ASSESSMENT OF COTTON-SEED (Gossypium species) MEAL AS INGREDIENT IN THE DIET OF *Clarias gariepinus* FINGERLINGS

BY

ASHIRU AMINU
B.SC ED.BIOLOGY, A.B.U
M.Sc/Scien/09268/2009-2010

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

DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY,
ZARIA-NIGERIA

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DECLARATION

I declare that the work in this thesis entitled "ASSESSMENT OF COTTON-SEED (Gossypium species) MEAL AS INGREDIENT IN THE DIET OF *Clarias gariepinus* FINGERLINGS" has been performed by me in the Department of Biological Sciences, under the supervision of Professor J.Auta and Dr. S.A. Abdullahi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Ashiru Aminu	Signature	Date

CERTIFICATION

This thesis entitled "ASSESSMENT OF COTTON-SEED (Gossypium species) MEAL AS INGREDIENT IN THE DIET OF *Clarias gariepinus* fingerlings", by Ashiru Aminu meets the regulations governing the award of the degree of Master of Science in Biology Education of Ahmadu Bello University Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

____________________ ____________________

Prof. J. AUTA Date
Chairman, Supervisory Committee

____________________ ____________________

DR. S.A ABDULLAHI Date
Member, Supervisory Committee

____________________ ____________________

Prof. J. AUTA Date
Head of Department Biological Sciences.

____________________ ____________________

Prof. A.A.JOSHUA Date
Dean, School of Postgraduate Studies.

DEDICATION

This project is dedicated to my late father Alhaji Muhammadu Bakafushi, may Allah be please with him.

ACKNOWLEDGEMENT

I wish to express my unquantifiable gratitude and appreciation to the Al-mighty Allah for the ability given to me. May his blessing be upon his beloved messenger prophet Muhammad (S.A.W) and his entire companions.

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ABSTRACT

The effect of feeding graded levels of cotton *Gossypium* Spp. seed meal as an inclusion in the diet of *Clarias gariepinus* juveniles for growth performance and feed utilization was analysed in comparison with the conventional commercial fish feed. Six experimental rations formulated were cotton-seed *Gossypium* Spp. meal replaced fish meal at graded levels of 20%, 30%, 50%, and 100% and were fed to *Clarias gariepinus* juveniles for 56 days, the experiment was conducted in six outdoor concrete tanks in the Department of Biological Sciences Garden, Ahmadu Bello University, Zaria, Nigeria. Data for each parameter were subjected to Analysis of variance (ANOVA) and the means for various experimental diets were compared for significant differences at 0.05% level of inclusion. The results showed that fish fed with D_1 which has 20% gave the best Body Weight Gain (81.28g), Specific Growth Rate (2.23), Feed Conversion Ratio (2.04), Protein Efficiency Ratio (2.21), Net Protein Utilization (35.14) and Condition Factor (1.49), while diet D_2 gave the least Weight Gain (49.67g), Specific Growth Rate (1.58), Feed Conversion Ratio (3.64), Protein Efficient Ratio (1.24), Net Protein Utilization (20.27) and Condition factor (0.79).The levels of Anti-nutritional Factors are negligible. The results were significantly different ($p<0.05$) for both growth and feed utilization parameters. Carcass crude protein was also significantly higher $p<0.05$ in fish fed $D_{1\ (20\%)}$ with (60.63%) and therefore, cotton-seed meal can be used as a replacement for fish meal at 20% level of inclusion. The results established significant reduction in production cost while optimal production is achieved.

TABLE OF CONTENTS

CHAPTER THREE

CHAPTER FOUR

CHAPTER ONE

1.0 INTRODUCTION

Fish plays significant role in the diets of people. Fish are the superior source of animal protein (62-72%) protein, depending on the species. Fish contribute about 40% of animal protein intake of an average person in Nigeria (Babbit, 1990)

Nigerians are large fish consumers with total consumption estimated at more than 1.3 million metric tones per year. With only some 450,000 MT of domestic catch, Nigeria is one of the largest importer of fish and import some 800,000 MT annually, contributing to a loss of jobs to overseas fishermen and this has unconstructive impact on the balance in trade (Miller and Atanda, 2004).

Essential Amino Acids (EAAs) cannot be synthesized by animal and often remain inadequate but are needed for growth and tissue development (Sogbesan, and Ugwumba, 2008). Fishmeal is known to contain complete Essential Amino Acids (EAAs) profile that is needed to meet the protein requirement of most fish species. Since fish meal is expensive as a feed ingredient, the use of non-conventional feedstuffs has been reported with good growth and better cost benefit values (Sogbesan, Ugwumba, 2008)

Fish feeds are used to amplify production and maximize profit, nonetheless fish feeds are often the most expensive item of most fish farming operations amounting to about 60% of the production cost (Akiyama, 1988).

Protein is a basic component of animal tissues and therefore an essential nutrient for both maintenance and growth. At maintenance level the fish requires it for replacement of worn out tissues, such as internal epithelial cell, enzymes and hormones, which are vital for proper functioning of the body and are recycled quite rapidly. The requirement of protein is therefore obvious since protein constitute 45-47% of tissue dry matter (Muria,1985).

The chronic shortage and escalating price of conventional fish meal leads to search for alternative unconventional agro-industrial by-products, for inclusion in fish meal; which are unsuitable for human consumption.

1.1 Research Problem

High cost of imported fish feed is a major problem to fish farmers in Nigeria and fish feed is generally considered as the most expensive single item in intensive fish farming. According to NRC (1993). Fish feed constitutes about 40-60% of the recurrent cost of most intensive fish farming ventures and can sometimes negate the economic viability of a farm if suitable feeds are not used. This problem has become a major source of fear and phobia to many prospective fish farmers in Nigeria and urgent solution most therefore be taken if fish farming is to be attractive, lucrative and sustainable.

Commercial fish feed are usually expensive because the traditional or conventional fish feed ingredient such as fishmeal, soyabean, guinea corn, vegetable oil, e.t.c, that are competing for human and livestock consumption. We must therefore, look resourcefully outward to identify, explore, and utilized other resources which are cheaper and less competative i.e, the use of non-conventional fish feed resources which are not usually common in the market, and are not the traditional ingredients used for commercial fish feed. These non-conventional resources could be of plant or animal origin (Madu *et al.,* 2003)

1.2 Justification

Due to the elevated cost of conventional fish feed which militate against aquaculture improvement in Nigeria, search of non–conventional feeds which are cheaper and less competative can be use in this direction.

Expensive feeds will marginalized or nullify the profitability of fish farming and so can incapacitate the expansion of farm, consequently low yield in terms of quality and quantity, thereby resulting in scarcity of commodity (fish) and eventually high cost of the few available ones to the disadvantage of the populace (Wee and Aminu, 1986).

The use of non-conventional feed resource is justifiable in the following ways:-

- They are non competitive in terms of human consumption.
- Their prices are relatively very low.
- They can a times be a by-product or waste products from agricultural and agro – allied industries.

- Their utilization as feed resource can sometimes help to reduce waste accumulation. This, enhance environmental protection, good sanitation and waste management (Madu *et al.,* 2003).

Cotton-seed meal is a non-conventional fish feed, it is an agro industrial by-product which is unsuitable for human consumption, cheap and efficient, and it could be used as an ingredient in the diet of fish.

Clarias gariepinus was selected for this research because it is one of the commercially important fish species use in aquaculture in Nigeria. It is also the number one fish presently used in aquaculture in Nigeria. It is hardy and withstands hash environmental conditions, fed on varieties of food items among many reasons.

1.3 Aim of the Study

The aim of this research is to assess the inclusion cotton-seed meal in the diet of *Clarias gariepinus* and the effects on the growth.

1.4 Objectives of the Study:

- Cotton-seed meal does not contain significant proximate components.
- To determine the proximate composition of cotton-seed meal.
- To assess growth performance and feed utilization by *Clarias gariepinus* fed with different graded levels of cotton–seed meal.
- To determine carcass composition of *Clarias gariepinus* fed with different graded levels of cotton-seed meal.

1.5 Hypotheses.

- Cotton-seed meal doesn't contain significant proximate components.
- There is no significant difference in the growth performance and feed utilization by *Clarias gariepinus* fed different graded level of cotton-seed meal.
- There is no significant difference of the carcass composition of *Clarias gariepinus* fed graded levels of cotton-seed meal.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Non-Conventional Fish Feed

Commercial fish feeds are usually expensive because the traditional or conventional protein source ingredient such as fish meal, soyabean meal, groundnut cake, maize, millet, guinea corn, vegetable oil e.t.c are competing for human and livestock consumption (Madu *et al.,* 2003). Fish feed constitute for about 60-80% of management cost in agricultural production (Igonifagha, 1979). Periodic scarcity and high cost of conventional fish feed cause a dramatic increase in cost of fish. For the production of conventional fish feed at cheap price and available, there is need for commercial fish feed producers in the country to use less expensive fish feed with good growth and better cost values (Oreshegun and Ayinla, 2001).

