GROWTH PERFORMANCE AT VARIOUS GROWTH STAGES OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) FED VARIOUS INCLUSION LEVELS OF PROCESSED WATER MELON (*CITRULLUS LANATUS*, MANSF.) SEED CAKE DIETS

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DECLARATION

I declare that the work in this thesis entitled: "GROWTH PERFORMANCE AT VARIOUS GROWTH STAGES OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) FED VARIOUS INCLUSION LEVELS OF PROCESSED WATER MELON (*CITRULLUS LANATUS*, MANSF.) SEED CAKE DIETS" has been carried out by me at the Department of Biological Sciencesunder the supervision of Professors J. Auta, S.A. Abdullahi and J.A. Adakole. 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 in any other institution.

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Signature Date

CERTIFICATION

This thesis entitled: ``THE GROWT PERFORMANCE AT VARIOUS GROWTH STAGES OF *CLARIAS GARIEPINUS* (BURCHELL,1822) FED VARIOUS INCLUSION LEVELS OF PROCESSED WATER MELON (*CITRULLUS LANATUS*, MANSF.) SEED CAKE DIETS" by Musa, Yoane BABALE meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D.) in Fisheries of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to Almighty God and all those who encouraged, supported and contributed to the success of this work.

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ABSTRACT

This study explored the utilization of extruded water melon seed cake (*Citrullus lanatus,*Mansf.)as an alternative source of protein in fish feeds formulation for sustainable production of *Clarias gariepinus*. The proximate analysis, anti-nutrients compounds, amino acids, some minerals, carcass proximate composition, apparent digestibility, haematology and economic viability were determined according to standard methods. Toasting for 20 minutes gave the best crude protein of 32.68% and the least fibre value of 5.48%. The best anti-nutritional compound was at 40 minutes. Toasting at 20 minutes gave the best essential and non essential amino acids.Toasting time for 20 minutes gave the best mineral concentration.The experimental diet with 25% inclusion level of WMC gave the best growth performance and feed utilization in the fingerlings, juveniles and adults respectively. The percentage weight gain (PWG), Specific growth rate (SGR), Feed conversion ratio (FCR) and Protein efficiency ratio (PER) in the fingerlings were 91.50%, 1.78g, 1.64g and 1.66g, juveniles were 48.00%, 2.33g, 1.12g and 1.66g and adults were 44.00%, 0.61%, 0.89g and 1.17g respectively. The apparent digestibility of the feed in fingerlings of *C.gariepinus* clearly shows that the 25% inclusion level was the best with apparent protein digestibility (APD) value of 62.58%, apparent lipid digestibility (ALD) 50.75%, apparent dry matter digestibility (ADD) 39.62% and apparent carbohydrate digestibility (ACD) 40.80%. Similarly, in the juveniles theapparent protein digestibility (APD)was 70.72%, apparent lipid digestibility (ALD) 58.67%, 48.58% and apparent carbohydrate digestibility (ACD) 40.98% and in adults were apparent protein digestibility (APD) 80.65%, apparent lipid digestibility (ALD) 69.54%, apparent dry matter digestibility (ADD) 64.43% and apparent carbohydrate digestibility (ACD) 66.61% respectively. The hamaetological parameters at 25%

inclusion of water melon seed cakein the fingerlings of *Clarias gariepinus* were parked cell volume (PCV) 27.80%, white blood cell (WBC) 24.80%, red blood cell (RBC) 6.88% and haemoglobin (Hb) 15.73%, juveniles were parked cell volume (PVC) 43.06%, white blood cell (WBC) 33.80%, red blood cell (RBC) 8.74% and haemoglobin (Hb) 17.71% . In adults the haemoglobin parameters were parked cell volume (PCV) 53.34%, white blood cell (WBC) 34.87%, red blood cell (RBC) 10.44% and haemoglobin (Hb) 21.84%. The carcass proximate composition in the fingerlings of *Clarias gariepinus* were crude protein 30.45% CP, 3.11% lipid, 4.67% ash and 2.19% fibre, while juveniles were 44.90% CP, 5.47% lipid, 6.70% ash and 2.66% fibre. The adults had 68.69% CP, 8.62% lipid, 13.55% ash and 3.14% fibre. The mean final weight, mean yield cost N 357.00k, mean net profit N 323.00k, benefit cost ratio N 1.00 and incidence of cost N 0.82 were best at 25% inclusion level in all the experimental diets.Feed formulated for experimentwere dietswhich contained water melon seed cake and soy bean as the control at 0%, 25%, 50%, 75% and 100% of the total dietary protein.Feed were formulated using Pearson'ssquarewith crude protein at 45%, 42% and 40% for the fingerlings, juveniles and adults growth stages.The experiment was conducted for a period of 180 days in plastic tankshaving water holding capacity of 360 litres, which had stocking density of 20 fingerlings per tank and three replicates. The fish were fed three times daily at 5% body weight. Data obtained from the completely randomized experiment were subjected to one way analysis of variance (ANOVA) using Duncan Multiple Range Test. Results suggested that water melon can be used to substitute soy bean meal up to 25% levels of dietary protein in *Clarias gariepinus* at fingerlings, juveniles and adults growth stages without serious retardation in growth. Based on the relative cost of diets per unit weight gain and protein, 25% water melon cake meal was most economical.

The differences observed in growth parameters such as MWG, PWG, SGR, PER and FCRwere attributed to the anti-nutrients present in the feed and these compound increased with increasing levels of water melon diets of the fish. Therefore, more studies need to be carried out to determine possible and simple methods tofurther reduce the anti-nutrients to a level that would enhance better growth performance of C*larias gariepinus* at fingerlings, juveniles and adults growth stages.

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ACRONYMS/ABBREVIATIONS

AA - Amino Acid

ANF - Anti Nutritional Factors

AOAC – Association of Analytical Chemists

BCR – Benefit Cost Ratio

CF – Commercial Feed

CF – Crude Fibre

CHO – Carbohydrate

CL – Crude Lipids

CP – Crude Protein

CWT – Clean Water Team

Cyst – Cysteine

DF – Degree of Freedom

DM – Dry Matter

DMRT – Duncan Multiple Range Test

EE – Ether Extract

FAO – Food and Agricultural Organization

FCR – Feed Conversion Ration

GE – Gross Energy

GLM – General Linear Model

GNC – Ground Nut Cake

GP – Gross Profit

IC – Incidental Cost

ICA – Investment Cost Analysis

LOS – Level of Significance

LSD – Least Significant Difference

Meth. – Methionine

MFG – Mean Final Weight

MFI – Mean Feed Intake

MIW – Mean Initial Weight

MSS – Mean Sum of Square

NFE – Nitrogen Free Extract

NM – Nitrogen Metabolism

NPV – Net Profit Value

PER – Protein Efficiency Ration

PI – Profit Index

PI – Protein Intake

PWG – Percentage Weight Gain

RFWA – Rural Fresh Water Manual

RWM – Raw Water Melon

SAS – Statistical Analysis System

SE – Standard Error

SEM – Standard Error of Means

SGR – Specific Growth Rate

TEAA - Total Essential Amino Acid

TNEAA - Total Non Essential Amino Acid

Vit. Premix – Vitamine Premix

WMC – Water Melon Cake

CHAPTER ONE

1.0 INTRODUCTION

1.1Background of the Study

Nigeria is endowed with an estimated surface area of 12.5m ha of fresh water, dams, lakes, reservoirs and ponds is capable of producing 105metric tonnes of fish annually. Effort by various succeeding government have not been able to meet fish demand for the increasing population of Nigerians (Omitoyin, 2007). Private entrepreneurs have invested in fish farming as an enterprise that can provide an alternative solution to viable and sustainablefish protein production (Ayinla, 2003). The low output which is reflected in low catch per unit effort in the inland water bodies are due to over exploitation as a result of overpopulation and indiscriminate fish farming practices (Abu *et al*., 2010).Some problems associated with reduction in fish catch includes the use of small mesh sizes which allow overexploitation of all sizes of fish, obnoxious use of fishing methods that destroy all living organism in the water body, pollution from agro-chemicals which invariably leads to massive mortality of fish in large water bodies from run-offs and the use of explosives which also leads to huge economic losses (Jia *et al*., 2003)

The estimated population of Nigeria is over 170 million,this has necessitated gross importation of frozen fish and fish product from foreign countries in an attempt to bridge the increasing gap between internal fish production and demand in the country.The annual fish demand in Nigeria was estimated at 1.5 million metric tonnes with an existing demand deficit of 1.0 million metric tonnes (F.A.O., 2014).

The human demand for food fish is expected to rise from the present consumption level of about 90 million metric tonnes to about 150 metric tonnes by the year 2016. The share of aquaculture in the total world food fish production is set to increase from

29% to 40% by the year 2016 (Aduku and Bolorunduro, 2016). Aquaculture has become the fastest growing food production sector of the world, with an average annual increase of about 10% in 2000 compared to 3% increase for livestock meat and 1.6% increase incapture fisheries (F.A.O., 2000). To sustain such high rates of increase in aquaculture production, a matching increase of global levels of aqua- feed production for the year 2000 vary widely from 4.5-16.8 million metric tonnes (F.A.O., 2014).

Consequently, one of the factors militating against fish farming in Nigeria has been lack of adequate feed that are formulated to meet the nutrient requirement of culturable fish species. As such fishes do not attain market size at the right age (Gabriel *et al*., 2007). Nigerian fish farmers hardly use good quality fish feed pellets due to high cost of fish feed ingredients and fish meal (Francis *et al*., 2001). Omitoyin (2007) reported that a lot of fish farmers in Nigeria depend on imported quality fish feeds which are usually expensive.An estimated 4,000 tonnes of quality fish feeds are imported into the country each year.Utilization of such commercially formulated feeds increases the cost of production, thereby reducing the profit margin of fish farmers, which ultimately translates to high cost of fish (Ayinla, 2003)

Fish has been known to be rich in protein, minerals and fat, therefore, there is the need to boost its production using indigenous feed (Aduku and Bolorunduro, 2016). In recent years, fish has been acknowledged as a functional food which plays an important role in the prevention and management of heart disorders. The health and nutritional status of young children, aged people, people living with HIV and AIDS, pregnant and lactating women is enhanced through regular consumption of fish (F.A.O., 2014). Present fish production in the country is inadequate and does not meet the demand of fish needed by the populace. Hence there is the impetus to step up the

aquaculture development in Nigeria.Lack of affordable and readily available nutritive fish feeds is hampering its development and this has continued to be a major constraint to the advancement of aquaculture in Nigeria (Fagbenro *et al.,* 2013).

Fish nutrition experts world over and particularly in Nigeria have considered the procurement of alternative protein sources for inclusion in fish diet to partially or completely replace fish and soybean meals (Stickey *et al.,*2006). Fish meal is the main animal protein source in fish feed due to its balanced amino acids and minerals, but it is expensive and similarly, soybean meal is the main source of plant protein and is also costly. This is because the two protein sources are competitively demanded by man, animals and industries, and this has led to the increase in their prices in the Nigerian markets,when these protein sources are combined with other feed ingredients they account for 60-70% total operational costs in the aquaculture industries (Collins *et al.,* 2007).

Though legumes and oil seeds have economic potential of being used as plant protein sources, but only little knowledge is available on their usage compared to animal protein sources in aquacultural practices. Studies have shown that vegetable protein sources have high potentials for supplying fish with required protein needed for maximum productivity *(*Sotolu and Faturoti, 2008).

Watermelon is from the family *Cucurbitacea* and *genus Citrullus lanatus*and it is an oil seed that is rich in 10 essentially indispensable amino acids, it contains 30.6 crude protein, some minerals and lipids among others (Vandermark, 2011). Hence it is one of the oil seeds that have the potential of being used as plant protein to reduce dependence on soy bean (Hertrampf and Pascal, 2003).

Watermelon is a fast growing oilseed, it originated from South Africa, but has been introduced to may tropical and sub-tropical countries. In Nigeria, it is grown in almost

every part of the country; the pulp is red, yellow and whitish in some species. The seeds and rind are mostly eaten by animals (Monoj, 2007). The seeds are rich source of protein but rarely eaten in most part of the country by man, which makes it a good alternative for feed production at an inexpensive amount due to less competition on the usage of the seeds by man and industries (Adewolu., 2008).

The use of watermelon in feed formulation is expected to provide all the indispensable amino acids required by *Clarias gariepinus (*Fagbemi *et al.*,2007). When there is an imbalance in the amino acid of feed, it reduces the growth rate of the fish, the protein content of the blood, organs and tissues. It will also lower the hormone secretion, resistance in many types of diseases, metabolic disorder and infections (Adeyemo*et al*., 2003).

Watermelon is rich in protein and some minerals which is needed by *Clarias gariepinus* for osmotic regulation, maintenance of body fluids, locomotion and stability in its environment. Watermelon has good amount of the following amino acids, such as arginine, methionine, leusine, lysine and valine.It is also rich in magnesium, phosphorous, calcium and zinc (Johnson *et al*., 2012).Water melon seeds are almost available in every ecological area in Nigeria and its seeds can be collected or bought in the market at a little cost (Braide*et al.*, 2012). Consequently, the use of water melon seeds as feed for fish farming maylead to profitable aquaculture, curb rural urban migration and provide youth employment. When water melon seeds which is easily cultivated, processed and cheaper to buy is used for fish production, it may generate employment to the teaming youths in Nigeria and elsewhere, thereby curbs violence, drug abuse, reduce rural migration and other vices in the society (Moelh, 2003, Omitoyin, 2007).

1.2Statement of the Research Problems

Clarias gariepinus is an important aquaculture fishin Africa and in Nigeria in particular, but its culture is being hampered and becoming unattractive by high cost of feeds and its scarcity (FAO, 2014). This is because despite the vast aquatic resources, oil seeds and legumes which Nigeria is endowed with, most fish feed manufacture in the country rely on imported feed ingredients and fish feeds from European countries, the importation of fish meal and other feed ingredients which makes fish farming expensive because fish feeds account for 70%-80% of the cost of production depending on the location in Nigeria (Aduku and Bolorunduro, 2016). The vast unconventional plants based proteins which could supplement fish meal only contribute to stunted growth, low harvest and non profitable fish farming, because they are not readily digested due to the presence of anti-nutritional factors.

1.3 Justification

Aquaculture production seems to be responding to the increased fish demand and have exclusively increased the world fish production by 20 million tonnes (F.A.O., 2014).Irrespective of the achievement of aquaculture, the high cost and scarcity of desirable feeds for aquaculture enterprises in Nigeria has become a serious constraint in the successful operation of intensive aquaculture enterprise.

Fish meal and soybean meal which are the major sources of protein are used as food, by human, it is in high demand by industries for the production of human, animal and fish feeds and this makes the products and feeds expensive. The need to reduce this cost is imperative in the aquaculture business. An inexpensive source of fish feeds will make fish farming attractive due to its profitability. Research and studies into cheaper alternative protein sources for the development of least cost feeds for a small-enterprise fish farmer is an utmost priority in developing countries.

For Nigeria to be successful in fish production, the quest to make it profitable by using low cost feed with good protein content that can be sourced locally will be of great value and interest to farmers. In addition, Nigeria spent over150 billion Naira on fish importation in 2014 (FAO,2014). If there are readily available fish feeds, locally produced and of high-quality, many people will be attracted to fish farming, thereby boosting fish production and reducing fish importation.

Plant base proteins are relatively cheap and abundant, although, the presence of anti - nutritional compounds have limited their usage, therefore, there is the need to evaluate simple processing methods to remove the anti-nutrients before small fish farmers can utilize these plant protein sources. Consequently, the use of water melon seeds as a fish feed may lead to profitable fish farming via reduction in the cost of feed.

1.4Aim and Objectives of the Study

The aim of this study was to evaluate the effect of partial or complete replacement of soybean meal with watermelon seeds cake on the growth performance, nutrient utilization, body composition and economic viabilityof *Clarias gariepinus* at different growth stages.

Objectives of the study are:

i. To determine the proximate composition, anti-nutritional factors, amino acid concentration and mineral content of raw and processed watermelon seeds.
ii. To determine the growth performance of *Clarias gariepinus* fed water melon seed cake to fingerlings, juveniles and adults*Clarias gariepinus*.
iii. To determine the apparent digestibility of water melon seed cake fed to fingerlings, juveniles and adult *Clarias gariepinus*.

iv. To determine the haematological parameters and carcass proximate composition of *Clarias gariepinus* fed water melon seed cake at fingerlings, juveniles and adult growth stages.

v. To determine the cost benefit and economic production of partial or complete replacement of soybean meal with water melon seed cake meal in the diets of *Clarias gariepinus*.

1.5Research Hypotheses of the study

i. There is no significant difference in the proximate composition, anti-nutritional factors, amino acids concentration and some mineral contents of raw and processed water melon seeds.
ii. There is no significant difference on the growth response and nutrient utilization of *Clarias gariepinus* at fingerlings, juveniles and adults growth stages.
iii. There is no significant difference in the co-efficient of digestibility of feeds fed at fingerlings, juvenile and adult growth stages.
iv. There is no significant difference in the haemotology and carcass composition of *Clarias gariepinus*fed water melon seed cake at the fingerlings, juvenile and adult growth stages.
v. There is no significant difference in the cost benefit analysis and economic viability of partially or complete replacement of soybean meal with water melon seed cake in the diet of *Clarias gariepinus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Background Information on Watermelon

Watermelon belong to the melon family Cucurbitaceae and genus *Citrullus lanatus*.In the same family are squash, cucumber, muskmelon, calabash and pumpkin. Cucurbitaceae, also called cucurbits and the gourd family are a major group of Angiosperms.It is mostly prostrate or climbing herbaceous annual or perennial creeping plants native to tropical and temperate areas with about 90 genera (Braide *et al.,* 2012).The angroecium is usually five fused petals and five stamens (male) or an inferior ovary(female) or combined to form central column syngenesious and anthers are bilobed which produces from 10-15 fruits, weighing from 3-10 kilogram, the "Florida giant" may weigh up to 20 kilogram (Sotolu and Faturotj, 2009, Johnson *et al.* 2012). Watermelon (*Citrullus lanatus*) fruit is round, oval or oblong, with a light green to very dark green skin with various pattern or stripe and red, yellow or orange flesh (Braide *et al.,* 2012). The fruit has a smooth green exterior rind, yellow, sometimes white and juicy, sweet interior flesh (Vandermark, 2011). The fruit is used in preserves, jellies and conserves and to make pickles. It can also be used for smoother, sorbets or granite depending on the texture whether smooth or coarse (Magdy *et al.*,2003, Babalola and Apata, 2006).

The rind is edible and is sometimes used as vegetable. In China, they are stir fried, stewed or more often pickled and could be used for cooking with olive oil. The de-skinned and de-fruited rind is cooked with olive oil, garlic, chili pepper, scallion sugar and rum (Fagbemi, 2007). Pickled *Citrullus lanatus* rind is also commonly consumed in the southern United States. The inner rind is usually light green or white and can be used for making wine (Braide *et al*., 2012). The seeds are flat and

smooth,varying in size and may be white tan, brown, black red green or mottled. The seeds are consumed as snacks in China, Israel and elsewhere. The seeds are rarely



eaten in

Plate I: Water Plant Showing Fruit
Source: Field Survey 2015

Sudan, Nigeria and Egypt except that it is eaten by animals(Johnson *et al.*,2012). In Africa, seed may be ground into coarse flour or oil may be extracted from them (Sotolu and Faturoti, 2008).



Plate II: Matured Water Melon Fruit Showing Red Pulp
Source: Field Survey 2015



Plate III: Water Melon Seeds
Source: Field Survey 2015

2.2Chemical Composition of Watermelon Seeds

The protein in watermelon seeds consists of several amino acids, such as arginine, tryptophan, glutamic acid, lysine, methionine etc (Johnson *et al.*, 2012).Several minerals abound in watermelon such as Magnesium, sodium, potassium, zinc, calcium, iron and phosphorus (Egbuonu *et al*., 2014). Water melon seeds also contain B vitamins, particularly folate, thiamin, riboflavin, vitamin B6 and pantothenic acid (Johnson *et al.*, 2012).

2.3 Proximate Composition of Water melon Seed

The proximate composition of water melon seeds as obtained by Braide *et al.* (2015) shows that the seeds contain crude protein (30.3±1.7%), crude lipid (5.6±3.5%), ash (10.0±1.2%), moisture (4.80±0.4%), crude fibre (3.30±0.6), carbohydrate (24.40±6.30%), Gross energy of value of 546.7±27 kcal./100g. The amino acid composition of water melon was also reported as lysine 10.13, histidine 2.84, orginine 4.40, aspartic acid 8.30, threonine 2.89, serine 2.07, glutamic acid 14.85, proline 2.54, glycine 8.86, phenylalanine 4.90, cysteine 2.62, valine 3.01, methionine 1.97, isoleucine 5.16, leucine 5.64 and trypsine 1.10g/100g.While the lipid, ash, moisture and fibre were 7.17, 5.25, 5.91 and 5.51 respectively (g/100g) and it also contain minerals such as calcium 72, magnesium 38, phosphorus 190, sodium 72, iron 1.55, copper 0.20, zinc 0.90, potassium 278, sulphur 191, silicon 4, arsenic 0.37 and iodine 0.15 g/100g respectively.

2.4Major Anti-nutrients in Water melon Seed

The major anti-nutrients mostly found in water melon seed are saponins, cyanogenic glucosides, tannins, phytic acid,alkaloid and oxalates (Braide et al.,2012).

2.4.1 Tannins

According to Tsukahara *et al.* (2002) tannins are a diverse group of polyphenolic compounds, but in contrast to gossypol and hydrolysis, tannis yield sugar residues, phenolearboxylic acids, and condensed tannins (Akande *et al.,* 2010). Tannins are widely distributed in nature, food crops such as sorghum contains up to 5% condensed tannins, while water melon contain 0.64g/100g of tannins.Legumes such as faba bean, lima bean, sunflower seed meal containing 1.2-2.7% chlorogenic acid (Abu *et al*., 2010). It is also speculated that, the content of condensed tannins (24g/kg-1) was the cause of observed growth depression in Tilapia and Rohu (*Labeo rohita*) fingerlings when feeding diets with 250g/ kg-1 or 200g /kg-1 copra (Adegunwa, 2012).

2.4.2Phytic acid

Phytic acid or the hexa phosphate of miniositol occurs naturally throughout the plant kingdom and water contain 2.63 as reported by Samia El-Safy *et al*. (2012). Many of the major legumes and oil seeds including soybean, rape seed and cottonseed, 62-73% and 46-73% of the total phosphorus within cereal grains and legume seeds being in form of organically bound phytic phosphorus respectively (Akande *et al.,* 2010). For example, phytic acid is reportedly known to reduce protein and mineral bio-availability, reduced chetale metal iron, such as calcium, magnesium, zinc, copper, iron, etc to form insoluble complexes that are not readily absorbed from the gastro intestinal tract (Valverde, 2011). Phytic acid reduced growth, feed efficiency, protein efficiency ratio, protein digestibility, hypertrophy and vacuolization of intestinal epithelium in common carp (Agboola, 2004, Amisah *et al.,* 2009).

