# EFFECTS OF REPLACING FISHMEAL WITH HEAT-PROCESSED *MORINGA OLEIFERA* (LAM.) KERNEL MEAL ON GROWTH AND FEED UTILIZATION IN *CLARIAS GARIEPINUS* (TEUGELS, 1986)

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**AHMADU BELLO UNIVERSITY, ZARIA.**

**JUNE, 2011**

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**A DISSERTATION SUBMITTED TO THE SCHOOL OF POST-GRADUATE STUDIES, AHMADU BELLO UNIVERISTY, ZARIA IN PARTIAL FULFILMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D) IN FISHERIES.**

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**JUNE, 2011**

## DECLARATION

I declare that the work in the dissertation entitled **"EFFECTS OF REPLACING FISHMEAL WITH HEAT-PROCESSED *MORINGA OLEIFERA* (LAM.) KERNEL MEAL ON GROWTH AND FEED UTILIZATION IN *CLARIAS GARIEPINUS* (TEUGELS, 1986)"** has been performed by me in the Department of Biological Sciences under the supervision of Professor S. J. Oniye, Professor J Auta and Dr. F. Abeke. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree at any University.

OPADEYI, VICTORIA ABOSEDE

…… ……. ……

Name of Student Signature Date

## CERTIFICATION

This dissertation titled **"EFFECTS OF REPLACING FISHMEAL WITH HEAT-PROCESSED *MORINGA OLEIFERA* (LAM.) KERNEL MEAL ON GROWTH AND FEED UTILIZATION IN *CLARIAS GARIEPINUS* (TEUGELS, 1986)"** by Opadeyi, Victoria Abosede meets the regulations governing the award of the Doctor of Philosophy (Ph.D Fisheries) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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## DEDICATION

Dedicated to GOD, the CREATOR OF FISHES who gave human beings the wisdom to multiply fishes.

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## ABSTRACT

The suitability of heat-processed *Moringa oleifera* (Lam.) kernel meal for partial replacement of fishmeal and its effects on the growth and feed utilization of *Clarias gariepinus* (Teugels) were determined. Boiling and toasting respectively, at 10, 20 and 30 minutes were employed to obtain the most nutritionally acceptable meals for replacing fishmeal in *Clarias* diets. The chemical compositions of the boiled and toasted kernels were determined and data analysed using Analysis of Variance (ANOVA) and means separated with Least Significant Difference (LSD) ($P < 0.05$). The results showed that there was no significant difference ($P > 0.05$) in the crude fibre (4.03, 4.79, and 4.99), ash (2.13, 2.10, 2.19) and moisture (3.09, 2.83, 2.92) for the *Moringa* kernels boiled at 10, 20 and 30 minutes duration respectively. However, the lipid content (37.01) was significantly ($P<0.05$) lowest in 30 minutes-boiled sample. The protein content reduced significantly ($P<0.05$) from 35.72% in 10-minutes boiled kernels to 27.87% in 30 minutes-boiled samples. The anti-nutrient detected was oxalate which was significantly ($P < 0.05$) lowest in the 30 minutes-boiled kernels (5.60 mg $g^{-1}$). Similarly, in the toasted samples, there was no significant difference ($P < 0.05$) in the crude protein, and lipid contents of toasted sample. Crude fibre was significantly lower in 30-minutes toasted kernels than that of 20 minutes. Consequently on the basis of amino acids profile, the lowest anti-nutrient, and 30 minutes-boiled and toasted *Moringa* kernels were used for replacing fishmeal at 25%, 50% and 75% levels in the diets of *Clarias gariepinus*. Seven diets were formulated, $D_1$ (100% fishmeal), $D_2$ (75% fishmeal, 25% toasted *Moringa* kernel meal), $D_3$ (50% fishmeal and toasted *Moringa* kernel meal respectively), $D_4$ (25% fishmeal, 75% toasted *Moringa* kernel meal), $D_5$ (75% fishmeal, 25% boiled Moringa

kernel meal), $D_6$ (50% fishmeal and boiled *Moringa* kernel meal respectively), $D_7$ (25% fishmeal 75% boiled *Moringa* kernel meal) and $D_8$ (commercial fish diet, Chi feed). Indoor static tanks were used and the experiment lasted 90 days. The research findings showed no significant difference ($P < 0.05$) in the mean feed intake by *Clarias* fed toasted and boiled *Moringa* kernel meal – based diets but feed intake was significantly higher ($P < 0.05$) in the fish fed commercial diets and the control (0.775and 0.506 respectively). Diet 7 (25% fishmeal and 75% boiled *Moringa* kernel meal) produced the best weight gain in *Clarias* (80.06%) while the least weight gain (46.43%) was obtained in Diet 4 (25% and 75% of *Moringa* kernel meal). The specific growth rate was best significantly ($P < 0.05$) in Diets 6 and 7 but lowest in Diet 4. The feed conversion ratio and protein efficiency ratio were significantly best in Diet 7 but worst in Diet 2. The control (D1) and commercial feed (D8) however proved best over the *Moringa* diets in terms of growth and feed utilization by *Clarias.* Diet 1 (control) had the least cost of feed per kilogram weight gain (₦541.58) and least cost of feed per kilogram protein produced (₦399.52) but Diet 7 had the least cost of feed per kilogram weight gain (₦619.08) among *Moringa* – based diets. Conclusively, diets containing boiled *Moringa* kernel meal produced better growth and feed utilization in *Clarias* than those with toasted kernel meal and up to 75% of fishmeal could be replaced with boiled *Moringa* kernel in the diet for *Clarias gariepinus.*

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## ABBREVIATIONS

| | | |
|---|---|---|
| ANOVA | - | Analysis of Variance |
| *Clarias* | - | *Clarias gariepinus* |
| CP | - | Crude Protein |
| D.O | - | Dissolved Oxygen |
| EAAI | - | Essential Amino Acids Index |
| FCR | - | Feed Conversion Ratio |
| $H_o$ | - | Null Hypothesis |
| LSD | - | Least Significant Difference |
| *Moringa* | - | *Moringa oleifera* |
| P.E.R | - | Protein Efficiency Ratio |
| P.P.M | - | *Pusarium* Petal Meal |
| SGR | - | Specific Growth Rate |

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background information

The daily protein intake of an average Nigerian is very low compared with the required value. The very common and available sources of plant and animal proteins such as beans, peas and livestock are at present beyond the reach of the common man. This has resulted in all sorts of malnutrition especially among the young people. Fish has been acknowledged to supply a good balance of protein, vitamins and minerals with very low carbohydrate content; hence its role in nutrition is recognized. It is also a rich source of essential nutrients required to supplement both infant and adult diets (Craig, 2009). Fish was once the cheapest and readily available source of animal protein in Nigeria but its present high cost has made it unavailable on the table of most Nigerian homes. Adesulu (2001) attributed the high cost of fish to the following:

- low productivity of large rivers and lakes which are already over exploited,
- destruction of aquatic organisms in small rivers and streams due to pollution, and
- availability of very few number of fish farms, some of which are not managed properly or already abandoned.

Fish feeds especially imported ones such as Coppens and Dizengoff which constitute the greatest determinant of profitability of fish farming are expensive. The high cost of these feeds is accounted for by the high cost of fishmeal, the main protein source, and other conventional protein sources such as soya bean and groundnut.

The science of fish nutrition has advanced rapidly over the past 15 years due to the development of commercial fish farming. For fish to attain their genetic potential for

growth, reproduction and longevity, proper nutrition is required. Feed represents the largest single cost items in intensive fish production as it is with other live stocks. Lall (1991) stated that the main objective of ration formulation is to utilize the knowledge of nutrient requirements, locally available feed ingredients and digestive capacity of fish to develop a nutritionally balanced mixture of feedstuffs which will be eaten in sufficient amount to provide optimum production at an acceptable cost, that is, helping the farmer to produce high quality and large quantity of fish at the lowest possible cost. Fish nutrition therefore involves ration formulation that would meet the exact or almost exact requirement of the fish species concerned. However it should be noted that no single feed ingredient contains all nutrients that are needed in correct proportions. It is therefore suggested that by selecting the various feedstuffs of plant and animals origin in proper amounts, a well balanced compound ration may be formulated (Lall, 1991).

The production of aquaculture feeds is expected to be rise from the current level of about 13 million metric tonnes to about 30 metric tonnes in 2010. It is estimated that a minimum of the 3 metric tonnes of fishmeal equivalent alternative protein sources will be required in the aquaculture industry yearly by the year 2010. The selection of plant-derived protein sources for use as animal feeds should take human food security interest into account. It will be highly desirable if products from plants that can grow on degraded soil and require low external energy subsidies could be used. Such plants include *Jatropha curcas, Moringa oleifera*, *Sesbania* species and *Mucuna pruriens.* Alongside their potentials in the production of feed ingredients, these species can help reclamation of degraded areas. Furthermore their development may be aided by national and international funding (Francis *et al., 2001*).

## 1.2 Research Problems

For effective production of fish on a large scale, fish feed is one of the essential factors to consider. Protein source constitutes 64 – 67% of the total cost of fish feeds therefore recent researches in aquaculture are focused on reducing its cost (Prendergast *et al.,* 1994). Though fish feed constitutes one of the major inputs in intensive fish farming, it can negate the economic viability of a farm if suitable feeds are not used. The use of commercial pelleted fish feed is so expensive that it accounts for about 60% - 80% of the recurrent cost of fish farming venture (Madu and Ufodike, 2003). Nigeria is blessed with variety of feed ingredients for human, livestock and fish, inspite of this, the nutrition of pond fishes is one of the major problems of fish culture in this country. Making qualitative supplementary feeds available to culture fish species at appropriate level and time still remains an objective yet to be accomplished. This would be accounted for by the fact that most of the feed stuffs like maize, sorghum, soybean, groundnut cake and fish meal used in preparing fish feed face serious competition with humans as food in Nigeria. In view of this serious problem, attempts are being made to assess the potentials of non-conventional sources of fish feeds ingredients which should possess qualities such as being readily available, cheap, high amino acid composition and able to produce fast growth in fish within a short duration.

In nutritional studies, priority is always given to the protein requirements because it is the single nutrient required in large quantity for growth (Lovell, 1987). The major source of protein in conventional fish feed is the fish meal which is actually the best source of fish protein but is relatively expensive. Its high cost and scarcity have prompted world-wide research into non-conventional but yet efficient protein sources of

feed stuff for fish. Efforts are therefore being directed at developing diets for fish in which the significant proportion of the fish meal is partially or wholly being substituted by readily available and cheap plant as well as non-conventional animal protein sources (F.A.O., 1997).

The plant sources of protein are mostly legumes and the most common legumes used in fish feed formulation are soybean (*Glycine max)* and groundnut (*Arachis hypogea*). However, these crops are presently facing competition for use by human and other livestock as food. This has resulted in other legumes such as Jack bean, Bambara nut, Sword bean, Yam bean and Lima bean being investigated for fish feed ingredients (Banyigyi *et al.*, 2001). A major hindrance to the use of conventional and non-conventional plant protein sources identified is the presence of anti-nutritional factors such as phytates, tannins, saponins and oxalates in these plant sources (Banyigyi *et al*., 2001; Olvera – Castillo *et al.,* 2011). Several treatment such as soaking, fermenting, sun drying, auto claving, boiling and toasting have been investigated to reduce or remove the anti-nutritional factors (Burel *et al.,* 2000; Adegoke *et al.,* 2010; Bamgbose *et al.*, 2010)

*Moringa oleifera* (Lam) (Zogale – Hausa) is a plant that has come under focus in the last two decades due to its medical and nutritional attributes which have been well documented (Fugile, 2001). It has been successfully used in combating malnutrition in humans in some developing countries due to its high nutritional value and low anti-nutritional contents (Fugile, 2001). In Nigeria *Moringa* leaves are consumed as vegetables, especially in the northern parts but the seeds are left unused. Only a few seeds are collected by farmers and planted as hedges around their farms. In spite of the plants' value for human uses, extensive research has not been carried out on its

usefulness in replacing fish meal as protein source in fish diet except for the work done by Richler *et al.* (2002) in which fishmeal was replaced with treated *Moringa* leaves.

## 1.3 Justifications

*Moringa oleifera* (Lam.) resembles a leguminous plant by the possession of bilaterally symmetrical flowers and pinnate leaves; some botanists, classify it as a member of Violet family while others advocate its placement in the Bignonia family based on its long woody pods and winged seeds (Fugile 2001). *Moringa oleifera* has come under focus in nutritional importance for human and animals due to the high protein content of its fresh leaves (25 – 29% C.P) and fresh fruit (20.7% C.P) (Makkar and Becker, 1997). The crude protein content is higher in leaves from which certain oils or growth promoting components have been extracted. Analyses showed that the crude protein content of extracted leaves is up to 43.5% while that of unextracted leaves is 25.1% (Fugile, 2001). Ahwange *et al.* (2004) reported that *Moringa oleifera* seeds collected in Zaria, Kaduna State contain 40.31% crude protein thus making it highly comparable to that of raw soybean (35.6 – 42.4%)(Giami, 2002). Various qualities such as the plant's availability, ease of propagation, fast growth, drought resistance and simple processing techniques (Ogunsina *et al.,* 2010) made *Moringa* a choice for investigating its suitability as a substitute for fishmeal in fish rations.

## 1.4.0 Aims and Objectives

### 1.4.1 Aim

The general aim is to determine the suitability of boiled or toasted *Moringa oleifera* kernel meal for replacing fishmeal in the feed of *Clarias gariepinus*. This aim will be achieved through the following objectives,

### 1.4.2 Objectives

The specific objectives are:

- to determine the best processing method and duration that produced *Moringa oleifera* kernel meal with the highest chemical composition particularly in terms of crude protein contents amino acids profile and anti-nutritional factors.
- to determine which treatment and the replacement level of fishmeal with *Moringa oleifera* kernel meal in the diet that produced the best growth and feed utilization in *Clarias gariepinus.*
- to assess the cost effectiveness of *Moringa oleifera* kernel meal based diets in comparison with the control diet and the commercial feed used for feeding *Clarias gariepinus*.

## 1.6 Research Hypotheses

The research hypotheses include:

$Ho_1$: There is no significant difference in the chemical composition of *Moringa* kernels boiled or toasted for 10, 20 and 30 minutes respectively.

$Ho_2$: There is no significant difference in the growth and feed utilization by *Clarias gariepinus* fed diets containing different levels of substituted boiled or toasted *Moringa* kernel meal.

$Ho_3$: There is no significant difference in the cost of feed per kilogram weight gain and cost of feed per kilogram protein gain in *Clarias gariepinus* for all formulated diets.

# CHAPTER TWO

## 2.2 LITERATURE REVIEW

## 2.3 Fish Feed Development in Africa

Fish feed is considered as one of the fundamental challenges facing the development of aquaculture in Africa. Hetch (2000) observed that in spite of a lot of researches on inexpensive feed ingredients, not much has been contributed to aquaculture development in Africa. He suggested that more research on how best plant protein can be used as fish feed should be carried out. Fish feed technology is one of the least developed sectors of aquaculture particularly in the developing countries (F.A.O. 2003). The need for such researches was reinterated by Jamu and Ayinla (2003) in view of the fact that feed accounts for at least 60% of the total cost of fish production in Africa. However, opportunities exist in Nigeria to reduce feed cost with the availability of local feed ingredients of plants and animal origins rich in crude protein (MSME, 2005). The purpose of fish culture is to produce fish with fast growth and economic viability. These artificial feeds are well compounded mixture of feed stuffs in marsh or pelleted form.

### 2.2.0 Dietary Needs of Fish

Ayinla (1991) stated that for efficient production and growth of fish in culture, provision of the best feeds at the level not exceeding dietary requirement is necessary. In nature, fish normally consume an array of food organisms which appear to provide various nutrients required by them. However under culture condition, the natural feeds are not sufficient for optimum growth giving rise to the needs for supplementary feeds. Fish nutrients requirement have been classified into:

a. Basal feed which is essentially carbohydrate-base containing less than 20% crude protein.

b. Protein concentrate such as fish meal, groundnut cake, soybean meal and others having more than 20% crude protein.

c. Fixed ingredients consisting of minerals, vitamins, salt and fat.

### 2.2.1 Crude Protein Requirements

Protein is regarded as the most important component of fish feed required for maximum fish growth and reproduction, and each species of fish has different protein requirement at specific growth stage or age, size and feeding habit (carnivorous, herbivorous or omnivorous). It is therefore required that any feed should be readily available, cost effective and nutritionally qualitative.

Various dietary needs have been indicated by various researchers. Jackson *et al.* (1982) stated that *Oreochromis niloticus* weighing 30 grammes require 30% crude protein in its diet. Lovell (1987) also stated that the percentage Crude protein (C.P.) required for *Tilapia* is 25%; *Clarias* fry, 38 – 38.5%, *Clarias* fingerlings 32.5% and adults, 37%; and that for a source or protein to provide good growth, such proteins must contain Essential Amino Acids (EAA) that cannot be synthesized by fish. The protein needs of *Clarias gariepinus* fingerlings range between 31 – 34%; juvenile, 31 – 34%; adult, and brood-stock, 40%. Ayinla (1988) and Eyo and Olatunde (2001) also indicated that the dietary protein requirement for catfish fingerlings and adults are 40% and 35% respectively.

Ayinla (1988) reported that with the exception of fish meal protein, there are no known animals or plant feed proteins available to the fish feed compounder whose essential amino acid profile alone approximate the dietary requirement of cultured fish. The major problem of these other protein sources is the imbalance of amino acids which render them unsuitable as solo source of dietary protein for fish. An example is the deficiency of methionine in pulses, oil seeds meat, bone meal and hydrolyzed feather and the deficiency of tryptophan in fish silage and hydrolyzed feather meal. Another problem is the presence of disproportionate levels of specific amino acids such as leucine – isoleucine antagonisms and argunine – lysine antagonisms. An example is blood meal, which is a rich source of valine, leucine and histidine but a poor source of methionine and isoleucine whose deficiency is as a result of the antagonistic effect of excess leucine. Sogbesan and Ugwumba (2008) suggested that protein in fish feed for effective utilization should be supplemented with other protein sources generally having low biological values so as to obtain the required essential amino acids profile for fish. There are two major sources of protein for substituting fish meal in fish diets. Those of animal origin include terrestrial and aquatic worms, fish silage, blood meal, tadpole meal, insects and their larvae, poultry by products meals, meat and bone meal, and dairy products such as whey powder. Various attempts to replace all or part of the fish meal components with these substitutes in fish feed have caused from good to fair or poor results in terms of growth and feed conversion efficiency (FCE). The plant protein sources include oil seed-plants such as groundnut, soybean, sesame, cereals and their by-products.

### 2.3.0 Plant Protein Sources as Alternatives to Fishmeal in Fish Feed.

Plant protein sources are known as the cheaper feed sources for fish as well as other livestock. Investigations have been carried out to replace animal protein (fish meal) by plant protein in the diet of fish. El-dahhar *et al.* (1993) reported that the replacement of animal protein by plant protein reduced the growth performance of fish in linear relationship to the rate of replacement. He attributed this to the deficiency of some amino acids in the plant protein and suggested that the deficient amino acids should be supplemented in order to improve the growth of the fish. FAO (1997) suggested the need to seek for other sources of protein to replace fishmeal in fish feed.