The effective production and growth of fish in culture system depend on feeding complete diet feed at level not exceeding the dietary requirement of the fish (Ayinla, 1992).There is an absolute need to intensify effort in order to supply culture species with adequate diets to supply their nutrients requirements qualitatively and quantitatively in order to achieve fast growth and high fish yield (Wee and Aminu,1986)

A wide range of Agricultural product and Agro-industrial by product has been studied and are known to posses nutrients for fish feeding (Okoye and

Eyo, 1989). Some of the product include; millet bran, groundnut cake, cotton-seed cake, wheat bran, cassava peel, blood meal, grasshopper, baobab leaves which are formed to be abundant and cheap in the northern zone of Nigeria (Okoye and Eyo., 1989).The non-conventional resource used for fish feed could be of plant or animal original and may be classified under the three major resource group:

i. Aquatic and terrestrial plant and animal (mainly macrophytes, invertebrates, and amphibians).
ii. Agricultural by-products of plants or animals origin.
iii. Industrial waste product (food processing and poultry industrial) (Madu *et al.,* 2003).

2.2 Proximate Composition of Some Non-conventional Fish Feeds

Proximate composition of some non-conventional fish feed resources of animal origin, shows that they are better than some of the conventional feed ingredient, poultry feather meal with crude lipid content of 84.9% can be very good source of lipid (Madu *et al.,* 2003).

Proximate composition of some non-conventional fish feed resources of plant origin shows that many of the unconventional sources are of very good nutrient profiles which when incorporated into feeds are able to meet part of the protein and energy requirements of the fish. For example, Duck weeds grown under favourable condition has protein content of 35% to 45% and fiber content of 05% to 15% depending on the species involved. Also it has very high content of carotenoids xanthophylls. It is therefore a good source of dietary protein in feed manufacturing (Madu *el al.,* 2003).

Large proportion of Nigerian fish supply is derived from capture fisheries, which has still not been able to satisfy fish demand in the country. In order to intensify fish production through aquaculture, adequate fish feed would be required for attaining high fish yield (Eyo, 1994). Therefore, there is a pressing need to search for alternative cheaper fish feed stuff from non-conventional sources. (Alegbeleye *et al*., 2001)

2.3 Feedstuffs and their Nutrient Content

Feedstuff used in fish feed formulation are composed mainly of natural product as well as many of the by-product of processing or milling industries but the proportion in which these components are present differs between feeds.

Foodstuffs can be classified as energy feedstuffs and protein supplement. The energy feedstuffs contain high amount of carbohydrates, low to high amount of ether extracts or lipid and low amount of protein. Carbohydrates have a sporting effect on protein in artificial feeds so that fish can utilize protein efficiently for growth rather than energy (Eyo, 1994).

In the selection of feedstuff for diet formulation, those with generally similar properties may be substituted, one for another and exchanges made within mixture in accordance with market price, local availability and chemical composition. In making substitution particular regard should also be paid to essential nutrients content and balance of the final diet. While carbohydrate feedstuffs are essential plant materials, protein supplements are either of plant or animal origin. Evidence from analytic data shows that, those of plants origins have crude protein content usually ranging between 4%-48% (Eyo, 1994). Apart from fishmeal, other animal proteins have high amino acid balance and digestibility than plant protein. Animal feedstuff also contains high concentration of phosphorus and calcium which are more digestible than those found in plant feedstuffs (Madu *el al.,* 2003).

2.5 Anti-Nutrient Factors in Some Local Feedstuffs

These growth inhibiting factors occur in virtually all available ingredient of plant origin. Therefore, in most cases form a "shield effect" on the protein molecule in the ingredient thereby, preventing proteases or similar digestive enzymes. This result in passing of the entire proteinaceous molecule with the faeces, undigested and hence unavailable to the fish for growth purposes. All plant feedstuffs such as cereals e.g. rice (*Oryza sativa*), sorghum (S*orghum bicolor)*, maize (*Zea mays*) oilseeds e.g. groundnut (*Arachis hypoge),* Soya beans (*Glycine max)* and cotton seed (*Gossypium* sp.*)* contain protease inhibitors and phytic acid. A Phytic acid is a hexaphosphoric acid derivative of mositol and exists in plant protein as a complex calcium-magnesium-potassium complex referred to as phytin. Both sorghum (Guinea corn) and cotton (*Gassypium* sp.) seed possess in addition tannin's or tannic acid and gossypol, respectively (Eyo, 1994)

The utilization of non-conventional feedstuffs of plant origin had been limited as a result of the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponegin, moosine, cyanoglycosides and linamarin (Sogbesan *et al.,* 2006). These anti-nutritional factors negate growth and other physiological activities (Oresegun and Alegbeleye, 2001).

2.6 Some Methods of Processing Anti-Nutritional Factors in Feedstuffs.

Feedstuffs are heat treated in order to destroy the anti-nutritional factors present in them and make them more digestible. Anti nutritional factors are substances which alter the nutritional values of the feedstuff or the health of the fish. They may be inherent in the feed stuffs or may have contaminated the feedstuffs from other sources. The latter are the pesticides and herbicides, which contaminate feedstuffs when in contact with them. Other contaminant includes toxins (mycotoxin) produce by mold, which grow on damp feeds during storage.

The anti-nutritional factors present in feedstuffs prevent digestion of the protein in feed e.g Tripsin, inhibitors in raw Soya beans and groundnut cake or forms complexes with other elements e.g. Gossypol in cotton seed-meal and phytate in Soya beans.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Research Site

This research was conducted at the outdoor hatchery, behind Fish Laboratory at the Department of Biological Sciences Department, Ahmadu Bello University, Zaria.

3.2 Fish Sources and Management

A total of seventy two (82) Juveniles of *Clarias gariepinus* were obtained from Bangiwa Farm, Funtua Local Government, Katsina State. The fish were transported in a fifty liters (50) half filled plastic container with water. In the laboratory they were acclimatized for two weeks in two larges water baths of 160 liters capacity at the rate of 36 fish per water bath. All fish were feed daily at 9.00 am and 3.00 pm at rate approximately 5% of their average body weight. After acclimatization the fish were distributed randomly into the experimental concrete tanks representing six treatments, each tank has twelve fish. At stocking, body weight and length of fish per tank was measured.

3.3 Experimental Set-up

The experimental set-up consists of six outdoor concrete tanks of 1.3mx0.9mx1m capacity ($1.17m^3$,) the concrete tanks were cleared and cleaned. Each tank was assigned to each of six experimental diets containing different level of cotton-seed meal, the sixth tank save as control. The water level was maintained at 0.6m throughout the experimental period and the waste water was replaced every four days.

3.4 Source of Cotton-seed Meal

Cotton-seed meal was purchase from Nigerian Oil Mills Company, Tafawa Balewa Road, Nassarawa Local Government, Kano State, Nigeria. The meal was then incorporated into the experimental diet according to the requirement of each treatment.

3.5 Method Used for Formulating Ration

Pearson's square method was used in formulating the six diets used for this research. Table 3.1 Shows the composition of each ingredient used, water was added, mixed properly, filtered and then sundried. (Falayi, 2003)

TABLE 3.1 Nutrient and Feed Formulation Table

	DIETS IN PERCENTAGE (%)					
	D1	**D2**	**D3**	**D4**	**D5**	**CONTR**
INGREDIENTS	**20%**	**30%**	**40%**	**50%**	**100%**	**00%**
Yellow maize	8.71	8.71	8.71	8.71	8.71	10.92
Wheat grain	13.71	13.53	13.53	13.53	13.53	16.95
Wheat offal	13.62	13.62	13.62	13.62	13.62	17.07
Cotton seed cake	12.82	17.25	25.65	32.07	64.14	00.0
Fish meal	51.31	44.89	49.41	32.07	00.0	55.06
Bone Meal	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin Premix	0.4	0.4	0.4	0.4	0.4	0.4
Lysine	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.2	0.2	0.2	0.2	0.2	0.2
$Nacl_2$	0.1	0.1	0.1	0.1	0.1	0.1

D1=20% Inclusion

D2=30% Inclusion

D3=40% Inclusion

D4=50% Inclusion

D5=100% Inclusion

3.6 Feed Analysis

The Association of Official Analytical Chemist AOAC (1990) methods of analyses were used to analyse the following parameters: Moisture content, Crude Protein content, crude fibre content, Oil/Lipid Content, Ash Content, and Free Nitrogen Extract for the Experimental diets.