2.4.3 Alkaloids

Alkaloids represent a large and structurally diverse group of compounds present within angiosperm plants. The majority of alkaloids usually containing nitrogen in the

form of a heterocyclic ring (Abdulwahab *et al*. 2011). Johnson *et al*., (2012) reported that water melon contain 1.42g/100g alkaloids.Some of the more well- known alkaloids include coniine (hemlock), nicotine (tobacco) atrapine (deadly night shade) cocaine (leaves of coca plant) quinine (cinchona bark) strychnine (seeds of *Nux vomica*) morphine (dried latex of opium poppy) and salanine (unripe potatoes and potato sprouts) (Barros *et al.,* 2000). Fish fed 100mg/kg diet of pyrrolizidine alkaloids displaying markedly reduced growth and mortality from the fourth month onwards. Fish fed lower dietary concentrations (20mg/kg diet) displayed marked liver abnormalities, including liver *Megalocytosis necrosis*, fibrous tissue scarring and veno occlusion of hepatic veins (Collins *et al.,* 2007).

2.4.4Hydrogen Cyanide

Hydrogen cyanide is toxic to animals and human beings and is toxicity stems from its ability to link with the co-factors iron, manganese or copper of various metallo-proteins especially cytochrome oxidase of the mitochondria respiratory chain (EFSA, 2007) uptake of HCN in fish can be through ingestion, the skin or the gill respiratory epithelium, following which it rapidly distribute throughout the body through binding to melallo-proteins (Fagbenro *et a.l.,* 2013). The enzyme cytochrome oxidase is the main site of rapid lethal cyanide action and the brain, the major target organ of the HCN induced cytotoxic target organ of the HCN induced cytotoxic hypoxia (F.A.O. 2014). HCN forms a stable complex with and inhibits the enzyme cytochrome oxidase blocking the mitochondrial electron transfer chain. The result is ceassation of cellular respiration causing cytotoxic hypoxia and tissue anoxia and depression of the central nervous system by combination of cytotoxic hypoxia and lactic acidosis is the cause of death (Dimitroski *et al.,* 2010). Braide *et al.,* 2012 reported that water melon contain 0.57d/100g of cyanide. The only report on exposure of fish to cyanide through

the diet is a growth study conducted with Nile Tilapia fed soaked and raw sun-dried cassava meal with total cyanide levels of 9.9 and 71.1mg/kg respectively. The cyanide did not depress growth in the fish as the two diets showed similar growth at similar levels of inclusion. No relevant information is available on maximum inclusion levels or interactions with gut micro-flora indices (Edward *et al.* 2003, Lim and Lee, 2009).

2.4.5 Saponin

Water melon seeds contain 0.72g/100g saponins which have a soap- like foaming property when they are added to liquid.Because of this property, saponins disrupt epithelial function and create other digestive issues. Saponins have been connected to damaging red blood cells, inhibiting engymes and interfering with thyroid function.

2.4.6Oxalates

Water melon seeds contain 0.75 oxalates. Oxalates interfere with calcium absorption and crystallize into tissues of fish if consumed regularly.

2.4.7Fibre

Fibres are referred to as non-starch polysaccharides (NSP), are also defined as the polysaccharides not digested by endogenous enzymes in mono gastric animals. Dietary fibre is a mixture of substances derived from the structural materials of the plant cell well and range of non—structural polysaccharides either present naturally to foods or derived from food additives (Capman, 2000, Lakshni and Kaul, 2011). Effects of semi—purified and soluble NSPs, such as guar gum and alginates in fish diets, have been investigated and found to result in decreased dry matter of the feces in both fish (Barrows *et al.*, 2000). The increased intestinal viscosity in catfish leading to decreased digestibility of main nutrients when including guar gum at 40g /kg-1 and 80g/ kg-1 to the diets. Generally, no clear effect on intestinal viscosity by the presence

of soluble NSPs in the diet observed (Rindaldi *et al*., 2000, Amirokolale *et al.* 2005). High dietary fibres alter flow, impair interactions, affects intestinal receptors, restrict nutrient diffusion, change microbial diversity and activities and change absorptive surfaces in fish. Depending on the nutrient profile of the matter reaching the distal compartments, microbial species will be stimulated differently. Fibre may decrease the numbers and diversity of bacterial strain present in the intestine of fish (Tsukahara *et al.,* 2002).

2.5 Back ground information on Clariid Catfishes

The African catfish belong to the family Claridae. The large family Claridae hosts nine (9) genera, viz: Channallabes, Clariallabes, Dinotepterus, Dolichallabes, Gymnallabes, Heterobranchus, Horaglonis, Urgitglanis and Clarias. The genus Clarias consists of forty-five (45) species ranging from Africa to South Asia having about 2000 species of catfishes in the world (about 10% of the total number of fishes) (Jia *et al.,2003.* Recent revision of the systematic of African catfish have revealed that there are several species being synonymised under the name Clarias mosambicus of Central Africa, *Clarias lazera* of West and North Africa and Asia minor (Steven, 2009). *Clarias gariepinues* has been placed in the subgenus Clarias together with the West African species, *Clarias anguillaris*, *Clarias senegalensis* and others. *Clarias gamensis* another Southern African species is placed in the Subgenus Clarias (Dinotopteroides, while *Heterobranchus longifilis* has been placed in its own genus (Sogbeson *et al., 2009*). According to Jia *et al*. (2003) catfishes constitute a large group of primarily fresh water fishes which are widely distributed throughout the world. They are especially diverse in larger rivers, such as the Amaazon and Zaire in each of which several hundred species are found.

2.5.1 Natural feeding in clariid catfishes

Numerous studies on the feeding habit of catfishes confirmed that, they are generally classified as omnivores. It has been discovered that they feed mainly on aquatic insects, fish, crustaceans, higher plant debris, terrestrial insects, mollucs, diatoms, arachnids and rotifers, hence referred to as euryphagic feeder (Sotolu and Faturoti, 2009). However the greater importance of zooplankton in the diet of large fish is believed to be due to the increased gape and number of gill rakers of the large fish (Terry *et al*., 2000, Ugwunba *et al*., 2001).Its propensity toward a carnivorous feeding habit suggest that *Clarias gariepinus* has a relatively high dietary protein requirement, in the order of 40- 50 % cp on dry matter basis.The fact that the animal also feeds on plant material reflects its ability to digest plant proteins and utilize carbohydrates as energy source (Fasari, 2009).From a farming perspective, euryphagy holds the benefits that a wide variety of feed ingredients of animal and plant origin may be considered in formulating feeds that will satisfy the fish dietary requirements (Francis et *al*., 2001,Robinson *et al*., 2001).

2.5.2 Natural feeding in larval clariid catfishes

In Clariid catfish larva, the first feeding is defined as the transitional phase, where the larval shift from yolk sac endogenous feeding to exogenous source of nourishment (F.A.O., 2014). The first natural foods of the young larva fish start from 3-4 days after hatching. They usually feed on phytoplankton and zooplankton. Among the zooplankton, the rooted Rotifers (*Branchionus plicatilus*) and paramecium are usually eaten first. Cladecerans (*Moina nicrura*) Copepods (*Dirplanosoma excisum*) and Daphnia have also been detected in the gut samples of larval catfishes (Collins, 2007; Adewolu, 2008).

The composition of the natural food will vary considerably depending on the age and feeding conditions of the larva. *Artemia salina* has been favourably accepted by the larva of catfishes (FAO, 2004).

Clariid catfish larva and juveniles did remarkably well in plastic bowls where there was abundant phytoplankton bloom of pleurococcus species, Navicula species and with good aeration. This was further confirmed utilizing data from numerical and frequency of occurrence methods (Lim and Lee, 2009). In the studies using volumetric method, insects and insects remains were found to be most important components of the diets of catfish fingerlings (Fagbemi, 2007)

2.5.3 Artificial feeding in clariid catfishes

Complete feeds contain all the essential nutrients (protein, energy source, vitamins and minerals) against supplementary feeds .Care must be taken when giving artificial feeds not to overfeed, as this may be harmful to the newly formed digestive system and spoil the water, since most artificial feed is inert (Rakpong, 2001, Amisah, 2009). It has been reported that, artificial feed should contain some portion of fishmeal, fish oil, oil seeds or fish products. In addition to the known advantages of the amino acid composition, the feed should be able to attract the catfish larva and acceptably to them (Adewolu, 2008).

2.5.4Protein requirement of *Clarias gariepinus*fingerlings

Fingerlings of*Clarias gariepinus* have a high protein demand of around 45-50% and a high lipid requirement of 9%(Aduku and Bolorunduro, 2016). The carbohydrate content can be as high as 25-35% of the diet. A minimum level of 0.5 – 1 % n-fatty acids has been recommended. *Clarias gariepinus* fingerlings grow better if at least 10% of the total lipid consists of fish oil (Lim and Lee, 2009). It is recommended that

minimum level is incorporated into the fingerlings feeds (Mugendi *et al.*,2010). Fatty acids requirement of n-3 and n-6 ratio of 1:1 appears to be 0ptimal for growth and body conditions. Olurin (2006) found that intestinal alpha – amylace activity increased dietary carbohydrate levels. This shows that *Clarias gariepinus* fingerlings are capable of digesting carbohydrates from early stage and this goes through out the life of the fish span.Valverde *et al*. (2011) obtained the effect of water melon on the growth of *Clarias gariepinus* fingerlings as follows: mean final weight (98.00g), specific growth rate (1.68.00g), feed conversion ratio of (1.46g), protein efficiency ratio (1.48.00g) and nitrogen metabolism (1482).

2.5.5 Protein requirement of *Clarias gariepinu*s juveniles

The nutrient requirement of *Clarias* gariepinus during the juvenile growth stage is from 40- 43% CP, 10-12% dietary lipid and between 15- 32 % for carbohydrates. Optimum digestible energy is between 14-16 kj/g and the protein to energy ratio is optimal between 26- 29 mg/kj of digestible energy (Olive-Teles, 2001; Pham *et al*., 2007). Both plant and animal proteins are well digested and can be used at varying degrees to replace fish meal. Least costing of the diet and appetite feeding schedules have shown that profitability can be optimised at dietary levels of between 35- 38% (Olukunle and Agboola, 2005; Olatunde, 2005).

It has been shown that growth is negatively affected if fish oil is used as the sole source of lipid, which clearly suggests that the species has a certain requirement for n-6 fatty acids. However, the dietary lipid of water melon source does not affect whole body composition or muscle lipid level in *Clarias gariepinus* juveniles (Robinson *at al.*,2001).

It would appear that the average permissible carbohydrate level is around 27% and that cannot utilize dietary carbohydrate levels above 26 %. On the other hand,

Sogbeson and Ugwumla (2006) found that dietary carbohydrate levels of between 26 – 32 % had significant protein sparing effect, advocating the greater use of carbohydrate in the diet formulation of juveniles of *Clarias gariepinus.*

The precise vitamin and mineral requirement of *Clarias gariepinus* juveniles are poorly understood. Experiment results, however, suggest that the requirements by Channel catfish are more than adequate to cater for the needs of North Afriacan catfish (Sotolu and Adamson,2009). Under practical farming conditions, the fish obtain a substantial proportion of their micro-nutrient requirements from the environment.Farmers have found that adding a general vitamin and mineral premix such that it makes up 1% of the diet is more adequate to fulfil micro nutrient requirements (Barros *et al*., 2000).A more recent study by FAO (2014) has shown that mineral supplementation of feed containing 27% fish meal had no beneficial effect on growth of*Clarias gariepinus* juveniles.They suggest that, it is not necessary to include a mineral mix into diets that contain a high proportion of fish meal.

Tlili *and Lenucci* (2011) reported values of the effect of water melon on the growth performance of *Clarias gariepinus*juveniles as follows: mean final weight (235.00g), specific growth rate (2.10g), feed conversion ratio (1.24g), protein efficiency ratio (1.61g) and nitrogen metabolism (2120).

2.5.6 Protein requirementof *Clarias gariepinus* adults

The protein requirement in catfish has been defined as the content in food which gives maximum growth, maximum economic profit and maximum protein deposition(Sogbeson and Ugwumla, 2006). The amount of protein required by catfish could be determined by giving diet containing graded levels of protein over some period. Ayinla and Nwadukwu (2003) reported the essential amino acids requirements of *Clarias gariepinus* juveniles as thus: arginine (5.8), histidine (3.9) isoleucine (5.3),

leucine (12.3), lysine (8.6), methionine and cysteine (-), phenylalanine and tyrosine (3.8), threonine (4.9), tryptophan (-), valine (3.5).

Sotolu (2008) reported the essential amino acids requirements of channel cat fish and *Oreochromis niloticus* as thus: arginine (4.3) (1.0), histidine (1.5) (0.4), isoleucine (2.6) (0.6), (leucine (3.5) (0.8), lysine (5.1) (1.2), methionine and cysteine (2.3) (0.6), phenylalanine and tyrosine (5.0) (0.6), threonine (4.9) (0.5), tryptophan (0.5) (10.12), valine (3.0) (0.7).

The energy food, vitamins and minerals are made constant throughout the feeding period(Fagbemi, 2007). The fish growth rate is maintained in different dietary protein content. Generally, the catfish growth rate increases with increasing dietary protein content until growth rate cease, indicating that the limit of response has occurred (Bolorunduro, 2002)

2.6Apparent Digestibility of *Clarias gariepinus*

Digestion can be defined as the breakdown of food by catalytic enzymes. Digestion of food in fish depends on the kind of food, the extent to which it is susceptible to the effects of the digestive enzymes, the activity of the digestive enzymes and the length of time the food is exposed to the action of the digestive enzymes (Hertrampt and Pascal, 2003). Each of these factors is affected by many secondary factors, some of which are associated with the fish itself, such as species, age, size, physiological condition and environmental condition, such as water temperature, composition of diet, particle size and quantity of diet consumed (Abu *et al.*, 2010).

Nutrient digestibility may vary among fish species due to differences in the digestive system and enzymes and foods consumed. Despite these differences and the lack of pepsin in fish without stomach, variation in the digestibility of proteins and lipids among species are very small. Much more pronounced are variations in digestibility

of carbohydrates, especially starch (Adewolu, 2008). According to Pham *et al.* (2007) carnivorous fish digest starch to a much lesser degree than omnivorous and herbivorous fish. Differences in digestibility of carbohydrates can also occur among different families of carnivorous fish within the same species (Perceival *et al.*, 2001). Differences in digestibility of carbohydrates can also occur among different families of carnivorous fish and even within the same species (Pham *et al.*, 2007). A study on the effect of digestive capabilities of African catfish by FAO (2014) obtained the following parameters: protein (86.60%), lipid (79.90%, dry matter (83.20%) and carbohydrate (87.0%) from poultry offal meal.Sotolu and Adamson (2010) obtained values of digestibility of *Clarias gariepinus* from plant protein fed with Leucaena leucocephala meal as follows: protein (66.48%), lipid (5.58%), ash (10.80%),nitrogen free extract (3.94).

2.7Haematological Parameters of *Clarias gariepinus*

Fish cultures are at risk due to the adverse effects of stress. Blood chemistry and haematological measurements can provide valuable physiological indices that may offer critical feedback on different stressors (Binukumari *et al.*, 2011). Haematological characteristics of most fish have been studied with the aim of establishing normal value range and any deviation from it may indicate a disturbance in the physiological process (Rainzapaiva *et al.*, 2000). Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. The red blood cells count, haematocrit and haemoglobin concentration vary with diet and strain as well as temperature, season of the year and nutritional status of the fish. Haematological studies have been widely used as means of assessing the state of health of fishes and the establishment of the haematological characteristics of fishes generally serves as a standard for

physiological, pathological or toxicological studies (Gabriel *et al*., 2001). Haematological parameters of different species of fish and screening test provide a bank of useful information from which valuable and informative conclusions could be drawn. Regular monitoring of haematological parameters of farmed fish can be used to prevent damage to farming activities (Fagbenro *et al.,* 2013).

TliliandLenucci(2011) reported the effect of water melon on the haematological parameters of *Clarias gariepinus* fingerlings, juveniles and adults as follows: packed cell volume (31.33%), (35.20%), (36.57%) white blood cell (12.18%), (34.10%) (35.12%) haemoglobin (16.82%), (20.34%), (22.41%) red blood cell (10.08%), (11.89%) (12.08%) mean cell haemoglobin (24.48%), (35.98%) (37.36%) (mean cell corpuscular haemoglobin (44.84%), (46.34%) (70.67%) (mean cell volume (70.14%), (72.03%) (74.61%) and lymphocytes (30.35%). (32.41%) (49.10%) (69.04%). Ochang *et al.* (2007) reported MCV, MCH and MCHC values of 132.8-308.40, 20.90-47.20pg and 10.90-38.10% respectively in their studies in relation to stocking and feeding conditions. The haematological and biochemical indices of farm fish as with other farm animals namely – haemoglobin, red blood cells, white blood cells, packed cell volume, plasma protein, blood glucose, specific gravity of blood plasma and whole blood, coagulation time etc. have been analyzed and variously reported as useful tools in assessing the performance, viability and health status of fish and animals (Ezeric, 2001). According to Osuigwe *et al.* (2007) the PCV, RBC count, WBC count, and HB concentration decreased significantly with increasing dietary jack bean seed meal, such that fish fed the control diet had the highest values that were significantly different from the value obtained from *Clarias gariepinus* fed other diets, and that jack bean seed has been (JBSM) shown to contain anti-nutritional factors known to cause some negative effects on some haematological parameters,

and concluded that, the increasing presence of anti-nutritional factors in increasing dietary JBSM caused the inferior haematological parameters observed in *Clarias gariepinus* fed such diets. It is also important to note that irrespective of the reduction in the levels of haematological values observed in *Clarias gariepinus*, they were still within the normal range reported by Ezeric, *(*2001), Gabriel *et al*.(2007).

Osuigwe *et al.* (2007) also reported that the erythrocytes count range of 2.17 – 2.6 x 106mm3 in *Clarias gariepinus* were within the range described by Gabriel *et al.* (2007) reported mean values of packed cell volume (PCV) in *Clarias gariepinue*s obtained from their study were within the range of the corresponding value described by Gabriel *et al.(*2007 reported a mean corpuscular volume (MCV, 73.11-108.71) and mean corpuscular volume haemoglobin (MCH, 24.40-36.20pg) obtained by Azeric(2001).

Amoo (2004) reported that, sex of the fish may also influence the blood parameters, males consistently had higher packed cell volume values than the females and this has been proposed as means of sexing fish. Blood cell responses are important indicators of changes in the internal and/or external environment of animals. Adegunwa (2004) stressed that, a number of haematological indices such as haematocrit (Ht), Haemoglobin (Hb), red blood cells (RBC) are used to assess the functional states of the oxygen carrying capacity of blood stream and have been used as indicator of the presence of metallic pollutants in aquatic environment. Adeyemo *et al.* (2003) reported that red blood cell (RBC) concentration of haemoglobin (MCH) and haematocrit (Ht) have been used to indicate secondary responses of an organism to irritants.Decrease in erythrocytes has been reported to be the major reliable indicators of various sources of stress in fish, and decrease in these cells indicates vulnerability to stress and infection (Rain-Paiva *et al.*,2000).

Drop in the red blood cells implies reduction in the level of oxygen that is being carried to the tissue and the level of carbon dioxide returned to the lungs will also be reduced. Reduction in mean haemoglobin concentration and mean corpuscular haemoglobin concentration indicates anaemia (Fagbenro *et al.*, 2013).

According to Ezeric (2001) PCV, RBC and mean cell concentration (MCHC) are used for diagnosing anaemic condition, while neutrophils and monocytes (differential counts) protects the body against bacterial invasion. Lymphocytes involved in immune process, producing antibodies against foreign organism protecting against microbes, low RBC indicates malnutrition and platelets helps in diagnosing problems associated with bruising or haemorrhage.

2.8Carcass Composition of *Clarias gariepinus*

Knowledge of the carcass composition of 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, proper nutrition is one of the essential factors that influence the ability of the fish to attain genetic potential, growth, reproduction and longevity (Abdullahi *et al*., 2001) reported studies on the carcass composition of *Clarias gariepinus* having 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, orginine 4.40, aspartic acid 8.30, threonine 2.89, serine 2.07, glutamic acid 14.85, proline 2.54, glycine 8.86, phenylalanine 4.90, cysteine 2.62, valine 3.01, methionine 1.97, isoleucine 5.16, leucine 5.64 and trypsine 1.10.While the lipid, ash, moisture and fibre were 7.17, 5.25, 5.91 and 5.51 respectively (g/100g) and it also contain minerals such as calcium 72, magnesium 38, phosphorus 190, sodium 72,

iron 1.55, copper 0.20, zinc 0.90, potassium 278, sulphur 191, silicon 4, arsenic 0.37 and iodine 0.15 g/100g respectively.

2.9 Water Quality Requirement in Catfish Production

Suitable water quality is important to the survival and health of fish at all stages of growth. Water quality requirement varies between species and between the different life stages as the fish develop. It is pertinent to note that interactions of water-quality characteristics and variation in any of the parameters can affect the other (Rural Fresh Water Manual, 2010).

Information on the physical and chemical properties of water in which aquaculture is to be practiced is of prime importance in deciding the suitability of the water body for successful fish culture (Barak *et al.*,2014). These characteristics would differ widely depending on the type of water body, such as pond, concrete tank, plastic tank, wooden tank, re-circulatory system, cages, lake, chosen for fish culture (Boyd, 2000). It becomes obvious that when information on environmental characteristics of the body of the water bodies are available, one could make proper choice only with the information on the tolerance and optimal ranges of these parameters for the species to be cultured (Onu and Okongwu, 2006).For each culture system chosen, fish have a range or zone within which it can live indefinitely, referred to as the tolerance zone (Eyo, 2003, Yovita, 2007). The area outside this zone is the lethal or resistance zone in which the fish will die in a fixed period of time (survival or resistance time). The tolerance zone is further restricted by ranges of the factors for optimal biological activities (OBA), growth and reproduction being the most important, but perhaps, more important are the range for behavioural activities (BA), such as feeding and swimming (Pandey and Shakia, 2007).

Inter-relationship between water quality and feeding in intensive aquaculture is complex, that is why no matter how clean the water used for fish culture is, with time it will become polluted because fish are completely dependent upon the water to breathe, feed, excrete wastes, maintain salt balance, growth and reproduction (Abu *et a l.,* 2010). Water temperature and dissolved oxygen influenced feeding activity, metabolism and growth, and hence are of fundamental importance in the determination of both the types and quantities of feeds used (Gabriel *et al*., 2007). Therefore not exceeding the carrying capacity of fish in ponds and tanks, this is because feeding fish in intensive culture system in turn leads to the release of potentially harmful organic and inorganic wastes into the water (Barak *et al.*2015). Nitrogenous wastes primarily dissolved ammonia or feces comprising indigestible materials are produced more or less continuously in actively feeding fish, this creates stressful condition and increases the risk of disease epidemic (Barak *et al*., 2014). Hassan (2001) reported that ammonia in its unionized form is highly toxic, which feces released into the water rapidly break up contributing to both the suspended and settleable solids loading of the water system. Regular water change is therefore important in keeping fish healthy and in stimulating fish to breed, water should however, never be change all at once, as this will be lethal to the synergistic bacteria which sustain the culture system and also put fish at risk of shock due to changes in water chemistry (Adewolu, 2008; DeTolla *et al.,* 2008). It is always needful to change 40-50% of the water in the culture receptacle bi-weekly, this is because fresh water replenishes trace minerals that are used up in the tanks by fish, flora and fauna (Mugendi *et al*., 2010).