Plant protein supplements, cereal grains and grain by-products are widely used in feeds for aquaculture species. Global availability and relatively low cost compared to ingredients of animal origin are their most obvious positive attributes

The inclusion of non-conventional plant fed stuffs (NCPF) in fish diets largely depends on the availability, nutrient level, processing technique, fish species and cultural farming pattern (Gabriel *et al*., 2007). Several parts of plants have been used in fish feed formulation as discussed in the followings:

### 2.3.1 Use of Leaves

The use of some leaves in fish feed formulation has also been investigated by some researchers. Bastatico and Baladia (1980) used undisclosed amounts of Ipil-Ipil (*Leucaena leucocephala*) leaf meal in formulating fish ration. Leaf meal of Ipil-Ipil has the advantage of low crude fibre and high protein in comparison with other forage legumes. The findings showed that inclusion level of up to 40% produced satisfactory growth performance in some commercial fish.

Edwards *et al.* (1985) also incorporated water hyancinth (*Eichornia crassipes*) in compost and dried forms into pelleted feed for Tilapia (*Oreochromis niloticus*). The levels of inclusion of water hyancinth in the feed were 100%, 75%, 50% and 25% for compost and 25%, 75% and 100% for dried form. The control was 100% commercial fish feed produced by the National Inland Fisheries Institute (NIFI) Bangkok. The results showed that significant yield of Nile Tilapia was obtained from 50% compost leaf incorporated meal. However it was observed that though the fish consumed some of the compost, much nutrition came from natural foods in the pond generated as a result of the fertilizing effect of the unconsumed compost of the water hyacinth leaf.

Falaye *et al.* (2001) also experimented with Ipil – Ipil (*Leucaena leucocephala*) leaf meal to determine the growth and survival rate of Tilapia (*Oreochromis niloticus*) fries. The leaf was processed by soaking in water for 24 hours. The dried leaves were ground and incorporated into the experimental dies at 25%, 50% and 75% replacement levels for groundnut cake while 100% groundnut cake diet was used as control. The results showed fish fed with the control diet had the best growth while those fed with 75% inclusion level of Ipil – Ipil leaf meal had the poorest growth performance. This was attributed to the presence of the anti-nutritional factors particularly mimosine present in the Ipil-Ipil leaf. It was suggested that the level of the anti-nutritional factor can be reduced using other appropriate method of treatment.

### 2.3.2 Use of Flower

Dried flowers of flamboyant tree (*Pusarium regia*) were used as ingredients to formulate fish feed rations. The dried flowers (crude protein 15.03%) were ground into

meal (Pusarium Petal Meal, P.P.M.) and included in fish ration at 15% and 20% levels. The results showed that the fish fed on control diet (0% P.P.M.) had significantly higher carcass protein than those with the test ingredients. However the ash content was higher in the diet with 20% level of inclusion of P.P.M. The weight gain noticed in the diets was not due to the inclusion of the P.P.M. but the normal increase as a result of tissue protein synthesis (Ipinjolu and Faturoti, 1999).

### 2.3.3 Use of Plant Seeds

Dabrowski and Kozack (1979) used commercial soybean meal (C.P. 29 – 40%) at 40 to 60 % level of inclusion. The result showed that the growth performance was lower in fish fed the test diet than those fed the fishmeal – based diet. The growth rate continued to decline with increasing soybean content. Soybean as indicated by Viola *et al.* (1981) has been major plant protein source for compounding fish ration, but of recent, the demand for it has been too high due to its use by human beings. Further researches on the use of soybean for fish feed have been carried out.

Abel *et al.* (1984) used thermally and hydrothermally treated full fat soybean meal (C.P. 44 – 50%) in the feed of carp at 50% level of inclusion. The result showed that fishmeal - based diet produced fish with the best performance while among the soybean meals, the hydrothermally and intensely thermally treated meals produced best growth performance.

Olvera-Novoa *et al.* (1988) used autoclaved, raw and water treated *Sesbania grandiflora* seedmeal (C.P 30%) at different inclusion levels of 9 to 33% to feed Tilapia. The results showed depression in feed intake and increased mortality with increased use

of *Sesbania* seed meal. Though the fish fed the water-treated seed meal performed better than other treatments, it was not as good as the control (fishmeal).

Wee and Shu (1989) utilized raw defatted and boiled soybean meal (C. P. 30%) as well as the mixture of both in the feed of Nile tilapia. The inclusion levels were 55% ratio 56 to 58% raw and boiled, 58% boiled and 52% defatted soybean. The findings showed that the fish fed diet with boiled or defatted soybean grew better than control (fishmeal) while increasing level of raw meal progressively depressed growth.

Sintayehu (1996) fed commercial soybean meal, cotton seed meal and sunflower seed meal to Tilapia at 21 – 34% levels of inclusion (C. P. 25.5) and 32% fish meal replacement level. The results indicated that fish fed the sun flower meal and cotton seed meal showed slightly better performance than control but the soybean meal-fed fish had inferior growth performance and feed conversion than the control.

Solomon *et al.* (1996) investigated the effects of replacing fish meal with soybean meal, groundnut cake and blood-meal at varied proportions on the growth and food utilization of *Clarias anguillaris* fingerlings in outdoor hapas. Results showed that fish meal gave the highest growth rate when compared with groundnut cake and blood-meal. However fish meal diet is not cost effective. Also the protein of groundnut cake is more utilized than that of Soybean meal though the latter has higher amino acids profile than the former.

Alegbeleye *et al.* (1996) assessed the use of Jack bean (*Canavalia ensiformis*) meal as an ingredient in the diets of *Clarias gariepinus* fingerlings at 15%. 30%, 45% and 60% level of substitution of soybean meal. The results obtained indicated that feed consumption was higher in control (full fat soybean meal) that in the test diet. The

survival rate and mean weight gain were also higher in control than the test diets. However the feed conversion ratio (F.C.R) was highest in diet with 60% Jack bean meal inclusion level where fish consumed more feed to produce significant ($P<0.05$) increase in weight, thereby making the feed uneconomical. Although the performance of fish fed on 15% and 30% inclusion level of Jack bean meal compared with the control, the fish mortality was higher in the later. The high dietary levels of legumes have been found to be deleterious to the performance of cultured fish due to the presence of toxins and nutrient imbalance associated with plant protein sources.

Unheated and heat treated *Jatropha curcas* meal (C. P. 35%) were separately included in common carp feed at 23% level of inclusion. The results showed that the body weight gain, protein efficiency ratio and feed conversion ratio of fish were significantly ($P<0.05$) similar for unheated and heated (15, 30, 45 minutes) *Jatropha* meal – containing diets. The 15 minutes-heated meal was however considered optimum for fish. The trypsin inhibitor and lectin activities decreased with increased heating duration (Makkar and Becker, 1999).

Fagbenro (1999) used autoclave to roast winged bean (C.P. 40%) at 50% level of inclusion in the feed of African cat fish. The findings showed no significant difference in the performance of the fish in the test diet and the control.

Banyigyi *et al.* (2001) also used another rich source of plant protein, Bambara nut (*Voandzeia subterranea* (L) thouars) meal which was heat treated to feed *Oreochromis niloticus* juveniles in order to determine their feed utilization and growth. The result indicated no significant difference in the growth and feed utilization of all the processed bambara nut meal based fed to *Oreochromis niloticus* but low growth performance was

noticed with the raw bambara nut meal. This could be attributed to trypsin inhibitory factors present in the nut. It was suggested that the duration of processing of the bambara nut be extended and other feed processing methods be employed to enhance better growth performance and nutrient utilization by the fish since the nut has high digestibility.

Cotton seed meal has been used in fish diets. Up to 15% of cotton seed meal could replace soybean meal and if supplemented with lysine, up to 30% of cotton seed meal could be used in the diets for channel catfish. Glanded cotton seed meal however caused depressed growth in channel catfish in contrast to fish fed glandless seed meal (Hertrampf and Piedad – Pascual, 2003).

The effects of substituting fish meal with sesame cake in fish diet on growth and food utilization and body composition of the Nile Tilapia (*Oreochromis niloticus)* were investigated by Ofojekwu and Kigbu *et al.* (2003). The results showed that the best growth and food utilization were obtained with 10% sesame and 40% fish meal (C. P. 36,22% and crude fibre 10.18%). The result further indicated that up to 20% sesame cake can be used with 30% fish meal in the diet of the juvenile of *Oreochromis niloticus* without affecting the over-all performance. However with high levels of sesame cake, the growth depression was significant as observed in the diet with 50% Sesame cake and 0% fish meal which resulted in the poorest growth rate of the test fish. The poor performance was attributed to the high fibre content and phytic acid present in the sesame cake.

Ofojekwu *et al.* (2003) also substituted palm kernel meal for cotton seed cake in the diets for Nile Tilapia (*Oreochromis niloticus* L.). Their findings showed that as the

level of palm kernel meal increased to 24% and higher, the weight gain and feed utilization declined significantly. It was recommended that when using cotton seed and palm kernel, the substitution levels should be at 20% and 16% respectively. Alegbeleye *et al.* (2004) used another plant protein source, rubber, (*Hevea brasiliensis*) seed cake with C.P. content of 36% to replace groundnut cake in the diets for Nile Tilapia (*Oreochromis niloticus).* The findings showed that fish fed on 30% inclusion level of rubber seed meal gave the best results for specific growth rate (S.G.R.) of 1.73, feed conversion ratio (F.C.R) of 1.69 and protein efficiency ratio (P.E.R.) of 1.97 which are significantly superior ($P> 0.05$) to values obtained in other diets. The result further showed that 60% inclusion level of rubber seed meal gave the poorest result showing conclusively that equal replacement of groundnut cake with rubber seed meal was the most suitable as protein dietary source for *Oreochromis niloticus*.

The nutritive values of fibre–reduced and high-fibre sun flower cakes as replacements for anchovy fishmeal in Tilapia diets were investigated. Diets based on the fibre – reduced cake had higher levels of all amino acids than the ones based on the high fibre cake. Lysine and threonine concentrations were lower in diets based on sunflower cakes. Feed intake, percent weight gain and feed conversion ratio were significantly ($P<0.05$) lower in the sunflower cake – based diets than the fish meal diets. Reduction in the fibre content of sunflower cake improved feed intake, growth rate and weight gain (Maina, *et al.,* 2007).

Raw and hydrothermally processed *Prosopis justifora* seeds meal were used as supplementary feed for the growth of *Labeo rohital* fingerlings. The weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio of hot water-

soaked seed meal at 20% replacement level were higher among the test diets (20%, 35% and 50% substitution levels) but lower than control. The 50% inclusion level diet produced decreased growth, carcass composition, digestion enzyme activity and digestibility when compared with 20% and 30% inclusion level diets. Hydrothermally processed seed meal produced improved growth when compared to raw but not up to the control (fishmeal inclusion diet) (Bhatt *et al*., 2011).

Sesame (*Sesamum indicum L.*) oil cake was used to replace fish meal in the diet for rainbow trout (*Onchoryhncus mykiss* W.). The growth rate of fish fed rations containing less than 50% sosame oil cake was significantly ($P<0.05$) better than the control but above this 50% replacement level produced significantly reduced protein intake, retention and efficiency in the fish (Nang Thu *et al.,* 2011).

Raw and heat treated cowpea (*Vigna unguiculata*) were used to substitute fishmeal in the diets for Nile Tilapia. (*O. niloticus*). Substitution of 20% raw cowpea had no significant effect ($P>0.05$) on production parameters but growth was negatively affected in 100% raw cowpea due to anti-nutrients present in the seed. Among the heat treated seeds (oven drying at 48$^{o}$C, hot air drying at 70$^{o}$C and autoclaving at 19$^{o}$C, only autoclaving eliminated trypsin – inhibitor and lectin contents independent of dehulling. It was suggested that heat treated cowpea mal can replace fishmeal in tilapia fingerlings diet up to 20% replacement level (Olvera – Castillo *et al*., 2011).

Soyabean meal was used in formulating diets for juvenile *Pangasianodon hypothalamus*. The findings showed that the growth performance and feed utilization decreased with increase in soya bean meal inclusion. The final weight and relative growth rate control diet (fishmeal inclusion) were significantly higher ($P< 0.05$) than

those fed test diet. Replacement of not more than 45% of soya bean meal was recommended without adverse effect on growth, feed utilization and body composition of the fish (Phunet *et al.,* 2011).

### 2.3.4 Anti-Nutritional factors in Plants

Makkar (1993) defined anti-nutrients as "substances which by themselves or through their metabolic products arising in living systems interfere with food utilization and affect the health and production of animals". He further classified these substances into four groups namely:

- Factors affecting protein utilization an digestion, for examples, protease inbitors, tannin and lectins.
- Factors affecting mineral utilization such as phytates, gossypol pigments, oxalates and glucosinates.
- Anti-vitamins,
- other substances such asmycotozins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents, phytoestrogens and saponin.

Rumsey *et al.* (1993) also classified anti-nutrients according to their ability to withstand thermal processing which is a common method for destroying them. Such 'heat-liable' factors include protease inhibitors, phytates, lectins, goitrogens and anti-vitamins. The 'heat-stable' factors include saponins non starch polysaccharides, antigenic protein, oestrogens and some phenolic compounds. Oligosaccharides become more digestible after heat treatment but the effect of heat on tannin is uncertain.

Francis *et al.* (2001) also stated that most of the alternative plant derived nutrients sources are known to contain a wide variety of anti-nutritional substances whose effects can be deleterious when included unprocessed in fish diet. He further identified the anti-nutritional substances present in some commonly used alternative sources of feed ingredients for fish. Soybeans meal contains protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, anti-vitamins and allergens. The anti-nutritional factors identified in a rape seed meal include tannins, glucosinolates, phytic acid and protease inhibitors. Saponins and alkaloids are also present in lupin seed meal while sun flower oil cake contains arginase inhibitors and saponins. Gossypol and cyclopropenic acid are present in cotton seed in leucaena leaf meal. Alfalfa leaf meal contains phytoestrogens (Francis *et al.,* 2001).

### 2.3.5 Use of Plants By-Products

Plants by-products have been used in formulating fish ration Mgbenka and Agua (1990) used plantain and yam peels in addition to shredded smoked fish wastes available in abundance in many restaurants in Nigeria, to produce feed for African cat fish. The results showed that the fish fed on fish wastes have the least weight gain while the ones fed plantain and yam peel meal had the highest significant weight gain. The difference in the growth of the fish feed the test diets was attributed to protein sparing as a result of the metabolism of carbohydrates from the feed stuffs containing yam and plantain peels.

Okoye and Salisu (2000) determined the effect of Tofu (soybean curd) as a replacement for fish meal in the diet of Tilapia (*O. niloticus*) fingerlings reared in a flow-through system. Tofu has a high protein content and is suitable for livestock feed. The results of this work showed that complete replacement was not nutritionally suitable for

the fish. The best result was obtained from the diet containing 20% Tofu and 10% fish meal.

### 2.3.6 Processing of Plant Derived Ingredients for Fish Diet Formulation

Various chemicals have been identified in plants (leaves, barks, roots, seeds, pods and fruits) that are toxic or caused detrimental effects to animals that consumes such. Some of these are tannins, alkaloids etc, which are referred to as anti-nutrients. Anti-nutrients are known to hinder the absorption of essential nutrients required for growth in animals and fish in particular. It is therefore necessary to process plant ingredients to reduce anti-nutrients to a safe level before incorporation into fish diets.

Heat treatment is commonly used to deactivate protease inhibitors in soybean. Liener (1994) reported that the extent to which trypsin-inhibitor is destroyed in soybean by heat is a function of temperature, duration of heating, particle size and moisture content of the ingredients. He further stated that though heat treatment will effectively eliminate most of these anti-nutrients, careful control of processing condition is essential to prevent the denaturing of the protein content by excessive heat treatment.

Heat treatment by cooking, sun drying or heating under high pressure have been used. Viola *et al.* (1983) observed that hydrothermal treatment of the raw full-fat soybean by cooking for only one hour destroyed 80% of the trypsin inhibitor. Autoclaving at 105°C for more than 60 minutes was found to be more effective than the boiling procedure as 95 - 100% of the trypsin inhibitor was removed. However, in view of the fact that autoclaving requires special apparatus, it may be unaffordable to the fish farmers.

Milling to remove the outer layer of seeds, particularly cereals, reduces the phytate content which is concentrated in the outer endosperm.

Toasting and de-fatting have been identified as processing methods for removing anti-nutrients of some leguminous and cereal ingredients for fish feed. However Rumsey *et al.* (1993) indicated that toasting and de-fatting did not significantly reduce anti-genicity levels in soyabean meal.

Makkar and Becker (1996) also suggested the treatment of tannin-containing feeds ingredients with oxidizing agents and supplementation with tannin - complexing agent, polytethylene glycol to mitigate their negative effects on animals. Makkar and Becker (1997) stated that glucosinate can be extracted from the full-fat and fat-free *Moringa oleifera* kernels by soaking in water. In addition, because of the high solubility of most saponins in water, soaking has been recommended provided it does not otherwise affect the nutritional quality of the material.

Aregheore *et al.* (1998) reduced the lectin content in Jatropha seed meal from 102 to 1.17 heamagglutination units by moist heating at 100°C for 10 minutes. Burel *et al.* (2000a) indicated that heat treatment is effective in reducing the glucosinate content of rape seed meal in rainbow trout diet from 40 to 20μniol/g after wet pressure cooking. Adegoke *et al.* (2010) applied soaking and boiling processing methods on avocado (*Persea Americana* Mill) seeds. The ash, crude protein, ether extract, crude fibre and carbohydrate contents as well as the gross energy and the anti-nutrients reduced as the boiling duration increased to 25 minutes. The amino acids especially methionine, lysine and tryptophan however increased as the boiling duration increased. The crude protein, ether extract and crude fibre on the other hand, increased with soaking duration.

The effects of processing on the mineral content, proximate composition and phytochemical factors of the seeds of *Bauhinia monandra* (kurz) was investigated by Agbugui *et al.* (2010). Boiling significantly ($P<0.05$) reduced the anti-nutrients such as phytate, tannin, hydrogen cyanide and saponin in the seed while increase in the boiling period resulted in the decreased in the ash content and the nitrogen feed extract and a slight decrease in the protein content. Bamgbose *et al.* (2010) reported the effect of processing methods on the proximate composition of *Moringa oleifera* leaves. The blanched leaves had significantly higher values of protein, vitamins C. B and carotene while the boiled with potash had the lowest retention of vitamin C. The effect of soaking, and boiling on the nutritional quality of M*ucuna flagellipes* "Ukpo" showed that soaking for 24 hours and boiling for 90 hours produced seeds with lower crude fibre (10%) and higher Nitrogen free extract. The fat and ash content also reduced as boiling time increased. The decrease in protein content in the soaked and boiled seeds was as a result of leaching of some water soluble nutrients (Udensi *et al.,* 2010). A similar result was reported for sesame (*Sesamum indicum)* with increased boiling duration. The anti-nutrients of the seeds also decreased with increased boiling duration (Jimoh *et al.*, 2011).

Fermentation has been reported by Kumar *et al*. (2011) to reduce the phytate content of grains.

## 2.4.0 Animal Protein Sources in Fish Feed

### 2.4.1 Use of Fishmeal

Fish meal has for so long been regarded as the best protein source for fish due to its high protein content (66%) and complete amino acid profile such as lysine, leucine, methionine, valine, histidine, isoleucine, phenylalanine, threonine, tryptophan and arginine which are required for fish growth and performance (Olukunle, 2006). However, because of the high cost and unavailability, attempts are being made at replacing it with unconventional protein sources.