3.6.1 **Moisture Content and Dry Matter Analyses**

The moisture and dry matter content was determined according to the method AOAC (1990). Five 2.5g of each diet was weighted using weighting balance and sample placed in a Petri dish. The samples were then put into a crucible. The crucible and the content were placed in the gallenkamp hot box oven with the temperature maintained at 100^0c. The sample was dried for twenty four hours. After drying, the sample was removed, cooled in desiccators for and weighed. The crude was returned to the oven for further drying to continue until a constant weight was obtained.

Formula used:

$$\text{Dry matter} = \frac{\text{wt of crucible with dried sample} - \text{wt of crucible}}{\text{Wt of crucible with initial sample-wt of crucible.}} \times 100$$

3.6.2 Crude Protein Content Analysis:

The crude protein content was analysed using semi-automated method using technical BD-20 block digester (AOAC. 1990) for the total analysis of kjeldal/nitrogen was used. O.5g sample was weighted on a filter paper, and placed in the BD-digester flask.15ml concentrated sulphuric acid, 8g of potassium sulphate and 1g selenium dioxide was added. The BD digester was placed in fume cupboard and heated for 2 hours at 410^{0}c until a clear solution was obtained. After cooling the colourless solution was transferred to a volumetric flask and made up to 250ml each with distilled water. 5ml of the made solution was pipette into a distillation chamber and 10ml of 35% sodium dioxide solution was added. The librated ammonia was distilled into 10ml of 3% boric acid. The ammonium borate formed was titrated against standard 0.05m hydrochloric acid until the original purple colour was restored.

$$\% \text{ crude protein} = \frac{Mx0.014xDFxVAx6.25}{WS \text{ x } VL} \text{ X } 100$$

Where M = morality of acid

0.14 = nitrogen content per 100ml

DF = dilution factor

VA = volume of acid

6.25 protein conservation factor/mg nitrogen = 6.25mg protein

WS = weight of sample

VL = volume of liquor distillate

3.6.3 Crude Fibre Content Analysis:

The crude fibre content was described according to the method of AOAC. (1990). 1 gram (1g) of grounded sample was poured to soxhlet apparatus and extracted with petroleum ether and di-ethyl ether. 100ml of 0.25m sulphuric acid solution was added and the content was boiled for thirty minute, the hot solution was immediately sieved and washed with hot water until it was free from acid, the insoluble matter was transferred into the initial flask. The 100ml of 0.314 sodium hydroxide solution was added and mixture was boiled for half and hour, after which the mixture was allowed to settle for one minute. It was then filtered and washed with hot water and become base free. It was then oven dried at 100^0c and weighted. The weighted sample was then transferred to a muffle furnace at 450^0c for two hours, cooled in desiccators and reweighed. The loss in weight was calculated as the percentage fibre content as shown below:

$$\text{Percentage crude fibre} = \frac{(A-B)}{P} \times 100$$

Where A = weight of ashless filter paper

B = weight of ash

P = Original sample weight

3.6.4 Oil/Lipid Content

The soxhlet method of extraction according to AOAC (1990) was used, it involves extraction of dried material with light petrol, the petrol evaporated from the solvent and residue weighted. 2.5g of the samples powder were placed in extractor thimbles and weighted. 200ml flask was used. $25cm^3$ of graded diethyl ether and graded petroleum ether was forward into flask. The flask and its content were connected to soxhlet siphon and condensed. The oil in sample is extracted under reflux on a heating mounting. The heating and extraction processing continued for eight hours, then the thimbles were removed, oven dried at 85^0c for two hour and weighted, the process repeated until constant weight was obtained, the lipid content was calculated from weight of material in the receiver flask.

Calculation:

$$\text{Lipid percentage} = \frac{\text{weight of lipid}}{\text{Weight of sample}}$$

(Pearson, 1976)

3.6.5 Ash Content Determination.

The method of AOAC (1990) was used in determining the Ash content. The sample was weighted and oven dried, cooled and weighted. 1g of the final ground sample was place into crucible than transferred to a furnace set at 560^0c for 14Hrs, after which the heated sample was allow to cooled. The Sample was re-heated and weighted for another 25 minutes

$$\text{Total Ash \%} = \frac{\text{weight of crucible + ash} - \text{weight of crucible}}{\text{Weight of sample}} \times 100$$

3.7 Determination of Anti-Nutritional Factors

The association of the official analytic chemist AOAC (1990) method was used to analysed the following anti-nutritional factors

3.7.1 Determination of Oxalate

Oxalate was determined by the method of (Oke, 1969). One gram of the sample was placed a 200ml volumetric flask, 150ml of distilled water and

10ml of 6m Hcl were added. The mixture was warmed on a water bath at 70^0c for 4 minutes. The supernatant was then diluted to 200ml. Three 50ml aliquots of the supernatants was evaporated 25ml, the brown precipitate was filtered off and washed. The combined solution and washings was titrated with concentrated ammonia solution in drops until salmon pink colour of methyl orange changed to faint yellow.

The solution was heated on water bath to 90^0c and the oxalate was precipitated with 10ml of 5% calcium chloride ($CaCl_2$) solution. The solution was allowed to stand overnight and then was centrifuged. The precipitate was washed into a beaker with hot 25% H_2SO_4 dilute to 125mls with distilled water and after warming to 90^0c, it was titrated against 0.05m $KMnO_4$.

Calculation: 1ml of 0.05m $KMnO_4$ = 2.2mg oxalate

3.7.2 Determination of Phytic acid/Phytate

Phytate phosphorus was determined by the method Wheeler and Ferrel (1971). Exactly one gram of the defatted sample was extracted for 1 hour with 100ml of 3% TCA with occasional swirling by hand. The suspension was centrifuged and a definite volume (10ml) of the supernatant was precipitated with 4ml of ferric chloride solution (containing 2mg of ferric ion ml^{-1} in 3% TCA). The precipitate of ferric Phytate was converted to ferric hydroxide with 4ml 1.5M NaOH. The ferric hydroxide was dissolved in hot 40ml 3.2M HN03 and transferred to 100ml volumetric flask. The volume was made up with distil water.

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

Standard Iron: This was prepared by dissolving 0.5g of iron III chloride ($FeCl_3$) in distilled water and the volume made up to 1 litre. This stock solution contained 3.4×10^5 ppm of iron.

Working Standard: 1ml of the stock solution was pipette into 100ml volumetric flask and diluted to mark with distilled water, thereby obtaining the concentration of 3ppm. Absorbance was measured at 480nm with 1.5M KSCN. Phytic acid content was calculated on the assumption that it contained 28.20% phosphorus by weight. The mole of iron to Phytate phosphorus is 1:1.

3.7.3 Determination of Tannin

The method of AOAC (1980) was used. 1.0g group sample was weighed into a conical flask and 100mlof distilled water added. This was boiled gently on a hot plate for one hour and filtrated through a NO. 44 What man filter paper into a 10ml volumetric flask. The paper was washed with distilled water and extract diluted to volume (this is the extract volume). Pipette 50ml of distilled water and 10ml of dilute extract (aliquot volume) into a 100ml conical flask followed by 5ml Folin-Denis reagent and 10ml of saturated Na_2CO_3 solution. Dilute to volume with distilled water.

After mixing the solution is allowed to standard for 30 minutes in a water bath (25^0c). Optical density is measured at 700mm and the absorbance compared on a standard tannic acid (tannin) curve.

The tannic acid standard curve was prepared by dissolving 0.2g of tannic acid in distilled to 200ml (1mg/ml). Varying concentration (0.1-1.0mg/ml) of standard tannic acid solutions are pipette into 10 different 100ml conical flask. 5ml Folin Denis reagent and 10ml saturated Na_2CO_3 solution is pipette into the test tubes. Then make up to the 100ml mark with distilled water. The solution is left to stand for 30mins and the optical density was measured at 700mm. A plot of optical density tannic acid concentration was made; with the line passing through the origin.

% Tannin content was calculated thus:

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

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

3.9.4 Determination of Saponin

The methods of AOAC (1980) was used, 5.0g ground was weighed into a thimble and transferred into the soxhlet extractor chamber filled with a condenser. Some quantity of acetone reflux was poured into the flask. The sample was exhaustively extracted at its lipid for 3 hours and the solvent distilled off. This is the first extraction.

