EVALUATION OF *LEUCAENA LEUCOCEPHALA* (LAM DE WIT) LEAFMEAL IN THE DIET OF *HETEROBRANCHUS LONGIFILIS* VALENCIENNES, 1840

BY

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DEPARTMENT OF BIOLOGICAL SCIENCES,
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DECLARATION

I hereby declare that this work entitled "EVALUATION OF *LEUCAENA LEUCOCEPHALA* (LAM DE WIT) LEAFMEAL IN THE DIET OF *HETEROBRANCHUS LONGIFILIS* VALENCIENNES, 1840" has been performed by me in the Department of Biological Sciences under the supervision of Prof. J.K. Balogun, Prof. S.J. Oniye and Prof. J. Auta. Any assistance or citation of other work has been duly acknowledged. I further declare that the results of this work have not been submitted for the award of any other degree or fellowship at any University.

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CERTIFICATION

This thesis entitled "EVALUATION OF *LEUCAENA LEUCOCEPHALA* (LAM DE WIT) LEAFMEAL IN THE DIET OF *HETEROBRANCHUS LONGIFILIS* VALENCIENNES, 1840" by Aderonke Nana AGBO meets the regulation governing the award of degree of Doctor of Philosophy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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This work is dedicated to the Almighty God who gave me the strength to carry out this research.

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ABSTRACT

This study evaluated the effects of *Leucaena* leafmeal in the diet of *Heterobranchus longifilis* fingerlings. *L. leucocephala* leaves were processed in three different ways to determine the best processing method that will reduce the level of anti-nutritional factors. The processing methods were sundrying of fresh leaves, soaking in water at room temperature and boiling in water (100°C) for five minutes. The treatments were sundried for 24 hours (SD_1), sundried for 48 hours (SD_2), sundried for 72 hours (SD_3), soaked in water for 24 hours (SW_1), soaked in water for 48 hours (SW_2), soaked in water for 72 hours (SW_3) and boiled for 5 minutes (SB_5). A feeding trial was then conducted for 36 weeks in which growth performance and nutrient utilization of *Heterobranchus longifilis* fed *Leucaena* leafmeal based diets were determined. Experimental diets of 42.5-45% crude protein were compounded with each diet containing 10%, 20%, 30% and 40% inclusion of *Leucaena* leafmeal while fishmeal based diet served as control. The trials were conducted in 1000 litres plastic tanks measuring 1mx1mx1m. A total of 20 fingerlings of *Heterobranchus longifilis* with mean weight 6.56g ± 0.47 were stocked per tank and fed twice a day at 3% body weight. Digestibilty of diets, haematological indices and carcass composition of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal were evaluated. The cost benefit of incorporating *Leucaena* leafmeal in the diet of *Heterobranchus longifilis* was determined.

Processing had effect on the proximate composition, amino acid content, mineral content and anti-nutrients. Soaking in water (at room temperature) for 72 hours (SW_3) led to 89, 96, 86, 97 and 70% reduction in the levels of phytate, cyanide, tannin, saponin and mimosine respectively. Since soaking in water (at room temperature) for 72 hours was more effective in reducing anti-nutrients when compared to other treatments, *Leucaena* leafmeal was prepared from this treatment. Replacement of fishmeal with *Leucaena* leafmeal at 20% led to an increase in mean weight gain (MWG) and specific growth rate

(SGR) while 30% and 40% led to a decrease in growth parameters. *Heterobranchus longifilis* fed commercial diet had the highest mean weight gain among all the treatments followed by 20% *Leucaena* leafmeal inclusion level. However there was no significant difference in the mean weight gain and specific growth rate for all the treatments. The feed utilization parameters also followed the same pattern. The apparent digestibility coefficient (ADC) of protein and lipid were within normal range however there was a progressive decrease with increasing inclusion of *Leucaena* leafmeal. This shows that apparent digestibility coefficient (ADC) was related to feed utilization. The carcass analysis of *Heterobranchus longifilis* increased from initial values showing that the feeds were utilized and converted to flesh. However, the carcass crude protein in the control diet and 20% *Leucaena* leafmeal inclusion level were not significantly different ($P>0.05$). The haematological indices of *Heterobranchus longifilis* fed the control diet was higher than all the other experimental diets for packed cell volume (PCV), red blood cell (RBC) and haemoglobin (HB) with values of 34.67%, 2.90 $\times 10^6$ mm^3/ l, 11.50 (g/100ml). However the control diet and 20% *Leucaena* leafmeal inclusion level were not significantly different ($P>0.05$) indicating that this inclusion level did not have adverse effect on haematology of *Heterobranchus longifilis*. The cost benefit analysis revealed that 20% *Leucaena* leafmeal inclusion level gave the highest profit of N22.77 when compared to the control which had a profit of N10.23. However the gross profit and the incidence cost showed no significant difference ($P>0.05$) for all treatments. The results of this study showed that replacing fishmeal with 20% *Leucaena* leafmeal in the diet of *Heterobranchus longifilis* had no adverse effect on the growth and haematological indices of the fish.

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ABBREVIATIONS

ADC- Apparent digestibility coefficient

ADCP- Aquaculture Development and Coordination Programme

ANPU-Apparent Net Protein Utilization

AOAC- Association of Official Analytical Chemist

CF- Crude Fibre

CP- Crude Protein

$Cr_2 O_3$- Chromic Oxide

DL- Decilitres

DM- Dry Matter

EAA- Essential Amino acid

EE- Ether extract

FCR- Food conversion ratio

FL- Femtolitres

FWT- Final weight

HB-Haemoglobin

LLM- *Leucaena* leafmeal

MCH-Mean corpuscular haemoglobin

MCHC-Mean corpuscular haemoglobin concentration

MCV- Mean corpuscular volume

MO- Moisture

NFE- Nitrogen free extract

NRC- National Research council

PCV- Packed Cell Volume

PG- Picograms

RBC- Red blood cell

SPSS- Statistical package for social sciences

SR- Survival rate

WBC- White blood cell

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Fish is an important component of human nutrition and a principal source of animal protein for sixty percent of the world's population especially in developing countries (FAO, 2008). In recent years fish has been classified as a functional food which helps in the prevention and management of heart disorders (Ameigheme, 2005). The health and nutritional status of young children, pregnant and lactating women is enhanced through consumption of fish because it provides high value protein and polyunsaturated omega-3-fatty acids (FAO, 2008).

As global population continues to grow there is an increased demand for fish and other aquatic products. Consumption of fish and other aquatic products globally is predicted to reach 186 million tonnes by the year 2030 (FAO, 2014). World fish production was estimated to be 158 million tonnes in 2012 and 42.2 % of this is from aquaculture which is expected to cushion the effect of increased demand for fish products and production from capture fisheries which remains stagnant (FAO, 2014).

In Africa, though aquaculture is still developing but it has contributed 0.68% to the world aquaculture production and the annual growth rate was 11.7% in 2012 (FAO, 2014). Apart from increasing fish production, aquaculture alleviates poverty among rural populace, contributes to food security, provides livelihood, it is a source of income and foreign exchange (Jia *et al.*, 2001).

In Nigeria projected fish demand for 2015 was estimated at 2.66 million tonnes while projected domestic supply was 0.66 million tonnes (FDF, 2007). The deficiency of about two million tonnes is usually bridged by importation which was valued at USD 594.4 million (FDF, 2007). Since fish contributes 49% of the animal protein in the country, there is the need to increase domestic fish production and this can be achieved through aquaculture (Sikoki, 2005). In the year 2007 aquaculture contributed 13.8% to the fish production, Nigeria is blessed with inland water surface area of 14 million hectares and 1.7 hectares of land for aquaculture development which is yet to be utilized (FDF, 2007). The most cultured fish genera in Nigeria include *Clarias* species, *Heterobranchus* species, *Tilapia* species and Carp (Ayinla, 2007). There are various small-scale fish-farmers all over the country using a variety of production systems with pond culture being dominant (Ayinla, 2007). Small scale fish farmers account for over 70% of fish farmers and most farming practices are mainly semi-intensive (Ayinla, 2007). There are various constraints to aquaculture development which include poor quality fish seed, poaching, lack of capital, faulty data collection, poor marketing structure, unstable government policies and the lack of low-cost quality feeds (Omitoyin, 2007).

Feed is the most expensive operating cost in fish farming accounting for over 50-70% of total cost (Adekoya, 2001; Eyo, 2003a; Omitoyin, 2005; Ayinla, 2007). The rapid expansion and success of commercial fish culture will depend on the availability of good quality feed which is affordable to the small scale fish farmer (Eyo, 2001; Madu *et al.*, 2003). Good quality feeds brings about rapid growth of fish, keeps fish healthy, brings about high fecundity and hatchability in brood stock, encourages high stocking density and brings quick returns to the fish farmer (Eyo, 2003a).

Fishmeal is the conventional protein source in fish feed because of its desirable qualities (Eyo 2003a; Schipp, 2008). The continuous use of fishmeal as the main protein source is not sustainable because capture fisheries which produces fishmeal is leveling off thereby increasing the cost of fishmeal and the FAO code of conduct for responsible fisheries promotes human consumption of fish rather than using fish to feed animals (Tacon *et al.,* 2006). It has been documented that fishmeal can be totally or substantially replaced by alternative protein sources such as soyabean meal, groundnut cake and bambara nut meal (Ovie and Ovie, 2007; Schipp, 2008; Atse *et al*., 2008; Enyidi and Mbenka, 2014). In Nigeria there are several underutilized legumes such as *Leucaena,* velvet beans and pigeon pea.

Leucaena leucocephala (Lam de wit) is one of the tropical legumes that has the potential of being used as plant protein to reduce dependence on fishmeal and other conventional proteins (Hetrampf and Piedad-pascual, 2003; Amisah *et al.,* 2009). *Leucaena* is a fast growing multipurpose legume that has been introduced to many tropical and subtropical countries (FACT, 1997). The cost effectiveness of *Leucaena* in feed formulation has been limited due to the presence of a toxic non-protein amino acid called mimosine (Hetrampf and Piedad-pascual, 2003). The level of mimosine can be reduced through processing (Sethi and Kulkani, 1995; Monoj and Bandyopadhay, 2007). *Leucaena* is available in every ecological area in Nigeria and its leaves can be collected at little or no cost (Papka, 1995).

Catfishes of the genera *Clarias* and *Heterobranchus* are important aquaculture candidates in Africa and Nigeria (Ayinla, 2007; Nwafili and Gao, 2007). These species have a great potential to contribute to fish production and are readily acceptable to fish farmers.

Heterobranchus longifilis is highly priced and has one of the fastest growth rate among the African catfishes (Legendre *et al.,* 1992). It grows to a very large size, commands high market value due to its taste and flavor. *Heterobranchus longifilis* is an omnivore and has the ability to use supplementary feed effectively. The Nigerian farmer has not been able to meet the demand for *H.longifilis* due to high cost of feeding hence there is the need to boost the production of this species for food sustainability and food security using least cost feeds.

1.2 Statement of Research Problems

The high cost of commercial feeds and conventional feed ingredients has made fish feed too expensive for the average fish farmer. The use of plant protein sources such as soyabeans and groundnut in fish diet may not be sustainable because they are good sources of protein for human and consequently it competes with human food. Therefore, there is an urgent need to identify other protein rich plant resources that could substitute or supplement conventional protein feed ingredients. Other non conventional plant-based proteins such as *Leucaena* are not fully explored and exploited due to inherent antinutritional factors which are known to depress performance. Thus simple and cost effective processing methods which the small-scale fishfarmers can adopt should be employed to reduce the levels of antinutritional factors.

1.3 Justification

Leucaena leucocephala is rich in protein, the leaves can be collected at little or no cost and it is abundant in Nigeria therefore its usage in fish feed needs evaluation (Monoj and Bandyopadhyay, 2007). There is the need to evaluate simple processing method of removing anti nutrients from *Leucaena* leaves which the small scale fishfarmer can use.

Heterobranchus longifilis is widely cultured in Nigeria, has a high growth rate, has the ability to use supplementary feed effectively in captivity, is hardy and is of economic importance hence the choice of the species for this research.

1.4 Aim and Objectives

The aim of this study was to evaluate the effects of *Leucaena leucocephala* leafmeal in the diet of *Heterobranchus longifilis*.

The specific objectives were

i. Determination of proximate composition, antinutrients, amino acid profile and mineral composition of differently processed *Leucaena* leafmeal.
ii. To determine the growth responses and nutrient utilization of *Heterobranchus longifilis* fed graded inclusion levels of *Leucaena* leafmeal.
iii. To determine the digestibility of *Leucaena* leafmeal based diets and its effect on carcass composition of *Heterobranchus longifilis.*
iv. To determine the effect of *Leucaena* leafmeal based diets on haematological indices of *Heterobranchus longifilis*.
v. To determine the cost-benefit of incorporating graded levels of *Leucaena* leafmeal in the diet of *Heterobranchus longifilis.*

1.5 Hypotheses

i. There is no significant difference in proximate composition, anti-nutrients, amino acid profile and mineral composition of differently processed *Leucaena* leafmeal.
ii. There is no significant difference in growth response and nutrient utilization of *Heterobranchus longifilis* fed graded inclusion levels of *Leucaena* leafmeal.

iii. There is no significant difference in digestibility of *Leucaena* leafmeal based diets and carcass composition of *Heterobranchus longifilis*.

iv. There is no significant difference in haematological indices of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

v. There is no significant difference in cost benefits when *Heterobranchus longifilis* is fed with graded inclusion levels of *Leucaena* leafmeal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Catfish Culture

Clariids are of great economic importance as food fish, aquaculture candidate and are cultured mostly in Europe, Asia and Africa especially Nigeria (Legendre *et al.*, 1992; Nwafili and Gao, 2007). In Nigeria, *Clarias* and *Heterobranchus* species are commonly cultured due to their good taste, good flavor, acceptability among fish farmers and consumers (Ayinla, 2007; Olagunju *et al.*, 2007).

2.1.1 Biology of *Heterobranchus longifilis*

Heterobranches longifilis has one of the fastest growth rates among African catfishes and grows to a very large size and the maximum reported size is 55kg (Skelton, 1993). *Heterobranchus* can be distinguished from *Clarias* species by possessing a second dorsal (adipose) fin (Legendre *et al*., 1992).

Heterobranchus longifilis has a single annual spawning season in the wild which corresponds with the onset of the rains and rising flood (May-October) but fingerlings are available all year round through artificial breeding (Legendre *et al.*, 1992). *Heterobranchus longifilis* is a freshwater omnivore which feeds on insects, detritus, benthic invertebrates, tadpoles, shrimps and small fishes (Sogbesan and Ugwumba, 2006). The mouth of this species has no teeth but an abrasive plate, a large buccal cavity and chemosensory babels which helps in locating food (Legendre *et al*., 1992). It has been reported that *Heterobranchus longifilis* has predatory tendencies in which it feeds on Tilapia species and is more active in the dark (Offem *et al.*, 2009). A crude protein value of 40-42.5% has been

reported to be optimal for *Heterobranchus longifilis* (Eyo and Olatunde, 2001; Keremah and Alfred-Ochkiya, 2013). The estimated amino acid requirement is similar to that of *Clarias* species arginine-44.5g/kg, histidine-21.5g/kg, isoleucine-30.6g/kg, leucine-52.2g/kg, lysine-57g/kg, methionine-18.3g/kg, phenylalanine-27.3g/kg, threonine-31.6g/kg, tryptophan-7.1g/kg and valine-29.0g/kg (Eyo, 2003a).

The culture of *Heterobranchus longifilis* in earthen ponds, concrete ponds, plastic aquaria and cages has been documented (Coulibaly *et al.*, 2007; Ofor, 2007; Imorou-toko *et al.*, 2007; Ataguba *et al.*, 2010; Nlewadim *et al.*, 2011). However rearing *Heterobranchus longifilis* in earthen ponds seems to give better results probably due to availability of natural feeds such as zooplankton, aquatic invertebrates and insects (Coulibaly *et al*., 2007)

H.longifilis requires adequate quantity and good quality water for optimum growth, survival rate, reproduction and yield of fish (Omitoyin, 2007). Regular monitouring of water quality parameters is important for successful fishculture. The waterquality parameters are hydrogen-ion concentration (pH), temperature, nitrite, ammonia, water hardness, alkalinity, and turbidity. Different fishes have their recommended limits, the recommended waterquality limits for catfishes is temperature- 25-32°C, hydrogen-ion concentration (pH) 7-9, carbon-dioxide not more than 5mg/l, nitrite- not more than 0.03mg/l, ammonia- not more than 0.05mg/l and nitrate not more than 50mg/l (Ajana *et al.*, 2006).

2.1.2 Use of supplementary feed

The ability of *Heterobranchus longifilis t*o use supplementary feeds and plant based proteins has been studied. Eyo *et al*. (2004) evaluated the use of diets containing beniseed meal and soyabean meal to feed *Heterobranchus longifilis* fingerlings. The workers

observed that diets containing beniseed meal performed better than diets containing soyabean meal, this they attributed to the high methionine and lipid content of beniseed meal. Coulibaly *et al*. (2007) observed that *Heterobranchus longifilis* reared in cages accepted commercial feeds efficiently.

Ofor (2007) observed that when *Heterobranchus longifilis* fingerlings cultured in earthen ponds were fed diets containing soyabean meal, rice bran and brewerywaste, it led to a reduction in cost of feeding. Ovie and Ovie (2007) included groundnut cake in the diet of *Heterobranchus longifilis* fingerlings and reported it led to a reduction in cost of feeding without affecting growth and health. Atse *et al*. (2008) replaced fishmeal with soyabean meal in the diet of *Heterobranchus longifilis* juveniles and stated that soyabean meal should not be fed without adequate heat treatment to increase digestibility. Effiong *et al*. (2009) evaluated the inclusion of duckweed meal in the diet of *Heterobranchus longifilis* fingerlings and observed that 10% inclusion rate gave the best weight gain, FCR, SGR and SR which was not significantly different from the control which had no duckweed meal.

2.2 Importance of Feeds in Aquaculture

Feed is the most expensive operating cost, accounting for over 50-70% of total cost, depending on whether the aquaculture is extensive or semi-intensive (Adekoya, 2001: Eyo, 2003b, Omitoyin, 2005, Ayinla, 2007). Quality feed is necessary for fish to grow, resist disease and make aquaculture profitable however the feed must also be cost effective. Adequate supply of feeds has to be ensured so that the nutrients and energy requirements of the species under cultivation are met and the production goals of the system are achieved (Hasan, 2001). The rapid expansion of the Aquaculture sub-sector will depend on the availability of good quality feeds that is affordable by the small-scale fish farmer

(Tacon, 1997a, Eyo, 2001, Madu *et al*., 2003). The nutrient requirements of fish include protein, lipids, carbohydrates, vitamins and minerals (De Silva and Anderson, 1995). Imbalance of these nutrients predisposes the fish to nutritional diseases. For example an imbalance in the protein – energy ratio causes the development of fatty liver, reduced feeding activity and failure to grow (De Silva and Anderson, 1995). Poor nutrition makes the fish supceptible to disease causing agents which may eventually cause mortality (Hasan, 2001).

2.3 Nutrient Requirements of Fish

2.3.1 Protein

Proteins are organic compounds which comprises of amino acids and a critical component of fishfeed (Robinson and Menghe, 2007). Proteins are generally the most expensive component of fishfeed accounting for over 50% of the total feed cost in intensive aquaculture (Adekoya, 2001). Protein provides the amino acid required for the synthesis and replacement of worn out tissues without which there will be no meaningful growth. In formulation of fish feed protein is given priority to prevent loss of weight, depressed appetite and disease (NRC, 1993). Fishes have a high requirement for protein because it is used as a major source of energy (Robinson and Menghe, 2007). The protein requirements evaluation of some commonly cultured fishes in Nigeria was found out to be between 35% -50% crude protein (Eyo, 2003a). Protein requirement of fishes has been linked to age; fingerlings and juveniles have a higher protein requirement than adult fish (Bureau and Cho, 1999).

Amino acids are the basic sub-units of protein which determine the quality of a protein ingredient (Robinson and Menghe, 2007). The level and proportion of amino acids

provided in the protein is important because there must be proper balance for the amino acid to be useful to the fish (Robinson and Menghe, 2007). Amino acids in fish could be essential amino acids or non-essential amino acids. Essential amino acids cannot be synthesized by the fish and has to be made available in the diet these essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (NRC, 1993). A deficiency in one or more of essential amino acids can result in reduced growth (NRC, 1993). It has been reported that some non-essential amino acid can partially replace some essential amino acid (Robinson and Menghe, 2007). Cysteine can replace 60% of methoinine while tyrosine can replace 50% of phenyalanine (Robinson and Menghe, 2007). The protein quality of a feedstuff is determined by proportion of essential amino acid and the biological availability of the amino acid to the fish (Robinson and Menghe, 2007). To date there is no single animal or plant protein available with an essential amino acid profile which can fulfil the dietary requirements of fish apart from fishmeal (Hasan, 2001). Generally plant proteins tend to be limiting in one or more essential amino acids. Example of plant proteins and their limiting amino acids are cowpea-cysteine, cotton seed- methionine and lysine, sunflower- lysine, sesame seed methionine, groundnut- methionine, soyabean- methionine, lysine and threonine (Hasan, 2001). The amino acid profile of plant proteins may be improved through supplementation with synthetic amino acids. Another practical approach is to formulate feeds that contain combinations of different protein sources with different amino acid profile (Hasan, 2001).

2.3.2 Carbohydrates

Carbohydrates are compounds which consist of starches, sugars and fibres. Carbohydrates can be soluble like sugars and starch or insoluble like fibres (Wilson and Moreau, 1996).

Carbohydrates are the least expensive form of dietary energy and are frequently used for protein sparing in formulated diets (Hasan, 2001). Carbohydrates also serve as binders and aids in pelleting of fish feed (Eyo, 2003a). Carbohydrates utilization in fish varies depending on the species cultivated. Warmwater omnivorous or herbivorous fish species such as common carp, channel catfish and eel have been found to be more tolerant of high dietary carbohydrate levels (De Silva and Anderson, 1995). The utilization of dietary carbohydrate has also been found to vary with the complexity or chemical structure of the carbohydrate. Channel catfish appear to utilize complex carbohydrates more readily than simple sugars (NRC, 1993). Ovie et *al*. (2005) reported the carbohydrate requirement of *H. longifilis* to be 19.5%.

In diets for fish, it is not desirable to have a fibre content exceeding 8-12% because high fibre content would lead to decrease in total dry matter and nutrient digestibility of the diet resulting in poor performance (Hasan, 2001). Fibre is indigestible therefore it adds to the faecal waste which adversly affects the water quality and hence fish performance (NRC, 1993).

2.3.3 Lipids

Lipids are source of energy and fatty acids required for normal functioning of the body (Eyo, 2003a). Lipids are sometimes stored in the body of the fish to be used at a later time. Lipids help in the absorption of fat-soluble vitamins and provide polyunsaturated fatty acids such as Omega-3 fatty acid (Wilson and Moreau, 1996). Dietary lipids provide essential fatty acids and polyunsaturated fatty acids (PUFAs) that fish, like all animals, cannot synthesise but require for the maintenance of cellular function. Plant oils are generally rich sources of linoleic series fatty acid (n-6) but contain little or no linolenic

series fatty acid (n-3) (Hasan, 2001). The implication of this is that lipids especially those containing n- 3 fatty acid series need to be provided for in the diet (Hasan, 2001).