### 2.4.2 Use of Non-conventional Animal Protein Sources

The suitability of toad meal (*Bufo regularis*) in the diets of catfish, *Clarias lazera* has also been investigated by Annune (1990). The growth performance of *Clarias* was significantly ($P<0.05$) better in diets with toad meal inclusion level of up to 40% than those fed with commercial diet. The problem of toad poison, he opined, might have been overcome by the denaturing effect of heat.

Furthermore, tadpole meal has also been used to replace fishmeal. The result showed that fishmeal was superior to tadpole meal in terms of the feed conversion efficiency and nutrient utilization but less cost effective that the later (Ayinla *et al.* 1991).

Idoniboye-Obu *et al.* (1993) investigated the nutritional value of Brachyuran decapod crustacean (*Callinestes latimus* and *Cardisoma armatum*) for partial replacement of fish meal in the feed. The proximate analysis of the crabs showed that the meal contains contains 32% crude protein. However, they mentioned that, despite their suitability for partial or total replacement, crabs are not viable due to unavailability of

economic harvesting methods and processing techniques. Also, crayfish waste meal containing 41.2% crude protein was considered a good potential for replacement but has the problem of difficulty in the collection of good quality to sustain the production of adequate quantity to fish feed.

Current works in Nigeria on the use of unconventional animal protein sources for formulating fish diet include that of Madu and Ufodike (2003). Maggots (housefly larvae) with live Tilapia were fed to juvenile of catfish (*Clarias angurilaris*) to assess their growth and survival. It is worthwhile to note that the diet of maggots only (Diet I) ranked second best over those of live Tilapia fry-diet feed, National Institute for Freshwater Fisheries Research (NIFFR) commercial cat fish feed (40% C. P.), and live Tilapia plus NIFFR catfish feed. The diet containing live maggot plus NIFFR feed ranked overall best with high specific growth rate, best weight gain and had the least cost in terms of relative cost of feed per fish weight gain. Similarly, Ogunji *et al.* (2008) evaluated the influence of housefly maggot meal (magmeal) diets on the performance of tilapia (*Oreochromis niloticus*). The percentage body weight gain, feed conversion ratio and standard growth rate in all feeding groups were not significantly different ($P<0.05$) indicating that magmeal was well utilized by the fish and can be used as an alternative protein source in tilapia fingerlings production.

Other non-conventional animal protein feed stuffs used as fishmeal supplement in fish feed include earthworm meal, snail meal, termite meal and tadpole meal (Sogbesan and Ugwumba, 2008). Their findings showed that the clupeid fishmeal had the highest crude protein (71.04%) and dried unskinned-dried tadpole meal had the lowest value (43.5%). Termite had the highest crude lipid (30.50%) while garden-snail meal had the

lowest value (7.85%). It was concluded that the essential amino acid, protein and lipid values of these animal supplements in their study could satisfy the nutritional requirements of catfish at all ages.

### 2.4.3 Use of Animal By-Products as Fish Ingredients

Animal by products have also been used by various researchers in efforts to reduce the cost of fish production as a result of high cost of feed. Poultry wastes were used by Reolofs and Landsing (1977) in the diets of Israeli carp. The findings showed that the growth rate of the test fish was found to be inversely proportional to the amount of poultry wastes in the diet ($r^2$=0.975). The growth rate of the control feed differed significantly from the test diets (P > 0.005) while the Feed Conversion Efficiency was also inversely related to the level of the poultry waste in the diet. Though the control was more expensive, it produced the most economic result as indicated from the economic analysis comprising of cost of feed per kilogram; cost of feed per weight gain and cost of feed per kilogram of protein produced. An addition of 4% and 5% soybean oil in the poultry waste included diet which resulted in improvement in the growth of carp by 3.2% and 3.5% respectively over the control diet was recommended.

Eyo (1994) also indicated that the inclusion of blood meal in fish ration beyond 25% for diet fed to *Orechromis niloticus* and beyond 50% for *Clarias anguillaris* diet was found to cause reduction in the growth rate of the fish. Eyo and Olatunde (1999) replaced soybean meal with blood meal in the diet of mud fish (*Clarias anguillaris* L.) fingerlings. The result indicated an increase in the weight gain and food utilization by *C. anguillaris* fingerlings up to 50% replacement level of blood meal. They however stated

that beyond this inclusion level, a significant reduction in growth performance of the fingerlings was observed. This was traced to imbalance in the amino acid composition of blood meal which is poor in isoleucine.

Animal's offals have also been used for formulating feed ration. Ufodike *et al.* (2003) used chicken gut which is a waste product of slaughter operation and duckweed (*Lemma minor*), an aquatic macrophyte. Results showed a linear relationship between fish growth and protein level up to 50% inclusion of the chicken gut and duckweed. However, protein digestibility decreased with increase in the amount of duckweed. They advised that fibre content above 8% should be avoided in diets of fish because it impairs digestibility.

Cooked and dried poultry waste meal (C. P. 62.82%) was used to substitute fishmeal at 0%, 25%, 75% and 100% levels in the diet of *Clarias gariepinus* fingerlings. Mean weight gain of 108.82kg was highest in $PHW_1$ (0%) but not statistically different from $PHW_2$ (25% level) ($P<0.05$). Feed conversion Ratio was best in $PHW_1$ (1.43) but not statistically different from $PHW_2$ (1.62)($P>0.05$). Net Protein Utilization was highest in $PHW_2$ (27.12%) but not statistically different from $PHW_1$ (25.00%)($P>0.05$). it was concluded that cooked and oven-dried poultry hatchery waste could conveniently replace 25% of Danish fish meal in the diet for *Clarias* (Obasa *et al*., 2009).

### 2.5.0 The Potentials of *Moringa oleifera* (Lam) for Nutritional Uses

#### 2.5.1 Origin of *Moringa oleifera*

*Moringa oleifera L.* (Miracle tree, Horse radish, Drumstick tree (English), Zogale (Hausa); Ewe igbale (Yoruba) and Ikwe oyibo (Igbo) belongs to a monogeneric family of

shrubs and tree *Moringaceae* and is said to have originated from Agra and Oudh in the northwest region of India, south of the Himalayas mountains (Morton, 1991). It is a pan tropical multipurpose tree characterized by high biomass yield and can tolerate unfavourable environmental conditions (Hoffmann, *et.al.*, 2003). *Moringa* is now widely cultivated particularly in the Middle East and Eastern and Western parts of Africa. It is very common in Northern Nigeria where it is cultivated as vegetable plant and garden fencing shrub. The tree leaves, fresh and dried pods, seeds and kernels of *Moringa* (Plates 2.1, 2.2, 2.3 and 2.4) have been extensively used for various purposes especially in other West African countries where the plant has been widely cultivated.

Plate 2.1

Plate 2.2

Plate 2.3a

Plate 2.3b

### 2.5.2 Multiple Uses of *Moringa oleifera*

Extensive work has been done on *Moringa oleifera* as regards its usefulness for human nutrition (Makkar and Becker, 1997). It has also been used for alley cropping due to rapid growth long tap root, and large production of high protein biomass. The leaves

are readily eaten by cattle, sheep, goats, pigs, and rabbits. It can also be used as food for carp and other fishes. Pre-heated seed cake and meal can be used. *Moringa* leaves also provides excellent material for biogas production as well as domestic cleaning agents in some part of Nigeria. Its wood yields a blue die. The seed cake can be used as protein-rich plant fertilizer and the powdered seeds can be used as a honey and sugar-cane clarifier. The plant can be used to make a wide variety of traditional medicine. The leaves can incorporated into the soil before planting to avoid damping-off disease among seedlings. Another major use of *Moringa* is for water purification. The powdered seed kernels act as natural flocculants for the most turbid water (Fugile, 2001).

### 2.5.3 Nutritional Values of *Moringa* Leaves

Makkar and Becker (1997) indicated that *Moringa leaves* are edible and are commonly cooked and eaten like spinach for making soup or salads. The leaves are regarded as exceptionally good source of pro-vitamins A, vitamins B and C, minerals (particularly iron) and the sulphur containing amino acids, methionine and cyctine. The mineral contents of *Moringa* leaves from three agro-climatic origins (Nicaragua, India and Niger) have been investigated by Makkar and Becker (1997). The calcium content were 17.5, 26.4, and 13.9g/kg dry matter (DM) for Nicaragua, India, Niger respectively. Phosphorous content was highest (1.6g/kg) in leaves from Nicaragua and lowest (1.22g/kg) in leaves from Niger. The magnesium content for the *Moringa* leaves from the three countries was 0.11 respectively, while the iron content was highest (528g/kg) in leaves from Nicaragua and lowest (178g/kg) leaves from India. Other minerals identified in the leaves include Manganese (47.1, 51.8 and 113.9 g/kg for Nicaragua, India and

Niger respectively), Zinc (13.5, 13.7 and 24.2 g/kg) and Copper (11.2, 7.1 and 10.6 g/kg) (Makkar and Becker, 1997). This result confirmed that *Moringa* leaves are very rich in minerals.

*Moringa* leaves are very rich in protein, carotene, iron, ascorbic acid while the pod is rich in amino acid (lysine). The chemical compositions of fresh *Moringa* leaves showed that the dry matter was 18.7%, crude protein was 29.0%, crude fibres, 19.1%, and ether extract 5.2%. *Moringa* fruit had 10.7% dry matter, 20.7% crude protein, 20.0% crude fibre and 1.0% ether extract (Makkar and Becker, 1996)

These figures agreed with those given by Gupta *et al.* (1989) whose crude protein, lipid and ash values were 26.4%, 6.5% and 12% respectively for unextracted leaves. However, the crude protein content of extracted leaves was 43.5% while the lipid content reduced to 1.4% (Makkar and Becker, 1996). The Amino acids profile of *Moringa* leaves has also been studied (Table 2.5). *Moringa* leaves contain a complete profile of essential amino acids namely lycine, leucine, isoleucine, methionine, phenylalanine, histidine, trytophan and valine in appropriate quantities for good growth of animals especially fish. The non-essential amino acids are also present in appreciable quantities. The amino acids composition of extracted and un-extracted *Moringa* leaves have been investigated (Appendix I).

*Moringa* leaves have been suggested as good sources of protein supplement especially for high production cows because it contains high crude protein content and low protein degradation value in ruminants. The digestion of the fibre of *Moringa* in the rumen has been considered high, though the degradation rates of extracted *Moringa*

leaves is significantly lower than that of the unextracted leaves due to high temperature ($80^oC$) for drying the leaves after extracting.

*Moringa* leaves have the advantage of containing negligible levels of tannins and saponins (Makkar and Becker, 1997). Trypsin inhibitors and lectins were not detected but the reported phytate content of 3.1% might decrease the availability of minerals in monogastris. It was suggested that extracted leaves with 80% aqueous ethanol would still be a better source of feed (protein supplement) because it is free of tannins, lectins, trypsin inhibitors, cynogenic glucosides, glucosinolates and flatus factors and low levels of saponins and phytates (Makkar and Becker, 1997). Yitzak (2008) further reiterated the benefits of *Moringa* leaves for lengthening the lactation period in women and in providing important micro nutrients for infants.

### 2.5.4 Nutritional Qualities of *Moringa* Seeds and Kernels

*Moringa* seeds are borne in pods which are green when fresh and brown when dried. The young pods are very tasty and can be boiled and eaten like green beans. The pods are very rich in free leucine. The seeds are best eaten when green before they change their colour to yellow. The dried grinded seed can be used as hot seasoning base.

Findings showed that the *Moringa* kernels contain 36.8% crude protein and 41.7% lipids (Makkar and Becker, 1997). However if the coagulant was extracted by soaking in water, the crude protein content is reduced to 35.3% in the kernel and 70.3% in meal. The higher crude protein content of the meals as compared to kernels together with the higher solubility of proteins from meal suggests that the coagulants used for water purification can be recovered efficiently from the meal. The residue left after

extraction of coagulants from the meal, had high crude protein content (approximately 70%) all of which is in the form of true protein high pepsin digestibility, virtual absence or negligible levels of anti-nutritional factors such as tannins, saponins, alkaloids, inhibitors of trypsin and amylase, lectin, cyanogenic glucosides and glucosinates. This suggests that most of the protein in the extracted kernel – meal would be available to animals when included in their diet (Nikolaus *et al.,* 2001). Also the residue contains a high concentration of sulphur – containing amino acids than that of recommended amino acid pattern of FAO/WHO/UNO reference for 2 – 5 years old child. The kernels, meal and water extracted residues were however found to be deficient in lysine, leucine, phenylalanine, tyrosine and threonine when compared to standard of FAO protein but the sulphur – containing amino acid were higher as seen in (Appendix II).

Nikolaus *et al*. (2001) stated that anti-nutrition factors such as tannins, trypsin and amylase inhibitors were not detected in treated or untreated *Moringa* kernel samples. The saponin content was also not high (0.5% and 0.6%) in unextracted and extracted meals respectively.

Soaking of *Moringa* kernels removed approximately 50% of the saponins, but the phytate contents of the kernel samples were higher than those in the vegetable parts suggesting that soaking was not effective in reducing phytate in the kernels. The high phytate level, though similar to levels in soybean and sunflower, has been noted to decrease the bio-availability of minerals in monogastris. The cyanogenic glucosides levels observed in kernel samples are much lower than those considered safe for cassava and almond cakes as fixed by FAO/WHO (1991) who regulated the amount of HCN as

50mg for livestock except poultry with safe limit of 10mg and for human, a level of not more than 10mg is also recommended.

Hoffmann *et al.* (2003) also studied the effects of the seed extract of *Moringa oleifera* on rumen fermentation in-vitro for dairy cattle. The normal result obtained when ruminants are fed on rations very rich in starch and high quality protein is rapid fermentation causing ruminal acidosis. This rapid breakdown of dietary protein to ammonia increases nitrogenous excretions rather than contributing directly to the animals nutrient requirements. In order to delay animal protein degradation, dietary proteins were denatured by treatment with formaldehyde or using antibiotics to suppress the bacterial population responsible for rapid protein fermentation. The uses of these synthetic additives have been condemned while emphases are being placed on natural additives. The results of using *Moringa* seeds extract showed that when the *Moringa* extract was mixed with rumen fluid, most of the small protein precipitated very quickly (> 10 min.) causing increased protein concentration. This indicated that there was a delay or inhibition in protein fermentation.

Kakengi *et al.* (2007) studied the effects of *Moringa oleifera* leaf meal as substitute for sunflower seed meal on the performance of laying hens in Tanzania. The levels of substitution were 20, 15, 10, and 0%. The result showed that egg weight was significantly highest in the control (100% sunflower seed meal). It was suggested that *Moringa* leaf meal could completely replace sunflower seed meal up to 20% without any detrimental effect in the laying chickens, but for better efficiency, 10% inclusion level of *Moringa* leaf meal was recommended. Asaolu *et al.* (2010) also determined the effects of *Moringa* and bamboo leaves on groundnut hay utilization by West African dwarf goats.

The results showed that feeding *Moringa* leaves in the same ratio with groundnut hay will significantly improve nitrogen intake and retention as well as total digestible nutrient in the goats.

### 2.5.5 The Use of *Moringa* Parts in Fish Feed Formulation

A lot of work has been done on *Moringa oleifera* for human medicinal and nutritional uses (Fugile 2001). However information on the utilization of *Moringa* in fish feed composition is very scarce or unavailable. The only reported works available to the researcher included that of Richer *et al.* (2003) on the evaluation of nutritional quality of Moringa leaves as an alternative protein source for Nile Tilapia. Results showed that diets with high inclusion levels (20% and 30%) of Moringa leaves depressed the growth performance of the fish (S.G.R.: 2.0% in both groups) compared to diet 2 (10% level of inclusion) and diet 1 (control). It was suggested that the relatively high total phenolic and non-heamolytic saponin and phytic acid in diets with inclusion levels of Moringa leaves at 20% and 30% cold have contributed to the poorer growth performance in these groups. These results suggest that *Moringa* leaf meal can be used to substitute only up to 10% of dietary protein in Nile Tilapia without significant reduction in weight.

Afuang *et al.* (2003) also compared the nutritional value of raw, methanol extracted residues and methanol extracts of *Moringa* leaves on growth performance and feed utilization of Nile Tilapia. They reported a reduction in the growth performance of fish fed increasing level of raw *Moringa* leaves, no significant difference ($P = 0.05$) in the growth of fish fed 11%, 22% and 33% methanol extracted *Moringa* leaf meal compared with the control diet (fish meal diet) and similar growth to that obtained from the control diet in fish fed 13%, 27% and 40% methanol soluble extracts of raw *Moringa* leaf. Also

a significant reduction in muscle cholesterol was noticed for fish fed these later diets. It was concluded that the solvent extracted *Moringa* leaf meal could replace about 30% of fish meal for Nile Tilapia diets.

In Nigeria, the chemical contents of *Moringa* seeds were determined by Ahwange *et al.* (2004). The protein value (40.31%), they indicated, compares favourably with that of soybeans (*Glycine max)* while the carbohydrate content is 9.11%. The crude fibre content was 3.28% while the oil content was 41.58%. The ash content which indicates the minerals contents of seed was 3.60%. The minerals contents of *Moringa* seed was also determined by Ahwange *et al.* (2004). The values obtained for calcium (20.50 g/kg) and potassium (27.40 g/kg) were considered adequate for fish growth. Other minerals determined were sodium (2.99%), sulphur (3.75%), phosphorus (1.36%) and iron (1.48%). The anti-nutritional factors identified in the *Moringa* seed were hydrogen cyanide (0.58 mg/100g), tannins (2.13%) and saponins (2.25%) (Ahwange *et al., 2004*).

## 2.6.0 Experimental Fish

### 2.6.1 Suitability of *Clarias gariepinus*) in Aquaculture

The choice of Afrcan catfish, *Clarias gariepinus* for aquaculture is dependent on its many qualities such as its ability to tolerate low dissolved oxygen (D.O) and its consequent ability to withstand handling stress, high disease resistance, rapid growth rate, high yield potential and its acceptability of a variety of feed items (Anetekhai *et al.*, 1996, Adewolu *et al.,* 2008).

Fagbenro and Davies (2003) indicated that *Clarias* spp. has a high propensity to consume a wide variety of supplementary feeds thereby making it possible to combine a variety of conventional and non-conventional ingredients for formulating its diet. In Nigeria, is a very important fresh water fish and enjoys wide acceptability in most parts of the country of its unique taste and less bones in contrast to Tilapia (Aiyeloja *et al.,* 2011).

### 2.6.2 Carcass Composition of *Clarias Gariepinus*

Proper nutrition is one of the essential factors that influence the ability of the fish to attain genetic potential, growth, reproduction and longevity. Knowledge of the carcass composition of the fish is a good measure for assessing the growth and quality of protein and its utilization, whether the diet promoted growth or simply accumulated only fat.

Studies have been carried out on the carcass composition of some indigenous fishes. Abdullahi *et al.* (2001) reported that *C. gariepinus* has the following nutrients: moisture 75.00%, ash 10.50%, carbohydrate 10.20%, crude lipid 27.10 and crude protein 52.20%. The amino acid composition (g/100g) was also reported as lysine 10.13 histidine 2.84, arginine 4.40, aspartic acid 8.30, threonine 2.89, serine 2/07, glutamic acid 14.85, praline 2.54, glycine 8.86, alanine 4.90, cystine 2.62, valine 3.01, methionine 1.97, isoleucine 5.16 leucine 5.64, trysine 1.10, phnylamine 2.00. The mineral content (mg/100g) was reported as calcium 3910, potassium 633, iron 53, magnesium 31, sodium 261, copper 2, phosphorus 16 and zinc 19. These indices were either higher or close to the reference values of the FAO/WHO (1991). Minerals are important in the metabolic and physiological activities and subsequent growth and development of any organism.

