost and benefits of feeding *Clarias gariepinus* with water washed neem seed cake diets and feeding with soya beans based diet.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aquaculture Development and Feeding of Fish

Fish has long been valued as a source of protein for human nutrition. Consumption of fish generally cuts across ecological, socio-economic, cultural and religious boundaries, leading to its predominant role as an animal protein. Presently, fish accounts for over 50% of total animal protein consumed in most developing countries and global estimate stands at 15.5% in 2003 (FAO, 2007). Fish is a first class high-quality animal protein and relatively the cheapest source (Tidwell and Allan, 2001). Global per capita fish consumption has increased over the past four decades, rising from 9.0 kg in 1961 to an estimated 16.5 kg in 2003 (FAO, 2007). Historically, the oceans were considered limitless and thought to harbor enough fish to feed an ever-increasing human population.

However, the demands of a growing population, particularly in poorer countries, now far outstrip the sustainable yield of the seas (Tidwell and Allan, 2001). Global capture fisheries production especially marine fisheries resources are being exploited to their maximum or beyond the level of sustainability, the worldwide demand for fish is increasing, raising the question of whether in the future the global demand for fish products can be met (ICTSD, 2003). In order to breach the gap between demand and supply, aquaculture is seen as the best solution.

Aquaculture is defined by FAO (1990) as “the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants with some sort of intervention

in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. Farming also implies individual or corporate ownership of the stock being cultivated". Aquaculture has been conducted since pre-historic times and, from a humble beginning, has spread all over the world gradually transforming from a traditional practice into science (FAO, 1990). It is now the fastest growing animal producing sector with an average world annual growth rate of 8.8% 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems (FAO, 2007). It is remarkable that one out of every three fish consumed in the world is now farm raised (Gatlin *et al.*, 2007). Despite the high growth rate of aquaculture (with a production of 67 mmt), there is still a negative balance between demand (110 mmt) and supply of fishery products (FAO, 2007). Increasing the production capacity of aquaculture resources through intensification seems to be the way forward to meet the ever increasing demand for fish. This entails increasing primary, intermediate and terminal productivity capacities of our natural aquatic ecosystem and creation of productive artificial aquatic ecosystems through proper planning, development and man agement (Sadiku and Jauncey, 1995).

A major determinant of successful growth and intensification of aquaculture production depends on aquafeed. Feed accounts for a major part (30-70%) of the total operation cost of an average fish farm (El-Sayed, 2004). Traditionally, animal protein sources, particularly fishmeal have been the major ingredients of aquafeeds (Glencross *et al.,* 2007). Ironically, fishmeal is one of the most expensive ingredients in formulated fish feeds. Although, fishmeal production has remained relatively stable,

averaging 6.07 mmt over the past two decades (Tacon *et al.*, 2006) its decline is likely and can no longer meet the demand from the expanding aquafeed industry.

The challenge facing the aquaculture industry is to reduce inclusion rate of fishmeal and fish oil in aquafeeds (especially for farmed carnivorous finfish and marine shrimp) and identify economically viable and environmentally friendly alternatives to fish meal and fish oil on which many present aquafeeds are largely based (Gatlin *et al.*, 2007). Replacement of fishmeal and fish oil with available and cheaper plant feedstuffs has been identified as an essential requirement for the future development of aquaculture (Tacon *et al.*, 2006).

2.2 Aquaculture Production

Global aquaculture production in 2006 was reported to be 67 mmt with a value of slightly over USD 86 million (FAO, 2007). Of the world total, China accounted for nearly 70% of the quantity and over half the global value of aquaculture production. Aquaculture production in developing countries increased at an annual rate of 11%, compared with 5% for China and about 2% for the developed countries (FAO, 2007). Most of the production came from extensive /semi-intensive systems in developing countries, particularly Asia, rearing mostly organisms low on the feed chain such as omnivores and herbivores (Hassan, 2001).

2.2.1 Aquaculture production in africa

Total aquaculture production in Africa in 2006 was estimated to be 760,036 mt which was about 81.5% increase over the last decade. Egypt alone contributed about 595,030 mt (78.3%) (FAO, 2007). Total aquaculture production in sub- Saharan Africa

(SSA) was 160,302 mt accounting for only 21.1% of African production (FAO, 2007). Aquaculture in SSA is dominated by Nigeria, contributing about 52.8% with the other four top producers together contributing about 34.3% of SSA production. Between 1997 and 2006, overall aquaculture production in SSA has increased by 68.4% from 50,609 mt to 160,302 mt (FAO, 2007).

FAO estimates that fish provides 22% of the protein intake in sub-Saharan Africa. This can, however, exceed 50% in the poorest countries, especially where other sources of animal protein are scarce or expensive (WorldFish Center, 2005). Per capita consumption of fish in sub-Saharan Africa is the lowest in all regions and continues to decline. This has been attributed to leveling off in capture fish production and increasing population growth (World Bank, 2004). In order to maintain the current level of per capita supply of fish in Sub-Saharan Africa (6.6 kg.yr-1) up to 2015, fish production (capture fisheries and aquaculture) must increase by 27.7% over this period. This assumes an average annual population growth of 1.9% over the period (2002–2015) (World Bank, 2004).

FAO projections show that with just 5% of the suitable areas used, Africa could meet its fish production target but it would need a lot of work. Based on 1997 levels of production, aquaculture would have to increase by 267% by 2020 to maintain the current fish consumption level in Africa (Delgado *et al*., 2003). According to Hecht (2007), about 70% of total aquaculture production in SSA comes from commercial farms, produced by less than 20% of farmers, while the remaining 30% is produced by small-scale farmers that represent over 80% of all farmers. The systems used by the commercial sector range from semi intensive to intensive pond, cage and tank culture

of catfish (*Clarias spp*.) and tilapia (*Oreochromis spp*.) and high value products such as shrimp (Madagascar and Mozambique) and abalone (South Africa) while noncommercial subsistence aquaculture primarily consists of small-scale pond culture of tilapia, catfish and common carp, *Cyprinus carpio* (Hecht, 2007). Machena and Moehl (2001) reported that the major aquaculture products in Africa are mainly fresh and brackish-water finfish.

According to Machena and Moehl (2001), the attributes of Sub Saharan Africa include underutilized water and land resources, available and inexpensive labour, high demand for fish and a climate that favours all year-round production. However, optimal use of these resources has frequently been curtailed by poor infrastructure and lack of production inputs. The potential for expansion is nevertheless considerable, but requires several enabling factors that include; a positive perception of aquaculture, sound policies at the national level, strong public institutions, availability of nutrient inputs, conducive investment policies to attract increased private-sector participation, and access to credit for commercial-scale enterprises (Machena and Moehl, 2001). Increasing demand for fish coupled with rapidly dwindling catches from capture fisheries, the implementation of new participatory approaches to technology development and transfer, and the emergence of a few successful large-scale tilapia culture operations directed at the export market offer opportunities for further expansion in both the small-scale and commercial sectors (Jamu and Brummet, 2004).

Aquaculture development in Africa is insignificant compared to the rest of the world (Changadeya *et al*., 2003). According to Hetcht (2000) the entire continent

contributed only 0.4% to the total world aquaculture production for the period 1994 to 1995. In the year 2000 it contributed a mere 0.97% of the total global aquaculture (FAO, 2003). Although the history of aquaculture is relatively recent in Sub-Saharan Africa compared to Asia, and some other parts of the world most known aquaculture systems have been introduced over the last 35 years (FAO, 1996).

In Sub-Saharan regions aquaculture in most places is still essentially a rural, secondary and part-time activity taking place in small farms with small fresh water ponds (FAO, 1996). The systems that are generally practiced range from extensive to semi-intensive cultural systems with limited fish yield, which are mostly consumed directly or sold locally (CIFA, 1998). Almost all fish farming is carried out by rural small scale operators in small fresh water ponds as a secondary activity to agriculture. Although there is abundant potential for the development and expansion of aquaculture in this region, factors such as the novelty of aquaculture, the general poor economic conditions in many countries and the relative paucity of entrepreneurial skills and credit facilities hamper its development (FAO, 1997).

Aquaculture development in most African countries is primarily focused on socio-economic objectives such as nutrition improvement in rural areas, income generation, diversification of farm activities (integrated farming) and creation of employment especially in rural communities where opportunities for aquaculture in Africa is limited (CIFA, 1998). This approach over the years has resulted in sustained aquaculture growth in some African countries such as Coté D'ivoire, Egypt, Ghana, Malawi, Nigeria and Zambia (Jamu and Ayinla, 2003).

While there is still room for enhancing aquaculture production in Africa through improved production systems, genetics and general farm management principles, the desired and expected growth of aquaculture to meet the ever increasing demand for fish, and to satisfy its socioeconomic functions, is only achievable through cost-effective and high quality fish feed.

2.2.2 Aquaculture production in nigeria

Despite its long history, aquaculture in Nigeria has only emerged as an industry, and has experienced tremendous growth in production during the last two decades. In 2007, production figures from aquaculture indicated a total (excluding aquatic plants) of 85,087 metric tons (FDF, 2007). Fish farming is the sub-set of aquaculture that focuses on rearing of fish under controlled or semi controlled conditions for economic and social benefits (Anthonio and Akinwumi, 2002). *Clarias gariepinus* is a highly esteemed fish in Nigeria because it commands high commercial value (Ugwumba and Ugwumba, 2007).The story of aquaculture in Nigeria is essentially the story of catfish culture and the hope of fish supply in Nigeria hangs on its development and culture (Okoye and Sule, 2001).

Food and Agriculture Organization (2002), stated that fisheries products represented a major source of export revenue for developing countries, amounting to over US $ 20 billion per annum in late 1990s. This exceeded the values obtained from the exports of meat, dairy, cereals, vegetables, fruit, sugar, coffee, tobacco and oilseeds in 1997 from developing countries (I.T.C, 2002). Food and Agriculture Organization in 2008 reported that out of total fish produced in Sub-saharan Africa, 40% was in Nigeria and *Clarias gariepinus* contributed substantially to the total fish

produced. However, F.A.O (2007), reported that Nigeria imports about 560,000 tonnes of fish estimated at about $400 million annually while annual domestic fish supply in Nigeria stands at about 400,000 tons. This makes Nigeria one of the largest importers of fish in the developing world.

Catfish production is important to the Nigerian economy. It serves as a source of income, reduces the rate of unemployment in the economy and increases the Gross Domestic Product (GDP) (Adebayo *et al*., 2013). In most countries it earn a higher price than tilapia as it can be sold live at the market and they have a market value two to three times that of Tilapia (Emokaro *et al.*, 2010). According to Olagunju *et al.* (2007), it requires less space, time, money and has a higher feed conserving rate.