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

$$\% \text{ Saponin} = \frac{\text{wt of saponin}}{\text{Wt of sample}} \times 100$$

3.7.5 Determination of Cyanogenic Glucosides

The method of AOAC (1980) was also used 1.0g ground sample was weighed into a 250ml flask. 200ml of distilled H_2O was added and allowed to stand for 2 hours. Full distillation was then carried out and 150-170ml of distillate (extractive volume) was collected in a 250ml conical flask containing 20ml of 2.5% NaOH. To 100ml of the distillate (align of volume) containing Cyanogenic glucoside, 8ml of 6N Ammonium hydroxide and 2ml 5% KI was added, mixed and titrated with 0.02N Ag No3 against a black background. Permanent turbidity indicates end point.

Cyanogenic glucoside content (mg/100g) =

$\underline{\text{Titre volume x 1.08 (g) extract volume}}$ x 100

Aliquot volume x sample weight (g)

3.8 Feeding Regime

The fish were fed with the meal on daily bases at the rate of 5% of the total biomass up to the end of experimental period, this was done twice daily at 9:00 am and 3:00pm.

3.9 Monitoring of Water Quality Parameters

Water quality parameters such as Temperature, Hydrogen ion concentration (pH) and Dissolve Oxygen (DO) were determined in all the tanks using standard methods described by APHA (1980).

Water temperature was taken with thermometer calibrated in ^{o}C. The thermometer was lowered into water and retained for about 2-5 minutes and the value recorded. pH – this was determined by using rent EIL model 7055 pH meter. The pH meter was lowered into the water sample for about 3-5 minutes and recorded. Dissolved oxygen was determined using YSI model 57 oxygen meter calibrated parts per million (ppm).

3.10 Sampling and Monitoring of Experimental Fish

Every two weeks, fish were taken from each aquarium/concrete tank, weighted, measures and the amount of feed was adjusted according to the changes in body weight through out the experimental period (56 weeks). The sampling exercise was conducted in the morning between 7:30am 8:30am prior to morning ration to minimize stress (Madu and Akilo, 2001).

During the experimental period analysis were made for the following parameters:

1. **Weight gain (g)** = Average final weight (g) -Average initial weight(g)

2. **Percentage Life wt Gain** (PWG%) = $PWG = 100 \frac{(Y-X)}{X}$

Where Y = final mean weight (g)

X = initial mean body weight (g)

3. **Specific Growth Rate** (SGR) = $\frac{LnW2-LnW1}{t} \times 100$

Where: Ln = the nature log, W1 = initial fish weight (g), W2 = the final fish weight in "grams" and t = period in days.

4. **Fish Survival** = 100x (number of fish - number of fish died) / no of fish stocked

5. **Feed Conversion Ratio (FCR)** = feed ingested/ weigh gain in gram (g)

(Adikwu, 2003)

6. **Protein Efficiency Ratio (PER)** = weigh gain (g) / protein ingested

7. **Feed Conversion Efficient (GFCE)** = weigh gain / feed intake x 100

8. **Apparent Net Protein Utilization (ANPU %)** = $\frac{Nb - Na}{N1 \times 100}$

Nb = Body protein at the end of feeding trial

Na = Body protein at the beginning of feeding trial

N1 = Amount of protein ingested

9. **Condition Factor (K)** = $(W/L^3) \times 100$

Where: W= weight of fish in "grams" L = total length of fish in "cm"

(Madu, and Akilo, 2001)

10. **Specific Growth Rate (SGR)** = $\frac{LnW2-LnW1}{t} \times 100$ (Brown, 1957)

Where: Ln = the nature log, W1 = fist fish weight, W2 = the following fish weight in "grams" and t = period in days.

11. **Nitrogen Metabolism (NM)** = (0.549) (b-a) h/2; a initial weight gain (g); b = final weight gain (g); h = time (days)

3.11 Statistical Analysis

The data obtained were subjected to analyses of variance (ANOVA) and means from the various Treatments were compared for significant different at 5% level using Duncan's Multiple Range Test (DMRT) of the system analytic statistic (SAS) statistical package.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Processed Cotton *Gossypium* Sp. Seed Meal

Table 1 shows the result for the proximate composition of the experimental diet fed to the *Clarias gariepinus*. The processed cotton-seed meal was subjected to chemical analysis; the result obtained shows the percentage proximate composition of cotton-seed meal as; crude protein 22.06%, crude fiber 17.01%, oil 20.90%, ash 5.93%, (carbohydrate) nitrogen free extract 34.40%.

Table 4.1 Proximate Composition of Processed Cotton *Gossypium* Sp. seed Meal.

Composition	Cotton-seed meal
Crude protein	22.06%
Crude fibre	17.01%
Oil/lipid	20.90%
Ash	5.93%
Nitrogen Free Extract (NFE)	34.10%

Figures represent mean values of proximate determinations

4.2 **Anti-Nutritional Factors**

Some anti-nutritional factors that were detected during the proximate analysis in cotton-seed meal are presented in Table 2 Tannin and Oxalate proximate were very low and the levels of phytic acid and cyanogenic glycoside were reduced during the feed heat treatment (Eyo, 2003).

Table 4.2 Anti-Nutritional Factors in Cotton-seed Meal *Gossypium* Spp.

ANTI-NUTRITIONAL	**MEAN CONTENT**
Phytic acid (mg/g)	4.06$\pm$ 0.53
Cyanogenic	2.10$\pm$ 0.72
Saponin (%)	1.38$\pm$ 0.35
Tannin (mg/g)	0.48$\pm$ 0.66
Oxalate (mg/g)	1.12$\pm$ 0.73

$\pm$ represent standard Deviation

4.3 Growth Assessment of *Clarias gariepinus*

Table 3 shows the growth assessment of *Clarias gariepinus* fed different graded levels of cotton-seed meal.

4.3.1 Mortality Rate

The number of fish was 12 in all the tanks at the beginning; Fish mortality was experience in tanks one, five, and control. The number of mortality are, 2,1,1 for the tanks respectively.

4.3.2 Body Weight (g) Measurement

Control which contains zero amount of cotton-seed meal recorded the highest average initial body weight followed by fish fed D_5, D_2, D_3, D_4, and D_1 respectively. Fish fed D_1 recorded the highest average final weight which was 118.0(g) followed by the fish in control D_5, D_4, D_3, and D_2 respectively. fish fed D_1 which has 20% inclusion of cotton-seed meal recorded the highest mean body weight gain of 87.28(g) followed by the fish fed D_4, 65.47(g) D_5, 63.83(g) control 61.66(g) D_3: 59.91(g) and D_2 recorded the lowest mean body weight gain of 49.69(g) respectively. The statistical analysis showed that there was significant difference ($p<0.05$) between the treatments in average fish body weight gain with fish fed D_1 which has 20% inclusion of cotton-seed meal been the best and D_2 which has 30% inclusion of cotton-seed meal was the least. Fish fed D_4 and D_5 performed better than the control. Fish fed D_2 has no significant difference ($p\geq0.05$) with the control treatment.

4.3.3 Average Increase in Standard Length

The average increase in standard length of fish among the treatments were 6.27 (cm), 6.90 (cm), 5.88 (cm), 6.61(cm), 8.38 (cm), and 7.66(cm), for Fish fed D_1, D_2, D_3, D_4, D_5 and, control respectively. However, statistically there was no significant difference ($p \leq 0.05$) between the treatments.

4.3.4 **Specific Growth Rates (SGR)**

The Specific Growth Rate shows that fish fed D_1 had the highest SGR of 2.23 while Fish fed D_2 and control had lowest of SGR 1.58.

The SGR for fish fed D_4, D_5, and D_3 had the SGR of 1.96, 1.87 and 1.80 respectively. The statistical analysis however showed significant different ($p \leq 0.05$) between Fish fed D_1 and D_2. Fish fed control, D_3, $D_{,4}$ and D_5. There was no significant difference ($p>0.05$) between Fish fed control and D_2.

4.3.5 Fish Survival Rate (FSR)

Fish Survival Rate among the treatments was generally high, ranging from 100% for fish fed D_2 D_3 and D_5 while Fish fed D_1, D_4 and control had 91% 97% and 97% respectively. Statistically, there was no significant difference ($p<0.05$) in the Survival Rate among Fish in different treatments tanks.