Eyo (2003) reported that all feedstuff namely maize, corn, palm kernel cake, blood meal, soya beans meal and fish meal contain a fair amount of calcium, potassium, sodium, phosphorus, magnesium and manganese.

Carcass composition of *C. gariepinus* and *O. niloticus* fed Castor seed as feed ingredient boiled for 0, 20, 35, 50 and 65 minutes showed the highest protein content in fish feed seeds boiled for 65 and 35 minutes respectively (Agboola, 2004). Similarly carcass nutrient consumption as reported by Balogun *et al.* (2004) for *O. niloticus* fingerlings fed boiled *Delonix regia* were significantly high.

The proximate and mineral composition in some selected freshwater fishes in Nigeria were studied. The result obtained for *C. gariepinus* was crude protein 44.28%; crude fibre, 0.10%; ether extract, 7.80%; Ash, 4.00%; moisture, 6.60% and dry matter, 93.40%. The findings also noted that the variation in the calcium and phosphorus content was due to an increase in the proportion of bore to flesh as the fish grew (Fawole *et al.,* 2007).

## 2.7.0 Least Cost Analysis

The least cost analysis of fish feed is the formulation of fish feed using computer based analysis of available scientific data (Lovell 1981). It is explained as a mathematical procedure by which limited resources are allocated, selected, scheduled or evaluated to achieve optimal solution to a particular objective. Such resources may be capital, raw material, man-power or production facilities and objectives may be minimum or maximum profit. The higher the cost of the sources of dietary protein the higher the least cost (Chow et. al, 1980). It is also explained as the linear programming analysis of

fish feed cost for intensive fish culture (Al-Duwais and Hebicha, 1998). Feed costs represents about 50% to 60% of production cost and has a major role in the economics of such enterprise.

The information needed for the computer formulation of least cost feed were stated by Lovell (1981) as the cost of feed ingredients, the nutrient content of the feed, and availability of feed materials. The critical nutrients required are essential amino acids, essential fatty acids, phosphorus digestible energy and protein ratio. The present trend of using the nutritional requirements of exotic species to determine the least cost for our local species does not give the correct formulation. Talabi (1986) reported similar standard procedure for producing cost effective feeds using linear programming.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Source of Fish and *Moringa* Seeds

Clarias *gariepinus* fingerlings were obtained from Alheri commercial fish farm in Kaduna, Kaduna State, Nigeria. The seeds of *Moringa oleifera* simply called *Moringa* were obtained from the farms around Zaria, Kaduna State. The dropped seeds were hand picked and some were obtained directly from mature dried pods. The collected seeds were shelled manually to obtain the cream coloured kernels.

## 3.2.0 Treatment of *Moringa* Kernels

Two heat treatments, toasting and boiling, were employed in this study.

### 3.2.1 Toasting Method

*Moringa* kernels were properly sorted out to remove stones and other dirt's. The clean kernels were toasted in an oven (Zanko Japan 50 t 6220) at 90$^{o}$C for 10, 20, and 30 minutes respectively. The toasted kernels at different time intervals were milled and their samples analysed for their proximate composition, amino acids and anti-nutrients contents.

### 3.2.2 Boiling Method

15 litres of clean water was heated to boiling point (100$^{o}$C), while boiling, 2.5kg of *Moringa* kernels were poured into it and allowed to boil again before the kernels were

removed with a colander to drain off the water at 10, 20, and 30 minutes intervals respectively. The drained kernels were air dried until a constant weight was obtained. The dried kernels for each treatment were milled and packed in cellophane bags while a sample of each treatment was analysed for the proximate composition, amino acid and anti-nutrient contents.

## 3.3 Control Ingredient

The control ingredient, fishmeal, was obtained by milling well dried sea clupeids obtained from Sabon - Gari market, Zaria and packaging it in cellophane bag. A sample of the fishmeal was anlysed for its proximate composition, amino acids profile and anti-nutrients contents.

### 3.4.0 Determination Of Proximate Composition Of Processed *Moringa* Seeds

The proximate composition (moisture, crude protein, crude lipid crude fibre, ash and nitrogen free extracts) of the raw and boiled seeds, experimental diets, experimental fish before and after the experiment were determined using the methods of the Association of Analytic Chemists (A.O.A.C., 1995). All chemical analyses were replicated twice.

### 3.4.1 Moisture Content and Dry Matter Content

Principle: This was done based on the difference between the net weight and the weight after drying to a constant (at 100$^{o}$C) for 24 hours. Empty clean beakers, one for each sample were dried in hot air oven at 100$^{o}$C for 30 minutes. Each beaker was then

weighed (W1). Five grammes of the sample were placed in the beaker and then reweighed (W2). The beaker with the sample was then dried in an oven at 60$^{o}$C for 72 hours, during which time a constant weight was attained, and then transferred to a desiccator to cool. The beaker was quickly weighed with minimum exposure to atmosphere (W3). The loss in weight of the sample during the drying represented the moisture content, while the dry matter was the weight obtained after drying the sample. This is shown as:

$$\% \text{ moisture} = \frac{W2 - W3}{W2 - W1} \times 100$$

$$\% \text{ dry matter} = \frac{W2 - W3}{W2 - W1} \times \frac{100}{1}$$

Where W1 = Weight of empty crucible

W2 = Weight of known amount of sample (fresh) + crucible

W3 = Weight of oven dried sample

### 3.4.2 Crude Protein Determination

**Principle:** Nitrogen is used as an index of the protein and so is termed crude protein because it represents all of the nitrogen that is in the form of non-protein nitrogen (NPN) such as nitrates, ammonia, urea and single amino acids, as well as the nitrogen present as true protein. The multiplication by a conversion factor of 6.25 is based on the assumption that true protein contains 16% nitrogen. Protein determination was carried out in three stages as follows:

**Digestion:** One gramme of sample was weighted into a 50ml digestion flask and the kjeldahl mixture which acts as a digestion catalyst was added with 5ml concentrated

tetraoxosulphate VI acid ($H_2SO_4$). Some pumice stones (anti-bumping granules) were also added. The flask containing the sample mixture was heated gently at an inclined angle in a kjeldahl digestion rack until forthing subsided. It was then boiled until the solution became colourless. Heating of the mixture released the nitrogen in the various samples which was then converted toammonia with the concentrated $H_2SO_4$. It was later allowed to cool. The sample was transferred to a 100ml volumetric flask and diluted with distilled water to the mark. It was then thoroughly mixed. The mixture was further allowed to cool before distillation. A blank containing only $H_2SO_4$ acid and catalyst was also heated.

**Distillation:** A known aliquot (10ml) was transferred to the sample addition funnel of the distillation apparatus and then introduced to the sample chamber. 10ml of 40% sodium hydroxide was added to the sample addition funnel and released to the sample chamber at a slow rate. The ammonia was entrapped in a receiving solution containing 10ml of 2% boric acid solution in which 4 drops of bromocresol green /2 drops of methyl red indicator had been added. Distillation continued until the pink colour turned greenish.

**Titration:** Titration with standard HCL acid (0.01N) was carried out and the percentage of crude protein determined with the following calculation:

$$\% \text{ Nitrogen} = \frac{\text{Titre value (A-B) x Normality of acid x Vol. of dilute N. x 100}}{\text{Sample of weight (mg) x volume of digest (aliquot)}}$$

$$= \frac{\text{Titre value x 0.01 x 100 x 14.007 x 100}}{\text{Sample weight (mg) x 10}}$$

$$\% \text{ Crude protein} = \% N_2 \times 6.25$$

$N_2$ – Nitrogen

### 3.4.3 Crude Lipid Determination

**Principle**: This is the continuous extraction of fat content from a sample using a suitable solvent in a soxhlet extractor. Two grammes of the dried sample (residue from the moisture determination was used) was placed into a pre-weighed extraction thimble (W1) and the weight recorded (W2). A dry 250ml round bottom quick-fit flask was weighted (W3) with a few anti-bumping granules inside. The thimble was fitted into the extraction unit of the soxhlet extraction apparatus using a pair of forceps. 300ml of petroleum ether (40 – 60$^{o}$C boiling point) was poured into the quick-fit flask. The apparatus was connected to a condenser. The heating rate was adjusted to give a condensation rate of 2 – 3 drops. After reflux extraction for 8 hours, the thimble was removed and the ether was reclaimed using the apparatus by distilling out some ether. The removal of the ether from the flask containing a mixture of ether and extracted fat was completed on a boiling water bath and the flask dried in an oven at 105$^{o}$C for 30 minutes. After this, the flask with the pure fat content was cooled in a desiccator and weighed (W4). The percentage crude fat was calculated as follows:

$$\% \text{ Crude fat} = \frac{W4 - W3}{W2 - W1} \times 100$$

$$= \frac{\text{fat}}{\text{Sample}} \times 100$$

### 3.4.4 Crude Fibre Determination

**Principle:** The bulk of roughage in foods is referred to as fibre. This is the non-digestible portion of the carbohydrate contained in the sample. Two grams of the

defatted dried sample obtained from crude fat determination were transferred into a 600ml beaker. The digestion mixture was prepared by mixing 500ml glacial acetic acid, 450ml distilled water and 50ml concentrated nitric acid, 20g of trichloro-acetic acid was dissolved in this mixture and mixed thoroughly. The quick-fit flask was attached to a condenser. It was brought to boil and refluxed for 40 minutes. Timing commenced from the time the mixture started to boil. Thereafter, the content of the flask was filtered through a weighed Whatman No. 4 ashless filter paper which was placed in a Buchner funnel over a conical flask. During filtration, residue from the digestion was washed six times with hot water and once with industrial methylated spirit to wash down particles adhering to the sides. The filtrate was dried at 105$^{o}$C for 12 hours, cooled and weighed to get the residue. The residue now made up of crude fibre and ash was incinerated in weighed crucibles at 600$^{o}$C for 12 hours. This was then cooled in a dessicator and weighed to obtain the weight of the ash. The loss on ignition was the crude fibre.

### 3.4.5 Ash Content Determination

**Principle:** To ash the sample, all the volatile organic constituents in the sample have to be burnt off to leave behind the non volatile mineral elements. An empty crucible was dried in the oven. After drying, it was cooled in the desiccators and weighed (W1), 2g of the already dried sample obtained after moisture extraction was put into the crucible and the weight recorded (W2). This was then transferred into a muffle furnace and incinerated at 550$^{o}$C for 24 hours. After this, it was placed in a desiccator for cooling and re-weighed (W3). Percentage ash content was computed by carrying out the following calculation:

$$\% \text{ Ash content} = \frac{W3 - W1}{W2 - W1} \times 100$$

$$= \frac{\text{Ash}}{\text{Sample}} \times 100$$

### 3.4.6 Nitrogen free Extract

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

## 3.5 Determination of Amino Acids Contents of Processed *Moringa* Kernel Samples

The amino acids content of the samples was determined using the Technicon Sequential Multi sample (TSM) amino acids analyzer (Model DNA 0209) at the Department of Zoology, University of Jos, Jos. The amino acids constitution is the indicator of the nutritive value of the protein source in a diet. The TSM involves the application of modern chromatographic techniques with automated procedures which allows relatively quick and convenient resolution of mixtures of amino acids. The mixture is produced through acid hydrolysis whose major set back is the destruction of practically all the tryptophan and a considerable portion of the cystine and methionine present (McDonald *et al.,* 1986). The tryptophan has to be released by a separate alkaline hydrolysis while cystine and methionine have to be oxidized to cysteric acid and methionine sulphone respectively to ensure their quantitative recovery.

The amino acid values obtained were used to determine the biological value which is the single numerical value ascribed to a protein which gives an estimate of the relationship between the essential amino acids balance of the sample and the amino acid

requirement of the animals (Smith, 2001). The Essential Amino Acids Index (EAAI) was obtained using the formula:

$$EAAI = \sqrt[n]{\frac{a}{ae} x \frac{b}{be} x \frac{c}{ce} x - - - - - \frac{j}{je}} \qquad \text{(Smith, 2001)}$$

when n = total number of amino acids

a, b, c - - - j = Individual essential amino acid value of the test ingredients.

a.e, b.e, c.e- - - j.e = individual essential amino acid value of egg used as standard.

### 3.6.0 Determination of Anti-Nutritional Factors in the Processed *Moringa* Kernels

Anti-nutritional factors were determined in the treated Moringa kernels as follows:

### 3.6.1 Cyanogenic Glycosides

This was determined by standard methods as described by AOAC (1995). 15g of the plant was weighed into 80 ml Kjeldahl flask; this was followed by addition of 100ml of distilled water and it was allowed to stand at room temperature (27.0 ± 0.2$^{o}$C) for 24 hours. Additional 100ml of distilled water was added to the same sample in the Kjeldahl flask, steam distilled and distillate collected in 20 ml 0.01N potassium thiocyanate using ferric alum as indictor. The hydrogen cyanide in the sample was then calculated by using the equation.

1ml 0.02N $AgNO_3$ = 0.4 mg HCN.

### 3.6.2 Tannin

Tannin was determined using standard methods described by AOAC (1995). This method was however slightly modified. About 2g of the ground plant was defatted for 2 hours using Soxhlet extraction apparatus. The residue was dried in oven for 12 hours at 100$^{o}$C, boiled with 300ml of distilled water, diluted to 500ml in standard volumetric flask and filtered through non-absorbent cotton wool. A volume of 25ml of the infusion was measured into 2 litre porecelin dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardizd against 0.1N oxalic acid) until blue solution changed green; then few drops of 0.1N potassium permanganate was added. The difference between the two titrations was multiplied by 0.006235 to obtain the amount of tannin in the sample using the equation.

0.1N oxalic acid = 0.006235g tannin.

### 3.6.3 Determination of Phytic Acid

Phytic acid (inositol hexaphosphate) content of each sample was determined by the modified method of Uppstrom and Svensson (1980). About 10g aliquot of the powdered sample was extracted overnight at room temperature with vigorous shaking in an MSE orbital shaker at 200rpm for 1 hour to ensure homogeneity an then neutralized by adding about 20ml of 20% sodium hydroxide: 50ml of 1% ferric chloride in 1N hydrochloric acid and the mixture brought just to boil. This resultant mixture was filtered through an ashless Whatman No. 0 filter paper. The resultant residue was dissolved in about 10ml of 2% sodium hydroxide. This procedure dissolves phytic acid while

precipating ferric hydroxide which was removed by filtration. The filtrate (containing phytic acid) was digested with a mixture of concentrated sulphuric acid and perchloric acid in the ratio 5:1 (v/v) in a Kjeldahl flask. The pH of the digest was adjusted to 4.5 and phosphate determined colorimetrically at the 710nm. Concentration of phytic phosphate was calculated from the standard inorganic phosphate curve.

### 3.6.4 Determination of Total Oxalate

The total oxalic acid of the powdered samples was determined using the method employed by Ilarslan (1997). About 2g aliquot of the plant materials was weighted into a 250ml flask; 190ml, distilled water and 10ml of 6M Hydrochloric acid were added. The mixture was digested for 1 hour on boiling water bath, cooled, transferred into a 250ml volumetric flask, diluted to volume and filtered. Four drops of methyl red indicator were added followed by concentrated ammonia until the solution turned faint yellow. It was the heated to 100$^{o}$C, allowed to cool and filtered to remove precipitate containing ferrous ions. The filtrate was boiled, 10ml of 5% calcium chloride added with constant stirring was allowed to stand overnight. The mixture was filtered through Whatman No. 40 filter paper. The precipitate was washed several times with distilled water. The precipitate was transferred quantitatively to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80$^{o}$C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was also run for the test sample. From the amount of potassium permanganate the total oxalate concentration (mg $g^{-1}$) was calculated by multiplying the titrate reading with 11.5.

### 3.7.0 Feed Composition

#### 3.7.1 Basal Ingredients

The basal ingredient for formulating the *Moringa* kernel based fish feed included yellow maize (zea mays C. P. 9%) obtained locally from Sabon Gari market in Zaria. This ingredient was milled using hammer mill grinder. Wheat bran was also used to supplement the energy base (Table 3.1).

#### 3.7.2 Protein Sources

The test ingredient (Moringa kernel meal) served as one of the main source of protein in the fish feed, this was used to substitute the fish meal at different levels of inclusion Soya bean cake was also used as one of the supplementary protein sources.

#### 3.7.3 Fixed Ingredients

The fixed ingredients consisted of cassava starch flour obtained locally from the market and used as the binder for the ingredients for easy pelleting. Other ingredients included vitamins and minerals pre-mix, palm oil, table salt, bone meal, wheat offal and soya bean cake meal which was used to balance the protein content in the feed. Methionine and Lysine were also used to balance the amino acid contents of the feed (Table 3.1).

### 3.8 Feed Formulation

Seven diets ($D_1$-$D_7$) were formulated using Least Cost Ration formulation which took into consideration the nutritive content of the major ingredients. All the feed ingredients were integrated into computing the required quantities to make up 100 units of the feed (Table 3.1). The eighth diet ($D_8$) was not formulated in this research but was a commercial pelleted feed purchased in Zaria.

**Table 3.1 Ingredients Composition of formulated diets (% Dry weight)**

| Ingridients | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ | $D_7$ |
|---|---|---|---|---|---|---|---|
| Maize | 25.75 | 20.00 | 14.00 | 7.00 | 19.00 | 12.00 | 4.00 |
| Soyabean | 17.00 | 23.45 | 30.00 | 38.00 | 25.00 | 32.30 | 41.15 |
| Wheat offal | 4.00 | 3.50 | 2.00 | 0.00 | 1.50 | 1.50 | 0.00 |
| Fishmeal | 40.00 | 30.00 | 20.00 | 10.00 | 30.00 | 20.00 | 10.00 |
| Moringa Kernel meal | - | 10.00 | 20.00 | 30.00 | 10.00 | 20.00 | 30.00 |
| Cassava | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Palm oil | 4.00 | 3.50 | 3.50 | 4.50 | 4.50 | 4.00 | 4.00 |
| Bone meal | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Salt | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Pre-mix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine | 2.00 | 2.00 | 2.45 | 2.45 | 2.45 | 2.45 | 2.60 |
| Lysine | 3.70 | 4.00 | 4.50 | 4.50 | 4.00 | 4.20 | 4.70 |
| TOTAL | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

KEY:

| | |
|---|---|
| D1 – 100% FSM | D5 - 75% FSM, 25% $MKM_b$ |
| D2 - 75% FSM, 25% $MKM_t$ | D6 - 50% FSM, and $MKM_b$ |
| D3 - 50% FSM, and $MKM_t$ | D7 - 25% FSM, 75% $MKM_b$ |
| D4 - 25% FSM, 75% $MKM_t$ | D8 – Chi-feed (commercial) |

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

### 3.9 Determination of Proximate Composition of Experimental Diets

The proximate compositions of the experimental diets were determined as stated in section 3.4.

