GROWTH AND NUTRIENT UTILIZATION OF AFRICAN CATFISH(*CLARIAS GARIEPINUS* BURCHELL, 1822) FED VARYING LEVELS OF *ALBIZIA LEBBECK* (BENTH) LEAF MEAL

By

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AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

MARCH, 2015

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LEBBECK(BENTH) LEAF MEAL

By

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M.Sc/Sci/ 8835/2011-2012

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,

AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT OF THE REQIUREMENTS FOR THE AWARD OF MASTERS DEGREE IN EDUCATIONAL BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

MARCH, 2015

DECLARATION

I declare that this research titled **"GROWTH AND NUTRIENT UTILIZATION OF AFRICAN CATFISH (*CLARIAS GARIEPINUS* BURCHELL, 1822) FED VARYING LEVELS OF *ALBIZIA LEBBECK* LEAF MEAL"** has been conducted by me in the Department of Biological Sciences. The information derived from literatures has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Elizabeth Adenike OYELERE _____ _____

Signature Date

CERTIFICATION

This thesis entitled **"GROWTH AND NUTRIENT UTILIZATION OF AFRICAN CATFISH (*CLARIAS GARIEPINUS* BURCHELL, 1822) FED VARYING LEVELS OF *ALBIZIA LEBBECK* LEAF MEAL"** by **Elizabeth AdenikeOYELERE** meets the regulations governing the award of the degree of **MASTERS OF SCIENCES IN EDUCATIONAL BIOLOGY** of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

I sincerely dedicate this work to God Almighty for the gift of life, grace, resources and success of this research work.

ACKNOWLEDGMENT

I sincerely acknowledge God Almighty for the gift of life, grace, resources and success of this research work. I acknowledge my supervisors, Prof. J.K. Balogun, Dr. B.Y. Abubakar for their immense effort towards the success of this research. I acknowledge Prof. J. Auta, Prof. S.A. Abdullahi, Dr. S.A. Abolude for their advice and contributions. I acknowledge my family, particularly my husband for his patience, advice and support morally, financially towards the successful completion of this research work, the Oyeleres', my mother and siblings for their prayers and encouragement. I acknowledge some of the Fishery and Hydrobiology Laboratory of Biological Sciences Department staff members, (Mr. W. Steven, Mr. S. Luka, Malam M. Musa) for their assistance all through the period of this research. My sincere appreciation goes to the Department of Biological Sciences for the enablement granted me to undergo this programme. I cannot leave out my friends and seniors who stood by me and for their advice and various input in this research work, just to mention a few, (Mrs. R. Agbo, Mr. Onimisi, Mr. C. Yaro for helping me with the analysis, Madam K. Amarachi, Malam M. Hassan, Dr. Marian, Mr. A. Jatau) may God reward you all luxuriantly.

ABSTRACT

This study examined the dietary sustainability of *Albizia lebbeck* leaf meal as alternative source of protein to soya bean meal in *Clarias gariepinus* diet. The leaf was treated by rinsing with water after plucking from the stalk and then soaked for twenty-four hours to reduce its bitterness and toxicity. It was then shade dried and ground into powder. Proximate analysis revealed the differences in the values of Crude protein of raw (28.06%), soaked and shade- dried (26.06%) as well as soaked and sundried (26.19%) *A. lebbeck* leaf. Analysis of anti- nutrient composition from the raw, soaked and shade- dried as well as soaked and sun-dried *A. lebbeck* leaf sample revealed the presence of alkaloid, flavonoid, tannin and saponin. Varying inclusion levels of *A. lebbeck* in the Diet formulated was 0%, 10%, 20% and 30% isonitrogenous feed (40% crude protein) and then a commercial Diet. Ten *C. gariepinus* fingerlings were allotted to fifteen (twenty litres) rectangular plastic tanks and cultured for ten weeks with the initial average weight of 4.11g at seven weeks where each Diet had three replicates. Growth performance and nutrient utilization parameters revealed that fish fed 10% *A. lebbeck* leaf meal gave the best weight gain (60.66g), the least was obtained in those fed Control (0%) Diet (29.17g). Similar trend occurred in specific growth rate where fish fed 10% *A. lebbeck* leaf had the highest (3.93g) and the lowest occurred in fish fed Control (0% *A. lebbeck* inclusion level) as 3.03g.Fish fed Diet containing 20% *A. lebbeck* inclusion level and those fed the Commercial Diet had close range values in their weight. Feed intake showed was significant difference $p<0.05$ where highest value was obtained in fish fed diet with 10% *A. lebbeck* inclusion level (58.18g) while the lowest was obtained in fish fed Control (0% *A. lebbeck* inclusion level) as 44.23g. There was significant difference $p<0.05$ in protein efficiency ratio where fish fed diet with 10% *A. lebbeck* inclusion level had the highest value (2.57g) while the fish fed control (0% *A. lebbeck* inclusion level) had the lowest (1.67g).Net protein utilization showed no significant difference $p<0.05$ though, fish fed diet containing 10% *A. lebbeck* had higher value (61.32g) among other diets while the those fed control Diet (0% *A. lebbeck* inclusion level) recorded lowest value (56.67g). Feed conversion ratio was significantly different among the diets where fish fed control diet (0% *A. lebbeck* inclusion level) had the highest (1.57%) while the lowest occurred in in fish fed diet containing 10% *A. lebbeck* (0.96%). The Carcass crude protein composition obtained by the fish was

significantly different $p<0.05$ between the initial (46.344%) and final value ranging from (51.07% to 54.95%) and also among the Diets, the highest value 54.95% was obtained in fish fed diet containing 10% *A. lebbeck* inclusion level while those fed the control Diet (0% *A. lebbeck* inclusion level) had the lowest value (51.07%). This result recommends that protein in the diet of *C. gariepinus* can be replaced up to 10% *A. lebbeck* inclusion level without compromise in the fish growth performance.

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ABBREVIATIONS

A. s. l. - Above sea level

ADG - Average daily growth

ANOVA - Analysis of variance

ANPU - Apparent Net Protein Utilization

AOAC - Association of Official Analytical Chemist

APHA - American Public Health Association

CF -Crude fibre

CP - Crude protein

CRD - Completely Randomized Design

D - Days of weighing

DM - Dry matter

DO - Dissolved oxygen

EE - Ether extract

FBW - Final body weight

FCR - Feed Conversion Ratio

FI - Feed Intake

IBW - Initial body weight

K - Condition factor

ln - Natural logarithm

MBWG - Mean body weight gain

MFA – Ministry of Food and Agriculture

MFBW- Mean final body weight

MFSL - mean final standard length

MIBW - Mean initial body weight

MISL- Mean initial standard length

NFE- Nitrogen Free Extract

NPU -Net Protein Utilization

PER - Protein Efficiency Ratio

pH - Hydrogen ion concentration

PI - Protein Intake

PWG - Percentage weight gain

PWG - Weight Gain

SD - Standard deviation

SGR - Specific Growth Rate Percentage

SPSS - Statistical Package for Social Sciences

SR- Survival rate

WTG - Mean

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Fish is very important to humans due to its very high quality protein as well as the essential amino acids required by the body for growth and maintenance of muscle tissue. Around the world fish protein makes up complete protein sources in many people's Diets. Proteins of high quality, as found in most fresh fish, can be used to maintain an active metabolism (Ayoola, 2011). Proteins of low quality do not contain all essential amino acids required for use in protein synthesis and as such the protein must either be used as energy or converted to fat. One of our most valuable protein sources is fish; about 25% of animal protein is obtained from fish as well as shell fish (Mahmoud *et al*., 2011). Fishes are rich in Omega-3 fatty acids, also fish plays a very important role for normal growth particularly for the blood vessels and the nerves as well as keeping our skin and other tissues youthful (Ayoola, 2011). In order to make a balanced protein diet (similar to fish Diet) affordable, locally available ingredients such as agricultural by-products and plant proteins should be included in the Diet or substituted for expensive animals and plant protein sources (Adeparusi and Agbede, 2005). The projected human population in Nigeria from year 2000 to year 2014 is 114.4 to 169.1 million while fish demand is 1,430.00 tonnes to 2,175.00 tonnes and supply is 467.098 to 730.248 tonnes (Ozigbo, 2014). Production of fish from aquaculture is therefore seen as the only remedy to this problem since capture from the wild is dwindling by the day as a result of depletion in stock while the population is on the increase (Akinrotimi *et al*., 2011).

The nutritional requirements for growth, reproduction and normal physiological functions of fish are similar to those of other animals, but generally more protein is

required in the Diet of fish. Due to this, the efficiency in the use and utilization of proteins is more significant for fish than for other animals, so feed mixtures with 25 to 45% of proteins are mainly used (Davies and Gouveia, 2010). Hence, the challenge is to bridge the wide gap between fish demand and supply. In order to meet the growing demand of fish in Nigeria aquaculture industry is growing. Catfish is a primary species produced at domestic fish farms and it may take quite some time before production can match consumer's demand (Ayoola, 2011). *Clarias gariepinus* is one of the important culturable fish in Nigerian fresh water zone (Ugwumba and Ugwumba, 2003).

Artificial or prepared Diets may be either complete or supplemental. Complete Diets supply all the ingredients (protein, carbohydrates, fats, vitamins, and minerals) necessary for the ideal growth and health of the fish (Steven and Helfrich, 2009). Most fish farmers use complete Diets, those containing all the required protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (< 8.5%), phosphorus (< 1.5%), water (< 10%), and trace amounts of vitamins as well as minerals (Steven and Helfrich, 2009). When fish are reared in high density indoor systems or confined in cages and cannot forage freely on natural feeds, they must be provided a complete Diet (Steven and Helfrich, 2009).

The need for plant protein in fish feed is essential because it is more available and affordable. The search for protein-rich plant source is an ongoing process, leaf meal inclusion in aquaculture feed also is fast gaining global attention over the years due to its availability, protein and mineral/vitamin contents and economic feasibility (Ali *et al*., 2003).Several studies have been conducted on the use of terrestrial and aquatic leaf meals as Dietary protein sources in fish feed. These include: Cassava leaf meal

(Bureau and De la Noue,1995; Madalla, 2008),*Carica papaya* leaf meal (Reyes and Fermin,2003), Alfalfa leaf meal (Ali *et al*., 2003), Leuceana leaf meal (Bairagi *et al*., 2004), Mallow (Abdelhamid *etal*., 2004), Gliricidia leaf (Adeparusi and Agbede, 2005), water hyacinth (Abdelhamid *etal*., 2006), Moringa leaf meal (Madalla, 2008) and potato leaf meal (Adewolu, 2008). Even the plant proteins are becoming more expensive because of the demand between man and his animals for these protein sources. Therefore, there is the need to continually search for new protein sources, especially among the lesser known plants, among which is *Albizia lebbeck* (Muhammad *et al.*,2010).

Albizia lebbeck is a multipurpose tree. As a fodder tree, its foliage, twigs, flowers and immature pods are relished by different classes of livestock (camels, cattle, and small ruminants like rabbits). *Albizia lebbeck*is also a source of firewood and timber wood. *Albizia lebbeck* is suitable for agroforestry regimes in which animal production benefits are combined with wood production (Lowry *et al*., 1998), and it is used for shelter belts and as shading tree in coffee or tea plantations (Orwa *et al*., 2009).It was shown by Lowry et al. (1992a) that rabbits had good growth performance when fed *A. lebbeck* leaves up to 50 % of the Diet.

The aquaculture industry has been globally recognized as the fastest growing food producing industry (NACA/FAO, 2000). Total world production of fish and fish products rose from 140 million tonnes in 2007 to 145 million tonnes in 2009, according to the FAO (2013) report. Much of the fish comes from aquaculture and the growing rate is almost seven percent a year (FAO, 2013). Therefore for aquaculture production to be expanded, more fish feed will in turn be required. As well, this will in turn require higher qualities of alternative protein sources to supplement, replace

partially or wholly the conventional protein feedstuffs particularly fishmeal and oil seeds whose prices have continued to increase in recent years, hence, constituting a major factor militating against adequate animal protein supply to Nigerians to meet 56 g per head per day (FAO, 1985).

