GROWTH PERFORMANCE AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* (TEUGELS) FED DIFFERENT DIETARY LEVELS OF SOAKED *BAUHINIA MONANDRA* (LINN.) SEED MEAL AND SUN-DRIED LOCUST MEAL (*SCHISTOCERCA GREGARIA*)

By

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DECLARATION

I declare that the work in the dissertation entitled **'GROWTH PERFORMANCE AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* (TEUGELS) FED DIFFERENT DIETARY LEVELS OF SOAKED *BAUHINIA MONANDRA* (LINN) SEED MEAL AND SUN-DRIED LOCUST MEAL (*SCHISTOCERCA GREGARIA*)'** was undertaken by me, in the Department of Biological Sciences under the supervision of Profs. S.J. Oniye, J. Auta, C.A.M. Lakpini and Dr. F.O. Abeke.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

This dissertation entitled "GROWTH PERFORMANCE AND FEED UTILIZATION OF *CLARIAS GARIEPINUS* (TEUGELS) FED DIFFERENT DIETARY LEVELS OF SOAKED *BAUHINIA MONANDRA* (LINN) SEED MEAL AND SUN-DRIED LOCUST MEAL (*SCHISTOCERCA GREGARIA*)" by Balogun, Bose Ibiloye meets the regulations governing the award of the degree of Doctor of philosophy (Ph.D Fisheries) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the loving and evergreen memory of my late father Prince Bisi Isioye. May Almighty Allah Grant him Aljanah Firdaus. Ameen.

ABSTRACT

This study was conducted to evaluate the suitability of soaked *Bauhinia* seed meal (*Bauhinia monandra* Linn.) and sun-dried locust meal as alternative protein sources for *Clarias gariepinus* and to determine whether the use of such unconventional diets could help in reducing the overhead cost of feed inputs in aquaculture. The proximate analysis of the diet, carcass composition of fish, amino acid assay and anti-nutrients were determined using standard methods. Data for each parameter were subjected to analysis of variance (ANOVA) while means of results were compared at 5% level of significance. A preliminary study was conducted to determine the processing method that reduced anti-nutrients to the minimum level. Boiled, toasted and soaked seeds were used. Soaked *Bauhinia* seed meal (SBSM) was discovered to have maximum reduction of anti-nutrients. The result of the proximate and anti-nutrients analysis differed significantly ($P<0.05$) in all the methods. Two experimental diets were formulated to contain SBSM and locust meal (LM) at 0%, 25%, 50% and 75% of the total dietary protein. All diets were isonitrogenous (40% crude protein) and isocaloric (3212kcal/kg). A 12 weeks feeding trial was conducted using juveniles which were randomly distributed among 24 improvised non-recirculatory and semi-flow through indoor plastic tanks of 52cm X 34cm X 33.5cm at a stocking rate of 10 fish per tank and three (3) replicates per treatment. The experimental design was complete randomized. The fish were fed at 5% body weight, twice daily. Diets with higher inclusion levels of SBSM and LM (diets 3 and 4) significantly depressed growth performance of fish [Specific growth rate (SGR): 1.48, 0.22] and SGR (0.57 and 0.56) respectively in both groups compared to diet 1, 2 and 5 (1.67, 0.85 and 1.84) respectively for both groups. The relative levels of anti-nutrients present in SBSM diet after processing may have contributed to the poorer growth performance of these groups, while the presence of chitin may have contributed to poorer growths in the group of fish fed LM.

The results suggested that SBSM and LM can be used to substitute up to 25% levels of dietary protein in *C. gariepinus* juveniles without significant reduction in growth. The results of the specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER), apparent net protein utilization (ANPU), protein efficiency ratio (PER), condition factor (K) and the percentage survival ratio (PSR) were significantly different ($P<0.05$) in both experimental diets. Based on the relative cost of diets per unit weight gain and protein gain, diet 2 (25% SBSM and 25% LM) were most economical. The variations observed in the SGR, FCR, FER, ANPU, GFCE and PER were associated with the anti-nutrients present in the diets, these parameters reduced with increasing levels of SBSM and LM in the diets of fish. Therefore, more investigations need to be carried out to determine feasible and simple methods for inactivating/reducing the anti-nutrients for efficient inclusion of SBSM and LM in practical diet for *C. gariepinus* at higher levels.

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ACRONYMS/ABBREVIATIONS

AA	-	Amino Acid Profile
ANF	-	Anti Nutritional Factors
EAAI	-	Essential Amino Acid Index
CP	-	Crude Protein
CF	-	Crude Fibre
L	-	Lipids
DM	-	Dry Matter
NFE	-	Nitrogen Free Extract
Raw BSM	-	Raw *Bauhinia* Seed Meal;
BBSMx	-	Boiled *Bauhinia* Seed Meal at Xminutes
TBSMx	-	Toasted *Bauhinia* Seed Meal at Xminutes
SBSMx	-	Soaked *Bauhinia* Seed Meal at Xhours
SGR	-	Specific Growth Rate
FCR	-	Feed Conversion Ratio
FER	-	Feed Efficiency Ratio
ANPU	-	Apparent Net Protein Utilization
PER	-	Protein Efficiency Ratio
K	-	K factor
PSR	-	Percentage Survival Ratio
GFCE	-	Gross Feed Conversion Efficiency
MFBW	-	Mean Final Body Weight
MWG	-	Mean Weight Gain
PLWG	-	Percentage Live Weight Gain
MFSL	-	Mean Final Standard Length
MLG	-	Mean Live Weight Gain
PLG	-	Percentage Live Weight Gain

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Nigeria with an estimated surface area of 12.5m.ha of freshwater, lakes, reservoirs, and ponds (Ita *et al.,* 1984) is capable of producing $512x10^3$ metric tonnes of fish annually unfortunately, the country has not succeeded in attaining fish–food sufficiency (FAO, 1990). Inspite of efforts by succeeding governments through several agricultural programmes, the desired objective of improving fisheries sub-sector has not been achieved by the government. However, private enterprenuers have invested in fish farming as the only enterprise that can provide an alternative solution to viable and sustainable fish protein production (Ayinla and Bekibele, 1992; Adesulu, 2001; Omitoyin, 2007). The low productivity which is reflected in low catch per unit effort in the inland water bodies (rivers and lakes) are due primarily to over exploitation as a result of overpopulation and indiscriminate fish farming practices (Adesulu, 2001). Some problems associated with decline in fish catch include:

(i) Utilization of unregulated mesh sizes, which allow overexploitation of all sizes and ages of fish,

(ii) Pollution due to the use of chemicals in fishing, which invariably leads to massive mortality of fish in large water bodies, and

(iii) The use of explosives which also leads to huge economic losses (Adesulu, 2001).

The Nigerian population of over 140 million has necessitated gross importation of frozen fish from foreign countries in an attempt to bridge the escalating gap between internal fish production and demand in the country. The annual fish demand in Nigeria was estimated at 1.5 million metric tonnes (FDF, 1998 and FMAWR, 2007) with an

exsiting demand-production gap of 1.0 million metric tonnes (Adekoya, 2001). The importation of fish has however become a very expensive venture considering the foreign exchange costs involved (Ajayi, 1979). For instance, Nigeria imported fish worth over 500 million US dollars in 2005 (FMAWR, 2007).

The human demand for food fish is expected to rise from the current consumption level of about 90 million metric tonnes (Mtn) to about 110Mtn by the year 2010. The share of aquaculture in the total world food fish production is set to increase from 29% in 1996 to 38% by the year 2010 (FAO, 1997). Aquaculture has become the fastest growing food production sector of the world, with an average annual increase of about 10% since 1984 as compared to 3% increase for livestock meat and 1.6% increase for capture fisheries (FAO, 1997). To sustain such high rates of increase in aquaculture production, a matching increase in the levels of production of fish feeds is required (Francis *et al;* 2001a). Estimates of the global levels of aqua-feed production for the year 2000 vary widely from 4.5 – 16.8Mtn (FAO, 1997).

One of the major factors militating against fish farming in Nigeria has been lack of adequate feed that are formulated to meet the nutrient requirements of culturable fish species (Olarinde, 2005; Olaniyi, 2009a). 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 (Jamu and Ayinla, 2003; Omitoyin, 2007) due to high cost of fish feed ingredients particularly fishmeal. 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 tons of quality fish feeds are imported into the country each year (AIFP, 2004). Utilization of such commercially formulated feeds increases the cost of production thereby reducing the profit margin of fish farmers. This ultimately translates to high cost of fish. For

instance, feed represents a high proportion (50-80%) of variable cost of production (Eyo, 1990; Helfrich and Craig, 2002)

The growth and nutritional status of fish depends on the quality and quantity of dietary protein in its meal. Fishmeal, the best conventional protein source in fish diet, is expensive and scarce. A balanced fish diet contains all the essential nutrients in the appropriate quantities with energy forming the bulk while protein constitutes the most expensive item in such formulated feed (Annune and Oniye, 1993). Nutritionists give priority to protein since it is the single ingredient needed in largest quantity for growth and development (Lovell, 1981). Thus in formulating fish feed, consideration is given to the protein requirements of the fish in question, the protein constituent and the amino acid profile of the feed stuffs to be incorporated in the feed. Fish meal compared with other commercially used protein sources have high biological value, it is highly palatable, contains high digestible energy, rich in lysine and methionine, rich in minerals and vitamins, and perhaps, unidentified growth factors required by fish (Lovell, 1981; Helfrich and Craig, 2002; Eyo, 2003).

1.2 Research Problems

In order to sustain the high growth of the aquaculture industry, it is imperative to increase fish feed production (Francis *et al*., 2001) because fish feed accounts for 60-80% of variable cost of production (New, 1993). The high cost and fluctuating price of fish meal as well as its uncertain availability has led to the need to identify alternative protein sources for fish feed. Numerous emphases have been directed at the use of conventional plant protein sources such as soybean (Shiau *et al.,* 1987; Eyo, 1990; Sadiku and Jauncey, 1995); groundnut (Eyo, 2003); cotton seed (El-Sayed, 1990); sun flower (Tacon *et al*., 1984). Soybean has been shown to be effectively utilized by fish than any other plant

protein sources (Lim and Dominy, 1989). Groundnut cake has also been reported to be highly utilized for efficient growth of fish (Eyo, 1994). However the scarcity of these plants sources and competition from other sectors for such conventional crops for livestock and human consumption as well as industrial use make their costs too high and places them far beyond the reach of fish farmers or producers of aqua-feeds (Ayinla, 1988; Fasakin *et al.,* 1999; Omitoyin*,* 2007). Therefore, in an attempt to attain a more economically sustainable and viable production, research interest has been directed towards the evaluation and use of unconventional or lesser utilized protein sources, particularly from plant products such as seeds, leaves and other agricultural by-products (Ayinla, 1988; El-Sayed, 1999; Banyigyi *et al.,* 2001a and b; Anhwange *et al*., 2004, 2005; Olaniyi, 2009a).

Previously feedstuffs like maize (*Zea mays*), sorghum (*Sorghum bicolor*), soybeans (*Glycine max*), groundnut (*Arachis hypogea*) and fishmeal were used in preparing fish pellets. However, now they face serious competition from man and his livestock as food sources in Nigeria. Since the use of fish meal in fish feed formulation is not cost effective, efforts are being directed globally towards discovering unconventional, cheaper, readily available and highly digestible alternative protein sources of feedstuff for fish. However attempts at searching for these unconventional sources as supplement or total replacement is a difficult task (Ayinla, 1988).

In Nigeria one of the alternative plant protein sources that have been extensively investigated for possible use as ingredients in fish feed formulation are legumes which include Jackbean (Alegbeleye *et al*., 2001); Bambara nut (Bayingyi *et al*., 2001a and b); Lablab bean (Adeparusi and Eleyinmi, 2004); Castor oil seed (Agboola, 2004); Senna seed (Umar, 2006) and Locust bean (Tamburawa, 2010).

Alternative animal protein sources used in Nigeria to supplement feeds for fish production include the use of maggots, termites and earthworms in formulating fish feed (Adekoya, 2001). In China, Silk worm pupae have been used traditionally to feed fish while in Western Thailand maggots produced from pig manure have been used as fish feed (Madu and Ufodike, 2003).

In view of all the aforementioned, this research intends to explore the potentials of using graded levels of *Bauhinia monandra* and locust, (*Schistocerca gregaria*) as protein sources in the diet of juvenile *Clarias gariepinus* (Teugels).