### 3.10 Feed Preparation

Feed preparation was involved milling the grain ingredients separately, sieving (using > 1 mm sieve), mixing of all dry ingredients, addition of oil before adding hot water ($90^oC – 95^oC$) and mixing to form a dough (Lall, 1991). The mixture was pelleted using a 3mm diameter pelletizer. The strands were sun dried and packaged in water-proof cellophane until usage time.

### 3.11 Experimental Design

Completely Randomized Design (CRD) was employed in this research. A group of 180 fingerlings of *Clarias gariepinus* acclimatized for 7 days using Chi commercial

pelleted feed 46%. After the period of acclimatization, 10 fish were assigned into each transparent 50 litre capacity plastic tank. A total of 16 static plastic tanks were used in-door and eight diets consisting of 7 formulated diets (Table 3.1) and one commercial feed (Chi feed) were fed to the culture fish.

## 3.12 Feeding of Experimental Fish

The fish were starved for 12 hours prior to the onset of the experiment, to empty their gut and, increase their appetite and reception of the new diet. They were thereafter fed with the assigned test diets at 5% biomass (Banyigyi *et al., 2001*). Feeding was done twice daily (08.00h and 09.00 h) and evening (17.00h and 19.00h). The feed quantity was adjusted fortnightly after weighing to obtain the new biomass. The fecal matter and food remains were siphoned out of the tanks and fresh water supplied from stored water daily. The water was aerated using electric aerator and the water quality parameters were measured weekly.

## 3.13 Monitoring of Water Quality Parameters

Water quality parameters such as dissolved oxygen (DO), hygrogen ion concentration (pH) and temperature were measured weekly using American Public Health Association (APHA) (1980) methods. The calibrated mercury thermometer was used for measuring water temperature; the pH and dissolved oxygen concentration were measured using the Jenway meters (model 3050 for D O and 9070 for the pH).

## 3.14 Monitoring of Experimental Fish

The experimental fish were weighed individually at the beginning of the experiment, while batch weight was taken fortnightly. One fish from each of the treatments was sacrificed and dried at the beginning and the end of the experiment for carcass and gross chemical analysis. The mean total length and standard length of the fish were determined fortnightly while the record of fish mortality in each treatment was recorded daily.

### 3.15 Statistical Analysis for Treated Kernels

The data obtained were analysed using analysis of variance (ANOVA) in respect of time intervals (10, 20 and 30 minutes) of roasting and boiling of the moring a kernel. Least Significant Difference (LSD) at 5% level of probability, was employed to separate means.

### 3.16 Analysis of Growth Performance and Feed Utilization of *Clarias* Fed Test Diets

The data obtained on the growth performance and feed utilization of Clarias fed on the formulated diets were analysed as follows:

a) **Growth Performance of Clarias**

i) % **Weight gain** = $\frac{Wf - Wi}{Wi} x100$

where

Wf = mean final body weight of fish

Wi = mean Initial body weight of fish

ii) **Specific Growth Rate** (SGR) (Ricker, 1975).

$$SGR = \frac{100(LnW_2 - LnW_1)}{Ti - To}$$

$$= \frac{100(2.303(\text{Log of } W_2 - 2.303(\text{Log of } W_1)}{\text{Time (days)}}$$

Where Ln = base of natural log

$W_2$ = mean final body weight of fish

$W_1$ = mean initial body weight of fish

$T_1$ = final time (in days)

$T_0$ = Initial time (in days)

b. **Feed Utilization Analysis** (Falayi, 2009)

i. **Food intake/day** = $\frac{\text{total unit of feed consumed}}{\text{total No. of days}}$

ii. **Feed Conversion Ratio (FCR)** (Adikwu, 2003)

$$= \frac{\text{Feed fed (dry weight basis)}}{\text{Weight gain}}$$

iii. **Protein Efficiency Ratio (PER)** (Osborne *et al.* 1919; Falayi, 2009)

$$PER = \frac{\text{Weight gained}}{} \times 100$$

Crude protein consumed

Where crude protein consumed =

$$\frac{\text{\% protein in feed x total diet consumed}}{100}$$

iv. **Net Protein Utilization (N.P.U)** (Miller and Bender, 1958; Falayi, 2009)

$$= \frac{\text{fish protein gained}}{\text{Protein consumed}} \times 100$$

Where fish protein gained = final body protein – initial total body protein.

### 3.17 Statistical Analysis of Growth and Feed Utilization Parameters by *Clarias*

The data for each of the parameter were subjected to ANOVA and the means were separated using LSD at 5% level of probability.

### 3.18 Economic Analysis of Formulated Diets

The feed cost (₦/kg) was obtained using Linear programming which took into consideration the various components of the different diets. The Least Cost Analysis determined included:

i. Cost of feeding/kg

ii. Cost of feed/kg weight gain

iii. Cost of feed/protein produced. (Tacon, 1987)

## CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Proximate Composition of Boiled *Moringa* Kernels

The mean proximate composition of boiled *Moringa* kernel at different time intervals and that of fishmeal are presented in Table 4.1. The crude protein (CP) of 30 minutes – boiled *Moringa* kernels was significantly (P<0.05) lower than that of kernels boiled for 10 and 20 minutes respectively while the CP of fishmeal was significantly (P<0.05) higher than those of the three *Moringa* kernels meals. The crude fibre content was significantly (P<0.05) lowest in the fish meal. Among the *Moringa* samples, the crude fibre content was highest in the 30 minutes – boiled kernels and lowest in 10 minutes – boiled samples. The lipid contents were high in the seeds boiled for 10 and 20 minutes respectively but significantly (P<0.05) decreased as the boiling time increased to 30 minutes. The fishmeal had the lowest lipid content in contrast to the boiled seeds. The ash value was significantly higher (P<0.05) in the fishmeal than the boiled *Moring*a seeds but there was no significant difference (P>0.05) in the ash values of the boiled *Moringa* kernels at the different time internals.

**Table 4.1 Proximate Composition of boiled *Moringa* kernels.**

| Time (Mins) | Crude Protein (CP) | Crude Fibre (CF) | Lipid | Ash | Dry Matter | Nitrogen Free Extract (NFE) |
|---|---|---|---|---|---|---|
| 10 | $35.72^{b}$ | $4.03^{b}$ | $38.77^{c}$ | $2.13^{a}$ | $96.91^{a}$ | $16.29^{b}$ |
| 20 | $34.96^{b}$ | $4.79^{b}$ | $38.98^{d}$ | $2.10^{a}$ | $97.17^{a}$ | $16.39^{b}$ |
| 30 | $27.87^{a}$ | $4.99^{b}$ | $37.01^{b}$ | $2.19^{a}$ | $97.08^{a}$ | $25.02^{c}$ |
| Control (fishmeal) | $46.74^{c}$ | $0.91^{a}$ | $14.75^{a}$ | $21.66^{b}$ | $94.53^{b}$ | $10.39^{a}$ |
| SED± | 1.14 | 0.26 | 0.17 | 0.42 | 0.40 | 1.70 |
| LSD (P<0.05) | 4.37 | 0.83 | 0.54 | 1.34 | 1.27 | 5.41 |

Means having the same superscript in same column are not significantly different (P> 0.05).

**4.2 Amino Acids Composition of Boiled *Moringa* Kernels**

The mean amino acids content of the boiled *Moringa* kernels are presented in Table 4.2. The values of sulphur-containing amino acids particularly lysine and methionine wee higher in the fishmeal than the boiled *Moringa* kernels. The values of Lysine decreased as the boiling time increased from 10 minutes to 30 minutes. The Essential Amino Acid Indices for the kernels boiled for 10, 20 and 30 minutes were not significantly different ($P > 0.05$) from each other but they were significantly ($P < 0.05$) lower than that of the control.

**Table 4.2**

**Amino acids content (g/100g protein) of boiled *Moringa* kernels.**

| Amino acid | Boiled Moringa kernel | | | Control |
|---|---|---|---|---|
| | 10 | 20 | 30 mins | (Fishmeal) |
| Arginine | 6.04 | 6.52 | 5.01 | 6.13 |
| Histidine | 2.43 | 2.49 | 2.05 | 2.65 |
| Isoleurine | 3.39 | 3.25 | 4.11 | 3.68 |
| Leucine | 7.59 | 7.97 | 8.36 | 7.02 |
| Lysine | 3.21 | 3.00 | 2.84 | 7.83 |
| Methionine | 1.15 | 1.35 | 1.02 | 2.87 |
| Phenylalanine | 4.50 | 4.22 | 4.05 | 4.54 |
| Threonine | 3.23 | 2.90 | 2.37 | 4.58 |
| Valine | 3.90 | 4.26 | 2.95 | 5.05 |
| Aspartic acid | 7.29 | 6.20 | 8.56 | 9.49 |
| Serine | 3.26 | 3.51 | 3.95 | 4.67 |
| Glutamic acid | 12.50 | 13.36 | 14.16 | 14.50 |
| Praline | 2.40 | 2.03 | 1.43 | 4.37 |
| Glycine | 3.30 | 4.55 | 3.29 | 7.05 |
| Alanine | 4.20 | 5.11 | 3.95 | 6.58 |
| Cystine | 0.89 | 1.00 | 1.85 | 0.92 |
| Tyrosine | 2.69 | 3.51 | 3.41 | 3.49 |

| | | | | |
|---|---|---|---|---|
| EAAI | 0.58 | 0.58 | 0.52 | 0.77 |

**KEY -** EAAI – Essential Amino Acid Index.

### 4.3 Anti-nutritional factors of Boiled *Moringa* Kernels

The non-detectable anti-nutrients in the boiled *Moringa* kernels were hydrogen cyanide, tannins and saponins. The only detected anti-nutrient was oxalate whose value was significantly higher ($P<0.05$) in kernels boiled for 10 minutes but significantly reduced as the boiling time increased to 30 minutes (Table 4.3). No anti-nutrients was detected in fishmeal .

**Table 4.3 Anti-nutritional composition of boiled *Moringa* kernels**

| Time (Mins) | Hydrogen Cyanide mg $g^{-1}$ | Tannins (%) | Saponins (%) | Oxalate $mgg^{-1}$ |
|---|---|---|---|---|
| 10 | ND | ND | ND | $14.56^{c}$ |
| 20 | ND | ND | ND | $8.96^{b}$ |
| 30 | ND | ND | ND | $5.60^{a}$ |
| Fishmeal | ND | ND | ND | ND |
| SED± | - | - | - | 0.10 |
| LSD ($P< 0.05$) | - | - | - | 0.430 |

Means followed by the same superscript in the same column are not significantly different (LSD $P<0.05$)
ND – Not Detectable

## 4.4 Proximate Composition of Toasted *Moringa* Kernels

The mean proximate composition of the toasted *Moringa* kernels at different processing time intervals and that of the control are presented in Table 4.4. The crude protein contents in the toasted kernels for 10, 20 and 30 minutes were not significantly different ($P<0.05$) from each other but were significantly lower than that of the control (fishmeal).

The crude fibre was significantly lowest ($P<0.05$) in the control (fishmeal) while among the toasted *Moringa* samples, the 10 minutes toasted samples had the lowest fibre content. The values of lipid content were not significantly different in the toasted kernels at different time intervals used but significantly ($P<0.05$) higher than that of fishmeal. The ash values was significantly higher ($P<0.05$) in fishmeal than the toasted *Moringa* kernels but there was no significant difference ($P<0.05$) in the ash content of the kernels toasted for 10, 20 and 30 minutes.

**Table 4.4 Proximate Composition of Toasted *Moringa* kernels**

| Time (Mins) | Crude Protein (CP) | Crude Fibre (CF) | Lipid | Ash | Dry Matter | NFE |
|---|---|---|---|---|---|---|
| 10 | $32.61^{a}$ | $3.72^{b}$ | $41.62^{b}$ | $2.28^{a}$ | $97.00^{a}$ | $16.78^{b}$ |
| 20 | $31.78^{a}$ | $3.98^{d}$ | $40.93^{b}$ | $2.31^{a}$ | $96.99^{a}$ | $17.00^{b}$ |
| 30 | $30.88^{a}$ | $3.87^{c}$ | $37.84^{b}$ | $2.25^{a}$ | $96.97^{a}$ | $22.15^{b}$ |
| Fishmeal Control | $46.74^{b}$ | $0.91^{a}$ | $14.75^{a}$ | $21.66^{b}$ | $94.53^{b}$ | $10.39^{a}$ |
| SED± | 1.01 | 0.00 | 1.03 | 0.47 | 0.14 | 1.81 |
| LSD ($P<0.05$) | 3.22 | 0.00 | 3.95 | 1.50 | 0.45 | 5.52 |

Means followed by the same superscript in same column are not significantly different according to Fishers LSD ($P< 0.05$).

## 4.5 Amino Acids Composition of Toasted *Moringa* kernels

The mean values of amino acid composition presented in Table 4.5. The values of sulphur-containing amino acids particularly lysine and methionine were higher in the fishmeal than the toasted *Moringa* kernels. However there was no significant difference in the Essential Amino Acid Indices (EAAI) among the different toasted samples but they were significantly lower than that of fishmeal.

**Table 4.5**

**Amino acids content (g/100g protein) of Toasted *Moringa* kernel.**

| Amino acid | Toasted Moringa kernel | | | Control |
|---|---|---|---|---|
| | 10 | 20 | 30 mins | (Fishmeal) |
| Arginine | 5.98 | 5.52 | 6.00 | 6.13 |
| Histidine | 2.40 | 2.10 | 2.05 | 2.65 |
| Isoleurine | 3.30 | 3.25 | 3.05 | 3.68 |
| Leucine | 7.45 | 7.34 | 7.68 | 7.02 |
| Lysine | 3.37 | 3.30 | 3.50 | 7.83 |
| Methionine | 1.15 | 1.56 | 1.00 | 2.87 |
| Phenylalanine | 4.50 | 3.88 | 4.14 | 4.54 |
| Threonine | 3.10 | 3.46 | 3.46 | 4.58 |
| Valine | 3.95 | 3.70 | 3.70 | 5.05 |
| Aspartic acid | 7.30 | 7.01 | 6.78 | 9.49 |

| | | | | |
|---|---|---|---|---|
| Serine | 3.56 | 3.76 | 2.89 | 4.67 |
| Glutamic acid | 12.67 | 14.06 | 13.16 | 14.50 |
| Praline | 2.04 | 2.00 | 1.90 | 4.37 |
| Glycine | 5.28 | 3.69 | 3.59 | 7.05 |
| Alanine | 4.10 | 4.71 | 4.67 | 6.58 |
| Cystine | 1.18 | 0.9 | 0.68 | 0.92 |
| Tyrosine | 2.65 | 2.70 | 2.80 | 3.49 |
| EAAI | 0.58 | 0.58 | 0.56 | 0.77 |

**KEY** EAAI – Essential Amino Acid Index

## 4.6 Anti-Nutritional Factors of Toasted *Moringa* Kernels

Hydrogen cyanide, tannins and saponins were non-detectable in the toasted samples except oxalate. The kernels toasted for 30 minutes has the lowest mean oxalate value while the kernels toasted for 10 minutes had the highest oxalate value (Table 4.6).

**Table 4.6 Anti-nutritional factors composition of toasted *Moringa* kernels**

| Time (Mins) | Hydrogen Cyanide mg $g^{-1}$ | Tannins (%) | Saponins (%) | Oxalate mg $g^{-1}$ |
|---|---|---|---|---|
| 10 | ND | ND | ND | $15.68^{c}$ |
| 20 | ND | ND | ND | $11.20^{b}$ |
| 30 | ND | ND | ND | $7.84^{a}$ |

| | | | | |
|---|---|---|---|---|
| Fishmeal | ND | ND | ND | ND |
| SED± | - | - | - | 0.223 |
| LSD (P< 0.05) | - | - | - | 0.096 |

Means followed by the same superscript in the same column are not significantly different (P>0.05).

ND - Non detectable

### 4.7 Physico – Chemical Parameters of Water for all Treatments

The lowest and the highest temperatures observed during the experiment were 22.0$^{o}$C and 30.3$^{o}$C respectively. The pH range was 6.83 – 7.75 while the Dissolved Oxygen (D.O) content ranged from 5.86 – 7.41 mgl$^{-1}$ (Table 4.7).

**Table 4.7 Physico-Chemical Parameters of Water during Experimental Period**

| Diets | Temperature Range ($^{o}$C) | pH range | Dissolved Oxygen (D.O) Range mgl$^{-1}$ |
|---|---|---|---|
| D1 | 22.0 – 30.2 | 6.92 – 7.75 | 6.33 – 7.31 |
| D2 | 22.0 – 30.3 | 6.85 – 7.36 | 6.01 – 7.32 |
| D3 | 22.1 – 30.2 | 6.85 – 7.51 | 6.21 – 7.35 |
| D4 | 22.0 – 30.1 | 6.83 – 7.65 | 6.14 – 7.33 |
| D5 | 22.0 – 30.1 | 6.85 – 7.54 | 5.86 – 7.32 |
| D6 | 22.0 – 30.1 | 6.85 – 7.61 | 6.21 – 7.41 |

| | | | |
|---|---|---|---|
| D7 | 22.1 – 30.1 | 6.84 – 7.62 | 6.31 – 7.35 |
| D8 | 22.1 – 30.1 | 6.86 – 7.57 | 6.33 – 7.40 |

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

## 4.8 Proximate Composition of Experimental Diets

The protein content ranged from 40.56 to 49.63% with the lowest mean value found in D3 (50% fishmeal and toasted Moringa kernel meal respectively) and the highest in 6 (50% fishmeal and boiled Moringa kernel meal respectively). The crude fibre content was lowest (0.78%) in the D8 (commercial, pelleted feed) and highest in D4 (25 fishmeal and 75% toasted Moringa kernel meal). Diet 4 had the highest lipid content (6.43%) while D2 (75% fishmeal and 25% toasted Moringa kernel meal) had the lowest. The ash content value was highest in Diet (11.40%) and lowest in Diet 8 (8.18%). The NFE ranged from 34.5% in D6 to 39.99% in D7 (Table 4.8).