The importance of catfish itself cannot be overemphasized. According to Anoop *et al.* (2009), it provides food for the populace, it allows for improved protein nutrition because it has a high biological value in terms of high protein retention in the body, higher protein assimilation as compared to other protein sources, low cholesterol content and one of the safest sources of animal protein (Adebayo *et al.,* 2013). Many species of fish are farm produced all over the world, but Catfish is taking the lead because of its uniqueness. The demand for Catfish in Nigeria is unprecedented so much so that no matter the quantity supplied into the market, it would be consumed by ready buyers.

2.3 Fish Feed Development

2.3.1 Overview

Fish feed technology is one of the least developed sectors of aquaculture particularly in Africa and other developing countries of the world (FAO, 2003). Feed is one of the major inputs in aquaculture production. It is one of the fundamental challenges facing the development and growth of aquaculture in the African continent. Fish feed development in Sub-Saharan Africa has not made a significant progress. Hecht (2000), noted that the research on inexpensive feed ingredients has not contributed greatly to aquaculture development in Africa and suggested that more research on how best plant protein can be used as fish feed. Development and management of fish feed, play very vital role in aquaculture growth and expansion, and it is a major factor that determines the profitability of aquaculture venture.

Jamu and Ayinla (2003) reported that feed accounts for at least 60% of the total cost of fish production in Africa, which to a large extent determines the viability and profitability of fish farming enterprise. As aquaculture becomes intensive, most farmers in Africa depend largely on imported fish feed from developed countries for the productivity and sustainability of the industry. For example, in Nigeria, an estimated 4,000 tons of quality fish feeds are imported in to the country each year (AIFP, 2004). This has contributed in no small way in increasing the total cost of production which will ultimately translate to high cost of fish, thereby making it expensive for the teeming population of the poor people living in Sub-Saharan Africa.

In some countries like Nigeria, Kenya, Namibia, Malawi, Uganda, Madagascar, Ghana and Cote D'ivoire, where little quantity of fish feeds are produced

locally, the quality of locally produced feed is very poor and production rate inconsistent. Jamu and Ayinla (2003) reported that the low quality of fish feed and its attendant high cost are the major factors limiting the development of aquaculture in Africa. Hence, research in fish nutrition that will utilize locally available ingredients and fabricated equipment without reducing the quality of the feed is urgent and crucial to the overall success of aquaculture development, growth and expansion in Africa. Aquaculture production in Africa involves both the intensive and semi-intensive system of production, which is daily gaining ground in the continent. For any aquaculture venture to be viable and profitable, it must have a regular and adequate supply of balanced artificial diets for the cultured fishes. This is so because the dissolved nutrients that promote primary and secondary production in the natural environment are seasonal and might be insufficient or may not occur in required proportions to meet the nutritional demand of cultured fishes (Ugwumba and Ugwumba, 2007).

There is therefore the need to develop and encourage fish farmers to make use of ideal pond fertilization programs, non-conventional feed resources, feed stuff processing, refinement and formulations that take cognizance of the requirements of the various species and their stages (Ibiyo and Olowosegun, 2004). In comparison to livestock feeds, fish feeds are unique in that they are pelleted and the size of the pellet depends to a large extent on size and age of the fish involved. Fish feed is very important in the overall performance of fish in the pond. This is why any attention

towards the production of rich and cheap feed will benefit fish farmers in Africa (Hecht, 2000)

2.3.2 Utilization of plant ingredients in aqua feeds

The need to increase aquaculture production requires corresponding increases in nutrition related inputs; i.e. intensifying culture practices by feeding more and better feedstuffs (Machena and Moehl, 2001). Feeds are mostly based on agricultural by-products available in an area and may be of modest quality but of a reliable quantity. Commercially produced feeds require cost-effective inputs and the industrial means to manufacture feeds, preferably pelletized feeds.

Therefore, countries that have expanding agricultural sectors and produce surpluses are often well placed for the economical production of commercial fish feeds (Machena and Moehl, 2001). In terms of aquafeed manufacturing in Sub Saharan Africa, Nigeria being the largest aquaculture producer also manufactures the largest amount of aquafeed. Production was estimated at 10,760 mt in 2000 and 2001 (Shipton and Hecht, 2005). This feed was, however, manufactured solely for the tilapia and catfish industries, accounting for only 30.3% of the country's aquatic feed production. The remainder, which represents the majority of the feeds used, were farm made feeds. Dependence on farm-made products to satisfy feed requirements of aquaculture organisms is prevalent in all the Sub Saharan African countries (Moehl and Halwart, 2005). Feeds are still mostly produced at the farm level and in most cases only one, or a mixture of two or more feed ingredients are used as

supplementary feed in pond culture. Farmers who were desperate to increase production have tried poultry feeds with little or no success (Amisah *et al*., 2009). The few commercial farms in Nigeria produce their own feed.

The feed produced and used widely in Africa are categorized into conventional and non conventional feed stuff; the categorization is based on the availability and acceptability of the feed stuff involved (Jamu and Ayinla, 2003)

2.3.3 Non conventional feed stuff

Unconventional fish feeds are potential feed ingredients, which have hitherto not been used in fish feed production for some reasons: They are not well known or understood, no effective study of the method of production with a view to commercializing them, they are not readily available and they can be toxic or poisonous (Moel and Harwart, 2005).

These feeds are generally referred to as unconventional feed ingredients. They could contain high quality feed nutrients that can compare favorably with conventional feed types. They are expected to be cheaper by virtue of non or less competition for human consumption. The feedstuffs can be of animal or plant source (Jamu and Ayinla, 2003).

They are locally available feed stuffs that are not standardized. The usage is not widely-spread and they are not consumed by man in most cases. Utilization in aqua feed is very common especially in the rural area of Sub-Saharan Africa, among low income group that are actively engaged in fish farming. Gabreal *et al*. (2007)

reported that these feeds normally come from kitchen wastes, plants and animal byproducts.

2.3.3.1 Kitchen wastes

This is being used at household level of aquaculture especially, in backyard fish farming where remnants from household wastes are used to feed the fish. These are used indiscriminately, without any standard. Examples of feed in this category are cassava and yam peels. Ubalua and Ezeronye (2008) recorded 60% growth performance with high level of economic performance when cassava peel was fed to tilapia *Oreochromis niloticus.* Oresegun and Alegbeleye (2001) recommended addition of 0.2% methionin with 20% inclusion of cassava peel. Also kitchen remnant like bread, cooked rice and yam are commonly used in the culture of fish.

2.3.3.2 Plant sources of feed

These are generally known as non-conventional plant feed stuff (NCPF). These are many and abundant, almost in every locality in Africa. Their potential and utilization in aquaculture feed have been reviewed (Ugwumba and Ugwumba 2003; Bichi and Ahmed, 2010; Agbabiaka *et al*., 20012). Their levels of inclusion in aquafeed varies and largely depends on their availability, nutrient level, processing technique, species of fish and cultural farming pattern prevalent in the locality. Nandeesha *et al*. (1991) recommended 30% level of inclusion of NCPF in aqua feed, due to many factors which limit its level of incorporation. These factors are: low protein content (Oresegun and Alegbeleye, 2001; Ibiyo and Olowosegun, 2004), amino acid imbalance (Eyo, 2001) and

presence of antinutritional factors (Oresegun and Alegbeleye, 2001, Bawa *et al.,* 2007).

2.3.3.3 Animal sources

The non conventional feed stuff of animal origin are high quality feed ingredients that could compare to some extent with the conventional types (Okoye and Sule, 2001). These are cheaper by virtue of the fact that there is less competition for human consumption. However, the only problem with these feed stuffs is their unavailability in large commercial quantities for the sustenance of aquaculture industry. In most parts of Africa, these are available in small quantities and their production is inconsistent and sporadic in nature. Examples include tadpole meal, maggots, earthworm meal, housefly larvae among others etc. (Ugwumba *et al*., 2001; Akinwande *et al*., 2002; Ibiyo and Olowosegun, 2004).

2.3.4 Conventional feed source

These are the feed stuff that are regularly used in the formulation of fish feed. Their usage is standardized and widely acceptable. Many of these are cheap and readily available in very large quantity. They are usually agro industrial by-products. Examples include wheat bran, groundnut cake and rice bran. Some are animal based (for example, fish meal, blood meal, shrimp meal), whereas others are plant based (for example, maize, soya bean meal, cotton seed meal (Okoye and Sule, 2001). Generally these materials are relatively cheap and available throughout the year (Gabreal *et al*.,

2007). The effectiveness of a feed is preferred as a determinant rather the cheapness. Hence, balance is therefore needed, if aquaculture is to be profitable (Falaye, 1992).

2.4 Biology of African Catfish

2.4.1 Food and feeding habits of *Clarias gariepinus*

C. gariepinus is equipped to feed on a wide variety of food items, from minute crustaceans to fish. Predation is more efficient on invertebrate prey. Most feeding takes place at night on active benthic organisms, but they may also feed during the day and at the water surface. Individual bottom foraging is the normal mode of feeding, although catfish may also feed in groups at the water surface (Bruton, 2010).

They are not specific in their food requirements. They are known to feed on insects, plankton, snails, crabs, shrimp, and other invertebrates. They are also capable of eating dead animals, birds, reptiles, amphibians, small mammals, other fishes, eggs, and plant matter such as fruit and seeds. Because they are mobile on land, they are able to prey on terrestrial organisms (Bruton, 2010). This species may also hunt in packs on occasion by herding and trapping smaller fish (Agbabiaka, 2010).The species is euryphagous and generally regarded as an opportunistic, omnivorous predator. It has the ability to efficiently utilize and/or switch between alternative food sources such as plants and detritus when prey animals become scarce (Dadebo, 2000).

Normally catfish are bottom feeders, but their feeding habits are adaptable and they occasionally filter feed in groups at the water surface. There are four recognized feeding modes, viz. individual foraging, individual shoveling, surface

feeding and formation feeding. Adoption of any one of the feeding modes depends on food availability (Bruton, 2010). Catfish in ponds have been observed to snatch sinking pellets before they reach the substratum (Agbabiaka, 2010).

The buccal cavity is capable of considerable vertical displacement that enables suction feeding. The teeth are numerous, small, cardiform and backwardly directed (Teugels, 1986). The premaxillary, mandibular and pharyngeal teeth are conical and sharp, whereas the vomerine band has mainly granular molar-like teeth with variable numbers of conical teeth, usually on the distal margin. The vomerine teeth band has no ventral partner, so that crushing and gripping of prey take place against the hyoid apparatus, which bulges upwards to form a tongue. *Clarias gariepinus* has long gillrakers on the anterior borders of the five bronchial arches, and additional gillrakers on the posterior margins of the third and fourth arches that interdigitate with those from the anterior row of the next arch (Anoop *et al*., 2009).The number of gillrakers increases with length. The mean width between gillrakers varies between 0.1 and 0.6 mm, but this increases with length. Despite this, larger fish are known to filter feed on phytoplankton, zooplankton and surface scum. The stomach is muscular, and the intestine is thin walled and relatively short, implying a dependence on high-protein foods. The stomach in North African catfish becomes functional 5–6 d (11 mm TL) after the start of exogenous feeding at 27.5 °C (Verreth *et al.*, 1992).