2.3.4 Vitamins

Vitamins are molecules required in small amounts in the diet (Eyo, 2003a). Vitamin requirements have been determined by feeding various vitamins under study in purified diets. Vitamin requirement is affected by other factors such as size, age, growth rate, stage of sexual maturity and environmental stressors (Wilson and Moreau, 1996). Vitamins can be divided into fat soluble (vitamins A, E, D and K) and water soluble vitamins which are B group, C and some specific cofactors (Hasan, 2001). Insufficient or lack of vitamins results in nutrient deficiency diseases, poor growth and susceptibility to infection (Eyo, 2001). The vitamin requirements of the majority of fish species in culture have not been determined but levels published by United States National Research Council for warm water fish is commonly used (De silva and Anderson, 1995). While natural food is usually rich in vitamins this may not be the case with supplementary feed therefore, in intensive culture systems most fishes require dietary supplementation of vitamins (Hasan, 2001). Most fresh ingredients used in feed formulation contain vitamins however processing affects these compounds. Ascorbic acid (vitamin c) is easily destroyed by cooking or improper storage therefore should be included 5-10 fold excess to take care of degradation during storage (Hasan, 2001).

2.3.5 Minerals

Mineral or inorganic elements are needed by animals to maintain many of their metabolic processes (Eyo, 2001). The minerals required can be divided into major and trace minerals. Major minerals required by fish include calcium, phosphorus, magnesium sodium, potassium, chlorine and sulphur (De silva and Anderson, 1995). While trace minerals are those required in small amounts these include iron, iodine, manganese, copper, cobalt, zinc, selenium, molybdenum, fluorine, aluminium, nickel, silicon, tin and chromium (Robinson and Menghe, 2007). Fish can absorb some minerals from water and these reduce their requirement (Eyo, 2003a). Phosphorus is required by fish in large quantities and feedstuff from plants are low in phosphorus therefore catfish diet is supplemented with Di-calcium phosphate (Robinson and Menghe, 2007). Determination of dietary mineral requirements of aquatic animals has been complicated by the fact that they have the ability to absorb minerals from the surrounding water in addition to the food ingested. The dietary requirement of a fish for a particular element thus depends to a large extent upon the concentration of the element in the water body (Hasan, 2001). At present, there is little information concerning the contribution of waterborne elements to the total mineral balance of fish (Hasan, 2001). Minerals available in feed ingredients are not always sufficient to meet the fish's requirements. Some of these minerals may leach out during processing of feed moreover many of the feed ingredients may be rich in particular minerals and deficient in others, or only partially available to the animal (Hasan, 2001). Since much of the mineral requirement is supplied by the food, partial mineral supplementation may be sufficient to meet dietary needs of fish.

2.4 Fishmeal as Protein Source

Fishmeal is a high quality animal protein. Fishmeal is made up of well balanced essential amino acids, is a good source of digestible energy, it is also rich in fatty acids, minerals and trace elements (Eyo, 2003a). It has also been reported that fishmeal is palatable, increase immunity against diseases, enhances growth and decreases incidence of deformity therefore an inclusion level of 10-20% was recommended for omnivorous fishes during their grow out stage (Tacon *et al.,* 2006; Schipp, 2008). However fishmeal production declined gradually since 2005 giving rise to a price increase of 206% between 2005 and 2013, this price increase and unstable supply made fishmeal unaffordable to the resource poor farmer but the price of soyabeans remained relatively stable (FAO, 2014). As aquaculture continues to grow there is an increased demand for fishmeal. In 2010, aquaculture used 73% of the global fishmeal production therefore the rise in global price of fishmeal had serious implications for aquaculture (FAO, 2014). The major challenge for the fishfeed industry is to find alternative protein sources that are sustainable and affordable (Tacon *et al.*, 2006).

2.4.1 Alternatives to fishmeal

In choosing an alternative feed ingredient to fishmeal the following factors should be considered, the effect of the alternative feed ingredient on growth of the fish, survival rate, feed conversion ratio, resistance to disease, safety of aquaculture product and price (Schipp, 2008). Furthermore the alternative protein ingredient should not be environment degrading in terms of nitrogen and phosphorus (Tacon 1997a). It has been demonstrated that fishmeal can be totally or substantially replaced by alternative protein sources (Schipp, 2008). Alternative protein sources can be divided into animal or plant sources.

Some of these protein sources are conventional that is those ingredients commonly used in the manufacture of fish feed while others are non conventional that is ingredients not commonly used in the manufacture of fishfeed (Madu *et al.*, 2003).

Animal based alternative protein ingredients that have been evaluated include grasshopper meal, earthworm meal, toad meal, termite meal, maggot meal, chicken offal meal, garden snail meal, fish waste, freshwater muzzle meal; silkworm pupa, crab meal and turkey meal (Nandeesha *et al.*,2000; Alatise *et al.*,2004; Nnaji and Okoye, 2004; Ogbe *et al.*,2004; Thompson *et al.*,2007; Ogunji *et al.*,2008; Sogbesan and Ugwumba, 2008; Soltan, 2009; Sotolu, 2009; Okanlawon and Sotolu, 2010; Ovie and Adejayan, 2010; Keremah and Gabriel, 2013).

A number of plant based protein ingredients have been incorporated into fish diet. These include duckweed leafmeal, mucuna seedmeal, water hyacinth leafmeal, pawpaw leafmeal, feed pea, lemna minor leafmeal, water spinach, pigeon peameal, swordbeans meal, jackbean meal, bambaranut meal, limabean meal, guar seedmeal, brewers yeast, fresh aquatic plants concentrate, azolla meal, spirogyra meal, alfafa leafmeal, moringa leafmeal, sweetpotato leafmeal, seaweed meal, banana leafmeal bamboo leafmeal, boiled tea leaves residue meal, mango seedmeal, *Leucaena* seedmeal, groundnut cake, soyabean meal, high protein distillers drygrain and yeast fermented Canola meal (Davies *et al.*, 1997; Al-hafedh and Siddigui, 1998; Oliva-teles and Goncalves, 2001; Ali *et al.*, 2003; Ritchter *et al.*,2003; Ogunji *et al.*,2005; Konyeme *et al.*,2006; Kalita *et al.*, 2006; Sheeno and Saha, 2006; Munguti *et al.*, 2006; Osuigwe, 2007; Osuigwe and Obiekezie, 2007; Adewolu, 2008; Dongmeza *et al.*, 2008; Sotolu and Faturoti, 2008; Atse *et al.*,2008; Effiong *et al.*, 2009; Koumi *et al.*, 2011; Barres *et al.*, 2012; Plaipetch and Yakupityage, 2012).

There are some studies on the partial replacement of fishmeal by plant proteins. Olivera-Novoa *et al.* (1990) reported the use of alfalfa leaf concentrate to replace 35% of fishmeal in the diet of tilapia (*Oreochromis niloticus*) fingerlings. It has been documented that 50% of fishmeal can be replaced by guar seed meal in the diet of *O. niloticus* (Al-hafedh and Siddigui, 1998). Mukhopadhyay (2000) reported the use of copra meal to replace 50% of fishmeal in the diet of Rohu (*Labeo rohita*) fingerlings and found out that growth was favourable. Nyirenda *et al.* (2000) found out that fishmeal can be totally replaced with soyabean meal in the diet of *Oreochromis karongae*. Oliva-Teles and Goncalves (2001) demonstrated the use of brewer's yeast to replace 50% of fishmeal in the diet of Seabass (*Dicentrarchus labrax*). Ritcher *et al.* (2003) tested the use of moringa leafmeal to replace 10% of fishmeal in the diet of tilapia (*O.niloticus*) and found out that growth was comparable to fishes fed control diet. Konyeme *et al.* (2006) reported the use of water hyacinth to replace 10 and 20% of fishmeal in the diet of *Clarias gariepinus* fingerlings. Sheeno and Saha (2006) replaced 10% of fishmeal with Azolla protein concentrate and Spirogyra powder in the diet of *L. rohita.* It was reported that boiled jackbean seedmeal can replace up to 40% fishmeal in the diet of *H. longfilis* fingerlings without compromising growth (Osuigwe and Obiekezie, 2007). Akintayo *et al.* (2008) demonstrated the use of sunflower seedmeal to replace 25% of fishmeal in the diet of C. *gariepinus* fingerlings. Cheng *et al*. (2010) reported that canola meal can replace up to 20% of fishmeal in the diet of Japanese seabass *(Lateolabrax japanicus)* without affecting growth of the fish. Fapohunda (2012) observed that boiled and roasted soyabean meal could replace 30, 35 and 40% of fish meal in the diet of *C. gariepinus*. Also the effect of soyabean meal obtained from roasted seeds compared favourable with the control diet. Plaipetch and Yakupityage (2012) reported 50% replacement of fishmeal with yeast fermented canola

meal in diet of Asian seabass *Lates calcarifer* without overall impact on growth. Falayi *et al.* (2013) reported the use of sesame seedcake to replace up to 67% of fishmeal in the diet of *C. gariepinus* and found out that growth was favourable. Ozovehe (2013) demonstrated the use of moringa leafmeal to substitute 10% of fishmeal in the diet of *C. gariepinus*. Enyidi and Mgbenka (2014) reported the use of bambaranut waste to replace 20% of fishmeal in the diet of Heteroclarias larva and found out that the growth was similar to the control. Plant based protein ingredients are cheap and available in large quantities however some have high fibre content, poor amino acid profile, poor palatability, poor digestibility and contain antinutritional factors as demonstrated by many authors (Francis *et al.,* 2001; Okoye, 2003; Agbede and Aletor, 2004).

2.5 *Leucaena leucocephala* (Lam de wit)

Leucaena leucocephala is one of the tropical legumes that have the potential of being used as a plant protein source to reduce dependency on fishmeal in fishfeed (Amisah *et al.,* 2009). *Leucaena leucocephala* is a fast growing evergreen multipurpose legume which is native to Mexico but has been introduced to many tropical and sub-tropical countries (FACT, 1997; Heuze and Tranc, 2012).

Leucaena leucocephala belongs to the family *Fabaceae*, sub-family *Mimosoidae* and genus *Leucaena*, it has 51 species but *Leucaena leucocephala* is the most common (Orwa *et al.,* 2009). *Leucaena leucocephala* tree has bipinnate leaves which are 15-20cm long, lanceolate leaflets, flat brown pods containing small seeds and white flowers (FACT, 1997; Orwa *et al.,* 2009). The picture of *L. leucocephala* leaves, pods and flowers are presented in Appendix I (Plate I, II and 111).

Leucaena leucocephala was one of the browse plants introduced to Nigeria in the 1950s and was recommended for large scale production (Onifade and Agishi, 1990).

2.5.1 Uses of *Leucaena leucocephala*

The pods seeds and leaves of *Leucaena leucocephala* have been used as food in some parts of Mexico (Simbaya, 2002). It has been documented that *Leucaena leucocephala* was used in dry season feeding of ruminants, agroforestation, wind breakers, live fence, alley farming, roadside landscaping, fuel production, paper production, timber production and nitrogen fixation (Simbaya, 2002; Hetrampf and Piedad-pascual, 2003; Orwa *et al*., 2009).

2.5.2 Propagation and establishment

Leucaena leucocephala is propagated by seeds which remain viable for several months. These seeds have hard waxy seed coat which needs scarification before planting (Shelton and Brewbaker, 1998). Scarification is achieved by nicking the seed, soaking the seed in boiling water for 30 minutes or soaking in sulphuric acid for 15 minutes (FACT, 1997). Nursery grown seedling and bare root transplants have also been used to propagate *L. leucocephala* (FACT, 1997). It has been reported that *L. leucocephala* takes 12-18 months to be fully established (FACT, 1997). *L. leucocephala* sheds it leaves through-out the year but more during the dry season and a leaf litter fall of 801.45kg/hectare/year was reported (Oladoye *et al.*, 2010).

2.5.3 Nutritional value of *Leucaena leucocephala* leaves

Leucaena leaf has crude protein values ranging from 15.2-34.3% dry matter (Sethi and Kulkarni, 1995; FACT, 1997; Simbaya 2002; Adeparusi and Agbede, 2005; Foroughbakhch *et al.*, 2007; Monoj and Bandyopadhay, 2007; Ayssiwede *et al.*, 2011,

Adedeji *et al.*, 2013). The nutrient value of *Leucaena* leaves depend on the soil, season, stage of cutting and cultivar even among the same species there is variation (Masafu, 2006; Monoj and Bandyopadhyay, 2007). *Leucaena* leaf contains vitamin A, Vitamin B, carotene and the 10 essential amino acids but like all plant based protein it is limited in methionine (Sethi and Kulkarni, 1995; Hetramphf and Piedda-Pascual, 2003; Monoj and Bandyopadhyay, 2007; Ayssiwede *et al.*, 2011; Adedeji *et al.*, 2013). Monoj and Bandyopadhyay (2007) reported the range of nutrients in *Leucaena* leaf to be crude protein 15.2-34.3%, ether extract 2.59-7.10%, crude fibre 7.33-16.55%, Ash-6.8-12%, Nitrogen free, extract 46.7 - 59.91%.

The use of *Leucaena* leaves to feed other animals such as livestock, goat, finishing pigs, laying hen, broilers and rabbits has been documented (Strivastava and Sharma, 1998; Zakayo *et al.,* 2000; Atawodi *et al.,* 2008; Fayemi *et al.,* 2011; Adedeji *et al.,* 2013).

The effect of *Leucaena* leafmeal in the diet of fish has been studied. Wee and Wang (1987) reported 25-50% inclusion of *Leucaena* leafmeal in the diet of the Nile tilapia (*Oreochromis niloticus*) without tit adversely affecting growth and body composition. Santiago *et al*. (1988) recommended the inclusion of 40% *Leucaena* leafmeal in the diet of Nile tilapia broodstock. When Hasan *et al*. (1990) partially substituted fishmeal with *Leuceana* leafmeal in the diet of India major carp (*Labeo rohita*) fry it led to a reduction in cost of feeding. Hasan *et al*. (1994) observed that 25% inclusion of *Leucaena* leafmeal in the diet of India major carp (*L. rohita)* fingerlings gave good results. Borlongan and Coloso, (1994) used a combination of soyabean meal and *Leucaena* leafmeal to replace 15% of fishmeal in the diet of milk fish (*Chanos chanos)* juveniles. Osman *et al*. (1996) reported the use of *Leucaena* leafmeal to replace 15% of Berseem leafmeal in the diet of

Nile tilapia and observed there was an increase in growth. Falaye *et al. (*1998) evaluated the use of *Leucaena* leafmeal to replace 25% of groundnut cake in the diet of *O. niloticus* juveniles. Nandeesha *et al*. (2000) recommended 15% inclusion of *Leucaena* leafmeal in the diet of *Catla catla*. Fingerlings of *L. rohita* were fed diets containing *Leucaena* leafmeal and it was observed that fishes fed *Leucaena* leafmeal diet performed better than the control therefore 30% inclusion rate was recommended (Baraigi *et al*., 2004). Amisah *et al.* (2009) reported that the use of *Leucaena* leafmeal in the diet of *C. gariepinus* at 20% inclusion level was cost effective and gave a better growth compared to the control which was fishmeal based.

2.5.4 Antinutritional compounds in *Leucaena leucocephala*

Despite the high protein content and cost effectiveness of *Leucaena* leafmeal in the diet of various animals and fishes, its usage in fish feed formulation is limited. *Leucaena* leaves has some antinutritional factors, the major one is mimosine a non-protein toxic amino-acid which is structurally similar to tyrosine (Monoj and Bandyopadhyay, 2007).

In cattle, sheep and goat, mimosine has been reported to cause loss of appetite, excessive salivation, enlargement of tyroid gland, reduced hair growth, goiter, and depressed growth (Shelton and Brewbaker, 1998; Monoj and Bandyopadhyay, 2007). In some ruminants mimosine is degraded to dihydropyridines which is less toxic by rumen bacteria (FACT, 1997). In fish high levels of mimosine has been reported to cause fin erosion and stunted growth (Sogbesan *et al.,* 2006).

Mimosine content of *Leucaena* leaves vary with season and maturity of plant, immature leaves contain higher mimosine than matured leaves (Masafu, 2006). Sethi and Kulkarni (1995) reported that matured leaves contain 2.6% mimosine while immature leaves have

5.1% mimosine. *Leucaena* is known to have other antinutritional factors such as tannins and phytic acid (Monoj and Bandyopadhyay, 2007). Reduction of mimosine will enhance usage of *Leucaena* leaves in fishfeed.

2.5.5 Reduction of mimosine

The amount of mimosine in *Leucaena* leaves can be reduced through processing (Monoj and Bandyopadhyay, 2007). Soaking leaves in water for 24 hours and 48 hours at 30°C respectively reduced mimosine content considerably (Wee and Wang, 1987; Hassan *et al.,* 1994; Amisah *et al.,* 2009). Soaking the leaves in hot water (70°C) for 10 minutes and soaking the leaves in 60°C water for 24 hours reduced mimosine content (Fayemi *et al.,* 2011; Adekojo *et al.,* 2014). Agbede (2005) also reported that sundrying the leaves for 72 hours reduced mimosine content. When Nuttaporn and Naiyatat (2009) autoclaved the leaves at 60°C for 24 hours and then soaked the leaves in water for 72 hours at room temperature 94% reduction of mimosine was observed. Fermenting *Leucaena* leaves for 5 days has been reported to reduce mimosine levels (Adekojo *et al.,* 2014). The use of metal ions to prevent absorption of mimosine in the intestine has been documented (Hertramph and Piedda-Pascual, 2003). The use of detoxifying bacteria (*Bacillus subtilis, Bacillus circulan* and *Trichodermis* species) to reduce mimosine content in *Leucaena* leaves has been reported (Baraigi *et al*., 2004; Yessirita *et al*., 2013).

2.6 Antinutritional Compounds

Antinutritional factors are defined as substances which themselves or their metabolite products arising in living system interfere with food utilization thus affecting the health and production of animals (Makkar *et al*., 2007). Most of the potential alternative, plant-derived nutrient sources are known to contain a wide variety of antinutritional substances.

Antinutritional factors could be broadly divided into four groups, the first are factors affecting protein utilisation and digestion, such as protease inhibitors, tannins, lectins. The second are factors affecting mineral utilisation which include phytates, gossypol pigments, oxalates and glucosinolates. While the third is antivitamins and the fourth are miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids and saponins (Francis *et al.,* 2001).

Antinutritional factor limits the inclusion of plant ingredients in fish diet. These factors causes poor palatability of fishfeed, poor feed intake, interferes with feed utilization and sometimes alters the gut morphology of fish (Francis *et al.,* 2002). An overview on the effects of some antinutrients present in oilseeds and pulses on fish has been reported by Francis and Becker (2002).

2.6.1 Tannins

Tannins are compounds which occur widely in the plant kingdom especially in legumes and can be divided into two major groups, hydrolysable and condensed tannins (Francis, *et al.,* 2001). Condensed tannins are not easily digested, bind to proteins, vitamins, minerals and digestive enzymes (Makkar *et al*., 2007). Contrary to condensed tannins, the hydrolysable tannins are easily degraded in biological systems, forming smaller compounds that can enter the blood stream and over a period of time cause toxicity to the organs for example liver and kidney (Kumar *et al*., 2010). Tannins have a bitter taste which affects palatability and depresses appetite thereby resulting in retarded growth (Kumar *et al*., 2010). Some common nutritional effects include reduced weight gain, poor feed conversion efficiency, reduced apparent digestibility of protein and reduced feed intake (Dongmeza *et al.,* 2008). These effects are not seen in all animals, and the extent is

variable depending on the source of the tannins, concentration, duration of exposure, diet composition, species of fish and age (Francis and Becker, 2002).

Tannins have the ability to bind to amide groups of enzymes and other proteins using hydrogen bonds thereby creating large blocks of amino acids that are resistant to digestive enzymes because of this factor it is important to consider the concentration of tannins in fish diets. Tannins are also known to interact with other antinutrients, for example interaction between tannins and lectins removed the inhibitory action of tannins on amylase while the interactions between tannins and cyanogenic glycosides reduced the deleterious effects of the latter (Francis *et al.*, 2001).

Dehulling of seeds, soaking in water and autoclaving has been reported to reduce level of tannins in plant proteins (Francis *et al.,* 2002). Limited literature on the effects of purified tannins on fish suggest that fish are sensitive to tannins and caution should be exercised in incorporating seeds and agro-industrial byproducts containing high amounts of tannins (Francis and Becker, 2002). Common carp has been shown to be able to tolerate a 2% addition of quebracho tannin powder condensed tannin without any effect on growth while a similar level of hydrolysable tannin tannic acid induced feed rejection after 28 days of feeding (Kumar *et al*., 2010).

2.6.2 Saponins

Saponins are steroids found in a wide range of plants, their chemical structure is made up of two portions i.e. sugar (glycone) and non–sugar(aglycone) which retards growth, damage intestinal macosa in fish and haemolysis of red blood cells (Francis and Becker, 2002). Dietary saponins are known to have several adverse effects on fish performance because they are characterized by bitter taste and reduce feed intake (Francis *et al.,* 2001).

Saponins have been reported to interfere with digestibility, reduce absorption of nutrients by forming complexes (Makkar *et al.,* 2007). Saponins sometimes form complexes with other antinutritional factors like tannins resulting in inactivation of toxic effects of both substances (Francis *et al.,* 2001). However they can also increase digestibility of carbohydrate rich feeds due to their detergent like activity which reduces viscosity thus preventing the normal obstruction action of such foods against digesta movement in the gut (Francis *et al*., 2001). The effect of saponin can easily be removed by extraction with water and values of 1g per kg are unlikely to affect fish growth (Francis and Becker, 2002; Dongmeza *et al.,* 2008)

2.6.3 Phytates

Phytates also known as phytic acid is composed of hexaphosphates of myo – inositol and it is the main storage form of phosphorous in plant tissues (Francis *et al.,* 2001). Phytic acid in salt form is referred to as phytate, phytic acid can make up to 6% by weight in cereals and oil seeds, up to 80% of total phosphorus in plants can be in phytate form. Phytate-bound phosphorous cannot be broken down by non ruminants including fish (Kumar *et al.,* 2010). The unavailability of this phosphorous results in negative impact on growth performance, nutrient digestibility, energy and mineral utilization (Kumar *et al.,* 2010). Phytic acid negatively affects the utilization of minerals which can be seen by its ability to bind up to 75% of all phosphorus (NRC, 1993). It can chelate di and trivalent metals including calcium, magnesium, zinc and iron into compounds that are less easily absorbed in the intestine (Francis *et al.,* 2001). Phytic acid also has the ability to non selectively bind to protein and inhibit activities of a number of digestive enzymes such as pepsin, trypsin, and alpha-amylase (Kumar *et al.,* 2010). Phytates can be reduced by dehulling, heating,

fermentation and supplementation with phytase (Kumar *et al.*, 2010). Phytate levels should be below 5g/kg in feed (Francis *et al.*, 2001).

2.6.4 Cyanogens

Cyanogens are compounds found in high concentrations in a number of pulses, root crops and some oil seeds which have been tried as fish feed ingredients. Cyanogens are not toxic but when hydrolysed produce toxic products such as hydrogen cyanide and probably other carbonyl compounds that suppress natural respiration and cause cardiac arrest (Francis *et al*., 2001). The enzyme required for this conversion is usually found in extracellular spaces in the plant tissues. Fish fed high concentration of cyanogens containing feed materials generally show reduced growth (Dongmeza, 2008).

High levels of HCN can be reduced significantly by soaking and/or thermal treatment (boiling, roasting and sun drying) and microbial fermentation. Physical damage/crushing of tissues facilitates the hydrolysis process by bringing together linamarin and linamarase. More studies are required to determine the level of tolerance of different cultured fishes

2.7 Digestibility in Fish

Digestibility has been defined as a relative measure of the extent to which ingested feed and its nutrients have been digested and absorbed by the fish (Kangombe, 2011). Only a proportion of ingested food is digested and its nutrients absorbed, the rest is passed out as feaces. The digestibility of a diet is influenced by various factors such as the methods of feacal collection, feeding rates, the size of the meal given, weight and age, dietary components, energy-protein ratio, temperature and water quality (Bureau and Cho, 1999 ; Belal, 2005). Digestibility in fish can be determined directly or indirectly (Lovell, 1989).

In the direct method, the quantity of feed ingested and the feaces voided are determined and the ratio gives the percentage digestibility. The indirect method involves the use of an indigestible marker, the difference between the quantity of marker in the feed and feaces gives the apparent digestibility coefficient (ADC). Chromic oxide is commonly used as an indigestible marker (Belal, 2005).