**Table 4.8 Proximate Composition of Experimental Diets**

| Diets | Dry matter | Crude Protein | Crude fibre | Lipid | Ash | NFE |
|---|---|---|---|---|---|---|
| $D_1$ | 93.16 | 46.85 | 1.85 | 4.14 | 11.40 | 35.66 |
| $D_2$ | 94.00 | 46.31 | 1.76 | 3.56 | 10.44 | 37.93 |

| | | | | | | |
|---|---|---|---|---|---|---|
| $D_3$ | 94.60 | 40.56 | 1.81 | 3.73 | 10.47 | 43.43 |
| $D_4$ | 93.17 | 43.80 | 3.28 | 6.43 | 8.33 | 38.16 |
| $D_5$ | 94.50 | 46.42 | 1.61 | 3.52 | 10.35 | 38.10 |
| $D_6$ | 92.57 | 49.63 | 2.75 | 4.65 | 8.41 | 34.56 |
| $D_7$ | 94.64 | 44.49 | 2.14 | 5.12 | 8.26 | 39.99 |
| $D_8$ | 99.98 | 46.63 | 0.78 | 4.67 | 8.18 | 39.75 |

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

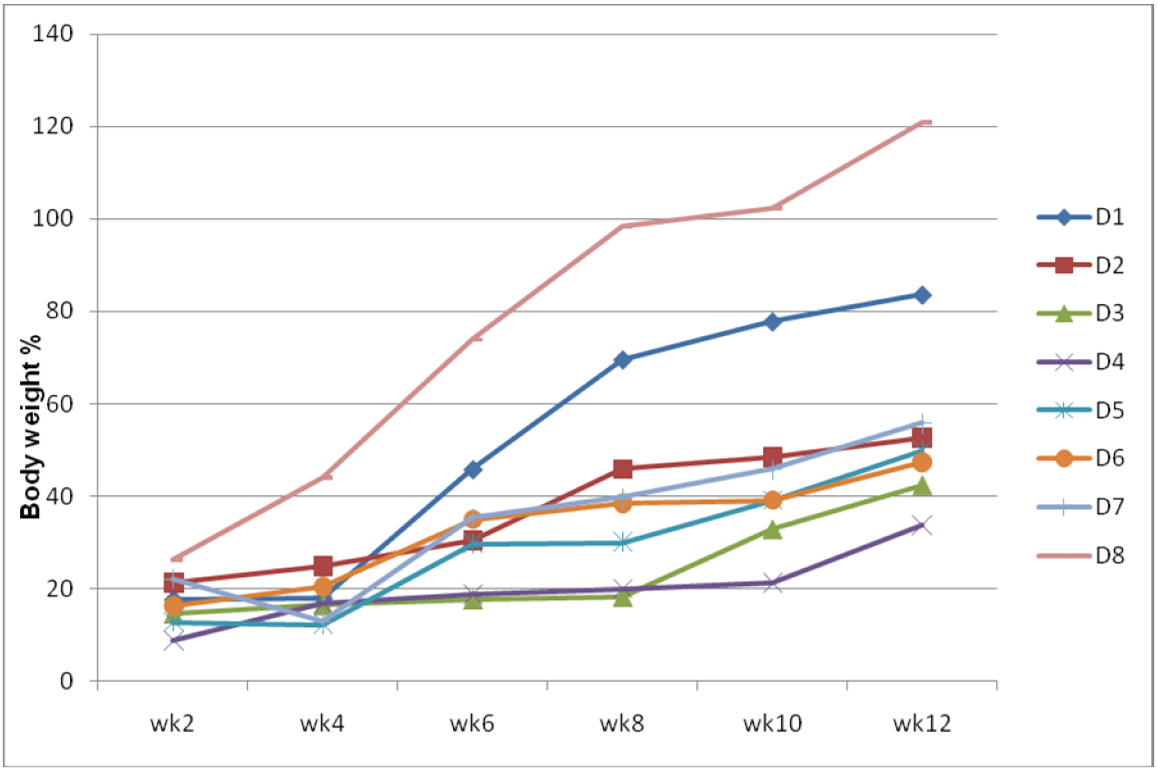
### 4.9.0 Analysis of Growth Performance and Survival of *Clarias Gariepinus* fed Experimental Diets

### 4.9.1 Weight and Length Increment in *Clarias*

The bi-weekly weight and length increments in *Clarias* are presented in Figures

4.1 and 4.2 respectively. The percent bi-weekly weight increment was highest in $D_8$ (commercial feed) and lowest in $D_4$ (25%FSM, 75%$MKM_t$).

**Figure 4.1 % Bi-weekly Body Weight Increment in *Clarias***




KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

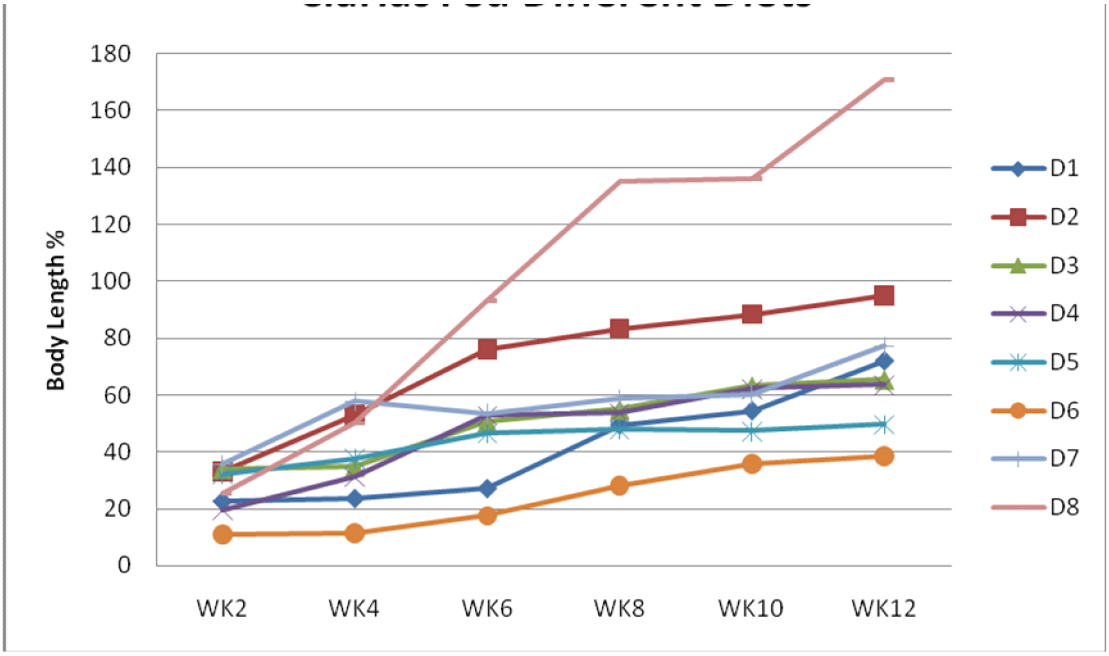
D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

**Figure 4.2 % Bi-weekly Body Length Increment in *Clarias***




KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

### 4.9.2 Analysis of Weight Gain and Specific Growth Rate

The analysis of the growth performance of *Clarias* in terms of percent weight gain showed that there were significant differences ($P<0.05$) between the different diets. The lowest mean weight gain was observed in the fish fed $D_4$ which contained 25% fishmeal and 75% toasted *Moringa* kernel meal. The weight gain was not significantly different ($P>0.05$) from the fish fed $D_3$ (50% fishmeal and toasted *Moringa* kernel meal) (Table 4.9.1). There was also no significant difference ($P>0.05$) in the weight gain of

fish fed $D_2$ (75% fish and 25% toasted *Moringa* kernel meal) and $D_3$. There was no significant difference ($P<0.05$) in the weight gain in fish fed $D_5$ (75% fishmeal and 25% boiled *Moringa* kernel meal) and $D_6$ (50% fishmeal and 50% boiled *Moringa* kernel meal). However there was a significant difference ($P<0.05$) in the weight gain in fish fed $D_1$, $D_7$ and $D_8$ (100% fishmeal, 25% fishmeal at 75% boiled *Moringa* kernel meal and commercial feed respectively). The best percent weight gain was obtained (46.43%) in the fish fed the commercial diet (281.12%) while the least percent weight gain was obtained in fish fed Diet 4 (Table 4.9.1). The specific growth rate indicates the percent rate of increase in body weight per day. Among all the diets, $D_8$ (Commercial diet) produced the best mean Specific Growth Rate in *Clarias* (Table 4.9.1). This was significantly higher than those of other test diets. This performance was closely followed by that obtained from fish fed $D_1$ (control 100% fishmeal). Among the *Moringa*-based diets, Diet 6 (50% fishmeal and boiled *Moringa* kernel meal respectively) and Diet 7 (75% boiled *Moringa* kernel meal and 25% fishmeal) were not significantly different ($P>0.05$) but gave better performance in *Clarias* than the toasted *Moringa*-based diet (Table 4.9.1). Diet 5 (75% fishmeal and 25% boiled *Moringa* kernel meal) was not significantly different from Diet 2 (75% fishmeal and 25% toasted *Moringa* kernel meal). The least specific growth rate was obtained in Diets 3 and 4 (Diets with 50% and 75% toasted *Moringa* kernel meal) which are also not significantly different ($P>0.05$) from each other.

**Table 4.9.1 Growth Performance of *Clarias gariepinus* fed Experimental Diets (Mean Values)**

| Diets | Mean Feed Intake | % Weight Gain | Specific Growth Rate (SGR) |
|---|---|---|---|
| $D_1$ | 0.506 [c] | 108.31 [e] | 0.814 [d] |
| $D_2$ | 0.397 [b] | 58.52 [bc] | 0.512 [b] |
| $D_3$ | 0.415 [b] | 47.69 [ab] | 0.433 [a] |
| $D_4$ | 0.397 [b] | 46.43 [a] | 0.425 [a] |
| $D_5$ | 0.397 [b] | 64.24 [c] | 0.519 [b] |
| $D_6$ | 0.372 [a] | 68.69 [cd] | 0.580 [c] |
| $D_7$ | 0.418 [b] | 80.06 [d] | 0.615 [c] |
| $D_8$ | 0.775 [d] | 281.12 [f] | 1.486 [e] |
| SED± | 0.017 | 5.05 | 0.02 |
| LSD (P<0.05) | 0.041 | 11.94 | 0.047 |

Means followed by same superscript in the same column are not significantly different (P>0.05).

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal.

### 4.9.3 Survival Rate of Test Fish

The percentage survival of the fish on weekly basis is presented (Table 4.5.2). The final survival rate was highest (85%) among fish fed $D_8$ (commercial feed) while the lowest rate (55%) was among fish fed. $D_5$.

**Table 4.9.2 Survival Rate of *Clarias Gariepinus* per treatment**

| Weeks | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ | $D_7$ | $D_8$ |
|---|---|---|---|---|---|---|---|---|
| 0 (initial) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2 | 100 | 100 | 100 | 100 | 100 | 95 | 95 | 100 |
| 4 | 95 | 95 | 100 | 100 | 90 | 80 | 95 | 95 |
| 6 | 70 | 95 | 90 | 85 | 65 | 65 | 75 | 85 |
| 8 | 60 | 85 | 80 | 85 | 65 | 60 | 70 | 85 |
| 10 | 60 | 85 | 65 | 80 | 55 | 60 | 70 | 85 |
| 12 (Final) | 60 | 80 | 65 | 80 | 55 | 60 | 70 | 85 |

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

### 4.10.0 Analysis of Feed Utilization in *Clarias Gariepinus* fed Experimental Diets

### 4.10.1 Feed intake

The lowest mean feed intake was in fish fed Diet 6 (50% boiled *Moringa* kernel, 50% fishmeal). The feed intake was significantly lower than those of other diets, however, there was no significant difference ($P>0.05$) in the feed intake of fish fed Diets 2, 3, 4, 5, and 7 (Table 4.10).

There was no significant difference ($P>0.05$) in the intake of toasted and boiled *Moringa* kernel meal – based diets. However the consumption of Diet I (100% fishmeal diet) was significantly different from those of *Moringa* based diets. The fish fed the commercial ration ($D_8$) had the best mean feed intake and this was significantly different ($P<0.05$) from the control diet ($D_1$) and the *Moringa*-based diets.

Mean feed intake was highest in the commercial ration ($D_8$) followed by that of $D_1$ (100% fishmeal). Among the *Moringa*-based diets there was no significant difference ($P>0.05$) in the feed intake by *Clarias* except for the $D_6$ which was least consumed by the fish.

### 4.10.2 Feed Conversion Ratio (FCR)

The best feed conversion ratio was obtained in the fish fed the commercial fish feed ($D_8$) which was significantly different from others (Table 4.6). The fish fed the $D_1$ (100% fishmeal) performed next to those fed $D_8$. Among the *Moringa*-based diets, there was no significant difference ($P>0.05$) between $D_2$, (toasted moringa) $D_5$, $D_6$, and $D_7$ (boiled *Moringa*) with respect to feed conversion ratio. Fish fed $D_3$ and $D_4$ which contained the higher levels (50% and 75% respectively) of toasted *Moringa* kernel meal were not significantly different ($P>0.05$) from each other.

### 4.10.3 Protein Efficiency Ratio (P.E.R.)

Among the experimental diets, the fish fed $D_8$ (the commercial diet) gave a significantly higher protein efficiency ratio than the other diets (Table 4.10). The fish fed the $D_1$ diet (100% fishmeal) also had significantly higher protein efficiency ratio than the *Moringa*-based diets but was significantly lower than that obtained from the commercial feed. The protein efficiency ratio in fish fed $D_7$ (25% fishmeal and 75% boiled *Moringa* kernel meal) was significantly different ($P>0.05$) from other boiled *Moringa*-based diets ($D_5$ and $D_6$) and $D_2$ (25% fishmeal and 25% toasted *Moringa* kernel meal). However $D_7$ was significantly different from $D_3$ and $D_4$ (diets containing 50% and 75% toasted *Moringa* kernel meal respectively). The feed that contained high level of inclusion of boiled *Moringa* kernel meal had higher protein efficiency ratio.

### 4.10.4 Net Protein Utilization (N.P.U)

The NPU is an indication of the amount of protein used in building up the fish carcass from the total protein consumed. Fish fed Diet 1 had a significantly higher NPU than all other diets. $D_2$ and $D_5$ with 75% inclusion level of fishmeal had higher N.P.U. values than the diets with higher inclusion level of processed *Moringa* kernel meal. The N.P.U. of the fish fed $D_5$ (75% fishmeal and 25% boiled *Moringa* kernel meal) was significantly higher ($P<0.05$) than those fed other *Moringa*-based diets. Fish fed $D_4$ (25% fishmeal and 75% toasted *Moringa* kernel meal) had the lowest N.P.U. among those fed with toasted *Moringa* kernel – based meals. The fish fed the commercial feed $D_8$ showed a significantly lower value of N.P.U. than $D_1$ (100% fish meal). Fish fed $D_8$ (commercial feed) performed better in terms of Weight Gain, SGR, Feed Intake, FCR and P.E.R. (Table 4.10)

**Table 4.10** **Feed utilization parameters in *Clarias* fed experimental diets. (Mean values)**

| Diet | Feed Intake | Feed Conversion Ratio (F.C.R) | Protein Efficiency Ratio (P.E.R) | Net Protein Utilization (N.P.U) |
|---|---|---|---|---|
| $D_1$ | $0.506^{b}$ | $4.525^{b}$ | $0.470^{c}$ | $73.17^{b}$ |
| $D_2$ | $0.397^{b}$ | $7.060^{c}$ | $0.310^{ab}$ | $59.21^{f}$ |
| $D_3$ | $0.415^{b}$ | $8.435^{d}$ | $0.295^{a}$ | $37.43^{e}$ |
| $D_4$ | $0.397^{b}$ | $8.620^{d}$ | $0.270^{a}$ | $34.93^{d}$ |
| $D_5$ | $0.397^{b}$ | $6.440^{c}$ | $0.340^{ab}$ | $64.59^{g}$ |
| $D_6$ | $0.372^{a}$ | $6.590^{c}$ | $0.310^{ab}$ | $18.66^{e}$ |

| | | | | |
|---|---|---|---|---|
| $D_7$ | $0.418^b$ | $6.035^c$ | $0.380^b$ | $27.72^c$ |
| $D_8$ | $0.775^d$ | $2.835^a$ | $0.760^d$ | $24.25^b$ |
| SED± | 0.017 | 0.533 | 0.032 | 0.29 |
| LSD (P = 0.05) | 0.041 | 1.261 | 0.076 | 0.069 |

Means followed by the same superscript in the same column are not significantly different (P> 0.05).

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

## 4.11 Carcass Composition of Fish fed Experimental Diets

The initial mean Dry Matter (DM) content of the fish was 84.94% for the diets. The final DM was highest (91.02%) in $D_1$ (100% fishmeal) and lowest (89.53%) in $D_7$ (25% fishmeal, 75% boiled Moringa kernel meal). The final crude protein content was highest (64.32%) in $D_1$ (100% fishmeal) and lowest (52.19%) in $D_7$ (35% fishmeal with 75% boiled Moringa kernel meal). The final crude fibre content of the carcass was nil while the final lipid content was highest (11.02%) in $D_5$ and lowest (8.57%) in $D_3$. the

final ash content was highest in fish fed $D_5$ (75% fishmeal with 25% boiled Moringa kernel meal) and lowest in fish fed Diet 6. $D_6$ had the highest final NFE (27.17%) while $D_1$ had the lowest (10.35%) (Table 4.11).

**Table 4.11** **Initial and final Carcass composition of *Clarias* fed Test Diets (% Mean values)**

| | Final Carcass Composition | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial carcass composition | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ | $D_7$ | $D_8$ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dry Matter | 84.94 | 91.02 | 89.97 | 90.09 | 90.49 | 89.68 | 90.90 | 89.53 |
| 90.97 | | | | | | | | |
| Crude Protein | 47.97 | 64.32 | 57.38 | 54.31 | 53.19 | 58.25 | 52.44 | 52.19 |
| 55.56 | | | | | | | | |
| Crude Fibre | 0.92 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| Lipid | 10.12 | 9.03 | 8.82 | 8.57 | 10.14 | 11.02 | 7.89 | 8.08 |
| Ash | 10.33 | 16.04 | 17.92 | 15.79 | 13.59 | 18.51 | 12.50 | 13.84 |
| 13.04 | | | | | | | | |
| Nitrogen free Extract | 20.27 | 10.35 | 15.88 | 21.33 | 23.08 | 12.21 | 27.17 | 25.09 |
| 23.01 | | | | | | | | |

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

## 4.12 Least Cost Analysis of Diets

The computerized least feed cost on the basis of the ingredients indicated that $D_8$ (Commercial feed) has the highest cost (₦320.00) per kilogram of feed. This was closely followed by $D_1$ (100% fishmeal) that cost ₦120.01. The Moringa based – diets had very close least feed costs per kilogram of feed for both toasted and boiled samples ($D_2$

₦117.12; $D_7$ ₦116.68; $D_4$ ₦112.84; $D_5$ ₦118.96; $D_6$ ₦116.84; $D_7$ ₦115.42;). The highest computed least feed cost was obtained in $D_4$ and the lowest in $D_7$ (Table 4.12).

**Table 4.12 Computed Feed Nutrients and Least Cost Analysis**

| Control | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ | $D_7$ | $D_8$ |
|---|---|---|---|---|---|---|---|---|
| % C.P | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | - |
| ME (Kcal/ kg) | 32.06 | 32.00 | 32.08 | 32.01 | 32.02 | 32.01 | 32.02 | - |
| % Lipid | 4.18 | 4.15 | 4.12 | 5.16 | 5.99 | 5.42 | 4.87 | - |
| % CF | 2.63 | 3.14 | 3.25 | 3.45 | 3.14 | 3.28 | 3.48 | - |
| % Ca | 1.72 | 1.68 | 1.42 | 1.14 | 1.44 | 1.44 | 1.42 | - |
| % P | 1.29 | 1.11 | 1.09 | 1.06 | 1.01 | 1.00 | 0.98 | - |
| % Lysine | 5.04 | 5.03 | 5.01 | 5.04 | 5.06 | 5.00 | 5.01 | - |
| % Methi-onine | 3.01 | 3.10 | 3.02 | 3.01 | 3.26 | 3.21 | 3.18 | |
| Least Feed Cost ₦/kg | 120.01 | 117.12 | 116.68 | 112.84 | 118.96 | 116.84 | 115.42 | 320.00 |

KEY: D1 – 100% FSM
D2 - 75% FSM, 25% $MKM_t$
D3 - 50% FSM, and $MKM_t$
D4 - 25% FSM, 75% $MKM_t$
D5 - 75% FSM, 25% $MKM_b$
D6 - 50% FSM, and $MKM_b$
D7 - 25% FSM, 75% $MKM_b$
D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

## 4.13 Economic Analysis of Experimental Diets Fed to *Clarias*

The cost of feed consumed by the experimental fish was highest ₦22.26 in $D_8$ (commercial feed) followed by $D_1$ (₦5.47) lowest while the lowest was $D_4$ (₦4.14). Among the Moringa – based diets, $D_5$ had the highest cost of feed consumed (₦4.96). (Table 4.13).