Predation is most efficient on relatively slow-moving benthic organisms, but fast prey such as fishes can also be caught individually or by using pack-hunting tactics (Merron, 1993). The percent composition of natural food is dependent on the availability and abundance of various food items within systems. Based on the

proximate composition of diets of wild populations, Hecht, (2000) predicted that the species would have a relatively high protein demand (>45 percent), a lipid demand of around 18.5 percent and a dietary energy requirement of around 18 kJ/g. The predictions were very close to the empirically determined requirements.

The species feeds mainly on insects, phyto- and zooplankton, invertebrates and fish, but also takes young birds, rotting flesh and plants (Yalcin *et al*, 2001). Frogs, snakes, fledgling birds, small mammals, seeds and fruits have also been recorded in the stomachs. The natural diet is determined largely by prey abundance in any particular habitat. During the larval and early juvenile phase, the natural diet is restricted mainly to zooplankton and chironomids. During this stage, the taste buds on the circumoral barbels play an important role in prey detection (Nyina-wanniza *et al*., 2010). The species is also able to detect electrical pulses and uses this ability to detect prey (Hanika and Kramer, 2000). With increasing size and development of the feeding apparatus, the diet becomes more diverse. Bruton (2010) recorded over 40 prey species in the stomachs of *C. gariepinus* in lakes Sibaya (South Africa) and Kinneret (Israel), with fish contributing 75 percent and 81 percent of the dry weight of the diet, respectively, followed by crustaceans. On the other hand, Hecht (2000) found that zooplankton becomes more important in the diet with increasing size. This simply illustrates the extraordinary ability of the species to switch diet.

Agonistic behavior and cannibalism in larvae and early juveniles under culture conditions is affected by light intensity, photoperiod, feeding method, density, food availability and feed type (Verreth *et al*., 1992). Van de Nieuwegiessen *et al*. (2009) suggested that welfare in fish up to 100 g is significantly improved with increasing

density, while welfare of larger fish is not negatively affected by increasing density. The behavioral effect of increasing density is that the fish assume a behavior pattern that is reminiscent of a “rolling bait ball” in which there is no sign of aggression, and this leads to improved feed consumption and improved food conversion ratios (Hecht and Uys, 1997).

2.4.2 Environmental requirement of *Clarias gariepinus*

Clarias gariepinus can endure extremely harsh conditions (Skelton, 2001). It is able to tolerate very low oxygen concentrations and even survive for considerable periods out of water, via the use of a specialized suprabranchial organ (Skelton, 2001). This organ is a large paired chamber with branches above the gill arches specifically adapted for air breathing (Bruton, 2010) and allows it to move over land even when not forced to do so by drought. Water temperatures between 8 and 35°C, salinities of 0 to 10‰ and a wide pH range are all tolerated (Agbabiaka, 2010). *C. gariepinus* exhibits high growth rates between 25 and 33 °C, with optimum growth recorded at 30°C.The ability of the fish to be able to tolerate these extreme conditions allows it to survive even in moist sand or in borrows with an air-water interface (Van der-Waal, 1998). Water quality requirement of African catfish is similar to that of other catfish species in egg and larvae stage. In first phase of nursing, it requires environment similar to that of carp fry (Vander-wall, 1998). After development of accessory respiratory organ, it can survive in extremely poor conditions from fingerling stage. North African catfish live in a variety of freshwater environments, including quiet waters like lakes, ponds, and

pools. They are also very prominent in flowing rivers, rapids, and around dams. They are very adaptive to extreme environmental conditions and can live in pH range of 6.5-8.0. They are able to live in very turbid waters and can tolerate temperatures of 8-35^{o}c. Their optimal temperature for growth is 28-30^{o}c (Agbabiaka, 2010).

2.4.3 Nutritional requirement of *Clarias gariepinus*

Although the species is euryphagic, it feeds predominantly on fish, its propensity toward a carnivorous feeding habit suggests that *C. gariepinus* has a relatively high dietary protein requirement, in the order of 40–50 percent of crude protein on a dry weight basis. The fact that the animal also feeds on plant material reflects its ability to digest plant proteins and utilize carbohydrates as an energy source (Van Weerd, 1995). From a farming perspective, euryphagy holds the benefit that a wide variety of feed ingredients of animal and plant origin may be considered in formulating feeds that will satisfy the fish's dietary requirements.

2.4.3.1 Larvae and early juveniles requirements

The nutritional requirements of *C. gariepinus* common to most species, the larvae and early juveniles (up to approximately 1 g) have a high protein demand of around 55 percent and a lipid requirement of 9 percent. The carbohydrates content can be as high as 21 percent of the diet. A minimum level of 0.5–1 percent dietary n-3 fatty acids has been recommended for *Heterobranchus longifilis* fry (Kerdchuen, 1992). In the absence of any quantitative information for *C. gariepinus* and the suggestion

made by Fagbenro, (1998) that early juveniles grow better if at least 10 percent of the total lipid consists of fish oil, it is recommended that the minimum level as suggested by Kerdchuen (1992) is incorporated into larval feeds. Some work has been undertaken on the qualitative amino acid requirements of larvae (Conceição *et al*., 1998), but the quantitative requirements of larvae, except for methionin are not known. Similarly, the fatty acid requirements are unknown, except that a 1:1 ratio of n3 and n6 fatty acids appears to be optimal for growth and body condition. The amino acid requirements of early juveniles and from >10 g bodyweight onwards are better understood (Fagbenro, 1998). Ali and Jauncey (2005) found that intestinal alpha-amylase activity increased with increasing dietary carbohydrate levels. These findings show that *C. gariepinus* is capable of digesting carbohydrates from an early stage and this persists throughout the animal's lifespan.

2.4.3.2 Grow-out phase

There is some evidence to suggest that the nutrient requirements change at around 5 g in weight and remain fairly constant thereafter. This is largely reflected by a decreasing dietary protein demand. Most of the evidence suggests that the basic nutritional requirements during the grow-out phase range from 40 to 43 percent for protein, 10 to 12 percent for dietary lipid and between 15 and 32 percent for carbohydrate (Ali and Juancy, 2005). Optimum digestible energy is between 14 and 16 kJ/g and the protein to energy ratio is optimal between 26 and 29 mg/kJ of digestible energy (Fagbenro, 1998).

Least costing of the diet and appetite feeding schedules have shown that profitability can be optimized at dietary protein levels of between 35 and 38 percent. It has been shown that growth is negatively affected if fish oil is used as the sole source of lipid (Ng, *et al,* 2003; Ng *et al*., 2004), which clearly suggests that the species has a certain requirement for n-6 fatty acids. However, the dietary lipid source does not affect whole-body composition or muscle lipid level in catfish, although fatty acid and alpha-tocopherol levels generally reflect the fatty acid profile and alpha-tocopherol concentration of the dietary lipids that are used (Ng, *et al,* 2003).

However, the basic nutrient requirements of catfish for normal growth was reported by Robinson *et al*., (2006) as 32% for crude protein, 8.5 – 9.5kcal/g protein for energy and 4-6% fats which increases as protein level increases. These authors recommended 20-30% carbohydrate and < 4% fiber as optimum for *C. gariepinus* Culture. El-Gaedy (2009) concluded that the dietary requirements of catfish fry were 40% crud protein and 8% oil and for fingerlings were 35% crud protein and 8% oil in terms of growth performance and economic evaluation.

2.5 Neem (*Azadirachta indica)* Tree Distribution and Seed Availability

Azadirachta indica has been introduced and established throughout the tropics and subtropics for its highly valued hardiness, it's almost year-round shade, and its multiple wood and non-wood products. Although the exact origin of *A. indica* is unknown, it is thought to have originated in the Myanmar region and to be distributed naturally throughout the Indian subcontinent (Panhwar, 2003).

It has long been cultivated in Malaysia, Indonesia and Thailand, where it is completely naturalized. In the 19th century, Southeastern Asian emigrants took it to Fiji, Mauritius and Guyana, and the British to Egypt, East Africa and sub-Sahelian West Africa, where it is widely grown and has become naturalized. The cultivation of neem spread to Africa in the 1920's when it was introduced to Ghana, Nigeria and the Sudan, and the species is now well established in more than 30 countries (Aruwayo *et al*., 2010). It has recently been introduced to tropical South and Central America. Now it is one of the fastest spreading trees and has become pan-tropic.

In Nigeria, Neem trees are planted in reforestation projects across the Sahel and Savanna zones aimed at controlling desert encroachment. Anon (2010) reported that over four millions of Neem trees have been planted in the northern part of Nigeria especially in Borno, Kano, Katsina and Sokoto states; and rich in protein. About 3,500 hectares of land is under cultivation in Kebbi, Sokoto and Zamfara states, with a density of about 1,200 trees per hectare (NARICT, 2004). In Nigeria, Neem form about 90% of forestry established in 12 states within the savanna zone under the Aforestation program (Nwokeabia, 1994). It may grow up to 15m tall under ideal conditions and is reported to live up to 200 years (TNT, 2003). It takes about five years to produce the first fruit crop but can produce a good yield in the third year (Panhwar, 2003).

The tree has the ability to withstand frost due to a very suberised back layer and can easily survive desert conditions of temperature above 50ºC and takes about five years to produce the first fruit crop but can produce a good yield in the third year (Panhwar 2003). Neem tree flowers between February and May, with profuse clusters

of small white flowers. The fruits are drupes, turning golden yellow on maturity, which occurs during June to August. A full grown tree can produce 30-100 kg fruit depending on rainfall, soil type and ecotype.50 kg of fruits yield 30 kg of seed giving 6 kg of oil and 24 kg of seed cake (Ramesh, 2000). The tree begins bearing fruit after 3 to 5 years and produces about 50 kg fruits annually when mature in. The seeds are about 2 cm long and 1.5cm diameter, kernel of about 1.5 cm length containing about 30 to 40 percent oil and 5 to 6 years old tree can yield 20 kg kernels (Panhwar, 2003). The yield of 20.5 kg fresh fruit for a fully grown neem tree was reported in Nigeria (NARICT, 2004) and an average yield of 17.71 kg in Nigeria (Sokoto and its environs) (Aruwayo, 2011).The large number of the neem trees and its yield in the northern part of Nigeria is an advantage for its use.

2.5.1 Anti-nutritional factors in neem seed cake as feed

The possibility of using NSC in livestock ration was explored by Christoper (1996) using the feeding practice of local farmers in Southern India. However, NSC pungent, odour and the bitter taste caused by the active principles isolated from different parts of the plant namely azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives of these principles constitute the hindrances to its use (Paul *et al*., 1996)

Despite the high CP content, its incorporation in animal diets was discouraged due to their adverse effect on their performance because of the presence of bitter and toxic triterpenoids mainly nimbin, nimbidin, azadrachtin and salanin (Odunsi *et al*., 2009). Biologically active principles isolated from different parts of the plant include: azadirachtin, meliacin, gedunin, salanin, nimbin, valassin and many other derivatives

of these principles. Meliacine forms the bitter principles of the neem oil; the seed also contain tignic acid (5-methyl-2-butanic acid) responsible for the distinctive odour of the oil (Uko and Kamalu, 2001). Neem seed cake is very unpalatable due to the presence of salanolide meliacin which has been found to be one of the active principles of neem seed oil (Garg and Bakhani,1993). These compounds are natural products called triterpenoids or more specifically, limnoids and they are concentrated in the seed despite their presence in other parts of neem tree.