The effects of water quality on feeding fish in intensive system has received considerable attention in recent years particularly in Northern America, United States

of America, China, Israel, Philippine, where intensive sectors have developed within rigidly enforced regulatory frame works for the control of water pollution (Terlizzi *et al.*, 2004; DeTolla *et al.*, 2008).

2.9.1 Water temperature

Temperature is often referred to as the master factor among environmental factors affecting fish. All fishes have temperature range, referred to as the "biokinetic range", within which they live all their life (PETCO, 2004). This range is limited by the upper and lower tolerance limit, and beyond these critical temperatures, the fish may live for a brief period (resistance time), but would eventually die (Lethal zone) (Pandey and Shakia, 2007).

Both the tolerance range and resistance time in lethal temperature are subject to the acclimation temperature, that is, the temperature to which the fish have been previously exposed (Belleingham, 2005).Therefore, it is a matter of great concern for aquaculturist not only to know the seasonal and daily changes in temperatures of the water body selected for aquaculture, but also the thermal tolerance range and thermal history of the fish to be introduced in the water body (Jia *et al.*, 2003; Sticky *et al.*, 2006).

The thermal tolerance of fin fishes and shell fishes vary greatly, those with a wide range of tolerance being referred to as "eurythermal" and those with narrow range as "stenothermal". Temperature range of water body obviously must depend on the latitude or geographical location and also the altitude at which it is located, both these aspects have to be considered while transporting and stocking fishes (Pandey and Shakia, 2007). Apparently, in keeping or handling fish, temperature is probably the most important water quality variable, largely due to the fact that, fish are not able to

regulate their own body temperature and therefore have a body temperature similar to that of the water around them (Barak *et al.*,2015).

All metabolic, physiological and life processes such as feeding, reproduction, growth, locomotion and distribution of fishes are greatly affected by temperature (Jia *et al*., 2003). These metabolic activities are slowest at colder temperatures and increases as eternal temperature rises.

All fish have a minimum and maximum lethal temperature limit, metabolic rates in fish double for each 18^{o}F rise in temperature (Olagunju *et al*., 2007; Olaleye, 2005). Sudden changes in temperature should be avoided as this would lead to high fish mortality, even though tropical fish can strive under stenothermal temperature range, temperatures slightly above or below this range will stress fish over time and could lead to disease or death (Rural Fresh Water Manual, 2010).

It is safer not to transport fish into water more that 2^{o}C different from that which they came from, if fish are moved between ponds they should never be moved without checking that the two ponds have the same temperature (Belleingham, 2005). If they are difference, it is important that they are acclimatized by allowing the container holding the fish to float in the new pond for at least 10 minutes in order to harmonize the temperature before allowing fish to swim on their own into the water (Omitoyin, 2007; Olukunle, 2009). It is safer to transport fish to slightly cooler water rather than warmer water, as it contain more oxygen which will assist the fish in overcoming the stress of handling as they gradually tolerate change in temperature (PETCO, 2004). Preferred temperature range for optimum catfish growth is 85^{o}F (29.4$^{o}_{C}$). Catfish, Tilapia and crap thrive in warm ponds of 33^{o}C. Tilapia are more adversely affected by too low temperatures and usually high mortality is recorded when the water

temperature goes below 12-13$^{o}_{C}$ for lengthy periods, catfish and carp are tolerant of wide range of temperature variations (Robinson, 2001).

2.9.2 Dissolved oxygen

Oxygen is essential for the production of energy, metabolic function, digestion, assimilation of food, reproduction and growth (Sogbesan and Ugwumba, 2006). Requirements for oxygen vary according to fish species, age, stage of development and size (Boyd, 2000) emphasized that, for any cultured species, knowledge of dissolved oxygen level and potential fluctuations at the farm site are important in optimizing feeding regime. Jia *et al.* (2003) stated that, since oxygen requirements are high and oxygen has a low solubility in water, occasion may arise where oxygen levels falls close to or below minimum level under low dissolved oxygen conditions fish show reduced feeding responses and abnormal swimming patterns. Also as the temperature of water rises, particularly in the tropics, its handling capacity for oxygen decreases, this may increase the metabolic rate of the fish (Amisah,2009).

Fish may be seen gathering at the water in let or gasping at the surface (Belleingham, 2005; Butler, 2006). Gaseous changes occur at the gills and any disease which affects the gills may interfere with the respiration of the fish (Boyd, 2000). The gills often respond to injury by increasing the mucus layer or by increasing the size (hypertrophy) or number of cells (hyperplasia) of the gills epithelium; (Barros and Hardy, 2000). This is an attempt to protective response, but in so doing, the distance for diffusion of gases between water and blood in the fish is effectively increased, thus increasing the efficiency of the gaseous exchange, in severe cases the fish may die from lack of oxygen even though the water contains adequate levels of oxygen (Capman, 2000).

It has been noted that dissolved salt content of the water affects its oxygen, hence there will be an increasing solubility of oxygen with increasing salinity (Clean Water Team, 2004). As a general guide, 5-6ppm (mg/L) of oxygen is considered to be an absolute minimum requirement for fish. Acute oxygen shortage can lead to a stressful and could predispose the fish to disease or otherwise affect their response to a variety of beneficial agents (Omitoyin, 2007; Sotolu and Adamson, 2008).

2.9.3 Ammonia

Ammonia is a very common pollutant, which is added to the surface water from aquaculture run-off. In addition, it is found naturally in ground and surface waters due to microbial breakdown of nitrogenous material. It is 40-90% of the nitrogenous waste form the metabolism of proteins and it is also excreted by fish from the gills in the urine Ammonia is present in water in its free (NH_3) and ionized (NH^+_4) forms, the relative proportions of each being dependent on the pH (acidity) and temperature of the water. In alkaline water, more ammonia is present in the free form (NH_3) and this is highly toxic. Under normal circumstances, however, where there is a good flow of water, the ammonia is quickly diluted and removed, causing a problem (Fish Doc., 2004).

Toxicity results therefore, under conditions of overcrowding or where alkaline water is being recycled without proper filtration so that a build-up of free ammonia (NH_3) occurs (Stevens, 2009). As ammonia in water increases, excretory processes are impaired and ammonia build-up in the fish and circulatory system is also affected (Barak *et al.*,2014). There is resultant elevation of blood pit and adverse effects on enzyme catalyzed reactions and the stability of cell membranes. High ammonia levels may increase oxygen consumption, cause gill damage and reduces the ability of the blood to transport oxygen, Mesner andGeiger(2005) reported that, long extreme

exposure of fish to unionized ammonia can result in loss of appetite, affect binding of haemoglobin with oxygen, thus causes an oxygen lack in the tissue, poor growth and in extreme cases death may occur. In general, young fish are more susceptible than old. For some species, levels of unionized ammonia (NH_3) of 0.02 – 0.05ppm are probably the maximum which can be tolerated for long periods of time without adverse effects (Capman, 2000, Butler, 2006).

2.9.4 Water pH

pH is a measure of the number of hydrogen ions and therefore a measure of acidity. The water is considered neutral, if the pH value is seven (7). Higher than seven (7) means that the water is alkaline, pH lower than seven (7) means that the water is acidic (Menser and Geiger, 2005). Most aquatic plants and animals have adopted to live in water with a measure of specific pH and may suffer from even a slight change. There is no normal pH that applies to all fish species because fish come from sources with different pH levels, however, almost all fresh water fish can inhabit waters with a pH form 6.5-9.0 (Butler, 2006). In such water productivity is generally highest. Deviation from the optimum range impedes the recirculation of nutrients by reducing the rate of decomposition of organic matter inhibit nitrogen fixation (Barak *et al.*,2014). A pH below the value of 4 or above 10 will be lethal to most fish. When moving fish from one farm to another, the pH of the water in both farms is recorded and fish are only moved when the pH of the receiving farm is very near or below, that of the transported fish farm (PETCO, 2004). Fish are more stressed and susceptible to disease when exposed to a change in pH greater than 0.3 for 24 hours period, they are prone to attack by parasites and diseases (Capman, 2000). Therefore, a low pH has a negative effect on the organism farming food of the fish and ultimately on the growth of the fish and high pH can hinder the growth of plankton (Jia *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Experimental Site

The study was carried out at the Nigerian Institute for Agriculture Extension Research Liaison Services (NAERLS), Skill Acquisition Centre, Ahmadu Bello University, Zaria, located within latitude 11^{o}9'48''N and longitude 7^{o}E at an altitude of 630m above sea level in the Northern guinea savannah.

3.2 Sources and Processing of Ingredients

Water melon seeds were purchased at Bara market in Bauchi State, Nigeria. Theseeds were certified at the Institute of Agriculture Research, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

The first 100g of dry raw water melon seeds were sundried for 3 days, ground using Q-link blender (model: QBL-18L40).Another 100g of the water melon seeds were soaked in a 1:3 (seed: water) i.e.100g/3 liter ratio for 10, 20, 30 and 40 hours; thereafter, the water was drained completely, seeds were evenly spread in thin layer on a slab to air and sun dried for 5 days.

The third 100g water melon seeds were boiled for 10, 20, 30 and 40 minutes at 100^{o}C and evenly spread on a slab to air and sun dried for 5 days and seeds were ground using a blender. The fourth 100g water melon seeds were cleaned and toasted for 10, 20, 30 and 40 minutes in an aluminum pan as described by Mugendi *et al.* (2010) and cooled under shade, seeds ground using the blender and coat removed through 0.2mm mesh size sieve.

The fifth100g of the watermelon seeds were toasted in an aluminum pan for 10, 20, 30 and 40 minutes at 100^{o}C and cooled under shade, ground into powder and seed coat removed using 0.2mm mesh sized sieve, oil extracted using manual method

according to the method of Ayinla (2003), thereafter the water melon cake produced after frying and ground using blender as described by Mugendi *et al*., (2010).

The feed was formulated according to Pearson's square method as described by Adewolu (2008). The ingredient was mixed thoroughly to form slurry before being compressed to produce pellets using a locally fabricated industrial pelletizing machine (Gabriel *et al*., 2007).The pelletized feeds were sundried to 10% moisture content, packed in polythene bags and kept safely. The five feed were used for preliminary studies and the 25% inclusion level of water melon feed gave the best growth performance and nutrient utilization and therefore was selected for this study.

3.3 Experimental Procedures

The design of the experiment was a completely randomize design with five treatments and three replications. The treatment groups were fed diets containing graded levels (0%, 25%, 50%, 75% and 100%) of water melon seed cake. The proximate composition of the water melon seed cake is shown in table 4.1.Commercial feed (Coppens) was used as a reference diet.

The fish were cultured in 18 cylindrical black plastic tanks of 20 x 15 x 1.2m dimension with a water holding capacity of three hundred and sixty liters (360L). Each tank was washed thoroughly with detergents, filled to 2/3 (240 liters) capacity and stocked with twenty fingerlings of *Clarias gariepinus* of average weight of 10g and covered with ½ inch mesh sized polyethelene netting material to prevent fish from leaping out and attack on the fish by predatory animals. Aeration was provided in the water tanks through air stones using aquarium water pumps.

3.4Experimental Fish

Four hundred (400) fingerlings of *Clarias gariepinus* were purchased from Bagauda farms, Kano State, Nigeria. The fish was harvested and kept overnight in jerry can for

transportation very early in the morning to the experimental site. Upon arrival at NAERLS Skill Acquisition Centre ABU Zaria, the fish were acclimatized in a 4mx5mx1.3m dimension for 2 weeks for the fingerlings to attained mean weight of 10g and on day one, the fish were given multi- vitamin in order to boost their appetite and to reduce stress. During this time, the fish were physically examined to see if they were in good condition and also to observe any mortality, incidence of shooters or jumpers and to select uniform size to be cultured in the trial tanks. The processed five watermelon seed diets were formulated according to Pearson's square method (Adewolu, 2008) to partially or completely replace soybean meal at 25%, 50%, 75% and 100% and 0% as the control. Each was calculated according to Pearson's square method at 45% CP, 42 % CP and 40% CP to feed the fish at fingerlings, juvenile and adult growth stages. Feed meal diet with 45% 42% and 40% soybean meal served as the control.

3.5 Feeding of Experimental Fish

Five experimental diets produced at different sizes of 2mm, 3mm, 4mm, 5mm and 6mm for $T_{1,}$ T_2, T_3, T_4 and $T_{5,}$ were used to feed the fish at 5% body weight for 6 months. Sampling of the fish was carried out in every 14 days using scoop nets to determine growth rate and feed weighed and quantity were adjusted according to changes in the weight gain of the fish. Feeding of the fish was carried out thrice a day, in the morning at 8:00- 9:00, 12:00-1:00pm and at 4:00-5:00 pm in all the eighteen experimental tanks as described by (Kataki *et al*., 2006).

3.6 Physico-Chemical Parameters of Experimental Water

Temperature was determined bi-weekly using combined pH and temperature pen meter model pH-009 (iii). The $_p$H and oxygen were measured using pH, conductivity and oxygen metre (model EC- 900). Ammonia was determined using the method

described by Steven (2009). Water in the culture tanks were drained and replaced bi-weekly to enhance better growth conditions (Hossain *et al*., 2003).

3.7Sampling of Fish

Fish in the various treatment groups were weighed forth nightly using digital weighing scale (model: EL-5KO2). Before the sampling days the fish were starved for 24 hours before the commencement of the exercise.

3.8 Bio-Chemical Analysis of Experimental Fish and Feed

The bio-chemical cSomposition of the experimental fish and feed were determined using methods of the Association of Official Analytical Chemists (2000), in the research laboratory of National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State. The analyzed bio-chemical components were the dry matter, crude protein, crude fibre, crude fibre, lipid, ash, moisture and nitrogen free extract. These were determined in triplicates.

3.9 Growth and Nutrient Utilization Parameters Analysis

The following growth and nutrient utilization parameter were calculated as described by Stickey (2006).

3.9.1 Mean weight gain (MWG) = $W_2 - W$, (g)

Where W_2 = Final mean weight (g)
W_1 = Initial mean weight (g)

3.9.2 Percentage Weight Gain (PWG (%)= $\frac{\text{MWG(g)X100}}{\text{IWG (g)}}$

3.9.3 Specific growth rate

Specific growth rate was calculated according to the formula :

$$\text{SGR} = \frac{\log W_2 - \log W_1 \times 100}{\text{Time}}$$

Where W_2 = Weight of fish at W_2 in days

W_1 = Weight of fish at time W_1 in days

Lo_e = Natural Log to base e

3.9.4 Food conversion ratio (FCR)

The Food Conversion Ratio (FCR) is expressed as the proportion of the food fed per unit live weight gain of fish.

$$FCR = \frac{\text{Total feed fed (g)}}{\text{Total wet weight Gain}}$$

3.9.5 Protein intake

The protein intake was calculated according to the formula:

P1 = Feed Intake (g) x % protein in the diet

3.9.6 Protein efficiency ratio (PER)

Protein efficiency ratio (PER) was calculated using the formula:

$$PER = \frac{\text{Wet weight gain (g)}}{\text{Protein Consumed (g)}}$$

The PER: Expresses the measure of dietary protein utilization by the fish.

3.9.7Nitrogen metabolism (NM)

This was calculated according to the formula:

$$NM \quad \frac{(0.549)\,(b-a)\,h}{2}$$

Where: a = Initial Weight of Fish

b = Final Weight of Fish

0.549 = Experimental Constant

h = Experimental Periods in Days

3.9.8Condition Factor (K) was calculated according to the formula:

$$K = \frac{100}{L^3}$$

Where: W = Final Mean Body Weight (g)

L = Mean Standard Length (CM)

3.9.9The carcass proximate composition

The carcass proximate composition of the fish samples were analyzed at NAPRI, ABU, Shika, Zaria, Kaduna State before and after the experiment by the methods of AOAC (2000) and gross energy were calculated by the methods described by Adewolu (2008).

3.10 Determination of Anti- Nutritional Factors

3.10.1 Determination of tannin

Tannin was determined using methods described by AOAC (2000). About 2g of ground water melon seeds was defatted for 2 hours using Soxhlet extraction apparatus the residue was dried in oven for 12 hours at 100^{0}_{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 litter porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until blue solution change 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.

3.10.2 Determination of phytic acid

Phytic acid (inosotol hexaphosphate) content of each sample was determined by the modified method of AOAC (2000). About 10g aliquot of the powdered sample was extracted overnight at room temperature with vigorous shaking to attain 200 rpm in 1 hour to ensure homogeneity and 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 ash less filter paper. The resultant residue was dissolved in about 10ml of 2% sodium hydroxide. This hydroxide 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 second and phosphate determined colorimetrically at 710nm.

3.10.3 Determination of total oxalate

The total acid of the powdered samples was determined by the AOAC (2006). About 2g aliquot of the plant materials was weighed 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 then heated to 100^0C, allowed to cool and filtered to remove precipitate containing ferric ions. The filtrate was boiled, 10ml of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through hatman 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^oC 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 used, the oxalate content of the unknown sample was calculated using equation.

1ml of KMnO4 = 2.2mg.

3.10.4 Alkaloid determination

The gravimetric method of Terry (2000) was adopted.

Procedure

5.0g of each sample was weight out and disperse into 50ml of 10% acetic acid solution in ethanol. This was shaken well and allowed to stand for 4hrs before filtering.

Evaporate the filtrate to one quarter (1/4) of its original volume.

Add drop wise conc. NH_4OH to precipitate the alkaloids.

Filter off the precipitate with a weighed filter paper and wash with 1% NH_4OH solution.

The filtering should be done with a weighed filter paper.

Dry the precipitate in filter paper in the oven at $60^{O}C$ for 30 min and reweigh.

By weight difference, the weight of alkaloid is determined and expressed as a percentage of the sample weight analyzed. Given by the formula:

Toxicants

$$\% \text{ Alkaloids } W = W_2 - W_1 x\ 100$$

Where:

W = Weight of sample

W_1 = Weight of empty filter paper

W_2 = Weight of paper plus precipitate

3.10.5Saponin was measured according to the methods of (AOAC, 2000)

A gravimetric method employing the use of soxhlet extractor and two different organic solvents was used to determine the saponin. The first solvent extracts lipids and interfering pigments, while the second solvent extracts the saponnins properly. 5g of the dried ground sample was weighed into a thimble and transferred into the

soxhlet extraction chamber fitted with a condenser and a round bottomed flask. The sample was exhaustively extracted of its lipids and interfering pigments for 3 hours by heating the flask. The quantity of the acetone, enough to cause a reflux were poured into the flask. The sample was exhaustively extracted of its lipids and interfering pigments for 3 hours by heating the flask on a hot plate and the solvent distilled off. For the second extraction, a pre-weighed round flask was fitted into the soxhlet apparatus which bore the thimber containing the sample. The menthol was poured into the flask which would be enough to cause reflux. The saponin was then exhaustively extracted for 3 hours by heating the flask on a hot plate after which the solvent was distilled off. The flash was reweighed. The difference between the final and initial weights of the flask represents the weight of the saponins extracted.

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

3.10.6 Determination of flavonoids

Flavonoids was determined using methods described by AOAC (2000). About 2g of ground water melon seeds was defatted for 2 hours using Soxhlet extraction apparatus the residue was dried in oven for 12 hours at 100°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 litter porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until blue solution change 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 flavonoids in the sample.

3.11 Determination of Amino Acids

(a) Determination of amino acid profile

The Amino Acids profile in the fish and feeds were determined using methods described by AOAC (2006). 1cm^3 of the samples and standard mixture were loaded with equal volume of internal standard (Norleucine) using a micropipette. The cartridges were then placed on a sample tray of the TSM sampler. Two chromatographic columns were packed with 75% resin slurry and mounted directly below TSM sampler. The front column was used for separation of the acidic and neutral amino acids. Buffer solutions were connected to the system via a peristatic valve.

(b) Preparation of buffers

(i) Buffer No 1 (PH3.25, 0.25N Na^+/0.1m citrate/6% methyl cellosolve). 400cm^3 of stock solution was measured and poured into a 4 litres beaker equipped with stirrer. About 3 litre of distilled water was added and agitated. 240cm^3 methyl cellosolve and 1.0cm^3 thiodiglycol were added after which 6N hydrochloric acid was used to titrate the solution to pH 3.25. It was then transferred to a volumetric flask and made up to a total volume of 4 litres.

(b) Buffer No 3 (PH5.25, 0.37N Na^+/0.1m citrate). This buffer was also prepared in the same way as the second buffer except that 40.0g sodium chloride was added. It was titrated to pH4.25 then the analyser was started, the buffers were delivered to the columns through the sampler from two high pressure pumps and selected by the buffer-changing valve (the valve sequences, the flow of the effluent from either column into analytical system or to the waste). The buffers washed the column, removing some of the amino acids were eluted from the column, they were collected individually partitioned by the bubble system into the analytical system. Here they

were mixed with hydrin solution supplied through the proportioning pump. The reaction mixture now formed, passed through a capillary tube immersed in a 100^{o}C water bath where amino acids reacted with ninhydrin to produce a coloured product.

The developed effluent (reaction steam) was led through the colorimeter where the absorbance was monitored continuously as it passed over a sensitive photo-electric unit. The signals from the colorimeter was finally fed into an automatic pen-recorder which was carefully preset. The type of amino acid was established on the basis of its position on the chromatogram and it was quantitatively estimated on the basis of the area under curve.

(c) Method of calculating amino acid value from the chromatogram peaks

The net height of individual peak was readily measured. T he half- height of the peak on the chart was locatewd and width of the peak at the half-height was accurately measured and recorded. The approximation of the area of each peak was obtained by multiplying the net height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula: NE= $\frac{\text{Area of norleucine}}{\text{Area of amino acid}}$

From the norleucine equivalent above, a constant S was calculated for each amino acid in the standard.

S standard = NE standard x mol. Weight x NM AA standard.