1.2 Statement of the Research Problems

Animal feed, today, is becoming costly due to the competition between man and animal for protein source (Adeparusi and Agbede, 2005). The source of fish feed is very expensive especially imported ones because of high rate of foreign exchange. The use of commercial pelleted fish feed expensive as it accounts for about 60% - 80% of the recurrent cost of fish farming venture (Madu and Ufodike 2003). The high cost of local feeds is accounted for by the high cost of fishmeal, the main protein source, and other conventional protein sources such as soya bean and groundnut. There is limited information on the use of *A. lebbeck* leaf meal as protein source for fish.

1.3 Justification

Albizialebbeck is a leguminous plant therefore, it may be a good source of acceptable plant protein for catfish. The constant growth of aquaculture cannot be sustained by dependence on fishmeal as major protein source, there is therefore need to reduce aquaculture dependence on fishmeal and also exorbitant plant protein (Enyidi, 2012). The conventional proteins (soybeans and groundnut) are being competed for by man and animals (Adesulu andMustapha, 2000). Therefore, the high level of protein (16-23%) in *A.lebbeck* indicates that it can be used as a cheap source of protein in fish feed formulation. Feed stuffs of plant origin have great potential for a wide application in fish feed industry: they are a cheaper source of high quality protein,

there is a global bioavailability and they are relatively easily renewed (Francis *et al*.,2001). It is important therefore to search for alternative ingredients of high nutritional value that are cheap, available and non - competitive for human, livestock or industrial uses, to be used for fish feed.

1.4 Aim

To assess the effect of varying inclusion levels of woman's tongue (A. *lebbeck*) leaf meal on the **growth and nutrient utilization of A**frican catfish(***C. gariepinus*)** fingerlings.

1.5 Objectives

1. To determine the proximate composition of *A. lebbeck* leaves (raw and processed).
2. To determine the antinutrient composition of *A. lebbeck* leaves(raw and processed).
3. To determine the **growth and nutrient utilization of *C. gariepinus* fingerlings fed varying inclusion levels of woman's tongue (*A. lebbeck*) leaf meal.**

1.6 Research Hypotheses

1. There is no significant difference between the proximate composition of raw and processed *A. lebbeck* leaf.
2. There is no significant difference between the antinutrient composition of raw and processed *A. lebbeck* leaf.
3. There is no significant difference between the **growth and nutrient utilization of *C. gariepinus* fingerlings fed varying inclusion levels of *A. lebbeck* leaf meal.**

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of *Clarias gariepinus*

Clarias gariepinus is a typical air-breathing catfish with a scaleless, bony elongated body with long dorsal and anal fins, and a helmet like head. Colour varies dorsally from dark to light brown and is often mottled with shades of olive and grey while the underside is a pale cream to white (Skelton, 2001).

It is a benthopelagic, dioecious, omnivorous fish and widely tolerant to extreme environmental conditions (Yalcin *et al.*, 2001). *C. gariepinus* family *Claridae* is generally considered to be one of the most important tropical catfish species for aquaculture in West Africa (Solomon and Taruwa 2011). One problem facing fish culturists is the need to obtain a balance between rapid fish growth and optimum use of the supplied feed (FAO, 2009). By the end of the sixties *C. gariepinus* was recognized as one of the most suitable species for aquaculture in Africa. The suitability of this species for pond culture is based on the following biological criteria (Hogendoorn, 1979): it matures and is relatively easy to reproduce in captivity, it can grow fast and efficiently, it supports high population densities. *Clarias gariepinus* fingerlings are efficient opportunists and survivors, equipped to exploit whatever resources are available, they have a wide tolerance to environmental extremes and grows fast (Esenowo and Ugwumba, 2010).

2.2 Habitat of *Clarias gariepinus*

Clarias gariepinus live in a variety of freshwater environments, including quiet waters like lakes, ponds, and pools, they are also very prominent in flowing rivers. 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 degrees

Celsius. Their optimal temperature for growth is 28-30 degrees Celsius The presence of an accessory breathing organ enables this species to breath air when very active or under very dry conditions (Teugels, 1986).

2.3 Food and Feeding Habits of *Clarias gariepinus*

The stomach examination of *Clarias* species typically comprise insects (adults and larvae), worms, gastropods, crustaceans, small fish, aquatic plants and debris, but terrestrial seeds and berries, and even birds and small mammals, have equally been observed. Larvae are almost exclusively dependent on zooplankton for the first week of exogenous feeding. *Clarias gariepinus* are bottom feeders which occasionally feed at the surface (Teugels, 1986).

In *C. gariepinus*, four modes of feeding behaviour have been observed as described by Bruton, (1977) which are:

- feeding by grasping from surface in group or individually (e.g. *C. gariepinus* in poultry-fish integrated ponds),
- group feeding by forming a circle (e.g. zooplankton grazing),
- individual foraging of food,
- individual shovelling of feed, slow methodical searching for food is their normal tactic.

When a suitable food item is found, *C. gariepinus* grasps it often by suction. A strong negative pressure (suction) is created by increasing the volume of the buccopharyngeal chamber.Many authors concluded that *C. gariepinus* is an omnivorous slow moving



Plate I: Dorso - lateral view of *Clarias gariepinus*

predatory fish which feeds on a wide variety of food items from zooplankton to fishes of half of its own length (Janssen, 1987). They show no parental care. They use their four pairs of barbels (Plate I), to feel their way around in the dark and find food detected by the array of sensitive taste buds covering the barbels and head. Research in South Africa showed that removal of the barbels reduced feeding efficiency in *C. gariepinus* by 23 %**.**

2.4 Brief Description of *Albizia lebbeck* Benth

Albizia lebbeck belongs to the family Fabaceae. It is a medium to large tree of multi-stemmed widely spreading habit (about 30 m in diameter) when grown in the open but capable of good log form in plantation (Everist, 1986). There are about 25 species of *Albizia*. They commonly have graceful, finely cut foliage and numerous feathery clusters of white, pink, red, or yellow 18 flowers in summer. Various species are grown in mild climates as ornamentals.

Albizia lebbeck is an introduced species in Nigeria (Keay *et al*., 1964). It is indigenous to the Indian sub-continent and those areas of Southeast Asia with a marked dry season, and to the monsoon areas of Southern Australia (Parrotta, 1988). Although, geographically widespread, it appears to have potential for increasing pastoral production in extensive systems in the wet-dry tropics where the major problem is low feed quality of the basal Diet. It has various names in various regions, for instance it is called ‘siris’, ‘koko’, ‘vagai’ (India), ‘tekik’ (Japanese), ‘kikote’, ‘tarisi’ (Sudanese), ‘khago’, ‘kase’ (Thai), walnut (East Indian) (Gupta, 1981). In Nigeria, it is called ‘sugar tara’ (Hausa) (Lackson, 2012), ‘igbagbo’ or ‘ayinre’ (Yoruba), ‘eshegeshege’ (Edo) (Umberto, 1947).

2.5 Distribution and Habitatof *Albizia lebbeck*

Albizia lebbeck is native to tropical Africa, Asia and northern Australia. It is widely naturalized within sub-humid and semi-arid tropics and subtropics where there is a marked dry season and a reliable rainy season. It is found from sea level up to 1800 m altitude (Lowry *et al*., 1992b). Optimal growth conditions are average day temperatures ranging from 19°C to 35°C, annual rainfall between 500 mm and 2500 mm and fertile, well-drained loamy soils. It may however withstand lower and more irregular rainfall conditions. It can also grow on a wide diversity of soils such as acid, alkaline or saline soils, eroded soils, and laterites except heavy clays (Lowry *et al*., 1992b, Orwa *et al.*, 2009). It is tolerant of heavy grazing and fire (Lowry *et al*., 1992b). Seedlings are sensitive to frost and heavy browsing but older plants can survive (NAS, 1980).

Albizia lebbeck (L) is found in dense deciduous forests in tropical and sub-tropical countries of Asia, such as Laos, Cambodia, Malaysia, Indonesia and Vietnam, Africa (Dy Phon, 2000) and Australia. It has been widely cultivated and is now pantropical. The species grows poorly on heavy clays, but grows well on fertile, well-drained, loamy soils, in areas that receive from 600-2500 mm of rain per year. However, this species is also capable of tolerating as little as 300 mm of rainfall in years. It is normally encountered below 1800 m above sea level (a.s.l.), and prefers mean annual temperatures from 20-35^{o}C. This tree is nitrogen-fixing, and tolerates acidity, alkalinity, heavy and eroded soils, waterlogged soils, and drought. Older trees can survive grass fires and intense night frost, and although these events will kill off aboveground growth of young trees, new growth normally follows (Dorthe, 2000). In Cambodia, it is found in Stung Treng, Ratanakiri, Koh Kong, Battambang, Preah Vihear and Kampong Thom (Khorn, 2002).



Plate II: Leaves and pods of *Albizia lebbeck* plant

2.6 Production of Fish Feed

The nutrients needed by fish include carbohydrates, fats, protein, vitamins and minerals. Other feed components are fibre and moisture as well and fish Diet must contain all the nutrients in the right proportions to bring about growth. Feeding increases fish growth and brings about fish farming profitability. The Ministry of Food and Agriculture (MFA) documented that nutritious and economical feeds are essential in any farming condition and must be appropriately managed to be effective (MFA, 2013). Another method that improves the sustainability of aquaculture is using vegetable products for fish feed (Snyder, 2012).

The rapid world-wide expansion of aquaculture and livestock production strongly indicates that a crisis will be precipitated in the livestock and aquaculture feed industries in the near future. This is because fish are fastidious eaters in that they require higher levels of Dietary protein. *Clarias gariepinus* exhibit very poor utilization of carbohydrate as an energy source, and certain evidence imply that the inclusion of certain types of carbohydrates in their Diet is in fact, detrimental. Hence, it has been recently reported that soya bean meal extracted with alcohol performed better in rations than unextracted meal. This improvement has been attributed to the removal of low molecular weight carbohydrates by the alcohol. The African catfish have digestive capacity to utilize most of the studied potential feed resources reasonably well (Phonekhampheng *et al*., 2008).

2.7 The Use of Plant Materials in Fish Feed Production

Plant materials (seed, leaves) are being used in production of fish feed as alternative to conventional plant based protein but is an underutilized protein source (Archimède *et al*., 2011). Olaniyi *et al*. (2013) fed 12.5% inclusion level of *Moringa oleifera* to *C.*

gariepinus which resulted in highest final weight gain of 32.10g. This study revealed that *C. gariepinus* can tolerate up to 12.5% level of replacement without any adverse effect, this showed that *M. oleifera* leaf meal enabled the fish to increase in weight. Also, the value of carcass composition obtained from fish fed experimental feed was higher compared to the one fed control Diet. This further proved that the locally produced Diet can bring about good growth performance in the fish.

Fish fed *Gliricidiasepium* leaf protein in evaluation of *Leucaena leucocephala* and *Gliricidia sepium* leaf protein concentrate as supplements to Bambara groundnut *Vignas subterranean* (L. Verdc) in the Diet of *Oreochromis niloticus*offered a better growth performance, nutrient utilization and digestibility than either groundnut or a mixture (1:4) of groundnut with Bambara groundnut. *Gliricidia sepium* Leaf Protein (LP) gave a higher growth performance in *O. niloticus* than *L. leucocephala* LP while fish fed *L. leucocephala* LP had a better protein gain. Conclusively, these leaf proteins would consequently be a good supplement in Bambara groundnut Diets for *O. niloticus* (Adeparusi and Agbede, 2005). Konyeme *et al*. (2006) elucidated that the highest total weight gain recorded was 52.0g in fish fed 10% and 20% water hyacinth supplemented Diets. The weekly changes in weight suggests that 30% water hyacinth meal inclusion levels gave the final highest weight increase based on the final week result sampled although, there was no significant difference $p>0.05$ between the growths of fingerlings fed Diets containing 0% - 40% water hyacinth meal which inferred that inclusion of water hyacinth up to 40% significantly improved the growth of *C. gariepinus* (Konyeme *et al*., 2006).