Studies have been carried out to determine the digestibility of ingredients commonly used in feed formulation for various fish species. Fagbenro (1998) evaluated the ADC protein for some oilseed meals such as soyabean meal, sunflower meal, peanut meal, roselle seedmeal, beniseed meal and cottonseed meal in the diet of *Clarias gariepinus*. The author reported that the ADC for protein of 80 – 87% for oilseed meals was lower than that of fishmeal. Oliva-Teles and Goncalves (2001) tested the effect of brewers yeast in the diet of seabass, it was reported that the ADC for protein of the control diet was higher than that of the 50% replacement level. The workers attributed this to the fact that brewers yeast was a plant protein. Similarly Baraigi *et al*. (2004) reported a higher ADC for protein of the reference diet when *Labeo rohita* was fed with *Leucaena* leafmeal based diets. Salim *et al.* (2004) compared the ADC for protein, drymatter, lipids of animal and plant ingredients in the diet of *Labeo rohita*. The workers reported higher values for animal ingredients than plant ingredient, they futher explained that the fibre, starch and imbalanced amino acids in plant ingredients might be the cause. The ADC protein, ADC drymatter and ADC lipids was evaluated for menhaden fishmeal and soyabean meal in the diet of sunshine bass, all the apparent digestibility coefficients were higher for fishmeal (Thompson *et al*., 2002). Olude *et al*. (2008) examined the effect of copra meal in the diet of *Oreochromis niloticus* and reported that ADC protein decreased with increasing inclusion level of copra meal. Sotolu and Sule (2011) reported a low ADC protein of 71.20% when *Clarias gariepinus*

was fed diets water hyacinth leafmeal. Mubarak *et al.* (2011) observed that when *Oreochromis niloticus* was fed water hyacinth based diets that the ADC protein, ADC drymatter and ADC lipids decreased as water hyacinth level increased. The authors reported that 20% inclusion level gave an acceptable ADC protein. Plaipetch and Yakuptiyage (2012) reported low ADC protein and ADC drymatter when Asian seabass *Lates calcarifer* was fed yeast fermented canola meal based diets. The low ADC protein was attributed to the level of phytic acid in canola meal. Generally apparent digestibility coefficients for most animal ingredients especially fishmeal are higher than plant ingredients (NRC, 1993).

2.8 Haematological Indices

Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Ighwela *et al*., 2013). It also provides a pathological reflection of the whole body, hence its usage in diagnosing the functional status of animals exposed to toxicants and sress induced conditions (Osuigwe *et al.,* 2005). Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviations directed towards the evaluation and use of non conventional sources of plant protein (Ogunji *et al.,* 2005). The inclusion of plant protein sources in the ration of fish requires investigation on limiting factors in the plant ingredients such as high crude fibre content and antinutritional factors as it may indicate a disturbance in the physiological processes of fish (Ogunji et al., 2005).

Seasonal variations, nutrition, size, age, sex, genetic properties, different management systems, population density, lack of food supply, diet and environmental stress have been reported to affect the haematological status of fish (Akinrotimi *et al*., 2010 ; Anyanwu *et al.,* 2011; Okorie-Kanu and Unakalamba, 2014). Other factors such as stress due to

transportation, acclimatization and sampling can also cause variations in haematological parameters (Akinrotimi *et al*., 2010). The haematological indices used for assessment include red blood cell count, haematocit (PCV), haemoglobin and white blood cell count.

2.8.1 Red blood cells

Red blood cells are the most abundant cells in the blood of a healthy animal. These cells are constantly produced and destroyed (when old or damaged). Red blood cells are important in assessing the oxygen carrying capacity of the blood (Diyaware *et al.*, 2013). Red blood cells contain haemoglobin and are responsible for carrying oxygen from the gills to the cells throughtout the body as well as carbon-dioxide from the tissues to the gills for excretion (Grupta *et al.*, 2013). A drop in the number of red blood cells implies a reduction in the level of oxygen that is been carried to the tissues and carbon-dioxide returned to the gills will be reduced. Low levels of red blood cells might be an indication of malnutrition and various sources of sress (Okorie-Kanu and Unakalamba, 2014). Grupta *et al.* (2013) reported that the number of red blood cells in fish is influenced by temperature and reproductive activities. Fishes are poikilothemic animals therefore increase in temperature leads to increased physiological activities which in turn increases the oxygen binding properties of the haemoblobin. There are other parameters which are derived from the red blood cell these include the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). High MCH shows that there is high concentration of haemoglobin in the cell which indicates good oxygen transport and healthy fish while low MCV and MCHC are indications of anemic conditions (Diyaware *et al*., 2013). The amount of red bloods cells most of the time shows an inverse relationship with the white blood cells this might be

because high red blood cell count lessens the requirement for large numbers of white blood cells (Grupta *et al.,* 2013).

2.8.2 Haemoglobin

Haemoglobin is the iron-containing metallo protein in the red blood cell that carries oxygen from the gills to the tissues (Grupta *et al.,* 2013). The oxygen carring capacity of blood is increased by the reversible binding of haemoglobin to oxygen, when binded with oxygen is referred to as oxy- haemoglobin. During physiological and environmental changes, the haemoglobin instead of binding with oxygen binds to other ions (hydrogen and carbon) thereby depressing haemoglobin-oxygen affinity (Gallaugher and Farrell, 1998).

2.8.3 Heamatocrit (PCV)

Heamatocrit is also knowed as packed cell volume and this is the percentage of red blood cell in circulation. The haematocrit is highly variable in fish and is influenced by a number of factors such as age and sex (Akinrotimi *et al*., 2010; Okorie-Kanu and Unakalamba, 2014). If the haematocrit is high it increases the total amount of oxygen the blood can carry (Diyaware *et al.,* 2013). Haematocrit is one of those parameters that showed a general correlation with fish activity, environmental changes such as water quality (Jawad et al., 2004). However both haemoglobin and haematocrit are dependent on the number of red blood cells (Diyaware *et al.,* 2013).

2.8.4 White blood cell

White blood cells are critical components of the immune system in a healthy animal. White blood cells may have a life span of few hours to years depending on the physiology of the animal. These cells are the first line of defence against infection and enables fish to fight stress (Diyaware *et al.,* 2013). There are different types of white blood cells namely

neutrophils, eosinophils, basophils, lymphocytes and monocytes (Grupta *et al.,* 2013). They protect the body by phargocytosis against foreign body and production of antibodies. Fish changes the number and proportion of white blood cells in response to enviromental fluctuations, unfavourable conditions and other stress related conditions (Akinrotimi *et al*., 2010; Anyanwu *et al.,* 2011; Okorie-Kanu and Unakalamba, 2014).

2.8.5 Haematology and fish diet

The effect of some non conventional plant ingredients on haematological parameters has been studied. Ogunji *et al.* (2005) evaluated the effects of pigeon pea based diets on the haematological parameters of *Clarias gariepinus*. The workers observed that the control diet had higher haematological parameters than the diets which contain pigeon pea. When jackbean meal was incorporated into the diet of hybrid catfish (*Heteroclarias*) it was observed that the haematological parameters decreased with increasing level of jackbean meal which the workers suggested might be due to nutrient deficiency (Osuigwe *et al.,* 2005). Sotolu and Faturoti (2009) fed *C. gariepinus* with *Leucaena* seedmeal based diets and observed there was a decrease in red blood cells, packed cell volume and haemoglobin as inclusion of *Leucaena* seedmeal increased. The authors inferred that lower inclusion level would give better health status. Aderolu *et al*. (2011) reported that the inclusion of sheabutter and palmoil at 1.5% inclusion level did not have negative impact on the haematological parameters of *C. gariepinus*. Anyanwu *et al.* (2011) fed hybrid catfish with diets containing *Carica papaya* leafmeal and observed that test diets compared favourably with control for red blood cells, pack cell volume and haemoglobin while the white blood cells showed wide variations. The effect of dietary maltose on the haematological parameters of tilapia was evaluated and it was reported that 35% maltose inclusion level

caused a slight decrease in these parameters (Ighwela *et al*., 2013). Fagbenro *et al*. (2013) incorporated sunflower meal and sesame meal into the diet of *C. garepinus* and the effect on haematological parameters was determined. The workers observed that there was no significant difference in haematological parameters of the control and test diets. When Dienye and Olumuyi (2014) fed *C. gariepinus* with *moringa* leafmeal based diets most of the haematological parameters decreased with increasing level of the meal except the white blood cells. The workers stated that 10% *Moringa* leafmeal inclusion level had no adverse toxicological effect on *C. gariepinus*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Location

The experiment was conducted in the indoor hatchery of Mando Fishfarm Kaduna which is within latitude 10° 31'North and longitude 7° 26'East in the northern guinea savannah zone of Nigeria

3.2 Collection and Processing of *Leucaena leucocephala* Leaves

Leucaena leaves were collected from trees planted at the forage section of the National Animal Production Research Institute (NAPRI), Zaria. The leaves were hand picked and leaflets were separated from the long leaf stalk. The leaves were divided into four portions of 500g each and subjected to three processing methods, namely sundrying, soaking in water at room temperature and immersed in boiled water for 5 minutes. The first 500g of leaves were spread on a on a concrete slab outside the hatchery and sundried for 24 hours, another 500g was sundried for 48 hours while the last 500g was sundried for 72 hours (Agbede, 2005; Ayssiwede *et al.*, 2011). These were coded SD_1, SD_2 and SD_3, respectively.

.The second set of leaf samples were soaked in water at room temperature (30° C). Ten litres of water was measured into a rubber bowl after which 500g of the leaf sample was soaked in it for 24 hours. Another 500g of leaf sample was soaked for 48 hours and the last 500g for 72 hours. The leaves were removed from the water and allowed to dry homogenously in the sun until they became crispy (Wee and Wang, 1987; Amisah *et al*., 2009). These samples were labeled SW_1, SW_2 and SW_3 respectively. Another leaf sample of 500g was immersed in1000 litres of boiled water (100° C) for 5 minutes. The boiled *Leucaena* leaf sample was then sundried for 24 hours until it became crispy and coded SB_5

while the last sample (unprocessed) consists of 500g of *Leucaena* leaves and was coded SU.

All the 8 leaf samples were ground into fine powder using a hammer mill, labeled and separately packed into polythene bags. These were stored for proximate analysis, amino acid analysis, antinutrients and mineral analysis.

3.3 Formulation of Experimental Diets

Imported Danish Fishmeal (72% crude-protein), vitamin premix, groundnut cake maize, soyabean cake and bonemeal were purchased from a reputable feed miller in Kaduna (Dasolag Nig Ltd) while salt and cassava starch were purchased from a local market in Kaduna. The proximate analysis of the ingredients was carried out and the composition is shown in Table 3.1. Each feed ingredient was weighed according to the formulation and grounded into fine powder to improve pellet quality using a hammer mill. The feed ingredients were mixed together and then pelleted into 2mm and 4mm using a locally fabricated industrial pelleting machine according to methods described by Gabriel *et al.* (2007) then the pellets were sundried. Five experimental diets containing 0, 10%, 20%, 30% and 40% *Leucaena* leaf meal were formulated. These diets were formulated to contain approximately 42.5-45% crude protein calculated according to Pearson's square method (ADCP, 1980). Fishmeal based diet and commercial feed served as control so were coded SWC and SWCF while 10%, 20%, 30% and 40% inclusion levels of *Leucaena* leaf meal were coded SW10 SW20, SW30 and SW40 respectively. Representative samples of each diet were kept for proximate analysis while the rest was packed into polythene bags. The gross composition of experimental diets is shown in Table 3.2.

Table 3.1: Proximate Composition of Ingredients used in Feed Formulation

Ingredient	Mo%	CP%	Ash%	CF%	EE%	NFE%
Fishmeal	6.7	71.9	12.4	0.9	6	2.1
Soyabean cake	7.92	44	6.65	6.5	7.6	27.33
Yellow maize	9.13	9	1.9	3.0	3.8	73.15
Groundnut cake	9.09	45.5	6.27	5.7	7.25	26.19
Leucaena leafmeal	8.15	22.08	4.56	21.5	5.37	38.33

Mo – Moisture
CP – Crude protein
EE – Ether Extract
CF – Crude fiber
NFE – Nitrogen free extract

Table 3.2: Gross Composition of Experimental Diets

Ingredients (g/100g)	SWC	SW10	SW20	SW30	SW40
Fishmeal	30	27	24	21	18
Yellow maize	20	20	20	20	20
Groundnut cake	10	10	10	10	10
Soyabean cake	38	38	38	38	38
Leucaena leafmeal	-	3	6	9	12
Bonemeal	0.5	0.5	0.5	0.5	0.5
Salt	0.1	0.1	0.1	0.1	0.1
Lysine	0.2	0.2	0.2	0.2	0.2
Methonine	0.3	0.3	0.3	0.3	0.3
Vitamin C	0.3	0.3	0.3	0.3	0.3
Premix	0.1	0.1	0.1	0.1	0.1
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

SWC – control diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level

3.4 Procurement of Experimental Fish

Five hundred fingerlings of *Heterobranchus longifis* (6.0g-7.0g) were purchased from National Institute for Freshwater Fisheries Research (NIFFR) New-Bussa and transported in 50 litres "Jerry-cans". The fingerlings were acclimatized in circular fiberglass tanks in Mando fishfarm indoor hatchery for 14 days and fed with coppens (an imported feed from Netherlands) then starved for 24 hours prior to the commencement of the experiment. The fingerlings were randomly sorted and stocked at 20 fingerlings per tank.

3.5 Experimental Design

The experiment was conducted using a completely randomized design (Appendix 1). A total of 18 white plastic (1000 litre) tanks with dimension 1m x 1m x 1m were used (Appendix 2). Water was supplied from a bore hole fitted with overhead plastic tanks of 5,000 litres capacity. The experimental tanks were washed and allowed to dry in the sun before taken inside the hatchery. The tanks were initially filled with water one-third the size of the tank and this level was increased to two-thirds as the experiment progressed. Each tank had an inlet pipe within holes bore in the pipe so that the water is sprinkled to aid aeration

3.6 Experimental Procedure

The experiment consisted of six treatments and each treatment had three replicates. The treatments are two controls, a commercial feed and a compounded feed, then feeds with *Leucaena* leafmeal at 10% 20% 30% and 40% inclusion levels.

Fishes were fed at 3% body weight (Lovell, 1989) and were fed twice daily between the hours of 9.00-10.00am and 5.00pm-6.00pm. The ration was adjusted after every two weeks

according to the weight increase recorded when the fishes were sampled. The fishes were removed from the tank with a scoop net after draining the water and transferred to a pre-weighed bowl. The bowl and the fishes were then weighed with a digital scale. The weight of the fish was determined by substracting the weight of the bowl from the total weight.

The water in the tank was reduced by one third daily and fresh water added. Left over feed and feaces were flushed out daily while any dead fish found was removed and recorded. The experiment lasted 252 days.

3.7 Determination of Waterquality Parameters of Experimental Tanks

Water quality parameters were taken twice a week, before feeding the fish. Water temperature was measured using mercury in-glass thermometer calibrated in degree centigrade. Oxygen was determined using a digital oxygen meter while pH was determined with a pH meter (pH-009) model.

3.8 Proximate Analysis

3.8.1 Determination of ash content (AOAC, 1990)

Principle

The ash content was determined from the loss in weight that occurs during igniting at 550°C in muffle furnace which is enough to allow all organic matter to burn off without permiting any appreciable decomposition of the ash constituents.

Procedure**:** Crucibles were cleaned and dried in the oven. After drying they were corked in the desiccator and weighed (W_1). After which 2g of the grounded sample was placed in the crucible and weighed (W_2). They were transferred into furnace and set to 550°C. The Sample was incinerated in the furnace for 8 hours. The crucible containing the

ash was removed and cooled in the desiccator and weighed (W_3). The weight of the residue in the crucible corresponds to the organic matter content.

% Ash was calculated as:

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times \frac{100}{1}$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

3.8.2 Determination of moisture content (AOAC, 1990)

Principle: This was done based on the difference between the net weight and the weight after drying to a constant (at 100°C for 24 hours).

Procedure: Crucibles were washed and dried to a constant weight in an air oven at 100°C, they were later removed and cooled in a desiccator and weighed (W_1). Two grams of the grounded sample was placed in the weighed moisture dish (W_2) the crucible containing the sample was kept in an oven at 100° C for 24 hours and weighed. It was kept back in the oven and reweighed after about 3 hours to ensure a constant weight (W_3). The moisture content was calculated as:

$$\%\text{Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

3.8.3 Determination of lipid content (AOAC, 1990)

Principle: This is the continuous extraction of fat content from the sample using suitable solvent like petroleum ether (40-60° C) in a soxhlet extractor. Principle is that nonpolar component of the sample are easily extracted into organic solvent ether.

Procedure: Two round bottom flasked were clean and few anti bump granules were added to prevent bumping. 300mls of petroleum ether (40-60° C) boiling point were poured into the flask. These were fitted into the soxhlet extraction units. Extraction thimbles were weighted and 20ml of the sample was placed into it and weighed (W_1), the thimble was fixed into the soxhlet extraction unit with forceps and cold water circulation put on.The heating mantle was switched on and solvent refluxing was adjusted at a steady rate. Extraction was carried out for eight hours. The thimble was removed and dried to constant weight in an oven at 70° C and was weighed (W_2). The extractible lipid was calculated as:

$$\% \text{ Lipid} = \frac{\text{Wt of lipid extracted}}{\text{Wt of dried sample}} \times \frac{100}{a}$$

Where the weight of lipid extracted is given by the loss in weight

W_1-W_2 of thimble content after extraction.

3.8.4 Determination of nitrogen and crude protein

Protein:-These are the major compounds containing nitrogen (minor nitrogenous ingredients of food include amino acids, purines, ammonium salts and thiamine). So nitrogen is used as an index of the protein termed “crude protein” as distinct from true protein.

a. Digestion: It involves oxidation of the organic matter with sulphuric acid and reduction of nitrogen to ammonium sulphate.

$$\text{Conc } H_2S0_4 \longrightarrow (NH_4)_2SO_4$$

(N-free)

$$C \xrightarrow{\text{Oxidation}} CO_2$$

(Organic matter)

b. Distillation**:-** It involves liberation of the ammonia by sodium hydroxide. The ammonia is trapped in an (excess) Boric acid and titrated with hydrochloric acid (HCl).

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2Na_2SO_4 + 2H_2O + 2NH_3$$

$$NH_3 + H_2O \longrightarrow NH_4OH$$

$$H_3BO_3 + 3NH_4OH \longrightarrow (NH_4)_3BO_3 + H_2O$$

c. Titration: The back titration method is employed i.e the ammonia reacts with the Boric acid in the receiving flask and the amount of excess acid is determined by titration with HCl.

$$(NH_4)_3BO_3 + 3HCl \longrightarrow NH_4CL + H_3BO_3$$

The percentage total nitrogen was calculated and crude protein was estimated by multiplying the percentage nitrogen with the standard conversion factor of 6.25.

METHOD**:-** Exactly 2g of the sample was weighted using Mettle 50 weighing balance into 100ml kjedahl flask and few antibump granules, one gram of catalyst ($K2SO_4$+anh $CuSO_4$) was added to speed up the reaction. The flask was fixed on the Kjeldahl digestion rack and heated slowly at first until fretting subsides and then more rigorously with occasional rotation of the flask to ensure even digestion and avoid over heating of content.

After a clear solution was obtained, the sample was transferred to 100ml volumetric flask and diluted to mark with distilled water after cooling. 10mls of the diluted sample or digest was pipetted into Markham semi macro nitrogen still and 10mls of 40% NaOH solution

added. The sample was distilled liberating NH_3 into 100ml conical flask containing 10ml of 40ml of 40% Boric acid and 2 drops of methyl red indicator. Distillation was continued until the pink colour of the indicator turned greenish.The control was titrated with 4% Boric acid with end point indicated by a change from greenish to pink colour. The volume of acid used for each sample distillate was noted as well as that of blank.

% total N per sample was calculated as:

$$\%N = \frac{V_1 - V_0 \times M \times 14 \times 100 \times 100}{0.2 \times 1000 \times 10 \times 1}$$

V_0 = vol. of HCl required for blank

V_1-vol. of the HCl required for 10ml sample solution.

M= Molarity of acid (0.1m)

14=atomic weight of N_2

100=total volume of digest

100=% conversion

10=volume of distillate

0.2= amount of sample taken in gram

1000=to convert to litre.

The crude protein was calculated as % crude protein (cp) = 6.25xN

NOTE**:** Protein contains 16% N_2. This makes the general conversion factor to be 6.25

3.8.5 Determination of crude fibre (AOAC, 1990)

Principle: The bulk of roughage in foods is referred to as the fibre. This is estimated as crude fibre.

Procedure**:** Two grams of the grounded sample was placed in a round bottom flask. 100ml of 0.25M H_2S0_4 was added and the mixture boiled under reflux for 30minutes. The insoluble matter was washed several times with hot water until it was acid free. Thereafter, it was transferred into a flask containing 100ml of hot 0.312M NaOH solution. The mixture was boiled again under reflux for 30 minutes and filtered under suction, the insoluble residue was washed with hot water until it was base free. It was dried to constant weight in an oven at 100°C, cooled in a desiccator and weighed (C_2). The weighed sample was incinerated in a furnace at 550°C for 2hours. It was put off and allowed to cool down. It was removed cooled in a desiccator and weighed (C_3). The crude fibre was calculated as the loss in weight on ashing:

Weight of the original sample = W

$$\% \text{ crude fibre} = \frac{C_2 - C_3}{W} \times \frac{100}{1}$$

3.8.6 Nitrogen free extract

The nitrogen-free extract (NFE) which is the digestible portion of the carbohydrate contained in the sample and was determined by subtracting the percentage sums of moisture, crude protein, crude fat, crude fibre and ash from 100.

3.8.7 Energy values

The energy values of the leaf samples and feed was calculated as described by Pauzenga (1985). Crude protein x 37 + ether extract x 81.8 + nitrogen free extract x 35.

3.9 Determination of Anti-nutritional Compounds

3.9.1 Cyanogenic glycoside

The procedure of AOAC (1990) was used in the determination of cyanogenic glycosides. To determine cyanogenic glycosides in the sample, the alkaline titration method was used. 5g of sample was placed in a 200ml conical flask. 50ml of distilled water was added. The content of the flask was stirred and allowed to stand for 4 hours.The filtrate was collected into a beaker using glass wool which was placed in a funnel. The filtrate was steam distilled into 20ml of 2.5% Sodium hydroxide. About 75ml of the distillate was collected. This was titrated with 0.02N Silver nitrate after the addition of 8ml of 6N Ammonium hydroxide and 2ml of 2% Potassium iodide. Permanent turbidity indicated end point.

1ml 0.02N $AgNO_3$ = 1.08 mg HCN

3.9.2 Phytic acid

Phytate phosphorus was determined by the method of Wheeler and Ferrel (1971). A known weight of each ground sample was soaked into 100ml of 2% HC1 and 5hours and filtered. $25cm^3$ of the filtrate was taken into a conical flask. $5cm^3$ of 0.3% potassium thiocynate solution was added. The mixture was titrated was titrated with standard solution of $FeC1_3$.

3.9.3 Tannin

Tannin was determined using the standard method described by AOAC (1990). Two grams of the dried sample was boiled with 300ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non-absorbent cotton wool. A volume of 25ml of the infusion was measured into a 2 litre porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution changed green, then few drops of 0.1N-9.005 potassium permanganate was added. The titre was multiplied by 0.0066235 to obtain the amount of tannin in the sample as shown in the equation is below:

0.1N oxalic acid = 0.0066235g tannin

3.9.4 Saponin

The standard method of AOAC (1990) was used to determined saponin in the samples. A gravimetric method employing the use of a soxhlet extractor and two different organic solvents was used. The first solvent extracts lipids and interfering pigments while the second solvent extracts the saponins proper.