3.12Determination of Minerals

Calcium, zinc, copper, phosphorus, iron, manganese, magnesium and sodium were determined according to the procedure of (AOAC, 2006). Approximately 10mg of phosphorus sample was extracted overnight in 25ml of concentrated nitric acid. This was followed by digestion in concentrated nitric acid and then perchloric acid on a hot

plate. Distilled water (20ml) was added to the sample and boiled until white fumes appeared. This was followed by addition of 5ml of solution and further boiled until crystals were formed. The crystals were then dissolved using 20ml of acidified water and 80ml of distilled water followed by 20ml of a mixed reagent for colour development. The mixed reagent consisted of 250ml sulphiric acid antimony, 250ml sodium molybdate, 500ml distilled water and 2g/l ascorbic acid. The sample was left for 15 minutes for colour to develop. The quantity of phosphorus was then determined using a spectrophotometer (Cecil Elegant Technology, UK). Other minerals (potassium, calcium, sodium, zinc, magnesium, manganese, copper and iron) were quantified using a Thermo X series 2 inductively coupled plasma mass spectrophotometer (ICP MS) (Thermo Scientific, USA). Samples weighing approximately 80mg were digested in nitric acid for 1 hour in a Mars X press microwave (CEM Corporation, USA) and then diluted to 10ml with distilled water ready for quantification.

3.13 Feed Formulation and Pellet Preparation

The experimental diets contained fish meal (FM), soybean meal (SM), yellow maize meal (YMM), ground nut cake meal (GNCM), watermelon seeds meal (WMSM) and additives. All ingredients were ground into a fine powder using a hammer mill and sieved by a 0.25mm sieve. Fish meal, soybean meal, ground nut cake meal.Yellow maize meal and additives were obtained from commercial suppliers.Prior to feed formulation,proximate, composition of these ingredients were determined. Five isonitrogenous diets and isocaloric (gross energy 3387 kcal kg) were prepared where the water melon seed meal replaced soybean meal at 0%, 25%, 50%, 75%, and 100%. While 0% soybean meal and commercially pelletized feed (coppens) served as the control.

All dietary ingredients were ground and mixed with vitamin-mineral premix and oil mixture. Water was added gradually until a desirable paste-like consistency was obtained after which the mixture was pelletized through 3mm disc and sun-dried.The pelletized feeds were then stored in air tight polythene bags, until required. Diet samples were subjected to proximate analysis, Pearsons square feed formulation for the *Clarias gariepinus* fingerlings, juveniles and adults and gross energy was computed using the equation of Olurin (2006) the results of which are presented in Tables 3.1, 3.2, 3.3 and 3.4.

Table 3.1: Proximate and Energy Composition of Ingredients Used in the Experimental Diets of Fingerlings, Juveniles and Adult *Clarias gariepinus*

INGREDIENTS	**CP**	**EE**	**MOIST**	**CF**	**ASH**	**NFE**	**GE**
Fish meal	74.23	8.10	8.81	-	10.12	3.96	1197
Soy meal	44.5	3.2	6.9	7.9	5.8	34	1009
Yellow corn	9.45	3.65	8.89	10.94	4.01	64.10	809
GNC Meal	44	5.36	8.99	6.10	6.01	31.00	2434
Watermelon	37.00	6.00	9.12	4.36	5.81	45.11	2074

CP-Crude protein
EE-Ether extract
CF-Crude fibre
NFE-Non fibre extract
GE-Gross energy
GNC- Ground nut cake

Table 3.1: Feed Stuff Ingredients used at different Percentages of Soybean replacement at 45% for of *Clarias gariepinus* fingerlings

INGREDIENTS	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**
Maize	31	31	31	31	31
Watermelon	-	5.5	11	16.5	22
G/nut	23.4	23.4	23.4	23.4	23.4
Soybean Cake	21	15.75	10.5	5.25	-
Fish meal	20	20	20	20	20
Oil	2	2	2	2	2
Salt	0.2	0.2	0.2	0.2	0.2
Lysine	0.2	0.2	0.2	0.2	0.2
Meth./Cyst.	0.2	0.2	0.2	0.2	0.2
Vit. Premix	0.1	0.1	0.1	0.1	0.1
Total	**97.90**	**98.15**	**98.40**	**98.65**	**98.90**

WMC- Water melon seed
G/nut-Ground nut
Meth-Methionie
Cyst-Cysteine
Vit, premix-Vitamin premix

Table 3.3: Feed Ingredients used at Different Percentages of Soybean replacement at 42% for Juveniles of *Clarias gariepinus*

INGREDIENTS	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**
Maize	31	31	31	31	31
Watermelon	-	5	10	15	20
G/nut	23.4	23.4	23.4	23.4	23.4
Soybean Cake	21	15.75	10.5	5.25	-
Fish meal	20	20	20	20	20
Oil	2	2	2	2	2
Salt	0.2	0.2	0.2	0.2	0.2
Lysine	0.2	0.2	0.2	0.2	0.2
Meth./Cyst.	0.20	0.20	0.20	0.20	0.20
Vit. Premix	0.1	0.1	0.1	0.1	0.1
Total	**98.10**	**97.85**	**97.60**	**92.10**	**97.10**

WMC- Water melon seed
G/nut-Ground nut
Meth-Methionie
Cyst-Cysteine
Vit, premix-Vitamin premix

Table 3.4: Feed Stuff Ingredients used at Different Levels of Soybean replacement at 40% for *Clarias gariepinus* adults

INGREDIENTS	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**
Maize	32	32	32	32	32
Watermelon	-	5.5	11	16.5	22
G/nut	22	22	22	22	22
Soybean Cake	21	15.75	10.5	5.25	-
Fish meal	20	20	20	20	20
Lysine	0.2	0.0	0.2	0.2	0.2
Oil	2	2	2	2	2
Salt	0.2	0.2	0.2	0.2	0.2
Meth./Cyst.	0.2	0.2	0.2	0.2	0.2
Vit. Premix	0.1	0.1	0.1	0.1	0.1
Total	**97.70**	**97.75**	**96.20**	**97.70**	**98.70**

WMC- Water melon seed
G/nut-Ground nut
Meth-Methionie
Cyst-Cysteine
Vit, premix-Vitamin premix

3.15 Estimation of Digestibility

Digestibility was determined by collection of feacal materials by dissection of the fish and removalof feaces from the reticulum (Percivel *et al*., 2008) and preserved prior to analysis. The indirect method as described by (Stickey, 2006) was used to measure apparent digestibility in which an inert material such as chromic oxide was added to feed at 0.5% of the feed. The chromic oxide level of both the feed and feaces was determined by the equation as follows:

Apparent digestibility coefficient = $10^2 – (10^2$ x id/if x Nf/Nd)

Where id = % Cr_2O_3 in diet

If = %Cr_2O_3 in feaces

Nf = Protein in feaces

Nd = Protein in diet

3.15.1 Feacal collection

Ten hours after feeding, three fish were sacrificed by partial decapitation by cutting through the vertebral column. The gastrointestinal tract was divided into four parts (Ugwumba,2001). These were the stomach, the fore-gut, the mid-gut and the hind-gut. To distinguish between the fore-gut, mid-gut and hind-gut when sampling for the faecal material and to keep the results constant, the total length of the gut was measured from the anus to the sphincter muscle leading into the stomach and this was divided by three. Each segment then represented the approximate appropriate section of the gastrointestinal tract (Sotolu and Faturoti, 2009). The stomach was considered the region between the cardiac and plyloric sphincters. A longitudinal incision was made along the length of the abdomen from the anus to the base of the mouth. The gastrointestinal tract was then carefully removed (Adewolu, 2008).

The hind part of the gastrointestinal tract was then severed from the mid-gut. A longitudinal incision was then made in the rectal or hind-gut to evacuate its feacal content and stored in a freezer. Before analysis, frozen faecal material was thawed overnight in a refrigerator. Thereafter, the faecal material was dried at 60^{o}C for 48hrs and then ground to a fine powder using mortar and pestle. Chromic oxide was quantified following the method of (Tsukahara, 2002). Approximately 50-100mg of sample was digested with 5ml of concentrated nitric acid followed by 3ml of perchloric acid which turned the digesta from green to yellow. This was the diluted to 100ml of distilled water and absorbance read using Uvikon 860 spectrophotometer (Kontroormun Instruments) at 350nm. The weight and percentage of chromic oxide was calculated using the following formulae:Weight of Cr2O3=Absorbance – 0.0032/0.2089

Digestibility coefficients of diets were estimated to determine bioavailability of nutrients. Apparent digestibility coefficients (ADC) of the feeds were computed using the formula described by Hossain (2003).ADC (%) = 100 x (% nutrient in feaces/% nutrient in feed) x (% marker in feed/marker in feces). Feacal material was placed in a sterile vial which was then placed in a drying oven at 40^{o}C until constant weight was achieved.

3.16Determination of Haematogical Parameters

Before the experiment and at the end of week 24 of feeding, fish from each treatment were randomly selected for haematological analyses. Live fish was put on a tray. A wet towel was used to cover the fish head. Blood were drawn from the caudal vein through the lateral line of fish into ethylene diamine tetra-acetic acid (EDTA) bottles as anti-coagulant by the use of needle and syringe and immediately taken to the

laboratory for haemotological analysis to determine the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC)(Sotolu, 2008).

3.16.1 Packed cell volume (PCV)

The heparinized capillary tubes were ¾ filled with whole blood and one end scaled with plasticine. The tubes were centrifuged for 5min in a microchaematocrit centrifuge at 12,000 rpm. The PCV was read by the use of haematocrit reader (Terry, 2000).

3.16.2Red Blood Cell (RBC) and White Blood Cell (WBC) Counts

The RBC and total WBC counts were carried out by the use of the Neubauer improved counting chamber as described by (Teixeira, 2000). For red blood cell counts, blood was diluted 1:200 with Dacies fluid (99ml of 3% aqueous solution of sodium citratic; and 1ml of 40% formaldehyde) which keeps and preserves the shape of the red blood cell for estimation in the counting chamber Shah and Altindag (2004).

3.16.3Total white blood cell counts

For white blood cell counts, the dilution was 1:20 using 2-3% aqueous solution of acetic acid of which tinge of Gentian violet was added. Thin blood smears were stained with Wright-Giemsa stain, Robinson *et al*. (2001). A total of 100 white blood cells were enumerated and differentiated.

3.17 Haemoglobin (Hb) Estimation

The cyanomet haemoglobin method as described by Osuigwe (2007) was used in the determination of haemoglobin concentration. Well-mixed blood of 0.02mL was added to 4mL of modified Dabkin's solution (potassium ferricyanide, 200mg; potassium cyanide, 50mg; potassium dihydrogen phosphate (140mg). The volume was made up

of 1L with distilled water at pH of 7.0. The mixture was allowed to stand for 3min and the Hb concentration was read photometrically by comparing with a cyanomet haemoglobin standard with a yellow-green filter at 625nm.

The mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated as follows (Fagbemi *et al.,* 2007) MCV (µm3 cell-1) = (Packed cell volume as percentage/RBC in millions cell mm-3) x 10. MCH (pg cell-1) = (Hb in g 100 mL-1/RBC in millions cell mm-3) x 10

MCHC (g 100m-1 Hct) = (Hb in g 100 ml-1/packed cell volume as percentage) x 10

3.18Determination of carcass

At the end of the 24 weeks of the experiment, random samples of the carcass were collected from the reticulum by placing the fish on a table and cutting through the anus up to the head region and samples evacuated into a plastic bottle and were taken to NAPRI for the determination of the proximate composition of the crude protein, lipid, ash, fibre, moisture and nitrogen free extract according to the method of AOAC (2006).

3.19Determination of Cost Benefit Analyses

The production cost in naira of the experiment was calculated following the method of Abu *et al*. (2010) base on the current market price of the ingredients used for formulating the diets. Economic evaluation in terms of Investment Cost Analysis (ICA), Net Profit Value (NPV), Gross Profit (GP), Profit Index (PI), Incidence of Cost (r) and Benefit Cost Ratio (BCR) of all the experimental diets were determined according to investment Cost Analysis (ICA) = Cost of feeding + cost of fingerlings

Net Production Value (NPU) = Final mean weight + Total no. of survival x Cost per kg

Gross Profit (GP) = Net Profit Value – Investment Cost Analysis

Profit Index (PI) = $\frac{\text{Net Profit Value}}{\text{Cost of Feeding}}$

Incidence of Cost (r) = $\frac{\text{Cost of feeding}}{\text{Weight of fish produced}}$

Benefit Cost Ratio (BCR) = $\frac{\text{Net Profit Value}}{\text{Investment Cost Analysis}}$

3.20Data Analyses

The data obtained were subjected to one way analysis of variance (ANOVA) using GLM procedure of (SAS, 2004).Mean separation was done using Duncan Multiple Range Test (DMRT) and to separate mean values at $p<0.05$ level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of toasting time on the proximate composition of water melon seed cake

The effect of toasting time on the proximate composition of water melon seeds cake is presented in Table 4.1.Water melon cake toastedfor 20 minutes gave the highest crude protein, Lipid and ash values of 32.68±0.52, 3.36±2.20 and 4.86±0.02 respectively, while the least crude protein, lipids and ash values of 28.34±0.52, 2.24±2.20 and 2.98±0.02 were recorded in raw water melon seeds. The raw water melon seeds had the highest dry matter, fibre, moisture and nitrogen free extract of 94.41±0.03, 7.12±0.12, 5.10±0.14 and 76.98±0.25 respectively. Similarly, the lowest values of fibre, moisture, dry matter and nitrogen free extract were observed in toasted water melon cake for 20 minutes with values of 93.26±0.03, 5.48±0.12, 3.80±0.14 and 73.76±0.25.

4.2 Effect of toasting time on the anti-nutrient content of water melon seed cake

The anti-nutritional composition of toasted water melon cake is presented in Table 4.2. The processing method of water melon cake toasted at 40 minutes gave the least values of tannin, oxalate, phytate, alkaloid cyanide and saponins of 0.51±0.01, 0.45±0.01, 0.44±0.01, 0.32±0.01, 0.25±0.01 and 0.70±0.01 respectively, while the raw water melon seeds gave the highest values of 2.01±0.01, 0.61±0.01, 1.08±0.01, 0.54±0.02, 0.68±0.03 and 1.46±0.10 of tannin, oxalate, phytate, alkaloid, cyanide and saponins respectively. There was progressive decrease in the anti-nutritional composition of toasted water melon cake as processing time increased.

Table 4.1: Effect of toasting time on the proximate composition of water melon seed cake

	TIME					
INDICES (%)	**RWM 0 MINS**	**WMC 10 MINS**	**WMC 20 MINS**	**WMC 30 MINS**	**WMC 40 MINS**	**SE**
Dry matter	94.41±0.03^{a}	93.45±0.03^{e}	93.26±0.03^{e}	93.54±0.03^{c}	93.67±0.03^{b}	0.60±0.02
CP	28.34±0.52^{e}	31.49±0.52^{c}	32.68±0.52^{a}	31.50±0.52^{b}	30.42±0.52^{d}	0.73±0.02
Lipid	2.24±2.20^{e}	3.30±2.20^{d}	3.36±2.20^{a}	3.34±2.20^{b}	3.31±2.20^{c}	0.22±0.01
Ash	2.98±0.02^{e}	4.70±0.02^{d}	4.86±0.02^{a}	4.83±0.02^{b}	4.82±0.02^{c}	0.36±0.01
CHO	58.84±0.01^{a}	54.21±0.01^{b}	38.54±0.01^{e}	46.51±0.01^{c}	46.48±0.01^{d}	3.50±0.04
Fibre	7.12±0.12^{a}	6.98±0.12^{b}	5.48±0.12^{e}	6.10±0.12^{d}	6.12±0.12^{c}	0.47±0.03
Moisture	5.10±0.14^{a}	4.12±0.14^{b}	3.80±0.14^{e}	4.08±0.14^{c}	4.04±0.14^{d}	0.22±0.02
NFE	76.98±0.26^{a}	74.35±0.25^{d}	73.76±0.25^{e}	75.21±0.25^{c}	75.38±0.25^{b}	0.55±0.02
GE(kcal/g)	1172.26^{a}	1207^{b}	1001.31^{e}	1055.00^{c}	1005.02^{b}	43.00±0.1

Means with different superscripts along the same row were significant different ($P<0.05$).

Key:
CP = Crude protein
NFE = Nitrogen Free Extract
RWM = Raw water melon seeds
GE=Gross energy
CHO = carbohydrate
SE= Standard error

Table 4.2: Effect of toasting time on anti-nutrient content of water melon seed cake

Indices	**RWM 0 Mins**	**WMC 10 Mins**	**WMC 20 Mins**	**WMC 30 Mins**	**WMC 40 Mins**	**SE**
Tannin/100g	2.01±0.01[a]	1.56±0.01[b]	0.53±0.01[c]	0.52±0.01[d]	0.51±0.01[e]	0.30±0.02
Oxalate/100g	0.61±0.01[a]	0.50±0.01[b]	0.48±0.01[c]	0.47±0.01[d]	0.45±0.01[e]	0.03±0.01
Phytate/100g	1.08±0.02[a]	0.68±0.02[b]	0.49±0.02[c]	0.46±0.02[d]	0.44±0.02[e]	0.12±0.03
Alkaloid/100g	0.54±0.02[a]	0.46±0.02[b]	0.35±0.02[c]	0.33±0.02[d]	0.32±0.02[e]	0.04±0.02
Cyanide/100g	0.68±0.03[a]	0.50±0.03[b]	0.28±0.03[c]	0.27±0.03[d]	0.25±0.03[e]	0.10±0.03
Saponins/100g	1.46±0.02[a]	1.36±0.02[b]	0.70±0.02[c]	0.70±0.02[d]d	0.70±0.02[e]	0.15±0.02

Means with different superscripts along the same row were significantly different ($p<0.05$)
Key:
Raw= Raw water melon
WMC=Water melon cake
SE=Standard error

4.3 Effect of toasting time on the essential amino acids concentration of water melon seed cake

The result of processing method of toasting water melon cake is presented in Table 4.3. The processing methods of toasting water melon cake for 20 minutes gave the best result in all the amino acids recorded, followed by 10 minutes, 30 minutes 40 minutes, while that of raw water melon seeds gave the lowest values. The values of methionine, arginine and tryptophan were higher in 20, 30 and 40 minutes the FAO value, while raw water melon seeds were lower. The values of valine and isoleucine were higher in the 20 minutes than the FAO values, but lower than the FAO values in the 10, 30 and 40 minutes values. The leucine value was lower than the FAO value in all the treatments. The histidine values were higher in 20, 10, 30 and 40 minutes and lower in the raw water cake compared to the FAO values.Treonine values were higher in the 20, 30 and 40 minutes, while it were lower in the 10 minutes and raw water melon seeds. The value of phenylalanine was higher in the 20, 10. 30 and 40 minutes than the FAO values, while it was less than the FAO value in the raw water melon seeds.

4.4Effect of toasting time on the non essential amino acids of water melon seed cake

All non-essential amino acids in water melon cake for 20 minutes were higher than those in 10 minutes, 30 minutes and 40 minutes, while those in raw water melon seeds had the lowest values. The values glutamic, proline and aspartic acids were higher in the 10, 20, 30, 40 minutes and the raw water melon seeds than the FAO values.The tyrosine, serine and glysine were not present in the FAO values.The alanine values were higher in the 20, 30 and 40 minutes than the FAO values, while it were less in the 10 minutes and raw water melon seeds than the FAO values as shown in Tab

Table 4.3: Effect of toasting time on the essential amino acids content of water melon seeds cake

		TIME					
INDICES (%)	**FAO**	**RWM 0MINS**	**10 MINS**	**20 MINS**	**30 MINS**	**40 MINS**	**SEM**
Methionine/100g	2.00	3.41^{e}	3.61^{b}	6.88^{a}	6.11^{c}	5.89^{d}	±0.23
Arginine/100g	5.20	2.40^{e}	4.16^{b}	6.17^{a}	5.15^{c}	4.64^{d}	±0.20
Tryptophan/100g	3.05	3.89^{e}	4.42^{b}	4.86^{a}	4.31^{c}	4.11^{d}	±0.35
Valine/100g	5.00	3.79^{e}	4.02^{b}	6.10^{a}	4.11^{c}	3.82^{d}	±0.45
Isolecine/100g	4.00	2.16^{e}	3.63^{b}	4.51^{a}	3.14^{c}	3.12^{d}	±0.31
Leucine/100g	7.00	3.38^{e}	3.72^{b}	6.84^{a}	6.64^{c}	6.48^{d}	±0.23
Lysine/100g	5.40	2.47^{e}	3.22^{b}	6.89^{a}	5.56^{c}	5.49^{d}	±0.32
Histidine/100g	2.40	2.15^{e}	3.91^{b}	4.73^{a}	3.84^{c}	3.46^{d}	±0.55
Thresnine/100g	4.00	2.61^{e}	3.23^{b}	4.53^{a}	4.12^{c}	4.01^{d}	±0.38
Phenylalanine/100g	3.05	3.02^{e}	4.43^{b}	4.48^{a}	4.32^{c}	4.16^{d}	±0.31
TAA	36.10	29.28	34.74	55.58	47.30	45.18	

Means with different superscripts along the same row were significant different ($P<0.05$)
Key:
RWM = Raw water melon seed
TAA-Total essential amino acids
FAO= Food and agricultural organization
SEM=Standard error of means

Table 4.4: Effect of toasting time on non-essential amino acids concentration of water melon seed cake

		TIME					
Indices (%)	**FAO**	**RWM 0MINS**	**10 MINS**	**20 MINS**	**30 MINS**	**40 MINS**	**SEM**
Tyrosine/100g	-	1.98^{e}	2.86^{b}	3.96^{a}	3.53^{c}	3.12^{d}	±0.36
Alanine/100g	6.10	4.68^{e}	5.50^{b}	7.35^{a}	6.48^{c}	6.26^{d}	±0.43
Cysteine/100g	3.00	2.11^{e}	2.82^{b}	3.04^{a}	2.73^{c}	2.64^{d}	±0.15
Glutamic/100g	14.70	8.20^{e}	12.30^{b}	12.60^{a}	12.42^{c}	12.38^{d}	±0.84
Proline/100g	10.70	6.17^{e}	8.60^{b}	10.12^{a}	8.26^{c}	8.13^{d}	±0.63
Aspartic/100g	7.70	4.36^{e}	5.41^{b}	7.45^{a}	7.10^{c}	7.02^{d}	±0.58
Serine/100g	-	3.12^{e}	4.69^{b}	6.94^{a}	6.04^{c}	6.02^{d}	±0.68
Glysine/100g	-	2.02^{e}	3.44^{b}	4.91^{a}	3.38^{c}	3.29^{d}	±0.45
TNAA	42.20	32.58	45.62	56.37	49.94	48.86	

Means with different superscripts along the same row were significant different ($P<0.05$).

Key:

RWM = Raw water melon seeds

TNAA= Total non essential amino acids

FAO= Food and agricultural organization

SEM= Standard error of means

4.5Effect of toasting time on some mineral concentration of water melon seed cake

Mineral content obtained from toasted water melon cake showed increasing values from 10 minutes to 20 minutes, while there was decrease in values from 20 minutes to 40 minutes with increase in time. The values in the 20, 30 and 40 minutes were higher than the FAO values in all the non essential amino acids, while the values of the 10 minutes and raw water melon seeds were lower than the FAO values(Table 4.5).