1.3 Justification

Despite the fact that most seeds are readily available, their use in animal feed formulation is usually restricted, due to the presence of one or more endogenous anti-nutrients (Udoessien and Ifon, 1992; Francis *et al*., 2001; Eyo, 2003; Ezekiel, 2004; Tamburawa, 2010). Non reduction or destruction of these components by processing normally have adverse effects on the nutritional value of the seeds (Udoessein and Ifon, 1992). *Bauhinia monandra* plants are readily available and there is no competition between man and livestock for the seeds. The plants are found existing in most places as ornamental plants. Large quantities of *Bauhinia monandra* seeds are available during the fruiting season throughout the savannah region of Nigeria, but underutilized since they drop off to the ground from explosive dispersal from dehisced mature pods during the fruiting season. There is no documentation regarding the use of processed seed of *B. monandra* in compounding animal diet. However, information regarding the anti-metabolic constituent of *Bauhinia* seeds in relation to effects of processing is scanty.

Lack of comprehensive compositional data regarding essential nutrients and anti-nutrient compositions of the seed of several wild indigenous plants have limited the

prospects of their utilization as livestock feed in Nigeria. Anhwange *et al.,* (2004; 2005) reported that the percentage composition of proteins, lipids, carbohydrates and fibre in *B. monandra* were 33.09, 28.70, 21.45 and 3.25 respectively. The amino acid composition in form of lysine, phenylalanine, leucine, isoleucine, methionine, valine, threonine and cysteine were 2.86, 3.77, 2.13, 2.31, 1.54, 3.54, 2.70 and 1.11g/100g protein respectively. The seed also contain 11.5mg/100g phytate, 0.32mg/100g hydrogen cyanide, 6.0% tannins and 2.05% saponins; and that based on the nutritional component of *Bauhinia* seed it offers tremendous potentials as an attractive substitute for traditional protein sources in the diet of man and his livestock provided the toxicants in the seed were adequately removed or reduced to a tolerable limit (Anhwange *et al*., 2004; 2005).

Grasshopper as animal protein have been used in fish feed formulation. However research work on locust as animal feed is scarce. Locust has approximately 24% crude protein which makes it a potential source of animal protein in formulation of fish diets (FAO, 2004). Locusts are usually present in periods of outbreaks and plagues. They are usually found in the north central states of Nigeria where they are sold in markets and consumed in large quantities. People and birds often eat locust but the level of consumption is not usually enough to significantly reduce populations over large areas since there could be as many as 40 million locusts per sq km in areas of invasion (FAO, 2004). Thus in order to avoid wastage, their potential for inclusion as protein source in animal feed with particular reference to fish could be exploited. The use of locust meal could therefore be economical and advantageous if incorporated in fish feeds without compromising growth and feed conversion (FAO, 2004).

The choice of *Clarias gariepinus* among other fish species was due to the fact that the fish specie is found in nearly all fresh water bodies in Nigeria. They could be cultured in small water bodies and they also have attributes of being good converters of feed

(FAO/IFAD, 1987). The fish are also cultured due to their tolerance to low dissolved oxygen, rapid growth rate, acceptability of a wide variety of food items, hardy and disease resistant, ability to spawn in captivity and respond to induced breeding (Madu, 1995; Adesulu, 2001; Omitoyin, 2007). The fish is in high demand, highly priced, and with high economic returns either as fresh or smoked/dried (Banyigyi *et al*., 2001).

1.4 Aim and Objectives of the Study

1.4.1 Aim

The aim of this study is to use soaked *Bauhinia* seed meal and sun-dried locust meal as alternative protein sources to partially replace fish meal in the diet of *Clarias gariepinus* (Teugels) and to evaluate their effects on growth performance and feed utilization of the fish

1.4.2 Objectives

The specific objectives of this work are

- To determine the effect of different processing methods on the proximate, amino acid and anti-nutrients contents of *Bauhinia monandra* seeds.
- To determine the growth response and feed utilization of *Clarias gariepinus* juveniles fed with graded dietary levels of locust and processed *Bauhinia* seed meal.
- To determine the effects of locust and the processed *Bauhinia* seed meal based diets on the carcass composition of fish.

1.5 Hypotheses

- Same quantities of anti-nutrients and proximate components exist in the raw and processed *Bauhinia* seeds.

- *Clarias gariepinus* shows the same growth response and feed utilization when fed different levels of *Bauhinia* seed and locust diets.
- Various levels of diets have no effect on the carcass composition of *Clarias gariepinus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General

In the wild, fish obtain food naturally from aquatic environment; this food may be phytoplankton or zooplanktons, insects, seeds and small fish. However, under culture condition the natural feeds are not adequate for optimum growth therefore there is need for supplementary feeds to help fortify the naturally available diet with extra protein, carbohydrate, lipid, minerals and vitamin (Alatise and Okoye, 1979; Lim and Dominy, 1989).

The quality and quantity of feed used in fish culture are the major factors in determining profitability because feed represents the largest single expenditure in semi-intensive or intensive culture operations. Economical production depends on availability of least-cost nutritionally balanced diet (Lim and Dominy, 1989). In Nigeria the high cost of feed imputs is a major problem of fish farmers in intensive and semi-intensive fish farming culture system (Ayinla, 1988; Fagbenro and Davis, 2003). Nutrition is critical in fish production since it accounts for 40-80% of production cost (Igeofagha, 1979; Eyo, 1990; New, 1993; Prendagast *et al.*, 1994).

2.2 Nutrient Requirements of Fish

Lall (1991) and Helfrich and Craig (2002) indicated that proper nutrition is one of the major factors influencing ability of fish to attain genetic potential for growth, reproduction and longevity. Efficient production and growth of fish in the culture systems depends entirely on feeding complete feed at appropriate rate with due considerations to the dietary requirements of the fish which should not be exceeded (Ayinla, 1991).

Formulated or artificial diets may either be complete or supplemental. Complete diets supply all the nutrients (proteins, carbohydrates, fats, vitamins and minerals) necessary for the optimal growth and health of fish. Generally, the basic nutrient composition of fish feed include protein (18-50%), lipid (10-25%), carbohydrate (basal diet) 15-20%, ash (< 8.5%), phosphorus (< 1.5%), water (< 10%), vitamins and minerals. Amongst these nutrients energy forms the bulk or basal diet while protein constitutes the most expensive item in formulated diets. These key nutrients determine the scale of production of a fish diet while the rest of the nutrients promote the efficiency of utilization of these two nutrients (Annune and Oniye, 1993).

Fish are normally provided with complete diets when reared in high density indoor systems or confined in cages and cannot forage freely on natural feeds (Helfrich and Craig, 2002). However, supplemental (incomplete, partial) diets are fed only to help support the natural food (insects, algae, small fish) that are naturally available in fish ponds or outdoor raceways. Supplemental diets do not contain full complement of vitamins and mineral, although they are used to help fortify the naturally available diet with extra protein carbohydrate and lipids (Helfrich and Craig, 2002).

The main objective of fish feed formulation is to put together raw materials (feed ingredients) that will provide nutritionally balanced feed for fish. This is actually aimed at providing nutrients for rapid fish growth so as to enhance optimal production at low feed cost (Ayinla, 1991; Annune and Oniye, 1993). The formulation of fish diets therefore needs an understanding of the nutrient requirement of different fish species in relation to age, feeding habits, production objectives and physical state; and nutrient composition of the feed stuffs and the level of associated anti-nutritional factors and feed contamination in such feed stuff.

2.3 Dietary Protein Requirement for fish

Fish require much higher dietary crude protein, 16-60%; EIFAC (1971); 20-60% Hastings (1976); 35-55%; NRC (1982); 24-27% Tacon (1987); 30-56% Lim and Dominy (1989) and 18-50% Helfrich and Craig (2002) and lower dietary energy in comparism to other animals (Lim and Dominy, 1989). Thus protein requirement is given high priority in any nutritional study since it is the single nutrient that is needed in the largest quantity for growth and development and also because it is the most expensive ingredient in the diet (Lovell, 1989 and NRC, 1993). This implies that fish feeds should be carefully formulated to ensure that the protein fraction does not exceed the optimum level required by the fish in order to minimize wastage. The protein requirements of fish vary for each fish species and with each life state (Lim and Dominy, 1989; Alcestes and Jory, 2000). Helfrich and Craig (2002) also indicated that wide variations in protein requirements of fish were due to differences in species (genetic composition), size of fish, water temperature, culture management (water exchange/water quality), feed allowance/rate, stocking rate, amount of non-protein energy, quality of the dietary protein. Availability of natural food and rearing environment.

Helfrich and Craig (2002) indicated that proteins are composed of carbon (50%), nitrogen (16%), oxygen (21.5%) and hydrogen (6.5%). The proteins are needed to supply amino acids and to make enzymes and hormones (Ensminger and Olentine, 1978). Helfrich and Craig (2002) however stated that protein is used for fish growth if adequate level of fats and carbohydrates are present in the diet. If not protein may be used for energy and life support rather than growth.The quality of protein is principally influenced by its amino acid composition. The findings of Helfrich and Craig (2002) indicated that protein level in aquaculture feeds generally average 28 – 32% for cat fish and 32 – 38% for tilapia.

Fry and fingerling fish require a diet higher in protein (which may frequently exceed 50% crude protein), lipids, vitamins and minerals and lower in carbohydrates because they are developing muscles, internal organs and bone with rapid growth (Alcestes and Jory, 2000).

Sub-adult fish require more calories of fats and carbohydrates for basal metabolism and a smaller percentage of protein for growth. Adult fish require lesser amount of protein however the amino acid which make up that protein need to be available in certain ratios. Maintainance diets may contain as little as 25 – 35% crude protein (Francis-Floyd, 2004) and food for grow-outs often approach or exceed 40% crude protein. Brood stock animals also require high protein and fat levels to increase the reproductive efficiency (Alcestes and Jory, 2000). However, the reports of Ayinla and Bekibele (1992), Alcestes and Jory (2000) and Francis and Floyd (2004) vary with the report of Ayinla (1988) which indicated that fingerling stages of *Clarias gariepinus* required 31 – 34% crude protein; juveniles 31 – 34% crude protein; adults, 40% crude protein and broodstock, 40% crude protein. Degani *et al.* (1989) and Machiels and Hunken (1985) also reported 40% crude protein requirement for broodstock of *Clarias gariepinus*.

2.4 Fishmeal/Alternative Protein Sources for Fish

Fishmeal a conventional animal protein source remains unequalled as a major source of protein in fish feeds because of its high nutritive value (rich amino acid profile) and palatability (Ayinla, 1988; Lim and Dominy, 1989). However, its escalating cost and unavailability has forced aquaculture nutritionists and feed manufacturers to use less expensive, readily available plant protein and animal protein as a substitute to fishmeal (Lim and Dominy 1989; Tacon, 1994). Alcestes and Jory (2000) and Abdel-Warith *et al.,* (2001) indicated that irrespective of associated set backs fishmeal still constitutes a

substantial part of the feed formula for tilapia and many other species cultivated at commercial levels.

Several plant and animal protein sources have been identified, investigated and utilized in domestic animal feeds but relatively few are used in fish feeds due to the high protein dietary requirements of fish (Lim and Dominy, 1989). Commercial aquaculture feeds for growouts contain 25-45% crude protein with a consequence that only high protein content plant feed stuffs such as oil seed residues are used in fish feed. The extent of plant protein utilization in commercial feeds as stated by Alcestes and Jory (2000) and Lim and Dominy (1989) depends on its availability, cost, acceptability by fish, ease of processing and nutritive value (adequate balance of essential nutrients), presence of toxins and antinutritional factors. Conventional plant protein sources include peanut (groundnut), sesame seed, soybean, sunflower seed and rapeseed. Non conventional plant protein sources that have been used in fish feed formulation include lima seed, jackbean seed, bambara nut, plants by-products and agro-industrial by-products. Based on the findings of several researchers (Ayinla, 1988; Lim and Dominy, 1989; Alcestes and Jory, 2000 and Eyo, 2003), soybean meal has been demonstrated to be the most commonly used and it often constitutes approximately 30-40% of fish feed.

Ayinla (1988) and Ayinla and Bekibele (1992) indicated that the major problems of alternative (plant and animal) protein sources are imbalanced amino acid contents and thus far the essential amino acid components of these protein sources are unequal to that of fish when used as single source of dietary protein in fish diets. Most plant proteins sources and animal protein sources particularly oil seeds, pulses (legumes), bone meal, blood meal are deficient in one amino acid or another. The presence of disproportionate levels of specific amino acids due to antagonisms like the leucine – isoleucine antagonisms as well as arginine – lysine antagonisms limits the utilization of the aforementioned protein

sources. Eventhough bloodmeal is rich in valine, lysine and histidine, the use of bloodmeal has shortcomings as a result of its poor methionine and isoleucine contents due to the antagonistic effect of excess leucine. Other alternative animal protein sources that have also been used to replace fish meal in the diet of fish include insects and their larvae, tadpole meal, bloodmeal, aquatic worms, fish silage, bone meal, poultry by-products, meat meal and dairy products. However attempts at using these alternative protein sources have generally yielded poor results in terms of poor growth and poor efficiency of feed utilization (Ayinla, 1988).