A known weight of the dried ground sample was weighed into a thimble and transferred into the soxhlet extraction chamber fitted with a condenser and a round bottomed flask. Some quantity of acetone enough to cause a reflux was poured into the flask. The sample was exhaustively extracted of its lipids and interfering pigments for 3 hours by heating the flask on a hotplate and the solvent was distilled off.

For the second extraction, a pre-weighed round-bottom flask was fitted into the soxhlet apparatus which bore the thimble containing the sample. Then methanol was poured into

the flask. The methanol was enough to cause reflux. The saponin was then exhaustively extracted for 3hours by heating the flask on a hotplate after which the solvent was distilled off. The flask was reweighed. The difference between the final and initial weights of the flask represented the weight of the saponins extracted.

$$\% \text{ Saponins} = \frac{\text{Weight of saponins}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.9.5 Determination of mimosine

The mimosine content of the leafmeal was determined by the rapid colorimetric method of Megarity (1978). To extract the mimosine, samples were digested with 0.1N HC1, filtered and clarified by removing plant pigments with activated carbon. Ferric chloride (0.5%) was then added to the mimosine extracts to develop a coloured compound, the intensity of which was estimated by measuring the absorbance at 535nm in a Pye Unicam PU 8650 visible spectrophotometer. The concentration of mimosine was then calculated from a standard curve using pure mimosine (Sigma Chem., Ltd.London).

3.10 Determination of Amino Acid Profile

The Amino Acid profile in the known sample was determined using methods described by Benitez (1984). The known sample was dried to weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM).

Defatting sample:

A known weight of the dried sample was weighed into extraction thimble and the fat was extracted with choloform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (1990) the extraction lasted for 15hrs.

Nitrogen determination

A small amount (200mg) of ground sample was weighed, wrapped in Whatman filter paper (01) and put in the Kjeldhal digestion flask. Concentrated suphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

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

The distillate was then titrated with standardize 0.01N hydrochloric acid to grey coloured end point, the percentage nitrogen in the original sample was calculated using the formula:

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a = Titre value of the digested sample

b = Titre value blank sample

v = Volume after dilution (100ml)

w = Weight of dried sample (mg)

C = Aliquot of the sample used (10ml)

14 = Nitrogen constant in mg

Hydrolysis of the sample

A defatted sampleof 1mg-500mg was weighed into glass ampoule. 7ml of 6 NHCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g (methionine and cystiene). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C for 22hours. The ampoule was allowed to cool before breaking it open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6N HCl during hydrolysis.

The filtrate was then evaporated to dryness at 40° C under vacuum in a rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into TSM analyzer

The amount loaded was between 5 to 10 microlitres. This was dispended into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an Analysis lasted for 76 minutes.

Method of Calculating Amino Acid Values from the Chromatogram Peaks

The net height of each peak produced by the chart recorder of TSM (each representing an Amino acid) was measured. The half-height of the peak on the chart was found and width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height with the width at half-height.

The norclcucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE: = \frac{\text{Area of Norcleucine Peak}}{\text{Area of each amino acid}}$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{std} = NE_{std} \times \text{Molecular weight} \times uMAA_{std}$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using following formular:

$$\text{Concentration (g/100g protein)} = NH \times W@NH/2 \times S_{std} \times C$$

$$\text{Where } C = \frac{\text{Dilution} \times 16 + NH \times W\ (nlcu)}{\text{Sample Wt (g)} \times N\% \times 10 \times \text{vol. loaded}}$$

Where: NH= Net height

W = Width at half height

nlcu = Norleucine

3.11 Mineral Analysis (AOAC, 1990)

Digestion:

One gram of each sample was weighted into a clean dried crucible and placed in a furnace. The temperature was raised to 550°C and allowed to ash for 3 hours. The contents were cooled, and 10ml of Aqua regia was added to the sample in crucible and then filtered into a 25ml volumetric flask to make up to volume with de ionised water. Calcium, magnesium and sodium were read with PG 990 Atomic Absorption Spectrophotometer and potassium was read using Jenway flame photometer.

Phosphorus

A sample of 1ml aliquot of the plant extract was pipette into a 25ml volumetric flask and 10ml distilled water was added, 4ml of reagent B was then added to make up to 25ml with distilled water and allowed to stand for 15minutes for the colour to develop. The phosphorus content in solution was then determined on a Jenway 6705 uv/vis spectrophotometer.

3.12 Growth Parameters and Nutrient Utilization

The following growth parameters were calculated as described by Castell and Tiews (1980).

a. Mean Weight Gain (MWG)

$= W_2 - W_1$ (g)

Where W_2 = Final mean weight (g)

W_1 = Initial mean weight (g)

b. Specific Growth Rate (SGR) = $\frac{LnW_2 - LnW_1}{T_2 - T_1} \times 100$

Where W_2 = Final weight

W_1 = Initial weight

$T_2 - T_1$ = Time interval between W_2 and W_1 in days

Ln = Natural log

c. Food Conversion Ratio (FCR)

$$= \frac{\text{Total Feed consumed (g)}}{\text{Weight gain by fish (g)}}$$

d. Survival Rate (SR) =

$$= \frac{\text{Total number of fishes harvested}}{\text{Initial number of fish stocked}} \times 100$$

e. Protein Efficiency Ratio (PER)

$$= \frac{\text{Weight gain by fish (g)}}{\text{Protein intake by fish (g)}}$$

Where protein intake

$$= \frac{\text{Total feed consumed}}{\text{Crude protein in feed}} \times 100$$

f. Apparent Net Protein Utilization (ANPU)

$$= \frac{\text{Fish protein gain}}{\text{Protein consumed}} \times 100$$

Where fish protein gain

= final body protein – initial body protein

3.13 Digestibility Determination

The indirect method which involves the use of an indigestible marker was used for the experiment (Lovell, 1989). Chromic oxide (Cr_2O_3) was used as an indigestible marker. It is assumed that all the chromic oxide consumed will pass through the digestive tract and appear in the feaces. Analysis of the difference of chromic oxide percentages in feed and feaces was used to calculate digestibility. Diets were formulated to accommodate 0.5kg/100kg chromic oxide and were fed to fishes for 14 days. Feaces were collected by dissecting the intestine as described by Belal (2005). Feaces were collected 10-11hours after feeding by dissecting the distal end of the intestine close to the anus, after which the feaces were gently squeezed out. Feaces from each treatment were pooled together to have enough feaces for analysis. Feaces were stored in labelled petri-dishes and frozen at -4° C. The feaces and feed was then analyzed for proximate composition and chromic oxide.

3.13.1 Chromic oxide determination

Chromic Oxide level was determined according to the methods described by Furukawa and Tsukahara (1966). Approximately 50-100mg of sample was digested with 5mls of concentrated nitric acid followed by 3mls of perchloric acid, turning the digesta from green to yellow. This was then diluted to 100mls with distilled water and absorbance measured at 350nm with a spectrophotometer.

Digestibility of the different experimental diets was estimated to determine bioavailability of nutrients. The Apparent digestibility coefficient of crude protein, lipid, carbohydrate and dry matter was determined according to standard formula (De silva and Anderson, 1995)

$$ADC\ \% = 100 - \left(100 \times \left\{\frac{\%\ \text{marker in feed}}{\%\ \text{marker in feaces}} \times \frac{\%\ \text{nutrient in feaces}}{\%\ \text{nutrient in diet}}\right\}\right.$$

3.14 Determination of Haematological Parameters

At the end of the feeding experiment 2 fishes were taken per tank and 6 per treatment. The average weight of the fishes was 150g. Blood samples were taken from the fishes according to methods described by Wedemeyer and Yasutake (1977). The fish was placed on its back in a trough and blood was collected from the posterior end of the abdomen towards the tail using no 21 needle. The blood was emptied into EDTA (Ethylene Diamine Tetra Acetate) treated bottles to prevent coagulation. After which the blood was analyzed according to standard haematological procedures as described by Blaxhall and Daisley (1973).

3.14.1 Haemoglobin count (Hb)

Haemoglobin count was determined using the cyanmethemoglobin method. Blood was mixed with Drabkin's reagent, which contain Ferricyanide and cyanide. The Ferricyanide oxidizes the iron in the haemoglobin. The methemoglobin unites with the cyanmethemoglobin which produced a colour which was measured in colorimeter.

Procedure: 5ml of Drabkin's solution was measured into a clean, dry test-tube. The blood was mixed thoroughly and exactly 0.02ml was transferred into the reagent. The tube was

allowed to stand for 10 minutes after the reading was done in the spectrophotometer at 540nm, using Drabkin's reagent as blank. The haemoglobin value was read in grams per deciliter (g/d1) from the standard table.

3.14.2 Haematocrit determination (PCV)

The haematocrit which is known as packed cell volume (PCV) of each fish was determined by centrifugation of blood using hematocrit centrifuge. By definition hematocrit is the percentage volume of packed red cells following centrifugation. The purpose of centrifugation was to obtain a maximum packing of the red blood cell so that little or no plasma remain trapped between the cells. The packed cell volume is a function of red blood cell size and the number of cells per unit volume. This was done by centrifuging the blood sample for 5 minutes at 10,000 (rpm) as described by Lagler *et al.* (1977). The force obtained by centrifugation depends upon the radius at which a particle is distant from the centre of revolution. The radius is the distance in centimeter from the centre of the shafts to the bottom of haematocrit tube.

3.14.3 Red blood cell count (RBCC)

The red blood cell was determined using the manual haematocytometer method. A small amount of whole blood was accurately diluted with a fluid, which is isotomic with blood. The diluted blood was mounted in a hematocytometer and the number of cells in a circumscribed volume was counted. Blood was drawn to the 0.5 mark in a clean dry red cell pipette then wiped and the level of blood was carefully adjusted to the 0.5 mark. The pipette was plunged quickly into the red cell-diluting mix the blood and diluents. The pipette was shaken for few seconds. The content of the pipette was well shaken before carrying out the count. The counting chamber and cover glass were cleared with 70%

alcohol and wiped dry with soft gauze before the count was mounted. The cover glass was placed over both ruled area dampen first to anchor. Immediately after shaking 6-8 drops were expelled from the pipette and the tip of the pipette was wiped.The tip of the pipette was held to the edge of the cover glass and enough fluid was allowed to flow into the cover glass to fill the chamber. One drop was used to fill the chamber. If the chamber is flooded, it must be cleaned and mounted again. The central square millimeter in the counting chamber was used for counting the erythrocytes. The cells contained in 80 of the 400 small squares or 0.2 squares millimeter were counted.

3.14.4 White blood cell count (WBCC)

A small amount of blood was accurately diluted with 2% acetic acid, which destroys the non nucleated erythrocyte and makes clearly visible the nuclei of the leukocytes. Nucleated erythrocytes remain intact. Blood was drawn to the 0.5 mark in a clean, dry WBC pipette, the outside of the pipette was wiped and the level of the blood carefully adjusted to the 0.5 mark. The pipette was plunged quickly into the 2% acetic acid, and the fluid was drawn to the 11 mark (1:20 dilution). The pipette was twirled to mix the blood and diluents. The pipette was removed from the diluents then the rubber tubing was well mixed by shaking before an accurate count was done. Holding the pipette in a horizontal position between the thumbs and index finger and shaking vigorously up and down for about 5 minutes performed proper mixing. The bead in the bulb was moved from one point to the other during the shaking. The counting chamber and cover glass were cleaned with 70% alcohol and wiped dry with soft gauze before the count was mounted. The cover glass placed over both ruled areas. It was anchored by dampening so that the chamber could be inverted without the cover glass falling. Immediately after shaking, 3 drops were rapidly expelled

from the pipette and the tip of the pipette was wiped. The tip of the pipette was held to the edge of the cover glass and enough fluid was allowed to flow under the cover slip to fill the chamber. The leukocytes were counted under low power in the four large squares. As in the red blood cell touching the left and upper margins were included: those on the right and lower margins were not checked within 10 cells, the central square was counted.

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by Dacie and Lewis (2001).

MCV (FL) = packed cell volume /RBC ($10^6 \mu L^{-1}$) x 10

MCH (pg) = Hb (gdL^{-1})/RBC ($10^6 \mu L^{-1}$) x 10

MCHC (%) = Hb ($gdL^{-1)}$/packed cell volume x 100

3.15 Carcass Analysis

The proximate composition of the fish carcass was carried out before and after the experiment (AOAC, 1990). Before the experiment five fingerlings were randomly chosen for proximate analysis. After the 252days experiment, 2 fishes per tank were sacrificed for proximate analysis.

All chemical analysis was carried out in triplicates. Differently processed *Leucaena* leaves and experimental feed were analyzed for proximate composition (AOAC, 1990)

3.16 Cost Benefit Evaluation

The feed production cost was based on current market prices of ingredients at Kaduna, Nigeria. The economic evaluations were calculated employing the methods described by New, (1989); Sogbesan and Ugwumba (2006).

Feed Cost (N/kg) = Total Cost of ingredients required to produce a kilogramme of each diet

$$\text{Profit Index} = \frac{\text{Value of fish (N)}}{\text{Cost of feed (N)}}$$

$$\text{Incidence Cost} = \frac{\text{Cost of feed (N)}}{\text{Weight of fish produced (kg)}}$$

$$\text{Benefit Cost Ratio} = \frac{\text{Total cost}}{\text{Total sales (N)}}$$

Where Total Cost = Total Fixed Cost (N) + Total Variable Cost (N)

3.17 Statistical Analysis

All data collected were subjected to one way analysis of variance (ANOVA) to test for significant difference among treatments using SPSS version 15.0 followed by Duncan's multiple range test (DMRT) which was used to separate significantly different means. The level of significance set for treatments was $P \leq 0.05$. Principal component analysis was carried out to establish the relationship between haematological indices and growth parameters. The growth parameters were subjected to trend analysis to determine their patterns at different inclusion levels.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Differently Processed *Leucaena* Leaves

The result of the proximate analysis of differently processed *Leucaena* leaves is as shown in Table 4.1.

4.1.1 Crude protein

The result of this study shows that the crude protein (Table 4.1) in the sundried treatments were higher than all the other treatments, with SD_3 (sundried for 72 hours) having the highest crude protein of 33.22% followed by SD_2 (sundried for 48 hours) with 31.83% and then SD_1 (sundried for 24 hours) 30.66%. The treatments that were soaked in water at room temperature had signjficantly lower crude protein. Soaking in water at room temperature for 72 hours (SW_3) was 22.08% followed by (SW_2) soaking in water at room temperature for 48 hours 16.97% and the least 16.46% in soaking in water at room temperature for 24 hours (SW_1). The crude protein value in the sundried treatments were significantly different ($P \leq 0.05$) from the treatments that were soaked in water at room temperature.

4.1.2 Ether extract

In the sundried treatments, the ether extact as shown in Table 4.1 was highest (5.68%) in SD_3 followed by SD_2 with (5.50%) and lowest (4.99%) in SD_1. Among the soaking in water at room temperature treatments, SW_1 had the highest (5.58%) followed by SW_3 (5.37%) and then SW_2 (5.23%). The (raw) SU and SB_5 (soaking in boiled water 100° C for

5 minutes) had values of 5.22% and 5.40%, respectively. The ether extract in the three processing methods did not follow any definite pattern.

4.1.3 Crude fibre

The crude fibre content of the sundried treatments were significantly different ($P \leq 0.05$) from the soaking in water at room temperature and soaking in boiled water 100° C for 5 minutes treatments as shown in Table 4.1. SW_3 had the highest (21.50%) among all the treatments while SD_3 gave the lowest (9.56%). Treatments SW_1, SW_2, SD_1, SD_2 and SB5 had values of 18.80%, 20.35%, 12.73%, 10.56% and 14.26%, respectively

4.1.4 Ash

The ash content (Table 4.1) of the sundried treatments were significantly different ($P \leq 0.05$) from the soaking in water at room temperature and soaking in boiled water 100°C for 5 minutes treatments. Treatments SD_1 and SD_2 had the highest (7.07%) then SD_3 which had 7.03%. The SB_5 treatment had a value of 6.22% while SW_1, SW_2 and SW_3 had values of 5.95%, 5.61% and 5.56 %, respectively.

Table 4.1 Proximate composition of differently processed *Leucaena* leaves

(g/100g)	Treatments							
Parameters	SU	SD_1	SD_2	SD_3	SW_1	SW_2	SW_3	SB_5
Moisture	8.76± 0.38^{b}	10.13±0.13^{a}	10.21±0.66^{a}	10.64±0.53^{a}	5.97±0.34^{c}	5.84±0.82^{c}	8.15±0.1.22^{b}	7.77±0.73^{b}
Ash	6.13±0.13^{ab}	7.07±0.88^{a}	7.07±0.17^{a}	7.03±0.16^{a}	5.95±0.28^{b}	5.61±0.16^{b}	5.56±0.81^{c}	6.22±0.49^{ab}
Ether extract	5.22±0.11^{bc}	4.99±0.03^{c}	5.50±0.18^{ab}	5.68±0.20^{a}	5.58±0.17^{ab}	5.23±0.15^{bc}	5.37±0.25^{ab}	5.40±0.36^{ab}
Crude protein	30.25±0.38^{c}	30.66±0.51^{bc}	31.83±0.82^{ab}	33.22±0.23^{a}	16.46±0.36^{f}	16.97±0.82^{f}	22.08±1.30^{e}	28.31±1.26^{d}
Crude fiber	15.35±1.07^{c}	12.73±0.28^{d}	10.56±0.29^{e}	9.56±0.20^{e}	18.80±0.08^{b}	20.35±1.21^{ab}	21.50±2.73^{a}	14.26±0.48^{cd}
Nitrogen free extract	35.23±1.51^{bcd}	34.42±1.32^{cd}	34.84±1.42^{bcd}	33.94±0.44^{d}	47.25±0.63^{a}	45.99±0.64^{a}	38.33±4.66^{b}	38.03±2.20^{bc}
Energy(Kcal)	2747.16± 96.84^{c}	2843.04± 27.63^{ab}	2881.54± 30.26^{a}	2724.93± 26.39^{c}	2665.48± 19.32^{d}	2597.62± 13.98^{d}	2807.50± 98.60^{abc}	2779.40± 40.35^{bc}

Note: Means with the same superscript within the same row were not significantly different (P>0.05)

Legend

SU – raw

SD_1 – Sundried for 24 hours

SD_2 – Sundried for 48 hours

SD_3 – Sundried for 72 hours

SW_1 –Soaking in water at room temperature for 24 hours

SW_2 –Soaking in water at room temperature for 48 hours

SW_3 –Soaking in water at room temperature for 72 hours

SB_5 – Soaking in boiled water (100° C) for 5 minutes

. 4.1.5 Nitrogen free extract (NFE)

The soaking in water at room temperature for 24 hours (SW_1) had the highest (47.25%) among all the treatments followed by (SW_2) soaking in water at room temperature for 48 hours which had 45.99% then (SW_3) soaking in water at room temperature for 72 hours with 38.33%. Soaking in boiled water 100° C for 5 minutes (SB_5) had higher nitrogen free extract (38.03%) than sundried for 48 hours (SD_2), sundried for 24 hours (SD_1) and (SD_3) sundried for 72 hours which had values of 34.42%, 34.84% and 33.94%. The nitrogen free extract values of the sundried treatments were significantly different ($P \leq 0.05$) from the soaking in water at room temperature treatments as shown in Table 4.1.

4.1.6 Energy (KCAL)

The highest energy value (Table 4.1) of 2881.54 kcal was recorded for SD_2 followed by SD_1 with 2843.04 kcal and then SW_3 had 2807.50 kcal. Treatment SD_3 had 2724.93 kcal, SW_2 and SW_1 had lower values of 2597.62 kcal and 2665.48 kcal while SB_5 had 2779.40 kcal. The energy levels of the different processing methods did not follow any definite pattern.

4.2 Amino Acid Composition of Differently processed *Leucaena* leaves

The Amino acid composition of differently processed *Leucaena* leaves as presented in Table 4.2 shows that there was a reduction in all the essential amino acid values for the processed *Leucaena* leaves when compared to the raw sample (SU). The sundried treatments had higher values for all essential amino acids followed by soaking in boiled water 100° C for 5 minutes then the soaking in water at room temperature treatments with

the least. There was significant difference ($P \leq 0.05$) in the amino acid values of the sundried treatments and the soaking in water at room temperature treatments.

4.3 Antinutrients composition of differently processed *Leucaena* leaves

The antinutrients composition of differently processed *Leucaena* leaves is presented in Table 4.3. This study shows that there was a reduction in antinutrients of processed leaves when compared with the raw sample (SU). Generally, the antinutrients were significantly higher ($P \leq 0.05$) in the sundried treatments followed by soaking in boiled water 100° C for 5 minutes then the soaking in water at room temperature treatments had lower values.

4.3.1 Phytate

The phytate content (Table 4.3) was highest (0.71mg/100g) in SD_3 followed by SD_2 0.66mg/100g, then SD_1 with 0.61mg/100g. Among the soaking in water at room temperature SW_1 had the highest value of 0.16mg/100g, followed by SW_2 with 0.13mg/100g while SW_3 had the least value of 0.10mg/100g. Treatment SB_5 gave a value of 0.25mg/100g. Treatments SW_1, SW_2, SW_3 gave 82-90% reduction of phytate while SD_1, SD_2 and SD_3 gave 23-33% reduction, then SB_5 gave 73% reduction.

4.3.2 Cyanide (Cynogenic glucoside)

Treatments SD_1, SD_3 were highest in cyanide followed by SD_2 and SB_5 with values of 0.07mg/100g and 0.05mg/100g, respectively. Among the soaking in water at room temperature treatments, SW_1 and SW_2 were highest with (0.04mg/100g) while SW_3 had the least value of 0.02mg/100g. The percentage reduction was 86-90 for SD_1, SD_2, SD_3 and SB_5 while SW_1, SW_2 and SW_3 gave 92-96% reduction as shown in Table 4.3.