4.6 Physico-chemical parameters of water in the experimental plastic tanks of *Clarias gariepinus* fingerlings

The physico-chemical parameters of some water quality indices such as temperature, dissolved oxygen, pH and ammonia are presented in Table 4.6. There was no significant difference ($p>0.05$) in the mean temperature of the experimental tanks which ranged from 27.30±0.02 – 8.01± 0.02 during the fingerlings growth stage. Similarly, there was no significant difference ($p>0.05$) in the dissolved oxygen values which ranged from 6.70±0.02 – 6.80±0.02. There was no significant difference ($P>0.05$) in the pH values which ranged from 7.64±0.03 -8.50±0.03 andthere also no significant difference ($P>0.05$) in the ammonia values which ranged from 0.04±0.06 – 0.05±06.

Table 4.5: Effect of toasting time on some mineral content of water melon seed cake

	TIME					
INDICES (%) FAO	**RWM 0MINS**	**WMC 10MINS**	**WMC 20MINS**	**30 MINS**	**40 MINS**	**SEM**
Magnesium/100g	8.44^{e}	8.71^{b}	8.80^{a}	8.69^{c}	8.56^{d}	8.54±0.06
Manganese/100g	8.42^{e}	9.60^{b}	9.80^{a}	9.50^{c}	9.46^{d}	8.80±0.25
Calcium/100g	9.39^{e}	9.65^{b}	9.78^{a}	9.60^{c}	9.59^{d}	9.56±0.06
Sodium/100g	5.58^{e}	6.12^{b}	6.15^{a}	6.10^{c}	6.08^{d}	6.13±0.43
Phosphorus/100g	7.30^{e}	8.13^{b}	8.19^{a}	8.10^{c}	8.04^{d}	8.15±0.16
Copper/100g	3.30^{e}	4.62^{b}	4.65^{a}	4.61^{c}	4.59^{d}	4.63±0.26
Zinc/100g	5.03^{e}	6.10^{b}	6.12^{a}	6.07^{c}	6.00^{d}	6.11±0.20
Iron/100g	2.60^{e}	3.64^{b}	3.68^{a}	3.61^{c}	3.60^{d}	3.65±0.21
Potassium/100g	9.32^{e}	9.84^{b}	9.89^{a}	9.82^{c}	9.81^{d}	9.85±16.67

Means with different superscripts along the same row were significant different ($P<0.05$).
Key:
RWM = Raw water melon seeds
WMC=Water melon cake
FAO= Food and agricultural organization

Table 4.6: Physico-chemical parameters of water in the experimental plastic tanks of *Clarias gariepinus* fingerlings

Parameters	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
Tempeture	27.3±0.02	28.2±0.02	28.3±0.02	28.4±0.02	28.3±0.02	28.10±0.02	±0.16
D.O.	6.80±0.04	6.70±0.04	6.80±0.04	6.80±0.04	6.73±0.04	6.72±0.04	±0.02
pH	8.50±0.03	7.82±0.03	7.84±0.03	7,81±0.03	7.66±0;03	7.63±0.03	±0.13
Ammonia	0.04±0.04	0.04±0.04	0.04±0.04	0.05±0.04	O.05±0.04	0.05±0.04	±0.10

Key:
CF =Commercial feeds
D.O= Dissolved oxygen
WMC=Water melon cake
SEM=Standard error of means

4.7 Physico-chemical parameters of water inthe experimental plastic tanks of *Clarias gariepinus* juveniles

The physico- chemical parameters of some water quality indices such as temperature, dissolved oxygen, pH and ammonia are presented in Table 4.7. There was no significant difference (p>0.05) in the mean temperature of the experimental tanks which ranged from 27.20±0.05 – 8.60± 0.05 during the fingerlings growth stage. Similarly, there was no significant difference (p>0.05) in the dissolved oxygen values which ranged from 6.26±0.24 – 6.40±0.24. There was no significant difference (p>0.05) in the pH values which ranged from 7.30±0.03 -7.50±0.03 and there also no significant difference (p0.05) in the ammonia values which ranged from 0.04±0.20 – 0.05±0.20.

4.8 Physico-chemical parameters of water in the experimental plastic tanks of *Clarias gariepinus*adults

The physico- chemical parameters of some water quality indices such as temperature, dissolved oxygen, pH and ammonia are presented in Table 4.8. There was no significant difference (p>0.05) in the mean temperature of the experimental tanks which ranged from 27.40±0.07 – 29.30± 0.07 during the adults growth stage. Similarly, there was no significant difference (p>0.05) in the dissolved oxygen values which ranged from 6.70±0.04 – 6.80±0.04. There was no significant difference (p>0.05) in the pH values which ranged from 7.63±10.03 -8.50±0.10 and there also no significant difference (p>0.05) in the ammonia values which ranged from 0.05±0.04 – 0.06±04.

Table 4.7: Physico-chemical parameters of water in the experimental plastic tankss of *Clarias gariepinus*juveniles

Parameters	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
Temperature	28.3±0.05	28.4±0.05	27.4±0.05	27.2±0.05	27.3±0.05	28.20±0.02	±0.06
D.O.	6.40±0.24	6.36±0.24	6.38±0.24	6.32±0.24	6.28±0.24	6.26±0.24	±0.02
pH	7.50±0.10	7.42±0.10	7.40±0.10	7,50±0.10	7.36±0.10	7.30±0.10	±0.13
Ammonia	0.04±0.20	0.04±0.20	0.05±0.20	0.05±0.20	O,45±0.20	0.05±0.20	±0.02

Key:
CF =Commercial feeds
D.O= Dissolved oxygen
WMC=Water melon cake
SEM=Standard error of means

Table 4.8: Physico-chemical parameters of water in the experimental plastic tanks of *Clarias gariepinus adults*

Parameters	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
Temperature	28.50±0.07	28.30±0.07	27.40±0.07	29.30±0.07	29.29±0.07	29.28±0.07	±0.06
D.O.	5.70±0.06	5.70±0.06	5.70±0.06	5.59±0.06	5.66±0.06	5.20±0.06	±0.102
pH	7.00±0.12	7.58±0.12	7.50±0.12	7,42±0.12	7.26±0.12	7.10±0.12	±0.04
Ammonia	0.05±0.10	0.05±0.10	0.05±0.10	0.05±0.10	O,06±0.10	0.06±0.10	±0.02

Key:
CF =Commercial feeds
D.O. =Dissolved oxygen
WMC=Water melon cake
SEM= Standard error

4.9Growth performance and nutrient utilization of *Clarias gariepinus* fingerlings

The nutrient utilization and growth performance of *Clarias* gariepinus fingerlings is presented in Table 4.9. From the results of Mean Final Weight (121.50g), Mean Weight Gain (111.50g), Mean Feed Intake (162.00g), Mean Protein Intake (73.00g), ADWG (1.86g), Percentage Weight Gain (92%), Specific Growth Rate (1.81g), Feed Conversion Ratio (1.63g), Protein Efficiency Ratio (1.52g), Nitrogen Metabolism (1836) and Condition Factor (0.02) of fish fed with commercial feed gave the best growth performance and nutrient utilization than all the other experimental diets. There was significant difference ($P<0.05$) in fish fed with commercial feed compared to all the other experimental diets.

However, the growth performance of fish fed with the experimental diet shows that 25% inclusion level is better than all the other diets with Mean Weight Gain (107.10g), percentage weight gain (91.50%), Specific Growth Rate (1.78g). Statistical test shows a significant difference ($P<0.05$) in the 25% inclusion level to all the others.A steady decrease of growth performance and nutrient utilization were recorded with increase of the seeds meal. At 25% inclusion, Mean Weight Gain (107.10g), 50% (90.00g), 75% (69.10g) and 100% (47.76g) respectively. Similarly, SGR 25% (1.78g), 50% (1.67g), 75% (1.50g) and 100% (1.30g).The same trend were observed in FCR and PER respectively. On the other hand, 100% inclusion level clearly gave the worst growth performance and nutrient utilization. Statistical analysis shows significant difference ($P<0.05$) between the 100%, 75%, 50%, 25% and 0% growth and nutrient utilization performances. The results of bi-weekly growth performance of fingerlings of *Clarias gariepinus* is presented in Figure 4.1.Within the first weeks in all the treatment, growth was very slow and a steady rate of increase was recorded within 2-4, while marked accretion between week 4-8.

Table 4.9: Growth Performance and feed utilization of *Clarias* gariepinus fingerlings

PARAMETERS	CF	WMC 0%	WMC 25%	WMC 50%	WMC 75%	WMC 100%	SEM
Mean Initial Weight (g)	10	10	10	10	10	10	±0.00
Mean Final Weight (g)	121.50[a]	110.30[c]	117.10[b]	100.0[d]	79.0[e]	57.76[f].	±0.08
Weight Gain (g)	111.50[a]	100.30[c]	107.10[b]	90.00[d]	69.10	47.76[f]	±10.07
Mean Feed Intake(g)	162a	146c	156b	134d	105e	78.00f	±9.00
Mean protein intake (g)	73a	66.18[c]	70.26[b]	60.30[d]	47.25[e]	35.10[f]	±4.10
Percentag Weight Gain (%)	92.00[a]	91.00[c]	91.50[b]	90.00[d]	87.35[e]	83.00[f]	±1.43
Specific Growth Rate (g)	1.81[a]	1.74[c]	1.78[b]	1.67[d]	1.50[e]	1.30[f]	±0.10
Feed Conversion Ratio (g)	1.63f	1.65[d]	1.64[e]	1.67[c]	1.68[b]	1.73[a]	±0.04
Protein Efficiency Ratio (g)	1.67[a]	1.65[c]	1.66[b]	1.54[d]	1.63[e]	1.62[f]	±0.03
Nitrogen Metabolism	1836[a]	1652[c]	1764[b]	1483[d]	1139[e]	787[f]	±165
Condition Factor	0.01[a]	0.01[a]	0.01[a]	0.01[a]	0.01[a]	0.01[a]	±0.00

Means with different superscripts along the same row were significantly different ($P<0.05$)

Key:
CF- Commercial feed
WMC-Water melon cake

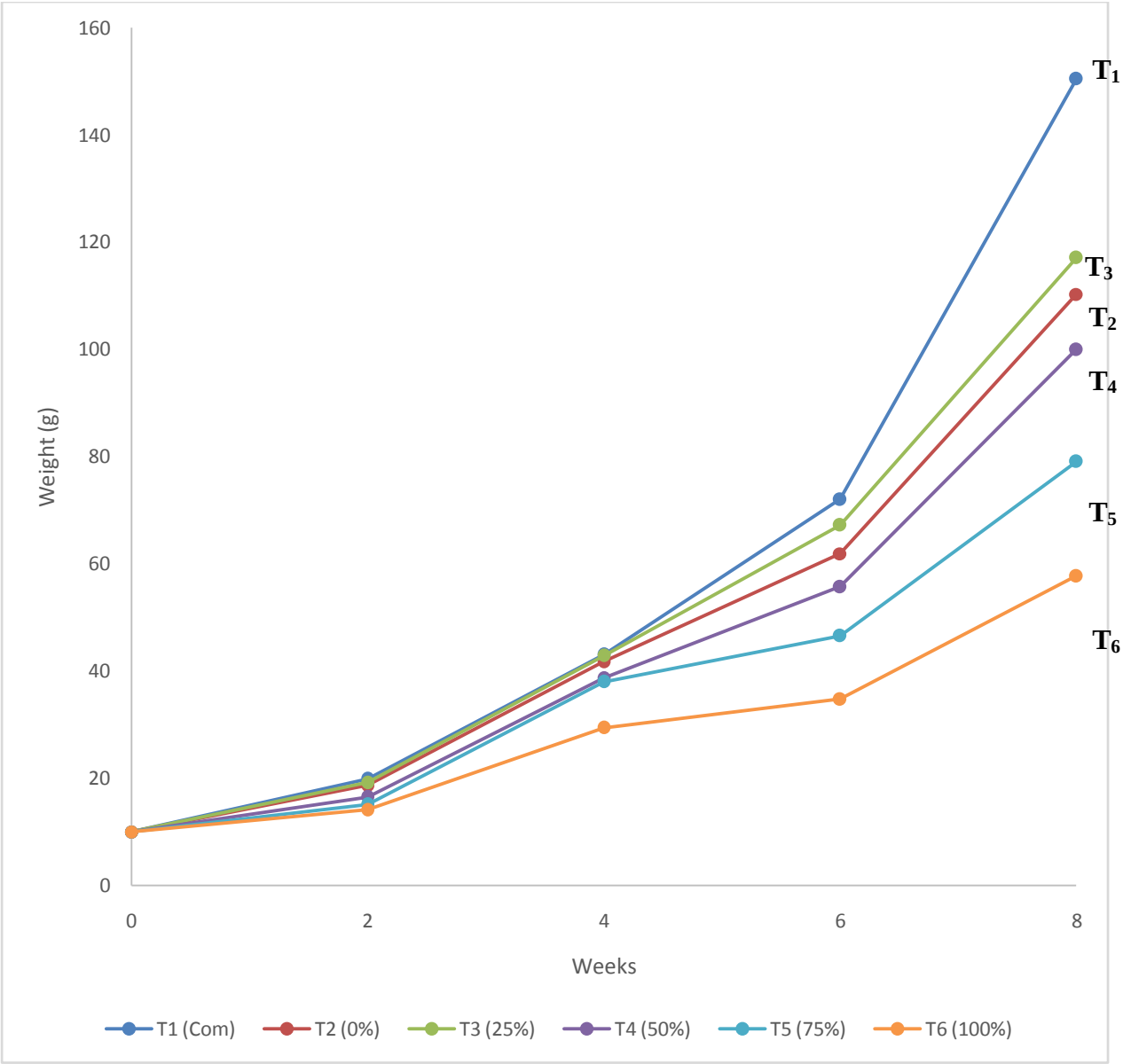


Figure 4.2: Biweekly growth pattern of *Clarias gariepinus* fingerlings fed graded levels of watermelon seeds cake

4.10Growth performance and nutrient utilization of *Clarias gariepinus* juveniles

From the results of Mean Final Weight (260.20g), Mean Weight Gain (138.70g), Mean Feed Intake (374g), Mean Protein Intake (156.47g), Percentage Weight Gain (53.31%), Specific Growth Rate (2.36g), Feed Conversion Ratio (1.10g), Protein Efficiency Ratio (1.62g), Nitrogen Metabolism (2284) and Condition Factor (0.01) fish fed with commercial feed gave the highest growth performance and nutrient utilization than all the other experimental diets. There was significant difference ($P<0.05$) in fish fed with commercial feed compared to all the other experimental diets.

However, the growth performance of fish fed with the experiment diet clearly shows that 25% inclusion level is better than all the other diets with Mean Weight Gain (133.10g), percentage Weight Gain (53.15%), SGR (2.33g), FCR (1.12g) and PER (1.63g). Statistical analysis shows a significant difference ($P<0.05$) of 25% inclusion level to all the others. Progressive decrease of growth performance and nutrient utilization were obtained with increase of the seeds meal.

At 25% inclusions Mean Weight Gain (138.70g), 50% (89.30), 75% (166.00g) and 100% (140.70g). The same trend were recorded in FCR and PER respectively. On the other hand, 100% inclusion level clearly gave the worst growth performance and nutrient utilization. Statistical test shows significant difference ($P<0.05$) between the 100%, 75%, 50%, 25% and 0% growth and nutrient utilization performances. The results of bi-weekly growth performance of juveniles *Clarias gariepinus* is presented in figure 4.8. Growth was very slow between week 14-20 and sharp increase between week 14-16 as shown in Table 4.10.

Table 4.10: Growth performance and feed utilization of *Clarias gariepinus* juveniles

PARAMETERS	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
Mean Initial Weight (g)	121.50[a]	110.30[c]	117.10[b]	100.00[d]	79.10[e]	57.76[f]	±11.20
Mean Final Weight (g)	260.20[a]	240.20[c]	250.40[b]	189.30[d]	166.00[e]	140.70[f]	±18.11
Mean Weight Gain (g)	138.70[a]	126.80[c]	130.30[b]	124.30[d]	118.00[e]	120.00[f]	±10..70
Mean Feed Intake(g)	274a	244c	258b	232d	220e	218f	±9.00
Mean protein intake (g)	157.80a	155.40c	156.24b	152.04[d]	143.00e	134.40f	±6.40
Percen. Weight Gain (%)	48.00	48.00[c]	48.00[b]	46.20[d]	45.00[e]	43.00[f]	±0.92
Specific Growth Rate (g)	2.36[a]	2.30[c]	2.33b	2.13[d]	2.03e	1.91[f]	±0.10
Feed Con. Ratio (g)	1.10a	1.11[c]	1.12[b]	1.18[d]	1.29[e]	1.78[f]	±0.07
Protein Effici. Ratio (g)	1.67[a]	1.65[c]	1.66[b]	1.64[d]	1.63[e]	1.62[f]	±0.03
Nitrogen Metabolism	2284[a]	2138[c]	2192[b]	1471[d]	1433[e]	1466[f]	±176
Condition Factor	0.01[b]	0.01[b]	0.01[b]	0.01[a]	0.01[a]	0.01[a]	±0.00

Mean on the same row with the same superscripts were significantly different ($P<0.05$)

Key:
CF-Commercial feed
WMC-Water melon cake

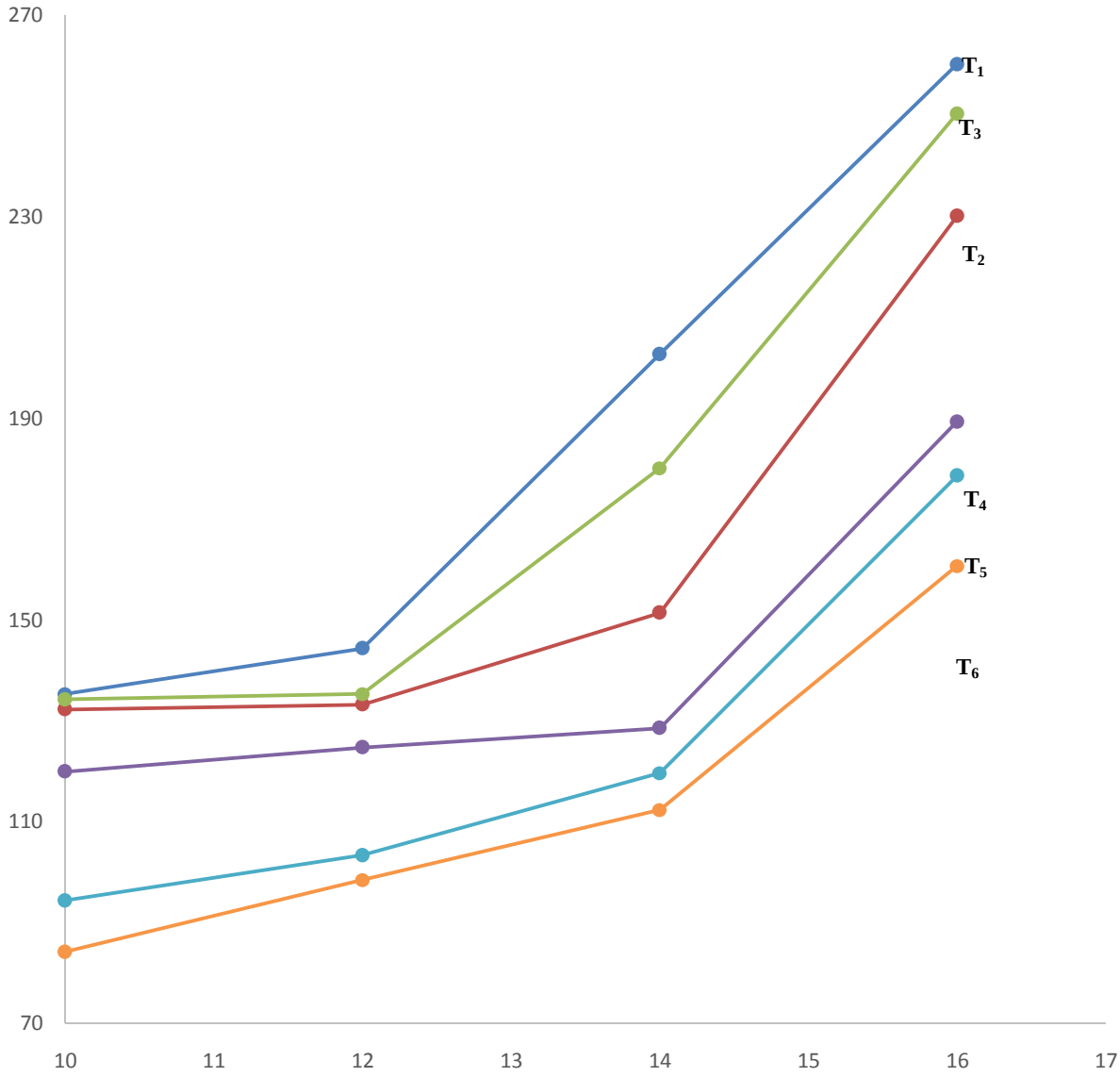


Figure 4.2: Biweekly growth pattern of *Clarias gariepinus* juveniles fed graded levels of water melon seed cake

4.11Growth performance and nutrient utilization of *Clarias gariepinus* adults

From the results of Mean Final Weight (550.00g), Mean Weight Gain (289.80g), Mean Feed Intake (610g), Mean Protein Intake (21.60g), ADWG (5.00g), Percentage Weight Gain (53%), Specific Growth Rate (0.60g), Feed Conversion Ratio (0.84g), Protein Efficiency Ratio (1.65g), Nitrogen Metabolism (3971) and Condition Factor (0.02) fish fed with commercial feed gave the best growth performance and nutrient utilization than all the other experiment diets. There was significant difference ($P<0.05$) in fish fed with commercial feed compared to all the other experiment diets.

Nevertheless, the growth performance of fish fed with the experiment diet shows that 25% inclusion level is better than all the other diets with Mean Weight Gain (260.00g), Percentage Weight Gain (57%), SGR (0.61g), FCR (0.87g) and PER (1.66g). Statistical test shows significant difference ($P<0.05$) of 25% inclusion level to all the others. A steady reduction in growth performance and nutrient utilization were recorded with increase of the seed meal.

At 25% inclusions Mean Weight Gain (260g), 50% (240), 75% (190) and 100% (171.70). The same trend were observed in FCR and PER respectively. Conversely, 100% inclusion level clearly gave the worst growth performance and nutrient utilization. Statistical analysis shows significant difference ($P<0.05$) between the 100%, 75%, 50%, 25% and 0% growth and nutrient utilization performances. The resultsof bi-weekly growth performance of adult *Clarias gariepinus* is presented in Figure 4.3. Growth was slow from week 18-20 and marked growth from 20-24 as shown in (Table 4.11).