**Table 4.13 Economic Analysis of experimental Diets**

| Parameters | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ | $D_6$ | $D_7$ | $D_8$ |
|---|---|---|---|---|---|---|---|---|
| A | 10.10 | 5.06 | 4.46 | 4.15 | 5.61 | 6.44 | 6.28 | 24.60 |
| B | 45.56 | 35.73 | 37.35 | 36.69 | 36.69 | 42.22 | 32.22 | 69.57 |
| C | 120.01 | 117.12 | 116.68 | 112.84 | 118.96 | 116.84 | 115.42 | 320.00 |
| D | 5.47 | 4.19 | 4.36 | 4.14 | 4.36 | 4.96 | 3.76 | 22.26 |
| E | 541.58 | 828.06 | 977.58 | 997.59 | 777.18 | 770.19 | 583.85 | 904.88 |
| F | 15.65 | 9.83 | 5.67 | 5.46 | 10.77 | 3.93 | 4.64 | 7.88 |
| G | 349.52 | 426.25 | 768.96 | 758.24 | 404.83 | 1,262.09 | 935.34 | 2,824.87 |

KEY:
A – Weight gain (g)
B – Total feed consumed (g)
C – Computer Least cost of feed (₦)
D – Cost of feed consumed (₦)
E – Cost of feed/kg weight gain (₦)
F – Fish protein gained (g)
G – Cost of feed/kg protein produced (₦)

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmeal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

# CHAPTER FIVE

## 5.0 DISCUSSION

The protein values obtained for 10, 20 and 30 minutes boiling durations in this study were 35.72%, 34.96% and 27.87% respectively. The lower value of the crude protein content (27.87%) obtained as the boiling duration increased to 30 minutes may be associated with denaturation of protein as the heat period increases (Table 4.1). Liener (1994) stated that denaturation of protein could occur due to excessive heat treatment which could further cause functional and nutritional changes in the protein source. It could also be due to leaching and solubilization of some protein content in the boiling water as the processing time progressed. Obasi and Wogu (2008), Udensi *et al.* (2010) and Jimoh *et al.* (2011) stated similar effects on different seeds experimented on. The protein contents of toasted *Moringa* kernels were not significantly different for each toasting period. The reason could be that the toasting temperature did not change significantly to bring about any significant reduction in the protein content.

The essential amino acid profiles on the boiled and toasted *Moringa* kernels could be considered complete (Table 4.2 and 4.5). The profile was similar to that obtained by

Sarkar and Peace (1994) and Ahwange *et al.* (2004). Amino acids are the basic structural units of all proteins and they play important role in the manufacturing of proteins. However, lysine and methionine which are very important for fish growth decreased with boiling duration. The implication is that, if 30 minutes boiled *Moringa* kernel meal would be used for fish ration as the major protein – source, the feed must be supplemented with crystalline lysine and methionine in order to promote optimal growth in fish as recommended by Craig (2009). The crude fibre content of the boiled *Moringa* kernels increased with increasing boiling duration but decreased as toasting duration increased to 30 minutes. The decrease in the latter could be due to dry heat being used which might have destroyed the fibre content. The low crude fibre of the control (fish meal) (0.91) could be accounted for by the fact that fishmeal is derived from fish which are animals and they do not efficiently digest fibre but eliminate it solid wastes (Afuang *et al*., 2000). The significant reduction in the lipid contents of the boiled and toasted *Moringa* kernels as the processing time increased from 10 minutes to 30 minutes could be accounted for by release of the lipid molecules into the boiling water or evaporated into the air from the cells in the case of toasting as the heating duration increased. Udensi *et al.* (2010) obtained a similar result with *Mucuna flagellipes*. The low lipid content in the 30 minutes processed *Moringa* kernels would make the meal acceptable for fish ration formulation since high lipid content in fish rations would affect the feed quality in terms of low floatation in water and possibility of high rancidity during storage (Lall, 1991). The ash contents in the boiled toasted kernels at different processing time were not significantly different from each other but the values wee significantly lower than that of control fishmeal (21.66%). The high value of the ash content which depicts the mineral

content of the fishmeal could be attributed to the presence of bones which is known to store mineral elements such as phosphorus, calcium, magnesium.

The main anti-nutrient identified in the chemical analysis of the boiled and toasted Moringa kernels was oxalate while others such as tannin, trypsin, saponin and others were not detectable (Tables 4.3 and 4.6). The elimination of most of these anti-nutrients could be due to the heat (wet or dry) treatment employed. The hydrothermal treatment of seeds was however more effective in reducing the anti-nutrients contents than dry heat (toasting) because of the hydrolysis of the chemical compounds that make up the anti-nutrients. The effectiveness of reducing anti-nutrients by boiling over toasting has been reported for other seeds such as soyabeen (Viola *et al.,* 1983), *Delonix regia* (Abdullahi and Abdullahi, 2004) and Avocado (*Persea Americana* Mill) (Adegoke *et al.*, 2010).

Among the treatments, the kernels boiled or toasted for 30 minutes duration had the significantly lowest anti-nutrient contents respectively. This implies that the longer the heat processing duration, the lower the oxalate content. Afuang *et al.* (2003) reported that processing techniques such as soaking drying and thermal treatments, particularly boiling, brought about reduction in the anti-nutrients in plant – based fish feed ingredients. The low levels of anti-nutrient content (oxalate) of boiled *Moringa* kernel (5.60%) and the toasted sample (7.84%) made them suitable for fish ration formulation. In view of the fact that high levels of anti-nutrients could result in low feed consumption and utilization by fish (Abdullahi and Abdullahi, 2004). The findings showed that the chemical composition of the *Moringa* kernels, boiled or toasted for 10, 20 and 30 minutes respectively were significant different ($P<0.05$) in their protein, ash and lipid contents at different processing time. in view of these findings, the null hypothesis (Ho) that stated

that "There is no significant difference in the chemical composition of *Moringa* kernels boiled or toasted for 10, 20 and 30 minutes respectively" should be rejected.

The physico-chemical parameters (Table 4.7) of the culture water in the semi static in-door tanks used for raising the lest fish were monitored throughout the experimental period for the optimum growth of the test fish. The temperature range obtained was 22.0$^{o}$C – 30.3$^{o}$C. The lower temperature 22.0$^{o}$C was obtained in all treatments during the harmattan season but the laboratory environment where the fish were raised was heated using 100W electric bulb. The higher temperature was obtained at the end of the harmattan in February. The temperature range in this experiment was acceptable range and did not affect the fish feeding negatively. Auta (1993) reported a temperature range of 25$^{o}$C – 30.0$^{o}$C in a research carried out in static tanks in Zaria.

The pH range of 6.83 – 7.75 and Dissolved Oxygen (D.O) range of 5.86 – 7.41 mgl$^{-1}$ recorded during this study suggest that they are within acceptable range for growth and maintenance of *Clarias gariepinus*. The pH range of 6 – 8.5 and D.O. of 4 – 8.0mg$^{-1}$ has been reported (Balogun *et al*., 2004). In view of the fact that all the physico-chemical parameters of the culture water were within acceptable ranges, the fish growth and feed utilization may be affected by other intrinsic and extrinsic factors.

The highest survival rate was noted in fish fed $D_8$ (commercial feed). Alegbeleye *et al.* (1996) obtained a similar result with commercial feed. The lowest revival was obtained from $D_5$ (75% fishmeal, 25% boiled Moringa kernel meal). The high fish mortality in $D_5$ was probably due to the water pollution by a dead rat found in the water which was replaced immediately with fresh water.

To determine the growth and feed utilization by *Clarias gariepinus* all the diets ($D_1$ – $D_7$) were formulated with 40% crude protein content. After compounding the rations, some slight increase was noted in the crude protein as observed from the proximate analysis. The increase might be due to the release of protein from some of the ingredients during feed formulation. Lall (1991) reported that the physical characteristics, milling and composition of feed ingredients may have significant effects on the processing and quality of the finished product. The crude fibre was also significantly higher in the diet containing high levels of *Moringa* kernel meal than the fish meal. This is because fishmeal has little or no fibre but high protein content in contrast to plant – based ingredients with higher fibre content (Afuang *et al.,* 2003).

The analysis of the feed intake by *Clarias* (Table 4.9.1) showed that there was no significant difference ($P<0.05$) in the intake of toasted and boiled *Moringa* kernel meal – based diets except $D_6$. This could be due to the fact that both sets of diets were produced from M*oringa* (a plant-based ingredient). However the mean feed intake was significantly lower in $D_6$ but highest in $D_8$ (commercial ration). The low feed intake in $D_6$ might be attributed to its high fibre content (2.75%) similar to what was observed by Ofojekwu and Kigbu (2003) and Maina *et al.* (2007) who indicated that crude fibre not only has no known dietary value for fish but it also dilutes digestible nutrient densities thus increasing the release of polluting wastes into the water environment. This could consequently reduce the feed intake nu fish. The higher feed intake in $D_8$ could be attributed to improved feed pelleting, longer pellet flotation and better aroma. This was followed by that of $D_1$ (100% fish meal).

Weight gain is an important index in measuring fish responses to experimental diets and a reliable indicator of growth (Balogun *et al.,* 2004). The best weight gain was obtained in fish fed Diet 8 (commercial feed) followed by those on $D_1$ (100% fishmeal). This could be as a result of better digestibility and utilization of the nutrients in the feed to produce good weight gain. The diet containing 100% fishmeal gave the best performance in many fish nutrition experiments (Solomon *et al.,*1996; Abel *et al.,* 1984). Among the *Moringa* kernel meal-based diets, $D_7$ (25% fishmeal and 75% boiled *Moringa*) produced a significantly ($P<0.05$) higher weight gain than others. This could be attributed to higher feed intake by the fish. The lower anti-nutrients in the boiled *Moringa* kernel than the toasted sample could have resulted in better feed utilization and growth in the fish. The works Eyo and Olatunde (1999) and Omafuvbe *et al.* (2004) indicated that boiling of soybean and locust bean seeds promoted growth in *Clarias gariepinus*. Hydrothermally processed *Proscopis justiflora* seeds also produced improved growth in *Labeo rohutal* fingerlings (Bhatt *et al.,* 2011). The Specific Growth Rate (SGR) values (1.486 and 0.814) were significantly higher ($P<0.05$) in $D_8$ (commercial feed) and $D_1$ (100% fish meal inclusion) respectively when compared with the *Moringa* based meal. This could be attributed to absence of anti-nutritional factors in the fishmeal used as major protein source in $D_1$ as well as the better quality of the commercial feed ($D_8$). The significantly higher SGR values ($P<0.05$) of Diet 6 and 7 (50% and 75% boiled *Moringa* kernel meal inclusion) could be attributed to lower anti-nutritional contents of the boiled *Moringa* kernel meal when compared with the toasted kernels whose anti-nutritional contents were higher. Rumsey *et al.* (1998) reported that processing methods like toasting and defatting did not reduce anti-genic compounds in

soyabean and this could trigger a variety of non specific and specific immune responses in fish body and consequently results in a reduction in fish growth.

The best Feed Conversion Ratio (2.84) was obtained in fish fed $D_8$ (commercial ration). This could be related to its highly nutritive qualities such as better ingredients improved processing and pelleting resulting in longer floatation of pellets and more stability in water . The fish fed $D_1$ (100% fishmeal) performed creditably next to those of the commercial feed with FCR of 4.53. This could be attributed to high crude protein content and absence of anti-nutrients in fishmeal which could have hindered the digestibility of the feed (Afuang *et al.,* 2003). Among the *Moringa* – based diets, the Feed Conversion Ratios in fish fed boiled *Moringa* kernel meal diets ($D_5$, 6.44; $D_6$, 6.59; $D_7$, 6.04) was not significantly ($P<0.05$) different from each other and were better than those obtained from other toasted kernel meal diets. Boiling as a processing method has been reported to enhance digestibility of feed (Agbugui *et al.,* 2010) and this could have resulted in better FCR than that obtained in toasted kernel based-diets. Diet 2 with the lowest inclusion level (25%) of toasted *Moringa* kernel meal that produced the best FCR among the toasted kernel based meal, further confirms that toasting was not as effective as boiling method for reducing anti-genic compounds present in the kernels.

The Protein Efficiency Ratio (PER) indicates the weight gained per crude protein consumed by the fish. Excess protein beyond the required level needed by the fish will be excreted as nitrogenous wastes in form of ammonia through the gills (Lovell, 1981). This suggests that a higher PER value indicates a better utilization of the crude protein consumed. The commercial diet ($D_8$) produced fish with significantly highest ($P<0.05$) PER while $D_1$ (100% fishmeal) performed next to $D_8$ (Table 4.10). This performance

could be accounted for by the use of fishmeal which has high crude protein content (46.4%) and complete amino acids required by fish in formulating these diets. Feedstuffs of vegetable origin are as a whole lower in protein content when compared with those of animal origin and none can perform very well as fishmeal on a protein equivalent basis. Among the *Moringa* kernel meal – based diets, $D_7$ (75% boiled *Moringa* kernel meal inclusion) produced fish with the best PER value ($0.38^{o}$) though significantly ($P<0.05$) lower than those for $D_1$ (100% fishmeal inclusion) and $D_8$ (commercial feed with PER values of 0.47 and 0.76 respectively. The result obtained in $D_7$ could be as a result of increased protein retention which made it more available for the fish growth. Kperegbeyi and Ikprite (2009) obtained a similar result using processed *Cajanus cajans* seed meal in the broiler starter chicks feed. The feed intake, Feed Conversion Ration and Protein Efficiency Ratio were better in the boiled than the toasted or raw seeds.

The better growth performances and feed utilization obtained in fish fed boiled *Moringa* based-diets generally support the fact that wet heat processing of plant-based protein sources is more effective in reducing or eliminating various anti-nutrients contained in them (Makkar and Becker, 1997; Aregheore *et al.,* 1998; Burel *et al.,* 2000a; Francis *et al*., 2001; Amaefule and Nwagbara, 2004; Bamgbose *et al.,* 2010).

The Net Protein Utilization (NPU) is an indication of the amount of protein used in building up the fish careass from the total protein consumed. Diets 1 (100% fish meal), 2 (75% fishmeal and 25% toasted *Moringa* kernel meal and 5 (75% fishmeal and 25% boiled *Moringa* kernel meal) had the highest NPU values (73.17%, 59.21% and 64.59% respectively). The higher inclusion level of fish meal in these diets might have been responsible for the better NPU values obtained because fishmeal has higher

nutritional value especially high levels of essential amino acids especially lysine methionine and cysteine which are generally deficient in plant ingredients, and higher digestibility. A similar result was obtained in fish fed diets with different replacement levels of fishmeal with soybean meal, groundnut cake and blood meals. The control diet (100% fishmeal) gave the best NPU (Solomon *et al.,* 1996).

Among the *Moringa*-based diets, the best NPU was obtained in Diet 5 (25% boiled *Moringa* kernel meal inclusion) which further confirms that the boiled kernel meal contributed a higher percentage of protein than the toasted kernel meal to the fish protein in addition to that obtained from fish meal.

The carcass analysis showed that all diets contributed to the increase in the dry matter, crude protein and ash contents of the fish carcass. The increase might have been brought about by the contribution of the protein and ash supplied by the fish meal and the *Moringa* kernel meal. The low lipid content in the carcass samples could also be a result of the low supply in the feed and the efficient use of the available lipid in the feed to supply energy to the fish. The low crude fibre obtained in all the fish carcass sample could also imply that the available fibre was well digested by the fish.

The initial null hypothesis ($Ho_2$) stated that "there is no significant difference in the growth and feed utilization by *Clarias* fed diets containing boiled or toasted *Moringa* kernel meal used at different levels to substitute fish meal". In view of the fact that the fish meal based diet produced a significantly ($P<0.05$) different result from the *Moringa*-based diets and the boiled *Moringa* kernel meal – based diets performed significantly ($P<0.05$) better than the toasted kernel-based diets for the growth and feed utilization parameters $Ho_2$ was reject.

The cost per kilogram of feed was highest in $D_8$ (commercial feed (₦320.00/kg) making it the most expensive of all the test diets. The high cost which could be due to high cost of protein ingredient particularly fishmeal, as well as cost of importation, has been the bane of researches on fish feed in order to produce cheaper but efficient feeds. The cost per kilogram of feed was ₦120.00 in $D_1$ which ranked next to the commercial feed. The high cost was also due to the cost of sea clupeids used in making the fish meal for the experimental diets clupeid. The sea clupeid is in high demand for both human consumption and other livestock feed production resulting in its increased cost.

Among the *Moringa*-based diets, the least cost values per kilogram feed were within the range of ₦112.84 and ₦118.96. The cost of feed consumed per fish was lowest in $D_6$ (50% fishmeal and 50% boiled *Moringa* kernel meal). The lowest cost ₦3.76 was as a result of lowest feed intake. However $D_6$ and $D_7$ had the best cost of feed per kilogram weight gain (₦583.85 and ₦619.08 respectively) making them more economical for the fish farmers when compared with ₦904.88 for commercial feed and other *Moringa* based diets though slightly higher than $D_1$ (100% fish meal). The economic analysis of the feeds is more relevant in terms of the weight gain in fish since it determines the selling cost to the buyers.

The protein content of the fish is one of the best indices of the nutritive quality of the fish sought by consumers especially in more developed countries. The cost of feed for producing one kilogram of protein for the consumer is also important to the fish farmers. Though the commercial diet produced the best growth and feed utilization results, (Table 4.13) the costs of feed to produce one kilogram weight gain and protein content respectively were ₦904.88 and ₦2,824.87 respectively and therefore were too

high for maximum profitability by the farmer. $D_1$ (100% fish meal) also had the least cost of feed per kilogram weight gain and cost of feed per kilogram protein produced (₦541.58 and ₦349.52 respectively) but the in-availability of the clupeids used for making local fishmeal due to its high demand by humans and in livestock feed production, renders it unsuitable. Among the *Moringa* – based diets the lowest cost (₦404.03) of feed to produce one kilogram of protein was obtained in $D_5$ (75% fish meal and 25% boiled *Moringa* meal) and highest in $D_6$ (₦1,262.09). This implies that the farmer would need to supply more feed when using $D_6$ in order to produce fish with high protein content. However if the fish farmer would consider the economics of production in terms of quantity and quality of fish produced using *Moringa*-based diets, $D_7$ (75% boiled *Moringa* meal and 25% fish meal) should be considered in view of its lower cost of feed per wt gain (₦619.08) while $D_5$ should be given consideration for its lowest cost for producing one kilogram protein produced.

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATIONS

### 6.1 Conclusion

*Moringa oleifera* has been successfully used by humans due to its high nutritive value and medicinal importance. Its high protein content and good amino acids profile led to its being considered in this research for its inclusion in fish feed. This research was therefore aimed at determining the suitability of heat treated *Moringa oleifera* kernels for replacing fishmeal partially in the diet of *Clarias gariepinus*.