Table 4.3 Growth Performance Indices of *Clarias gariepinus* Fed Different Graded Levels of Cotton Seed-Meal

PARAMETERS	TREATMENTS IN PERCENTAGE (%)					
	D_1 20%	D_2 30%	D_3 40%	D_4 50%	D_5 100%	D_6 00%
No of fish	12	12	12	12	12	12
Mortality rate(%)	09	00	00	03	00	03
Average Initial weight (g)	33.72	34.63	33.96	32.65	34.40	37.25
Average Final weight (g)	118.0	84.3	93.87	98.12	98.33	98.91
Weight gain (g)	87.28^{a}	49.67^{d}	59.91^{cc}	65.47^{b}	63.83^{bc}	61.66^{c}
Percentage life Weight Gain (PWG%)	284.11	143.43	176.41	200.52	187.91	165.53
Average Initial Standard Length. (cm)	13.63^{d}	15.10^{bd}	16.06^{a}	15.69^{b}	14.52^{c}	15.33^{bc}
Average Final Standard Length (cm)	19.90	22.00	21.94	22.30	22.90	22.99
Average Increase in Standard length (cm)	6.27^{c}	6.90^{c}	5.88^{d}	6.61^{d}	8.38^{a}	7.66^{b}
Average Initial Total Length (cm)	13.63	16.20	17.26	17.05	15.83	16.88
Average Final Total Length (cm)	22.24	24.18	22.73	24.56	25.48	25.27
Average Increase in Total Length (cm)	8.61	7.98	5.47	7.51	9.65	8.39
SGR	2.23^{a}	1.58^{d}	1.80^{a}	1.96^{b}	1.87^{c}	1.58^{d}
Fish survival rate	91	100	100	97	100	97

Averages within each raw followed by different superscript are significantly different $p<0.05$

4.4 Growth Performance and Feed Utilization

Table 4 shows the growth and nutrient utilization of *Clarias gariepinus* fed different graded levels of cottonseed-meal as inclusion in *Clarias gariepinus* feed, fed for 8 weeks.

4.4.1 Feed Conversion Ratios (FCR_S)

Feed conversion ratios were 2.04, 3.64, 3.12, 3.21, 3.40 and 3.16 for Fish fed D_1, D_2, D_3, D_4, D_5 and control respectively. Fish fed D_2 had the highest (least) and Fish fed D_1 had the lowest (best). FCR statistically there was significant different ($p<0.05$) among the treatments.

4.4.2 Protein Efficiency Ratio (PER)

Protein Efficiency Ratio was highest with Fish fed D_1 (2.21) and lowest with Fish fed D_2 (1.24) while Fish fed D_3, D_4, D_5 and control had 1.45, 1.65, 1.33 and 1.43 respectively. There was significant different ($P<0.05$) between the treatments.

4.4.3 Gross Feed Conversion Efficiency (GFCE)

Gross feed conversion efficient (GFCE) was highest in Fish fed D_1 with values 48.80 followed by Fish fed D_4 36.53, D_3 32.02, control 31.75, D_5 29.38, then lastly by the Fish fed D_2 27.45.

4.4.4 Apparent Net Protein Utilization

Apparent net protein utilization was 35.14, 20.47, 8.75, 27.15, 29.44, and 2.73 for Fish fed D_1, D_2, D_3, D_4, D_5, and control respectively. Fish fed D_1 with values 35.14 was the highest while control had the lowest with values 2.73, statistically there was significant difference ($p<0.05$) between the treatments.

4.4.5 Condition Factor (k)

The condition factor (k) show that Fish fed D_1 was the highest with values 1.49 and Fish fed D_2 with values 0.79 as the lowest. Fish fed $D_{3,}$ D_4, D_5 and control show no significant difference among ($P \geq 0.05$). Fish fed D_1 had values 1.49 being significantly different ($p<0.05$) from the remaining treatments.

4.4.6 Specific Growth Rate

The Specific Growth Rate (SGR) shows that fish fed D_1 had the highest SGR of 2.23 while Fish fed D_2 and control had lowest of SGR 1.58. The SGR for fish fed D_1, D_5, and D_3 had the SGR of 1.96, 1.87 and 1.80 respectively. The statistical analysis however showed significant different ($p<0.05$) between D_1 and D_2, control, D_3, D_4 and D_5. While there was no significant difference ($p>0.05$) between Fish fed control and D_2.

4.4.7 Nitrogen Metabolism (NM)

Nitrogen metabolism (NM) was highest with fish fed D_1 with values 1,604.37 and lowest with Fish fed D_2 with values 763.52 while fish fed D_3, D_4, D_5 and control had the values 920.93 1,006.40 and 947.37 respectively. Statistically, there was significant different ($p<0.05$) among the treatments.

Table 4.4 Growth and Nutrient Utilization of C*larias gariepinus* Fed Different Graded Levels of Cotton Seed-meal.

TREATMENT **(%)**	PARAMETERS					
	FCR	PER	GFCE	NPU%	K	SGR	NM

Trt. 1 (20%)	2.04^{d}	2.21^{a}	48.80^{a}	35.14^{a}	1.49^{a}	2.23^{a}	$1,604.37^{a}$
Trt. 2 (30%)	3.64^{a}	1.24^{d}	27.45^{d}	20.47^{c}	0.79^{c}	1.58^{c}	763.52^{d}
Trt 3 (40%)	3.12b^{c}	1.45^{c}	32.02^{c}	8.75^{d}	0.88^{b}	1.60^{c}	920.93^{d}
Trt 4 (50%)	3.21^{bc}	1.65^{b}	36.53^{b}	27.15^{b}	0.88^{b}	1.96^{b}	$1,006.40^{d}$
Trt 5 (100%)	3.40^{b}	1.33^{d}	29.38^{c}	29.44^{b}	0.81^{c}	1.87^{b}	981.34^{c}
Ctr (00%)	3.16^{bc}	1.43^{c}	31.75^{c}	2.73^{d}	0.83^{c}	1.58^{c}	947.37^{d}

Values on the same column with the different superscript are significantly different $P<0.05$

FCR = Fed conversion Ratio

PER = Protein Efficient Ration

GFCE = Gross Feed Conversion ration

ANNU = Apparent Net Nitrogen Utalization

K = Condition factor

SGR = Specific Growth Rate

NM = Nitrogen Metabolism

4.5 Initial and Final Carcass Composition of Experimental Fish (*Clarias gariepinus)*

Table 5 gives the proximate composition of the experimental fish *Clarias gariepinus* juveniles at the beginning and end of feeding trials.

4.5.1 Dry Matter (DM)

The dry matter (DM) contents of the fish before the experimental diet were 94.04, 93.73, 93.44, 94.46, 93.57 and 93.66 for the control, D_1, D_2, D_3, D_4, and D_5 respectively. The percentage dry matter for all treatment was nevertheless significantly different ($p<0.05$) than that of the fish at the beginning.

4.5.2 Crude Protein (CP)

Proximate analysis shows that, the crude protein in all fish fed the experimental feed was higher than that of the beginning. The fish fed on D_5 had the highest percentage of 60.88% while fish fed on control had the least protein content of 50.38%. Fish feed on D_1, D_2, and D_4 had the following percentage 60.63%, 54.94%, 57.50% respectively. Statistically, the crude protein content of D_5 was significantly higher ($p<0.05$) than those of Fish fed D_2, D_3, and D_4 and control.

4.5.3 Crude Fibre (CF)

The crude fibre values of control and treatment are 0.02g/100g, 0.06 g/100g, 0.10 g/100g, 0.03 g/100g, 0.08 g/100g and 0.06 g/100g for control, D_1, D_2, D_3, D_4 and D_5 respectively. Fish fed D_2 showed significant difference

($p<0.05$) between the treatments. The crude fiber content of the fish before the commencement of the feeding trial was significantly higher ($p<0.05$) than those of the fish at the end of the experiment.

4.5.4 Fat/Oil

The result also shows the oil values of fish treated with diets as 8.06 g/100g, 7.77g/100g, 7.84g/100g, 7.69g/100g, 8.01g/100g, 7.66g/100g for control, D_1, D_2, D_3, D_4 and D_5 respectively. Fish fed control had the highest values of 8.06 followed by Fish fed D_5 which recorded the lowest. Statistically, oil values of fish at the beginning were significant higher ($p<0.05$) than those at the end of treatment.

4.5.5 Ash Content

The ash content values had the highest values of 10.28g/100g for Fish fed D_4 and lowest values of 7.61g/100g for Fish fed $D_{5.}$ Statistically, there were significant difference ($p<0.05$) between the treatment, and no significant difference ($p\geq0.05$) between the treatment at the beginning and at the end of feeding trial**.**

4.5.6 Nitrogen Free Extraction (NFE)

Nitrogen Free Extract (NFE) of the fish at the beginning was 22.35g/100g and that of the fish fed the experimental diets had the values of 35.41g/100g to be highest for Fish fed control, while Fish fed D_2 (30%) had the lowest NFE values of 23.21g/100g.