Table 4.11:Growth performance and feed utilization of *Clarias gariepinus* adults

PARAMETERS	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
Mean Init. Weight (g)	260.20^{a}	245.10^{c}	250.40^{b}	230.30^{d}	218.00^{e}	210.00^{f}	±41.15
Mean Final weight (g)	550.00^{a}	500.20^{c}	510.00^{b}	460.30^{d}	370.10^{e}	321.40^{f}	±41.74
Mean Weight Gain (g)	289.80^{a}	258.90^{c}	260.00^{b}	240.00^{d}	190.00^{e}	171.70^{f}	±28.08
Mean Feed Intake (g)	330.00^{a}	318.00^{c}	324.00^{b}	310.00^{d}	302.00^{e}	280.00^{f}	±16.73
Mean Prot. Intake (g)	132.00	127.30^{c}	130.00^{b}	124.00^{d}	121.00^{e}	112.00^{f}	±14.00
Spec. Growth Rate (g)	0.61^{a}	0.60^{c}	0.61^{b}	0.58^{d}	0.40^{e}	0.34^{f}	±7.32
Per. Weight Gain (%)	45.00^{a}	43.00^{c}	44.00^{b}	42.00^{d}	41.00^{e}	40.26^{f}	±2.90
Feed con. Ratio(g)	0.84^{f}	0.89^{d}	0.87^{e}	0.92^{c}	1.11^{b}	1.22^{a}	±0.36
ProteinEffi. Ratio (g)	1.82^{a}	1.70^{c}	1.71^{b}	1.67^{d}	1.66^{e}	1.65^{f}	±0.06
Nitrogen Metabolism	4473^{a}	3971^{c}	4282^{b}	3953^{d}	3129^{e}	2828^{f}	±652
Condition Factor	0.02^{a}	0.02^{a}	0.02^{a}	0.01^{b}	0.01^{b}	0.01^{b}	±0.00

Mean on the same row with the same superscripts were significantly different ($P<0.05$)

Key:
CF=Commercial feed
WMC= Water melon cake
SEM= Standard error of means

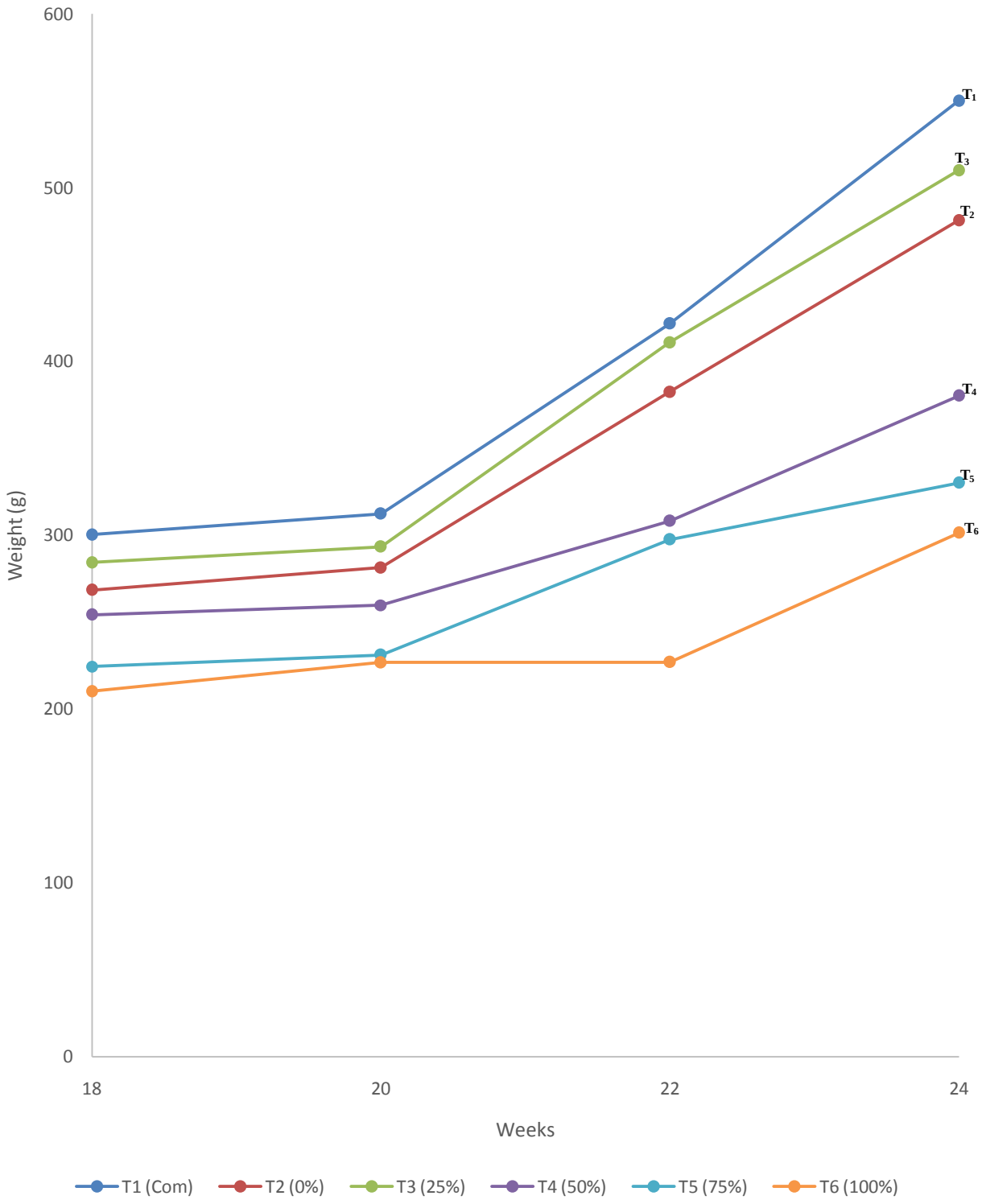


Figure 4.3: Biweekly growth pattern of *Clarias gariepinus* adults fed graded levels of watermelon seeds cake

4.12 Growth performance and nutrient utilization of *Clarias gariepinus* from fingerlings to adult

The figure shows bi-weekly growth pattern of *Clarias gariepinus* from fingerlings to adult growth stages.The figure result indicated that growth was highest with the commercial feed, followed by by the 25% water melon inclusion level to replace soy bean meal.The 0% replacement came third and was followed by 50%, 75% and 100% respectively.There was significant difference in the growth pattern exhibited by the six different types of feed used during the experiment (Figure 4.12).

4.13Trend line for final weight of *Clarias gariepinus* fed water melon seeds cake diet

From the results of the mean final weight, the highest growth performance and nutrient utilization of 550g was attained by the fish fed the commercial feed, followed by fish feed 25% with 510g water seeds cake inclusion level. Further increase in water melon diets to 50% (460.30g), 75% (370.10g) and 100% (321.40g) resulted in reduction in growth and nutrient utilization by the fish at all stages .The trend line of *Clarias gariepinus* fed water melon was 25% inclusion level beyond which growth continued to decline(Figure 4.13).

4.14Apparent digestibility of feed fed to *Clarias gariepinus* fingerlings

There is significant difference ($P<0.05$) in the APD, ADD and ACD of the commercial feed with that of 25% inclusion. In the experimental diets the values of APD, ALD, ADD and ACD reduces with increase of inclusion levels with significant difference ($P<0.05$) (Table4.14).

4.15Apparent digestibility offeed fed to*Clarias gariepinus* juveniles

The results obtained shows significant difference ($P<0.05$) in the APD, ADD and ACD of the commercial feed with that of 25% inclusion. In the experimental diets the values of APD, ALD, ADD and ACD reduces with increase of inclusion levels with significant difference ($P<0.05$) (Table 4.15).

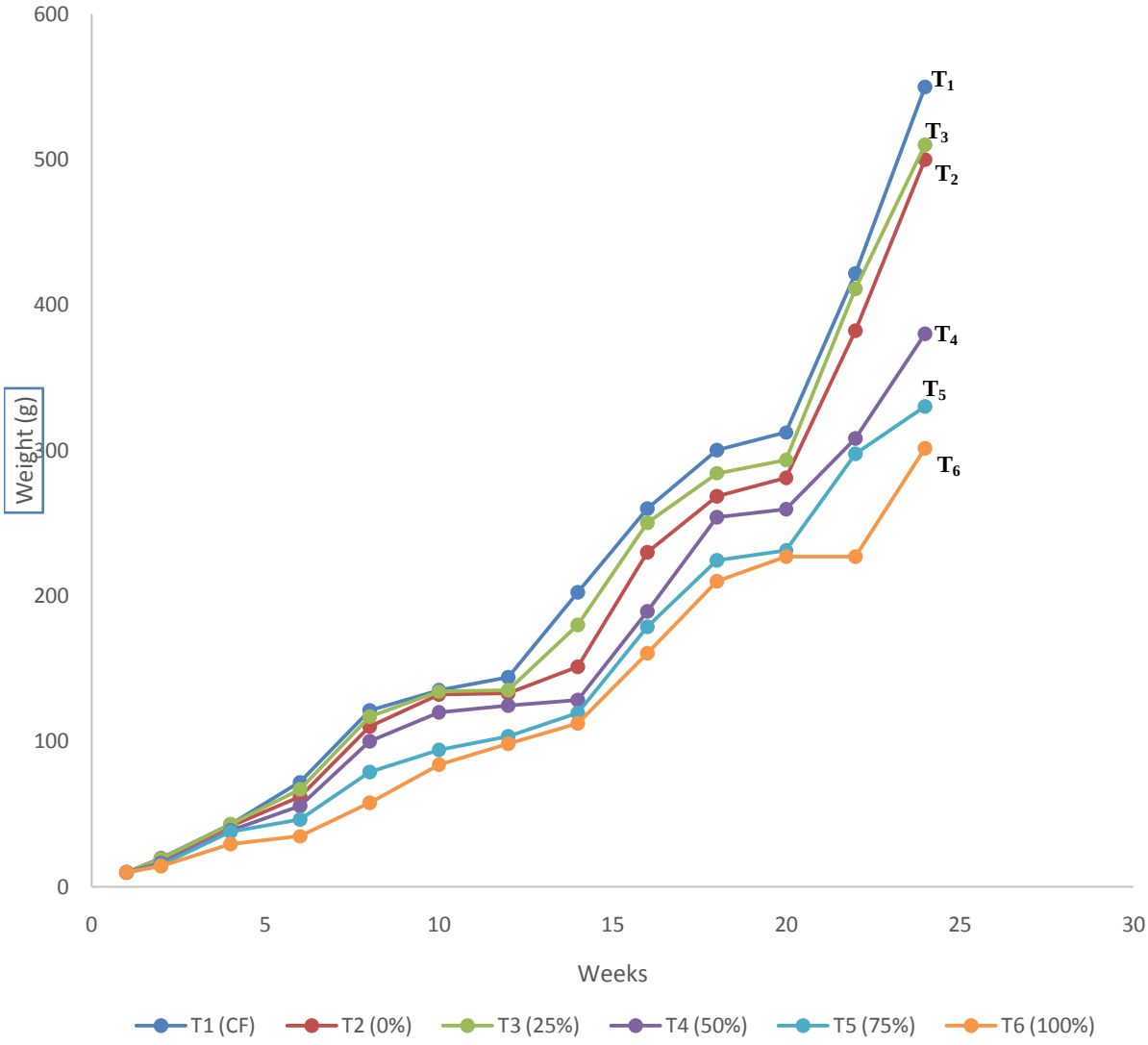


Figure 4.4:Biweekly mean growth pattern of *Clarias gariepinus* from fingerlings to adults stage fed graded levels of watermelon seeds cake

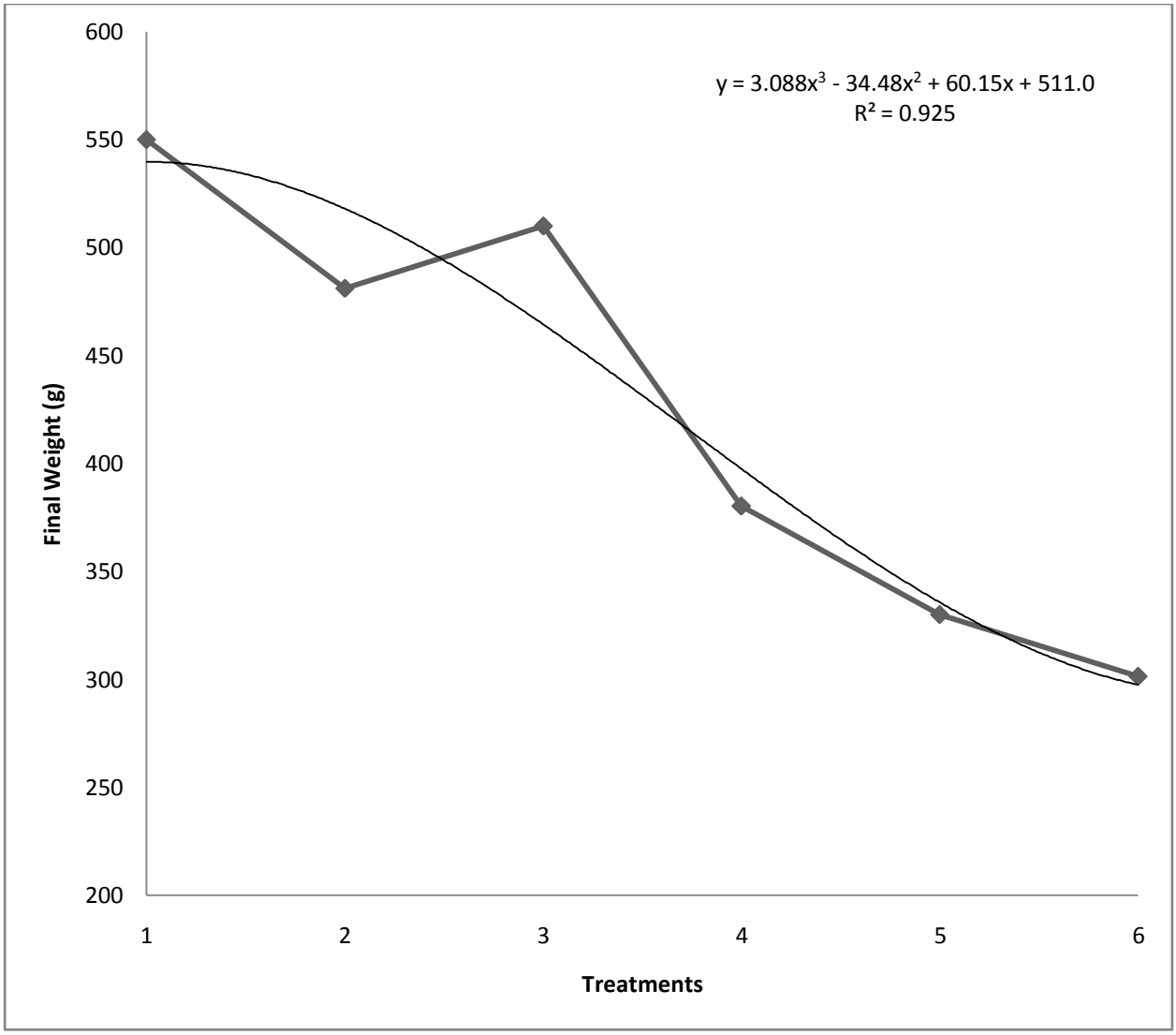


Figure 4.5: Trend line of mean final weight of *Clarias gariepinus*

Table 4.14: Apparent digestibility of the feed fed to *Clariasgariepinus* fingerlings

INDICES	**CF**	**0%**	**25%**	**50%**	**75%**	**100%**	**SEM**
APD	62.68^{a}	62.51^{ac}	62.58^{ab}	51.75^{d}	50.59^{e}	49.27^{f}	±0.56
ALD	51.76^{a}	50.74^{ac}	50.75^{ab}	50.65^{d}	49.34^{e}	49.12^{f}	±0.41
ADD	39.69^{a}	39.55^{c}	39.62^{b}	28.86^{d}	26.71^{e}	26.25^{f}	±0.64
ACD	40.81^{a}	40.36^{c}	40.80^{b}	38.37^{cd}	38.33^{de}	38.24^{ef}	±0.53

Means on the same row along the same superscripts were significantly different ($P<0.05$)
Key:
CF=Commercial Feeds
APD=Apparent Protein Digestibility
ALD= Apparent Lipids Digestibility
ADD= Apparent Dry matter Digestibility
ACD= Apparent Carbohydrate Digestibility

Table 4.15: Apparent digestibility of the feed fed to *Clariasgariepinus* juveniles

INDICES	**CF**	**0%**	**25%**	**50%**	**75%**	**100%**	**SEM**
APD	70.73a	70.68^{c}	70.72^{b}	68.95^{d}	68.63^{e}	68.38^{f}	±0.46
ALD	58.83^{a}	58.62^{c}	58.67^{b}	57.26^{d}	46.18^{e}	46.10^{f}	±0.52
ADD	49.68^{a}	47.54^{c}	48.58^{b}	37.73^{d}	35.68^{e}	33.96^{f}	±0.87
ACD	40.99^{a}	40.96^{c}	40.98^{b}	39.16^{d}	38.23^{e}	38.16^{f}	±0.57

Means on the same row along the same superscripts were significantly different ($P<0.05$)
Key:
CF= Commercial Feeds
APD= Apparent Protein Digestibility
ALD= Apparent Lipids Digestibility
ADD= Apparent Dry matter Digestibility
ACD= Apparent Carbohydrate Digestibility

4.16Apparent digestibility of feed fed to *Clarias gariepinus* adults

Values obtained revealed that, there is significant difference (P<0.05) in the APD, ADD and ACD of the commercial feed with that of 25% inclusion. In the experimental diets the values of APD, ALD, ADD and ACD reduces with increase of inclusion levels with significant difference (P<0.05) (Table 4.16)

4.17 Haematological parameters of *Clarias gariepinus* fingerlings

The result of heamatological parameters of *Clarias* gariepinus fingerlings is presented inTable.4.15. PCV, WBC and Hb shows there was no significant difference (P>0.05) in CF, 0% and25% respectively. Similarly, there was no significant difference (P>0.05) in RBC in the followingdiets, CF, 0% and 25%. In the experimental diets, inclusion level reduces the values of MCHCand MCH, while the values of LYM and MCV increases at 25% > 50% > 75% and > 100%respectively as shown in (Table 4.17).

4.18Haematological parameters of C*larias gariepinus* juveniles

The heamatological results shows that PCV, WBC and Hb had no significant difference (P>0.05) in the values obtained in CF, 0% and 25% respectively. Similarly, there was no significant difference (P>0.05) in RBC in the following diets, CF, 0% and 25%. In the experimental diets, inclusion level reduces the values of MCHC and MCH, while the values for LYM and MCV increased at 25% > 50% > 75% and > 100% respectively as shown in (Table 4.18).

Table 4.16: Apparent digestibility of the feed fed to *Clariasgariepinus* adult

	CF	0%	25%	50%	75%	100%	SEM
APD	80.66[a]	80.59[c]	80.65[b]	79.68[d]	78.54[e]	76.26[f]	±0.71
ALD	69.58[a]	69.38[c]	69.54[b]	58.77[d]	57.64[e]	56.79[f]	±0.46
ADD	67.82[a]	62.14[c]	64.43[b]	60.58[d]	59.18[e]	56.44[f]	±1.11
ACD	66.64[a]	66.43[c]	66.61[b]	57.48[d]	56.42[e]	55.35[f]	±2.05

Means on the same row along the same superscripts were significantly different ($P<0.05$)

Key:

CF=Commercial Feeds

APD= Apparent Protein Digestibility

ALD=Apparent Lipids Digestibility

ADD= Apparent Dry matter Digestibility

ACD=Apparent Carbohydrate Digestibility

Table 4.17: Haematological parameters of *Clarias gariepinus* fingerlings

INDICES (%)	**INI.VAL.**	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
PCV (%)	20.08	28.00^{a}	27.02^{ac}	27.80^{ab}	26.04^{d}	25.03^{e}	22.02^{f}	±0.65
WBC ($x10^{3}$)	10.64	24.82^{a}	24.80^{ac}	24.81^{ab}	23.80^{d}	23.20^{e}	23.14^{f}	±0.07
RBC ($10^{6}mm^{3}$)	2.82	6.98^{a}	6.81^{ac}	6.88^{ab}	5.34^{d}	5.22^{e}	4.01^{f}	±0.16
Hb (gdL-1)	12.10	15.79^{a}	15.73^{ac}	15.74^{ab}	14.71^{d}	13.68^{e}	13.60^{f}	±0.42
MCHC(gdL-1)	18.10	20.84^{a}	20.75^{ac}	20.78^{ab}	19.44^{d}	19.29^{e}	18.45^{f}	±0.53
MCH ($x10^{3}$)	17.12	21.70^{a}	21.66^{c}	21.68^{b}	20.38^{d}	19.36^{e}	18.24^{f}	±0.90
LYM (%)	15.21	36.65^{f}	36.64^{de}	36.65^{cd}	46.64^{c}	48.26^{b}	50.16^{a}	±0.73
MCV (gd-L-1)	16.42	42.66^{f}	42.48^{d}	42.52^{e}	52.45^{c}	52.36^{b}	52.75^{a}	±0.40

Means with different superscripts along the same row were significantly different ($P<0.05$)

Key:
CF=Commercial Feeds
PCV=Packed Cell Volume
WBC=White Blood Cell
RBC=Red Blood Cell
HB=Haemoglobin
MCHC=Mean Corpuscular Haemoglobin
MCH=Mean Cell Haemoglobin
MCV= Mean Cell Volume
LYM= Lymphocyte

Table 4.18: Haematological parameters of *Clarias gariepinus* juvenile

INDICES (%)	**INN.VAL.**	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
PCV (%)	20.08	44.01^{a}	41.12^{ac}	43.06^{ab}	40.10^{d}	38.04^{e}	34.23^{f}	±0.76
WBC ($10^{3)}$	10.64	33.81^{a}	33.70^{ac}	33.80^{ab}	32.27^{d}	32.23^{e}	22.27^{f}	±0.10
RBC ($10^{6}mm^{3}$)	2.82	8.76^{a}	8.66^{ac}	8.74^{ab}	8.45^{d}	7.37^{e}	7.18^{f}	±0.11
Hb (gdL-1)	12.10	17.78^{a}	17.54^{c}	17.71^{ab}	16.65^{d}	15.82^{e}	15.56^{f}	±0.42
MCHC(gdL-1)	18.10	34.62^{a}	34.28^{c}	34.31^{b}	23.13^{d}	21.54^{e}	20.48^{f}	±0.58
MCH ($10^{3)}$	17.12	35.45^{a}	33.67^{c}	34.23^{b}	32.23^{d}	21.46^{e}	20.21^{f}	±1.51
LYM (%)	15.21	45.56^{f}	45.59^{d}	45.54^{e}	55.50^{c}	59.37^{b}	60.14^{a}	±0.81
MCV (gd-L-1)	16.42	48.57^{f}	48.52^{ce}	48.55^{cd}	68.38^{c}	66.28^{b}	70.19^{a}	±0.37

Means with different superscripts along the same row were significantly different ($P<0.05$)

CF=Commercial Feeds
PCV=Packed Cell Volume
WBC=White Blood Cell
RBC=Red Blood Cell
HB= Haemoglobin
MCHC= Mean Corpuscular Haemoglobin
MCH=Mean Cell Haemoglobin
MCV= Mean Cell Volume
LYM= Lymphocyte

4.19 Haematological parameters of *Clarias gariepinus* adults

Values of PCV, WBC and Hb shows no significant difference (P>0.05) in the values of CF (40.74), 0% (40.56) and 25% (40.58) respectively. Similarly, there was no significant difference (P>0.05) in RBC in the following diets, CF (4.46), 0% (4.28) and 25% (4.44). In the experimental diets, inclusion level reduces the values of MCHC and MCH, while the LYM and MCV values increased at 25% > 50% > 75% and >100% respectively as shown in (Table 4.19).