Alestes and Jory (2000) indicated that the use of plant proteins (a cheaper source of protein) in fish culture will continue to increase as aquaculture industries keep expanding with more focus on cheaper unconventional sources as against conventional sources like soybean.

2.5 Amino Acid Requirements for fish

All fish species require the 10 essential amino acids namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Feed stuff deficient in any of the 10 essential amino acids cause depressed appetite and consequently depressed growth rate of fish (Ayinla, 1988; Ayinla and Bekibele, 1992).

Among all the amino acids lysine and methionine are often the first limiting amino acids. Fish feeds prepared with plant protein (particularly soybean are typically low in methionine. Thus, extra methionine must be added to plant protein sources as supplements to augment the inadequacy and to promote optimal growth and good health. It is also important to note and match the protein requirements and amino acid requirements of each fish species reared for attainment of optimal growth (Helfrich and Craig, 2002).

Lall (1991) reported the level of amino acids in channel catfish (Clarias) and *Oreochromis niloticus* thus: phenylalanine 5.0 (1.2); tryptophan 0.5 (10.12); histidine 1.5 (0.4); arginine 4.3 (1.0); lysine 5.1 (1.2); valine 3.0 (0.71); isoleucine 2.6 (0.6); leucine 3.5 (0.8); threonine 2.5 (0.5) and methionine 2.3 (0.6) respectively.

Ayinla (1988) reported the essential amino acid requirements of *Clarias gariepinus juveniles* thus: arginine (5.8); histidine (3.9); isoleucine (5.3); leucine (12.3); lysine (8.6); methionine and cystine (-); phenylalanine and tryrosine (3.8); threonine (4.9); tryptophan (-) and valine (3.5).

In certain cases, the presence of non essential amino acids in diets represents a sparing effect which reduces the need for fish to synthesize them. (Lim and Dominy, 1989). The indispensible amino acid phenylalanine is readily converted to dispensible tyrosine in fish. Thus its dietary level requirement is dependent on tyrosine concentration (Wilson and Halver 1986).

Studies on utilization of several dietary sulphur compounds in channel catfish showed that D-L methionine was utilized as effectively as L-methionine by channel catfish (Robinson *et al.,* 1978). Wilson *et al*. (1978) also reported that channel catfish fed a tryptophan deficient diet (0.05%) for eight weeks had significantly poorer weight gain and feed conversion efficiency than individuals fed diets containing as low as 0.12% tryptophan (0.5% dietary protein).

Eyo (1988) reported that 0.2% L-Soybean diet gave the best growth performance in *Clarias anguillaris*. Similarly Eyo and Olatunde (1998) demonstrated that D-L methionine can substitute L-methionine in soybean diet for mud fish *Clarias anguillaris* fingerlings. Viola *et al.* (1982) reported that supplementation of soybean meal with both methionine and lysine improved Carp diets

Robinson *et al.* (1980) reports on growth study of channel catfish fingerlings indicated that tyrosine can replace or spare 50% of the total phenylalanine requirements. NRC (1983) however indicated that since most protein sources used in practical fish feed contain adequate levels of phenylalanine and tyrosine, the sum of the two amino acids normally exceed dietary needs.

Oresegun and Alegbeleye (2001) reported that the supplementation of 0.2% methionine improved the FCR and PER of diet containing 20% cassava peels fed to tilapia (*Oreochromis niloticus*).

2.6 Carbohydrates/Energy Requirements for fish

Carbohydrates (starches and sugar) are the most economical and inexpensive sources of energy for fish diets. Carbohydrates are included in aquaculture diets to reduce feed costs and for their binding activity during feed manufacturing. They are also used due to their natural abundance. In fish, carbohydrates are stored as glycogen that are mobilized when necessary to satisfy energy demands (Annune and Oniye 1993; Helfrich and Craig, 2002).

Helfrich and Craig, (2002) indicated that fish have lower dietary energy requirements because they exert relatively less energy to maintain position and move in water than do mammals and birds and because they excrete most of their nitrogenous wastes as ammonia (through the gills) instead of urea or uric acid thus loosing less energy in protein catabolism and excretion of nitrogenous wastes (Goldstein and Forster; 1970). Fish also have a lower dietary energy requirement because they do not have to maintain a constant body temeperature. Therefore maintenance energy requirement and heat increament are lower for fish than for land animals; with the implication that carbohydrates are not efficiently used by fish (Lovell, 1981; Helfrich and Craig 2002).

Helfrich and Craig (2002) stated that mammals can extract about 4Kcal of energy from 1gram of carbohydrate whereas fish can only extract 1.6Kcal from the same amount of carbohydrates. Helfrich and Craig (2002) further indicated that up to 20% dietary carbohydrates can be used by fish as earlier indicated by Buhler and Halver (1961).

Most research efforts towards provision of adequate feed for fish have been centered on manipulation of dietary protein used in feed formulation. Generally, fish nutritionists have given priority to meeting the requirements for protein, major minerals and vitamins thereby allowing energy, to take care of itself (Lovell, 1988). Lovell (1988) further stressed that fish uses protein efficiently as a source of energy, it was also maintained that a high percentage of digested energy in protein is metabolisable in fish than land animals. However, a ration poor in carbohydrate entails the use of either lipid or protein to provide necessary calories (Cowey and Sargent, 1972).

2.7 Lipids Requirement for Fish

Lipids are non-protein calorie sources which are often neglected in fish feed preparation, they are generally more digestible than some carbohydrates (Hilton, 1982). Due to the high energy content of fats they can be utilized to partially spare or substitute for protein in aquaculture feeds (Helfrich and Criag, 2002). The protein sparing effects of lipids varies between species but appear to be optimal at about 15 – 18% of the diet (De-Silva *et al.,* 1995). Lipids supply about twice the amount of energy as proteins and carbohydrates and typically lipids comprise about 15% of fish diets, lipids supply essential fatty acids (EFA) and serve as transporters of fat-soluble vitamins.

Eyo (2003) indicated that lipids and fatty acids perform three different functions in the organism, as they serve as energy carriers, metabolic regulators and structural elements in the cell.

Helfrich and Craig (2002) indicated that a recent trend in fish feed is to use higher levels of lipids in the diet. They further asserted that increasing lipids can help reduce the high costs of feeds by partially sparing protein in the feed, it was also noted that problems such as excessive fat deposition in the liver can decrease the health and market quality of fish.

Helfrich and Craig (2002) indicated that simple lipids include fatty acids and triacylglycerols and that fish typically require fatty acids of the Omega 3 and 6 (n – 3 and n-6) families for maximum growth and efficient utilization.

2.8 Vitamins Requirements for Fish

Vitamins are organic compounds necessary in the diet for normal fish growth and health. They are not often synthesized by fish and thus must be supplied in the diet (Helfrich and Craig, 2002). Many vitamins function as co-enzymes (Cowey and Sargent, 1972). Vitamins are normally categorized into two groups namely: the water – soluble and fat soluble vitamins.

Fishes are extremely sensitive to vitamin deficiencies. Deficiency of each vitamin has certain specific symptoms with retarded growth being the most common deficiency symptom (Helfrich and Craig, 2002). Other signs common to several vitamin deficiencies were identified as abnormal skin pigmentation, ataxia, hypersensitivity haemmorrhage, fatty livers and increased susceptibility to bacterial infection.

Supplemental vitamins which comprises of all the aforementioned vitamins are usually part of commercially formulated diets for intensively cultured fish with the exception of inositol and biotin which are usually found in sufficient quantities in fish feed ingredients (Helfrich and Craig, 2002).

Lall (1991) summarized the dietary vitamin requirements for optimum growth of Channel catfish thus: vitamin A, 5,500-iu; D,500-4,000iu; E. 50-100; K.10; Thiamin 1-20mg; Riboflavin 9-20mg; Pyridoxine 3-20mg; Pantothenic acid 10-50mg; Niacin 14mg; Folic acid 5mg; B_{12} 0.02mg; Choline 400; Inositol; not required (N.R.) ; C (N.R.) or 100mg.

2.9 Mineral Requirement for Fish

Minerals are inorganic elements necessary in the diets for normal body functions. There are two (2) categories of minerals, macro-minerals and micro-minerals. Macro-minerals are required in large quantities. Common macro-minerals include sodium, chloride, potassium and phosphorus. These minerals regulate the osmotic balance and aid in bone formation and integrity. Micro-minerals (trace minerals) include manganese, iron, zinc, copper, iodine, cobalt and selenium. Micro minerals exist as components of enzyme and hormone systems (Helfrich and Craig, 2002)

Fish can absorb many minerals directly from the water through their gill membranes and skin, this phenomenon allows them to compensate to some extent for mineral deficiencies in their diet. Thus their minerial requirements are reduced (Helfrich and Craig, 2002). The dietary requirements for several minerals by Channel catfish include calcium <0.1% (Lim and Dominy, 1989), Phosphorus 0.45%, (Lovell, 1978); Magnesium 0.05% (Gatlin *et al.,* 1982), Zinc, mg/kg 20-150, (Gatlin and Wilson, 1983). Twenty milligrams per kilogram (20mg/kg) is the basal requirement for Zinc, however, 150mg/kg is recommended in practical fish feeds to compensate for mineral and phytate binding of zinc. Selenium, 0.25 mg/kg; Manganese, 2.40mg/kg; Copper, 5.00mg/kg and Iron, 30.00mg/kg (Gatlin and Wilson, 1984; 1986a; 1986b; 1986c). Gallagher (1993) indicated that dietary supplementation is necessary in some fishes. Since there is no

information on mineral requirements for Tilapia the requirements for channel catfish could be used for both *O. niloticus* and *Clarias gariepinus* (Lim and Dominy, 1989).

Blood meal, bone meal, fish meal, limestone, red pepper and wood ash were reported by Aduku (1993) as natural sources of macro and micro minerals used in feed formulation for animals.

2.10 Anti-Nutrients

Growth suppressors or anti-nutritional factors do occur in all available ingredients of plant origin. They may be inherent in the feed stuffs or may have contaminated the feedstuffs from other sources such as pesticides and herbicides when they come in contact with them. Other forms of contaminants include toxins (mycotoxins) produced by mould, which grow on damp feeds during storage (Eyo, 2003). Anti-nutritional factors normally form a "shield effect" on the protein molecule in the ingredients thereby preventing the proteases or similar digestive enzymes from getting to them. The resultant effect is the passing out of all the proteinous molecules along with the faeces undigested, and thus rendered unavailable for fish body uses which include growth purposes. Anti-nutritional factors alter the nutritional value of feedstuffs thereby causing poor nutrient utilization and impaired growth as well as poor health in fish (Eyo, 2003). This dreadful phenomenon or trend in plant proteins utilization has initiated a lot of research work on the utilization of plant food sources. Considering that the cost of artificial feeds (commercial formulated feeds) are high due to the competition between man and his livestock for feedstuff (cereals and legumes) the need therefore arises to exploit the potentials of plant protein sources as animal feed by subjecting them to processing in order to get qualitative growth and maximum production.

The inhibitory effect of anti-nutritional factors can be reduced by subjecting the seeds to controlled heat treatments which include toasting, parboiling, extrusion methods, soaking and sun drying (Balogun and Ologhobo 1989, Banyigi *et al.,* 2001; Adeparusi, 2001; Eyo 2001; Francis, 2002; Vijayakumari, *et al.*, 2007; Vadivel and Pugalenthi, 2007; Olaniyi, 2009a; Tamburawa, 2010).

Phytates (hexaphosphate of myoinositol) are common in plant seeds, and account for most of their phosphorus content (60% in soybean meal). Phytates are resistant to fish enzymes and they reduce the availability of dietary phosphorus. They also chelate with divalent and trivalent mineral ions, resulting in unavailability of these ions. Phytates form complexes with dietary protein and reduce their digestibility.

High phytate diets can retard growth and cause abnormalities in the intestinal histology of various commonly cultured fish species. Phytate can be reduced by dehulling cereals. In addition, heating, fermentation, addition of enzymes phytase, and supplementation with Zinc can also remedy high phytate content in fish diets.

Dehulling seeds, soaking and autoclaving, treatment with alkali, fermentation with lactic acid bacteria, treatment with oxidizing agents, and supplementation with the tannin-complexing agent polyethylene glycol are all measures that counteract tannins.