Table 4.2 Essential amino acid composition of differently processed *Leucaena* leaves

Amino acid (g/16gN)	Treatments SU	SD1	SD2	SD3	SW1	SW2	SW3	SB5	FAO Ref value[1]
Lysine	6.05±0.01^{a}	5.50±0.5^{b}	5.62±0.01^{b}	5.73±0.01^{b}	4.86±0.01^{d}	4.97±0.01^{cd}	5.79±0.01^{c}	5.56±0.01^{b}	5.80
Histidine	2.97±0.02^{a}	2.74±0.1^{b}	2.37±0.03^{e}	2.59±0.01^{c}	2.21±0.01^{g}	2.27±0.01^{fg}	2.32±0.02^{ef}	2.45±0.05^{d}	2.50
Arginine	6.37±0.02^{a}	6.11±0.1^{b}	5.87±0.01^{c}	5.87±0.01^{c}	5.18±0.01^{f}	5.35±0.01^{e}	5.70±0.02^{d}	5.71±0.01^{d}	5.20
Valine	6.02±0.02^{a}	5.97±0.02^{b}	5.33±0.01^{c}	5.27±0.01^{d}	4.52±0.02^{b}	4.63±0.01^{g}	4.80±0.01^{f}	4.92±0.01^{e}	5.00
Methionine	1.77±0.02^{a}	1.67±0.01^{b}	1.55±0.01^{d}	1.62±0.01^{c}	1.35±0.01^{g}	1.41±0.01^{f}	1.46±0.01^{e}	1.41±0.01^{f}	2.50
Isoleucine	5.91±0.03^{a}	5.64±0.01^{b}	4.76±0.01^{d}	4.88±0.01^{c}	4.19±0.01^{f}	4.31±0.01^{e}	4.31±0.01^{e}	4.31±0.01^{e}	2.80
Leucine	9.72±0.02^{a}	9.26±0.06^{b}	8.58±0.02^{d}	8.90±0.01^{c}	7.48±0.01^{b}	7.56±0.03^{g}	7.91±0.01^{f}	8.19±0.01^{e}	1.10
Threonine	5.72±0.01^{a}	5.08±0.1^{c}	5.08±0.02^{c}	5.40±0.01^{b}	3.49±0.01^{g}	4.16±0.01^{f}	4.44±0.01^{e}	4.77±0.01^{d}	3.40
Phenyalanine	6.66±0.05^{a}	6.07±0,01^{b}	5.75±0.02^{c}	5.74±0.02^{c}	4.72±0.02^{g}	4.89±0.01^{f}	5.06±0.01^{e}	5.40±0.02^{d}	6.30

1 -Reference value for good quality protein (FAO, 1990)

Note: Means with the same superscript within the same row were not significantly different (P>0.05)

Legend

SU – raw

SD_1 – Sundried for 24 hours

SD_2 – Sundried for 48 hours

SD_3 – Sundried for 72 hours

SW_1 –Soaking in water at room temperature for 24 hours

SW_2 –Soaking in water at room temperature for 48 hours

SW_3 –Soaking in water at room temperature for 72 hours

SB_5 – Soaking in boiled water (100° C) for 5 minutes

Table 4.3 Antinutrients composition of differently processed *Leucaena* leaves

Antinutrients	Treatments									Recommended
(mg/100g)	SU	SD_1	SD_2	SD_3	SW_1	SW_2	SW_3	SB_5	SEM	Limit[1]
Phytate	0.91±0.2[a]	0.61±0.05[b]	0.66±0.02[b]	0.71±0.09[b]	0.16±0.01[c]	0.13±0.01[c]	0.10±0.02[c]	0.25±0.03[c]	0.06	5g/kg
% Reduction	-	33	28	22	82	86	89	73		
Cyanide	0.50±0.1[a]	0.07±0.01[b]	0.05±0.01[b]	0.07±0.01[b]	0.04±0.01[b]	0.04±0.02[b]	0.02±0.00[b]	0.05±0.01[b]	0.03	5-50mg/kg
% Reduction	-	86	90	86	92	92	96	90		
Tannin	1.03±0.33[a]	0.61±0.01[b]	0.51±0.23[b]	0.62±0.01[b]	0.16±0.02[c]	0.13±0.01[c]	0.14±0.07[c]	0.2±0.02[c]	0.07	15mg/kg
% Reduction	-	41	50	40	85	87	86	81		
Saponin	1.43 ±0.48[a]	0.64±0.01[b]	0.46±0.04[b]	0.62±0.04[b]	0.36± 0.10[c]	0.05±0.02[c]	0.04±0.01[c]	0.05±0.01[c]	0.10	1g/kg
% Reduction	-	55	68	57	75	97	97	97		
Mimosine (%)	1.66±0.02[a]	1.25±0.01[b]	1.22±0.01[b]	1.28±0.03[b]	0.87±0.01[d]	0.72±0.12[e]	0.50±0.12[f]	1.07±0.04[c]	0.07	0.2g/kg
% Reduction	-	25	27	23	48	57	70	36		

1- Francis *et al*. (2001)

Note: Means with the same superscript within the same row were not significantly different (P>0.05)

Legend: SU – raw

SD_1 – Sundried for 24 hours

SD_2 – Sundried for 48 hours

SD_3 – Sundried for 72 hours

SW_1 –Soaking in water at room temperature for 24 hours

SW_2 –Soaking in water at room temperature for 48 hours

SW_3 –Soaking in water at room temperature for 72 hours

SB_5 – Soaking in boiled water (100° C) for 5 minutes

4.3.3 Tannin

The Tannin content (Table 4.3) was highest in SD_3 with a value of 0.62mg/100g, SD_1 had 0.61mg/100g and SD_2 with 0.51mg/100g while SW_2 had the least 0.13mg/100g. The sundried treatments gave a tannin reduction of 41, 50 and 40% for SD_1, SD_2 and SD_3 respectively. The soaking in water at room temperature treatments gave a tannin reduction of 85- 87% while SB_5 gave 81%.

4.3.4 Saponin

The highest value of 0.64mg/100g for saponin for was recorded in SD_1 while SW_3 had the least (0.04mg/100g). The percentage reduction of saponin in the soaking in water at room temperature and soaking in boiled water 100° C for 5 minutes treatments were 75-97% while that of the sundried was 55-57% as shown in Table 4.3.

4.3.5 Mimosine

The mimosine content of the sundried treatments was highest (1.28%) in SD_3 and lowest (0.5%) in SW_3. The percentage reduction of mimosine was 23-27% for SD_1, SD_2 and SD_3. The soaking in water at room temperature had higher percentage (48-70) reduction of mimosine than all other treatments. There was significant difference ($P \leq 0.05$) in the mimosine values of the sundried treatments and the soaking in water at room temperature treatments as shown in Table 4.3.

4.4 Mineral Composition of Differently Processed *Leucaena* Leaves

The mineral composition of differently processed *Leucaena* leaves as presented in Table 4.4 shows that processing led to a reduction in the minerals. The sundried treatments had

higher values for all the minerals evaluated in this study than the soaking in water at room temperature treatments.

4.4.1 Phosphorus

The phosphorus content as shown in Table 4.4 was highest (12.63mg/kg) in SD_3 followed by SD_1 (10.46mg/kg) then SD_2 (10.06mg/kg). SW_1 and SB_5 had 9.85mg/kg while SW_3 had 9.04mg/kg and the least (8.85mg) in SW_2. There was significant difference ($P \leq 0.05$) in the phosphorus content of the sundried treatments and the soaking in water at room temperature treatments.

4.4.2 Potassium

The highest potassium value (Table 4.4) of 30.63mg/kg was recorded for SD_3 followed by SD_1 (30.38mg/kg) then SD_2 with 29.38mg/kg. SW_2 gave 14.38mg/kg, SW_3 14.94mg/kg, SW_2 had 15.50mg/kg while SB_5 had 16.25mg/kg. The potassium values of the sundried treatments were significantly different ($P \leq 0.05$) from the soaking in water at room temperature treatments.

4.4.3 Magnesium

Magnesium content as shown in Table 4.4 was highest (11.61mg/kg) in SD_2 followed by SD_1 with 11.58 mg/kg and then SD_3 had 11.51mg/kg. SB_5 was the next highest with a value of 11.03mg/kg. The soaking in water at room temperature treatments were lower and decreased with increase in hours of soaking, SW_1 was 10.64mg/kg, SW_2 had 10.52 mg/kg and SW_3 10.41mg/kg.

4.4.4 Calcium

Calcium had highest value (Table 4.4) of 64.47mg/kg in SD_1 followed by SD_2 with 61.52mg/kg then SD_3 with a value of 53.10mg/kg. The calcium content of the soaking in water at room temperature treatments was highest in SW_1, 50.69mg/kg followed by SW_2

with 48.82mg/kg and least in $SW_{3,}$ 47.35mg/kg. Treatment SB_5 gave a value of 51.25mg/kg which was lower than the sundried treatments and higher than the soaking in water at room temperature treatments.

4.4.5 Sodium

The sodium content as shown in Table 4.4 was highest in SD_3 with 7.73mg/kg and followed by SD_2 (7.29mg/kg) and then SD_1 which gave 7.04mg/kg. Among the soaking in water at room temperature treatments SW_2 had 7.43mg/kg followed by SW_3 with 7.27mg/kg then SW_1 had 6.33mg/kg. SB_5 gave the lowest sodium value of 5.25mg/kg among all the treatments.

Table 4.4 Mineral composition of differently processed *Leucaena* leaves

Minerals (mg/kg)	Treatment								Mineral requirement of catfish[1]
	SU	SD_1	SD_2	SD_3	SW_1	SW_2	SW_3	SB_5	
Phosphorus	10.02±0.01 ^{d}	10.46±0.01 ^{b}	10.06±0.04 ^{c}	12.63±0.02 ^{a}	9.85±0.03 ^{e}	8.85±0.01 ^{e}	9.04±0.01 ^{f}	9.85±0.01 ^{e}	4500
Potassium	30.50±0.14 ^{a}	30.38±0.13 ^{a}	29.38±0.38 ^{b}	30.63±0.13 ^{a}	14.38±0.13 ^{f}	15.50±0.00 ^{d}	14.94±0.00 ^{e}	16.25±0.00 ^{c}	2600
Magnesium	11.59±0.56 ^{a}	11.58±0.48 ^{a}	11.61±0.49 ^{a}	11.51±0.54 ^{a}	10.64±0.44 ^{ab}	10.52±0.46 ^{b}	10.41±0.48 ^{b}	11.03±0.55 ^{b}	400
Calcium	61.96±1.77 ^{a}	64.47±3.20 ^{a}	61.52±3.13 ^{a}	53.10±2.49 ^{b}	50.69±7.30 ^{b}	48.82±2.61 ^{b}	47.35±2.47 ^{b}	51.25±1.81 ^{b}	4500
Sodium	6.88±0.36 ^{d}	7.04±0.03 ^{cd}	7.29±0.02 ^{b}	7.73±0.04 ^{a}	6.33±0.10 ^{e}	7.43±0.05 ^{b}	7.27±0.00 ^{bc}	5.25±0.07 ^{f}	-

1-Source: Wilson and Moreau (1996) Note: Means with the same superscript within the same row were not significantly different (P>0.05)

Legend: SU – raw
SW_1 –Soaking in water at room temperature for 24 hours
SW_2 –Soaking in water at room temperature for 48 hours
SW_3 –Soaking in water at room temperature for 72 hours
SB_5 – Soaking in boiled water (100°C) for 5 minutes
SD_1 – Sundried for 24 hours
SD_2 – Sundried for 48 hours
SD_3 – Sundried for 72 hours

4.5 Growth Performance and Nutrient Utilization of *Heterobranchus longifilis*

The proximate composition of experimental diets is as shown in Table 4.5 while Table 4.6 shows the growth performance and nutrient utilization of *Heterobranchus longfils* fed graded levels of *Leucaena leucocephala* leafmeal.

The mean initial weight of all the *Heterobranchus longfils* fingerlings was 6.57±0.47g and there was no significant difference among all the treatments (P>0.05).

4.5.1 Final weight

At the end of the experimental period as shown in Table 4.6 fishes fed commercial diet (SWCF) recorded the highest mean final weight (FWT) of 166.62g which was marginally different from fishes fed 20% *Leucaena* leafmeal inclusion level (SW20) which recorded 166.56g. Fishes fed control diet (SWC) recorded a lower mean final weight of 165.74g followed by 10% *Leucaena* leafmeal inclusion level (SW10) with a value 139.00g. While 30% *Leucaena* leafmeal inclusion level (SW30) recorded a mean final weight of 109.84g and 40% *Leucaena* leafmeal inclusion level (SW40) gave the lowest value of 105.56g. There was no significant difference in weight among all the treatments (P>0.05). The biweekly increase in final weight of *Heterobranchus longfils* fed graded levels of *Leucaena leucocephala* leafmeal is presented in Figure 4.1.

Table 4.5: Proximate composition of experimental diets

(g/100g)	Treatment					
Parameter	SWC	SW10	SW20	SW30	SW40	SWCF
Moisture	6.69^{b}	6.62^{b}	6.64^{b}	6.66^{b}	6.79^{b}	9.37^{a}
Ash	8.52^{b}	8.56^{b}	8.87^{b}	8.69^{b}	8.51^{b}	11.39^{a}
Ether extract	10.55^{b}	9.89^{c}	9.11^{d}	9.00^{d}	9.08^{d}	12.02^{a}
Crude protein	44.94^{b}	43.62^{ab}	43.27^{ab}	42.55^{b}	42.55^{b}	45.69^{a}
Crude fibre	1.47^{d}	1.58^{cd}	1.67^{c}	1.90^{b}	2.17^{a}	1.43^{d}
Nitrogen free extract	27.83^{a}	30.66^{a}	30.44^{a}	31.19^{a}	30.91^{a}	20.10^{b}
Energy(Kcal)	3500^{a}	3459.77^{ab}	3481.13^{ab}	3402.44^{abc}	3395.64^{b}	3377.57^{c}
Cost of feed N/Kg	205.73	191.25	176.57	162.96	148.80	360.00

Composition of vitamin premix per 5kg: Aqua biomix fish premix Vitamin A, 20,000,000.00 IU; Vitamin D, 32,000,000.00 IU; Vitamin E, 20,000.00 IU; Vitamin K3, 8,000.00 mg; VitaminB1 30,000.00 mg; Vitamin B2, 150,000.00 mg; Niacin, 50,000.00 mg; Pantothenic acid, 12,000.00 mg; Vitamin B6, 119 50.00 mg; Vitamin B12, 500,000.00 mg; Vitamin C 4,000.00 mg; Biotin $H_{2,}$ 800.00 mg; Choline chloride, 600,000 mg; Cobalt, 2,000.00 mg; Copper, 4,000.00 mg; Iodine, 5,000.00 mg; Inositol 200,000.00mg; Iron,40,000.00 mg; Manganese,30,000 mg; Selenium 200.00 mg; Zinc,40,000.00 mg; Lysine,100,000.00 mg; Methionine,100,000.00 mg; Antioxidant, 100,00.00 mg.

Note: Means with same superscript within the same row were not significantly different (P>0.05)
Legend
SWC – control diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level
SWCF - Commercial feed.

Table 4.6: Growth parameters and nutrient utilization of *Heterobranchus longfilis* fed graded levels of *Leucaena* leafmeal

	Treatment					
Parameter	SWC	SW 10	SW 20	SW 30	SW 40	SWCF
IWT(g)	6.33[a]	6.53[a]	6.27[a]	6.93[a]	6.67[a]	6.67[a]
FWT(g)	165.74 ±58.26 [a]	139.00 ±53.95 [a]	166.56 ±42.88 [a]	109.84 ±24.95 [a]	105.56 ±25.72 [a]	166.62 ±52.58 [a]
MWG(g)	159.41 ±57.97 [a]	132.47 ±53.78 [a]	160.29 ±43.20 [a]	102.91 ±24.88 [a]	98.65 ±25.40 [a]	159.95 ±52.35 [a]
MF I (g)	410.67 ±23.40 [a]	403.33 ±33.60 [a]	418.00 ±22.00 [a]	381.33 ±67.21 [b]	418.00 ±22.00 [a]	317.07 ±10.33 [b]
MPI(g)	184.55 ±11.42 [a]	170.12 ±6.13 [a]	180.88 ±9.94 [a]	162.20 ±28.13 [b]	177.86 ±9.36 [a]	142.68 ±4.65 [b]
SGR (%/day)	1.28±0.15 [a]	1.20±0.14 [a]	1.29±0.13 [a]	1.09±0.81[a]	1.09±1.18 [a]	1.27±0.61 [a]
FCR	2.87±1.23 [ab]	3.29±0.91[ab]	2.71±0.63 [ab]	3.81±0.08 [ab]	4.43±0.07 [a]	2.11±0.11 [b]
PER	0.84±0.02 [ab]	0.77±0.02 [ab]	0.88±0.02 [ab]	0.64±0.02 [ab]	0.56±0.02 [b]	1.13±0.02 [a]
SR (%)	93.33±0.33[a]	91.67±0.29[a]	95.00±0.21[a]	86.66±0.14 [a]	95.00±0.15[a]	86.67±0.38 [a]
ANPU (%)	51.78±18.80 [ab]	45.67±17.40 [ab]	53.15±12.32[ab]	37.29±7.58[b]	33.36 ±9.78[b]	69.06±23.10[a]

Note: Means with same superscript within the same row were not significantly different (P>0.05)

Legend-

SWC – control diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level
SWCF - Commercial feed

IWT- Initial weight
FWT- Finalweight
MWG-Mean weight gain
SGR-Specific growth rate
FCR- Food conversion ratio
SR-Survival rate
MFI-Mean feed intake
MPI-Mean protein intake
PER-Protein efficiency
ANPU-Apparent net protein utilization

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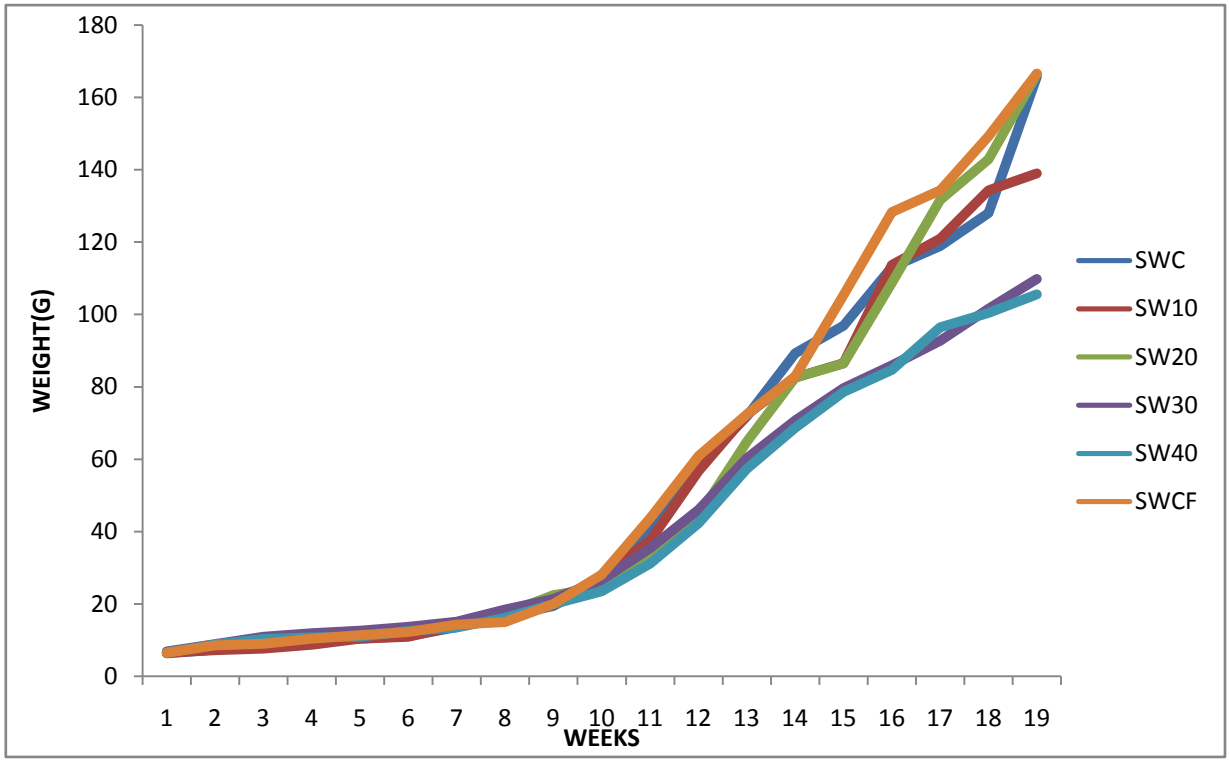


Figure 4.1: The biweekly increase in weight of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal

4.5.2 Mean weight gain (MWG)

The mean weight gain as shown in Table 4.6 was highest in treatment SW20 which had 160.29g followed by SWCF with a mean weight gain of 159.95g and then SWC with159.41g. SW10, SW30 and SW40 gave lower values of 132.47g, 102.91g and 98.65g respectively. However there was no significant difference among the treatments ($P>0.05$).

4.5.3 Specific growth rate (SGR)

The specific growth rate (Table 4.6) was highest in treatment SW20 with a value of 1.29% followed by SWC with 1.28% and then SWCF which had 1.27%. Lower SGR was observed in treatments SW30 and SW40 with a value of 1.09% while SW10 gave 1.20%. However there was no significant difference in specific growth rate among all the treatments ($P>0.05$).

4.5.4 Feed conversion ratio (FCR)

The feed conversion ratio (Table 4.6) was highest in treatment SW40 with a value of 4.43 and lowest in SWCF which gave 2.11. Treatments SW30 and SW10 had values of 3.81 and 3.29 respectively while SWC and SW20 gave lower values 2.87 and 2.71. Treatments SWC SW10 SW20 and SW30 showed no significant difference ($P>0.05$).

4.5.5 Apparent net protein utilization (ANPU)

The apparent net protein utilization as shown in Table 4.6 decreased as the inclusion level of *Leucaena* leafmeal increased. Treatment SWCF gave the highest value of 69.06% while SW40 had the lowest apparent net protein utilization of 33.36%. Treatments SWC, SW10, SW20 and SW30 gave apparent net protein utilization values of 51.78%, 45.67%, 53.15% and 37.29%

respectively. Treatments SWC, SW10 and SW20 showed no significant difference ($P>0.05$) while treatments SW30 and SW40 were significantly different ($P\leq0.05$) when compared to SWC.

4.5.6 Protein efficiency ratio (PER)

The highest protein efficiency ratio (Table 4.6) was recorded in treatment SWCF with a value of 1.13 followed by SW20 with a value of 0.88 then SWC which gave 0.84. Treatment SW40 had the lowest PER value of 0.56 while SW10 and SW30 had moderate values of 0.77 and 0.64 respectively. Treatments SWC, SW10, SW20 and SW30 were not significantly different ($P>0.05$).

4.5.7 Mean feed intake (MFI)

The mean feed intake as shown in Table 4.6 was highest in treatments SW20 and SW40 with 418.00g while SWCF gave the lowest value of 317.07g. Treatment SWC had a mean feed intake of 410.67g followed by SW10 with a value of 403.33g while SW30 had 381.33g. Treatment SWCF was significantly lower among all the treatments ($P\leq0.05$).

4.5.8 Mean protein intake (MPI)

Treatment SWC had the highest mean protein intake value (Table 4.6) of 184.55g followed by SW20 with 180.88g then SW40 with 177.86g. SWCF gave the lowest mean protein intake of 142.68g while SW10 and SW30 gave values of 170.12g and 162.20g respectively.

Increasing levels of *Leucaena* leafmeal inclusion level above 20% led to a reduction in all growth and nutrient utilization parameters.

4.5.9 Survival rate (SR)

Treatments SW20 and SW40 recorded the highest survival rate (Table 4.6) of 95% followed by SWC with a value of 93.33% then SW10 which gave 91.67% while SW30 and SWCF recorded survival rates of 86.66% and 86.67% respectively. The survival rate for all the treatments showed no significant difference (P>0.05).

The mean dissolved oxygen, mean hydrogen ion concentration and mean temperature in the experimental tanks is presented in Table 4.7.

The result of the trend analysis for the growth parameters is presented in Figures 4.2 – 4.10. The trend line for final weight, mean weight gain, specific growth rate, protein efficiency ratio, and apparent net protein utilization of of *H. longifilis* fed graded inclusion levels of *Leucaena* leafmeal based diets displayed a decreasing trend as the inclusion level of *Leucaena* leafmeal increased. While the trend line for the food conversion ratio increased as *Leucaena* leafmeal increased in the experimental diets. The trend line for mean feed intake, mean protein intake and survival rate did not display any definite pattern.

4.6 Apparent Digestibility Coefficient of Feed

The digestibility of graded levels of *Leucaena* leafmeal based diets is presented in Table 4.8. Generally the study showed that there was a reduction in apparent digestibility co-efficient as the inclusion levels of *Leucaena* leafmeal increased.

4.6.1 Dry matter apparent digestibility coefficient (Dry matter ADC).

The dry matter apparent digestibility coefficient as shown in Table 4.8 was highest in the control diet (SWC) 57.75% and lowest in treatment SW40 (40% *Leucaena* leafmeal inclusion level) which recorded 42.17%. Treatments SW10 (10% *Leucaena* leafmeal inclusion level),

SW20 (20% *Leucaena* leafmeal inclusion level) and SW30 (30% inclusion level) recorded similar values of 54.20%, 55.24% and 53.40%, respectively. The dry matter ADC was significantly different from each other in all the treatments ($P \leq 0.05$).

4.6.2 Protein apparent digestibility coefficient (Protein ADC)

The protein ADC as shown in Table 4.8 was highest in the control treatment (SWC) 79.71% and closely followed by SW10 with a value of 79.68% while SW40 recorded the lowest value of 69.62%. Treatments SW20 and SW30 had values of 77.69% and 73.41% respectively. The protein ADC in all the treatments was significantly different ($P \leq 0.05$).