Growth performance of the African catfish, *C. gariepinus*, fed varying inclusion levels of *L. leucocephala* leaf meal has established that, wide-ranging, L. *leucocephala* leaf meal could be incorporated up to 30% level in *C. gariepinus* Diets

without any deleterious effects on the growth (Amisah*et al*., 2009). Conversely, the best inclusion level was found in the Diet containing the 20% *L. leucocephala* leaf meal; therefore, it is more cost-effective to partly replace fishmeal with *L. leucocephala* leaf meal at the 20% inclusion level without reduction in growth. Amisah*et al*. (2009) also recommended the use of catfish fingerlings of about 25g in other feeding trial to elucidate response on early growth and the length class sizes that would best respond to growth of the *L. leucocephala* leaf meal. Amisah*et al*. (2009) observed that all the experimental Diets were accepted by *C. gariepinus* juveniles. This indicated that the levels of incorporation of *L. leucocephala* leaf meal did not affect the palatability of the Diets. This might be attributed to the processing technique which involved soaking and drying techniques that might have reduced the antinutrient in *L. leucocephala* leaf meal, thereby increasing its palatability in *C. gariepinus.* The leaves of *L. leucocephala* thus have the potential to make considerable contributions to the growth of the African catfish. It also has the potential to partially replace fish meal in a feeding regime and, thereby, reduce feed cost to the fish farmer whose most important production cost comes from feed.

Amanullah et al. (2006), Ndemanisho *et al*. (2006) and Raghuvansi et al. (2007)concluded in their various researches that *A. lebbeck* leaf meal had similar potential to the other leaf meal based supplements studied and can be included in roughage-based Diets to improve the protein nutrition status of animals.

According to Akinfala et al. (2003) fresh foliage and whole plant meal of cassava could be included in the Diet of weaned rabbits up to 45 % so as to replace maize grain, without any adverse effects on performances or apparent nutrient digestibility.

The best growth response was obtained in isonitrogenous cassava leaf Diet fed to *Clarias gariepinus* (20% of the total Diet) (Bichiand Ahmad, 2010).

Opadeyi (2011) explained that among the *Moringaoleifera* kernel meal-based Diets, 25% fishmeal and 75% boiled *M. oleifera* produced a significantly ($P<0.05$) higher weight gain which could be attributed to higher feed intake by the fish. The lower anti-nutrients in the boiled *M. oleifera* kernel than the toasted sample could have resulted in better feed utilization and growth in the fish. Opadeyi (2011) on the effects of replacing fishmeal with heat processed *M. oleifera* (Lam.) kernel meal on growth and feed utilization in *C. gariepinus* (Teugels, 1986) recommended that the use of *M. oleifera* kernels fish Diets formulation for *Clarias* could replace fishmeal up to 75% with 30 minutes boiled kernels meal so asto cut down productions cost by fish farmers. The better growth performances and feed utilization obtained in fish fed boiled *M. oleifera* based-Diets generally support the fact that wet heat processing of plant-based protein sources is more effective in reducing or eliminating various anti-nutrients contained in them (Amaefule and Nwagbara, 2004; Bamgbose *et al.*,2010).

Fapohunda (2012) reported on evaluation of processed soya bean meal in the feeding of *C. gariepinus* fingerlings and established that roasted soybean meal has comparable quality with fishmeal at an inclusion level between 35% and 40% which reflected in the overall growth performance. Therefore for optimum performance of the fish, 40% level of soya bean meal with 60% level of inclusion of fishmeal was most appropriate. Fagbenro *et al*. (2010)stated in the nutritional evaluation of sunflower and sesame seed meal in *C. gariepinus* that replacement of soya bean meal in *C. gariepinus* Diet with raw sunflower and sesame seed meal showed optimum growth response at a 30% replacement level. There was reduction in growth and feeding conversion ratio as the

raw plant protein increased beyond 30%. The growth reduction observed at higher inclusion levels of sunflower meal and sesame seed meal was related not only to Dietary amino acid profile but also the presence of antinutritional factors, hence lower level of inclusion of both sunflower and sesame meals did not affect the growth and nutrient utilization of fish (Fagbenro *et al*., 2010).

Keremah *et al*.(2013) reported that the test fish groups fed the different commercial (Aluu, Roone and Coppens feed purchased from the African Regional Aquaculture Centre, in Abuloma, Port Harcourt, Nigeria) and locally compounded Diets gained body weights and lengths progressively to the end of the experiment, thus showing that the fish responded positively to the Diets. High growth performance was obtained in fish fed Coppens compared to fish fed with other Diets (Aluu, Roone and locally compounded Diet). This was attributed to the adequate consumption and utilization of the feed by the test fish and recommended 40% and 35% crude protein (CP) for good growth performance in *Heterobranchus longifilis* fingerlings and juveniles in growth and feed utilization of catfish hybrid (*Heterobranchus longifilis* x *Clarias gariepinus*) fingerlings fed experimental Diets (Aluu, Roone, Coppens and locally compounded Diets).

Fagbenro *et al.* (2003) reported that *C. gariepinus, Heterobranchus bidorsalis, H. longifilis and Cyprinus carpio* obtained high growth performance when fed Diets containing 40 – 50% CP which suggested that catfish fingerlings should be fed with Diets containing 40% CP therefore, good growth and survival of hybrid catfish fingerlings may require feeds containing a qualitative Dietary protein of 45.37%.

Oso *et al*. (2013) observed in growth response and feed utilization of *C. gariepinus* fingerlings fed with Bambara groundnut as protein source that fish fed 25%, 50%,

75% and 100% Bambara groundnut meal actively grew efficiently without external sign of nutritional deficiency. Since growth performance of fish fed Bambara groundnut meal Diets at various levels up to 75% replacement were similar to that obtained by fish fed the fish meal (control Diet), Bambara groundnut meal therefore contained all the necessary growth factors required by *C. gariepinus*. The fish showed good appetite to all the treatment Diets (25%, 50%, 75% and 100% Bambara groundnut meal) as attested to by the increase in body weight and standard length. The result obtained by Oso *et al*. (2013) therefore showed that the highest replacement of fish meal Diet with plant protein source up to 75% could replace the conventional fish meal in the absence of growth promoter without having significant effect on the overall growth performance and feed utilization of fish. *C.gariepinus* fingerlings were able to efficiently utilize Bambara nut Diet like conventional fish meal, as the fish showed high performance which may be as a result of better acceptance and digestibility of the Diet during the experimental period. Though, fish fed Diet containing 100% Bambara groundnut meal showed reduction in growth performance; this proved that Bambara groundnut meal cannot be used as a sole protein source for *C. gariepinus.* Also, according to Agbo *et al*. (2011) the growth of fish fed 75% Cotton seed meal was lower than those of control and the other Cotton seed meal based Diets. He reported that up to 50% Cotton seed meal protein could be used to replace fish meal protein in the Diet of Tilapia without affecting overall growth and feed utilization of fish. However, any replacement beyond that level will cause depression in growth. Mbahinzireki *et al*. (2001) reported that there was depressed growth and even mortality of Tilapia when they were fed up to 100% Cotton seed meal of the Diet and thus recommended an inclusion level of about 50%.

Alegbeleye*et al*. (2012) explained that all feeds were accepted at the start of the feeding trial but feed intake was significantly higher in fish fed Diets with higher levels of fermented meal. The final weight of the fingerlings fed the 100% replacement Diet was significantly higher than those fed the control, 25%, and 50% Diets. In growth performance and nutrient utilization of African mud catfish (*C. gariepinus*) fingerlings fed different levels of fermented pigeon pea (*Cajanus cajan*) meal, all food efficiency factors were best in fish fed the 100% Diet but survival was lower than in fish fed the Diets containing a lower amount of fermented meal. Also, there was increase in carcass protein (33.3%) and lipid in all treatments compared to the initial contents and the highest and lipid (51.6%) gains were recorded in the group fed the 100% Diet. The group fed the 100% Diet had the highest weight gain (7.33 g), significantly superior to the control, while the group that received the 25% Diet had the poorest weight gain (5.29 g). He noted that the process of fermentation can be easily adopted by rural small-scale fish farmers who could extend the method to other non-conventional resources of plant origin.

Anyanwu *et al*. (2012) established in growth and nutrient utilization of *C. gariepinus* fed Dietary levels of jack bean (*Canavalia ensiformis*) meal that the Dietary levels up to 30% nutritionally measured well in improving the performance of the fish, also protein efficiency ratio of the 30% Jack Bean Meal (JBM) Dietary treatment was higher than other treatments (Abd Elhamid *et al*., 2009; Schuchardt *et al*., 2008) and therefore recommended that Jack bean meal up to 30% inclusion level under good processing, using the cracked, soaked and cooked (CAC) techniques, can be used in the Diet of *C. gariepinus*.

Anwa (2006) in the effect of processed *A. lebbeck s*eed meal on the growth and feed utilization of *C. gariepinus* (Teugels) juveniles explainedthat*A. lebbeck* seed protein was enhanced in feeding value for *Clarias* juveniles by sufficient heat treatment which showed increased growth rate from Diet one (*A. lebbeck* raw seed) to Diet four (*A. lebbeck* seed boiled for one hour). Changes could have occurred in the protein molecule itself due to heat application which increases the availability of the essential amino acid as observed in fish fed Diet four (*A. lebbeck* boiled 1 hour) which had the highest body weights compared to other Diets (Anwa, 2006, Eyo, 1999). Balogun (2011) explained that the growth performance and feed utilization of *Clarias gariepinus* fed different Dietary levels of soaked *Bauhinia monandra* (Linn.) seed meal and sun-dried locust meal (*Schistocerca gregaria*)with 25% locust meal Diets, 25% *B. monandra*seed meal Diet and 75% fish meal Diet appeared as the best compared to others. Similarly in comparison to the *B. monandra* Diet meal, 25% locust meal Diet was the best Diet that supported the growth of *C. gariepinus* and also had the highest carcass crude protein.

2.8 Antinutrient Components in *Albizia lebbeck*

Abdu *et al* .(2012)reported that the presence of anti-nutritional substances, in particular condensed tannins, in fodder of some trees and shrubs can limit animal performance, especially when tree/shrub foliages are fed in large quantities. Lowry *et al*. (1998) reported that leaves and flowers of *A. lebbeck* contains no adverse constituents, pods contain saponins that may limit intake but appear to have no other adverse effect. This is contrary to the results of phytochemical screening reported by Chulet *et al*. (2010); ethyl acetate maceration extract of *A. lebbeck* leaves shows presence of tannin, flavanoids, proteins and amino acids, ethanolic maceration extract

shows presence of alkaloids, tannin and flavanoids, while water maceration extract shows presence of alkaloids, tannin, saponins and flavonoids.

2.9 The Use of Animal Source in Fish Feed Production

Ogunji *et al*. (2008) in growth performance, nutrient utilization of Nile Tilapia (*Oreochromis niloticus*)fed housefly maggot meal (Magmeal) Diets**,** stated that the study showed an excellent overall growth performances and status of tilapia which recommend the suitability of magmeal in Diets of Nile Tilapia (*O. niloticus)* fingerlings.