Poor palatability was accompanied by either poor weight gain or loss of body weight along with lowered nutrient digestibility in crossbred calves fed concentrate mixture containing NSC that contributed 12.5, 25, or 50% of Crude Protein (CP) requirement (Ogbuewu *et al*., 2011). The authors suggested that NSC was unsuitable for animal feeding even for maintenance. This then makes the processing of the cake before being fed to animals very important. The processing of the neem seed in to palatable seed cake poses a challenge to its use as animal feed (Aruwayo *et al.,* 2010).

2.5.2 Methods of processing and removal of the active principles of neem seed

Single seeded mature Neem fruit contains 23.8% skin, 47.5% pulp, 18.6% shell and 10.1% kernel. The decortications of depulped seed yields about 26% kernel, which gives 45 to 50% oil leaving the rest as Neem kernel Cake (NKC). Another authors, Bawa *et al*. (2007), prepared NSC by soaking the dried neem fruits in water for 3-4 days and depulped using depulper machine. The seeds were dried for 7 days before being decorticated using a winnowing machine and then crushed after further drying for 3

days. Similarly, Aruwayo (2011) processed Neem seed in which the Neem fruit were spread in the sun for fifteen days and then soaked in water for three days and then depulped. The depulped seeds were washed and sun dried for a period of ten days. The dry seeds were decorticated, further dried for five days, crushed and the oil removed manually to produce the Neem kernel cake.

Smita *et al*. (2005) reported that Water Washed Neem Seed Cake was prepared by soaking the cake in water (W/V; 1:2) for 24 hour, followed by sun drying and grinding. Another method of preparing Neem Kernel Cake was described by Odunsi *et al*. (2009) which involves spreading the seeds in the sun to obtain a constant weight. The dried seeds were soaked in water in an open basin for 72 h. The seed were poured into a jute bag to drain the water and later sun-dried to constant weight. Then, the water soaked and untreated seeds were taken to the mill separately for oil extraction. Cake obtained from the oil-extraction was then ground in a hammer mill.

The active principles have been reported (James, *et al*., 2007, Bawa *et al*., 2007) to be slightly hydrophilic but freely lipophilic and highly soluble in organic solvents like hydrocarbon, alcohols, ketones and esters. There are various methods of removing the oil from the neem seed cake. For the expeller NSC, the crushed kernel will be steamed and the oil is pressed out using expeller machine. The hydraulic press NSC processing is devoid of heat. The milled kernel was cold-pressed using the hydraulic press machine until the oil content of the residue (cake) is minimal. To reduce the oil content of the cake, it is further defatted using hexane (James *et al*., 2007).

2.5.3 Nutritional composition of neem seed cake

The nutritional compositions of Neem seed cake have evaluated by many researchers and have been reported to be highly nutritious. Gowda and Sastry (2000) stated that the cake is a good source of protein (45–50%), phosphorus, calcium and iron. Trypsin inhibitory activity was detected in the cake, but it was low (15–17 TIU/mg protein). Lysine content of the cake protein was higher than most of the cereal proteins but lower than that of legume proteins. Sulphur-containing amino acids were the limiting amino acids of the cake protein. The problems of putting the cake in to uses still remains, this is because neem trees scattered around the world are genetically distinct and its nutritional potential is affected by climatic conditions, method of processing and, to lesser extent, the genetic makeup of the animals (NRC, 1992).

Interesting nutrient profile of *Azadirachta indica* have being reported by Gowda and Sastry (2000) in Alcohol and Urea treated Neem Kernel Cake. They reported crude protein values of 36.01 and 40.06 %, Fats 4.8 and 4.9 respectively with appreciable quantity of calcium and phosphorus.

James *et al*. (2005) reported that protein efficiency ratio (PER) for rats fed methanol processed neem seed cake compared favourably with soybean protein diet. Sastry and Agrawal, (2005) reported that water washed neen seed kernel cake can be incorporated in diets of growing goats up to 25% without deleterious effects on nutrient utilization and metabolism. Aruwayo *et al,* (2010) reported comparable crude protein digestibility and efficiency; and live weight changes in Uda lambs and rams when fed alkali treated neem kernel cake soaked overnight in water and dried in the

sun. Vasanthakumar *et al.* (1999) reported that dietary incorporation of neem seed kernel cake had no effects on the digestibility of various nutrients.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Experimental Site

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

3.2 Experimental Fish

A total number of 180 fingerlings of *Clarias gariepinus* of average weight (6.5g) and initial length 7.8 cm were procured from commercial hatchery (Miracle Fish Farm) Zaria. The fish were transported in 50L plastic container, where they were acclimatized and fed control diet for two weeks.

3.3 Experimental Feed Ingredients

All the dietary ingredients were separately processed and milled to fine particle size. The soybeans were thoroughly sorted to remove all extraneous materials. The clean beans were toasted for about 15 minutes and then dehulled, followed by winnowing, milling and sieving (Ademulegun and Koleosho, 2012). The fish meal and the yellow corn were separately milled. The neem seed cake was purchased from local women who usually extract the oil for medicinal and other uses. The cake was processed using the method of Smita *et al.* (2005), in which the cake was soaked in water (W/V 1; 2) for seven (7) days, during which the water in the container was changed every day. On the seventh day after removing the water, the remaining dough was poured on to a cotton material and hung to an object for two (2) days. Thereafter, it was spread in the sun to dry for another twelve (12) days. This was due to humid

and cold weather recorded at the period of the drying. The dried cake was then milled to obtain the fine material used for the formulation.

3.4 Proximate Analysis

Proximate analysis of dietary ingredients (Table 3.1), experimental diets, fish whole body and feacal samples were carried out according to AOAC (1990) procedures Whole body proximate analysis was used to determine body composition of fish. The proximate analysis followed methods described by the AOAC (1990). Components such as moisture, crude protein, crude lipid and ash were analyzed.

3.4.1 Crude protein

The micro-kjeldahl method according to AOAC (1990) was used for the crude protein determination in triplicate as follows; 200g of sample was digested in concentrated sulphuric acid and ammonia from the digest is released which reacts with 40% sodium hydroxide and distilled water, trapped in 2% boric acid and quantified by titration against 0.2M hydrochloric acid. Crude protein was calculated by multiplying the nitrogen by a factor 6.25 based on the assumption that most plant protein contains 16% nitrogen. Thus, 1mg nitrogen = 6.25 protein.

3.4.2 Moisture

Moisture determinations are used to convert all other nutrients to a dry matter basis. Sample containing 2 g dry material was dried to constant weight at 95-100 C under pressure <100 mm Hg. loss in weight was reported as moisture. This was achieved by

Table 3.1 Proximate composition of experimental feed ingredients

Ingredients	DM	CP	CF	OIL	Ash	NFE
Fish meal	88.70	60.00	0.00	10,56	9.22	20.10
Soya bean	93.19	40.19	6.17	8.69	5.32	39.63
Neem Cake	89.88	31.81	8.06	5.11	8.26	46.76
Maize	90.73	10.00	2.70	4.00	1.30	82.00

Key;

NSC= neem seed cake

DM = dry matter

CP = crude protein

CF= crude fibre

EE = Ether extract

NFE = nitrogen free extract

weighing the sample before and after drying. Moisture content was calculated using the formula bellow. (AOAC, 1990).

$$\text{Moisture} = \frac{\text{Initial Mass} - \text{Final Mass}}{\text{Initial Mass}} \text{ x } 100$$

3.4.3 Ash content

To determine the ash content, samples were weighed before and after being placed in a muffle furnace for approximately 6 h or untill powdery white. Then cooled, and then place in a desiccators for further cooling to room temperature before the final mass was taken. The formula bellow was used to calculate the ash content.

ASH Initial Mass–– Final Mass ×100/Initial Mass

3.4.4 Lipid

To determine the crude lipid content, the Soxhlet method (AOAC, 1990) was used to extract the lipid from the samples. Approximately 1 g of sample was placed in the cellulose thimble and extracted using 150 ml of acetone solvent. The system was heated in a water bath for approximately 10 h after which time the solvent was evaporated using a rotary evaporator. The flask was placed in a drying oven for 1 h to remove water. After cooling, the flask was weighed and the lipid content calculated using the following formula:

$$\text{LIPID} = \frac{\text{Final Flask Mass} - \text{Intial Flask Mass}}{\text{Initial Flask Mass}} \times 100$$

3.5 Determination of Anti-nutritional Factors

Triplicate samples of the raw and treated neem seed cake were taken to the department of animals of the Ahmadu Bello University Zaria for analysis of an-nutritional factors according to method described by AOAC (1990):

3.5.1 Tannin

Two grams (2g) of the ground sample was defatted for 2hours using soxhlet extraction apparatus. The residue was placed in an oven for 24hours, retrieved and boiled at 100^{o} c with 300ml of distilled water, diluted to 500ml in a standard volumetric and filtered through non absorbent cotton wool.

A volume of 25ml of the infusion was measured in to 2litre porcelain dish and titrated with 0.1N oxalic acid until blue solution changed to green, then few drops of 0.1 potassium permanganates was added. The difference between the two titration was multiplied by 0.006235 to obtain the amount of tannin in sample, since 0.1N oxalic acid =0.006235g tannin.

3.5.2 Oxalate

Two gram (2g) of aliquot of the ground neem seed cake was weighed to a 250 ml flask; 190ml distilled water and 10ml of 6m hydrochloric acid were added. The mixture was digested for 1hour on boiling water bath, then cooled, transferred in to a 250ml volumetric flask, diluted to volume and filtered. Four drops of methyl indicator were added followed by concentrated

ammonia until the solution turn to faint yellow. It was then heated to 100^{o}c, allowed to cool and filtered. The filtrate was boiled 10ml of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through whatman No. 40 filter paper. The precipitate was rinsed several time with distilled water, transferred to a beaker and 5ml of 25% sulphoric acid was added to dissolve the precipitate. The resultant solution was maintained at 80^{0}c then cooled and titrated against 0.5% potassium permanganate until the pink color persisted for approximately one minute. Blank test was also run for the test sample. From the amount potassium permanganate the oxalate was calculated. Thus;

1ml of potassium permanganate =2.24mg oxalate.

3.5.3 Determination of saponin

The standard method of A.O.A.C (1990) was used to determine Saponin in the samples. A gravimetric method employing the use of Soxhlet extractor and two different organic solvent was used. The first solvent extracted lipids and interfering pigments while the second solvent extracted the Saponin proper.