Table 4.5 Initial and Final Carcass Compositions of *Clarias gariepinus.*

Composition	Initial	Control	Diet i	Diet ii	Diet iii	Diet iv	Diet v
Dry Matter (DM)	95.86	94.04	92.73	93.44	94.46	93.57	93.66
Crude Protein (CP)	46.77	47.94	60.63	54.94	50.38	57.50	60.88
Crude Fibre	0.45	0.02	0.06	0.10	0.08	0.03	0.06
Crude Fat/Oil	15.24	8.06	7.77	7.84	7.69	8.01	7.66
Ash	11.05	8.57	8.33	7.69	10.18	10.28	7.61
Carbohydrate	22.35	35.41	23.21	29.43	31.67	24.28	23.79

Figures are means of duplicate determinations

4.6 Water Quality in the Experimental System

The average values of water quality parameters in the experimental system are shown in Table 6. Temperature fluctuates with little variation among the experimental tanks. The highest average temperature was recorded in tank 3 with temperature values of 27.50^{o}c. The hydrogen ions concentration showed no significant difference ($p \geq 0.05$) among the tanks.

Table 4.6 Physico-chemical Parameters of Experimental Tank Water.

	DIET IN %					
PARAMETERS	TANK 1	TANK2	TANK3	TANK4	TANK5	CTRL
Temperature (^{o}C)	26.40^{0}	26.60^{b}	27.50^{a}	27.01^{ab}	26.01^{c}	27.10^{ba}
P^{H}	5.97^{cb}	6.01^{ac}	6.67^{ab}	6.74^{a}	5.92^{cb}	5.82^{c}
Dissolve Oxygen (DO) Mg/L	6.76	$7.29^{c\ c}$	8.23^{a}	7.54^{c}	$7.82^{b\ c}$	7.99^{b}
Dissolve solid	59.00	60.00	61.00	62.00	57.00	59.00

Values with the same raw with different superscript are significantly difference ($p<0.05$)

CHAPTER FIVE

5.0 DISCUSSION

The crude protein of cotton-seed meal in this study was 22.06%. This value is higher than what was reported by Mujahid *et al.,* (2000) of 17.74%. The variations in crude protein of cotton-seed have been attributed to the environmental condition in addition to varieties. The crude protein of cotton-seed meal of Chinese source (international feed number. 5-01-619) obtained by grinding the residual press-cake after removal of most of the oil was 47% crude protein (Simon *et al*., 2008). In this study, the crude protein was is in the same range with that of (Mujahid *et al*., 2000). The importance of crude protein to *Clarias gariepinus* is for tissue building, body defense, growth, transportation of oxygen and reproduction.

The crude protein of some non-conventional fish feed of plant source are: locust bean seed range from 22.88%-25.81% (Duniya, 2006). Albizzia seed range from 38.04%-33.19% (Anwa, 2006). The crude protein of some non-conventional fish feed of animal source are: 63.0 ± 4.5, 63.8 ± 3.6, $46.3 \pm$,3.2 43.5 ± 3.4, 45.9 ± 2.5 47.8 ± 4.1 for earthworm meal, garden snail meal, Termite Meal, Un-skinned dried tadpole, un-skinned-boiled tadpole, skinned tadpole meal respectively (Sogbesan and Ugwumba, 2008). The crude fibre according to the proximate analysis of the *Gossypium* Spp. used in this study was 17.01, while Simon *et al.,* (2008) reported the crude fibre content of cottonseed meal of Chinese source as 12.5%. The crude fibre content of some non-conventional fish feed of plant origin were: locust bean seed, range from 2.04% and 1.13% (Duniya, 2006); Albizzia seed, range from 19.03%-11.63% (Anwa, 2006). The crude fibre of some non-conventional fish feed of animal source are: 1.9$\pm$0.2, 4.1$\pm$0.9, 7.3$\pm$1.2 3.8$\pm$2.1, 3.8$\pm$1.8, 3.0$\pm$1.9 for Earthworm meal, Garden, Snail meal, Termite meal, Un-skinned dried tadpole, Un-skinned-boiled tadpole, Skinned tadpole meal respectively. Fibre is used by *Clarias gariepinus* to aids digestion and prevents many disease of digestion tract.

The Crude Ash (CA) content of cottonseed meal according to this study was 5.93%. According to Mujahid *et al*., (2000) the average crude ash (CA) content of cottonseed meal of different varieties was 4.27%. Albizzia seed, range from 7.84-4.20 (Anwa, 2006). The crude ash content of some non-conventional animal protein feedstuffs were: 8.9$\pm$2.1, 6.5$\pm$0.5, 3.6$\pm$0.6 26.5$\pm$4.3, 26.1$\pm$2.3, 27.7$\pm$3.1, for Earthworm meal, garden snail meal, Termite meal, Un-skinned dried tadpole, un-skinned-boiled tadpole, skinned tadpole meal respectively. The Crude ash content contains minerals which are essential to the maintenance of life e.g calcium, chlorine, iron, phosphorous, potassium, sodium, and sulfur.

The standard lipid requirement of most fishes range from 8-10% (Adikwu, 1987). This report is not in conformity with the lipid contents 20.09% of this study. The crude protein content and carbohydrate contents shows progressive increase in values in the fish after the experiment compared to that at the beginning. The crude fibre, lipid, and ash content shows progressive decrease in values in the fish after the experiment compared to the fish before the experiment.

The level of anti-nutritional factors present in the cotton-seed meal used for this study was significantly low ($p<0.05$) due to processing method in the oil mill industry. Eyo, (2003) reported that; growth inhibiting factors occur in virtually all-available ingredient of plant origin. Pompa (1982) reported that, the higher the anti-nutritional factor the lower the palatability and could lead to low feed consumption and utilization. Eyo (2003) reported that, feedstuffs are heat treated in order to destroy anti-nutritional factors present in them and make them more digestible. This aggress with this study in which as a result of heat treatment of cottonseed (processing) the levels of anti-nutritional factors becomes low, that was the anti-nutritional factors reduce significantly ($p<0.05$) with processing.

This was because toxicity effects are expected when high concentration of free gossypol rationed. In the case of this research the cottonseed had undergone some processing treatments which contributed in the lack of visible clinical signs of gossypol toxicity in this study. These anti-nutritional factors negate growth and other physiological activities at the higher inclusion levels (Oresegun and Alegbeleye, 2001).

According to this study, fish fed D_1 had significantly greater mean body weight gain (87.28g) than those of $D_{2,,}$ D_3 D_4 D_5 and control that had 49.67g, 59.19g, 65.47g, 63.83g, 61.66g, respectively. This suggested greater protein utilization in Fish fed D_1 which had 20% inclusion of cotton-seed meal in the diet of *Clarias gariepinus* juvenile. This means were significant indicating the possibility of partial replacement of fish meal by cottonseed meal at 20% with the adverse effect on final body weight of *Clarias gariepinus* and similar trained was also observed for Specific Growth Rate and Feed Conversion Ratio. Simon *et al.,* (2008).

Obtained 91.29(g) for fish feed with 15% inclusion level of cotton-seed meal of *Clarias gariepinus* juveniles also recorded mean body weight gain of 67.18(g) and 48.13(g) for *Clarias* fed 45% and 60% inclusion of level of cotton-seed meal respectively.

Balogun *et al.,* (2004) reported that, weight gain and standard length increases are known to be the most essential parameters for measuring fish responses to experimental treatments and liable indicator of growth, using *Dlonix regia.*

Sanz *et al*., (1994) evaluated the nutritive potential of sunflower meal protein as compared to soyabean meal and fish meal protein in trout diets and they found that, sunflower meal protein could be replace up to 40% of fish meal protein or soybean meal protein in the diet at the same replacing percentage in trout diets without any negative impact on body weight. In another study, Abdul-Aziz *et al*., (1999) showed the possibility of partial substitution of soybean protein with sunflower protein up to 50% without adverse effect on body weight of Nile tilapia fingerlings.

Some attempts were made to replace the high cost animal protein source with sunflower meal (low costs plant protein). Fagbenro and Davies (2000) stated that, replacement of 67% of fish meal by sunflower meal in tilapia diets did not significantly altered the final weight.

Olvera-Novoa *et al.*, (2002) showed the possibility to replace animal protein source in tilapia fry diets with sunflower seed meal up to 20% without significant effect in body weight of Nile tilapia fry while the highest replacing levels significantly decrease the body weight.

In another study, El-Saidy and Gaber (2002) found that up to 50% de-hulled sunflower meal protein could be used to replace fish meal as a protein source in the diet of Nile tilapia, *Oreochromis niloticus* without significant effect on the Body Weight.

Abbas *et al.*, (2005) reported that the gradual rise in replacement level of fish meal by sunflower meal negatively affected growth performance of major carps and the minimum decrease in fish production was recorded at 25% replacement level while the maximum decrease was recorded at 75% replacing level of fish meal by sunflower meal, this is reverse of our findings in this study.