It was stated by Orire and Fawole, (2012) that there was a better growth performance of *C. gariepinus* fed milk fat at the highest inclusion level of 20% compared with other Diets including the control in growth performance of *C. gariepinus* fed Dietary milk fat and so concluded that milk fat can be utilized and converted for muscle development and growth of *C. gariepinus*. Milk fat percentage inclusion up to 20% was considered optimum for good feed conversion and growth in terms of weight. Also, Pie *et al*. (2004) established that Dietary lipid played essential role in the growth and development of fish. The fish on the experimental Diets showed satisfactory Diet acceptance even at high (20%) milk fat inclusion level which indicated that there was neither palatability problem nor feed intake depression. The inclusion at 50% snail offal meal in fish Diets offers high potential for profitable fish production (Sule and Sotolu, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Study Site

The experiment was carried out at the Fisheries Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

3.2 Experimental Design / Set Up

Completely Randomized Design (CRD) was employed in this research. Fifteen rectangular plastic aquaria with a dimension 50×31×32cm was used. Each plastic aquarium had capacity for twenty litres of water. Ten *Clarias gariepinus* fingerlings were allotted into each of the fifteen aquaria and fed for a period of ten weeks. Each of the five Diets had three replicates.

3.3 Collection and Identification of Plant Material

The leaves and pods of *Albizialebbeck* were collected from Ahmadu Bello University, Samaru campus, Zaria- Kaduna State, Nigeria. Leaves and pods of the plant was taken to the Herbarium of Biological Sciences Department for proper identification by comparing the *A.lebbeck* leaves and pods with that in the plant press (Plate II).

3.4 Preparation of Leaves

The leaves were plucked out from the plant stalk, rinsed with water then divided into two parts (the raw and the soaked leaf sample). The raw was rinsed and then shade dried for three days. The other part was soaked for 24 hours, the water was then drained and the leaves were rinsed with water and then divided into two parts, which was shade-dried and sundried for three days respectively using the method of Balgees *et al*. (2009), Yakubu *et al*. (2012) and Adejoro *et al*. (2013). The dry leaves was

ground to fine powder with an electric grinder, packaged in air tight glass jar and stored at room temperature until needed for analysis.

3.5 Chemical Analyses

The proximate composition of various dried leaf samples (raw, shade -dried and sun-dried leaf sample), Diet ingredients and whole body fish was determined using the standard methods of the Association of Official Analytical Chemist (AOAC, 2006).

3.5.1 Determination of moisture content and Dry Matter (AOAC, 2006)

Principle: This is based on the difference between the net weight and the weight after drying. This also determines the weight of dry matter.

Procedure: A clean crucible was dried to a constant weight in an air oven at 110^{o}C, cooled in a dessicator and weighed (W_1). 2g of finely pulverised sample was weighed in the crucible and then re-weighed (W_2). The crucible and its content were dried in an oven to a constant weight (W_3). The percentage moisture was calculated thus:

$$\% \text{ Moisture content} = \{(W_2-W_3)/ (W_2-W_1)\} \times 100 \text{ x}$$

3.5.2 Determination of ash content (AOAC, 2006)

Principle: The ash content was determined from loss in weight that occurred during igniting the sample at 550^{o}C in muffle furnace which is enough to allow all organic matter to burn off permitting any appreciable decomposition of the ash constituent.

Procedure: A porcelain crucible was dried in an oven at 100^{o}C for 10 minutes, cooled in a dessicator and weighed (W_1). 2g of finely pulverised sample was weighed (W_2) into the previously weighed clean crucible which was ignited in the muffle furnace at 550^{o}C for 1 hour and cooled in a dessicator. The crucible and its content were then transferred into the muffle furnace and the temperature shall be gradually increased until it reached 550^{o}C, the sample was left in the furnace for 8 hours to ensure proper

ashing. The crucible containing the ash was allowed to cool to 200^{o}C; the crucible was then removed and cooled in a desiccator until constant weight is obtained (W_3).

% Ash content = $\{(W_2-W_3) / (W_2-W_1)\}$ x 100

3.5.3 Determination of crude lipid content (AOAC, 2006)

Principle: This is the continuous extraction of fat content from the sample using suitable solvent e.g. petroleum ether in soxhlet, since non-polar component of the sample are easily extracted into the organic solvent (ether).

Procedure: Four grams (4g) of sample was weighed (W_1) into a clean, dried 500ml round bottom flask containing few anti-bumping granules was weighed (W_2) and 300ml of petroleum ether (40-60^{o}C) for the extraction was poured into the flask fitted with soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet extractor, and cold water circulation was put on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 hours. The solvent was recovered and the oil was dried in the oven at 70^{o}C for 1 hour. The round bottom flask and oil was cooled and then weighed (W_3).

% Crude Lipid Content = $\{(W_2-W_3)/ W_2-W_1\}$ x 100

3.5.4 Determination of crude fibre (AOAC, 2006)

Principle: This involves sequential digestion of the sample with dilute acid and alkaline solution. The residue obtained was ignited to obtain crude fibre.

Procedure: Two grams (2g) of finely pulverized was weighed into an extraction apparatus fat was extracted with liquid petroleum spirit (40-60%) the extracted was removed and dried at 105^{o}C for 30 minutes. Two grams (2g) of the defatted sample was weighed into a dry 600cm^3 round bottom flask. 100cm^3 of (0.023M) sulphuric acid was

added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it is acid free. This was quantitatively transferred into the flask and 100cm^3 of hot (0.312) sodium hydroxide solution was added and the mixture boiled under reflux for 30 minutes and quickly filtered under suction. The insoluble residue was washed with boiling water until it was base free. It was dried to constant weight in the oven set at 100^{o}C. Cooled in a dessicator and weighed (C_2). The weighed residue was then incinerated in a muffle furnace at 550^{o}C for 2 hours, cooled in a dessicator and reweighed (C_3).

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

Weight of original sample = W

% Crude Fibre = {(C_2-C_3)/W} x 100

3.5.5 Determination of crude protein by Kjeldahl method (AOAC, 2006)

Principle: The principle of this method is to digest the organic matter with sulphuric acid in the presence of a catalyst to render the reaction alkaline, and then distil and titrate the liberated ammonia.

Procedure: Two grams (2g) of the sample was weighed into 100cm^3 Kjeldahl digestion flask and about 1g of catalyst mixture (K_2SO_4 and $CuSO_4$) was added to speed up the reaction. 25ml of concentrated sulphuric acid was added into the flask. The content in the Kjeldahl digestion flask was heated slowly at first in Kjeldahl heating unit until frotting subsides and then more vigorously with occasional rotation of the flask to ensure even digestion and avoid over heating of the content. The heating continued until a clear solution was obtained. After cooling, the solution was transferred into 100 cm^3 volumetric flask and diluted to mark with distilled water. 10ml aliquot of the diluted solution or digest was pipetted into Markham semi macro nitrogen steel and

$10cm^3$ of 4% sodium hydroxide solution was added. The liberated ammonia was trapped in a $100cm^3$ conical flask containing $10cm^3$ of 40% boric acid and 2 drops of methyl red indicator. Distillation was allowed to continue until pink colour of the indicator turns green. The content of the conical flask was titrated with 0.1M HCl, with end point indicated by a change from green to pink colour. The volume of the acid used for the distillate as well as the blank was noted.

% Nitrogen = {(0.014 x M x (V_1-V_0))}/ weight of test sample} x 100

Where M= actual molarity of acid,

V_1 = volume of HCl required for 10ml sample solution,

V_0 = volume of HCl required for the blank

Atomic weight of nitrogen = 0.014

% Crude Protein = % Nitrogen (N_2) X 6.25

3.5.6 Determination of nitrogen free extract (NFE) (AOAC, 2006)

The total carbohydrate content was determined by difference method. The sum of the percentage moisture, % ash, % crude lipid, % crude protein and % crude fibre was subtracted from 100.

NFE= 100- (ash + crude lipid + crude protein + crude fibre)

3.6 Test for Anti-Nutrient Properties

Test for the quantitative presence of anti-nutrient properties (saponins, tanins, alkaloid and flavonoid) was carried out on the various dried leaf samples (raw, shade -dried and sun- dried leaf samples).

3.6.1 Determination of saponins (Hudson and El-difrawi, 1979)

Ten grams (10g) of pulverised sample was taken into $100cm^3$ of 20% aqueous ethanol in water agitated with magnetic stirrer for 12 hours at 55^oC. The solution was filtered

using Whitmann No. 1 filter paper and the residue was re-extracted with 300cm^3 of 20% aqueous ethanol. The extract was combined and reduced to about 40cm^3 under vacuum using a rotary evaporator. The extract and 20cm^3 Diethyl was transferred into 250cm^3 separatory funnel and shaken vigorously. The aqueous layer was discarded. The process of purification continued until a colourless aqueous extract is obtained. The pH of the remaining aqueous solution was adjusted to about 4.5 by adding 4g of sodium chloride and the solution then shaken successively with butanol. The butanolic extract was washed twice with 10cm^3 of 5% (W/V) sodium chloride and evaporated to dryness in a fume cupboard to give the saponin, which was weighed and expressed as a percentage.

3.6.2 Determination of tanins

One gram of each sample A and B was dissolved in 10 ml distilled water and agitated, left to stand for 30 min. at room temperature. Each sample was centrifuged and the extract recovered 2.5 ml of the supernatant was dispersed into 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50 ml flask. A 1.0 ml folin- dennis reagent was measured in each flask followed by 2.5 ml of saturated Na_2CO_3 solution. The mixture was diluted to 50 ml in the flask and incubated for 90 min at room temperature. The absorbance of each sample was measured at 250 nm with the reagent blank at zero. The % tanin was calculated (Jaffe, 2003).

3.6.3 Test for alkaloids (Harborne, 1973)

About 5.0g of the dry powdered sample was placed into a 100ml conical flask, containing 2ml of 5% H_2SO_4 in ethanol. The mixture was heated to boiling in a water bath, left to cool and then tested for the presence of alkaloids. Two (2) ml of the filtrate of the heated samples were then used to test for colour change using 2 drops of Mayer's

reagent for yellow precipitate and 2 drops of Wangner's reagent for reddish-brown precipitate.

3.6.4 Test for flavonoid (Vaya and Mahmood, 2006)

One gram of the dried sample was chopped into small pieces and then extracted with 20ml solvent (10% w/v) for 3 days at room temperature (25° C). Solvents used for extraction were distillated water and ethanol 70%.

3.7 Materials Used for Feed Formulation

Albizia lebbeck leaves, fishmeal, maize as main source of energy, soya beans, salt, premixes, and oil palm were the ingredients used. The control Diet do not contain *A. lebbeck* leaf meal. The percentage composition of the experimental diets formulated at varying inclusion levels of A. lebbeck was calculated (Table 3.1).

3.8 Preparation of the Feed

After grinding the ingredients, *A. lebbeck* leaves, fishmeal, maize and soya beans to fine powder, they were thoroughly mixed with a wooden rod and warm water (60ºC) was added to it to make it into dough using the method of Amisah *et al.* (2009). Dough was pelleted using a fabricated hammer mill to form pellets. The pelleted feeds were spread on plastic surfaces and sundried after which it was air-tightly packed, labelled and stored in a dry place.

3.9 Fish Source

One hundred and fifty *C. gariepinus* fingerlings with average body weight 4.11g, 7.44cm standard length and seven weeks old were obtained from a commercial fish hatchery in Kaduna. They were transported to the fisheries laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria in plastic containers and were allowed to acclimatize for seven days while they were fed 2mm floating pelleted commercial Diet before the commencement of the experiment.