A known weight of the dried ground sample was weighed and fitted unto the soxhlet apparatus (bearing the sample containing thimble) and methanol poured into the flask. The methanol was enough to cause a reflux. The saponin was then exhaustively extracted for 3hours. The flask was re-weighed. The difference in weight represents the weight of saponin extracted.

3.5.4 Phytic acid (phytate)

Phytic phosphorus was determined by the method of (1990). A known weight of each ground sample was soaked into 100m/of 2% HCl in a conical flask, 50$cm^3$of 0.3% potassium thiocynate solution was added. The mixture was titrated in a standard solution of $FeCl_3$ until a brownish-yellow colour persisted for 5 minutes. The concentration of the $FeCl_3$ was 1.04% w/v.

Mole ratio of Fe to Phytate = 1:1

$$\text{Conc. of phytatephosporus} = \frac{\text{Titrevalues}}{1000} \times \text{weight ofsample}$$

3.5.4 Hydrogen cyanide

The alkaline titration method was used to determine the cyanide content of the cake. 5g of sample was placed in a2000ml conical flask and 50ml of distilled water was added. The content of the flask was stirred and allowed to stand for 4hrs. The filtrate was collected in to a beaker using a glass wool which was placed in a funnel and steamed distilled in to 20ml of 2.5% sodium hydroxide. About 75ml of the distilled water was collected. This was titrated with 0.02N silver nitrate after the addition of 8ml of 6N ammonium hydroxide and 2ml of 2% potassium iodide.

3.6 Feed Formulation and Compounding

Four (4) iso nitrogenous diets were formulated according to the nutritional requirements of the experimental fish (*Clarias gariepinus)* at 40% crude protein and energy levels of 380.99kcal/kg – 394.79kcal/kg. The protein level was chosen on the basis of some previous studies (Fagbenro, 1998), where 40% protein in formulated feed demonstrated better growth of Catfish. Feed ingredients; soya bean, corn, fish meal and the Neem cake were separately milled, screened to fine particle size (<250 μm).

The dry ingredients were then weighed out according the formulation (Table 3.2) following Pearson Square Method. The ingredients were mixed until uniformly blended. Water was added (20%-30%) slowly to the mixture with continuous stirring

Table 3.2 Gross Compositions of Experimental Diets

	Graded Levels of Neem Seed Cake			
Ingredients	$D_{1(0\%)}$	$D_{2(9.62\%NSC)}$	$D_{3(17.35\%NSC)}$	**$D_{4(28.58\%NSC)}$**
Maize	24.25	23.69	25.51	**22.49**
Soya bean	36.37	27.44	17.55	**9.52**
Fish meal	36.37	36.37	36.37	**36.37**
Neem cake	-	9.62	17.55	**28.58**
Premixes	1.0	1.0	1.0	**1.0**
Cassava Starch	0.5	0.5	0.5	**0.5**
Lysine	0.5	0.5	0.5	**0.5**
Methionin	0.5	0.5	0.5	**0.5**
Salt	0.5	0.5	0.5	**0.5**
Fish oil	0.5	0.5	0.5	**0.5**

until dough was formed. This was followed by pelleting using a die size of 2mm. The pelleted feeds were sun dried and packaged in two layers of plastic bags measuring two kilogram (2kg) each and stored in a well ventilated room under ambient temperatures.

3.7 Experimental Design

The experimental fish were acclimated for two weeks, during which they were fed commercial feed (40%cp) at a daily rate of 5% of biomass with half of the daily ration fed in the morning (8:00am) and the other half in the evening (5:00pm). Thereafter, batch weighing and length measurement of fish was done to ascertain their initial mean weight in grams and initial mean length in centimeters, using top-load weighing balance (Meter Tolardo 567) and fish measuring board, respectively, before the commencement of the experiment and subsequently after every two weeks. The fingerlings were randomly grouped into five treatments of ten fish per fifty liter (50L) plastic tank. The treatments were allocated as, T1 (0%NSC), T2 (9.62%NSC), T3 (17.55%NSC), T4 (28.58%NSC) and T5 (commercial feed) respectively. Each treatment was in triplicate. Treatments T1 and T5 served as negative and positive control respectively (Table 3.2). The experiment lasted for fifty six (56) days. These substitution levels were based on previous experiment (Smita *et al.,* 2005) on *Ciprinus carpio* fed different level of water washed neem seed cake.

3.7.1 Feeding procedures

The fish were fed at five percent (5%) of their body weight twice daily during the week. To monitor the amount of feed administered, each tank had its own labeled container.

3.7.2 Determination of water quality parameters

Water quality parameters such as Temperature, Dissolved Oxygen and pH were monitored daily using mercury -in- glass Thermometer and automatic DO/pH Analyzer (model jpb 607) respectively.

3.8 Growth Measurements

Batch weighing (g) and body length (cm) of individual fish of each experimental tank were recorded every 2 weeks (14 days) during the experimental period using weighing balance and fish measuring Board respectively. Mortalities were recorded as they occurred. Parameters used in evaluating growth performance in this study were, weight gain by fish and specific growth rate (SGR). SGR is the most commonly used expression of fish growth. All these parameters were measured for all the treatments and their replicates including the control diets.

Weight gain (WG): - Is the difference between the final body weight and the initial body weight of fish over a period of time.

WG W2 – W1

Percentage weight gain was calculated using the formula

$$\mathrm{PWG} = \frac{\mathrm{FBW} - \mathrm{IBW}}{\mathrm{FBW}} \times 100$$

Where, FBW is final mean body weight (g), IBW is initial mean body weight (g).

Specific growth rate is the instantaneous change in weight of fish expressed as the percentage increase in body weight per day over any given time interval. It is calculated by taking natural logarithms of body weight, and expresses growth as %.day-1.

$$\mathrm{SGR}\,\frac{\mathrm{Ln\ FBW} - \mathrm{LnIW}}{\mathrm{D}}$$

Where D is the number of days of the experimental period.

Survival rates of fish**:**

$$SR = \frac{\text{number survived}}{Initial\ number\ stocked} \times 100$$

Condition factor (K) is information relating to the physiological status of the fish relating to its health and welfare (Balogun *et al.,* 2004). Cf was calculated as:

$$\mathrm{K} = \frac{\mathrm{W100}}{\mathrm{L}^3}$$

(Oni *et al*., 1983)

Where: W = Weight of fish. L = Standard length of fish

3.9 Nutrient Utilization Parameters

The following indices were used to calculate the efficiency of the experimental diets in relation to nutrient utilizations.

Feed conversion ratio (FCR)

FCR is defined as the amount of dry feed fed per unit live weight gain. It was calculated using the formulae bellow.

$$\text{FCR} = \frac{\text{Feed Fed}}{\text{Weight gain}}$$ (Balfour, 1988)

Protein Efficiency Ratio (PER**)** is defined as the ratio between the weight gain of fish and the amount of protein fed (De Silva and Anderson, 1995).

$$\text{PER} = \frac{\text{Weight gain (g)}}{\text{Crude Protein Fed (g)}}$$

(Balfour, 1988)

Net Protein Utilization (NPU): sometimes also called efficiency of protein utilization, evaluates the protein in the diet by the ratio between the protein retained in fish tissues and the dietary protein fed. NPU is determined by carcass analyses of samples of fish taken before and after feeding with the evaluated protein, and generally expressed as a percentage of the protein fed.

$$\text{NPU} = \frac{\text{Retained Protein in Tissue} \times 100}{\text{Dietary Protein Fed}}$$

(Balfour, 1988)

3.10 Cost and benefit analysis of experimental diets

A simple economic analysis was conducted to assess the cost effectiveness of the experimental diets. Only the cost of feed was used in the calculations with the assumption that all other operating costs remained constant. Costs of the feeds were calculated using market prices of ingredients as at the time of the experiment. Economic evaluation of the experimental diets was calculated by evaluation the feed cost in Nigerian naira (FC) needed to produce 1 kg of live weight gain of each experimental fish group.

Feed cost (₦) = (feed cost/kg) X (food consumption) (Eleamer and Sharon, 1984)

Price of one kg gain in weight (₦) (₦/gain "kg") = (feed cost/kg) X FCR

Miller (1976) also suggested another simple parameter called the Profit Index which was also used to evaluate the profitability of the diets.

$$\text{Profit Index} = \frac{\text{Value of Fish}(₦)}{\text{Cost of Feeding}(₦)}$$

3.11 Statistical Analysis

The data was subjected to analysis of variance (ANOVA) to test the significance among treatment means. Where there was significant difference, Duncan multiple range test was applied to rank treatment means ($P< 0.05$). All statistical analyses were computed using SPSS (IBM) Statistical package Version 20 for Windows.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Raw and Water Washed Neem Seed Cake

The proximate composition of raw and treated neem cake is presented in Table 4.1. The crude protein, fat and ash content of treated neem seedcake was higher than those obtained for untreated neem seed cake. The crude protein compositions were 25.38 and 31.81 for the raw and the water washed neem seed cakes, respectively. Dry matter, crude fiber and nitrogen free extract decreased after the treatment of the cake. Dry matter was 89.88 and 90.08 for the treated and raw neem seed cakes, respectively. The crude fiber was 8.06 and 8.56 for water washed and raw Neem Cakes, respectively.

4.2 Anti-nutritional Factors of Raw and Water Washed Neem Seed Cake

Analysis of anti-nutritional factors as presented in Table 4.2 indicates that all the components determined were greatly reduced after water washing of the cake. However, cyanide content tends not to be effectively removed by the treatment method as it was almost the same in the treated cake compared to the value obtained in the untreated cake.

4.3 Proximate Composition of Experimental Diets

The proximate composition of the experimental diets fed to *C. gariepinus* is shown in Table 4.3. The diets were iso nitrogenous as there was no significant difference ($p>0.05$) in the protein composition of the diets at 40% crude protein and they all met the set target specification for the experiment. The crude protein ranged from 40.50 to

Table 4.1 Proximate Composition of Raw and Water Washed Neem Seed Cake

	DM	CP	EE	ASH	FB	NFE
RNSC	90.08	25.38	4.56	4.20	8.56	57.30
WNSC	89.88	31.81	5.11	8.26	8.06	46.76

Key:

DM= Dry matter

CP = Crude protein

EE = Ether extract

FB = Fiber

NFE = Nitrogen free extract.

Table 4.2 Anti-nutritional Factors of Raw and Treated Neem Seed Cake (mg/100g)

	Saponin	Oxalate	Tannin	Cyanide	Phytate
RNSC	0.48±0.11[a]	1.59±0.20[a]	1.18±0.11[a]	0.65±0.06[a]	6.61±0.05[a]
WNSC	0.21±0.05[b]	0.19±0.08[b]	0.58±o.05[b]	0.66±0.05[a]	3.21±0.05[b]

Means with different superscript along the same column are not significantly different ($p<0.05$).