Table 4.19: **Haematological parameters of *Clarias gariepinus* adult**

INDICES (%)	**INI.VAL.**	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
PCV (%)	20.08	55.21^{a}	51.12^{c}	53.34^{b}	40.62^{d}	38.54^{e}	36.45^{f}	±0.76
WBC ($x10^{3)}$	10.64	34.88^{a}	34.86^{ac}	34.87^{ab}	34.43^{d}	33.25^{e}	33.14^{f}	±0.14
RBC ($10^{6}mm^{3}$)	2.82	10.46^{a}	10.28^{ac}	10.44^{ab}	9.17^{d}	8.10^{e}	8.03^{f}	±0.10
Hb (gdL-1)	12.10	21.89^{a}	21.63^{c}	21.84^{ab}	20.49^{d}	19.72^{c}	19.31^{f}	±0.47
MCHC(gdL-1)	20.10	36.61^{a}	36.52^{bc}	36.54^{b}	35.62^{d}	34.63^{e}	33.85^{f}	±0.60
MCH ($x10^{3)}$	20.12	48.65^{a}	48.62^{ac}	48.64^{ab}	39.24^{d}	30.12^{de}	30.10^{df}	±1.56
LYM (%)	15.21	$50..10^{f}$	50.16^{e}	64.23^{d}	65.14^{c}	66.41^{b}	76.80^{a}	±0.92
MCV (gd-L-1)	16.42	64.71^{f}	69.18^{e}	68.50^{d}	84.53^{c}	89.83^{b}	90.38^{a}	±0.38

Means with different superscripts along the same row were significantly different ($P<0.05$)

Key:

CF=Commercial Feeds
PCV=Packed Cell Volume
WBC=White Blood Cell
RBC=Red Blood Cell
HB=Haemoglobin
MCHC=Mean Corpuscular Haemoglobin
MCH=Mean Cell Haemoglobin
MCV= Mean Cell Volume
LYM= Lymphocyte

4.20Carcass proximate composition of *Clarias gariepinus* fingerlings

Results of carcass composition of *Clarias gariepinus* fingerlings is presented in Table 4.20. The values of crude protein (30.48%), Lipid (3.12%) and NFE (5.79%) in fish fed with commercial feed were higher than in all the experimental diets. There was significant difference (P<0.05) of these values in the commercial feed compared to the other experimental feeds. Similarly, the crude protein (30.45%) and NFE (5.78%) in 25% inclusion level were higher than at 0%, 50%, 75% and 100% inclusion levels respectively. Crude protein (30.45%), Lipid (3.11%), ash (4.67%) and NFE (5.78%) decreases with increased inclusion level from 25% inclusion level as follows < 50 % < 75% and < 100%. On the other hand, the values of fibre (2.09%) and moisture (3.48%) increases with increased inclusion level as follow 25% > 50% > 75% and > 100% respectively as shown in (Table 4.16).

4.21Carcass proximate composition of *Clarias gariepinus* juveniles

The values of crude protein (44.92%), Lipid (5.56%) and NFE (11.56%) in fish fed with commercial feed were higher than in all the experimental diets. There was significant difference (P<0.05) of these values in the commercial feed compared to the other experimental feeds. Similarly, the crude protein (44.90%), Lipid (5.47%), ash (6.70%) and NFE (11.55%) in 25% inclusion level were higher than at 0%, 50%, 75% and 100% inclusion levels respectively. Crude protein (44.90%), Lipid (5.47%), ash (6.70%) and NFE (11.55%) decreases with increased inclusion level from 25% inclusion level as follows < 50 % < 75% and < 100%. On the other hand, the values of dry matter (17.97%), fibre (2.66%) and moisture (6.28%) increases with increased inclusion level as follow 25% >50% >75% and >100% respectively as shown in (Table 4.21).

Table 4.20: Carcass proximate composition of *Clarias gariepinus fingerlings*

Indices (%)	**Initial val.**	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
DM	12.14	15.05^{f}	15.16^{d}	15.10^{de}	15.73^{bc}	15.78^{b}	15.81^{a}	±0.15
C P	28.26	30.48^{a}	30.35^{c}	30.45^{b}	30.31^{d}	28.54^{e}	28.27^{f}	±0.42
Lipid	1.40	3.12^{a}	3.10^{ac}	3.11^{ab}	2.61^{d}	1.88^{e}	1.67^{f}	±0.27
Ash	3.86	4.69^{a}	4.46^{ac}	4.67^{ab}	3.69^{d}	3.51^{e}	3.48^{f}	±0.25
Fibre	2.14	2.18^{cf}	2.20^{ce}	2.19^{ed}	2.25c	2.38b	2.69a	±0.10
Moisture	3.25	3.46f	3.53d	3.48e	4.34c	4.42b	4.49a	±0.21
NFE	4.48	5.77^{a}	5.74^{ac}	5.76^{ab}	4.84^{d}	4.62^{e}	4.59f	±0.24

Means with different superscripts along the rows were significantly different (P<0.05).

Key:
DM = Dry matter
CP = Crude protein
CF= Commercial Feeds
NFE = Nitogen free extract

Table 4.21: Carcass proximate composition of *Clarias gariepinus Juveniles*

Indices (%)	**Initial Value**	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
DM	12.14	14.68^{ef}	14.70^{d}	14.69^{e}	15.75^{bc}	15.88^{b}	16.45^{a}	±0.31
C P	28.26	44.92^{a}	44.89^{c}	44.90^{ab}	43.68^{d}	42.42^{e}	41.72^{f}	±0.57
Lipid	3.40	5.56^{a}	5.35^{c}	5.47^{b}	4.29^{d}	3.26^{e}	3.19^{f}	±0.45
Ash	3.86	5.73^{a}	6.32^{c}	6.70^{ab}	5.57^{c}	5.53^{ce}	5.50^{f}	±0.20
Fibre	2.34	2.60f	2.76d	2.66e	2.84c	2.90^{ab}	2.98a	±0.06
Moisture	4.25	6.25f	6.32d	6.28e	6.78c	6.82^{ab}	6.85a	±0.12
NFE	8.48	11.56^{a}	11.27^{c}	11.55^{ab}	11.34^{d}	10.57^{e}	10.48^{f}	±0.20

Means with different superscripts along the rows were significantly different (P<0.0 5).

Key:

DM = Dry matter

CP = Crude protein

CF = Commercial Feeds

NFE = Nitogen free extract

4.22Carcass proximate composition of *Clarias gariepinus adults*

Values of crude protein (68.81%), Lipid (8.63%) and NFE (12.71%) in fish fed with commercial feed were higher than in all the experimental diets. There was significant difference ($P<0.05$) of these values in the commercial feed compared to the other experimental feeds. Similarly, the crude protein (68.69%), Lipid (8.62%) and NFE (12.69%) in 25% inclusion level were higherthan at 0%, 50%, 75% and 100% inclusion levels respectively. Crude protein (68.69%), Lipid (8.62%), ash (13.55%) and NFE (12.69%) decreases with increased inclusion level from 25% inclusion level as follows < 50 % < 75 % and < 100 %. On the other hand, the values of dry matter (17.56%), fibre (3.14%) and moisture (8.14%) increases with increased inclusion level as follow 25% >50% >75% and >100% respectively as shown in (Table 4.22).

4.23 Economic indices of water melon seed cake diets feed to *Clarias gariepinus*

The cost benefit ratio and net profit (1.00 and 323) were significantly higher in fish fed 25% water melon diet compared to the O%, 50%, 75% and 100%. The commercial feed was economically better than all the experimental diets.The 25% inclusion level of water melon diet could be used to replace soy bean meal due to the higher level of economic benefit obtained at the 25% replacement level in the diet of *Clarias gariepinus* in this study as shown in (Table 4.23).

Table 4.22: Carcass proximate composition of *Clarias gariepinus* adult

Indices (%)	**Initial value**	**CF**	**WMC 0%**	**WMC 25%**	**WMC 50%**	**WMC 75%**	**WMC 100%**	**SEM**
D M	12.14	17.54f	17.75^{d}	17.56^{de}	18.65^{c}	19.13^{ab}	19.15^{a}	±0.31
C P	28.26	68.81^{a}	68.42^{c}	68.69^{b}	67.78^{cd}	66.77^{e}	66.27^{f}	±0.43
Lipid	3.40	8.63^{a}	8.61^{ac}	8.62^{ab}	6.10^{d}	5.58^{e}	5.49^{f}	±0.65
Ash	3.86	13.56^{a}	13.50^{ac}	13.55^{ab}	12.81^{f}	12.93^{e}	12.95^{d}	±0.14
Fibre	2.34	3.07f	3.18d	3.14e	3.20^{ac}	3.20^{ab}	3.36a	±0.04
Moisture	4.25	8.10^{f}	8.18d	8.14e	8.56c	8.78b	8.46a	±0.11
NFE	8.48	12.71^{a}	12.53^{c}	12.69^{b}	11.45^{d}	11.43^{de}	11.39^{f}	±0.27

Means with different superscripts along the rows were significantly different (P<0.05)

Key:

CF =Commercial Feeds

NFE = Nitrogen free extract

DM=Dry matter

CP= crude protein

Table 4.23:Economic indices of Water melon Diets fed to *Clarias gariepinus*

PARAMETERS	**CF**	**0%**	**25%**	**50%**	**75%**	**100%**
Mean Cost of Feed/kg (₦)	320	320	300.	280	240	200
Mean Feed Intake (g)	330	318	320	310	302	280
Cost of Fingerlings (₦)	30	30	30	30	30	30
Mean Total Expenses(₦)	350	350	320	310	270	230
Mean final Weight (g)	550	500	510	380	330.10	301.40
Cost of Fish/kg (₦)	700	700	700	700	700	700
Mean Yield Cost (₦)	385	350	357	266	231	211
Mean Net Profit (₦)	350	315	323	236	204.10	188
Benefit Cost Ratio	1.00	1.00	1.00	0.68	0.67	0.66
Incidence of Cost (₦)	0.84	0.72	0.82	0.61	0.59	0.58

CF: Commercial Feed

CHAPTER FIVE

5.0 DISCUSSION

5.1Proximate Composition of Water Melon Seed Cake

Analyses obtained shows that the water melon seed cake have higher values of crude protein, lipid and ash than the raw water melon seeds meals. This could be attributed to the release of volatile materials in the cake which led to the concentration of the high protein, lipid and ash and reduction in dry matter, moisture, carbohydrate and fibre. This findings disagrees with that of Samiya El-Safy *et al.* (2012) who reported that toasted water melon seed cake have highercrude protein (32.68±32) crude lipid (14.05±12, crude fibre (3.75±0.09) ash (3.75±11) and carbohydrate (17.62±02). The Increase in the hours of thermal application to 40 minutes in the water melon cake resulted in reduction of crude protein, ash and nitrogen free extract due to the intense heat application which is associated with the denaturing of nutritional contents of water melon cake. This finding is buttressed by Fagbenro*et al.* (2013) who reported reduction of crude protein (27.48%), lipid (3.80%), ash (5.50%),nitrogen free extract (16.45%) compared to raw pumpkin seeds. The raw water melon seed meal had the lowest crude protein (28.34%, ash (2.98%) nitrogen free extract (75.91%) and lipid (2.24%) but highest fibre (7.12%) which could be due to the lack of application of any processing methods to reduce their contents. Braide *et al.* (2012) made similar findings on the lack of thermal application on water melon seeds. The moisture content of the water melon cake reduced substantially from raw water melon seeds which suggest better keeping quality of the feed which may be associated to the gradual removal of moisture by heat. The value was highest in raw water melon seeds (5.10%) and this might be due to lack of thermal treatment. These

findingagreed with that of Fagbenro *et al.* (2013) who reported similar findings on processed and raw sun flower and sesame seeds.

5.2Anti-Nutritional factors of Water Melon Seed Cake

The anti-nutritional compound in water melon seed cake shows that tannins, oxalate, phytate, alkaloid, cyanide and saponins were higher in the raw water melon seeds compared to the values recorded in the 20, 30 and 40 minutes. These variations could be due to differences in thermal treatment of the water melon seeds.Tannin in the 20 minutes water melon seeds cake sample was lower than in the raw water melon seeds reported by Johnson *et al.* (2012). This could be due to the fact that, heat application in water melon seeds reduces the level of tannin. Tannin is known to have the ability to reduce the digestibility and palatability of protein because they have form insoluble complexes. The lowest level of cyanide was obtained from water melon cake compared to the raw water melon seeds. Low concentration of the cyanide (0.25±0.03g) could be attributed to the inhibitory ability of heat on the cyanogens. Braide *et al*.(2012) reported lower concentration of cyanide (0.03±0.00g) in water melon seeds when heated for 60 minutes. The significant level of reduction in the oxalate (0.47±0.01g) composition of water melon cake compared to the raw water melon seeds (0.61±0.01g) could be due to reduction in the oxalate activities of the seed by heat application. Fagbenro *et al.* (2013) reported areduction in the concentration of oxalate due to heat application in sunflower and sesame meal based diets. The significant reduction in phytic acid (0.44±0.02g) composition in the water melon cake sample in comparison with raw (1.08±0.02g) samples could be associated with reduction of phytate through the heating process. Similar findings was reported by Abdulwahab *et al.* (2011). Saponins have bitter taste

and can reduce plant palatability, some of which are toxic to cold blooded animals up to 3-4g/100g as reported by FAO.(2014). However a value of 0.70±0.02g was obtained in the water melon cake which was lower than the toxic level reported by FAO.(2014).

5.3 Essential and Non Essential Amino Acids of Watermelon Cake

There were significant difference in the essential amino acids among the water melon seeds cake and raw water melon seeds. The water melon seed cake have the best essential amino acids. The methionine was (6.88g/100g), Leusine (6.84g/100g) and Lysine (6.89g/100g) were the most concentrated amino acids in water melon cake toasted for 20 minutes. Samiya-El-Safy *et al*.(2012) reported lower values for methionine, leusine and lysine as 5.82g/100g, 1.29g/100g and 3.41g/100g respectively.These variations could be as a result of the different processing methods used in the various studies. The lowest concentration of the essential amino acids in water melon seed were those of phenylalanine (4.20g/100g) and threonine (3.38g/100g) and this result is similar to that of Erbas, (2008) who reported reduced concentration of phenylalanine (4.21g/100g) and threonine (3.39g/100g) in lupin seeds.. Raw water melon seeds had the lowest essential amino acids which could be due to its inert nature and this finding is similar to that of Johnson *et al.* (2012) who reported lower concentration of essential amino acids in unprocessed water melon seeds.The sulfur amino acids, though a bit high (methionine and cysteine) but were found to be insufficient to meet the requirement for protein nutrition of *Clarias gariepinus*, hence, this indicates that there was the need for supplementation of the meals containing water melon seeds with methionine + cysteine due to low sulphur amino acids contents. This position is supported by Scipp, (2008) who

reported the need for supplementation of feed containing low concentration of methionine and cysteine.

Glutamic acid, proline and aspartic acids are the most concentrated non-essential amino acids in water melon cake. Similar result was also reported by Martins *et al.*(2007) who observed high level of glutamic, proline and aspartic acids due to mild heat treatment on the water melon seeds. The raw water melon seeds had the lowest non-essential amino acid due to its inert nature and this finding was supported by Gabriel *et al.* (2007) on locally produced fish feed.

5.4 Some Mineral Contents of Water Melon Seeds Cake

The significant difference in the mineral contents observed in the water melon cake processed for 20 minutes compared to that of the raw water melon seeds could be as a result of the mild heat treatment which did not denature the mineral content of the seeds (Johnson *et al.*, 2012). *C. gariepinus* growth at fingerlings, juveniles and adults growth stages were enhanced due to high levels of potassium (9.89g/100g), calcium (9.78g/100g), manganese (9.80g/100g), magnesium (8.80g/100g), sodium (6.15g/100g) and phosphorus (8.19g/100g) which met their minerals requirement levels compared to the raw water melon seeds which had lower values of potassium (9.32g/100g), calcium (9.39g/100g), manganese (8.42g/100g), magnesium (8.44g/100g), sodium (5.58g/100g) and phosphorus (7.30g/100g) which affected the growth of the fish (Braide *et al.*, 2012). Micro- minerals such as copper (4.90g/100g), zinc (5.62g/100g, and iron (3.68g/100g) were within the physiological requirement of *C.gariepinus* at all stages of growth compared to the raw water melon seeds which had lower values of copper (3.30g/100g),

zinc (5.03g/100g) and iron (2.60g/100g) which did not meet the needs of the fish (Valverde,2011).

5.5 Physico-Chemical Water Quality Parameters

The physico-chemical parameters of water in the fingerlings, juveniles andadults*Clarias gariepinus*tanks shows that, dissolved oxygen, pH, temperature and ammonia are within the recommended ranges for rearingcat fishes, particularly *Clarias* spp.For instance the optimum pH range of 5-9, temperature range of 27-30, dissolved oxygen of 5mg/l and above and not more than 0.06mg/l ammonia in water culture for fingerlings, juveniles and adult*Clarias gariepinus* observed in this study has been an acceptable range for the culture of *Clarias* sp.(Fish Doc.,2004; Mesner and Geiger, 2005; Steven, 2009).

5.6 Growth Performance and Nutrient Utilization in *Clarias gariepinus*

The growth performance of fish fed the commercial feed in the fingerlings, juveniles and adults growth stages of *Clarias gariepinus* gave the highest values (121.50g, 260.20g and 550. 00g) of fish growth in comparison with the experimental diets. Mean weight gain and protein efficiency ratio are known to be the most important indices for measuring fish response to experimental diets and very reliable indicators of growth (Adewolu (2008).Fish fed 25% water melon seed cake inclusion level had the best Mean Weight Gain (MWG) and Protein Efficiency Ratio (PER)of 117.10g and 1.56g in fingerlings, 250.40g and 1.66g in juveniles and, 510.00g and 1.71g in adults in comparison to other inclusion levels. The reduction in growth in the 50%, 75% and 100%inclusion levels of water melon seed cake diets could be due to improper balance of essential nutrients such as amino acids, minerals andpresence of some anti-nutritional compounds in the diet.Decrease in palatability and indigestibility due to high fibre content which may have

led to reduced growth and poor food efficiency (Sogbeson and Ugwumba, 2006; Sotolu, 2008). Hossain *et al.* (2003)and Adewolu (2008) reported that high inclusion of plant protein in the diets of *Clarias gariepinus* suppressed growth significantly.The fish fed 25% water melon seed cake has higher growth performance in comparison to the soy bean used as control. This could be due to the high level of methionine (6.88/100g), leucine (6.84g/100g), lysine (6.89g/100g) and some minerals such as potassium (9.89g/100g), sodium (6.15g/100g) and magnesium (8.80g/100g) which are higher in water melon cake and are known to boost growth compared to soy bean meal methionine (0.54g/100g), leucine (2.16g/100g), lysine (2.60g/100g) and some minerals such as potassium (0.07g/100g), sodium (0.45g/100g) magnesium (2.68g/100g) (Ayinla, 2003). The good performance of the feed in terms SGR values, FCR,MPI andNM values in the 25% water melon seed cake diets in fingerlings, juveniles and adults of *Clarias gariepinus* confers it with better advantages over the rest of the experimental diets. This findings are similar to that of Chapman (2000) and Babalola and Apata (2006) who reported that the higher SGR and the lower feed conversion ratio (FCR).Chien and Chiu (2003)reported that a lower FCR value implies poor feed utilization by fish.The fish fed 25% water melon diets showed overall good performance in terms of Mean Final Weight (MFW), Mean Weight Gain (MWG), Percentage Weight Gain (PWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio(PER), Mean Protein Intake (MPI) and Nitrogen metabolism (NM) which could be attributed to low molecular weight of metabolites, improved palatability of the feed,better digestibility of the feed and lowerfibre content. This indicates that 25% water melon cake diets could replace soy bean meal in the diet of *Clarias gariepinus* fingerlings, juveniles and adults without

any deleterious effect on the growth performance. Mean final weight, MWG, PWG, SGR, FCR, PER, MPI and NM in fish fed 50%, 75% and 100% water melon diets decreased significantly ($p>0.05$) with increase in water melon diets in fingerlings, juveniles and adults growth stages,this implies that fish fed 50%, 75% and 100% the water melon diets generally had gradual decline in growth in comparison to those fed 25% inclusion levels. This could be due to the presence of anti-nutrients and high fibre content of the feed as the percentages of water melon increased in the diets. Fagbemi (2007) reported similar findings on fluted pumpkin seeds flour and Akande *et al.* (2010) on plant protein sources.

5.7Water Melon Digestibility in*Clarias gariepinus*

A higher percentage of crude protein digestibility (62.58%) was observed in fingerlings of C*larias gariepinus*fed water melon seed cakeat 25% inclusion level. Similarly, lipid digestibility, dry matter and carbohydrate were higher in the 25% than in 50%, 75% and 100%.This implies that greater amount of nutrients were available to the fish from the WMC at 25%.This finding is similar to the report of Phonekhampheng and Lindberg(2008).

The higher inclusion of WMC in the diet of C*larias gariepinus* at fingerlings, juveniles and adult growth stages decreased lipid digestibility.This may be associated with the increase in non- starch polysaccharides content, which reduces absorption of fat into the gastro intestinal tract. This agrees with the report of Yuan *et al*. (2010). The low carbohydrates digestibility at higher inclusion levels of water melon seed cake may be attributed to the presence of non-starch polysaccharides content and poor digestibility by the fish.It may also be due to the reduction in transit time of the feed as they pass through

the gastro intestinal tract due to the presence of dietary fibre, which is part of the carbohydrate component of plant ingredients and most fish cannot utilize it. This finding is similar to that of Valverde (2011).