Table 3.1: Percentage Composition of Various Inclusion Levels of *A. lebbeck* in the Experimental Diets

Ingredients	Diet one (control) 0%	Diet Two (10 %)	Diet Three 20%	Diet Four 30%	Ingredients for Diet Five	Diet five (Commercial)
Fish Meal	(360g)36%	(360g) 36%	(360g) 36%	(360g) 36%		
Albizia lebbeck leaf meal	0	(32.8g) 3.28	(65.6g) 6.56	(98.4g) 9.84		
Soya bean meal	(328g) 32.8	(295.2g)29.52	(262.4g)26.24	(229.6g)22.96		
Yellow maize	(277g) 27.7	(277g) 27.7	(277g) 27.7	(277g) 27.7	phosphorous	1.1%
Vitamin premix	(15g) 1.5	(15g) 1.5	(15g) 1.5	(15g) 1.5	Calcium	1.7%
Methionine	(5g) 0.5	(5g) 0.5	(5g) 0.5	(5g) 0.5	Vit. A	1200UI/Kg
Lysine	(5g) 0.5	(5g) 0.5	(5g) 0.5	(5g) 0.5	Vit. E	160mg/kg
Palm oil	(5g) 0.5	(5g) 0.5	(5g) 0.5	(5g) 0.5	Vit.C	120mg/Kg
Salt	(5g) 0.5	(5g) 0.5	(5g) 0.5	(5g) 0.5	Mn	50ppm
Total	1000g (100%)	1000g (100%)	1000g (100%)	1000g (100%)		
Crude protein	40.20%	40.15%	40.06%	40.10%	Crude protein	45.00%
Crude fibre	3.8	4.3	4.8	5.29	Crude fibre	2.00%
Ether extract	6.48	6.62	6.78	6.82	Lipid	12.00%
M.E.	2854	2858	2862	2859	Ash	7.00%

3.10 Weighing of Fish

Weighing of fish was done using an electrical weighing balance (Sartorius, CP8201) before the experiment commences as well as once in two weeks during the period of the experiment in order to adjust the feeding level by using 5% of the fish body weight for the subsequent weeks. Fingerlings were randomly selected from all the replicates in each group weekly and weighed. At the end of the experiment, the individual weights of all the surviving fingerlings from all the groups were measured to obtain their final mean weight after evacuation of feed by starving process.

3.11 Feeding of Experimental Fish

Fish were fed morning and evening at 5% of their body weight as they grew all through the period of the experiment and the inclusion level was: 0% (control) i.e. without *A. lebbeck* leaf, 10%, 20%, and 30% of the *A. lebbeck* leaf and a commercial Diet. The experiments were performed in replicates of three and feed was administered to tanks manually at the specified quantity to avoid pollution of water.

3.12 Determination of Physico- Chemical Parameters

Dissolved oxygen (DO), hydrogen ion concentration (pH) and temperature were measured weekly using American Public Health Association (APHA) methods (APHA, 1980). Hannan Instrument (Model H190129) was used to measure water temperature and pH while dissolved oxygen concentration was measured by Winkler (1888) titrimetric method.

3.13 Determination of Studies on Growth Parameters

Various parameters used in evaluation of growth performance were carried out using the formula documented by Adewolu and Adamson (2011) which are: Mean weight

gain (WTG), Specific Growth Rate (SGR), Percentage Weight Gain (PWG), Average daily growth (ADG).

a) Mean Weight Gain (MWTG)

MWTG = $Mean\ final\ Body\ weight - Mean\ Initial\ Body\ Weight$

b) Percentage Weight Gain (PWG)

PWG = $\frac{Mean\ \ Weight\ Gain}{Mean\ \ Initial\ \ Weight}\ x\ 100$

c) Specific Growth Rate (SGR)

SGR = $\frac{\ln FBW\ - \ln IBW}{D} \times 100$

Where:

ln = natural logarithm

D = number of days between weighing

FBW = Final body weight

IBW= Initial body weight

d) Average daily growth (ADG) (g/day)

ADG = $\frac{Mean\ \ Weight\ Gain}{Duration\ \ of\ feeding\ \ trials}$

3.14 Determination of Feed Utilization

Protein Efficiency Ratio (PER), Apparent Net Protein Utilization (ANPU),Feed Intake (FI), Protein Intake (PI), Feed Conversion Ratio (FCR), Survival rate (S) and were calculated as follows:

a) Feed Conversion Ratio (FCR)

FCR = $\frac{Weight\ of\ Dry\ Feed\ Fed\ (g)}{Weight\ Gain\ of\ Fish\ (g)}$

b) Net Protein Utilization (NPU)

(N.P.U) $\frac{fish\ protein\ \ gain}{protein\ \ consumed} \times 100$

c) Protein Efficiency Ratio (PER)

$$\text{PER} = \frac{Gain\ in\ Weight\ of\ Fish\ (g)}{Protein\ \ intake\ \ (PI)(g)}$$

d) Protein intake (PI) = $Feed\ Intake\ (FI) x\ \%Protein\ in\ Diet$

Where:

FI = $5\%\ Body\ Weight\ of\ Fish\ per\ Day$

e) Survival (%) = $\frac{SI}{S2}\ x\ 100$

Where:

SI = Number of fish at the end of experiment

S2 = Number of fish at the beginning of experiment (Steel and Torne, 1981).

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

Where:

W = Final Body Weight (g)

L = Final Standard Length (cm)

3.15 Statistical Analysis

Data on growth parameters were subjected to one - way analysis of variance (ANOVA) to test for significant difference in the means while means where significantly different were separated using Duncan's multiple range test. Analysis was performed using the SPSS (Statistical Package for Social Sciences) version 21. Significant level was set at $p \leq 0.05$ and values were expressed as Means±SD.

CHAPTER FOUR

4.0 RESULTS

4.1 Antinutrient Composition of *Albizia lebbeck* Leaf

The antinutritional factors of *Albizia lebbeck* leaf obtained for the raw, soaked and shade - dried as well as soaked and sun - dried showed highly significant differences ($P \leq 0.01$) in their means (Table 4.1). The raw *A. lebbeck* leaf had the highest values of alkaloid (6.31%), flavonoid (5.45%), saponin (16.15%) and tanin (0.93%) while the lowest antinutrient values were obtained in the soaked and shade-dried giving the flowing values: alkaloid (3.02%), flavonoid (2.58%), saponin (7.02%), and tanin (0.20%).

4.2 Percentage Proximate Composition of *Albizia lebbeck* Leaf

The proximate composition of *A. lebbeck* leaf obtained for the raw, soaked and shade -dried as well as soaked and sun - dried showed highly significant differences in their means ($P \leq 0.01$) in the dry matter, crude protein, crude fibre, ether extract, Ash content and Nitrogen Free Extract (NFE) (Table 4.2). The highest values for the dry matter (94.23%), Crude Fibre (CF) (24.23%) and Ether Extract (E.E) (11.46%) were obtained in the soaked and sundried leaves, the highest NFE (25.55%) was obtained in the soaked and shade dried leaves, while the highest Crude Protein (CP) (28.06%) and Ash content (14.16%) were obtained in the raw leaf sample. It was observed also that the lowest dry matter (92.02%), crude protein (26.06%), E.E (11.23%) and Ash content (13.32%) were obtained in soaked and shade dried leaves while the lowest CF (22.03%) was observed in the raw leaves.

Table: 4.1Antinutrient Composition of *Albizia lebbeck* leaf

Antinutritional Factor	Alkaloid%	Flavonoid%	Saponin%	Tanin%
Raw	6.31 ± 0.01^{a}	5.45 ± 0.01^{a}	16.15 ± 0.01^{a}	0.93 ± 0.01^{a}
Soaked and Shade Dried	3.02 ± 0.01^{c}	3.58 ± 0.01^{c}	7.02 ± 0.01^{c}	0.20 ± 0.00^{c}
Soaked and Sundried	3.67 ± 0.01^{b}	4.08 ± 0.01^{b}	12.09 ± 0.01^{b}	0.69 ± 0.01^{b}

Means with different superscripts along same column are significantly different ($p\leq 0.05$).

Table 4.2: Percentage Proximate Composition of *Albizia lebbeck* leaf

	D.M%	C.P.%	C.F.%	E.E%	ASH%	NFE%
Raw	93.42±0.07[b]	28.06±0.01[a]	22.03±0.03[c]	11.31±0.03[b]	14.16±0.01[a]	24.46±0.07[b]
Soaked and Shade-dried	92.02±0.01[c]	26.06±0.01[c]	23.86±0.01[b]	11.23±0.01[c]	13.32±0.01[c]	25.55±0.02[a]
Soaked and Sun-dried	94.23±0.01[a]	26.19±0.00[b]	24.23±0.01[a]	11.46±0.01[a]	13.82±0.01[b]	24.32±0.02[b]

Means with different superscripts along same column are significantly different ($p \leq 0.05$).

KEY:

D.M= Dry matter, C.P= Crude protein, C.F= Crude fibre, E.E= Ether extract, NFE= Nitrogen free extract

4.3 PercentageProximate Composition of Experimental Diets

The percentage proximate composition values of the experimental Diets was significantly different ($p \leq 0.05$) among the Diets except those of Crude protein as shown in Table 4.3 The C.P. (41.25%) and NFE (41.43%) were not significantly different ($p > 0.05$) among the Diets with the highest occurring in Diet five and Diet three respectively. Although, it was observed that the NFE content in Diets one and two were not significantly different ($p > 0.05$) from Diet three. D.M., was highly significantly different ($p \leq 0.01$) among the Diets where Diet 5 had the highest values of 93.20%. Also, the E.E. was significantly different ($p \leq 0.05$) among the Diets where Diet 5 had the highest value of 12.21%. The C.F. values obtained was highly significantly different ($p < 0.01$) among the various Diets where Diet four had the highest value of 8.58%.

4.4 Physico - Chemical Parametersof *Clarias gariepinus* Fed experimental Diets

The values obtained in the physico - chemical parameters was not significantly different $p > 0.05$ among the various Diets (Table 4.4). The highest hydrogen ion concentration (pH) value obtained was 7.00 in Diet one, the highest temperature was 26.61ºC in Diet three while the highest Dissolved oxygen (DO) 4.05 mg/L was obtained in Diet one.

Table 4.3 Percentage Proximate Compositions of Experimental Diets

DIET	Dry Matter (DM) %	Crude Protein (C.P) %	Crude Fibre (C.F) %	Ether Extract (E.E) %	ASH%	NFE%
Diet One	91.85±0.01^{b}	39.95±0.07^{a}	5.00±0.51^{b}	7.82±0.70^{b}	8.00±0.22^{b}	39.24±1.35^{a}
Diet Two	91.86±0.05^{b}	40.51±2.07^{a}	5.17±0.30^{b}	7.76±0.48^{b}	7.94±0.36^{b}	38.64±2.25^{a}
Diet Three	91.63±0.05^{c}	39.72±0.84^{a}	4.21±0.18^{b}	7.81±0.26^{b}	7.85±0.16^{b}	41.43±0.91^{a}
Diet Four	91.62±0.02^{c}	39.46±0.65^{a}	8.58±0.43^{a}	7.75±0.70^{b}	8.15±0.13^{b}	36.08±1.90^{ab}
Diet Five	93.20±0.01^{a}	41.56±0.01^{a}	4.47±0.02^{b}	12.15±0.05^{a}	9.50±0.10^{a}	32.33±0.16^{b}

Means with different superscripts along same column are significantly different ($p \leq 0.05$).

Table 4.4 Physico- Chemical Parameters of *Clarias gariepinus* Fed Experimental Diets

DIET	pH	Temperature (°C)	DO (mg/L)
Diet One	7.00±0.08[a]	26.04±0.22[a]	4.05±0.19[a]
Diet Two	7.18±0.07[a]	26.54±0.21[a]	4.35±0.18[a]
Diet Three	6.92±0.07[a]	26.61±0.30[a]	3.99±0.08[a]
Diet Four	6.90±0.08[a]	26.56±0.30[a]	3.96±0.19[a]
Diet Five	6.96±0.09[a]	26.13±0.14[a]	3.96±0.19[a]

Means with different superscripts along same column are significantly different ($p \leq 0.05$).

4.5 Growth Performance of *Clarias gariepinus* Fed Experimental Diets

There was no significant difference ($p>0.05$) in the Mean Initial Standard Length(MISL) and Mean Initial Body Weight (MIBW) of fish (Plate I) fed experimental Diets. The highest MISL (7.57cm) was obtained in fish fed Diet four (30% *A. lebbeck* inclusion) and MIBW in Diet five (Commercial Diet) (4.39g) (Table 4.5).