Key:

DM = dry matter

CP = crude protein

EE = Ether extract

NFE = nitrogen free extract

RNS = raw neem seed cake

WNSC = water washed neem seed cake

Table 4.3 Proximate Composition of Experimental Diets

DM	91.36±0.78[a]	92.04±1.22[a]	91.90±1.09[a]	91.11±0.57[a]	93.20±1.84[a]
CP	41.12±0.57[a]	40.50±0.15[a]	40.51±0.34[a]	40.56±0.32[a]	41.56±0.57[a]
CF	5.15±0.54[a]	4.23±0.13[bc]	3.12±0.06[c]	3.89±0.57[bc]	4.51±0.00[ab]
EE	6.39±0.8[a]	6.08±0.04[b]	7.00±0.86[b]	6.99±0.57[b]	12..21±1.15b
Ash	7.62±0.58[a]	7.90±0.57[a]	6.17±0.09[a]	6.17±0.57[a]	9.50±2.51[a]
NFE	39.75±0.43[a]	41.41±0.81[a]	43.33±0.19[a]	42.41±1.15[a]	53.92±0.00[a]
Kcal/kg	380.99	382.38	398.32	394.79	489.92

Means with same superscript across a raw are not significantly different (p> 0.05)

Key;

NSC= neem seed cake

DM = dry matter

CP = crude protein

CF= crude fibre

EE = Ether extract

NFE = nitrogen free extract

Kcal= kilo calorie

KG = Kilogram

41.56. The oil contents in the feeds ranged from 6.00 to 12.21 while the Nitrogen Free Extract ranged from 39.75 to 53.92. The energy contents ranged from 380.99kcal/kg to 394.79kcal/kg.

4.4 Water Quality Parameters

The water quality characteristics of the experimental Tanks monitored during the feeding trial are presented in Table 4.4.The result indicates an insignificant variations ($p>0.05$) among the experimental Tanks. Water temperature ranged from 26.0^{0}c - 26.3^{o}c. Hydrogen ion concentration (pH) values ranged from 7.23-7.96. Dissolved oxygen (DO) ranged from 5.0 – 5.50mg/l.

4.5 Growth Performance and Survival of *Clarias gariepinus* Fed Experimental Diets

The results of growth performance in terms of Final weight gain (FWG), Specific Growth Rate (SGR), Condition Factor (CF) and Survival Rate (SR) of *Clarias gariepinus* fingerlings fed experimental diets are presented in Table 4.5

4.5.1 Initial weight of fish

Result of the statistical analysis as presented in Table 4.5, Fig 1 revealed that average initial weight at the experimental start had ranged between 5.95 g and 6.06 g. There was no significant difference ($p>0.05$) among the experimental treatments, indicating the complete random distribution of individual fish at the start of the experiment.

Table 4.4 Mean Water Quality Parameters Recorded During Experimental Period

Parameters	$1_{(0\%soya}$	$2_{(9.62\%NSC)}$	$3_{(17..35\%NSC)}$	$4_{(28.58\%NSC)}$	$5_{(MULTIFEEDS)}$
Temp.	26.33±0.33	26.00±0.57	26.33±0.33	26.33±0.3	26.33±0.33
pH.	7.23±0.14	7.96±0.06	7.96±0.03	7.38±0.03	7.70±0.11
DO	5.46±0.27	5.37±0.15	5 .00±0.33	5.10±0.23	5.50±0.11

Key:

Temp. = Temperature

pH = Hydrogen ion concentration

DO = Dissolved oxygen

Table 4.5 Growth performance of *Clarias gariepinus* fed experimental diets

DIETS	MIL	MFIL	MIW	MFW	WG	PWG	SGR	CF	SR
Diet1	8.19	13.95	6.00±0.93	29.75±0.96^{a}	23.24±0.96^{a}	395.46±17.08^{a}	0.024±0.0005^{a}	1.24±0.22^{a}	90.00±5.77
Diet2	8.17	12.79	5.97±0.88	26.83±0.99^{ab}	20.80±0.98^{ab}	349.63±16.32^{ab}	0.023±0.0005^{ab}	1.04±0.13^{a}	90.00±5.77
Diet3	8.22	11.80	6.06±0.87	23.90±2.0^{b}	17.83±2.0^{b}	291.77±35.56^{bc}	0.022±0.0008^{b}	1.45±0.18^{a}	83.33±0.57
Diet4	8.46	12.89	6.06±0.16	22.70±0.72^{b}	16.63±0.05^{b}	273.92±2.98^{c}	0.021±0.003^{b}	1.07±0.008^{a}	90.00±5.77
Diet5	8.57	13.25	5.93±0.04	27.20±1.7^{ab}	21.27±1.7^{ab}	360.34±27.43^{ab}	0.023±0.006^{ab}	1.19±0.17^{a}	92.00±1.00

Means with same letter in the same column are not significantly different ($p>0.05$).

Key:

MIL= Mean initial length, MFIL= Mean final length, MIW = Initial weight, MFW=Mean final weight , WG = Weight gain,

PWG = Percentage weight gain, SGR = Specific growth rate, CF = Condition factor, SR = Survival rate

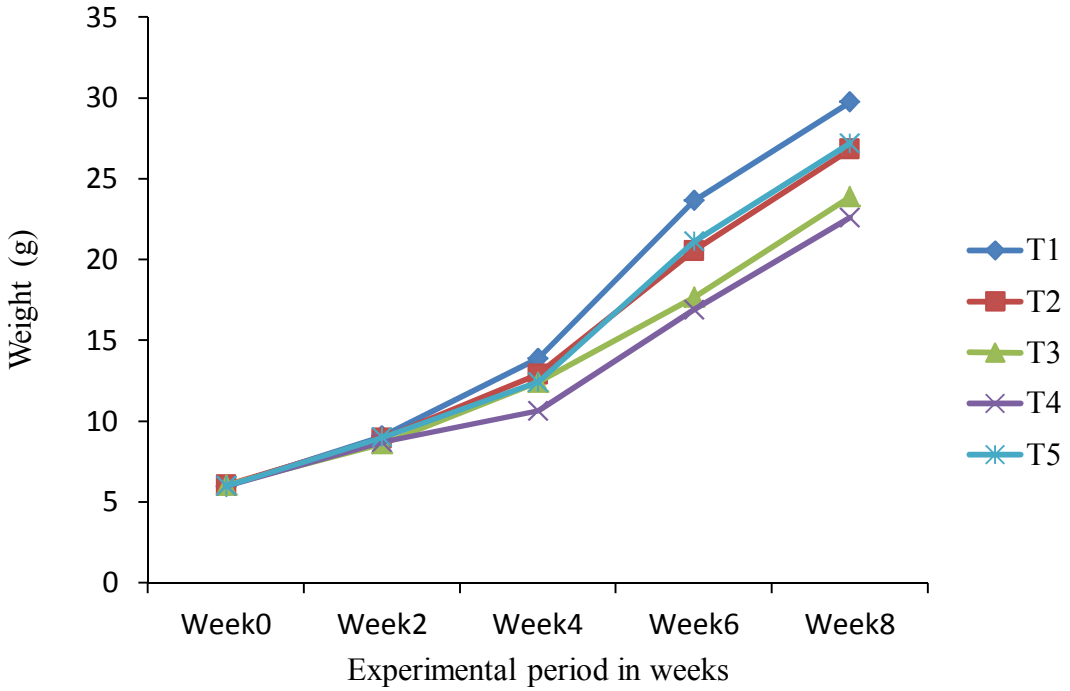


Fig1:Fouthnightly increase in weight (g/fish) of *Clarias gariepinus* fed experimental diets

4.5.2 Weight gain of fish

The results of the mean final weight gain of *Clarias gariepinus* fed the experimental diets are presented in Table 4.5. The mean weight gains ranged from 16.63g to 23.24g. There were no significant difference ($p>0.05$) between the weight gain of fish fed diets 2, 3, 4 and the control (diet5). However, the weight gain of fish fed diet1 (0%NSC) was significantly higher than those of 3 and 4, but did not show any significant difference ($p>0.05$) with those of diet2 and reference diet5. Fish in all the experimental groups increased significantly in weights over the eight (8) week feeding trial (fig1).

4.5.3 Percentage weight gain of fish

Table 4.5 presents the results of the percentage weight gain of *Clarias gariepinus* fed the experimental diet. The percentage weight gains ranged from 273% to 395.46%. The highest value was recorded in treatment1 (control) and the lowest value was observed in T4 (%NSC). There were significant differences ($p< 0.05$) between T3 and T4 compared to the control (T1). However, T2 (9.62%NSC) did not differ significantly ($P>0.05$) with T3 and the control diets.

4.5.4 Specific growth rate of fish

The specific of growth rate of *Clarias gariepinus* fed the experimental diets as presented in Table 4.5, ranged from 0.024g/fish/day, 0.021g/fish/day to 0.24g/day. The results revealed no significant difference ($p>0.05$) among the dietary levels of neem seed cake compared to control

(Commercial feed). However, T2 (9.68%NSC) did not differ significantly compared to the control diets. T3 (17.55%NSC) and T4 (28.58%NSC) were significantly lower than the control T1 (control). The control recorded the highest specific growth rate (0.024g) while T4 (28.58%NSC) had the lowest specific growth rate (0.021g)

4.5.5 Condition factor of fish

The condition factor which determines the welfare and health of the experimental fish (*C. gariepinus)* fed the experimental diets is presented Table 4.5. No significant differences ($p<0.05$) among experimental diets and the control. The condition factor ranged from 1.07 to 1.45.

4.5.6 Survival rates of fish

The survival rate of the fish throughout the experimental period as presented in Table 4.5 indicates that fish were not affected by treatments as there were no significant differences ($p>0.05$) among the dietary treatments compared to the control. The survival rate ranged from 83.33% to 92%. The highest value (92%) was recorded in T5 and the lowest (87%) was obtained in T3.

4.6 Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets

4.6.1 Acceptability of experimental diets

Diet acceptability was assessed subjectively by direct observation of fish behavior and feeding responses. Fish from all treatments adapted to the experimental diets within the first week of the experiment. Acceptability, however, varied for the different diets. The control diets and treatment2 were generally more acceptable since fish were observed to be actively feeding and the activity ceased in less than 5 minutes. The other diets were less readily accepted by fish taking longer periods (about 5-15 minutes). The mean total feed intakes (Table 4.6) in gram per fish ranged from 29.77g to 30.72 g. Feed intake did not show any significant ($p<0.05$) variation among test diets and the control, except for T4 (28.55%NSC) which was significantly lower than the control diets. Feed intake decreased with increasing level of NSC.

4.6.2 Protein efficiency ratio (PER)

The results of the protein efficiency ratio are presented in Table 4.6. The PER ranged from 1.6 to 1.88. Statistical analysis indicates that there is no significant variation among the experimental diets. However, the highest value recorded in treatment1 (0%NSC) and lowest value was recorded in treatment4 (28.55%NSC). PER decreased with increasing level of NSC in the diet.

4.6.3 Net protein utilization (NPU)

Net protein utilization values are presented in Table 4.6. The net protein utilization ranged from 87 to 100. Net protein utilization did not differ significantly ($p<0.05$) among the experimental diets and the control. Treatment5 has the highest NPU (100) and Treatment4 resulted in the lowest value (87).