Saponins occur as steroid or triterpenoid glycosides in many plants such as legumes, with concentrations of about 50 mg/kg in various legume seeds. Dietary saponins above a level of 1.5g/kg can retard growth and damage intestinal mucosa in fish. Extraction with water or gamma ray irradiation aid in the removal or neutralization of saponins (Francis, 2002).

The presence of high saponins levels in water are highly toxic to fish due to the damage caused to the respiratory epithelium of the gills by the detergent action of the saponins. Saponins have also been reported to affect protein availability. Endogenous

saponins have been shown to reduce the protein digestibility of soybean (Shimoyamada *et al.,* 1998) due to the formation of sparingly digestible saponin - protein complexes (Potter *et al*., 1993). The presence of high levels of saponins indicate damage of intestinal mucosa in fish. The intestinal mucosa of Chinook salmon fed diets with purified extracts of soybean meal (extracted to isolate soy saponins) had intestinal morphology resembling that of a fasting fish probably due to the detergent action of saponins on feeding (Bureau *et al.,* 1998). Extensive damage to the intestinal mucosa was observed when Chinook salmon and rainbow trout were fed at a dietary level of 1.5g/kg Quillaja bark saponin. However saponins have beneficial effect at lower levels.

Hydrogen cyanide (HCN) are cyanogenic compounds which are found in high concentration in root crops like cassava and some oil seeds which have been used as fish feed ingredients. Fish fed cyanogen-containing feed material have generally shown reduced growth when compared to the respective control (Hossain and Jauncey, 1989b; Ufodike and Matty, 1983). Dietary cyanide did not depress growth in Nile tilapia when fish were fed sundried cassava leaf meal at inclusion level of 9.9ppm total cyanide.

The presence of oxalate in fish feed ingredients is an indicator of reduced bio-availability of iron and calcium since oxalate impacts its effects by its attachment to these divalent minerals (Ezekiel, 2004).

2.10.1 Processing Methods of Reducing and Detoxifying of Anti-Nutrients in Feed Ingredients

Fermentation has been shown to reduce the phytate content of seeds because of the action of phytases produced by yeast or lactic acid bacteria (Duffus and Duffus, 1991). Abu (2005) reported the reduction in phytate contents of fermeted locust bean seed (*Parkia filicoidea*) by 17.7%. Kumar *et al*. (1978) reported that cooking decreased both water and acid extractable phytate phosphorus in legumes. Vidavel and Pungalenthi

(2007) indicated that cooking and autoclaving substantially reduced the level of phytate in Muncuna seed meal. Similarly Tamburawa (2010) indicated that soaking locust seed meal for 3days considerably reduced the levels of anti-nutrients particularly tannins and phytates (1.08% in the raw to 0.17%; 0.71% to 0.27% respectively)

Due to the concentration of phytate in the outer endosperm of seeds particularly cereals, milling has been employed to remove the outer layers of seeds in order to reduce the phytate content to considerable levels.

Liener (1980) reported that the extent of trypsin inhibitor destruction in soyabean by heat is a function of temperature, duration of heating, particle size and moisture content of the ingredients. The report further asserted that eventhough heat treatment effectively eliminated most of the anti-nutrients, careful control of processing conditions was required to prevent functional and nutritional alteration of the protein resulting as a result of denaturation associated with excessive heat treatment.

Heat treatment (autoclaving) was also found to reduce phytic acid in linseed and sesame meals by up to 72% and 74% respectively (Hossain and Jauncey, 1990). Rumsey *et al.* (1993) identified toasting and de-fatting as processing methods that could be used for removal of antinutrients of some leguminous and cereal ingredients for fish feeds. Bangoula *et al.* (1993) and Burd *et al.* (2000a) indicated the extrusion at high temperature has the potential to improve the carbohydrate digestibility of legume seeds because of a higher break up of cell walls.

Viola (1983) reported that hydrothermal treatment of raw full-fat soyabean cooked for 1hour destroyed 80% of trypsin inhibitor. Autoclaving at 105°C for more than 60 minutes was observed to be more effective than the boiling procedure because 95-100% of the trypsin inhibitor was removed. Due to the special apparatus required for autoclaving it is unaffordable to most fish farmers.

Norton (1991) indicated that moist heat treatment (and autoclaving for 15 – 30 minutes) is recommended as means of reducing the amount of trypsin inhibitors (TI) below the critical levels. However (Norton, 1991) indicated that the use of heating process should be carefully regulated to minimize the loss of nutritional quality of feed material, such as the loss of available amino acids like lysine and decrease in protein degradability due to excess heat denaturation.

Grant (1991) indicated that lectins can be removed by aqueous heat treatments by using boiling water at 100°C for 10minutes. Lectin content of Jantropha seed meal has been reduced from 102 to 1.17 heamagglutination units by moist heating at 100°C for 10minutes (Aregheore *et al.,* 1998). Heat treatment has been reported to be effective in reduction of glucosinolate contents of rape seed meal from 40 to 26μ mol/g after wet pressure cooking (Burel *et al.*, 2000a).

The reduction of tannins to tolerable levels by boiling was earlier reported by previous workers including Dakare (2005) for Parkia seeds. Due to the presence of high levels of tannin in the seed coats of many seeds, the boiling processing technique has been reported to reduce tannin to tolerable levels. Previous workers, Dakare (2005); Vidavel and Pungalenthi (2007); Tamburawa (2010) who worked on parkia seed, mucuna and parkia respectively indicated that a considerable level of tannins and other anti-nutrient contens of seeds evaluated were reduced when subjected to boiling.

Griffiths (1991) recommended the removal of condensed tannins by de-hulling the seed to remove the tannin-rich outer layer. Makkar *et al.* (1995b) and Makkar and Becker (1996) also indicated that treatment of tannin-containing feeds with oxidizing agents and supplementation with a tannin complexing agent, polyethylene glycol could mitigate their negative effect on animals. A reduction in tannin content of sesame seed meal from 20 to

10g/kg after fermentation with lactic acid has been observed (Mukhopadhyay and Ray, 1999a).

D'Mello *et al*. (1978) suggested the improvement of the nutritional value of Leucaena by soaking it in ferrous sulphate solution for one week prior to usage in feed formulation. Pascal and Penaflorida (1979) indicated that the nutritive value of Leucena leucocephala leaf meal can be improved if the mimosine present in it is degraded by soaking in water for 36hours. Wee and Wang (1987) also suggested the use of soaking as an efficient method in removal of mimosine from *Leucaena leucocephala* leaf meal.

Makkar and Becker (1997) indicated that glycosinolates could be extracted with water from full-fat and fat-free *Moringa oleifera* kernels. Based on the high solubility of most saponins in water, aqueous extraction (soaking) have been recommended for removal of saponins provided it does not otherwise affect the nutritional quality of the material (Francis *et al.*, 2001a).

2.10.2 Effects of Processing on Anti-Nutrient Contents of Leguminous Plant Seeds

The nutritive value of African locust bean seed had been reported to be improved by cooking, removal of the tough leathery testa on the seed as well as fermentation which inactivate, reduce and destroy the inherent anti-nutrient component (Fetuga *et al.,* 1974).

Olaniyi *et al.* (2009a) assessed the effect of feeding different dietary levels of processed Mucuna seed meal on the growth performance of African catfish (*Clarias gariepinus*) fingerlings. The inclusion levels were 0%, 5%, 10% and 15%. The results revealed that mean weight gain values were significantly different ($P<0.05$) and highest value (21.74g) was recorded for fish fed cooked mucuna seed meal (CMSM) diet at 10% inclusion level. The Specific Growth Rate (SGR) values of all processing methods were significantly different ($P>0.05$) except the control diet which was significantly different ($P<0.05$) and the highest Specific Growth Rate (SGR) value was recorded for CMSM at

5%, 10% and 15% inclusion levels. The Protein Efficiency Ratio (PER) values showed no significant different (P>0.05) across the treatment.

However, the fish fed diet CMSM (10% inclusion level) had the highest PER value of 63.9%, PPV values were significantly different (P>0.05). The protein contents in the carcass of the fish before and after the experiments were significantly different (P<0.05) but the fish fed soaked mucuna prior to cooking had the highest carcass protein value (42.90%). In all parameters measured fish fed CMSM diet had the best performance while the least performance was shown by fish fed control diet of 0% inclusion level. The result therefore indicated that cooked mucuna seed meal can be included in the diet of *Clarias gariepinus* fingerlings at 5-15% inclusion levels.

Olaniyi *et al.* (2009b) investigated the effects of feeding locust bean meal (LBM) based diets on the growth performance of African catfish in a feeding trial that lasted 84 days. Locust bean meal (*Parkia biglobosa*) was included to replace 0%, 25%, 50%, 75% and 100% fishmeal in the experimental diets. Significant increase (P<0.05) in final weight mean weight gain, feed intake, percentage mean weight gain, average daily weight gain and specific growth rate were observed from diet 2 to 3 as replacement level increased. The feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) were not significantly different (P>0.05) eventhough, the FCR (2.6) was very low, showing good performance in terms of feed conversion and utilization of diets by fingerlings. The performance in terms of growth and feed utilization of fingerlings fed 75% LBM and 100% LBM were observed to be poor compared with the fingerlings fed 50% LBM. The result therefore indicated that up to 50% LBM could replace fishmeal in the diet of African catfish fingerlings without any adverse effect on the growth performance.

The effect of duration of soaking of Rattle box seeds (*Crotalaria retusa L.*) on the level of some chemical composition and minerals was conducted by Yashim *et al.* (2009). The results indicated that duration of soaking had significant effect on Crude Protein (CP) Ether Extract (EE), Ash, Nitrogen Free Extract (NFE) and Crude Fibre (CF) but had no significant effect on DM. The Anti-nutritional factors (ANFs) analyzed showed highly significant decrease with increase in soaking days.

Tamburawa (2010) determined the effects of processing on the proximate and anti-nutrient compositions of differently processed locust bean seed meals. The four processing methods adopted included; cooking LBSM for 1hour, 2hours, 3hours and 4hours respectively; soaking LBSM for 1 day; 2 days; 3days and soaking and fermentation for 3days as well as toasting LBSM.

The results of the proximate determination indicated that DM, NFE and CF decreased progressively in all processing methods with increased duration of processing. The Crude Protein (CP) however increased in all the processing methods with increased duration of time; EE however did not show any particular trend in the cooked LBSM. However, it was higher in cooked LBSM for 2 hours; EE was noted to generally increase with increased duration of soaking; it declined with toasting. The result of the study further indicated that ash content increased with increased duration of processing.

The result of the anti-nutritent determination of the same study also indicated that processing reduced anti-nutritional factors (Tannin, phytate, saponin, oxalate, trypsin inhibitor) progressively with increased duration of processing time. Toasting was also found to considerably reduce percentage of anti-nutrient present in the seed when compared to the raw.

2.11 Plants Protein Utilization

Non-conventional plant feed stuff (NCPF) sources are of relative abundance almost in every locality in Africa, Nigeria inclusive (Gabriel *et al.*, 2007).

The extent of plant protein utilization depends on availability; acceptability by fish, ease of processing, and nutritive value. However, high inclusion levels of plant proteins in fish diets have in some cases resulted in reduced growth and poor feed efficiency, probably the result of improper balance of essential nutrients, such as low protein contents, amino acids and minerals, presence of toxic substances or anti-nutritional factors or decreased palatability and pellet water stability value (Gohl, 1981; Devendra, 1985; Lim and Dominy, 1989; Oresegun and Alegbeleye 2001; Ibiyo and Oluwasegun, 2004).

The use of plant derived materials such as legume seeds, different types of oil seed cake, leaf meals, leaf protein concentrates and root tuber meals as fish feed ingredient is limited by the presence of a wide variety of antinutritional substances, important among which are: protease inhibitors, phytates, glucosinolates, saponins, tannins, lectins, oligosaccharides and non-starch polysaccharides, phytoestrogens, alkaloids, antigenic compounds, gossypols, cyanogens, mimosine, cyclopropenoid fatty acids, canavanine, antivitamins and phorbol esters (Francis *et al.,* 2001a). Francis (2002) indicated that additional research needs to be carried out to better utilize the potentials of plant-derived materials in aquaculture feeds.

Considerable work has been conducted to evaluate the nutritive value of soybean meal as a substitute to fishmeal. Soybean meal actually appeared to be better utilized by most fish farmers due to their nutritional quality and initial lower cost compared to fish meal as well as their availability compared to other plant protein sources.

Presently, the high cost of soybeans has shifted the attention of researchers and feed compounders into searching for alternative cheaper sources of plant protein (Viola *et*

al., 1982). The high demand for soybean by man has led to its astronomic hike in price and thus an expensive component of animal feed.