5.8 Haematological Response of *Clarias gariepinus*to Water Melon Diets

The haematological data obtained in this study were within the recommended physiological requirement for C*larias gariepinus*. The packed cell volume (PCV), red blood cells (RBC) and haemoglobin (Hb) of fishes fed the commercial, control and 25% water melon seed diet were significantly different (P>0.05) in comparison to those fed 50%, 75% and 100%. The red blood cells (RBC) decreased as the level of water melon feed increased in the diet.Similar finding was reported by Pharm *et al.* (2007) and Adewolu and Adamson (2012). The commercial feed and diets containing 25% WMC had the highest RBC value of 51.12% and 53.34% respectively in comparison to the values of 40.62%, 38.54% and 36.45% in fish feed containing 50%, 75% and 100% respectively. The lymphocyte count increased as the level of water melon increased in the diet. The highest count of 76.80% was observed in fish fed diet containing 100% WMC and lowest value of 50.10% and 50.16% were observed in the fish fed the commercial and 25% diets. The mean corpuscular haemoglobin concentration (MCHC) increased with increase in WMC in the diet of all the experiments. The mean corpuscular volume (MCV) increased with increase in WMC in the diets with the values of 69.18%, 68.50%, 84.53%, 89.83% and 90.38% in 0%, 25%, 50%, 75% and 100% respectively.

White blood cells and lymphocytes increased as the inclusion levels of water melon diets increased at fingerlings, juvenile and adult growth stages. The high count of WBC and lymphocytes are responses to microbial infection or the presence of foreign body or

antigen in the circulating system(Brainde *et al.*,2012, and Barak *et al.*, 2014). Furthermore,the increase in WBC and lymphocytes as inclusion levels of water melon diets increased in the fingerlings, juveniles and adults growth stages of *Clarias gariepinus* could be due to residual anti-nutrients compounds present in the feed and also increase in indigestible substances in the feed as a means of combating stress in the body system of the fish. This may consequently stressed the fish thereby suppressing the immune system and results into increased susceptibility to diseases(Johnson *et al*., 2012).

The mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) of fish in this study at the fingerlings, juvenile and adult growth stages are lower in fishes fed 50%, 75% and 100% water melon diets in comparison to 25% inclusion level. Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) are useful in the diagnosis of anaemia in most animals (Madu *et al*., 2000, Ochang *et al.*, 2007 and Fagbemi *et al*., 2013).

5.9 Carcass Composition of *Clarias Gariepinus*

The proximate composition of the carcass of C*larias gariepinus* in this study indicates that the crude protein, lipid, dry matter, ash, fibre, moisture and nitrogen free extract were higher in all the treatments at fingerlings, juveniles and adult growth stages in comparison with the initial values.The water melon feed fed at 25% to the fish fingerlings gave the highest dry matter (14.13%), crude protein (30.45%), lipid (3.11%), ash (4.67%) and nitrogen free extract (5.78%) in comparison to the other inclusion levels. Sotolu and Faturoti (2010) reported higher carcass proximate composition of *Clarias gariepinus* fingerlings values of protein (44.44%) and lipid when fed with *Leucana leucocephal*.

This difference could be due to the use of fish oil and vegetable oil in the conduct of the experiment. Similarly, the water melon fed at 25% to juveniles gave the highest dry matter (17.97%, crude protein 44.90%, lipid 5.47% and ash 6.67%.Phonekhampheng *et al*., 2008) reported that the carcass proximate composition of *Clarias gariepinus* fingerlingsincludeprotein (66.06%), lipid (11.89%), ash (0.48) and dry matter (82.51%).This difference could be due to the type of plant protein sources used in the two experiments. The carcass composition was higher in the adult *Clarias gariepinus* fed 25% water melon feed..This finding disagrees with Abu*et al*. (2010) who reported thevalue of protein value of (65.29%), lipid (6.69%), ash (13.27%) and dry matter (20.10%). The moisture content in this study is closely related to the weight gain and inversely related to the percentage of body fat content. Fish fed the higher water melon meals inclusions levels of 50%, 75% and 100% at the fingerlings, juveniles and adult growth stages recorded the lowest weight gain, least fat and protein contents. This showed that the feed was poorly digested due to high levels of residual anti-nutrients.Similar finding was reported by Babalola and Apata (2000) on growth pattern of *Heterobranchus longifilis*fed varying inclusion levels of protein and lipids.

Fish fed meals 50%, 75% and 100% inclusion levelof water melon diets had lower body moisture and lipid contents. The reduction in lipid content is probably due to poor feed intake which resulted in starvation and in turn led to mobilization of body lipid reserves to meet energy needed for vital body functions(Adewolu, 2008). The presence of residual saponin and other inhibiting compounds may also have aided to inhibit pancreatic lipase activity and therefore delayed intestinal absorption of dietary fat (Adeyemo 2003).

5.10 Economic viability of WatermelonSeed Cake Diets in the growth of*Clarias gariepinus*

The total cost of producing water melon diets and purchasing other ingredients systematically reduced from N320.00k to N230.00k from 25% to 100%, while it was N350.00k in the control which had no water melon ingredients. This clearly shows a reduction in the cost of the diets *Clarias gariepinus* when fed with alternative plant sources such as water melon diets. Similarly, Abu *et al.*(2010) reported a reduction in the cost of diets of *Clarias gariepinus* fed cassava feed meal.Mean gross profit of fish was considerably higher (N357.00k) in the 25% water melon inclusion level diets when compared to the control (N350.00K), 50% (N266.00K), 75% (N231.10K) and 100% (N211.00K) inclusion levels. These findings may be associated with better growth and nutrient utilization of water melon diets at 25% replacement of soy bean meal than at other inclusion levels. Johnson *et al*., (2012) reported similar findings on the evaluation of anti-nutrients of water melon. The net profit parameters indicated that, the highest accruable profit of N323:00k was at 25% water melon inclusion level diets and the least was at 100% with N188:00k. The chance of obtaining a more variable difference of higher value of net profit at 25% inclusion level over and above those of the control, 50%, 75% and 100% levels indicate that, more profit would be feasible from fish produced at 25% water melon inclusion level. The best cost benefit obtained in diet containing 25% water melon diet is an indication that the economic viability of feeding *Clarias gariepinus* is cheaper at 25% replacement of soy bean meal with water melon diets. The benefit cost ratio (BCR) value of 1.00 portends that *Clarias gariepinus* production accrues more profit at 25% inclusion level of water melon diet. The value of (N1.00) simply means that every N1.00 invested in *Clarias gariepinus* production will

yield N1.73k. According to Olagunju *et al.* (2007) when benefit cost ratio is greater than one or equal to one indicates profit, but less than one shows lack of viability or unprofitability of the venture. Since the ratio in the diet is equal to one, therefore it is considered that, replacing soybean meal with water melon meal is a profitable venture, especially at 25% replacement level.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendations

6.1 Summary

The various processing methods employed for making water melon seedcake, toasting, soaking and boiling imparted some chemical changes in the nutritional composition of water melon seed meal and resulted in a significant decrease in the anti-nutritional factors. The water melon seeds thermal treatment at 20 minutes was recommended as suitable for use after it was established that the processing method reduced the anti-nutritional factors to a significant level and had better nutrient compounds such as amino acids, lipids, minerals, ash and nitrogen free extracts when compared to toasted, soaked boiled and raw seeds. In addition, the water melon seedswould not need prolonged thermal treatment before it can be suitable for use as fish feed.

The water quality parameters were within the range recommended for optimum growth of*Clarias gariepinus*. There were no significant differences (P>0.05) in nutrient utilization between the commercial feed and 25% inclusion level. The commercial, 25% and 0% groups had the highest mean weight gain (500g to 550g) than the groups that received replacement inclusion levels at 50%, 75%, while the group that received 100% water melon meal had the lowest mean weight gain (321.40g).

Feed conversion ratios (FRC) was 0.86g at the 25% inclusion level, 0.89g at the 0% level, 0.92g at 50%, 1.11g at 75% and 1.22g at the 100%; these values increased with the increase in inclusion levels.There were no significant difference (p> 0.05) between the commercial feeds, 25% and 0% water melon meal, but significant (P<0.05) between groups with 50%, 75% and 100% levels of diet. The best feed conversion ratio (FCR)

(0.86g) was recorded for *Clarias gariepinus* fed 25% water melon meal, which indicated a superior level of nutrient utilization of the water melon inclusion levels by the fish. There was no significant difference ($p>0.05$) in the protein efficiency ratio of *Clarias gariepinus* fed commercial feeds, 0% and 25% water melon, but significant differences ($P<0.05$) was established in fish fed 50%, 75% and 100% water melon meal.

There was no significant difference ($P>0.05$) in the SGR of fish fed commercial feed, 0%, and 25% water melon seed meal; the opposite was the case with fish fed 50%, 75% and 100% inclusion levels.

There was no significant difference ($P>0.05$) in the nitrogen metabolism of fish fed commercial feed, 0%, and 25% water melon meal; while fish fed 50%, 75% and 100% inclusion levels varied considerably..

There was no significant difference ($P>0.05$) in the apparent digestibility co-efficient of protein, lipid, dry matter and carbohydrates of the commercial feeds, control and 25% inclusion levels of water melon seed cake diets. There was significant difference ($P<0.05$) in the apparent digestibility co-efficient of protein, lipid, dry matter and carbohydrates as the inclusion levels of water melon meal in the diets increased from 50%-100% levels.

The packed cell volume (PCV) ranged from 27.80g/100g-53.34g/100g, white blood cells (24.81g/100g-34.88g/100g, red blood cells (RBC) ranged from 6.88g/100g10.44g/100g and haemoglobin 15.74g/100-21.84g/100g, and these reduced as the level of water melon inclusion in *Clarias gariepinus* increased in the diet and are significantly different

(P<0.05) mean cell volume and lymphocytes count increased as the level of water melon inclusion levels increased in the diet and are also significantly different (P<0.05).

The MCHC and MCH values in this study was their highest in *Clarias garienpinus* fed 25% watermelon meals in comparison to inclusion at higher levels (50, 75 and 100%).There was a significant difference (P<0.05) in the effects of the inclusion levels of water melon seed diets on the final carcass composition, such as protein, lipid, ash and dry matter amongst all the treatments fed various replacement of soy bean meals.

The highest cost benefit ratio and net profit (1.00 and N323.00k) in *Clarias gariepinus* fed 25% water melon inclusion varied significantly(P<0.05) from *Clarias gariepinus* fed 50%, (N236.00k) 75% (N204.10k) and 100% (N188.00k). Water melon seed cake could replace soybean meal at 25% inclusion level due to the highest economic return achieved at 25% replacement level in the diet of *Clarias gairepinus* culture from fingerlings to adult growth stages in 180 days as revealed by these research findings.

6.2 Conclusions

i. The processing method of toasting water melon seed for 20 minutes gave the highest crude protein value of 32.68% and reduced fibre content to 5.48%. There was reduction in the following anti-nutritional compounds such as tannin by 74%, oxalate (95%), phytate (89%), alkaloid (93%), cyanide (63.24%) and saponin (52%). Similarly, water melon seed toasted at 20 minutes gave higher values for essential and non essential amino acids, and minerals.

ii. Thermal treatment of water melon seed and feeding it at 25% inclusion levelenhanced the apparent digestibility coefficient by 80.65% in adults, 70.72% in juveniles and 62.85% in fingerlings.

iii. The growth performance and feed utilization of C*larias gariepinus* fingerlings, juveniles and adults fed at 25% inclusion levelgave the best weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio,with percentage weight gain of 91.50%, in fingerlings, 48.00% juveniles and 44.00% in adults respectively.

iv. Best haematological parameters were observed at inclusions in 25% level in the fingerlings, juveniles and adults growth stages.This was a clear indication that water melon at that inclusion level had no deleterious effect on the physiology of the fish. Similarly, the best crude protein value of 30.45% fingerlings, 44.90% juveniles and 68.69% in adults were recorded at 25% inclusion level.

v. The following economic indices, mean final weight (510.00g), mean net profit N 323:00k,benefit cost ratio1.00, incidence of cost N 0.82k, clearly indicated that the 25% inclusion level was the best.

6.3RECOMMENDATIONS

i. Due to soft seed coats of water melon seeds, it is recommended that thermaltreatment should not exceed 20 minutes in order to obtain better proximate composition, anti nutritive content, amino acids and minerals concentration for fish feed production.

ii. Frequent monitoring of water quality parameters and provision of aeration would ensure good water quality for fish production in plastic tanks.

iii. For best growth, the inclusion of water melon seed meals in the diet of C*larias gariepinus*should not exceed 25% inclusion level of substitution with soybean meals.

iv. The use of water melon seed meals to replace soybean meals at 25% gave better haemotology and carcass composition of *Clarias gariepinus.*

v. Economic analyses showed that 25% replacement of soybean with water melon gave highest profit compared to the rest of the inclusion levels.

vi. Additional studies on water melon thermal time as from 25-29 minutes is to further reduced the anti-nuritional factors is recommended.

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APPENDICES

Appendix 1: Analysis of variance for proximate composition of water melon cake

Sources of variation	df	MS	P-value		LOS
Dry matter	5	9.876	0.0001		*
Crude protein	5	7.654	0.0001	*	*
Crude lipid	5	8.450	0.0001		*
Ash	5	8.531	0.0001		*
Carbohydrate	5	8.452	0.0001		*
Moisture	5	0.862	0.0001		*
Nitrogen free extract	5	8.873	0.0001		*

df = degree of freedom, Ms = mean sum of square, * = P<0.05, LOS = level of significance

Appendix 2: Analysis of variance for anti-nutrient content of water melon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Tannin	5	0.456	0.0001	*
Oxalate	5	0.438	0.0001	*
Phytate	5	0.354	0.0001	*
Alkaloid	5	0.286	0.0001	*
Cyamide	5	0.357	0.0001	*
Saponins	5	0.785	0.0001	*

df = degree of freedom, Ms = mean sum of square, * = P<0.05, LOS = level of significance

Appendix 3: Analysis of variance for essential amino acids concentration of water melon cake

Sources of variation	**df**	**dfMS**		**P-value**	**LOS**
Methionine	5	5	5.631	0.0001	*
Arginine tryptophan	5	5	4.524	0.0001	*
Crude lipid 5	5	5	3.462	0.0001	*
Valine	5	5	5.723	0.0001	*
Isoleucine	5	5	3.545	0.0001	*
Leucine	5	5	5.866	0.0001	*
Lysine	5	5	5.876	0.0001	*
Histidine	5	5	3.682	0.0001	*
Threonine	5	5	3.548	0.0001	*
Phenylalanine	5	5	3.349	0.0001	*

df = degree of freedom, Ms = mean sum of square, * = P<0.05, LOS = level of significance

Appendix 4: Analysis of variance for non-essential amino acids concentration of water melon cake

Sources of variation	df	df	MS	P-value	LOS
Tyrisine	5	5	3.861	0.0001	*
Alanine	5	5	6.350	0.0001	*
Cysteine		5	2.304	0.0001	*
Glutamic	5	5	9.260	0.0001	*
Proline	5	5	8.126	0.0001	*
Aspartic	5	5		0.0001	*
		6.745			
Serine	5	5	5.694	0.0001	*
Glusine	5	5	3.491	0.0001	*

df = degree of freedom, Ms = mean sum of square, * = P<0.05, LOS = level of significance

Appendix 5: Analysis of variance for some mineral concentration of water melon seed cake

Sources of variation	df	df	MS	P-value	LOS
Magnesium	5	5	7.864	0.0001	*
Manganese	5	5	7.893	0.0001	*
Calcium	5	5	8.843	0.0001	*
Sodium	5	5	5.609	0.0001	*
Phosphorus	5	5	6.678	0.0001	*
Copper	5	5	3.490	0.0001	*
Zinc	5	5	3.360	0.0001	*
Iron	5	5	3.352	0.0001	*
Potassium	5	5	8.970	0.0001	*

df = degree of freedom, Ms = mean sum of square, * =P<0.05, LOS = level of significance

Appendix 6: Analysis of variance for growth performance of *Clarias gariepinus* fingerlings fed various inclusion levels of water melon cake

Sources of variation	**df**	**df**	**MS**	**P-value**	**LOS**
Initial mean weight	5	5	0.00026	0.0001	NS
Final mean weight	5	5	10.604	0.0001	*
Mean weight gain	5	5	11.208	0.0001	*
Percentage weight gain	5	5	0.0537	0.0001	*
Mean feed intake	5	5	0.2104	0.0001	*
Mean protein intake	5	5	0.2001	0.0001	*
Specific growth rate	5	5	0.0379	0.0001	*
Feed conversion ratio	5	5	0.0898	0.0001	*
Protein efficiency ratio	5	5	0.0679	0.0001	*
Nitrogen metabolism	5	5	8.9702	0.0001	*
Condition factor	5	5	0.0319	0.0001	NS

df = degree of freedom, Ms = mean sum of square, * = P<0.05, NS =Not significant, LOS=Level of significance

Appendix 7: Analysis of variance for growth performance of *Clarias gariepinus* juveniles fed various inclusion levels of water melon cake

Sources of variation	**df**	**df**	**MS**	**P-value**	**LOS**
Initial mean weight	5	5	1.317	0.0001	*
Final mean weight	5	5	3.919	0.0001	*
Mean weight gain	5	5	3.470	0.0001	*
Percentage weight gain	5	5	2.847	0.0001	*
Mean feed intake	5	5	3.464	0.0001	*
Mean protein intake	5	5	2.415	0.0001	*
Specific growth rate	5	5	2.652	0.0001	*
Feed conversion ratio	5	5	2.436	0.0001	*
Protein efficiency ratio	5	5	2.257	0.0001	*
Nitrogen metabolism	5	5		0.0001	*
		30.677			
Condition factor	5	5	0.0004	0.0001	NS

df = degree of freedom, Ms = mean sum of square, * = P<0.05, NS = Not significant, LOS=Level of significance

Appendix 8: Analysis of variance for growth performance of *Clarias gariepinus* adults fed various inclusion levels of water melon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Initial mean weight	5	3.230	0.0001	*
Final mean weight	5	7.210	0.0001	*
Mean weight gain	5	5.560	0.0001	*
Percentage weight gain	5	4.350	0.0001	*
Mean feed intake	5	3.760	0.0001	*
Mean protein intake	5	3.140	0.0001	*
Specific growth rate	5	2.680	0.0001	*
Feed conversion ratio	5	2.120	0.0001	*
Protein efficiency ratio	5	2.076	0.0001	*
Nitrogen metabolism	5	4.268	0.0002	*
Condition factor	5	0.002	0.0001	NS

df = degree of freedom, Ms = mean sum of square, * = P<0.05, NS = Not significant, LOS= Level of significance

Appendix 9: Analysis of variance for apparent digestibility of *Clarias gariepinus* fingerlings fed various inclusion levels of water melon seed cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Apparent protein digestibility	5	8.142	0.0001	*
Apparent lipid digestibility	5	6.431	0.0001	*
Apparent dry matter digestibility	5	5.672	0.0001	*
Apparent carbohydrate digestibility	5	4.846	0.0001	*

df = degree of freedom, Ms = mean sum of square, * = P<0.05, LOS = level of significance

Appendix 10: Analysis of variance for apparent digestibility of *Clarias gariepinus* juveniles fed various inclusion levels of water melon seed cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Apparent protein digestibility	5	8.336	0.0001	*
Apparent lipid digestibility	5	6.642	0.0001	*
Apparent dry matter digestibility	5	5.783	0.0001	*
Apparent carbohydrate digestibility	5	4.932	0.0001	*

df = degree of freedom, Ms = mean sum of square, * = P<0.05, LOS = level of significance

Appendix 11: Analysis of variance for apparent digestibility of *Clarias gariepinus* adults fed various inclusion levels of water melon seed cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Apparent protein digestibility	5	8.678	0.0001	*
Apparent lipid digestibility	5	7.452	0.0001	*
Apparent dry matter digestibility	5	6.648	0.0001	*
Apparent carbohydrate digestibility	5	5.434	0.0001	*

df = degree of freedom, Ms – mean sum of square, * - P<0.05, LOS = level of significance

Appendix 12: Analysis of variance for haematological composition of *Clarias gariepinus* fingerlings fed various inclusion levels of water melon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Packed cell volume	5	4.123	0.0001	*
White blood cell	5	4.018	0.0001	*
Red blood cell	5	1.086	0.0001	*
Mean corpuscular haemoglobin cell	5	2.243	0.0001	*
Mean cell haemoglobin	5	3.867	0.0001	*
Mean cell volume	5	5.886	0.0001	*
Lymphocytes	5	6.682	0.0001	*

df = degree of freedom, Ms – mean sum of square, * - P<0.05, L0S = level of significance

Appendix 13: Analysis of variance for haematological composition of *Clarias gariepinus* juveniles fed various inclusion levels of water melon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Packed cell volume	5	5.342	0.0001	*
White blood cell	5	5.156	0.0001	*
Red blood cell	5	2.110	0.0001	*
Mean corpuscular haemoglobin cell	5	3.645	0.0001	*
Mean cell haemoglobin	5	4.224	0.0001	*
Mean cell volume	5	5.897	0.0001	*
Lymphocytes	5	6.886	0.0001	*

df = degree of freedom, Ms – mean sum of square, * - P<0.05 , LOS = level of significance

Appendix 14: Analysis of variance for haematological composition of *Clarias gariepinus* adults fed various inclusion levels of watermelon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Packed cell volume	5	6.786	0.0001	*
White blood cell	5	6.243	0.0001	*
Red blood cell	5	2.378	0.0001	*
Mean corpuscular haemoglobin cell	5	4.562	0.0001	*
Mean cell haemoglobin	5	5.338	0.0001	*
Mean cell volume	5	6.078	0.0001	*
Lymphocytes	5	6.009	0.0001	*

df = degree of freedom, Ms – mean sum of square, * - P<0.05 , LOS = level of significance

Appendix 15: Analysis of variance for proximate carcass composition of *Clarias gariepinus* fingerlings fed various inclusion levels of watermelon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Dry matter	5	8.094	0.0001	*
Crude protein	5	9.896	0.0001	*
Crude lipid	5	3.202	0.0001	*
Ash	5	6.480	0.0001	*
Crude fibre	5	2.046	0.0001	*
Moisture	5	2.968	0.0001	*
Nitrogen free extract	5	3.475	0.0001	*

df = degree of freedom, Ms – mean sum of square, * - $P<0.05$, LOS = level of significance

Appendix 16: Analysis of variance for proximate carcass composition of *Clarias gariepinus* juveniles fed various inclusion levels of watermelon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Dry matter	5	8.478	0.0001	*
Crude protein	5	9.986	0.0001	*
Crude lipid	5	3.482	0.0001	*
Ash	5	6.590	0.0001	*
Crude fibre	5	2.857	0.0001	*
Moisture	5	2.979	0.0001	*
Nitrogen free extract	5	3.568	0.0001	*

df = degree of freedom, Ms – mean sum of square, * - $P<0.05$, LOS = level of significance

Appendix 17: Analysis of variance for proximate carcass composition of *Clarias gariepinus* adults fed various inclusion levels of watermelon cake

Sources of variation	**df**	**MS**	**P-value**	**LOS**
Dry matter	5	8.645	0.0001	*
Crude protein	5	9.995	0.0001	*
Crude lipid	5	3.678	0.0001	*
Ash	5	7.123	0.0001	*
Crude fibre	5	3.244	0.0001	*
Moisture	5	4.086	0.0001	*
Nitrogen free extract	5	4.562	0.0001	*

df = degree of freedom, Ms – mean sum of square, * - $P<0.05$ LOS = level of significance