4.6.4 Feed conversion ratio

The values of feed conversion ratio are presented in Table 4.6. The value ranged from 1.29 to 1.53. No significant variation ($p>0.05$) was observed among the experimental diets and the control. T1 has the lowest FCR (1.29) and the highest (1.53) were Table

4.6 Nutrient Utilizations of *Clarias gariepinus* Fed Experimental Diets

Diets	WG	FI	PER	NPU	FCR
Diet1	23.24±0.96^{a}	30.72±0.54^{a}	1.86±0.09^{a}	99.00±1.52^{a}	1.29±0.06^{a}
Diet2	20.80±0.98^{ab}	29.77±1.10^{a}	1.72±0.10^{a}	96.66±3.71^{a}	1.43±0.08^{a}
Diet3	17.83±2.0^{b}	26.85±0.93^{ab}	1.64±0.14^{a}	87.00±3.08^{a}	1.53.15^{a}
Diet4	16.63±0.05^{b}	25.66±0.59^{b}	1.59±0.02^{a}	90.33±2.40^{a}	1.53.04^{a}
Diet5	21.27±1.7^{ab}	30.36±1.94^{a}	1.68±0.03^{a}	100.00±6.83^{a}	1.43.02^{a}

Means with same letter in column are not significantly different ($p > 0.05$).

Key :

WG= Weight gain

FI= Feed Intake

PER= Protein Efficiency Ratio

NPU= Net Protein Utilization

FCR= Feed Conversion Ratio

observed in T3 and T4 respectively. The FCR increased with increasing level of NSC in the diet.

4.6.5 Carcass composition

Fish body proximate compositions analysed before and after feeding with the experimental diets are presented in Table 4.7. Crude protein (Cp) and ether extract (Oil) differed significantly ($p>0.05$) among the experimental diets and the control. Crude Protein ranged from 50.2 to 54.18, Fats ranged from 7.10 to 12.33. However, dry matter, ash and nitrogen free extract did not differ significantly ($p<0.05$) among the fish fed experimental diets and the control. Dry matter ranged from 85.68 to 88.50, ash ranged from 7.4 - 9.39 and nitrogen free extract was between 27.77 and 30.37. Apart from ash which is significantly higher in the initial carcass composition than after feeding with the experimental diets, all other variables increased after the feeding trial. The carcass crude protein and lipid increased while ash decreased with increasing level of neem seed cake in the diet.

4.7 Cost and Benefit Analyses of Experimental Diets

The cost of the diets decreased with dietary levels of Neem Seed Cake (Table 4.8). Cost per kilogram of the experimental diets ranged from ₦199.99 to ₦450. Treatment5 recorded the highest cost while Treatment4 had the lowest. Cost of feed required to produce unit weight of fish ranged from ₦170 to ₦630. Treatment5 was the highest while Treatment4 recorded the lowest. Profit index ranged from1.16 to 4.18, in which treatment5 was the lowest while Treatment4 recorded the highest profit index.

Table 4.7 Carcass Composition of Experimental Fish Before and After Feeding with the Experimental diets

Diets	DM	CP	Lipid	Ash	NFE
Initial	86.88±0.50[a]	46.03±0.57[c]	5.24±0.57[c]	19.9±0.01[a]	29.47±0.00[a]
Diet1	87.78±0.57[a]	53.63±0.58[a]	7.10±0.05[b]	8.45±0.45[b]	30.16±0.16[a]
Diet2	85.68±0.57[a]	52.29±0.57[ab]	13.46±0.57[a]	8.16±0.16[b]	30.43±0.43[a]
Diet3	88.18±0.10[a]	50.20±0.57[b]	12.29±0.57[a]	7.40±0.6[b]	29.78±0.00[a]
Diet4	88.41±0.00[a]	50.41±1.39[b]	12.35±0.56[a]	9.39±1.3[b]	27.77±0.23[a]
Diet5	88.50±0.60[a]	54.18±0.57[a]	7.24±0.13[b]	8.23±1.0[b]	30.37±0.35[a]

Means with same letter in column are not significantly different ($p>0.05$).

Key:

DM= Dry matter,

CP= Crude protein

NFE= Nitrogen free extract.

Table 4.8 Cost and Benefit Analysis of Experimental Diets

Diets	Profit index	Cost/Kg/fish(₦)	Cost/Kg/Feed(₦)
Diet1	2.61±0.030[a]	292	226.66
Diet2	2.52±0.146[b]	290	219.00
Diet3	2.40±0.216[b]	310	211.23
Diet4	4.18±0.093[a]	170	199.99
Diet5	1.16±0.021[c]	630	450.00

Means with the same letter in a column are not significantly different ($p>0.05$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition and Anti-nutritional Factors

The proximate composition of the raw and water washed neem seed cake (Table3) indicates a significant increase in the crude protein composition of the cake from 25.30% to 31.81% respectively. The crude protein obtained in this experiment is higher than 23.06% reported by Bawa *et al*. (2007) for alkaline treated neem seed cake, but is lower than 40.91% and 47.89% reported by Gowda and Sastry (2000) for raw and treated neem seed cake respectively. James *et al.* (2007) reported that processing generally affects the crude protein contents of Neem seed cake. The differences observed in this experiment may be as result of differences in geographical location, harvesting time and the processing methods employed. The increased in crude protein recorded (31.87%) may be due to the removal of the triterpenoids and other substances present in the cake as noted by James *et al.* (2007) which is an indication that water washing can be a better method of improving the nutritive value of neem seed cake.

The results of the anti-nutritional factors of the raw and water washed neem seed cake showed significant reduction of these factors following the treatment of the cake. Tannin, phytate, saponin and oxalate were reduced by 50.8%, 51.4%, 56.25% and 88%, respectively. This significant reduction of the anti nutritional factors may be as result of efficacy of water washing as method of removing anti-nutrient in the cake as suggested Bawa *et al.* (2007). However, the cyanide content was slightly higher in the treated neem seed cake than in the raw neem seed cake. This may be due to the insolubility of this compound in water, although the value are still

within the ranges 1.5- 2.2% reported as tolerable by African catfish (*Clarias gariepinus*) (Van weerd, 1995). Similarly Mubarak *et al.* (2012) reported significantly higher levels of anti-nutritional factors in biologically treated neem seed cake compared to the a raw neem seed cake.

5.2 Growth Performances

The growth performance of *Clarias gariepinus* fed water washed Neem cake indicates that mean initial weight (5.97g -- 6.0g) was not significantly different ($p>0.05$) among the experimental treatments, showing uniformity in size at onset of the experiment as recommended by previous studies (Jegede and Fagbenro, 2008).

Mean final weight gain generally decreased with increasing levels of neem seed cake in diets. The control diet recorded the best weight gain (23.24g) which was not significantly different ($p>0.05$) from the weight gain (20.8g) of fish fed diet containing 9.62% neem seed cake. Similarly, the specific growth rate (SGR) also displayed a decreasing trend with increasing levels of neem seed cake in the diets. The best performance (0.024g) was obtained in fish fed the control diet1 (0%nsc) followed by fish fed 9.62%nsc diet (0.023g) while the lowest value (0.021g) was recorded in fish fed on diet4 (28.38%nsc). Percentage weight gain, also revealed similar trend with those of final weight gain and specific growth rate.

The decreasing trend in growth performance may be due to residual anti nutritional factors inherent in the cake. The better growth performances recorded in dietary treatment2 may be due to lower level of inclusion which enhanced its nutritional composition, palatability and bioavailability. This agrees with findings of Agbabiaka (2010) who stated that the principal growth retarding principles of Neem seed cake were believed to be water soluble. In another experiment

Odunsi *et al.* (2009) also attributed the improved consumption of water washed Neem cake at both treatment and inclusion levels to the removal of bitter component during soaking and concluded that treated neem cake can be used as feed ingredient in poultry diet. Similarly, Jegede and Fagbenro (2008) did not observe any significant variation in growth parameters and feed conversion ratio of mixed sex *tilapia zilli* fed neem leaf meal supplemented diets

Generally the results suggest that the experimental diets were all adequate in nutrients and other dietary requirements of the experimental fish (*clarias gariepinus*) although analysis of amino acid profile of the neem seed cake was not carried out, literature (NRC, 1992) showed that neem seed cake is balanced in its amino acid composition.

Survival rate of *C. gariepinus* fed the experimental diets (Table 4.5) showed similar performances (87% - 90%) among the experimental treatments as no significant differences ($p>0.05$) was observed. In their findings, Talpur and Ikwunidin (2013) reported that fish fed Neem leaf-supplemented diet improved the immune system and increased survival rate in *L. calcarifer* fingerlings against *V. harveyi* infection. Mortality recorded in this experiment agrees with report of many scholars involving feeding trial with fish (Ashref *et al.,* 2010; Bichi and Ahmed 2010; Johny *et al.,* 2011,).

Condition factor is information relating to the physiological status of the fish relating to its welfare and health, fatness or wellbeing of fish (Nath *et al*., 2006). It is based on the hypothesis that heavier fish of a particular length are in a better physiological condition (Ndemele *et al.,* 2010). Condition factor is also a useful index for monitoring of feeding intensity, age, and growth rates in fish (Ndimele *et al.,* 2010). The condition factors, (1.07–1.45) of fish fed the dietary treatments were not significantly different ($P>0.05$) indicating that dietary inclusion of neem seed cake did not influenced the welfare of the experimental fish.

5.3 Feed Intake and Nutrient Utilization

All the experimental diets were accepted by the fish as displayed by their feed intake which did not show any significant variation between the experimental treatments except for treatment4 (28.58%NSC) which was significantly lower than the other diets. The decreased dry matter intake observed in fish fed diet containing 28.58% levels of NSC may be as a result of decreased palatability of the diets.

The control diets and the diets containing 9.62% neem seed cake were generally more acceptable since fish were observed to be actively feeding and the activity ceased in less than 5 minutes and no left over feed was observed. The other diet was less readily accepted by fish, taking longer periods (about 5-15 minutes). Similar observations were made by Vasanthakumar *et al*. (1999) and Gowda and Sastry (2000). Vasanthakumar *et al.* (1999) observed lowered feed intake, nutrient digestibility and growth in rabbits fed 20% raw neem seed meal.

Gowda and Sastry (2000) reported that neem seed cake is toxic and bitter to taste due to triterpenoids, which restricts its safe inclusion in livestock diets. Generally, all experimental diets were well accepted and no pathological signs were observed during the trial. This observation is in consonance with that of Agbabiaka *et al*. (2011) who stated that palatability can be determined by the rate of ingestion of feed particles and visual observation can be useful provided the feed and the fish are visible to be seen with naked eyes. In a related experiment, James *et al*. (2007) reported significant ($p<0.05$) reduction in daily feed intake and daily protein intake compared with standard protein diet, for rats fed with processed neem seed cakes.