Jackson *et al*. (1982) observed growth reduction in *Sarotherodon mossambicus* fed a diet in which 50% or more fishmeal was replaced by soybean meal. This was attributed to the low level of sulfur amino acids and the presence of other factors such as trypsin inhibitor or haemagglutinins.

Viola and Arieli (1983) reported that soybean meal could be used to replace up to half of the fishmeal in tilapia feeds having 25% crude protein content without requiring any supplementation. However, complete substitution of fishmeal by soybean meal resulted in significant reduction in weight gain and feed efficiency which were not overcome by supplementation of oil, lysine, methionine and vitamins. Tacon *et al.* (1983) reported that supplementation of 0.8% DL-methionine to a diet in which 75% of brown fishmeal was replaced by soybean meal, improved the growth performance of *Oreochromis niloticus* to a level comparable to that of a fishmeal diet.

Viola *et al.* (1986) using a 30% crude protein diet reported that isonitrogenous substitution of fishmeal with a 24% soybean meal reduced the growth of tilapia (*Oreochromis aureus* and *Oreochromis niloticus*). It was further explained that on addition of 2 – 3% dicalcium phosphate, the growth rate of fish was comparable to that of all fishmeal diet.

Shiau *et al.* (1987) demonstrated that fishmeal can be partially replaced by soybean meal when the dietary protein level is below optimum for growth of *Oreochromis niloticus* and *Oreochromis aureus* (24%). They further reported that at the optimum level of dietary protein (32%) replacement of 30% catfish meal with soybean meal there was significant depression of growth and feed efficiency. However, it was indicated that these trends could be reversed by addition of methionine to the level of the control diet.

Solomon *et al.* (1996) investigated the effects of replacing fishmeal with soybean meal, groundnut cake meal and blood meal at varied proportions on the growth and food utilization of *Clarias anguillaris* fingerlings. Results indicated that eventhough fishmeal gave the highest growth rate it was not cost effective compared to the other test ingredients. Although the results further indicated that groundnut cake meal was more efficiently utilized than soybean meal, the amino acid composition of groundnut cake was however lower than that of soybean.

Recently, the analysis of inclusion value of wild plant materials attracted attention because they contain significant amount of essential nutrients that can be used for both human consumption and in formulation of animal feeds. The proximate composition of seed protein of some wild plants of Nigerian origin showed that they could be adequately utilized in the formulation of animal feeds provided the level of their toxic components are known (Eromosele and Eromosele, 1993).

Fagbenro (1999) used autoclaved or roasted winged (40% CP) bean in the diets of African cat fish at inclusion rate of 50% to replace fish up to 80% level. The results indicate that the fish performance was not significantly different from control.

Alegbeleye *et al.* (2001) evaluated the use of Jack bean (*Canavalia ensiformis*) meal as an ingredient in feed formulation for *Clarias gariepinus* (Burchell, 1822) fingerlings at 10%, 15%, 30%, 45% and 60% levels of replacement for full fat soybean meal (FFSM) for a 56 day period on iso-nitrogenous and iso-calorific basis. Results indicated that feed consumption was higher in control (full fat soybean meal) compared to the test diets. The percentage survival rate and mean weight gains were observed to be higher in the control than the test diets. Feed Conversion Ratio (FCR) was highest in diet containing 60% inclusion level of jack bean meal where fish consumed more feed to produce unit increase in weight thereby rendering the feed uneconomical. The findings

also revealed that the performance of the fish fed 15% and 30% inclusion level of Jack bean meal compared favourably with the control; however higher fish mortality was recorded in the control. This trend which is deleterious to performance was attributed to the high dietary levels of toxins and anti-nutrients present in the leguminous seed. The presence of L-Canavanine (a thermostaple amino acid and arginine antagonist) with high inclusion levels of JBM was suggested to have been responsible for the poor feed utilization and poor survival rate observed.

A lot of investigative work has been conducted with regards to substituting plant protein sources for fishmeal in fish diets with Bambara groundnuts. Banyigyi *et al.* (2001) used heat treated (oven toasted) Bambara groundnut (*Vigna subterranean verde L.*) meal to feed *Clarias gariepinus* juveniles to determine its effect on growth and feed utilization. Three (3) of the BGM were oven toasted for 15, 30 and 45 minutes, the fourth meal was traditionally toasted while the fifth was raw BGM meal. The result of the study indicated high digestibility of experimental diets which suggests that Bambara groundnut meal have the potential of replacing or competing with soybean in fish feed formulation. Banyigyi *et al*. (2001) suggested that extension of the duration of heat processing of bambaranut or employment of feed processing methods such as boiling or fermentation may enhance better growth and feed utilization. The result also indicated that there was no significant difference ($P > 0.05$) in the growth performance and feed utilization of *Clarias gariepinus* fed Bambara heat treated diet. The fish fed diet containing oven toasted BGM for 45 minutes recorded highest percentage weight gain, FCE and PER. This was attributed to the deactivation of growth inhibiting substances in the meal.

The growth of tilapia was improved when 25% of fishmeal was replaced by cotton seed meal. The growth rate was however lower than the control when tested at 100% inclusion level (Jackson *et al*., 1982). In contrast Ofojekwu and Ejike (1984) reported a

much lower weight gain and feed efficiency of *Oreochromis niloticus* fed cotton seed cake diet compared to the tilapia fed fishmeal control diet. It was further reported that low gossypol cottonseed meal (0.03%) was a good protein source for *S. mossambicus*. Falaye (1992) also reported that cotton seed meal yielded good results when fed up to 100% in tilapia diets. Although cotton seed cake is an important protein source for animals, its use in commercial aquaculture feeds is very limited due to the presence of gossypol and the low level of available lysine (Alcestes and Jory, 2000). Thus it was further stressed that the level of cottonseed meal that can be incorporated in fish diets depends mainly on the gossypol content of the meal.

The use of rapeseed meal has been documented (Jackson *et al.,* 1982). Rapeseed meal is comparable in protein quality to soybean meal but has higher crude fibre content. The use of rapeseed meal has been limited due to the anti-nutritional factors such as tannin, phytic acid, glucosinolates and enzyme myrosinase. However, great improvements have been made in processing techniques to inactivate or remove the myrosinase and glucosinolates.

Jackson *et al.* (1982) reported good growth performance of *Oreochromis mossambicus* fed diets in which 50% of fishmeal was substituted by low glucosinolate rapeseed meal. Decreased growth rate was observed at higher levels of substitution.

Sunflower seed meal has been reported to contain a variety of endogenous anti-nutritional factors such as protease inhibitor, an arginase inhibitor and the polyphenolic tannin chlorogenic acid (Tacon *et al*., 1984). It has also been reported to have relatively high crude fibre content, which reduces the pelleting quality of the feed if included at high levels. According to Alcestes and Jory, (2000) inspite of these limitations, sunflower seed meal has been reported to be a good protein source for *Oreochromis mossambicus* even at 69.6% inclusion levels of the diet.

Lovell (1988) reported that peanut meal is highly palatable with better binding properties for pelleting than soyabean meal. It was also noted that peanut meal can replace 25% of fish meal in the diet of *Oreochromis mossambicus* without affecting growth performance. At higher inclusion levels growth rate decreased rapidly, probably due to its low methionine content.

Eyo (1990 and 1994) substituted soyabean for groundnut in the diet for *Clarias anguillaris* fingerlings and documented that groundnut cake was highly utilized and supported proper growth of fish.

Ofojekwu and Kigbu (2002) investigated the effects of substituting fish meal with sesame cake in fish feed diet to determine the effect on growth and feed utilization as well as body composition of the Nile tilapia (*Oreochromis niloticus*). The results in terms of best growth and food utilization were obtained from 10% sesame cake meal and 40% fishmeal. It was further indicated that up to 20% sesame cake can be used with 30% fishmeal in the diet of juvenile *Oreochromis niloticus* without any adverse effect on the overall performance. However significant growth depression was associated with high levels of sesame cake inclusion in the diet as observed in the diet with 50% sesame cake and 0% fishmeal which gave the poorest growth rate in fish. The poor performance was attributed to the high fibre content and phytic acid present in the sesame cake.

The use of rubberseed cake (*Hevea brasiliensis*) in fish feed formulation has been investigated by Alegbeleye *et al*. (2004). In the study the seed with 36% crude protein was used to replace groundnut cake in the diets of Nile tilapia (*Oreochromis niloticus*). The findings revealed that fish fed with 30% inclusion level of rubberseed meal gave the best results with the highest specific growth rate (S.G.R.) 1.73; feed conversion ratio (FCR) 1.69 and protein efficiency ratio (PER) 1.97 which were statistically significant ($P>0.05$) compared to the other diets. The results further indicated that 60% inclusion level of

rubberseed meal gave the poorest results suggesting that equal replacement levels of groundnut cake and rubberseed meal was optimum as protein dietary source for growth performance and efficiency of feed utilization for Nile tilapia (*Oreochromis niloticus*).

Balogun *et al.* (2004) replaced dietary protein with *Delonix regia* at different levels and fish showed appreciable performance and efficient feed utilization on completion of the feeding trial experiment.

The use of castor oil seed has also been reported. Agboola (2004) used diets containing graded levels of boiled castor oil seed (*Ricinus communis* L.) meal (CSM) as feed for *Clarais gariepinus* and *Oreochromis niloticus*. The seeds were processed at different time intervals (20, 35, 50 and 65 minutes). The raw seed served as control. The digestibility study indicated that the processed CS meals were highly digested by both species. This was attributed to reduction of the effects of anti-nutrients by heat treatment. However, based on the values of mean weight gains Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Net Protein Utilization (NPU) and digestibility, *Oreochromis niloticus* was able to utilize and convert CS meal better than *Clarias gariepinus* due to its vegetarian nature which enables it to produce enzyme (cellulase) for cellulose digestion. The poor performance of fish fed raw CS meal (control) could be attributed to high concentration of ricin which is a limiting factor to the use of castor oil seed in animal feed formulation.

Adeparusi and Eleyinmi (2004) evaluated the effect of processed Lablab bean (*Lablab purpureus*) meal supplementation on the digestibility and growth response of Common carp (*Cyrinus carpio*). Soybean was substituted for Raw Lablab (RLB) and processed Lablab (PLB) bean meals on iso-nitrogenous basis. The processed Lablab bean meal included Toasted Lablab (TLB) meal; Fermented Lablab (FLB) meal and Cooked Lablab (CLB) meal. The results indicated that fishes fed on the control (no Lablab) and

cooked lablab bean diets had the highest specific growth rate and feed conversion ratio. The CP digestibility values obtained in the study (85.4% – 86.6%) compared favourably with earlier reports of Adeparusi and Jimoh (2002) on protein digestibility of Nile tilapia (*Oreochromis niloticus*) fed on toasted lima bean diets (84.8%). The result thus shows that heat processing (cooking and toasting) can significantly improve the digestibility of LB beans. The low digestibility recorded in fish fed on RLB based diets could be due to the presence of anti-nutrients such as tannin, phytates and trypsin inhibitors. It also suggested that the presence of cyanogenic glucosides could decrease palatability.

The uses of *Senna occidentalis* (Linn) seed diet have also been documented in the search for alternative cheaper and unconventional sources of plant protein in fish diet. Umar (2006) studied the growth performance and feed utilization of *Oreochromis niloticus* fed different levels of Senna occidentalis seed. The results indicated that parboiled Senna seeds could serve as a good alternative source of protein in fish feed formulation. The growth indices indicated that diet containing 25% parboiled Senna diet was the best. The diet also had the highest carcass crude protein content.

2.12 Use of Conventional Animal Protein Sources in Fish Feed

Animal protein sources that have been tested in partial or total replacement of fish meal component of feed used in aquaculture include terrestrial and aquatic worms, fish silage, blood meal, bone meal, meat meal, poultry by-products meal, tadpole meal and whey powder. However attempts to partially or wholly replace fish meal in fish feed formulations with these sources have resulted in either good, fair, or poor results in terms of growth and food conversion efficiency as indicated respectively by Pfetter and Berkers (1977); Cropp *et al*. (1976) and Ayinla (1991).

Conventional animal protein sources are the feedstuffs that are regularly and conventionally used in formulation of fish feed. These include fish meal, fish silage, shrimpmeal and bloodmeal.

The use of animal protein sources have been documented by several fish nutritionists Akiyama (1988); Lim and Dominy (1989); Eyo (1990); Osuigwe (1995) and Solomon *et al.* (1996) reported the use of fish meal as the main protein source and as a control diet in different experiments to evaluate the performance of mud fish (*Clarias angullaris*) fingerlings and *Oreochromis niloticus* and the best growth performance and food conversion efficiency was obtained. Similarly Eyo (2001) reported that fishmeal in fish diets had higher values for the ash crude protein, essential amino acid (EAA), particularly lysine and methionine (the two indispensable amino acids which are in short supply in plant protein feedstuffs). The exceptionally well balanced amino acid composition has made fishmeal a traditional component of fish feeds.