However, the MFSL value of fish fed experimental Diets was significantly different ($p\leq0.05$) among the Diets where the highest occurred in fish fed Diets two (20% *A. lebbeck* inclusion) (17.06g), and lowest occurred in fish fed Diet one (control) (13.69cm).The MFBW, MBWG and PWG of fish fed experimental Diets was highly significantly different ($p\leq0.01$) where fish fed Diet two had the highest values of 64.81g, 60.66g and 1472% respectively. While, fish fed Diet one (control) had the lowest for MFBW (33.09g), MBWG (29.17g) and PWG (740%).

4.6 Specific Growth Rate, Condition Factor, Average Daily Growth and Survival Rate of *Clarias gariepinus* Fed Experimental Diets

The Condition factor and Survival rate of *C. gariepinus* fed experimental Diets obtained was not significantly different ($p>0.05$) among the Diets though highest value obtained was 1.32(g/cm^3) in Diet two (10% *A. lebbeck* leaf meal) and Diet four (30% *A. lebbeck* leaf meal) respectively. (Table 4.6) Meanwhile, the SGR, ADG of the experimental fish were highly significantly different ($p\leq0.01$) among the Diets. Diet two had the highest SGR (3.93%), ADG (0.87g), while Diet four had the highest SR (96.67%). The lowest SGR (3.03%) and ADG (0.42g) were observed in Diet one while the lowest SR (90.00%) was noticed in Diets two and five.

DIET	Mean Initial Standard length (M I SL) (cm)	Mean Final Standard length (MFSL) (cm)	Mean Initial Body weight (MIBW) (g)	Mean Final Body weight (MFBW) (g)	Mean Body Weight Gain (MBWG) (g)	Percentage weight Gain (PWG) (%)
Diet One	7..25±0.14[a]	13.69±0.48[b]	3.92±0.10[a]	33.09±3.52[c]	29.17±3.43[c]	740.96±72.69[c]
Diet Two	7.57±0.20[a]	17.06±0.70[a]	4.15±0.33[a]	64.81±3.47[a]	60.66±3.30[a]	1472.07±97.51[a]
Diet Three	7.53±0.10[a]	16.34±0.62[a]	4.35±0.06[a]	54.50±5.49[ab]	50.15±5.47[ab]	1153.06±122.61[b]
Diet Four	7.57±0.23[a]	15.53±0.41[a]	4.24±0.34[a]	44.12±3.74[bc]	39.88±3.42[bc]	940.68±26.84[bc]
Diet Five	7.55±0.13[a]	16.33±0.59[a]	4.39±0.07[a]	54.49±5.47[ab]	50.11±5.45[ab]	1141.70±121.95[b]

Table 4.5 Growth Performance of *Clarias gariepinus* Fed Experimental Diets

Means with different superscripts along same column are significantly different ($p \leq 0.05$).

Table 4.6 Specific Growth Rate, Condition Factor, Average Daily Growth and Survival Rate of *Clarias gariepinus* Fed Experimental Diets

DIET	Specific Growth Rate (SGR) (%)	Condition Factor (CF) (g/cm^3)	Average Daily Growth (ADG) (g)	Survival Rate (SR) (%)
Diet One	3.03 ± 0.13^{c}	1.28 ± 0.02^{a}	0.42 ± 0.05^{d}	90.00 ± 5.77^{a}
Diet Two	3.93 ± 0.09^{a}	1.32 ± 0.12^{a}	0.87 ± 0.05^{a}	90.00 ± 0.00^{a}
Diet Three	3.60 ± 0.14^{ab}	1.24 ± 0.04^{a}	0.72 ± 0.08^{ab}	93.33 ± 3.33^{a}
Diet Four	3.34 ± 0.04^{bc}	1.18 ± 0.10^{a}	0.57 ± 0.05^{c}	96.67 ± 3.33^{a}
Diet Five	3.58 ± 0.14^{ab}	1.24 ± 0.03^{a}	0.72 ± 0.08^{ab}	90.00 ± 0.00^{a}

Means with different superscripts along same column are significantly different ($p\leq 0.05$).

4.7 Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets

The Feed Intake (FI) of *C. gariepinus* was significantly different ($p \leq 0.05$) with the highest value of 58.18g recorded in fish fed the Diet containing 10% *A. lebbeck* leaf meal (Diet two) while the lowest was 44.23g in control Diet (Diet one) (Table 4.7). The Protein Efficiency Ratio (PER) and Feed Conversion Ratio (FCR) of *C. gariepinus* fed experimental Diets were significantly different ($p \leq 0.05$) among the Diets with Diet two (10% *A. lebbeck)* having the highest PER (2.57g) and FCR (0.96g) apart from the commercial Diet. The Net Protein Utilization (NPU) of *C. gariepinus* fed the experimental Diets was not significantly different $p>0.05$ among the Diets though fish fed Diet with inclusion level of 10% *A. lebbeck* had the highest NPU (61.32%).

4.8 Carcass Composition of *Clarias gariepinus* Fed Experimental Diets

The D.M and EE of Carcass Composition of fishfed experimentalDiets were not significantly different ($p>0.05$) among the Diets (Table 4.8) although the highest D.M obtained was 90.88% found in fish fed Diet three (20% *A. lebbeck* leaf meal) and 11.89% Diet five respectively while the lowest D.M and oil was obtained in fish fed Diets four (30% *A. lebbeck* leaf meal) and two (20% *A. lebbeck* leaf meal), respectively.

The C.P, Ash and the NFE of the experimental fish were highly significantly different $p<0.01$ across treatment. Fish fed Diet two had the highest C.P. (54.95%) while those fed Diet one had the lowest C.P (51.07%). The highest Ash value was obtained in fish fed Diet five (16.35%) while the lowest was recorded in those fed Diet four (14.81%). The highest NFE value was obtained in fish fed Diet one (22.84%) while those fed Diet five had the least value (18.61%).

Table: 4.7 Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets

DIET	Feed Intake (FI)(g)	Protein Efficiency Ratio (PER) (g)	Net Protein utilization (NPU) (%)	Feed Conversion Ratio (FCR) (g)
Diet One	44.23±3.59^{c}	1.67±0.25^{b}	56.67±4.67^{a}	1.57±0.24^{a}
Diet Two	58.18±1.23^{a}	2.57±0.13^{a}	61.32±1.30^{a}	0.96±0.05^{b}
Diet Three	55.36±4.57^{ab}	2.36±0.29^{ab}	59.85±5.32^{a}	1.13±0.15^{ab}
Diet Four	55.72±0.74^{ab}	1.81±0.14^{ab}	59.88±0.80^{a}	1.41±0.11^{ab}
Diet Five	47.17±0.36^{bc}	2.58±0.30^{a}	61.22±0.48^{a}	0.97±0.10^{b}

Means with different superscripts along same column are significantly different ($p \leq 0.05$).

Table: 4.8 Carcass Composition *Clarias gariepinus* Fed Experimental Diets

DIET	D.M %	C.P. %	E.E. %	ASH %	NFE %
Initial	91.05±0.00	46.34±0.71^{d}	10.88±0.24^{a}	12.03±0.48^{c}	30.76±1.43^{a}
Diet One	89.93±0.00	51.07±0.16^{c}	10.61±0.46^{a}	15.49±0.17^{ab}	22.84±0.44^{b}
Diet Two	90.52±0.00	54.95±0.05^{a}	10.40±0.84^{a}	15.16±0.29^{ab}	19.50±0.50^{cd}
Diet Three	90.88±0.00	51.36±0.75^{c}	11.50±0.64^{a}	14.92±0.47^{b}	22.23±0.36^{b}
Diet Four	89.61±0.00	52.62±0.47^{bc}	11.09±0.48^{a}	14.81±0.26^{b}	21.50±0.22^{bc}
Diet Five	90.19±0.00	53.16±0.08^{b}	11.89±0.21^{a}	16.35±0.52^{a}	18.61±0.40^{d}

Means with different superscripts along same column are significantly different ($p \leq 0.05$).

KEY:

D.M= Dry matter, C.F= Crude fibre, NFE= Nitrogen free extract, C.P= Crude protein

4.9 Bi- weekly Growth Performance of *Clarias gariepinus* Fed Experimental Diets

The bi-weekly growth performance of *C. gariepinus* fed the experimental Diets is depicted in Figure 4.1. There was no significant difference ($p>0.05$) in the growth of *C. gariepinus* across treatments at theinitial stage, the fish performance for week two also was not significantly different ($p>0.05$) among the Diets, but the difference in fish weight across treatments as from weeks four, six, and eight became significant ($p\leq0.05$). The weight gain was highly significantly different across treatments at week ten with fish fed Diet two having the highest weight (64.81g), fish fed Diets three and five had similar weights (54.50g) and (54.49g), respectively while, those fed Diet one had the lowest weight.

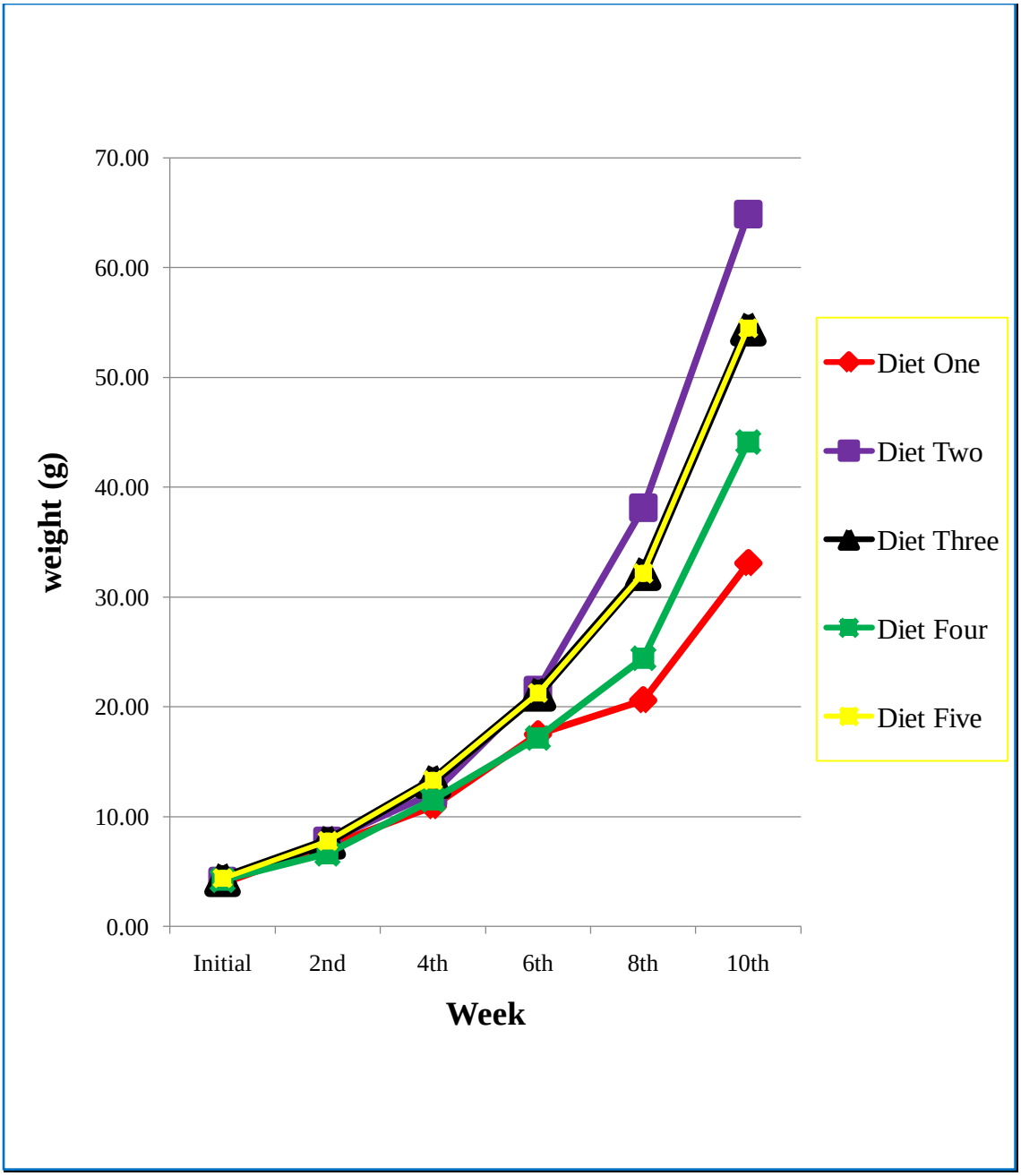


Figure: 4.1 Bi- weekly Growth Performance of *Clarias gariepinus* Fed Experimental Diets

CHAPTER FIVE

5.0 DISCUSSION

5.1 Antinutrient Composition of *Albizia lebbeck* leaf

Anti-nutrient factors were discovered in the leaf of *Albizia lebbeck* and include alkaloid, flavonoid, saponin and tanin. This was contrary to the findings of (Bhatta et al., 2005; Garcia and Medina 2005; Rai and Samanta 2007)who stated that the leaves of *A. lebbeck* are free of toxins and tannins but this agrees with the study of Chulet *et al.* (2010) and Andzouana and Moubuli (2011) who through a phytochemical screening of ethyl acetate successive extract discovered that *A. lebbeck* leaves showed presence of glycoside, tannins, saponins, flavanoids, alkaloids, steroids, carbohydrates, proteins and amino acids respectively in ethanol- water extraction.