4.6.3 Lipid apparent digestibility coefficient (Lipid ADC)

Treatment SWC had the highest Lipid ADC (Table 4.8) with a value of 82.84% while SW30 had the lowest which was 73.33%. Treatments SW10, SW20 and SW40 gave moderate values of 81.92%, 77.77% and 74.20%, respectively.

4.6.4 Carbohydrate apparent digestibility coefficient (Carbohydrate ADC)

The Carbohydrates ADC as shown in Table 4.8 was generally low in all the treatments. SWC recorded the highest with a value of 26.95%, followed by SW10 with 23.06% then SW20 which gave 22.87%. Similarly SW30 and SW40 gave values of 21.43% and 20.03%, respectively.

Table 4.7: Mean weekly water quality parameters of the experimental tanks

	Treatments					
Parameter	SWC	SW 10	SW 20	SW 30	SW 40	SWCF
DO	4.45±0.90	4.30±0.44	4.00±0.01	4.24±0.76	4.30±0.87	3.80±0.00
pH	6.69±0.01	6.68±0.01	6.72±0.00	6.72±0.01	6.68±0.01	6.64±0.01
Temperature	23.88±0.01	24.24±0.00	24.02±0.01	23.85±0.01	23.85±0.01	23.95±0.01

SWC – control diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level
SWCF - Commercial feed.

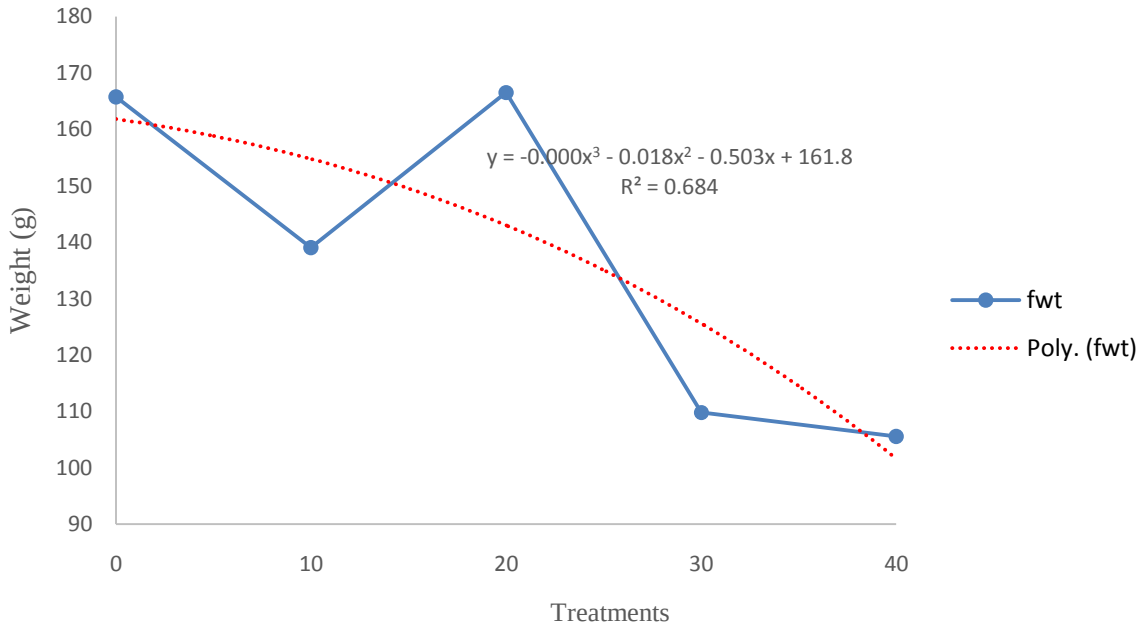


Figure 4.2: Trend line for final weight of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

fwt – final weight

poly -polynomial line.

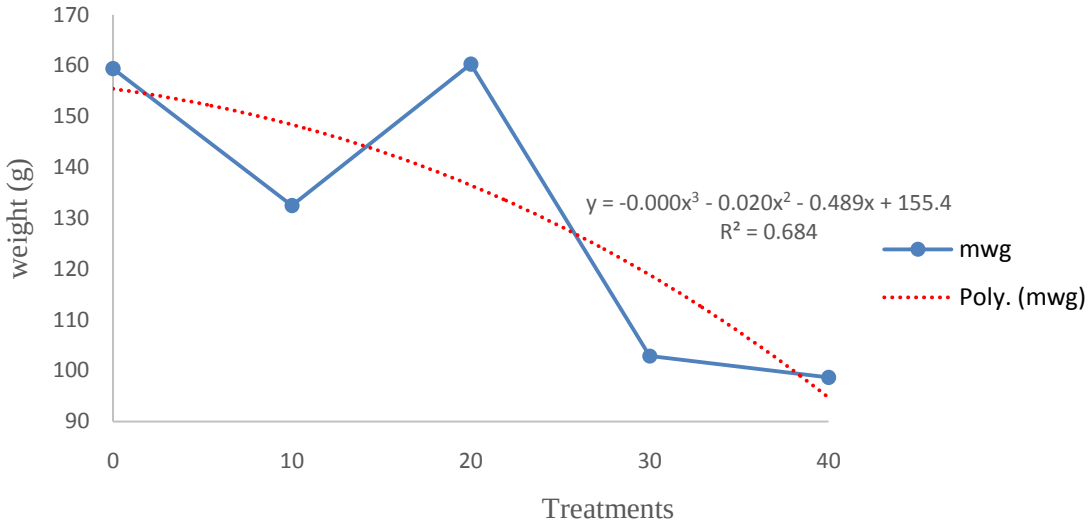


Figure 4.3: Trend line for mean weight gain of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

mwg –mean weight gain

poly -polynomial line.

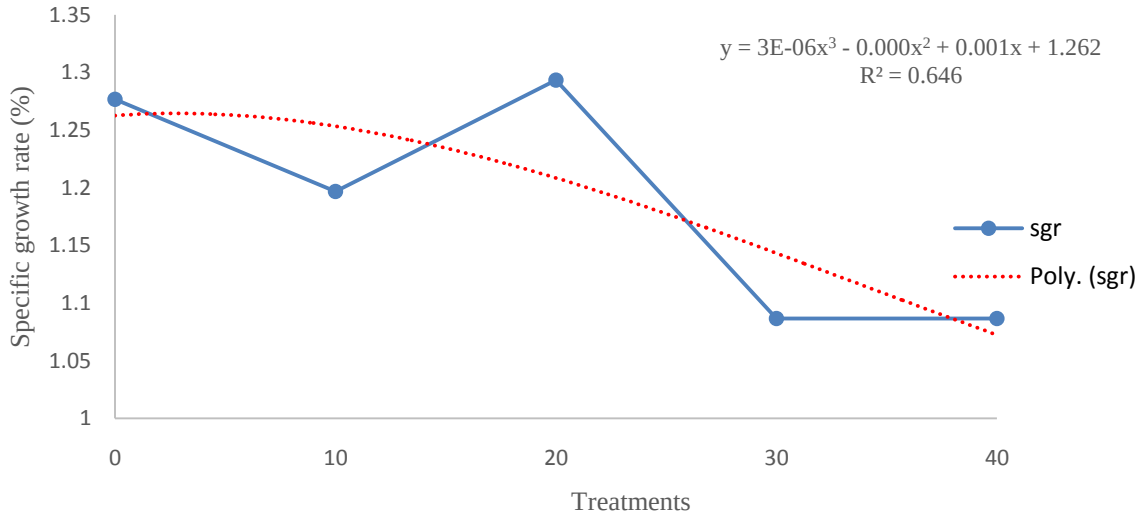


Figure 4.4: Trend line for specific growth rate of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

sgr –specific growth rate

poly -polynomial line.

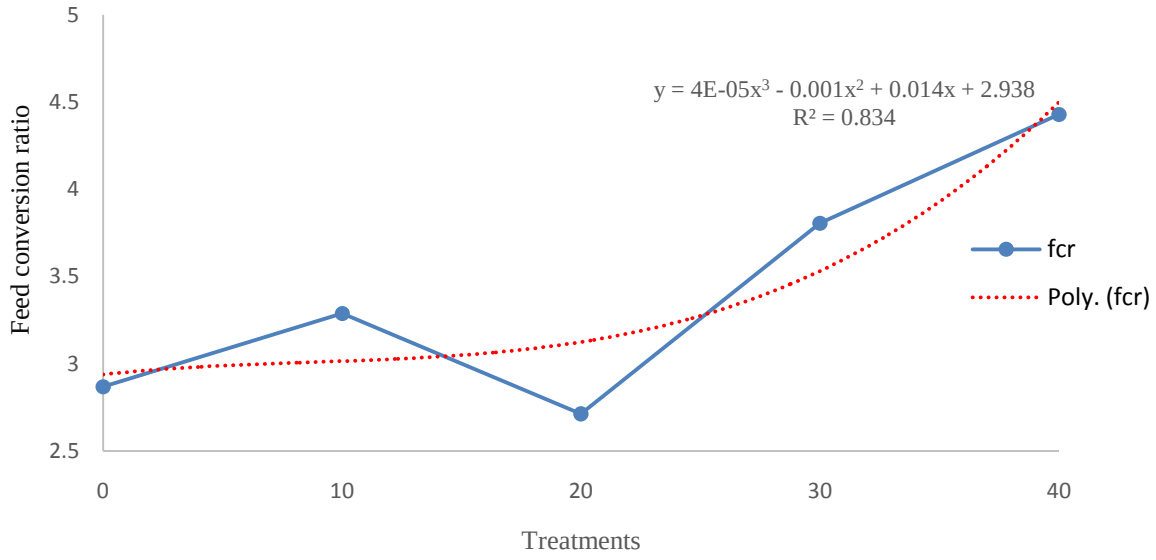


Figure 4.5: Trend line for feed conversion ratio of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

Fcr-feed conversion ratio

poly -polynomial line.

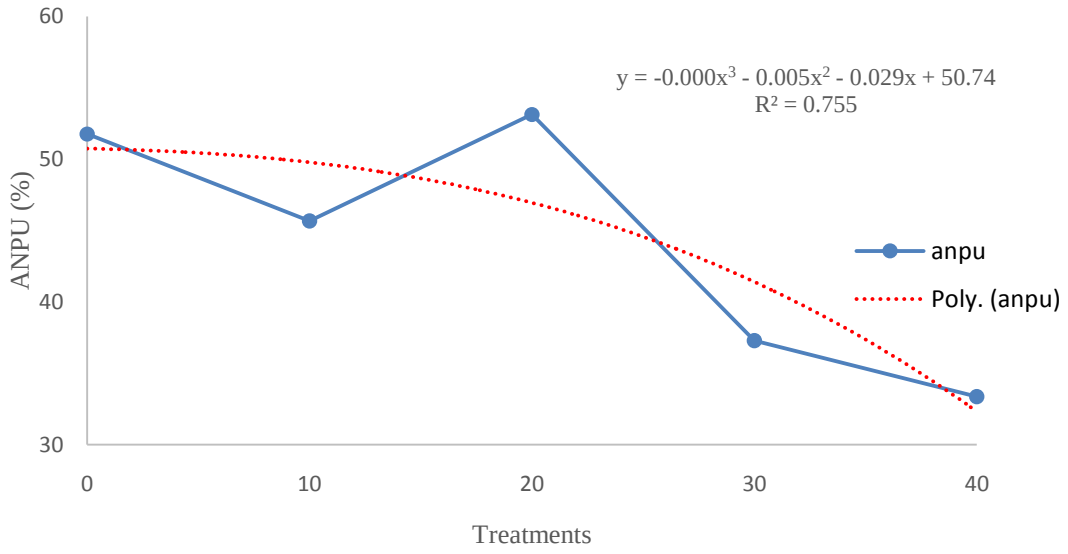


Figure 4.6: Trend line for apparent net protein utilization of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

anpu-apparent net protein utilization

poly -polynomial line.

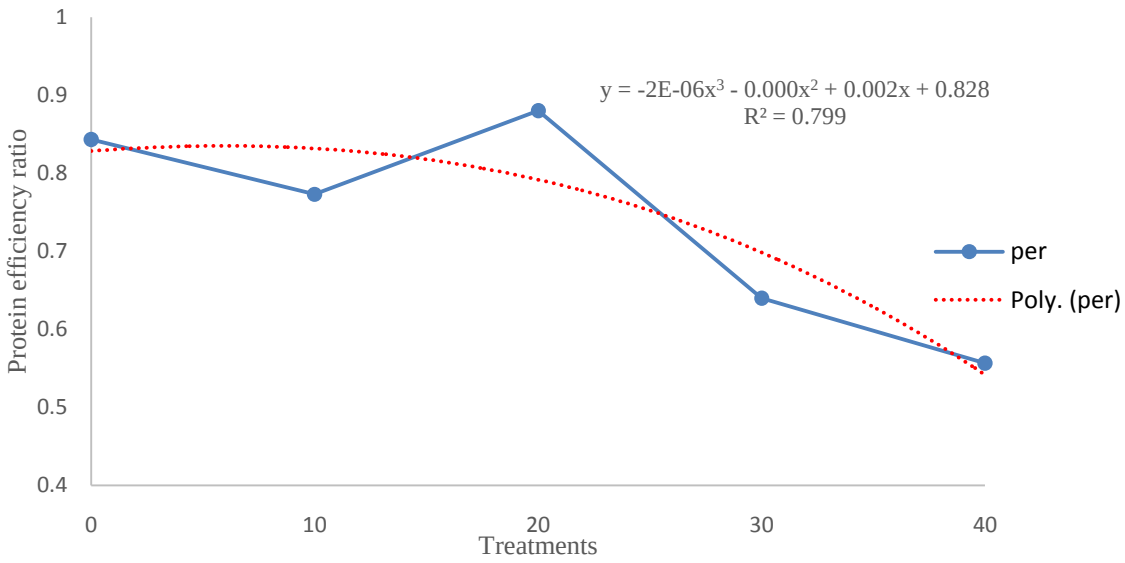


Figure 4.7: Trend line for protein efficiency ratio of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

per –protein efficiency ratio

poly -polynomial line.

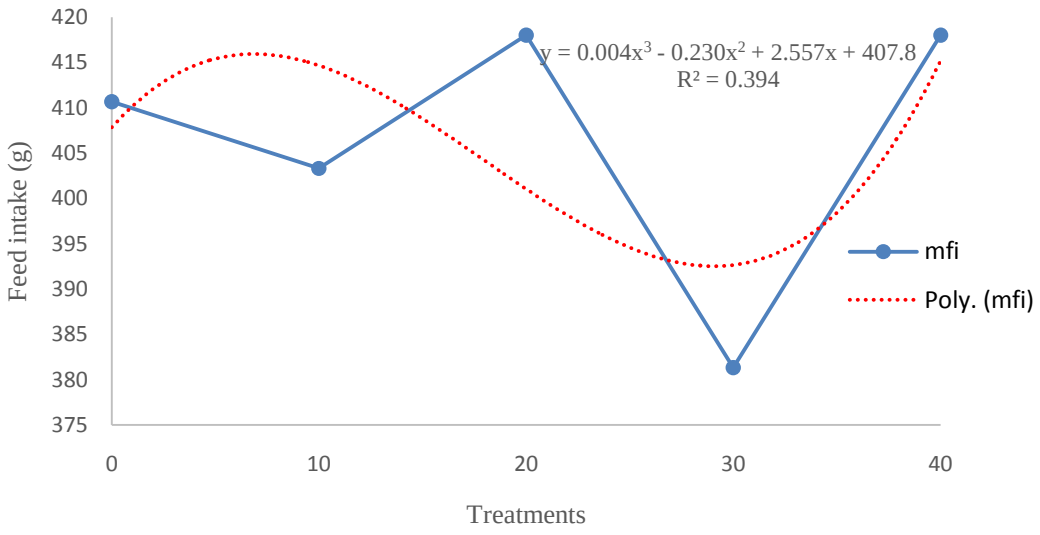


Figure 4.8: Trend line for mean feed intake of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

mfi–mean feed intake

poly -polynomial line.

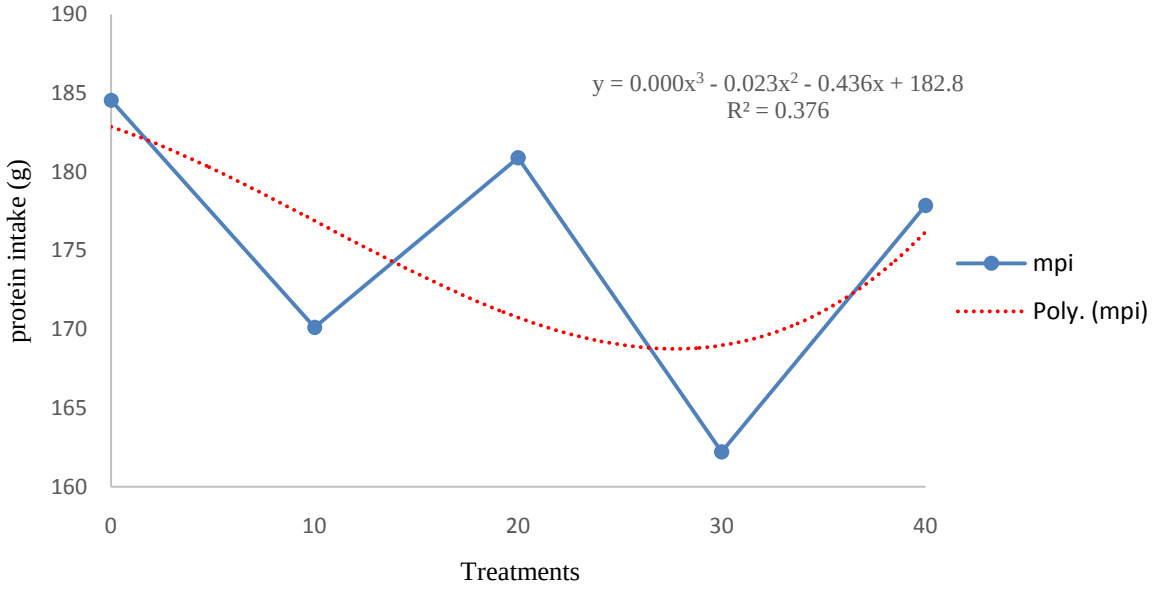


Figure 4.9: Trend line for mean protein intake of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

mpi–mean protein intake

poly -polynomial line.

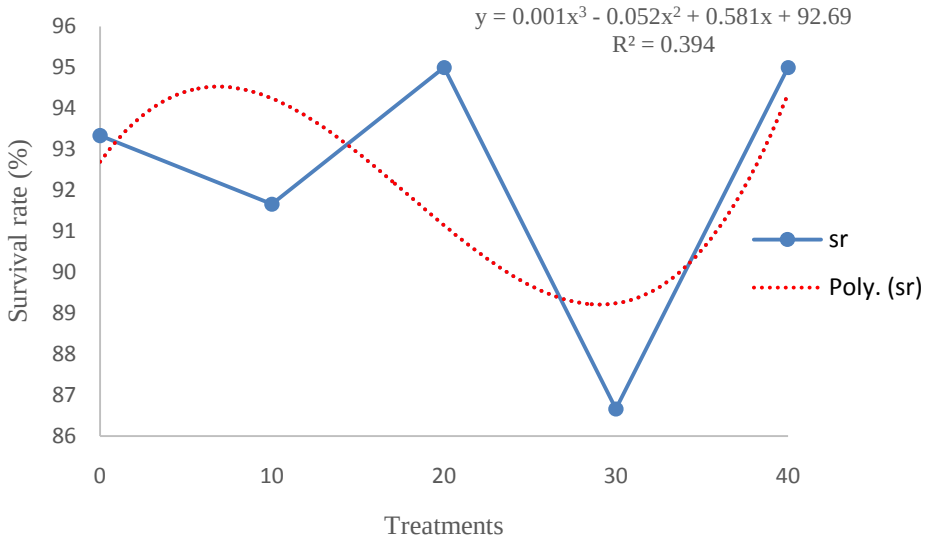


Figure 4.10: Trend line for survival rate of *Heterobranchus longifilis* fed graded levels of *Leucaena* leafmeal based diets.

sr –survival rate

poly -polynomial line.

Table 4.8: The apparent digestibility coefficient of graded levels of *Leucaena* leafmeal in the diet of *Heterobranchus longfilis*

	Treatments				
Coefficients	SWC	SW 10	SW 20	SW 30	SW 40
APD	79.71±0.01[a]	79.68±0.01[c]	77.69±0.01[b]	73.41±0.01[d]	69.62±0.01[e]
ALD	82.84±0.01[a]	81.92±0.01[b]	77.77±0.01[c]	73.33±0.01[d]	74.20±0.01[e]
ADMD	57.75±0.01[a]	54.20±0.01[b]	55.24±0.01[c]	53.40±0.01[e]	42.17±0.01[d]
ACD	26.95±0.01[a]	23.06±0.01[b]	22.80±0.01[c]	21.43±0.01[d]	20.03±0.01[e]

Note: Means with similar superscript within the same row were not significantly different at P>0.05.
Legend

APD - Apparent protein digestibility
ALD - Apparent lipids digestibility
ADMD - Apparent dry matter digestibility
ACD - Apparent carbohydrates digestibility
SWC - Control Diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level

4.7 Carcass Composition

The carcass composition of *Heterobranchus longifilis* before and after the feeding trials is presented in Table 4.9.

4.7.1 Crude protein

The carcass crude protein as shown in Table 4.9 increased in all the treatments when compared to the initial value 57.54% before the feeding trial. Treatment SWCF gave the highest value of 60.77% followed by SWC and SW20 which had the same value of 60.12%. SW10, SW30 gave values of 59.06%, 58.81% and while SW40 gave the least 58.72%, respectively. Treatments SWC and SW20 were not significantly different ($P>0.05$) with respect to carcass crude protein. The carcass crude protein seems to be correlated with the nutrient utilization.

4.7.2 Ether extract

The ether extract in the carcass as shown in Table 4.9 increased in all the treatments and was higher in treatments SW10, SW20, SW30 and SW40 than in the control.

4.7.3 Ash

The carcass ash value (Table 4.9) ranged from 11.03-11.48% and was higher in all treatments than the initial ash value of 9.41%.

4.7.4 Nitrogen free extract

The initial carcass nitrogen free extract as shown in Table 4.9 was 14.40%, this value was higher than the nitrogen free extract of all other treatments which ranged from 4.94-8.28%.

4.7.5 Crude fibre

The carcass analysis (Table 4.9) showed that crude fibre was not detectable in all the samples.

Table 4.9: Carcass composition of *H. longfilis* fed graded levels of *Leucaena* leafmeal based diets

			Treatments				
Parameter	Initial	SWC	SW 10	SW 20	SW 30	SW 40	SWCF
Moisture	10.75 ±0.05 [a]	11.41 ±0.19 [a]	10.60 ±1.19 [a]	10.36 ±0.12 [a]	11.13 ±0.67 [a]	11.41 ±0.67 [a]	10.75 ±0.28 [a]
Crude Protein	57.54 ±0.01 [c]	60.12 ±0.02 [b]	59.06 ±0.06 [c]	60.12 ±0.01 [b]	58.81 ±0.01 [d]	58.72 ±0.03 [e]	60.77 ±0.08 [a]
Crude Fibre	ND	ND	ND	ND	ND	ND	ND
Ether Extract	8.00 ±0.10 [e]	9.62 ±0.16 [d]	11.12 ±0.01 [b]	10.56 ±0.01 [c]	10.51 ±0.01 [c]	I0.53 ±0.02 [c]	12.06 ±0.01 [a]
Ash	9.41 ±0.01 [b]	11.06 ±0.33 [a]	11.42 ±0.43 [a]	11.18 ±0.38 [a]	11.39 ±0.85 [a]	11.03 ±0.01 [a]	11.48 ±0.38 [a]
Nitrogen Free Extract	14.40 ±0.09 [a]	7.79 ±0.47 [b]	7.80 ±1.42 [b]	7.78 ±0.33 [b]	8.17 ±1.33 [b]	8.28 ±0.66 [b]	4.94 ±0.57 [c]

Note: Means with similar superscript within the same row were not significantly different (P>0.05)
Legend: SWC – Control diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level
SWCF - Commercial feed.
Initial – At beginning of experiment

4.8 Haematological Indices

The haematological indices of *H. longifilis* fed graded levels of *Leucaena* leafmeal is shown in Table 4.10.