Ayinla and Akande (1988) reported that *Clarias gariepinus* fed with fish silage at 11%, 33.5% and 41% inclusion levels demonstrated feed acceptability without any symptoms of nutritional deficiency. However, they confirmed the superiority of fishmeal over fish silage as a protein source. High blood meal inclusion in the diet was found to cause a decrease in the growth rate of Tilapia, *Oreochromis niloticus* (Otubusin, 1987) and mudfish *Clarias anguillaris* (Eyo, 1990). Eyo (1994) indicated that the inclusion of blood meal in fish ration beyond 25% level of inclusion in diet for *Oreochromis niloticus* and beyond 50% for *Clarias anguillaris* resulted in reduced growth rate in both fish species.

Eyo and Olatunde (1996) also replaced soybean meal with blood meal in the diet of mud fish (*Clarias anguillaris* L.) fingerlings. The results indicated an increase in weight gain and food utilization when soybean meal was substituted with 50% blood meal in the diet of *Clarias anguillaris*. The result further indicated that there was a significant

reduction in growth performance of the test fish at inclusion levels higher than 50% blood meal substitution. This was attributed to imbalance in the amino acid composition of blood meal which reflected in its low contents of isoleucine and methionine.

Similarly, Eyo (2001) also reported that bloodmeal has very high value for crude protein content and low value for ether extract, however it was further reported to have low contents of essential amino acid (EAA), methionine and isoleucine.

2.13 Use of Unconventional Animal Protein Sources

The non-conventional feedstuff of animal origin are high quality feed ingredients which could compare to certain extent with the conventional types. They are cheaper by virtue of the fact that there is no competition for human consumption. However, the only problem with these feedstuffs is their unavailability in large commercial quantities for the sustainance of aquaculture industry in most parts of Africa, Nigeria inclusive (Gabriel *et al,* 2007).

Spinelli (1980) working on the larvae of housefly (*Musca domestica*) meal in the diet of *Salmo gairdineri* and *Oreochromis kitsutu* revealed that maggot meal was nutritionally equivalent to fish meal protein, effecting similar growth rate and feed efficiency values. It was further revealed that the only limitation to the use of maggots was presence of 90% moisture which implied that large quantities of maggots would be required to produce sufficient maggot meal and thus such meal may not be economically viable.

Similarly, Akinwande *et al.* (2002) also reported that maggot meal can successfully replace up to 50% fishmeal in the diet of *Clarias gariepinus* fingerlings for optimal growth and nutrient utilization.

Madu and Ufodike (2003) investigated the use of live maggot and live tilapia fry as unconventional diets for juveniles of the catfish (*Clarias anguillaris* L.). The result revealed that the greatest increase in body weight was achieved with diet containing maggot and artificial feed (99.7g) followed by diet in which maggot was fed exclusively (77.5g). The least increase in body weight (57.5g) was realized with diet with artificial feed alone. The combination of maggot with artificial feed thus formed a better balanced diet for the juveniles of catfish and thus the use of maggot to supplement artificial feeds in catfish production farms was recommended.

The use of toad meal (*Bufo regularis*) in the diets of catfish *Clarias lazera* has been investigated. Annune (1990) indicated that the growth performance of Clarias increased with increase in dietary inclusion of toad meal up to 40% than the fish placed on commercial feed ($P>0.05$). The limitation of toad meal in fish feed formulation is toad poison which was reported to have been destroyed by the denaturing effect of heat.

Idoniboye – Obu and Ayinla (1991) indicated that crayfish wastes with 41.2% crude protein have good potential for replacement of fishmeal. However this potential may not be realized due to difficulty involved in collection of enough quantities to sustain large quantities of fish feed production.

Falaye (1992) reported an efficient growth performance on inclusion of earthworm meal in *Oreochromis niloticus* diets. The inclusion of hydrolysed feathermeal in the diets of *Oreochromis niloticus* fingerlings was also reported. It was discovered that it could replace 30% of the expensive fishmeal without deleterious effects on fish growth and food conversion efficiency.

Idoniboye-Obu *et al.* (1993) evaluated the nutritional value of two (2) species of Brachyuran decapod crustacean (*Callinestes latimus* and *Cardisoma armatum*) for partial replacement of fishmeal in fish feed. It was reported that despite the good protein content

of the crab meal (32%) and their potentials for use as partial or total replacement of fishmeal the use of crabmeal may however be limited except economical methods of its harvesting and processing are developed.

Nnaji and Okoye (2004) investigated the effect of inclusion of grasshopper meal on growth, feed conversion efficiency and survival of *Clarias gariepinus* fingerlings and discovered that best survival of 100% was observed in diet containing 30% grasshopper meal and 100% fishmeal while the worst survival of 73.3% was observed in the diet containing 25% grasshopper meal and 15% fishmeal. It was also revealed that fish fed 10% grasshopper and 30% fishmeal performed better

2.14 Distribution/Cultivation, Nutritive Value and Uses of *Bauhinia* Seeds

Bauhinia monandra Linn is a member of the Caesalpiniaceae family. *Bauhinia* monandra Linn is a well known ornamental tree (Keay *et al.,* 1989). The tree is also commonly known as orchid tree, butterfly bauhinia, Jerusalem-date, Napoleon's plume, Poorman's orchid, St. Thomas tree (USDA, 1974) Though a native of South Eastern Asia, *B. monandra* is also found in West Africa (Daziel, 1955). In Nigeria it is common in the dry savanna especially along river banks. In Hausa it is known as "Jirga". About 600 species of *Bauhinia* grow in tropical regions of the world (Larsen, 1974 In: USDA, 1974). The genus includes, trees, vines and shrubs that are frequently planted for their showy flowers and ornamental foliage (Bailey, 1941, Neal, 1965 In: USDA 1974). *B. monandra* is a small fast growing evergreen tree or shrub that commonly reaches 3-15.2m in height and 0.5m in diameter (Little and Wadsworth, 1964 In: USDA, 1974). The leaves of *B. monandra* are shaped like butterfly wings rounded and split $^{1}/_{3}^{rd}$ to ½ their length forming two equal lobes. They are dissected by 11-13 main veins. *B. monandra* blooms in 3-4 years (Bailey, 1941, In: USDA, 1974). The petals are long-clawed 4-5.5 X 2-3cm,

white to pink, with red blotches or dots, the uppermost one splotched with deeper red, yellow or it may be yellow-margined. Its flowers and fruits most of the year (Little and Wadsworth, 1964 In: USDA, 1974). The plant fruits November – April. It is often planted along road sides, it requires moderate water with full sun to light shade for growth (USDA, 1974) and may be easily recognized by its large leaves, pink and white flowers with one large anther and elongated sharply pointed very persistent pods which split open explosively (Keay *et al.,* 1989). Pods are flattened, leathery, rather shiny brownish or black, seeds are ovate, compressed and dull brown (Stone, 1970). The fruits are dark, dehiscent pods 2.5cm wide, 15.2 – 30.5cm long and pointed at the apex, while still on the tree, they splite open with force scattering the seeds (USDA, 1974). The seeds are flat and about 1cm long. The pod ripeness is determined by a colour change from green to light or dark brown. *Bauhinia* tree is easily propagated by seeds (USDA, 1974).

Plate I: *Bauhinia monandra* seeds
Source: Field Survey, 2011

Plate II: *Bauhinia monandra* plant showing the dehisced pods
Source: Field Survey, 2011

Plate III: *Bauhinia monandra* plant showing the flowers and leaves
Source: Field Survey, 2011

Plate IV: *Bauhinia monandra* plant showing the bilobed leaves
Source: Field Survey, 2011

Plate V: *Bauhinia monandra* plant
Source: Field Survey, 2011

2.14.1 Uses of *B. monandra*

Bauhinia monandra (Linn.) is of considerable economic importance in human nutrition (Abrew *et al.,* 1990). Hutchinson (1967) also observed that the pods and leaves were eaten as vegetable in China and India, while in Africa, the pods and seeds were sources of black and blue dyes. In Eastern Sudan the pods are pounded and boiled in water to produce a laxative drink, while the crumbled back form a source of fibre for cordage (Irvine, 1961). According to Shashinia (1989) the pod is used as an astringent for diarrhoea, dysentery and cure for fever, a decoction of the root and bark is believed to cure leprosy and small pox, while the leave extracts are used to cure eye ailments. Anti-inflammatory balm is made from the bark of *Bauhinia monandra* (Keay, 1989). Anhwange *et al*. (2004) analyzed the *Bauhinia monandra* seed for amino acid contents and the result indicated that the seed contained 33.09% crude protein (Appendix 2a and b) which makes it a potential source of supplementary amino acids for both man and livestock provided the toxicants present in them are removed.

2. 14.2 Nutritive value

Although there is limited information on the use of *Bauhinia* as livestock feed with particular reference to fish. An extensive research was carried out by Anhwange *et al.,* (2004 and 2005) on the nutritional potentials and the anti-nutrient contents of *B. monandra* with a view of exploitation the seeds of the plant as an alternative plant protein source in livestock feed formulation (fish inclusive).

The nutritional potentials of *Bauhinia monandra* (Linn) are indicated in Appendices 1, 2a, 2b and 3. Among the essential elements (K, Ca, Na, Mg, S, P, Fe) shown in Appendix 1, the concentration of calcium was highest (77.90mg/g) followed by potassium (74.20mg/g). Concentration of phosphorus was the lowest (1.59mg/g). These elements are needed by the body because they constitute parts of the rigid body structure,

soft tissue and body fluids. Generally, minerals work in combination with each other and with other nutrients; therefore deficiency of any minerals may cause health problems. Smith *et al*. (1996) stated that essential elements such as calcium and phosphorus are needed for the building of healthy bones and teeth, while Fe assist blood formation and their deficiency cause muscle weakness and bones pain. Na and K maintain water balance in cells, transmit nerve impulses and stimulate normal movement of the intestinal tract. Glew *et al.* (1997) reported that Mg is essential because it maintains, repairs cell and provides energy and its deficiency may result in vertigo, convulsions, nervousness and heat palpitation, iron assists the muscles to keep reservoir of oxygen and increases the body resistance to infection. Its deficiency results in anaemia, tiredness, insominia and palpitations. The seeds are a useful source of vitamin A (Essien *et al.*, 1989).

The results of the nutritional and anti nutritional potential of *Bauhinia* is presented in Appendix 2b, the lipid content was 28.70% a value that compared favourably with other oil seeds like soya bean (27%) (Ezeagu *et al*., 1996). The search for new sources of lipids is been encouraged because lipids provide the body with the maximum energy, insulate the body from cold, prevent heat loss through the skin and gives a pleasant taste and texture to food (Anhwange *et al.,* 2005). Essential fatty acid prevents dryness and scaling of skin, regulates the action of hormones and facilitates transmission of nerve impulses (Badifu, 1993).

The acid value and free fatty acid value were 6.77 and 3.74 respectively. The low acid value is an indication that the oil has a low deteriorating rate and high edibility. The free fatty acid value shows that the rate of development of objectionable flavour or odour (rancidity) of this oil is low (Ekpa and Ekpa, 1996). In general *Bauhinia* seeds contain high amounts of linoleic and oleic fatty acids and low amounts of myristic linolenic fatty acids (Ramasastri and Shenolikar, 1974).

According to Young and Pellet (1994) proteins are essential constituents of all body tissue and are important in the formation of new tissues during growth, during pregnancy and during healing of wounds. The protein content of *Bauhinia monandra* was 33.09% and this compares fairly with Soya beans (Glycine max), and groundnut (*Arachis hypogaea*) which have 51.4 and 51.3% respectively. This indicates that the plant is a good source of protein.

The concentration of the essential amino acids (Appendix 3), lysine, phenyalanine, leucine, isoleucine, methionine, valine, thereonine and cystine were 2.86, 3.77, 2.13, 2.3, 1.58, 3.54, 2.70 and 1.11 respectively. The concentrations of the non-essential amino acids, glutamic acid, arginine, aspartic acid, and serine were 11.75, 6.74, 6.02 and 4.58 (g/100g protein) respectively were reported (Anhwange *et al.,* 2005). The result compliments the report of Di-Ciero *et al*. (1988) that *B. monandra* seeds are good source of essential amino acid.