The average physico-chemical parameters range are Temperature (26.01^{o}C – 27.50^{o}C,) P^{H} (5.82-6.7;) Dissolve oxygen, (7.29-8.23mg/l). These physico-chemical parameters are inconformity with the standard ones, they fall within the normal range, Temperature (25^0C-30^0C) P^H (6.0-9.0,) Dissolve oxygen (7.00-9.00)

According to Balogun *et al*., (2004) for good growth to be achieved in juvenile fishes, optimum water parameters such as P^H, Temperature and Dissolve Oxygen along with inherent factors of growth and species differences are essential. Auta, (1993) recorded a P^H range of 6.7-9.0, temperature range 25^oc – 30^oc, and dissolve oxygen of 5.0mg/l in an experiment carry out in tanks.

Anwa (2006) in her research reported that, average temperature range of $26.7\underline{+}0.15^o{}_c$ – $27.5\underline{+}0.32$, P^H $5.98\underline{+}0.07$-$7.74\underline{+}0.24$, dissolve oxygen $4.94\underline{+}0.24$-$8.75\underline{+}0.36$mg/l for *Clarias gariepinus* cultured in out door tanks. Duniya (2006) reported temperature range of 25.00^oc-27.20^oc, P^H range of 6.5-7.5, dissolve oxygen range of 6.0mg/l-9.3mg/l. Balogun *et al*., (2004) also reported temperature range of 23^oc – 27^oc, P^H 6.3-7.8 and dissolve oxygen 6.3-9.6mg/l for the optimal growth and development of *Oreochromis* Sp. cultured under aquaria conditions. In this regard since that the physico-chemical parameters fall within the recommend range the only factor that can affect the experimental fish is formulation of balance diet fed for growth and reproduction.

Proper processing takes care of anti-nutritional factors of feedstuffs. However, no obvious physiological defect that may lead to death was detected in the fish fed the experimental diets.

Alegbeleye *et al*., (2004) reported a relatively high mortality rate for fish fed raw locust bean diet, due to the presence of toxin in the raw diet. This is contrary to the result obtained in this study, in which a minimal mortality rate of (00%) was recorded, due to proper handling, and processing of the *Gossypium* Sp.Meal to take care of anti-nutritional factors. Duniya (2006) recorded low mortality due to proper handling of experimental procedure. According to Eyo, (2003) Anti-nutritional factors are being destroyed by heat treatment.

Fish feed the experimental diets shows that, the crude protein content in all the fish was significantly higher ($p<0.05$) than that of the fish at the beginning. The crude protein content at the beginning was 46.77% while the least crude protein content of the fish fed experimental diet was 50.38% and the highest was 60.88% this indicates reasonable protein accumulation due to optimal utilization for growth, reproduction and survival (Alegbeleye *el al.,* 2004).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

Juvenile African Catfish *Clarias gariepinus* fed 20% inclusion of cottonseed meal had the highest, crude protein content of about 60.63%, highest Specific Growth Rate, Feed Conversion Ratio, highest Percentage Weight Gain, best Feed Conversion Ratio, highest Protein Utilization highest Condition Factor and highest amount of Nitrogen Metabolism.

Recommendations

i. Cotton-seed meal in the diets of juvenile African catfish should be within the range of 20%-30% inclusion levels.

ii. Cotton-seed meal inclusion of 20% level should be maintained in fish feed because of its high fibre content and high protein content of about 40% for the later

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APPENDIX 1

GROWTH AND FEED UTILIZATION PARAMETERS ANALYSIS

Weight Gain (WTG)

Diet 1(20%) Cottonseed meal inclusion

Weight Gain (WTG) = final mean weight – initial mean weight gain:

WTG (D1) = FMWT – IMWT

FMWT = 118.0g

IMWT = 30.72g

WTG (D1) = 118.0-30.72 = 87.28g

Diet 2

WTG (D1) = FMWT – IMWT

FMWT = 84.30g

IMWT = 34.63g

WTG (D2) = 84.30g – 34.63g = 49.67g

Diet 3

WTG (D3) = FMWT – IMWT

FMWT = 93.87g

IMWT = 33.96g

WTG (D3) = 93.87g – 33.96g = 59.91g

Diet 4

WTG (D4) = FMWT – IMWT

FMWT = 1079.4g

IMWT = 32.65g

WTG (D4) = 1079.4g – 32.65g = 65.47g

Diet 5

WTG (D5) = FMWT – IMWT

FMWT = 98.33g

IMWT = 34.50g

WTG (D5) = 98.33g – 34.50g = 63.83g

Diet 6 (Control)

WTG (D6) = FMWT – IMWT

FMWT = 98.91g

IMWT = 37.25

WTG (6) = 98.91g – 37.25 = 61.66g

Percentage Weight Gain

$$(PWG\%) = 100 \times \frac{y-x}{X}$$

Where = y = FMWT

X = IMWT

Diet 1

y = 118.0

x = 30.72

$$PGW\ (\%) = \frac{118.0 - 30.72}{30.72} \times 100$$

= 284.11

Diet 2

Y = 84.3

X = 34.63

$$PWG\ (\%) = \frac{84.3 - 34.63}{34.63} \times 100$$

= 143.43

Diet 3

Y = 93.87

X = 33.96

$$PWG\ (\%) = \frac{93.87 - 33.96}{33.96} \times 100$$

= 176.41

Diet 4

Y = 98.12

X = 32.65

$$PWG(\%) = \frac{98.12 - 32.65}{32.65} \times 100$$

= 200.52

Diet 5

Y = 98.33

X = 34.50

PWG (%) = $\underline{98.33 - 34.50}$ x 100

$$\frac{\quad}{37.25}$$

= 187.91

Diet 6

Y = 98.91

X = 37.25

$$PWG\ (\%) = \frac{98.37 - 37.25}{37.25} \times 100$$

= 165.53.

Specific Growth Rate

SGR = 100 Lnw2 – Lnw1

W2 = final body weight

W1 = Initial body weight

T = Duration in days

Ln = Natural 10g

Diet 1

W2 =1180.0 Lnw2 = 7.07

W1 = 337.95 Lnw1 = 5.82

t. = 56

SGR = 2.23

Diet 2

W2 = 1011.6 Lnw2 = 6.91

W1 = 415.6 Lnw1 = 6.02

t. = 56

SGR = 1.58

Diet 3

W2 = 1126.5 Lnw2 = 7.02

W1 = 407.6 Lnw2 = 6.01

t. = 56

SGR = 1.80

Diet 4

W2 = 1079 Lnw2 = 6.98

W1 = 359.2 Lnw1 = 5.88

t. = 56

SGR = 1.96

Diet 5

W2 = 1180.1 Lnw2 = 7.07

W1 = 414.1 Lnw1 = 6.02

t. = 56

SGR = 1.87

Diet 6 (control)

W2 = 1088.1 Lnw2 = 6.99

W1 = 447.4 Lnw1 = 6.10

t. = 56

SGR = 1.58

Feed Conversion Ration

$$FCR = \frac{\text{wt of dry Feed Fed(g)}}{\text{Live weight gain(g)}}$$

Diet 1

$$FCR = \frac{178.86}{87.28} = 2.04$$

Diet 2

$$FCR = \frac{180.9}{49.67} = 3.64$$

Diet 3

$$FCR = \frac{187.02}{59.91} = 3.12$$

Diet 4

$$FCR = \frac{210.69}{65.47} = 3.21$$

Diet 5

$$FCR = \frac{217.26}{63.83} = 3.40$$

Diet 6 (Control)

$$FCR = \frac{194.22}{61.66} = 3.16$$

Protein Efficiency Ratio

PER DIET 1

$$PER = \frac{\text{Weight gain}}{\text{Protein}}$$

Where:

$$\text{Protein Fed} = \frac{\%\text{protein in diet x total}}{\text{Diet consumed}}$$

Diet 1

$$\text{PER} = \frac{87.28}{\frac{22.06 \text{ x } 178.81}{100}}$$

$$= \frac{87.28}{39.44}$$

$$= 2.212982$$

Diet 2

$$\text{PER} = \frac{49.67}{\frac{22.06 \text{ x } 180.90}{100}}$$

$$= 1.24$$

Diet 3

$$\text{PER} = \frac{59.91}{\frac{22.06 \text{ X } 187.02}{100}}$$

$$= \frac{59.91}{41.25}$$

$$= 1.45$$

Diet 4

$$\text{PER} = \frac{65.47}{\frac{22.06 \text{ x } 179.19}{100}}$$

$$= \frac{65.47}{39.52}$$

$$= 1.65$$

Diet 5

$$\text{PER} = \frac{63.83}{\frac{22.06 \text{ x } 212.24}{100}}$$

$$= \frac{63.83}{47.92}$$

$$= 1.33$$

Diet 6 (Control)

$$PER = \frac{61.660}{\frac{22.06 \times 194.20}{100}}$$

$$= \frac{61.66}{42.84}$$

$$= 1.43$$

Feed Conversion Efficient (%)

$$FCE = \frac{\text{Weight gain (g)}}{\text{Feed intake}} \times 100$$

Diet 1

$$\frac{87.22}{178.81} \times 100$$

$$= 48.80$$

Diet 2

$$\frac{49.67}{180.90} \times 100$$

$$= 27.45$$

Diet 3

$$\frac{59.91}{187.02} \times 100$$

$$= 32.53$$

Diet 4

$$\frac{65.47}{179.19} \times 100$$

$$= 36.53$$

Diet 5

$$\frac{63.83}{217.24} \times 100$$

$$= 29.38$$

Diet 6 (control)

$$\frac{61.66}{194.20} \times 100$$

$$= 31.75$$

Apparent Net Protein Utilization NPU (%)