At the beginning of this work the following objectives were set to:

- determine the best processing method and duration in terms of the physical qualities and chemical composition especially the crude protein contents, amino acids profile and anti-nutritional factors of *Moringa* kernel.
- determine which kernel treatment and the replacement level of fishmeal with the *Moringa* kernel meal in the diet that would produce the best growth and feed utilization in *Clarias gariepinus*.
- assess the cost effectiveness of diets produced from the processed *Moringa* kernel meal used for substituting the fishmeal at different levels for *Clarias gariepinus*.

Two thermal treatments – toasting and boiling were employed for reducing the anti-nutrients of *Moringa oleifera* kernels obtained locally around Zaria, Kaduna State, Nigeria. The durations used for toasting and boiling respectively were 10, 20 and 30 minutes. The treated seeds were physically observed and chemically analysed. The findings showed that 30 minutes-toasted and boiled kernels respectively were the best in terms of low fat and fibre content, high ash content and acceptable protein and essential amino acids profile as well low anti-nutrients.

The kernels were milled into meal and used for replacing fishmeal at 25%, 50%, 75% levels in 6 formulated diets (3 diets each using boiled and toasted *Moringa* kernels meal respectively). The main control was the diet containing. 100% fishmeal and a sub-control particularly for cost comparative purpose was the commercial feed (Chi-feeds). All formulated rations were with an average of 40% crude protein. The test diets were fed to *Clarias gariepinus* fingerlings for 12 weeks.

The research findings showed that the best growth in terms of Mean Weight Gain (MWG) and S. G.R., was obtained from the fish fed commercial diet (Diet 8 – Chi feed). The feed utilization in terms of mean Feed Intake, Feed Conversion Ratio and Protein Efficiency Ratio was also significantly higher in fish fed Diet 8. However it's N.P.U. was significantly lower than other formulated feeds except Diet 6 (50% fishmeal and boiled *Moringa* kernels meal respectively).

However among the formulated diets, fish fed Diet 1 (100% fish meal) which was the main control performed credibly next to those fed with commercial diet in terms of the growth and feed utilization by *Clarias*. Among the *Moringa*- based diets, Diet 7 (25% fish meal and 75% boiled *Moringa* kernels meal) gave the best result, in terms of the growth, feed utilization by *Clarias gariepinus*.

Though among all feeds in this research, Diet 8 (Commercial feed) gave the best result, the economic analysis renders it uneconomical in comparison with other diets. Diet 1 (100% fish) had the least cost in terms of cost of feed per kilogram weight gain and cost of feed per kilogram protein produced. It is important to note that the fishmeal used was not the imported commercial fishmeal. The meal was obtained from dried, milled cluipeds sold in local markets.

In view of the fact that the dried sea clupeids are in high demand by humans who consume them and animal feed millers who use them for making animal feeds, it is often not readily available. In view of this, the *Moringa* kernel meal – based diet would be of great value. Among the *Moringa*-based diets, D7 (75% boiled *Moringa* kernel meal, 25% fishmeal) which had the least cost of feed per 1 kilogram weight of fish could be considered as the most efficient of the test diets.

The findings in this research have justified the need to search for non-conventional sources of protein ingredients of both plant and animal origin for fish feed. The commercial pelleted feeds are expensive for many fish farmers. This has discouraged many prospective fish farmers from launching into the business at both small and large scale levels. However, a fish farmer can formulated fish rations by himself using local ingredients.

*Moringa oleifera* is a common plant in Northern Nigeria and will also thrive in the Southern parts. The leaves are widely consumed by people particularly in the northern parts but the kernels are left to waste. These kernels which have complete amino acid profile can be used for making fish feed. The method of processing (boiling) is simple and can be carried out by any fish farmer. Other ingredients are also locally available. At 75% replacement level of fishmeal with the meal from boiled *Moringa* kernel as in the cost of production will be highly reduced growth in this work, a significant increase and feed utilization in Clarias obtainable and as well acceptable level of protein will be available to the consumers. *Moringa* is well acceptable by humans with no residual-effects when consumed by people who eat fish fed on Moringa-included diets.

## 6.4 Recommendations

From the findings of this research, it could be recommended that fish farmers intending to use Moringa kernels for formulating fish diets for *Clarias* to cut down the cost of productions could replace fishmeal up to 75% with 30 minutes boiled kernels meal.

There are also no large plantations of *Moringa* as are available in other West African countries where the value of *Moringa* for humans has been recognized. Therefore Moringa plantations should be encouraged in Nigeria. Further research works are required for using other processing methods such as soaking and fermentation, and incorporating the processed kernels in the feeds for other species of fish.

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## APPENDIX I

### 1.0 Amino acid composition of extracted and unextracted *Moringa* leaves

| Amino acid | Amino acid composition (extracted leaves) g/kg DM | Amino acid composition (unextracted leaves) g/kg |
|---|---|---|
| Lysine | 26.77 | 14.06 |
| Leucine | 42.89 | 21.84 |
| Isoleucine | 22.53 | 11.30 |
| Methionine | 8.96 | 4.97 |
| Cystine | 5.18 | 3.39 |
| Phenylalanine | 27.14 | 15.51 |
| Tyrosine | 18.88 | 9.71 |
| Valine | 27.14 | 15.51 |
| Histidine | 13.57 | 7.50 |
| Threonine | 21.92 | 11.70 |
| Serine | 20.79 | 10.34 |
| Gluatamic acid | 50.87 | 25.65 |
| Aspartic acid | 46.11 | 22.61 |
| Proline | 25.75 | 13.63 |

| | | |
|---|---|---|
| Gylcine | 26.62 | 13.73 |
| Alanine | 28.67 | 18.37 |
| Arginine | 30.28 | 15.64 |
| Tryptophan | 9.26 | 5.27 |

**SOURCE: Sarkar and Peace (1994)**

## APPENDIX II

**2.0 Amino Acid Composition (g/kg DM) of Unextracted and Extracted *Moringa* Kernel Meal**

| Amino Acid | Unextracted Meal | Extracted Meal |
|---|---|---|
| Lysine | 6.394 | 6.437 |
| Leucine | 22.921 | 25.400 |
| Isoleucine | 13.266 | 15.179 |
| Methionine | 8.264 | 9.264 |
| Cyctine | 18.354 | 20.529 |
| Phenylalanine | 17.267 | 18.529 |
| Thyrosine | 6.524 | 6.089 |
| Valine | 15.092 | 15.788 |
| Histidine | 9.873 | 9.917 |
| Treonine | 9.786 | 9.917 |
| Serine | 11.961 | 12.396 |
| Glutamic acid | 84.161 | 85.379 |
| Aspartic acid | 17.267 | 16.354 |

| | | |
|---|---|---|
| Proline | 24.001 | 26.270 |
| Gylcine | 21.312 | 19.137 |

**SOURCE: Fugile (2001)**

## APPENDIX III

### 3.0 ANALYSIS OF DATA FOR BOILED *MORINGA* KERNELS

### 3.1 Mean Proximate Composition of boiled *Moringa* kernel meal at different time interval.

| Sample | % Moisture Content | % Ash | % Crude protein | % Crude fibre | % Ether extract |
|---|---|---|---|---|---|
| M $KM_{b10}$ | 3.09 | 2.13 | 35.72 | 4.03 | 38.77 |
| M $KM_{b20}$ | 2.83 | 2.10 | 2.10 | 4.48 | 39.98 |
| M $KM_{b30}$ | 2.92 | 2.19 | 27.86 | 4.99 | 37.01 |
| Fishmeal (Control) | 5.46 | 21.48 | 46.74 | 0.91 | 14.75 |

M KM = Moringa Kernel meal

b10, 20, 30 = boiled at 10, 20 and 30 minutes respectively

**3.2 Mean Anti-nutrient content in boiled *Moringa* kernel meal at different time intervals.**

| Sample | Mean Oxalate content |
|---|---|
| M $Km_{b10}$ | 14.56 ± 0.01 |
| M $Km_{b10}$ | 8.96 ± 0.01 |
| M $Km_{b10}$ | 5.60 ± 0.01 |
| Fishmeal (Control) | - |

**3.3 Mean essential amino acid index of boiled *Moringa* kernel meal at different time intervals**

| Sample | Essential Amino Acids Index (EAAI) |
|---|---|
| M $Km_{b10}$ | 0.587 ± 0.001 |
| M $Km_{b20}$ | 0.595 ± 0.001 |
| M $Km_{b30}$ | 0.526 ± 0.001 |
| Fishmeal (Control) | 0.774 ± 0.001 |

**3.4 ANOVA Table for Fat Content of Boiled *Moringa* Kernels at 10, 20, 30 Minutes Respectively**

| Source | Df | SS | MS | f-Ratio |
|---|---|---|---|---|
| Rep | 1 | 0.3 | 0.3 | 10 |
| Treatment | 3 | 833.29 | 277.76 | 9258.67 |
| Residual (Treatment – 1)(Time -1) | 3 | 0.08 | 0.03 | |
| Total | 7 | 833.37 | | |

**3.5 ANOVA Table for Protein Content of Boiled *Moringa* Kernels at 10, 20, 30 Minutes Respectively**

| Source | Df | SS | MS | F-Ratio |
|---|---|---|---|---|
| Rep | 1 | 1.49 | 1.49 | 1.16 |
| Treatment | 3 | 364.8 | 121.6 | 94.26 |
| Residual | 3 | 3.86 | 1.29 | |
| Total | 7 | 370.15 | | |

**3.6 ANOVA Table for Crude Fibre Content of Boiled Moringa Kernel at 10, 20, 30 Minutes Respectively**

| Source | Df | SS | MS | F-Ratio |
|---|---|---|---|---|
| Rep | 1 | 0.01 | 0.01 | 0.14 |
| Treatment | 3 | 21.49 | 7.16 | 102.29 |
| Residual | 3 | 0.02 | 0.007 | |
| Total | 7 | 833.37 | | |

### 3.7 ANOVA Table for Ash Content of Boiled *Moringa* Kernels at 10, 20, 30 Minutes Respectively

| Source | Df | SS | MS | F-Ratio |
|---|---|---|---|---|
| Rep | 1 | 0.51 | 0.51 | 2.8 |
| Treatment | 3 | 571.36 | 19.12 | 106.22 |
| Residual | 3 | 0.53 | 0.18 | |
| Total | 7 | 572.4 | | |

### 3.8 ANOVA Table for Essential Amino Acids Index of Boiled *Moringa* Kernels at 10, 20, 30 Minutes Respectively

| Source | Df | SS | MS | F-Ratio |
|---|---|---|---|---|
| Rep | 1 | 0 | 0 | |
| Treatment | 3 | 0.07 | 0.02 | 0.00 |
| Residual | 3 | 0.00 | 0.00 | 0.00 |
| Total | 7 | 0.07 | | |

### 3.9 ANOVA Table for Anti-nutritional factors of Boiled *Moringa* Kernels at 10, 20, 30 Minutes Respectively

| Source | Df | SS | MS | F-Ratio |
|---|---|---|---|---|
| Rep | 1 | 0 | 0 | |
| Treatment | 3 | 81.95 | 40.98 | 40.98 |

| | | | |
|---|---|---|---|
| Residual | 3 | 0.02 | 0.01 |
| Total | 7 | 81.97 | |

## APPENDIX IV

## 4.0 ANALYSIS OF DATA FOR TOASTED *MORINGA* KERNELS

### 4.1 Mean Proximate Composition of toasted *Moringa* kernel meal at different time interval

| Sample | % Moisture Content | % Ash | % Crude protein | % Crude fibre | % Ether extract |
|---|---|---|---|---|---|
| M $KM_{t10}$ | 3.03 | 2.28 | 32.61 | 3.72 | 41.62 |
| M $KM_{t20}$ | 3.01 | 3.13 | 31.78 | 3.98 | 40.93 |
| M $KM_{t30}$ | 3.00 | 2.25 | 30.88 | 3.87 | 37.84 |
| Fishmeal (Control) | 5.46 | 21.48 | 46.74 | 0.91 | 14.75 |

M K $M_t$ = toasted Moringa kernel meal

t10, 20, 30 = toasted at 10, 20 and 30 minutes respectively.

### 4.2 Mean essential amino acid index content of toasted *Moringa* kernel meal at different time interval

| Sample | Essential Amino Acids Index (EAAI) |
|---|---|
| M $Km_{t10}$ | 0.584 ± 0.001 |
| M $Km_{t20}$ | 0.581 ± 0.001 |
| M $Km_{t30}$ | 0.562 ± 0.001 |
| Fishmeal (Control) | 0.774 ± 0.001 |

**4.3 Mean Anti-nutritional content in toasted *Moringa* kernel meal at different time intervals**

| Sample | Mean Oxalate content |
|---|---|
| M $Km_{b10}$ | 15.68 ± 0.07 |
| M $Km_{b10}$ | 11.20 ± 0.02 |
| M $Km_{b10}$ | 7.84 ± 0.01 |
| Fishmeal (Control) | - |

**4.4 ANOVA Table for Fat Content of Toasted *Moringa* Kernels at 10, 20, 30 Minutes Respectively**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 0.27 | 0.27 | |
| Treatment | 3 | 82.65 | 327.55 | 306.12 |
| Residual | 3 | 3.22 | 1.07 | |
| Total | 7 | 986.14 | | |

**4.5 ANOVA Table for Crude Protein Content of Toasted *Moringa* Kernel at 10. 20. 30 Minutes Respectively.**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 3.36 | 3.36 | |
| Treatment | 3 | 341.02 | 113.67 | 110.36 |
| Residual | 3 | 3.10 | 1.03 | |

| | | | | |
|---|---|---|---|---|
| Total | 7 | 347.75 | | |

**4.6 ANOVA Table for Ash Content of Toasted *Moringa* Kernel at 10. 20. 30 Minutes Respectively.**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 0.30 | 0.30 | |
| Treatment | 3 | 545.52 | 181.84 | 826.13 |
| Residual | 3 | 0.67 | 0.22 | |
| Total | 7 | 546.49 | | |

**4.7 ANOVA Table for Essential Amino Acids Index of Toasted *Moringa* Kernel at 10. 20. 30 Minutes Respectively.**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 0.00 | 0.00 | |
| Treatment | 3 | 0.06 | 0.06 | 0.00 |
| Residual | 3 | 0.00 | 0.00 | |
| Total | 7 | 0.06 | | |

**4.8 ANOVA Table for Anti-Nutritional Content of Toasted *Moringa* Kernel at 10, 20, 30 Minutes Respectively.**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Rep | 1 | 0.00 | 0.30 | |
| Treatment | 2 | 61.80 | 30.90 | 618 |
| Residual | 2 | 0.10 | 0.05 | |
| Total | 7 | 61.9 | | |

## APPENDIX V

### 5.0 STATISTICAL ANALYSIS OF THE GROWTH AND FEED UTILIZATION BY *CLARIAS* FED THE FORMULATED DIETS

The obtained data were analysed using analysis of variance (ANOVA) (Table 4.7.1 to 4.7.5 and LSD at 5% level of probability was used to separate the means.

### 5.1 ANOVA Table for % mean weight gain in *Clarias* fed the test diets

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 14.29 | 148.29 | 5.82 |
| Treatment | 7 | 85206.80 | 12172.38 | 477.54 |
| Residual | 7 | 178.43 | 25.49 | |
| Total | 15 | 85533.40 | | |

### 5.2 ANOVA Table for Specific Growth Rate (SGR) of *Clarias* fed the test diets

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 0.010 | 0.01 | 0.00 |

| | | | | |
|---|---|---|---|---|
| Treatment | 7 | 1.723 | 0.246 | 615 |
| Residual | 7 | 0.003 | 0.0004 | |
| Total | 15 | 1.736 | | |

**5.3 ANOVA Table for mean feed Intake by *Clarias* fed the test diets**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 0.00 | 0.00 | 0.00 |
| Treatment | 7 | 0.231 | 0.033 | 110 |
| Residual | 7 | 0.003 | 0.0003 | |
| Total | 15 | 0.233 | | |

**5.4 ANOVA Table for Feed Conversion Efficiency (FCR) by *Clarias* fed the test diets**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 0.346 | 0.356 | 1.254 |
| Treatment | 7 | 51.696 | 7.385 | 26.0 |
| Residual | 7 | 1.987 | 0.284 | |
| Total | 15 | 54.039 | | |

**5.5 ANOVA Table for Protein Efficiency Ratio (PER) of *Clarias* fed the test diets**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 1 | 0.002 | 0.002 | 2.00 |
| Treatment | 7 | 0.363 | 0.052 | 52.00 |
| Residual | 7 | 0.005 | 0.001 | |
| Total | 15 | 0.37 | | |

**5.6 ANOVA Table for Net Protein Utilization by *Clarias* fed the test diets**

| Source | Df | SS | MS | F-ratio |
|---|---|---|---|---|
| Rep | 7 | 5857.045 | 836.72 | 976336.06 |
| Treatment | 1 | 0.0033 | - | |
| Residual | 7 | 0.006 | 0.000857 | |
| Total | 15 | 5857.0544 | | |

## APPENDIX VI

## 6.0 BI-WEEKLY BODY WEIGHT AND LEENGHTINCREAMENT IN *CLARIAS* FED FORRMULATED DIETS

### 6.1 Bi-weekly Body Weight Increment in *Clarias gariepinus* (%)

| | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 |
|---|---|---|---|---|---|---|---|---|
| wk2 | 17.58 | 21.16 | 14.68 | 8.72 | 12.69 | 16.29 | 22.2 | 26.37 |
| wk4 | 17.79 | 24.86 | 16.54 | 16.71 | 12.23 | 20.55 | 12.99 | 44.18 |
| wk6 | 45.77 | 30.52 | 17.79 | 18.76 | 29.71 | 34.93 | 35.42 | 73.92 |
| wk8 | 69.56 | 45.9 | 18.33 | 20.02 | 30.06 | 38.34 | 39.67 | 98.63 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| wk10 | 77.81 | 48.55 | 32.9 | 21.36 | 39.09 | 39.08 | 45.81 | 102.39 |
| wk12 | 83.6 | 52.72 | 42.44 | 33.89 | 49.83 | 47.28 | 55.84 | 121 |

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal

## 6.2 Bi-weekly Body Lenght Increment in *Clarias gariepinus* (%)

| | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 |
|---|---|---|---|---|---|---|---|---|
| WK2 | 22.79 | 33.05 | 33.83 | 19.38 | 32 | 11.11 | 35.59 | 25.4 |
| WK4 | 23.78 | 52.89 | 34.57 | 31.01 | 37.6 | 11.76 | 57.63 | 50 |
| WK6 | 27.27 | 76.03 | 50.38 | 52.71 | 46.4 | 17.65 | 53.39 | 92.86 |
| WK8 | 49.65 | 83.47 | 54.89 | 53.64 | 48 | 28.1 | 58.47 | 134.92 |
| WK10 | 54.55 | 88.43 | 63.16 | 62.02 | 47.2 | 35.95 | 60.17 | 135.71 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| WK12 | 72.03 | 95.04 | 65.41 | 63.57 | 49.6 | 38.56 | 77.12 | 170.63 |

KEY:

D1 – 100% FSM

D2 - 75% FSM, 25% $MKM_t$

D3 - 50% FSM, and $MKM_t$

D4 - 25% FSM, 75% $MKM_t$

D5 - 75% FSM, 25% $MKM_b$

D6 - 50% FSM, and $MKM_b$

D7 - 25% FSM, 75% $MKM_b$

D8 – Chi-feed (commercial)

FSM – Fishmal, $MKM_t$- toasted *Moringa* Kernel meal, $MKM_b$ – boiled *Moringa* Kernel meal