Bauhinia monandra seed had 21.45% soluble carbohydrate and 3.25%fibre content. The carbohydrate of the seed compares favorably with that of Soya bean (Glycine max) 20.7% and peanut (Arachis hypogaea) 24.6% (Neilsen *et al*., 1996). According to Madubuike *et al*. (1994), fibre diet prevents constipation and also reduces cholesterol level in the blood.

2.14.3 Anti-nutritional factors in *Bauhinia monandra* seed

According to the report of Anhwange *et al*. (2005) the phytates contents of *B. monandra* was 11.57mg/100g (Appendix 2), the presence of phytic acid causes phosphorus deficiency in monogastric animals by binding and forming indigestible phytates that make this element unavailable (Biehl and Baker 1992), it also affects the availability of Ca, Mg, Fe that are used in forming the phytin complex. Phytic acid is also

known to decrease bio-avaliability of zinc and other trace elements in humans and animals (Ezekiel, 2004).

The hydrogen cyanide content was 0.32mg. Chandra *et al*. (1980) noted that chronic exposures to hydrogen cyanide diet cause neurological respiratory, cardiovascular and thyroid debilities. It is also causes focal necrosis in liver and kidney (Okolie and Osagie, 1999). The tannin content of *B. monandra* was 60%. Tannins are known to impair the functioning to the rumen, liveweight gain, and reduce gastrointestinal parasitism (Chang *et al.,* 1994). It also affects protein utilization by binding to lysine and making it unavailable to monogastric animals (Bagepallis *et al*., 1982). Saponin content of *B. monandra* was 2.052% this indicates that the plant has low saponin content. Saponin are haemolytic and are known to interfere with the metabolism of vitamin E and also causes gastroenteritis, manifestated by diarrhoea and dysentery (Radositits *et al.,* 1997).

The results of Anhwange *et al*. (2004) generally indicated that *Bauhinia monandra* seeds from the work he carried out in Zaria are rich in essential nutrients and low in anti-nutritional factors.

Anhwange *et al.* (2004) therefore suggested that inclusion of *Bauhinia monandra* seeds in animal or human diet may require further processing especially in reducing the cyanide and phytate levels to what the body can tolerate.

2.15 Distribution, Collection and Nutritive Value of Locust

2.15.1 Distribution and collection

The desert locust *Schistocerca gregaria* are agricultural pests collected by people in several countries for food using large nets and other means. Locusts are usually stir fried and eaten (FAO, 2004). In Nigeria they are sold at markets in various parts of northern Nigeria. Locust invades these regions at a particular season of the year (FAO,

2004). They are part of a large group of insects commonly called grasshoppers which have hind legs for jumping.

Locust belongs to the family Acrididae. Locusts differ from grasshopper in that they have the ability to change their behavior and habits and migrate over long distances.

Extensive research is in progress on biological control and other means of non-chemical control of locusts. Thus, far control by natural predators and parasites is limited since locusts can quickly migrate away from most natural enemies. Although giant nets, flamethrowers, lasers and huge vacuum have been proposed in the past, they are not in use for locust control (FAO, 2004). The combating of desert locusts is extremely difficult due to the large surface area (16-30 million sq km) they occupy when there is outbreak and plaques in an area.

They are usually restricted to the semi-arid and arid deserts in Africa, the East and South- West Asia that receive less than 200mm of rain annually. This is an area of 16 million square kilometer consisting about 30 countries (FAO, 2004). And during plaques the out break extends to about 29 million sq km and 60 countries (FAO, 2004).

During out breaks desert locusts has potential to damage the livelihood of the people in the area and its surrounding environs. The activities of locust could be referred to as lethal cocktail, a situation that could be characterized as catastrophic. It is not only humans that risk going hungry, animals are also affected thus the livelihoods of nomadic pastoralist are also threatened leaving them desperately short of grazing land for their animals. Locust swarms can vary from one square kilometer to several hundred square kilometers. There can be at least 40 million and sometimes as many as 80 million locust adults in each square kilometer of swarm. Desert locusts usually fly with the wind at a speed of about 16–19km/h, thus depending on wind swarms travel 5-130km or more in a

day. Solitary desert locust adults usually fly at night while gregarious adults fly during the day (FAO, 2004).

A desert locust can consume roughly its own weight in fresh food per day that is about two grams every day. A very small part of an average swarm (or about one tonne of locusts) eats the same amount of food in one day as about 10 elephants or 25 camels or 2,500 people (FAO, 2004).

Locusts do not attack people or humans. There is no evidence that suggests that locust carry diseases that could harm humans. At present, the primary method of controlling desert locust swarms and hopper bands is with mainly organophosphate chemicals applied in small concentrated doses (referred to as Ultra Low Volume) ULV formulation by vehicle mounted aerial sprayers and to lesser extent by knapsack and hand-held sprayers (FAO, 2004) .

In South Africa, Ledger (1987) suggested that serious consideration be given to attempting harvest of the brown locust, *Locustana pardalina* (walker) as human and animal food (as indigenous people have done for centuries) in order to eliminate or reduce the use of insecticides on the pest. The idea was however dismissed as impracticable by agricultural officials. Nevertheless, local officials in Thailand launched a campaign to combine grasshopper harvest and sale with pest control when conventional control procedures proved unsuccessful (Deforliart, 1989).

2.15.2 Nutritive value

According to FAO (2004) locusts are rich in protein. About 62% of the dry weight of desert locust consists of protein (>50), fats and the remainder are inorganic constituents (Si, Cu, Fe, Mn, Na, Mg, K, Ca, Ti, Ni, P and S). The protein contents of grasshoppers 30 – 37% CP; (Nnaji and Okoye, 2004) are comparable to that of locust. Sutton (1988) stated that locusts have approximately 24% crude protein.

Banjo *et al.* (2006) reported that *Zonocerus variegatus* has CP 26.8; EE 3.80; Ash 1.20; CF 2.40; DM 92.18; M 2.61; NFE 63.2; Vitamin A (Ug/100g) 6.82; Vitamin B_2 (mg/100g) 0.07; Vitamin C (mg/100) 8.64; Ca (mg/100g) 42.16; P (mg/100g) 131.2; FC (mg/100g) 1.96; Mg (mg/100g) 8.21.

2.15.3 Uses of locust

In Japan, processed grasshoppers are the most commercially available. The widely eaten imago (the grasshopper, oxyya velox F.) is preserved by boiling in soy sauce. The product appears as a luxury item in supermarkets throughout the country, including Tokyo. (Mitsushashi, 1984)

Locusts are used by various African groups consistently as food. The locust individuals are gathered in the early day before they are active then boiled before being cleaned and salted. Even the legs are used by grinding and combining them with peanut butter and salt. Locusts are also becoming a food item in South Korea where rice farmers are beginning to gather and sell them to supplement their income from rice production (Encyclopedia Smithsonian, 1980).

In Nigeria they are consumed in the arid savannah and Northern parts that are prone to locust infestation. The adult state of variegated grasshopper *Zonocerus variegates* (Linn) (Order – Orthopthera: Family – Pyrgomorphidae) which have large dry season population in southwestern Nigeria is reported eaten in Akoko area of Ondo state (Fasoranti and Ajiboye, 1993).

People and birds often eat locusts but usually not enough to significantly reduce population levels over large areas. Considering its high protein contents (>50%). Its potential for inclusion as protein source in animal feed with particular reference to fish could be explored. The use of locust meal could thus be economical and advantageous if incorporated in fish feeds without compromising growth and feeds conversion.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at the fishery laboratory of the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria. Zaria is in the Northern Guinea Savannah of Nigeria, latitude $11^0$12'N and longitude 7^0 33'E and at an altitude of 610m above sea level. The mean average annual rainfall of Zaria ranges from 700-1400mm. The mean maximum temperature ranges from 27-35^{0}C (Balogun, 1997).

Three (3) sets of experiments were conducted. The first experiment was conducted to process the *Bauhinia monandra* seeds using different processing methods and to determine proximate analysis, Amino acid profile, and Anti-nutritional factors of the processed seeds. The other two (2) sets of experiments (feeding trials) ran concurrently. Similar materials and methodologies were adopted in these two (2) sets of experiments. However, formulation of test diets differed on the bases of the different graded level of test ingredients utilized. The entire experiments were conducted in two phases.

3.2 Sample Collection/Identification

Bauhinia monandra seeds from dehiscenced mature pods of the plants within the main campus of Ahmadu Bello University, Zaria, Nigeria, were collected and used for this research. The plant pod and seed samples were identified at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria.

3.3 Sample Preparation

The collected seed samples were sorted, screened and distributed into four batches. The first batch was the untreated seeds, the second, third and fourth batches were toasted, boiled and soaked respectively.

3.3.1 Processing of Untreated (Raw) Seeds

Two hundred grams (200g) of raw, sorted *Bauhinia* seeds were used for proximate analysis, amino acid assay and anti-nutrient determination.

3.3.1.1 Boiling

Raw *Bauhinia* seed samples were boiled with tap water in seed to water ratio of 1:10(w/v) at the rate of 5kg:10litres (Vadivel and Pugalenthi, 2007) in a 15litre metal cooking pot for a duration of 10 minutes, 20 minutes, 30 minutes and 40 minutes. The water was brought to boil at a temperature of 100^0C before the seeds were poured into it. A portion (1kg) of the original seed samples were removed from the boiling water with a sieve at 10, 20, 30 and 40 minutes intervals respectively using a stopwatch while the boiling continued. Dense ox-brown exudates were noticed and this became more pronounced as duration of boiling increased. The boiled samples at the stipulated intervals were allowed to cool and sun-dried to a constant weight on clean trays placed on concrete slabs. The seed samples were then packed in tightly sealed polythene bags.

3.3.1.2 Toasting

5kg of raw *Bauhinia* seeds were placed in a hot open pan which was pre-heated on fire for 5minutes to ensure that sufficient and uniform heat was obtained for toasting. The heating processing continued and toasting was accomplished by constant stirring of seeds to ensure uniform application of heat and to prevent charring (Eyo, 2001). Toasted *Bauhinia* seeds were removed at 10, 20, 30 and 40 minutes intervals then spread and

allowed to cool on clean trays placed on concrete slabs. The samples were then stored separately in tightly sealed labelled polythene bags.

3.3.1.3 Soaking in water

Raw *Bauhinia* seed samples were soaked in a bowl containing tap water at room temperature (30 ± 2^0C) in seed to water ratio of 1:10(w/v) at the rate of 5kg to 10litres which completely submerged the seeds (Vadivel and Pugalenthi, 2007). Dense ox-brown exudate was observed and was more pronounced as duration of soaking increased. The soaked seed samples were removed from the soaking water at the rate of 1kg with a sieve at 24, 48, 72 and 96 hours respectively and then spread separately on clean trays to sun-dry to a constant weight. The seed samples were then placed in appropriately labeled and tightly sealed polythene bags and stored in a cool dry place.

3.4 Chemical Analysis

The raw seed, toasted, boiled and soaked seeds that were properly labeled were taken to the Biochemistry Laboratory of the Department of Zoology, University of Jos, Plateau State to determine the Crude protein (CP), Crude fibre (CF), Nitrogen free extract (NFE), Ash, Moisture/Dry matter content, Lipid, Amino acids and the Anti-nutritional factors.