4.8.1 Haematocrit (PCV)

The Haematocrit (PCV) for the control diet (SWC) and the commercial feed (SWCF) as shown in Table 4.10 had the highest value of 34.67% followed by SW20 (20% *Leucaena* leaf meal inclusion level) with a value of 34.33%. Similarly SW10 (10% *Leucaena* leaf meal inclusion level) and SW40 (40% inclusion level) had 30.00% while SW30 (30% *Leucaena* leaf meal inclusion level) had the lowest value of 29.00%. Treatments SWC and SWCF were significantly different ($P \leq 0.05$) from all other treatments.

4.8.2 Red blood cell

The control diet SWC and SWCF had higher values (Table 4.10) for red blood cell than all other treatments. SWC gave 2.9 $x10^6/mm^3$ while SWCF had 2.88 x $10^6/mm^3$ followed by SW20 2.85 $x10^6/mm^3$. These three treatments were significantly different ($P \leq 0.05$) from SW10, SW40 and SW30 which gave 2.50 x $10^6/mm^3$, 2.48 x $10^6/mm^3$ and 2.40 x $10^6/mm^3$ respectively.

Table 4.10: Haematological indicies of *Heterobranchus longfilis* fed graded levels of *Leucaena* leafmeal

	Treatments					
Indices	SWC	SW 10	SW 20	SW 30	SW 40	SWCF
PCV	34.67±2.52 [a]	30.00±1.00 [bc]	34.33±1.53 [ab]	29.00±4.00 [c]	30.00±2.65 [bc]	34.67±0.58 [a]
RBC $\times 10^6$ mm^3 / l	2.90±0.18 [a]	2.50±0.10 [a]	2.85±0.13 [a]	2.40±0.35 [b]	2.48±0.01 [b]	2.88±0.03 [a]
WBC $\times 10^3$ mm^3 / l	19.17±0.79 [ab]	19.51±9.96 [ab]	19.19±0.09 [ab]	20.13±0.81 [a]	20.06±0.95 [a]	18.96±8.29 [b]
HB (g/100ml)	11.50±0.85 [a]	9.97±0.35 [bc]	11.43±0.51 [ab]	9.63±1.35 [c]	9.97±0.85 [bc]	11.40±0.17 [ab]
MCH	39.64±0.60 [a]	39.87±0.23 [a]	40.12±0.21 [a]	40.16±0.28 [a]	40.13±0.23 [a]	39.77±0.20 [a]
MCV	119.49±1.58 [a]	120.02±0.80 [a]	120.47±0.81 [a]	120.93±0.98 [a]	120.77±0.78 [a]	120.22±0.80 [a]
MCHC (%)	33.17±0.06 [a]	33.22±0.11 [a]	33.30±0.06 [a]	33.21±0.12 [a]	33.23±0.10 [a]	32.89±0.52 [a]
LYMPH	91.67±0.58 [ab]	82.67±11.02 [b]	90.67±8.06 [ab]	93.67[a]±2.52	90.67[ab]±4.92	89.67±2.52 [ab]

Note: Means with similar superscript within the same row were not significantly different (P>0.05)
Legend: SWC – control diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level
SWCF - Commercial feed.

4.8.3 White blood cell (WBC)

Treatment SW30 had the highest (Table 4.10) white blood cell value of 20.14 x $10^3/mm^3$ followed by SW40 with 20.05 x $10^3/mm^3$. Similarly SWC, SW10 and SW20 gave 19.17 x $10^3/mm^3$ 19.51 x $10^3/mm^3$ and 19.20 x $10^3/mm^3$ respectively, while SWCF recorded the lowest value of 18.96 x 10^3/mm. Treatments SWC, SW10 and SW20 did not significantly differ ($P>0.05$) with respect to WBC values obtained.

4.8.4 Haemoglobin (HB)

The haemoglobin was highest (11.50g/dl) in treatment SWC and lowest in SW30 which had 9.63g/dl as shown in Table 4.10. Treatments SWCF and SW20 were not significantly different ($P>0.05$) with values of 11.40g/dl and 11.43g/dl respectively, while SW10 and SW40 gave 9.97g/dl. Haemoglobin value in SWC was significantly different ($P\leq0.05$) from that of all other treatments.

4.8.5 Mean corpuscular haemoglobin (MCH)

The MCH was highest (40.16pg) in SW30 and lowest (39.64pg) in treatment SWC as shown in Table 4.10. Values of 39.77pg, 39.87pg, 40.12pg and 40.13pg for SWCF, SW10, SW20 and SW40 respectively were obtained. MCH values in all the treatments were not significantly different ($P>0.05$).

4.8.6 Mean corpuscular volume (MCV)

Treatment SW30 gave the highest value of 120.93fl while SWC gave the lowest 119.49fl. SW10, SW20, SW40 and SWCF gave values of 120.02fl, 120.47fl, 120.77fl and 120.22fl respectively (Table 4.10). There was no significant difference ($P>0.05$) among the treatments with respect to MCV.

4.8.7 Mean corpuscular haemoglobin concentration (MCHC)

There was no significant difference (P>0.05) in the values of MCHC obtained for all treatments as shown in Table 4.10.

The results of the principal component analysis to establish the relationship between the haematological indices and the growth parameters is shown in Figure 4.3. The principal component analysis revealed that a strong correlation exists between FWT, MWG, SGR, HB and RBC. Principal Component Analysis (PCA) Component 1 (61%) and component 2 (24%) combined to give the joint representation of the two main factors accounting for 85% of the variance.

4.9 Cost Benefit Analysis

The cost benefit analysis of feeding *Heterobranchus longifilis* with graded levels of *Leucaena* leafmeal based diets is presented in Table 4.11. Treatment SWCF (Commercial Feed) had the highest feed cost of ₦116.25 while SW30 gave the lowest ₦62.14. SWC (control diet) had a value of ₦84.35 with SW10 and SW20 recording similar values of ₦77.14 and ₦73.81. Treatment SW40 gave a value of ₦62.20 which was not significantly different (P>0.05) from the feed cost of SW30. There was no significant difference (P>0.05) among the values obtained for gross profit, profit index, benefit cost ratio and incidence cost.

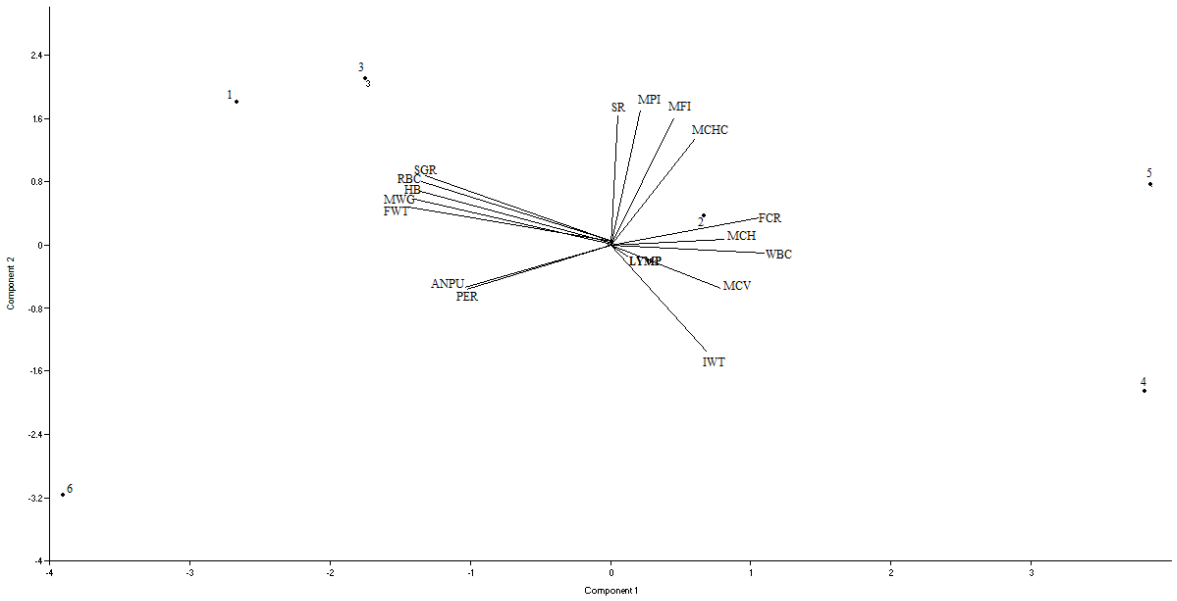


Figure 4.11: Principal Components Analysis (PCA) of the relationship between the haematological indices and the growth parameters. Component 1 (61%) and component 2 (24%) accounting for 85% of the variance.

ANPU-Apparent Net Protein Utilization FCR- Food conversion ratio, FWT- Final weight, HB-Haemoglobin, MCH-Mean corpuscular haemoglobin MCHC-Mean corpuscular haemoglobin concentration MCV- Mean corpuscular volume PCV- Packed Cell Volume RBC- Red blood cell, SR- Survival rate WBC- White blood cell LY-Lymph IWT- Initial weight MWG-Mean weight gain, MPI-Mean protein intake MFI-Mean feed intake SGR-Specific growth rate PER-Protein efficiency ratio

Table 4.11: Cost benefit analysis of feeding *Heterobranchus longfilis* with graded levels of *Leucaena* leafmeal

Indices	Treatments SWC	SW 10	SW 20	SW 30	SW 40	SWCF
Cost of feeding (N)	84.35±5.22[b]	77.14±6.43[b]	73.81±3.58[b]	62.14±10.95[c]	62.20±3.27[c]	116.25±3.80[a]
Total cost (N)	104.35±5.22[b]	97.14±6.43[b]	93.81±3.58[b]	82.14±10.95[c]	82.20±3.27[c]	136.25±3.80[a]
Mean yield cost (N)	116.25±40.99[a]	97.30±37.77[a]	116.59±30.01[a]	76.50±17.39[a]	73.90±18.00[a]	128.30±56.94[a]
Gross profit / loss (N)	10.23±41.60.[a]	0.16±31.47[a]	22.77±27.77[a]	- 5.53±15.85[a]	- 8.47±19.22[a]	- 7.95±58.71[a]
Incidence cost	0.56±0.23[a]	0.59±0.15[a]	0.46±0.10[a]	0.58±0.12[a]	0.61±0.16[a]	0.74±0.21[a]
Profit index	1.37±0.46[a]	1.24±0.37[a]	1.57±0.35[a]	1.24±0.25[a]	1.19±0.30[a]	1.11±0.51[a]
Benefit cost ratio	1.10±0.41[a]	0.98±0.31[a]	1.24±0.28[a]	0.93±0.19[a]	0.90±0.23[a]	0.94±0.44[a]
Cost of feed (N/kg)	205.73	191.25	176.57	162.96±0.01	148.80	360.00

Note: Means with similar superscript within the same row were not significantly different (P>0.05)
Legend
SWC – control diet
SW10 - 10% *Leucaena* leafmeal inclusion level
SW20 - 20% *Leucaena* leafmeal inclusion level
SW30 - 30% *Leucaena* leafmeal inclusion level
SW40 - 40% *Leucaena* leafmeal inclusion level
SWCF - Commercial feed.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of processing method on the Proximate Composition of *L. leucocephala* Leaves

The results of this study showed that processing methods (sundrying, soaking in water at room temperature and soaking in boiled water 100° C for 5 minutes) affected the proximate composition of *Leucaena* leaves.

The high value of crude protein in the sundried *Leucaena* leaves showed that sundrying method was superior to other methods employed in this study. Sundrying *Leucaena* leaves led to concentration of soluble solids thereby increasing the drymatter. Processing of *Leucaena* leaves by soaking in water led to a reduction of nutrients which may be due to leaching of soluble nutrients. Leaching of nutrients especially crude protein during soaking was reported by Adekojo *et al*. (2014). The workers observed that the *Leucaena* leaves soaked in water at room temperature for 36 hours and soaked in 60° C water for 24 hours had lower crude protein values when compared to the airdried leaf samples. The low crude protein value observed in the leaves that were processed by soaking in water could also be associated with loss of amino acids especially sulphur amino acids methionine + cysteine (Makkar *et al*., 2007). This study is contrary to the findings of Fayemi *et al.* (2011) where an increase in crude protein values was reported when *Leucaena* leaves were submerged in hot water (70°C) for 10 minutes compared to the leaves that were sundried for 36 hours. The variation in the crude protein values of the different processing methods in the current study and that of previous studies could be attributed to differences in duration of sundrying, hours of soaking, temperature of water and method of soaking (Masafu, 2006).

The range of crude protein value of *Leucaena* leaves in this study was less than the protein requirement of *Heterobranchus longifilis* indicating that *Leucaena* leaves will be better as a supplement to other conventional protein (Agbede, 2005).

The type of processing method employed affected the crude fibre values of *Leucaena* leaves. The high values of crude fibre in the soaked in water at room temperature treatments, may be due to loss of nutrients that are soluble in water leaving the insoluble fibre. The report of Falaye *et al.* (1998) when *Leucaena* leaves were soaked in water for 48 hours followed a similar trend. The crude fibre value (9.56%-21.5%) in the current study was higher than the crude fibre requirement of catfish which is 8-12% (DeSilva and Anderson 1995). This implies that *Leucaena* leafmeal would be better at low inclusion level to reduce fibre level in diets since fish cannot effectively utilize crude fibre.

The nitrogen free extract values in this study showed that *Leucaena* leaves in all the treatments has the potential to contribute energy so that protein will be used for growth. Ovie *et al.* (2005) stated that when there is limited energy in fish diet other nutrients will be broken down and used for body maintainance. The nitrogen free extract values were higher in the *Leucaena* leaves soaked in water at room temperature than the sundried *Leucaena* leaves. This observation may be due to the lower levels of other proximate components (crude protein, crude fibre, ash and ether extract) since nitrogen free extract values are obtained by subtracting other components from 100. Previous studies also reported high nitrogen free extract values for soaked and cooked *Leucaena* leaves (Osman *et al*., 1996; Adekojo *et al*., 2014).

The low energy levels recorded in the *Leucaena* leaves soaked in water at room temperature may be due to their higher crude fibre content (Ayssiwede *et al.*, 2011). Energy levels in all treatments indicate that *Leucaena* leafmeal can be a good source energy in catfish feed.

5.2 Effect of Processing Method on the Essential Amino Acid Composition of *L. leucocephala* leaves

The difference in the essential amino acid composition showed that processing methods (sundrying, boiling and soaking) has effect on the amino acids composition of *Leucaena* leaves. Essential amino acids cannot be synthesized by the fish and has to be made available in the diet, these essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The low amino acid content in the soaking in water at room temperature treatments may be attributed to loss of free water soluble amino acids. It has been reported that when there is loss of amino acids in water, the sulphur-containing amino acids (methoinine + cystiene) are the most affected (Hasan, 2001). These sulphur-containing amino acids are important in initiating synthesis of other proteins and forming di-sulphide bonds (Brosnan and Brosnan, 2006). Similarly Madalla *et al*. (2013) reported a reduction in values of methionine and cystiene by 67% when moringa leaves were soaked in water overnight, and indicate that the nutritive quality of soaked leaves appears to be limited by other nutritional factors, such as inbalance of certain amino acids.

The implication of this for catfish culture is that there is the need for supplementation with methionine and cysteine or ingredients that are rich in these amino acids. However the amino acid profile of *Leucaena* leaves is comparable to the FAO standard of amino acids in good quality protein (FAO, 1990).

5.3 Effect of Processing Method on the Antinutritional compounds of *L. leucocephala* leaves

The positive effects of the processing methods in the reduction of most anti-nutritional compounds are in line with the reports of Fayemi *et al*. (2011) and Adekojo *et al*. (2014). Similar reduction of various anti-nutritional compounds were reported when *Leucaena* leaves were subjected to sundrying, soaking and fermentation (Baragi *et al*., 2004; Fayemi *et al*., 2011; Adekojo *et al*., 2014). The presence of tannin, cyanogens, phytate, saponins and mimosine in the current study confirms the reports of earlier workers on antinutrients in *Leucaena* leaves (Wee and Wang, 1987; Monoj and Bandyopadhyay, 2007). Among the various processing methods employed, the soaking in water at room temperature was able to reduce the levels of various anti-nutritional compounds better than sundrying and boiling in water (100°C) for 5 minutes. The lowest quantities of mimosine and other anti-nutritional compounds recorded in soaking in water at room temperature for 72 hours processing method agreed with the report of Amisah *et al*. (2009) that soaking in water is more effective for the reduction and removal of anti-nutritional compounds in *Leucaena* leaves. Nuttaporn and Naiyatat (2009) reported a 94% reduction in mimosine when *Leucaena* leaves were soaked in water for 72 hours. The percentage reduction in the level of antinutritional factors (tannin, cyanogens, phytate, saponins and mimosine) of the sundried and boiled *Leucaena* leaves makes them unsuitable for fishfeed formulation (Makkar, 1993; Francis and Becker, 2001). Since the soaking in water at room temperature for 72 hours processing method was able to reduce the anti-nutritional compounds to the bearest minimum it was considered as the best in this study. The tannin, cyanogens, phytate, saponins and mimosine in the study were within tolerable limits. The recommended limit is tannin-15mg/kg, cyanogen-5-50mg/kg, phytate-

5g/kg, saponins-1g/kg and mimosine-0.02g/kg/day as documented by Francis *et al.* (2001) and Wee and wang (1988).

5.4 Effect of Processing Method on the mineral compostion of *L. leucocephala* leaves

The minerals such as phosphorus, potassium, magnesium and calcium were higher in concentration in the sundried *Leucaena* leaves when compared with the soaking in water at room temperature. This observation could be due to the reduction in mineral content as a result of leaching (Adekojo *et al.* 2014). Madalla *et al.* (2013) also reported a reduction in the concentration of potassium and calcium when moringa leaves was soaked overnight. Among all the minerals tested for in this study calcium had the highest value, this observation agrees with the report of Hertramph and Piedad-Pascal (2003) that *Leucaena* leaves has high calcium content. The sodium content was low in all treatments this was in agreement with the report of Hertrampf and Piedad-Pascal (2003).

The mineral composition of *Leucaena* leaves was lower than the mineral requirement of catfish (Wilson and Moreau, 1996). This indicates that there might be the need to supplement *Leucaena* leaves with other ingredients rich in minerals.

5.5 Growth Performance and Nutrient Utilization of *Heterobranchus longifilis* fed Graded Levels of *Leucaena* leafmeal

The diet formulated for growth trials met the crude protein requirement (40-42.5%) of *H. longifilis* (Eyo and olatunde, 2001). All fishes fed actively on the experimental diets which demonstrate that *H. longifilis* was able to tolerate up to 40% inclusion level of *Leucaena* leafmeal and was able to utilize it for growth. In terms of growth performance and nutrient utilization, commercial diet (SWCF) had the best performance followed by 20% *Leucaena*

leafmeal inclusion level (SW20) then the control diet (SWC). The slight difference between SWC and SW20 might be due to the synergistic effect of combining two or more biological compounds to have a superior effect such as fishmeal, groundnut cake and *Leucaena* leafmeal. Sogbesan and Ugwumba (2008) reported a similar effect when *H. longifilis* was fed with fishmeal, groundnut cake and termite meal. The low mean weight gain in SW30 and SW40 might be attributed to the lack of certain growth promoting amino acids such as methionine, improper balance of amino acids and increase in carbohydrates in the diet. The reports of Eyo *et al.* (2004); Sogbesan and Madu (2008) when *Clarias gariepinus* was fed with beniseed and earthworm meal also indicated that improper balance of amino acids in these ingredients resulted in reduced growth. The report of Ovie and Ovie (2007) when fishmeal was replaced with groundnut cake in the diet of *H. longifilis* showed that low levels of methionine resulted in low weight gain. Similarly, there was reduced growth of *Tilapia* when fed *Leucaena* leafmeal above 25% inclusion level (Wee and Wang, 1987) Furthermore Falaye *et al.* (1998) observed that substitution of *Leucaena* leafmeal for groundnut cake in the diet of Tilapia led to reduction in growth performance and nutrient utilization. Amisah *et al.* (2009) reported increased weight gain of *Clarias gariepinus* with increase in inclusion levels of *Leucaena* leafmeal. In this study the low mean weight gain in 30% and 40% *Leucaena* leafmeal inclusion level might not be due to palatability since fishes were seen to accept feed and fed actively but may be due to the fact that *H. longifilis* has the tendency of being carnivorous. The acceptance of *Leucaena* leafmeal based diets might be due to the effectiveness of the processing method in reducing antinutrients thereby increasing palatability. The report of Ayoola *et al.* (2013) when *Clarias gariepinus* was fed with moringa leafmeal followed a similar trend. The feed intake by *Heterobranchus longifils* in this study was not directly linked to palatability because 40% *Leucaena* leafmeal inclusion had the same feed intake with those

fed 20% inclusion level. The protein intake was related to the feed intake and the crude protein level of the experimental diets. The high FCR observed in this study can be attributed to imbalance of amino-acid and negative impact of high fibre content of *Leucaena* leafmeal. The report of Sotolu and Sule (2011) on the use of water hyacinth as protein supplement corroborates the fact that plant proteins result in high FCR due to their fibre content.

The quality of a protein is determined by its ability to support growth and development of the animal this in turn depends on the amino acid composition. Protein efficiency ratio (PER) and Apparent net protein utilization (ANPU) shows the protein quality and utilization (Sogbesan and Madu, 2008). The survival rate was high for all treatments (86.66-95.00%) therefore mortality may be due to other reasons not the diet. The implication of these findings is that incorporation of *Leucaena* leafmeal should be at low inclusion level.

The trend line for final weight, mean weight gain, specific growth rate, protein efficiency ratio, and apparent net protein utilization of of *Heterobranchus longifilis* fed graded inclusion levels of *Leucaena* leafmeal based diets could not explain the peak at the 20% inclusion level. The peak may be as a result of some extraneous factors during the experiment. This is in line with the work of Adesina *et al.* (2013) when *Clarias gariepinus* was fed with graded levels of boiled sunflower seedmeal that there were some other extraneous factors that made 30% boiled sunflower seedmeal inclusion level record higher mean weight gain than the control diet.

5.6 Apparent Digestibility Coefficient of Graded Levels of *Leucaena* leafmeal in the Diet of *Heterobranchus longfilis*

The apparent digestibility coefficients (ADC) in this study had an inverse relationship with the inclusion of *Leucaena* leafmeal. This observation could be attributed fact that plant proteins have high crude fibre, undigestible plant material and anti-nutrient factors. Sotolu and Faturoti

(2008) stated that slight differences in digestibility coefficient could result in significant difference in weight gain, SGR, FCR, PER and ANPU.

The ADC for protein is a key factor in the evaluation of the quality of a diet for fish and the potential of the diet to synthesize new tissues. The ADC for protein in this study corroborates fact that inclusion of plant proteins in fish diets leads to lower apparent digestibility coefficients when compared to fishmeal. The lower ADC for protein might be attributed to imbalance of amino acids in plant protein and high insoluble fibre which affects absorption of proteins in the intestine (Mubarak *et al*., 2011). The ADC for protein in this study was in line with the values (73-85%) reported by Baraigi *et al*. (2004) when *Labeo rohita* was fed with *Leucaena* leafmeal based diets. Similarly, Sotolu and Sule (2011) reported a low ADC protein of 71.20% when *Clarias gariepinus* was fed diets water hyacinth leafmeal. However the ADC protein was close to the range (75-95%) recommended by NRC (1993). The low ADC protein implies that *Leucaena* being a non convetional protein would be better as a supplement.