This acceptability of the diets containing 9.62% and 17.55% NSC may be attributed to the good processing techniques which involved soaking and drying that might have reduced the anti nutritional factors and bitter principles of the cake, and improved its compositions and palatability. This observation corroborates with the works of Oresegun and Alegbeleye (2001), Amisah *et al.,* (2009) and Olaniyi *et al*. (2009)

The nutrient utilization of *C. gariepinus* fed diets containing different dietary levels of Neem cake did not show any significant differences ($p<0.05$) in feed conversion ratio (FCR), Protein efficiency ratio (PER) and Net Protein Utilization among the experimental diets compared to the control. The net proein utilization which ranged from 87-100% (NPU), were within the ranges (>70) recommended for good food protein and dietary mixture as reported by Oresegun and Alegbeleye (2001).

The feed conversion ratio recorded in this experiment (1.29–1.53) in which the control diet had the lowest (best) and diets 3 and 4 had the highest (poor) was similar to the value on cotton seed meal (1.55) reported by Jabeen *et al*. (2004) when major cap (*Cirrhinus mrigala*) fingerlings were fed cotton seed, fish meal and barley. Contrary to this, Smita *et al*. (2005) reported significant increase ($p < 0.05$) in feed conversion ratio and decreased specific growth rate and protein efficiency ratio in fish fed water washed neem seed cake supplemented diets to *Cyprinus capio.*

This experiment suggests that even though growth depression occurred with fish fed water processed neem seed cake diet, the processed neem seed cake was still adequate and can meet the basic physiological needs of the experimental fish. This is in agreement with earlier findings of Jegede and Fagbenro (2008) that when Neem seed cake contains low amount of fat

and devoid of smell its protein efficiency ratio (PER) and net protein utilization (NPU) are comparable to any other oil seed cake.

Another reason for this appreciable nutrient utilization may be due to the good composition of essential and non essential amino acids in Neem cake which have been praised by many scholars (Gowda and Sastry, 2000) as complete in its amino acid compositions. Bairagi *et al.* (2004) disclosed that anti nutritional factors markedly reduce nutrient utilization. The reduction of these factors recorded in the present study could have facilitated better digestion and improved nutrient utilization.

5.4 Carcass Composition

Generally, the carcass crude protein *C. gariepinus* in this experiment increased significantly ($p<0.05$) after the feeding trial. The mean initial crude protein (46.03%) was significantly ($p<0.05$) lower than the values obtained after the feeding trial. However, apart from treatment2 (9.62%NSC) which recorded 52.29% CP, the other two treatments were significantly ($p<0.05$) lower than the control diets which had 53.63 and 54.18.

Similar result was reported by Smita *et al.* (2005) who reported increased carcass crude protein in *C. carpio* fed water washed neem seed cake. Odunsi *et al*. (2009) in terrestrial animal reported that soaking of neem seed cake improved dressing percentage of the experimental birds and will be a better improvement technique for neem seed cake. Also, James *et al.* (2007) reported that Rats fed methanol processed Neem seed cake compared favorably with soybean protein diets in their carcass composition. The decreased crude protein with increasing level of neem seed cake observed in this experiment may be due to the reduced feed intake observed.

5.5 Water Quality Parameters

The water quality parameters (Temperature, Dissolved Oxygen, Hydrogen ion concentration) monitored during the experimental period were not significantly (P>0.05) different among the experimental tanks. The Dissolved Oxygen ranged from 5.0 - 5.5 mg/l, temperature ranged from 26.0^0c – 26.3^0c while the hydrogen ion concentration (pH) ranged from (7.23 - 7.96). Ayoola and Fedrick (2012) stated that, 3 - 8mg/l of Dissolved Oxygen is recommended for fresh water fish culture. Smita *et al.* (2005) also did not report any variation in water quality condition when water washed Neem cake was fed to *C. carpio* as a A replacement to ground nut cake.

The water quality parameters recorded in this experiment is an indication that optimal water quality was maintained by frequently changing the water through the flowthrough system; this guaranteed that metabolic products did not accumulate and also indicate that use of Neem Seed cake in aquaculture diet did not have any negative influence on water quality as the values were all within the range generally recommended for the culture of Catfish.

5.6 Cost and Benefit Evaluation of the Experimental Diets

Cost-effectiveness analysis of the present study generally indicated that the cost of feed per kg of feed reduced from ₦226.66 in the control to ₦199.99 in treatment4. The cost required to produce a kilogram of fish decreased from ₦630 to ₦170 in treatment5 and treatment4 respectively treatment5 was significantly higher than all the other diets in terms of cost of feed per kilogram (₦450) and in lowest profit index (PI)(1.16). Experimental diet containing 28.58% neem seed cake

which had lowest cost per kilogram of diet and the lowest cost to produce a kg fish and the highest PI (4.18) was therefore more profitable than the other diets. Cheaper NSC replacement for soya beans could improve cost effectiveness of the diets.

The economic superiority of treatment4 in this study could be attributed to the fact that it was the cheapest diet since it contains the highest level of neem seed cake. The relatively higher cost factors of the treatment2 and treatment3 may be due to their higher feed conversion ratio and lower growth rate. When evaluating cost effectiveness of a diet, the feed conversion ratio and the cost of the feed should be taken in to account simultaneously and not independent of each other. Using the cheapest feed available more often than not does not mean the lowest cost to produce a kilo gram of fish.

Moreover, the use of neem seed cake in fish feed production may be more economical taken in to consideration it availability and less competition by humans. Owens *et al*. (2009) noted that the use of these ingredients (soya beans) for animal nutrition when human needs have not been met introduced the issue of moral and economic justification. Another group of researchers (Amisa *et al.* 2009) stated that when selecting alternative feed ingredients it is important that selected protein source do not conflict with human food security interest.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. The result of this experiment revealed that water washing can be a reliable method of debitterizing and detoxification of neem seed cake which improved the crude protein composition cake by 25.33% and reduced the antinutritional contents by 50.8%, 51.4%, 56.25% and 88% for tannin, phytate, saponin and oxalate, respectively.

2. Growth performance of the experimental fish were comparable to those of the control diets but decreased with increasing level of WNSC in the diet, suggesting that WNSC can be used to partially replaced soya beans in *C. gariepinus* diet.

3. The cost of feed per kg was reduced increased inclusion of WNSC in the diet and profitability enhanced at 28.58% WNSC inclusion.

6.2 Recommendations

1. There is a possibility that the fingerlings *clarias gariepinus* used in this investigation are more sensitive to the anti-nutritional factors than larger fish would be. Therefore, long term studies should be conducted using fish of a larger size range than those used in the present study.

2. This study was carried out under laboratory conditions. It would be necessary to investigate if the findings could be applicable to field conditions, especially the semi-intensive system commonly practiced in ponds.

3. From the results it was observed that the higher the inclusion level of neem seed cake protein the lower the growth performance and nutrient, and therefore the longer it could take fish to reach marketable. The additional days for culture could probably lead to increased production cost. Therefore, further studies aimed at enhanced nutrient utilization of neem seed cake is recommended.

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Appendices

Appendix 1 One way Analysis of Variance for Growth Performance

Single factor ANOVA

Source of variation		Sum of Squares	df	Mean Square	F	Sig.
IW	Between Groups	.042	4	.011	.291	.878
	Within Groups	.362	10	.036		
	Total	.404	14			

Single factor ANOVA for final weight of fish

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
FW	Between Groups	94.394	4	23.598		.030
	Within Groups	56.415	10	5.642		
	Total	150.809	14			

Single factor ANOVA for Final weight gain of fish

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
WG	Between Groups	97.073	4	24.268	4.272	0.028
	Within Groups	56.802	10	5.68		
	Total	153.875	14			

Single factor ANOVA for Percentage weight gain

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
PWG	Between Groups	30322.046	4	7580.511	4.884	.019
	Within Groups	15520.144	10	1552.014		
	Total	45842.190	14			

Single factor ANOVA for specific growth rate

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
SGR	Between Groups	.000	4	.000	3.194	.062
	Within Groups	.000	10	.000		

	Total	.000	14			

Single factor ANOVA for Relative growth rate

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
RGR	Between Groups	.088	4	.022	2.707	.092
	Within Groups	.081	10	.008		
	Total	.169	14			

Single factor ANOVA for Condition factor

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
CF	Between Groups	.314	4	.078	.981	.460
	Within Groups	.800	10	.080		
	Total	1.113	14			

Appendix 2 One way Analysis of Variance for Nutrient Utilization

Single factor ANOVA for Protein efficiency ratio

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
PER	Between Groups	.144	4	.036	1.400	.303
	Within Groups	.256	10	.026		
	Total	.400	14			

Single factor ANOVA for Net Protein Utilization

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
NPU	Between Groups	397.333	4	99.333	2.123	.153
	Within Groups	468.000	10	46.800		

	Total	865.333	14			

Single factor ANOVA for feed conversion ratio

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
FCR	Between Groups	.118	4	.030	1.299	.334
	Within Groups	.228	10	.023		
	Total	.346	14			

Single factor ANOVA for Profit index of diets

	Source of variation	Sum of Squares	df	Mean Square	F	Sig.
PI	Between Groups	13.871	4	3.468	73.458	.000
	Within Groups	.472	10	.047		
	Total	14.343	14			

Appendix 3 One way ANOVA for Proximate Composition Experimental Diets

		Sum of Squares	df	Mean Square	F	Sig.
DM	Between Groups	8.463	4	2.116	.499	.737
	Within Groups	42.389	10	4.239		
	Total	50.852	14			
CP	Between Groups	2.825	4	.706	1.286	.339
	Within Groups	5.493	10	.549		
	Total	8.318	14			

	Between Groups	6.780	4	1.695	4.302	.028
CF	Within Groups	3.940	10	.394		
	Total	10.720	14			
	Between Groups	77.007	4	19.252	10.476	.001
EE	Within Groups	18.377	10	1.838		
	Total	95.384	14			
	Between Groups	8.221	4	2.055	.466	.760
ASH	Within Groups	44.098	10	4.410		
	Total	52.319	14			
	Between Groups	378.024	4	94.506	70.743	.000
NFE	Within Groups	13.359	10	1.336		
	Total	391.383	14			

Appendix 4 Oneway Analysis of Variance for Carcass Composition

Single factor ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
DM	Between Groups	17.680	5	3.536	.634	.678
	Within Groups	61.312	11	5.574		
	Total	78.992	16			
CP	Between Groups	193.653	5	38.731	83.933	.000
	Within Groups	5.537	12	.461		
	Total	199.190	17			
Oil	Between Groups	62.290	5	12.458	5.520	.007
	Within Groups	27.085	12	2.257		
	Total	89.375	17			
Ash	Between Groups	341.134	5	68.227	11.040	.000
	Within Groups	74.161	12	6.180		
	Total	415.295	17			
NFE	Between Groups	14.901	5	2.980	.155	.974
	Within Groups	230.395	12	19.200		
	Total	245.296	17			