The content of alkaloid obtained contradicts that of Oseni andOkoye (2013) in the seed (33.77%), mesocarp (71.2%) and whole fruit (50.8%) of watermelon. Njidda and Ikhimioya (2012) affirmed that saponin have effect on erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, and bloat (ruminant), inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption. Also, it alters cell wall permeability and therefore produces some toxic effect when ingested.

However, it was documented by Shi *et al.* (2004) and Njidda and Ikhimioya (2012) that saponins are heat sensitive as portions of saponins were lost during soaking or when dissolved in water. Also as reported by Abu *et al.* (2005) the levels of various anti-nutritional factors in most legumes prevent the digestion of proteins. It was noted that using sun-dried Malawi grown Leucaena leaf meal caused marked depressions in growth and feed intake of chickens fed on a 5% Leucaena leaf-meal Diet (Meulen *et*

al., 1979). Njidda and Ikhimioya (2012) stated that the ultimate effect of high condensed tannin concentrations is to make the animal both energy and protein deficient, which thereby leads to reduced growth or weight loss and poor reproduction. The tannin content obtained in this study is lower compared to that found in leaves of *Solanum monocarnum* (Garden egg Leaf), *Abelmoschus esculentus* (Okro leaf), *Moringa Oleifera* (Drum Stick), *Ocimum gratissimum* (Scent leaf) but same with *Androgen citrates* (Lemon grass)(Agbaire, 2012). Since tannins are water soluble phenolic compounds (Agbaire, 2012), the method of processing employed in this study (soaking method) was effective in reducing tannin content in the leaf of *A. lebbeck*. The values obtained in this study contradict that of Garcia *and* Medina (2005) saponin being as low as 1.48%, alkaloids 0.61%, tannin precipitates 0.55%. Possible reason could be due to different environmental conditions but within the range documented by Anwa *et al.* (2007).

5.2 Proximate Composition of *Albizia lebbeck* Leaf

The D.M., C.P., C.F., E.E, Ash and NFE parameters of the experimental Diet were significantly different $p \leq 0.05$ among the raw, soaked and sundried as well as soaked and shade dried in the proximate composition of *A. lebbeck* leaf. The higher values obtained in the soaked and sun- dried method could be as a result of exposure to sunlight especially in the D.M., C.F. and E.E. The reduction in the content of D.M., C.P., C.F., E.E, and Ash in soaked and shade dried method could be as result of the method of processing used. The crude protein obtained in the raw leaf of *A. lebbeck* is higher compared to those of the processed, this is in accordance with Anwa *et al*. (2007) who observed highest crude protein in the proximate for raw *A. lebbeck* seed and thereby stated that processing techniques has an influence on the levels of proximate components. The value of CP obtained was higher than that of Prinsen

(1986) and Balgees *et al*. (2009); this could be due to difference in climatic condition, location, soil type, time of harvest and availability of nutrient. It was documented that *A. lebbeck* is not an oil seed (Anwa *et al.*, 2007), this could be the reason why the ether extract content obtained in the leaf was not so high (11.23 -11.46%). Oil seeds have a range of lipid contents from about 18% in soyabean to as high as 43% in groundnut (Anwa *et al.*, 2007).

The DM of *A. lebbeck* leaf (92.02- 94.23%) is high which shows that it can be shelved over a long period of time. The crude fibre in the processed leaves was higher than in the raw, and the reason could be due to the method of processing. There was reduction in Ash value obtained in soaked leaves than in the raw, the substantial reduction of the ash content in the soaked leaf samples compared to the raw leaf sample might be due to leaching of both micro and macro elements into the soaking medium through the enhanced permeability of the leaf during the soaking treatment Wee and Wang (1987) reported the loss of certain nutrients in water during soaking of *Leucaena leucocephala* leaf. The least ash value of the soaked seeds makes it a better energy source among other processing methods since ash does not contribute to the total digestible nutrient (Akinmutimi, 2004). The ash content obtained was higher compared to 17.50% that was reported by Balgees *et al*. (2009), this could be due to the processing method employed.

The NFE obtained in this study was found to be lower than that of Hussain *et al.* (2010) in Pakistan and Balgees *et al*. (2009) in Sudan, this could be due to difference in location (Zaria, Kaduna state- Nigeria), soil type and Climatic condition.

5.3 Percentage Proximate Composition of Experimental Diets

There was no significant difference ($p>0.05$) in C.P. among the Diets. The moisture content (ranging from 6.8 - 8.38 %) in Diet was not high, this showed that the feed were properly dried to prevent fungal growth. There was significant difference ($p\leq0.01$) in the C.F. among the various Diets due to the various inclusion level of *A. lebbeck* leaf. The usual approach to formulating Diets for simple-stomach animals is to use ingredients that will maintain Dietary fiber levels below acceptable maximum levels. These levels would be in the range of 3- 6% crude fiber for catfish Diets (Robinson *et al*., 2001). The crude fibre obtained in the experimental Diets were within this range except for the 30% inclusion level of *A. lebbeck* (Diet four) which had 8.58% fibre content. This showed that higher inclusion level of *A. lebbeck* can increase the fibre contentin the Diet of catfish.

The C.P. of the various Diets was isonitrogenous, and this is ideal for the study because the essential component in fish feed is the protein. All the experimental Diets were accepted by the fish which showed that the incorporation of *Albizia lebbeck* leaf meal in the Diets had no adverse effect on the palatability of the Diets. Reason could be the method of processing employed which made the feed palatable. This corroborates with the findings of Francis *et al.* (2001) who reported that reduction in antinutrient by different processing techniques resulted in better palatability and growth in fish.

5.4 Physico- chemical Parameters of *Clarias gariepinus* Fed experimental Diets

Physico- chemical parameters such as temperature, pH and dissolved oxygen showed no significant difference $p>0.05$ among the various Diets. They are as well within the acceptable limit for *C. gariepinus* culture (Ajani, 2006)**.** The water was changed once

in a week to prevent accumulation of wastes in the tanks. The range of mean dissolved oxygen of the treatments wasbetween 3.96-4.35mg/L; mean temperature, 26.13-26.61°C and mean pH, 6.9-7.18. These values falls within the ranges of 3-9 mg/L for dissolved oxygen, 4 - 32°C for temperature and 6.5-9 for pH reported by Ndimele and Owodeinde (2012) as the best for tropical fishes.

5.5 Growth Performance of *Clarias gariepinus* Fed Experimental Diets

The experimental fish within all the treatments showed great increase in weight, which indicates that the fishes were able to convert feed protein to extra muscles. Weight gain and species growth rate are usually considered as the most important measurement of productivity of Diets (Adesina *et al*., 2013).

There was decrease in average final weight gained (FWG), average weight gained (AWG), and specific growth rate (SGR) of the experimental fish as the inclusion level of *A. lebbeck* leaf increased across Diets. This may be due to fibre content in the experimental Diets which increase with increase in inclusion level. Lawal *et al.* (2013) reported decrease in weight gain at high fibre level inclusion when they fed Sun-hemp seed to *C. gariepinus* juveniles.

Experimental fish fed 10% *A. lebbeck* (Diet two) had the highest values for MFSL (17.06cm), MFBW (64.81g), MBWG (60.66g) and PWG (1472.07%) which were lower than the highest values documented by Fagbenro *et al*. (2010). Experimental fish fed 10% *A. lebbeck* (Diet two) performed better than those fed control Diet. Reason could be that the fish used the available protein in the Diet efficiently than others which led to increase in weight and this findings concur with that of Farahi *et al*.(2012) whose fish fed experimental Diets performed better than the control in the Dietary supplementation (of lemon balm and *Aloe vera*)fed to Rainbow trout in Iran.

Also, the fish fed the control Diet (Diet one) had the least growth performance probably because of the effect of heat on the bioavailability of nutrients or probably by the presence of alcohol-soluble carbohydrates in soybean meal which affected better digestion of the meal. Thermal treatment can reduce the solubility of nutrients through the formation of protein complexes with polyphenols, carbohydrates, and general denaturation of protein. The combination of these factors may be partly responsible for the comparatively low digestibility of the control Diet (Fagbenro *et al.*,1994 and Alegbeleye *et al.*, 2012). Osuigwe *et al*. (2002) in preliminary evaluation of jack bean (*Canavalia ensiformis*) seed meal where fishmeal was substituted in Diets of *C. gariepinus* (Burchell, 1822) affirmed that adverse effect of heat treatment on some essential amino acids possessed by the legumes might have resulted in the poor growth and food utilization of the fish that was fed higher levels of jack bean seed meal. Bressani *et al*. (1987) observed reduced lysine and methionine level in *C. ensiformis* through pressure cooking and roasting in nutrient utilization and growth responses of *C. gariepinus* fingerlings fed Dietary levels of jack bean (*C. ensiformis*) meal. Nnaji et al. (2010) reported that cassava leaf meal included at 10% in the Diet of tilapia fingerlings gave the best growth, feed conversion ratio and survival compared to the control Diet and other test Diets (leaf meals of *Gliricidia sepium* (mouse killer) and *Stylosanthes humilis* (Townsville stylo).

5.6 Specific Growth Rate, Condition Factor Average Daily Growth and Survival Rate of *Clarias gariepinus* Fed Experimental Diets

There was significant difference $p \leq 0.05$ in specific growth rate (SGR) of the fish fed experimental Diets in this study. Fish fed 10% *A. lebbeck* had the highest SGR (3.93%) this might be due to different percentages of inclusion in the Diets. The

finding of this study with respect to the aforementioned support that of Aderolu *et al*. (2011) but disagrees with that of Alegbeleye *et al.* (2012). Also, the SGR recorded by Zamal *et al*. (2008) (6.52% /day) is higher than the one obtained in this study.

The condition factor (K) of the experimental fish fed different Diets was not significantly different $p>0.05$ among the Diets. The mean condition factor proved that fish in all the treatments were in good condition throughout the period of this study, reason being that they were stable all through. The essence of measuring fish condition factor is to ensure that the fish were healthy all through the experiment. The values obtained contradict those of Adesina *et al.* (2013). Olurin and Aderibigbe (2006) and Edward *et al.* (2010) documented that a fish well fed will obtain a higher condition factor than one poorly fed. The condition factors obtained in this study were all above 1.0, this established that the feeds were properly utilized for better growth and sound health.