The apparent digestibility coefficient (ADC) for lipid was high in this study. This may be attributed to the fact that fish can effectively utilize lipids as a source of energy (Thompson *et al*., 2002). The apparent digestibility coefficient (ADC) for dry matter in this study was low due to the fact that ADC drymatter is higher in animal ingredients than plant ingredients. It can also be attributed to the high carbohydrate contents of plant materials and inability of fish to effectively utilize starch and fibre. The apparent digestibility coefficient (ADC) for carbohydrates was low in this study. This observation may be attributed to the fact that *Heterobranchus longfilis* is an omnivore but with high carnivorous tendency and lacks adequate enzymes for digestion of carbohydrates.

5.7 **Carcass composition of *Heterobranchus longfilis* Fed Graded levels of *Leucaena* leafmeal**

The carcass composition indicates that there was increase in all the nutrients in the body of the fish when compared to the initial composition. This shows that *Heterobranchus longifilis* was able to utilize all the diets however the difference in the values for carcass protein and lipids confirms the fact that there was different levels of utilization. The works of Sotolu (2010); Sotolu and Sule (2011) when *Clarias* was fed with *Leucaena* seedmeal and water hyacinth leafmeal followed the same trend. However Osman *et al.* (1996) observed a decrease in carcass protein and ether extract of *Tilapia* fed *Leucaena* leafmeal to be associated with low feed intake at higher inclusion levels. The increase in carcass lipid when compared with the control could be associated with phosphorus deficiency which is caused by anti-nutrients because of forming complexes with other compounds. This agrees with the report of Osuigwe and Obiekezie (2007) when *H. longifilis* was fed with jackbean meal and carcass lipid increased at higher inclusion levels. Increased carcass lipid could also be as result of high carbohydrates in the diet as reported by Sotolu (2010).

5.8 Haematological Indicies of *Heterobranchus longfilis* Fed Graded levels of *Leucaena* Leafmeal

In this study the haematological parameters such as haematocrit, haemoglobin and red blood cell were higher in the control than *Leucaena* leafmeal based diets. This may be attributed to the effect of nutrient deficient-diets or nutrient toxicity. Ogunji *et al.* (2005) reported similar findings when *Clarias gariepinus* larva was fed pigeon pea diets. The reduction of red blood cells, haematocrit and haemoglobin may be related to the effect of antinutrients. For instance, Osuigwe *et al*. (2003) reported that anti-nutrients in raw Jackbean caused a reduction in red blood cells, white blood cells, haematocrit and haemoglobin. Saponins cause erthrocyte

haemolysis which leads to a reduction in red blood cells count thereby causing anemia (Osuigwe *et al*., 2003). Anyawu *et al.* (2011) observed that the haemoglobin, red blood cells and packed cell volume of *Heterobranchus* hybrid fed dietary inclusion levels of *Carica papaya* compared favorably with the control diet. The white blood cell count was higher in 30% and 40% inclusion level than the other treatments this could be due to increased in antibody production as a result of stress. Ayoola *et al*. (2013) reported an increase in white blood cell values when moringa leaves were included in the diet of *Clarias gariepinus* and this they attributed to stress. Anyawu *et al.* (2011) stated that there are usually wide fluctuations associated with white blood cell values which maybe due to endogenous factors such as experimental conditions, diet and anti-nutrients.

The mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) showed no significant difference ($P>0.05$). However the work of Sotolu and Faturoti (2009) on *Leucaena* seedmeal in the diet *Clarias gariepinus* suggests that lower inclusion level of *Leucaena* leafmeal is better for health status of *H. longifilis*. The haematological parameters in this study were within the range reported for catfishes as documented by Nwabueze *et al.* (2011) which indicates the fishes were not overstressed.

The positive relationship between the haematological indices (HB and RBC) and growth parameters (SGR, MWG and FWT) in this study showed that blood parameters were affected by the growth of the fish which in turn depends on protein rich diets. The works of Akinwande *et al*. (2004) on haematology of *Heterobranchus longifilis* fed varying dietary protein levels and Gupta *et al.* (2013) on seasonal variations in haematological parameters of golden mahseer *Tor putitora* were in agreement with this observation.

5.9 Cost Benefit Analysis of feeding *Heterobranchus longfilis* with graded levels of *Leucaena* Leafmeal

The cost benefit analysis showed that commercial feed (SWCF) and control diet (SWC) had high feed cost this may be due to the high cost of imported fishmeal in the diets. However treatments containing *Leucaena* leafmeal had lower feed cost (₦62.20 - ₦77.14) this can be attributed to the fact that *Leucaena* leaves only involves cost of collection and processing. This agrees with the report of Sogbesan and Ugwumba (2008) when *Clarias gariepinus* was fed with garden snail meal that alternatives to fishmeal especially non-conventional feed ingredients are cheaper. Morever, the farmer will benefit economically through the utilization of the cheaper ingredient (*Leucaena* leafmeal) at 20% inclusion level, although 30% inclusion level had the least cost of feed. The significantly higher value of profit index and benefit cost ratio at 20% inclusion level above those of the control indicates that more profit would be generated from fish produced on 20% *Leucaena* leafmeal inclusion level. The report of Amisah *et al*. (2009) when *Clarias gariepinus* juveniles were fed with *Leucaena* leafmeal followed a similar trend.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

Aquaculture has huge potentials of alleviating poverty among rural populace, contributes to feed security, generates income and improves livelihood. However, the lack of affordable quality fishfeed is one of the major constraints to aquaculture development in Nigeria. The escalating prices of conventional feed ingredient and commercial feeds has made feeds costly beyond the reach of the average fish farmer. This has also reduced the profit margin of the fish farmer. In order for aquaculture to continue to grow with the use of affordable quality feeds there is the need to reduce dependence on fishmeal and other conventional proteins. Therefore this study evaluated the effects of incorporating *Leucaena* leafmeal with other conventional proteins in the diet of *Heterobranchus longifilis*.

The choice of *Leucaena* leafmeal was based on the fact that it has high protein content, it is abundant in Nigeria, available all year round and leaves can be collected at little or no cost. While *Heterobranchus longifilis* was chosen because of its economic importance and acceptability among fish farmers.

Leucaena leaves however contain several anti-nutritional factors which tend to interfere with nutrient utilization and digestibility. Therefore various processing methods which small scale fish farmers can employ were evaluated with the intention of removing the most significant anti-nutritional factor.

6.2 CONCLUSIONS

The following conclusions were drawn from the study:

i. There was difference in the proximate composition, amino acids composition, mineral composition and anti-nutients of the sundried (24 hours,48 hours, 48 hours) *Leucaena* leaves and the soaking in water (at room temperature for 24 hours, 48 hours 72 hours) *Leucaena* leaves. Soaking in water (room temperature for 72 hours gave the best reduction in anti-nutrients.

ii. Improper balance of nutrients and residual anti-nutritional factors reduced growth performance and nutrient utilization at higher inclusion levels.While the apparent digestibility coefficients for protein, drymatter, lipids and carbohydrates were significantly higher in the control diet than *Leucaena* leafmeal based diets.

iii. The final carcass analysis shows there was no significant difference in crude protein of fishes fed control diet and 20% *Leucaena* leafmeal inclusion diet, therefore *Leucaena* leafmeal at 20% inclusion level did not affect carcass composition of *Heterobranchus longifilis* .

iv. The haematological parameters shows that at 20% *Leucaena* leafmeal inclusion level there was no adverse effect on blood parameters of *Heterobranchus longifilis.*

v. The addition of *Leucaena* leafmeal at 20% inclusion level brought about 12.5% reduction in cost of feed and enhanced profitability.

6.3 RECOMMENDATIONS

i. More effective methods of processing that will enhance nutritional value of *Leucaena* leaves should be evaluated.

ii. Evaluation of experimental diets in farming conditions especially earthen pond to ascertain whether inclusion levels can be increased.

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APPENDIX I

PARTS OF *LEUCAENA LEUCOCEPHALA* PLANTS



Plate I. *Leucaena leucocephala* leaves



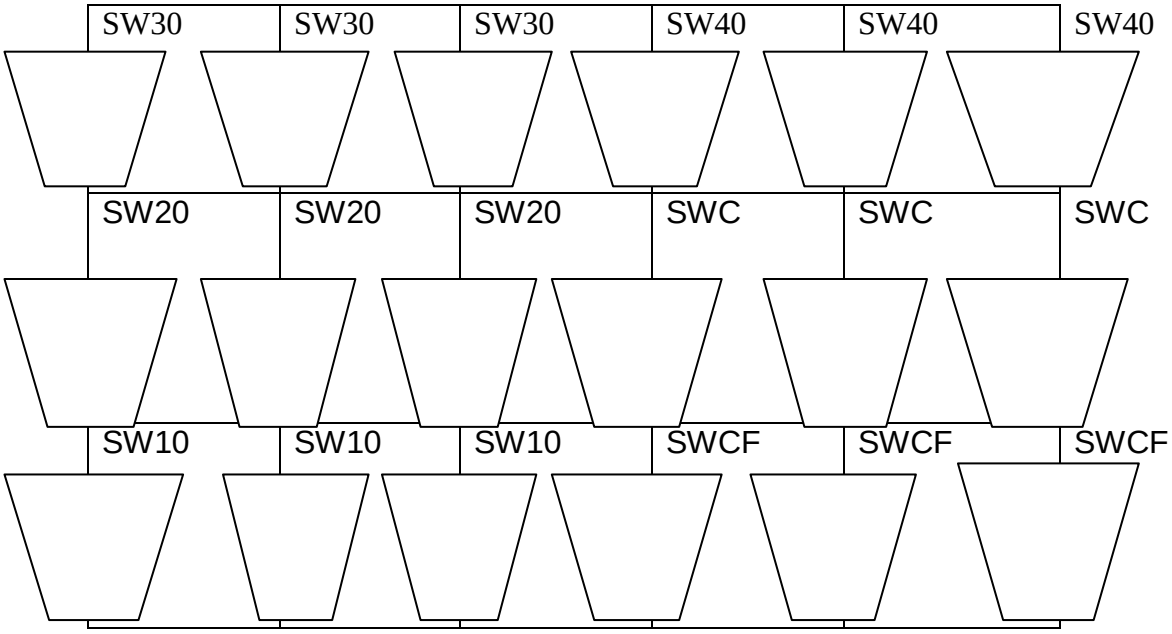
Plate II. *Leucaena leucocephala* pods



Plate III. *Leucaena leucocephala* flowers

APPENDIX II

EXPERIMENTAL TANKS IN A COMPLETELY RANDOMIZED DESIGN



APPENDIX III

EXPERIMENTAL TANKS



APPENDIX IV

WATER QUALITY PARAMETERS OF BORE-HOLE

Parameter	**Unit**	**Result**
PH	-	6.4
Total dissolved solid	PPM	90
Conductivity	NS/CM	170
Total Hardness	mg/l	420
Ammonia nitrogen	mg/l	16
Alkalinity	mg/l	Nil
Dissolved Oxygen	mg/l	7.2

Minerals	**Unit**	**Result**
Iron (FE)	mg/l	0.11
Magnesium (MG)	mg/l	3.0
Manganese (Mn)	mg/l	0.10
Calcium (Ca)	mg/l	62.0
Sodium (Na)	mg/l	108.0
Potassium (K)	mg/l	13.5
Zinc (Zn)	mg/l	<0.001
Lead (Pb)	mg/l	<0.01
Nitrate (No3)	mg/l	0.14

APPENDIX V

NON-ESSENTIAL AMINO ACIDS COMPOSITION OF DIFFENTLY PROCESSED *LEUCAENA* LEAVES (g/16gN**)**

-

TREATMENT/ Amino acid	SU	SD1	SD2	SD3	SW1	SW2	SW3	SB5
Aspartic acid	9.86	9.74	9.43	9.43	8.68	8.68	8.93	9.00
Serine	4.99	4.94	4.29	4.47	3.60	3.85	3.88	4.02
Glutamic acid	12.72	12.57	11.97	12.42	11.36	11.51	11.66	11.66
Proline	4.48	4.07	3.46	3.36	3.05	3.26	3.26	3.46
Glycine	5.91	5.67	5.09	5.28	4.37	4.42	4.52	4.8
Alanine	6.31	5.70	5.01	5.39	4.18	4.23	4.40	4.48
Cysteine	1.32	1.06	0.93	0.93	0.66	0.79	0.86	0.93

APPENDIX VI

CONCENTRATION OF CHROMIUM (PPM)

Treatment	Feed	Feaces
SWC	0.166	0.305
SW10	0.166	0.305
SW20	0.098	0.152
SW30	0.166	0.275
SW40	0.228	0.329

APPENDIX VII

PROXIMATE COMPOSITION OF FEACES

TREATMENT	SWC	SW10	SW20	SW30	SW40
Moisture	21.17^{e}	21.07^{d}	22.71^{c}	27.65^{a}	23.00^{b}
Ash	13.92^{a}	11.00^{c}	3.42^{d}	1.66^{e}	11.23^{b}
Ether Extract	3.62-	3.31^{d}	3.75^{b}	4.03^{a}	3.33^{d}
Crude Protein	18.23c	16.41^{e}	17.87^{d}	18.86^{a}	18.59^{b}
Crude Fiber	2.41^{e}	4.52^{d}	5.92^{c}	6.96^{b}	8.84^{a}
Nitrogen Free Extract	40.64^{d}	43.68^{b}	46.31^{a}	40.84^{c}	35.00^{e}

APPENDIX VIII

CURRENT PRICES OF FEED INGREDIENTS PER KILOGRAMME

Fishmeal	=	N500
Soyabean cake	=	N85
Groundnut cake	=	N40
Maize	=	N35
Premix and vitamin C	=	N1700

APPENDIX IX

ANOVA: PROXIMATE COMPOSITION OF DIFFERENTLY PROCESSED *LEUCAENA* LEAVES

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	956.116	7	136.588	210.332	.000
CP	Within Groups	10.390	16	.649		
	Total	966.506	23			
	Between Groups	15.864	7	2.266	8.956	.000
ASH	Within Groups	4.049	16	.253		
	Total	19.913	23			
	Between Groups	1.026	7	.147	3.609	.016
EE	Within Groups	.650	16	.041		
	Total	1.676	23			
	Between Groups	418.045	7	59.721	45.511	.000
CF	Within Groups	20.996	16	1.312		
	Total	439.041	23			
	Between Groups	72.897	7	10.414	22.655	.000
MO	Within Groups	7.355	16	.460		
	Total	80.251	23			
	Between Groups	583.146	7	83.307	19.852	.000
NFE	Within Groups	67.141	16	4.196		
	Total	650.287	23			
	Between Groups	687002.901	7	98143.272	18.919	.000
KCAL	Within Groups	83000.565	16	5187.535		
	Total	770003.466	23			

APPENDIX X

ANOVA: AMINO ACID COMPOSITION OF DIFFERENTLY PROCESSED *LEUCAENA* LEAVES

		Sum of Squares	df	Mean Square	F	Sig.
LY	Between Groups	3.373	7	.482	15.379	.000
	Within Groups	.501	16	.031		
	Total	3.874	23			
HI	Between Groups	1.411	7	.202	113.818	.000
	Within Groups	.028	16	.002		
	Total	1.439	23			
AR	Between Groups	3.066	7	.438	291.971	.000
	Within Groups	.024	16	.002		
	Total	3.090	23			
TH	Between Groups	7.425	7	1.061	729.438	.000
	Within Groups	.023	16	.001		
	Total	7.448	23			
CY	Between Groups	.801	7	.114	624.519	.000
	Within Groups	.003	16	.000		
	Total	.804	23			
VA	Between Groups	6.176	7	.882	5293.911	.000
	Within Groups	.003	16	.000		
	Total	6.179	23			
ME	Between Groups	.455	7	.065	519.881	.000
	Within Groups	.002	16	.000		
	Total	.457	23			
IS	Between Groups	9.106	7	1.301	9182.517	.000
	Within Groups	.002	16	.000		
	Total	9.108	23			
L.E	Between Groups	13.703	7	1.958	3404.571	.000
	Within Groups	.009	16	.001		
	Total	13.713	23			
TY	Between Groups	10.846	7	1.549	509.397	.000
	Within Groups	.049	16	.003		
	Total	10.895	23			
PH	Between Groups	8.898	7	1.271	2328.775	.000
	Within Groups	.009	16	.001		
	Total	8.907	23			

APPENDIX XI

ANOVA: ANTINUTRIENTS COMPOSITION OF DIFFERENTLY PROCESSED *LEUCAENA* LEAVES

		Sum of Squares	df	Mean Square	F	Sig.
PHYTATE	Between Groups	2.108	7	.301	44.969	.000
	Within Groups	.107	16	.007		
	Total	2.215	23			
CYANIDE	Between Groups	.540	7	.077	50.983	.000
	Within Groups	.024	16	.002		
	Total	.564	23			
TANNIN	Between Groups	2.195	7	.314	14.370	.000
	Within Groups	.349	16	.022		
	Total	2.545	23			
SAPONIN	Between Groups	4.576	7	.654	20.731	.000
	Within Groups	.505	16	.032		
	Total	5.080	23			
MIMOSINE	Between Groups	2.778	7	.397	96.296	.000
	Within Groups	.066	16	.004		
	Total	2.844	23			

APPENDIX XII

ANOVA: MINERAL COMPOSITION OF DIFFERENTLY PROCESSED *LEUCAENA* LEAVES

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	27.888	7	3.984	9466.835	.000
P	Within Groups	.007	16	.000		
	Total	27.894	23			
	Between Groups	1350.312	7	192.902	6172.033	.000
K	Within Groups	.500	16	.031		
	Total	1350.812	23			
	Between Groups	5.774	7	.825	3.310	.022
Mg	Within Groups	3.988	16	.249		
	Total	9.762	23			
	Between Groups	940.829	7	134.404	11.084	.000
Ca	Within Groups	194.018	16	12.126		
	Total	1134.848	23			
	Between Groups	12.954	7	1.851	102.476	.000
NA	Within Groups	.289	16	.018		
	Total	13.243	23			

APPENDIX XIII

ANOVA: GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF *HETEROBRANCHUS LONGIFILIS*

		Sum of Squares	df	Mean Square	F	Sig.
IWT	Between Groups	.900	5	.180	.750	.602
	Within Groups	2.880	12	.240		
	Total	3.780	17			
FWT	Between Groups	12428.903	5	2485.781	1.223	.357
	Within Groups	24384.181	12	2032.015		
	Total	36813.085	17			
MWG	Between Groups	12639.771	5	2527.954	1.251	.346
	Within Groups	24249.468	12	2020.789		
	Total	36889.239	17			
MFI	Between Groups	22666.533	5	4533.307	3.692	.030
	Within Groups	14733.607	12	1227.801		
	Total	37400.140	17			
MPI	Between Groups	3595.789	5	719.158	3.696	.030
	Within Groups	2335.235	12	194.603		
	Total	5931.025	17			
SGR	Between Groups	.134	5	.027	2.010	.149
	Within Groups	.160	12	.013		
	Total	.294	17			
FCR	Between Groups	10.247	5	2.049	2.392	.100
	Within Groups	10.283	12	.857		

	Total	20.529	17			
	Between Groups	.601	5	.120	1.703	.208
PER	Within Groups	.847	12	.071		
	Total	1.449	17			
	Between Groups	223.611	5	44.722	.700	.634
SR	Within Groups	766.667	12	63.889		
	Total	990.278	17			
	Between Groups	2453.604	5	490.721	2.008	.150
ANPU	Within Groups	2933.092	12	244.424		
	Total	5386.696	17			

APPENDIX XIV

ANOVA: APPARENT DIGESTIBILITY COEFFICIENT OF EXPERIMENTAL DIETS

		Sum of Squares	df	Mean Square	F	Sig.
DM	Between Groups	436.396	4	109.099	1090991.100	.000
	Within Groups	.001	10	.000		
	Total	436.397	14			
APD	Between Groups	232.718	4	58.180	581795.100	.000
	Within Groups	.001	10	.000		
	Total	232.719	14			
ALD	Between Groups	225.279	4	56.320	563198.100	.000
	Within Groups	.001	10	.000		
	Total	225.280	14			
ACD	Between Groups	80.493	4	20.123	274408.318	.000
	Within Groups	.001	10	.000		
	Total	80.494	14			

APPENDIX XV

ANOVA: CARCASS ANALYSIS OF *HETEROBRANCHUS LONGIFILIS* FED GRADED LEVELS OF *LEUCAENA*

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	2.999	6	.500	1.424	.273
MO	Within Groups	4.913	14	.351		
	Total	7.911	20			
	Between Groups	21.729	6	3.622	2399.116	.000
CP	Within Groups	.021	14	.002		
	Total	21.750	20			
	Between Groups	.000	6	.000	.	.
CF	Within Groups	.000	14	.000		
	Total	.000	20			
	Between Groups	29.002	6	4.834	866.830	.000
EE	Within Groups	.078	14	.006		
	Total	29.080	20			
	Between Groups	9.363	6	1.560	8.309	.001
ASH	Within Groups	2.629	14	.188		
	Total	11.992	20			
	Between Groups	147.458	6	24.576	35.379	.000
NFE	Within Groups	9.725	14	.695		
	Total	157.183	20			

APPENDIX XVI

ANOVA: HAEMATOLOGICAL INDICES OF *HETEROBRANCHUS LONGIFILIS* FED GRADED LEVELS OF *LEUCAENA*

		Sum of Squares	df	Mean Square	F	Sig.
PCV	Between Groups	109.778	5	21.956	3.992	.023
	Within Groups	66.000	12	5.500		
	Total	175.778	17			
RBC	Between Groups	.802	5	.160	4.248	.019
	Within Groups	.453	12	.038		
	Total	1.256	17			
HB	Between Groups	11.598	5	2.320	3.768	.028
	Within Groups	7.387	12	.616		
	Total	18.985	17			
MCH	Between Groups	.735	5	.147	1.407	.290
	Within Groups	1.254	12	.104		
	Total	1.989	17			
MCV	Between Groups	4.167	5	.833	.831	.552
	Within Groups	12.041	12	1.003		
	Total	16.208	17			
MCHC	Between Groups	.311	5	.062	1.191	.370
	Within Groups	.626	12	.052		
	Total	.937	17			
WBC	Between Groups	360.075	5	72.015	2.534	.087
	Within Groups	341.019	12	28.418		
	Total	701.095	17			
	Total	548.500	17			

APPENDIX XVII

PRINCIPAL COMPONENT ANALYSIS AND %VARIANCE

	Eige	%variance
1	111.021	61.228
2	4.37191	24.288
3	1.46665	8.148
4	0.81236	4.5131
5	0.328043	1.8225
6	1.07055E-27	5.9475E-27
7	3.77764E-61	2.0987E-60
8	00	
9	00	
10	00	
11	00	

APPENDIX XVIII

ANOVA: COST BENEFIT EVALUATION OF *HETEROBRANCHUS LONGIFILIS* FED GRADED LEVELS OF *LEUCAENA*

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	6037.678	5	1207.536	31.995	.000
FC	Within Groups	452.890	12	37.741		
	Total	6490.568	17			
	Between Groups	6037.678	5	1207.536	31.995	.000
TC	Within Groups	452.890	12	37.741		
	Total	6490.568	17			
	Between Groups	7703.272	5	1540.654	1.174	.377
MYC	Within Groups	15752.455	12	1312.705		
	Total	23455.727	17			
	Between Groups	2304.175	5	460.835	.366	.862
GP/L	Within Groups	15121.700	12	1260.142		
	Total	17425.875	17			
	Between Groups	.125	5	.025	.885	.520
IC	Within Groups	.339	12	.028		
	Total	.464	17			
	Between Groups	.406	5	.081	.546	.739
PI	Within Groups	1.787	12	.149		
	Total	2.194	17			
	Between Groups	.248	5	.050	.479	.785
BCR	Within Groups	1.244	12	.104		
	Total	1.492	17			