Nb = Body protein at end of feeding trial

Na = Body protein at the beginning of feeding trial

N1 = Amount of protein ingested

Diet 1

$$NPU(\%) \ \frac{60.63 - 46.77}{39.44} \times 100$$

$$= 35.14$$

Diet 2

$$NPU(\%) = \frac{54.94 - 46.77}{39.90} \times 100$$

$$= 20.47$$

Diet 3

$$NPU(\%) \ \frac{50.38 - 46.77}{41.25} \times 100$$

$$= 27.15$$

Diet 4

$$NPU(\%) = \frac{57.50 - 46.77}{39.52} \times 100$$

$$= 27.15$$

Diet 5

$$NPU(\%) = \frac{60.88 - 46.77}{47.92} \times 100$$

$$= 29.44$$

Diet 6 (control)

$$NPU(\%) = \frac{47.94 - 46.77}{42.84} \times 100$$

$$= 2.73$$

Condition Factors (k)

K = 100w/:L3

W = final mean body weight (g)

L = final mean standard length (cm)

Diet 1	FMBW	FMSL
	$K = \frac{100x118.00}{19.90^3}$	$\frac{11,800.00}{7,880.59}$

$$= 1.49$$

Diet 2

$$K = \frac{100x84.3}{22.00^3} \qquad \frac{8430.00}{10,648.00}$$

$= 0.97$

Diet 3

$$K = \frac{100x93.87}{21.94^3} \qquad \frac{9,387.00}{10,561.11}$$

$= 0.88$

Diet 4

$$K = \frac{100x98.12}{22.97^3} \qquad \frac{9,812.00}{12,119.45}$$

$= 0.81$

Diet 5

$$K = \frac{100x98.33}{22.97^3} \qquad \frac{9,833.00}{12,119.45}$$

$= 0.81$

Diet 6 (control)

$$K = \frac{100x98.91}{22.79^3} \qquad \frac{9,891.00}{11,836.67}$$

$= 0.83$

Mean Increase in Standard Length

MIISL = L2 – L1

Where: L2 final mean standard length (cm)

L1 initial mean standard length (cm)

Diet 1 L2 – L1 = 19.90 – 13.63 = 6.27

Diet 2 L2 – L1 = 22.00 – 15.100 = 6.90cm

Diet 3 L2 – L1 = 21.94 – 16.06 = 5.88cm

Diet 4 L2 – L1 = 22.30 – 15.69 = 6.61cm

Diet 5 L2 – L1 = 22.90 – 14.52 = 8.38cm

Diet 6 L2 – L1 = 22.99 – 15.33 = 7.66cm

Mean Increase in Total Length cm = L2- L1

Diet 1 L2 – L1 = 22.24 – 13.63 = 8.61cm

Diet 2 L2 – L1 = 24.18 – 16.20 = 7.98cm

Diet 3 L2 – L1 = 22.73 - 17.26 = 5.47cm

Diet 4 L2 – L1 = 24.56 – 17.05 = 7.51cm

Diet 5 L2 – L1 = 25.48 – 15.83 = 9.65cm#

Diet 6 L2 – L1 = 25.27 – 16.88 = 8.39cm

Fish Survival Rate

FS = $\frac{\text{No of fish harvest}}{\text{No of fish stocked}}$

Diet 1 FS = 10/11 x 100 = 90.5%

Diet 2 FS = 12/12 x 100 = 100%

Diet 3 FS = 12/12 x 100 = 100%

Diet 4 FS = 1/12 x 100 = 96.7%

Diet 5 FS = 11/12 x 100 = 100%

Diet 6 Fs = 11/12 x 100 = 96.7

Mean Initial Standard Length cm

Mean Final Standard Length cm

Mean Initial Total Length cm

Mean Final Total Length cm

Nitrogen Metabolism Nm

Nm = (b-a) ½

a. = Initial Weight (g)

b. = Final Weight (g)

h. = Time in days

Nitrogen metabolism

Nm = (0.549) (b-a)1/2

Diet 1

Nm = (0.549) (1180-337.95) 56/2

Nm = 0.549x 104.37

= 1,604.37

Diet 2

Nm = 0.549 (84.30 – 34.63) 56/2

0.549 x 49.67 x 56/2

= 763.52

Diet 3

Nm = 0.549 (93.87 – 33.96) 56/2

0.649 x 59.91 x 56/2

= 920.93

Diet 4

$$Nm = (0.549)\,(98.12 - 32.65)\,56/2$$
$$0.549 \times 65.47 \times 56/2$$
$$= 1{,}006.40$$

Diet 5

$$Nm = (0.549)\,(98.24 - 34.50)\,56/2$$
$$0.549 \times 63.84 \times 56/2$$
$$= 918.34$$

Diet 6 (control)

$$Nm = (0.549)\,(98.91 - 37.28)\,56/2$$
$$0.549 \times 61.63 \times 56/2$$
$$= 947.37$$

APPENDIX 2

SUMMARY OF GROWTH ASSESSMENT, NUTRIENT UTILIZATION, SURVIVAL PERCENTAGE OF *Clarias gariepinus* JUVENILES FED DIFFERENT LEVELS (%) OF COTTON-SEED MEAL. *Gossypium Spp*

ANALYSED PARAMETERS	TREATMENTS					
	D I	***DII***	***DIII***	***DIV***	***DV***	***DVI***
	20%	***30%***	***40%***	***50%***	***100%***	***CONTROL***
Initial Mean Weight (g)	30.72	34.63	33.96	32.65	34.40	37.25
Final Mean Weight (g)	118.0	84.3	93.87	98.12	98.33	98.91
Mean wt Gain (g)	87.28^{a}	49.67^{d}	59.91^{cc}	65.47^{b}	63.83^{bc}	61.66^{c}
Percentage life Weight Gain (PWG%)	284.11	143.43	176.41	200.52	187.91	165.53
Initial Mean Standard Length (cm)	13.63^{d}	15.10^{bd}	16.06^{a}	15.69^{b}	14.52^{c}	15.33^{bc}
Final Mean Standard Length (cm)	19.90^{b}	22.00^{b}	21.94^{a}	22.30^{b}	22.90^{a}	22.99^{a}
Mean Increase in Standard Length (cm)	6.27^{c}	6.90^{c}	5.88^{d}	6.61^{d}	8.38^{a}	7.66^{b}
Initial Mean Total Length (cm)	13.63d	16.20bd	17.26	17.05	15.83	16.88
Final Mean Total Length (cm)	22.24	24.18	22.73	24.56	25.48	25.27
Mean Increase in Total Length (cm)	8.61	7.98	5.47	7.51	9.65	8.39
Specific Growth Rate(%)	2.23	1.58	1.80	1.96	1.87	1.58
Feed Conversion Ratio (FCR)	2.04^{d}	3.64^{a}	3.12^{bc}	3.21^{bc}	3.40^{b}	$3.16b^{c}$
Protein Efficiency Ratio (PER)	2.21^{a}	1.24^{d}	1.45^{c}	1.65^{b}	1.33^{d}	1.43^{c}
Feed Conversion Efficiency (FCE)	48.80^{a}	27.45^{d}	32.02^{c}	36.53^{b}	29.38^{c}	31.75^{c}
Apparent Net Protein	35.14^{a}	20.47^{c}	8.75d	27.15^{b}	29.44^{b}	2.73^{d}

Utilization (ANPU%)						
Condition Factor (K)	1.49^{a}	0.79^{c}	0.88b	0.88^{b}	0.81^{c}	0.83^{c}
Survival Percentage	91	100	100	97	100	97
Nitrogen Metabolism	$1,604.37^{a}$	763.52^{d}	920.93^{d}	1006.40^{b}	981.34^{c}	947.37^{d}

In the same line Values with the different superscript are significantly different $P<0.05$