The Average Daily Growth (ADR) of fish recorded in this study was higher than that of Jegede and Fagbenro (2008), Ndimele and Owodeinde (2012) who induced *C. gariepinus* and *Heterobranchus bidorsalis* with synthetic hormone (ovaprim) and pituitary glands of male and female *C. gariepinus.* The average daily growth rate obtained in this study was contrary to that recorded by Zamal *et al*. (2008) of 14.00% in 15% Ipil-Ipil leaf meal.

There was no significant difference $p>0.05$ in survival rate (SR) of experimental fish fed different Diets. The Survival rate observed in this study was higher than that of Dayal *et al*. (2012) in India who had 62% as the highest S.R. and Adewolu and Adamson (2011) as well as that documented by Dedeke *et al.* (2013). According to Idi-Ogede (2012) who had 39% mortality in tap water set-up in a study of stream and

tap water effect on *C. gariepinus*, the high mortality in tap water may be due to sharp changes in water quality parameters.

5.7 Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets

Feed utilization expressed as FCR is known to be affected by body weight, ration size and temperature (Keremah and Beregha, 2014).The feed conversion ratio of 0.96g and 0.97g obtained by fingerlings fed Diet containing 10% inclusion level of *A. lebbeck* (Diet two) and commercial Diet (Diet five) respectively are lower in comparison with others, this is an indication that the Diet with 10% inclusion level of *A. lebbeck* (Diet two) apart from the commercial Diet is of better quality than the other Diets. This finding corroborates with that of Olele *et al.* (2013) who stated that lower food conversion ratio indicate higher protein conversion efficiency thereby resulting in better growth, the result obtained contradicts that of Olorunsanya *et al.* (2010). Adikwu (2003) documented that the lower the FCR, the better the feed utilization by the fish. The lowest FCR of fish obtained in this present study indicated better feed utilization by the fish and this obviously accounted for better growth performance of *C. gariepinus* fed 10% *A. lebbeck* leaf Diet among other Diets. This result also supports the observation made by Shabbir *et al.* (2003) and Jabeen *et al.* (2004) in related studies. The best feed conversion ratio (FCR) of fish among the treatments (0.96%) recorded at 10% *A. lebbeck* inclusion level indicated a superior level of utilization of the *A. lebbeck* Diet by the fish.

Protein Efficiency Ratio (PER) is known to be regulated by the non-protein energy input of the Diet and is a good measure of the protein-sparing effect of lipid and/or carbohydrate (Tibbets *et al*., 2005). The PER of the experimental fish obtained in this study exhibited significant differences $p \leq 0.05$ in all treatments. The PER values

increased among the experimental fish with respect to the quantity of total feed intake. Similar observations were made by Sotolu (2008). The significant difference $p \leq 0.05$ in PER value obtained at 10% *A. lebbeck* leaf treatment indicated maximum utilization of inherent nutrients in the Diet at this level which was not possible at higher inclusion level (30%). This agrees with the observation made by Adejumo (2005) who reported highest PER value at 20% inclusion level of replacing maize with fermented millet.

The NPU is an indication of the amount of protein used in building up the fish carcass from the total protein consumed or a factor of the quality, digestibility and utilization of the protein fed to the fish. The difference in NPU values of fish fed different experimental Diet in this study was not- significant ($p>0.05$) though the value obtained in Diet 2 (10% *A. lebbeck* inclusion level) was higher (61.32%) than other treatments. This observation is comparable to the report of Sogbesan *et al*. (2007) who recorded highest NPU at 25% inclusion level of earthworm meal. Feed intake was highly significant $p \leq 0.01$in fish fed10% *A. lebbeck* (Diet 2) compared to other Diets, this may be due to lower inclusion level of *A. lebbeck*.

5.8 Carcass Composition of *Clarias gariepinus* Fed Experimental Diets

High priority is given to protein requirement in any nutritional study mainly because it is the single nutrient that is required in the largest quantity for growth, development and the most expensive ingredient in formulation of Diet (Ajonina and Nyambi, 2013). Dietary lipids function as a ready source of energy for fish and also provide essential fatty acids which are needed for fish growth and survival. Fish generally require omega-3 fatty acids rather than omega-6 fatty acids in contrast to terrestrial animals which require omega- 6 fatty acids.

Carcass composition of fish shows the amount of protein retained in the fish body. The dry matter of the initial carcass composition was 91.05% which reduced in all the experimental Diets, though not significantly different $p>0.05$. The initial carcass crude protein of the fish was 46.34%. The final carcass crude protein values obtained by the experimental fish increased but the fish fed 10% *A. lebbeck* (Diet two) showed a highly significant $p\leq0.01$ trait among the treatments. Diet one and three showed the lowest carcass crude protein which could be due to the improper usage of the nutrient by the fish. The final oil value obtained by the fish in this study show no significant difference $p>0.05$from the initial. Though the value ranges among the Diets, as such, the fish gained little oil, increase in protein content and decrease in lipid content may be an indication of the quality flesh as documented by Diyaware *et al*. (2011).

The initial carcass composition value of NFE obtained by the fish was higher than those obtained in the final carcass composition values and this agrees with the work of Ozovehe (2013).

5.9 Bi-weekly Growth Performance of *Clarias gariepinus* Fed Experimental Diets

Fish had similar initial weight at the beginning of the experiment which progressively changed as the fishes were administered Diets. After eight weeks of feeding, fish fed Diet two obtained a higher weight compared to others and this trend continued until the terminal end (week ten), this may be due to the low level of fibre in the Diet. Fish fed Diet three and five had similar weight but fishes fed Diet one (control) had the least weight among all the Diets. Fish fed Diet four had a lower weight compared to Diet two, three and five probably because of the presence of high fibre in the Diet, as such; fish could not properly digest their feed. The least weight obtained by fish fed

Diet one (control) may be due to protein reduction in the Diet. It was documented by Ogunji *et al*. (2001) that some levels of amino acids in cracked soybean seed decreased after heat treatment**.** This result corresponds with that of Alegbeleye*et al*. (2012).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATION

6.1 Summary

The Diets fed to *Clarias gariepinus* had significant effect on their growth and nutrient utilization ($p \leq 0.05$). Mean final body weight, mean final standard length, mean average daily growth, mean percentage weight gain, mean specific growth rate were highest in fish fed fish fed 10% *Albizia lebbeck* inclusion level. Fish fed 30% inclusion level of *A. lebbeck* had reduced growth. Also, Feed conversion ratio, apparent net protein utilization was highest in fish fed 10% *A. lebbeck* inclusion level compared with those fed on other diets (0%, 20% and 30% *A. lebbeck* inclusion level). Though inclusion of leaves of various types had been researched upon in the Diet of *C. gariepinus*, to date; there is dearth of information on the incorporation of *A. lebbeck* leaf meal in Diet of *C. gariepinus.* The result of this study may therefore serve as baseline data having discovered that the leaf of *A. lebbeck* improved growth of fish since *C. gariepinus* performed better in various growth and nutrient parameters as stated above.

6.2 Conclusions

Based on the findings of this study and with respect to the earlier stated hypotheses, there was significant difference between the proximate composition as well as antinutrient composition of raw and processed *A. lebbeck* leaf**. Also, there was significant difference between** growth and nutrient utilization of *C. gariepinus* **fingerlings fed varying inclusion levels of *A. lebbeck* leaf meal. Therefore, the null hypotheses is hereby rejected and it** is inferred that the usage of *A. lebbeck* leaves do not in any way conflict with human food security interests. This study has

demonstrated that the leaves have the potential to partly replace soya beans at 10%. This would considerably reduce expenditure on soya beans without compromising growth performance and feed utilization of the African catfish.

6.3 Recommendation

Based on the findings in this study, it is therefore recommended that 10% inclusion level of *A. lebbeck* leaf meal can be adopted in the formulation of feed for *C. gariepinus* fingerlings using the soaked and shade dried method of processing.

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APPENDICES

Appendix 1: ANOVA Table for Physico- Chemical Parameters of *Clarias gariepinus* Fed Experimental Diets

		Sum of Squares	df	Mean Square	F	Sig.
HYDROGEN ION CONCENTRATION	Between Groups	.544	4	.136	2.083	.097
	Within Groups	3.264	50	.065		
	Total	3.807	54			
TEMPERATURE	Between Groups	3.180	4	.795	1.247	.303
	Within Groups	31.869	50	.637		
	Total	35.049	54			
DISSOLVED OXYGEN	Between Groups	1.155	4	.289	.907	.467
	Within Groups	15.915	50	.318		
	Total	17.070	54			

Appendix 2: ANOVA Table for Growth Performance of *Clarias gariepinus* Fed Experimental Diets

		Sum of Squares	df	Mean Square	F	Sig.
MEAN INITIAL STANDARD LENGTH	Between Groups	.222	4	.055	.684	.619
	Within Groups	.810	10	.081		
	Total	1.032	14			
MEAN FINAL STANDARD LENGTH	Between Groups	20.064	4	5.016	5.155	.016
	Within Groups	9.730	10	.973		
	Total	29.795	14			
MEAN INITIAL BODY WEIGHT	Between Groups	.414	4	.104	.721	.597
	Within Groups	1.437	10	.144		
	Total	1.851	14			
MEAN FINAL BODY WEIGHT	Between Groups	1740.572	4	435.143	7.370	.005
	Within Groups	590.435	10	59.043		
	Total	2331.007	14			
MEAN BODY WEIGHT GAIN	Between Groups	1709.201	4	427.300	7.582	.004
	Within Groups	563.546	10	56.355		
	Total	2272.747	14			
PERCENTAGE WEIGHT GAIN	Between Groups	890250.223	4	222562.556	8.167	.003
	Within Groups	272517.930	10	27251.793		
	Total	1162768.153	14			

Appendix 3: ANOVA Table for Specific Growth Rate, Condition Factor, Average Daily Growth and Survival Rate of *Clarias gariepinus* Fed Experimental Diets

		Sum of Squares	Df	Mean Square	F	Sig.
SGR	Between Groups	1.342	4	.336	8.771	.003
	Within Groups	.383	10	.038		
	Total	1.725	14			
CF	Between Groups	.029	4	.007	.466	.760
	Within Groups	.155	10	.016		
	Total	.184	14			
ADG	Between Groups	.349	4	.087	7.582	.004
	Within Groups	.115	10	.012		
	Total	.464	14			
SR	Between Groups	106.667	4	26.667	.800	.552
	Within Groups	333.333	10	33.333		
	Total	440.000	14			

Appendix 4: ANOVA Table for Nutrient Utilization of *Clarias gariepinus* Fed Experimental Diets

		Sum of Squares	Df	Mean Square	F	Sig.
NET PROTEIN UTILIZATION	Between Groups	42.514	4	10.629	.337	.847

	Within Groups	315.750	10	31.575		
	Total	358.264	14			
FEED INTAKE	Between Groups	440.554	4	110.138	5.104	.017
	Within Groups	215.803	10	21.580		
	Total	656.357	14			
PROTEIN EFFECIENCY RATIO	Between Groups	2.220	4	.555	3.432	.052
	Within Groups	1.617	10	.162		
	Total	3.837	14			
FCR	Between Groups	.887	4	.222	3.497	.049
	Within Groups	.634		.063		
	Total	1.520	14			

Appendix 5: ANOVA for Carcass composition of *Clarias gariepinus* Fed Experimental Diets

		Sum of Squares	df	Mean Square	F	Sig.
DRY MATTER	Between Groups	3.097	5	.619	.	.
	Within Groups	.000	6	.000		

	Total	3.097	11			
CRUDE PROTEIN	Between Groups	85.391	5	17.078	38.940	.000
	Within Groups	2.631	6	.439		
	Total	88.023	11			
ETHER EXTRACT	Between Groups	3.104	5	.621	1.141	.431
	Within Groups	3.265	6	.544		
	Total	6.370	11			
ASH	Between Groups	21.438	5	4.288	14.656	.003
	Within Groups	1.755	6	.293		
	Total	23.194	11			
NITROGEN FREE EXTRACT	Between Groups	186.976	5	37.395	40.031	.000
	Within Groups	5.605	6	.934		
	Total	192.581	11			