3.4.1 Determination 0f Moisture and Dry Matter

The moisture content was determined as described by AOAC (1980).Two grams (2.0g) of each finely ground *Bauhinia* seed sample were weighed with the salter analytical balance and placed into a preweighed clean petridish (W_1) and then the petridish and its contents were weighed (W_2). Each petridish and its content were placed in Gallenkamp hotbox air circulated oven at a constant temperature of 105±2ºC. The samples were left in the oven for 24 hours to dry, then transferred into a desiccator and left for 24 hours and

weighed again. The drying procedure continued until a constant weight was obtained (W_3). The percentage moisture content was determined using the expression: % moisture content =

$$\frac{\text{Wt of dish with initial sample } (W_2) - \text{constant weight of petridish + contents } (W_3)}{\text{Wt of dish with initial sample } (W_2) - \text{Wt of petridish } (W_1)} \times 100$$

3.4.2 Ash Determination

Two (2g) grams of the thoroughly mixed and finely ground *Bauhinia* seeds sample (2g) was placed into a dry evaporating dish (W_1) and the evaporating dish plus the seed sample were reweighed (W_2) and then charred over a low Bunsen Burner flame, then ashed at 500 – 570 ºC in a carbolite furnace for 24hours. When ashing was completed the evaporating dish was removed, cooled in a desiccator and weighed (AOAC, 1980). The sample was replaced in the furnace and reweighed at 30minutes intervals until a constant weight was obtained (W_3) the percentage ash content was obtained by using the expression

% Ash content =

$$\frac{\text{Wt of evaporating dish + Ash } (W_3) - \text{Wt of evaporating dish } (W_1)}{\text{Wt of sample } (W_2 - W_1)} \times 100$$

3.4.3 Determination of Lipid

The lipid content was determined as described by AOAC (1980) using the Soxhlet apparatus for continuous extraction. A clean dry round bottom flask (500ml) containing anti-bumping granules was weighed and about 210cm^3 of petroleum ether (B.P. 60-80ºC) was poured into the flask fitted with soxhlet extraction unit. The weighed sample (5g) was transferred into the thimble which was already fixed into the soxhlet extraction unit. Cold water circulation and the heating mantle was switched on, the heating rate adjusted until the solvent was refluxing at a steady rate. The extraction process was carried out for 8hours. The solvent was recovered by evaporation and the thimble fitted to the central

siphon portion of the extractor of the soxhlet apparatus. The flask and its content were placed in the oven at 70º±2C for one hour. The flask was cooled in a desiccator and weighed. The flask and the content were then replaced in the oven for 30minutes after which it was reweighed. This was repeated until the sample was dried to a constant weight. From the weight of the material residue in the receiver flask the percentage lipid content was determined as given below:

$$\% \text{ lipid content} = \frac{\text{Wt of lipid extracted}}{\text{Wt of sample}} \times 100$$

3.4.4 Determination of Nitrogen/Crude Protein Content

The method of Pearson's (1976) was used. 0.5g of the sample was weighed into 100ml Kjedahl flask and 1g of anti-bumping granules was added. One gram (1g) of the digesting mixed catalyst ($CUSO_4$ and K_2SO_4, ratios 8:1) and 15ml of concentrated sulphuric acid (H_2SO_4) was added. The flask was placed on a Kjedahl digestion rack and heated until a clear solution was obtained. At the end of the digestion, the flask was cooled and the sample was quantitatively transferred to a 100ml volumetric flask and made up to the mark with distilled water.

Ten milliliters (10ml) of the digest was pipetted into Markham steam distillation chamber and 10ml of 40% sodium hydroxide added. The sample was steam distilled, librating ammonia into a 100ml conical flask containing 10ml of 4% boric acid and a drop of mixed indicator (methyl red and methyl blue, ratios 2:1) until indicator changed colour from pink to green and 30ml volume of sample (distillate) was collected. The content of the conical flask (ammonium borate) was titrated against standard 0.1M HCl until the end point was indicated by a changed from greenish to pink colour. The volume of the acid used for each distillate was noted.

% Total nitrogen per gram samples was calculated using the formula

$$\% \text{ Nitrogen} = \frac{M \times V \times 14 \times 100 \times 100}{Wt \times 1000 \times 10}$$

Where:

M = Molarity of HCl

V = Volume of acid used

Wt = Weight of sample

14 = Atomic weight of nitrogen

100 = Total volume of digest

100 = % Conversion

1000 = Conversion to litre

10 = Volume of digest taken

% Crude protein = 6.25 × % N_2

Where 6.25 is the protein conversion factor since it is assumed that protein contains 16% nitrogen. Thus 1mg nitrogen = 6.25 protein

3.4.5 Determination of Crude Fibre

The AOAC (1980) method was used. Two grammes (2g) of the finely ground sample was placed into a round bottom flask, 100ml of 0.25m H_2SO_4 was added and the mixture boiled under reflux for 30minutes. The hot solution was quickly filtered under suction. The insoluble residue was washed several times with hot water until it was acid free. This was quantitatively transferred into the flask and 100cm^3 of hot (0.312M) NaOH solution was added and the mixture boiled again under reflux for 30minutes and quickly filtered under suction. The insoluble residue was washed with boiling water until it was base free. It was dried to a constant weight in an oven at 100°C, cooled in a decsicator and weighed (C_2). The weighed residue was then incinerated in a muffle furnace at 550°C for 2hours, cooled in a decsicator for 24hours and reweighed (C_3). The crude fibre was calculated as the wieght lost on ashing:

Weight loss on ashing (incineration) = $C_2 - C_3$

Weight of original sample = W

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

3.4.6 Determination of Nitrogen Free Extract (NFE)

This was determined by difference:

% Carbohydrate = 100 – (% Crude protein + % Lipid + %Crude fibre + %Ash + % Moisture)

3.4.7 Determination of Amino Acid Profile

The amino acid profile of the sample was determined by using the method described by Spackman *et al.* (1958). Five grams (5g) of the sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM)-1model DNA 0209 as indicated in the manual of Technicon Instrument Company (1973)

Defatting sample: Three (3.0g) grams of dried sdample was weighed into the extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (1980). The extraction lasted for 15 hours.

Hydrolysis of the sample: Two (2.0g) grams of the defatted sample was weighed into a glass ampoule. Seven millilitres (7ml) of 6 NHCl was added and oxygen was expelled by passing nitrogen into the ampoule (this was to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoule was then sealed with Bunsen burner flame and placed in an oven preset at 105ºC ±5ºC for 22hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins.

The filtrate was then evaporated to dryness at 40±2ºC under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles at 2±1°C in a freezer.

Loading of the hydrolysate into the TSM Analyzer (DNA 0209): Five to ten (5–10) microlitres of the hydrolysate was loaded. This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydolysate. The analysis lasted for 76 minutes.

Method of calculating Amino Acid values from the chromatogram peaks: The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and the width of the peak on the half height was measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half height.

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

$$NE = \frac{\text{Area of Norleucine peak}}{\text{Area of each amino acid}}$$

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

$S_{std} = NE_{std} \times \text{Mol. Weight} \times \mu MMA_{std}$

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

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

$$\text{Where: } C = \frac{\text{Dilution} \times 16}{\text{Sample Wt(g)} \times N\% \times 10 \times \text{Vol. Loaded}} \div NH \times W \text{ (nleu)}$$

Where: NH = Net height

W = Width @ half height

nleu = Norleucine

Technicon TSM-1 Model DNA 0209 had the capacity to analyse the following Amino Acids on g/100g protein basis: - Lysine, Histidine, Arginine, Aspartic Acid,

Threonine, Serine, Glutamic acid, Proline, Glycine, Alanine, Cystine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine.

3.5 Determination of Anti-Nutrients

Samples of the differently processed seeds were taken to Food Science Laboratory Faculty of Agriculture Ahmadu Bello University Zaria for the determination of anti-nutritional Factors. The anti-nutrients that were determined included Tannin, Total Oxalate, Hydrogen Cyanide (HCN), Saponin, and Phytic acid (Phytate).

3.5.1 Determination of Tannin

Tannin was determined using the method described by AOAC (1980). Two grams (2.0g) of the ground sample was defatted for 2 hours using soxhlet extraction apparatus. The residue was placed in an oven for 12 hours, retrieved and boiled at 100^0C, with 300ml of distilled water, diluted to 500ml in a standard volumetric flask and filtered through non-absorbent cotton wool.

A volume of 25ml of the infusion was measured into 2 litre porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution changed to green, then few drops of 0.1N potassium permanganate was added. The difference between the two titration was multiplied by 0.006235 to obtain the amount of tannin in the sample since 0.1N oxalic acid = 0.006235g tannin.

3.5.2 Determination of Total Oxalate

The total oxalic acid of the powdered samples was determined as described by Abeza *et al.* (1968). Two grams (2.0g) aliquot of the ground seed 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 then 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. The filtrate was boiled, 10ml of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through Whatman No. 40 filter paper. The precipitate was rinsed several times with distilled water. The precipitate was transferred to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80^0C then cooled 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 thus:

1ml of potassium permanganate = 2.24mg oxalate

3.5.3 Determination of Hydrogen Cyanide (HCN)

Hydrogen cyanide was determined by the alkaline titration procedure (AOAC, 1995). 10g of ground sample was soaked in a mixture of 200cm^3 of the distilled water and 10cm^3 of orthophosphoric acid. The mixture was left overnight to release all bounded hydrocyanic acid. The mixture was then distilled until 150cm^3 of the distillate was collected. 20cm^3 of distillate was taken into a conical flask containing 40cm^3 of distilled water. 8cm^3 of 6mol dm^3 aqueous ammonia and 2cm^3 of 5% potassium iodide solutions were added. The mixture was titrated with 0.02 mol dm^3 silver nitrate to a faint end point with permanent turbidity.

1ml 0.02N $AgNO_3$ = 1.08mg HCN

3.5.4 Determination of Saponin

10g of the ground sample was poured into a conical flask containing 100cm^3 of 20% aqueous ethanol and agitated with a magnetic stirrer for twelve (12) hours at 55^0C.

The solution was filtered using whatman No. 1 filter paper and a residue was re-extracted with 200cm^3 of 20% aqueous ethanol. The extracts were combined and reduced to about 40cm^3 under vacuum. The extract and 200cm^{3}diethyl ether were transferred into a 250cm^3 separatory funnel and shaken vigorously. The aqueous layer was discarded. The process of purification continued until a colourless aqueous extract was obtained. The pH of the remaining aqueous solution was adjusted to 4.5 by adding 4g NaCl, and the solution was then shaken successively with 60cm^3 and 30cm^3 portions of n-butanol. The butanolic extract was washed twice with 10cm^3 of 5% aqueous sodium chloride evaporated to dryness in a fume cupboard, to give the saponin, which was weighed and expressed as percentage (Hudson and El-Difrawi, 1979)

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

3.5.5 Determination of Percentage Phytate

A known weight of each ground sample was soaked into 100ml of 2% HCl for 5 hours and filtered. Twenty-five cubic centimetres (25cm^3) of the filtrate was taken into a conical flask and five cubic centimetres (5cm^3) of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of $FeCl_3$ until a brownish – yellow colour persisted for 5 minutes (Reddy *et al.,* 1982).

The concentration of the $FeCl_3$ was 1.04% W/V

Calculation mole ratio of Fe to phytate = 1:1

Concentration of phytate phosphorous

$$= \frac{\text{Titre value} \times 0.064}{1000 \times \text{Weight of sample}}$$

Phytic acid content was calculated on assumption that it contains 28.20% phosphorus by weight

3.6 Experimental Fish

Clarias gariepinus (Teugels) used in the experiment were obtained from a homogenous source in a standardized hatchery, Mairuwa fish farm, along Funtua-Gusau road, Katsina State. Three hundred and fifty (350) *Clarias juveniles* were transported in two (50litres) jerrycans between 7.00am and 8.00am, to an outdoor concrete tank (100cm x 150cm x 120cm) at the Department of Biological Sciences A.B.U, Zaria for two weeks to acclimatize prior to feeding trials. Fish were fed during the acclimatization period with 42% crude protein commercial feed (control diet) at 5% body weight twice daily. During the acclimatization period, the water temperature, pH and dissolved oxygen (D.O) were monitored. Water in the outdoor concrete tank was replaced at two days interval with dechlorinated water, stored for 24 to 48 hours prior to utilization.

3.7 Experimental Facilities/Design

3.7.1 Experimental Facilities

At the end of the acclimatization period of twenty-four (24) hours improvised non-recirculatory but semi-flow through indoor plastic tanks (52cm x 34cm x 33.5cm) were used for conducting the experiments at the rate of twelve (12) tanks per experiment. The total water holding capacity of each plastic tank was 54litres and each plastic tank constantly contained 40litres of dechlorinated water which served as water environment for the fish throughout the experimental period. The setup was covered with wire mesh to prevent fish from jumping out of the tank.

3.7.2 Experimental Design

The experimental design was a complete randomized design. Out of the three hundred and fifty (350) *Clarias* juveniles obtained from the hatchery, a total of two hundred and forty (240) juvenile fish of mean weight 20g were randomly selected and

distributed into the tanks at a stocking rate of 10 fish per tank. The twenty-four (24) tanks of 10 fish each were randomly assigned to 8 treatments (control inclusive) at the rate of one treatment per experimental diet with 3 replicates per treatment. In the first experiment Soaked *Bauhinia* Seed Meal ($SBSM_{96}$) soaked for 96 hours was used as protein source to progressively replace fish meal on an isonitrogenous (40% CP) and isocaloric (3212Kcal/Kg) basis at graded inclusion levels of 0% (Treatment 1), 25% (Treatment 2), 50% (Treatment 3) and 75% (Treatment 4) in the ration formulated. Ration 5, comprised of the imported fish diet (Coppens) which served as the control diet.

Table 3.1: Gross Composition of Experimental Diets (%)

Dietary treatments

Ingredients	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Replacement levels	**fishmeal 100%**	**75%**	**50%**	**25%**	**(Copens)**
Of Soaked Bauhinia Seed Meal	**$SBSM_{96}$-0%**	**25%**	**50%**	**75%**	**100%**
Maize	25	18	8	3	
Soyacake	12	21	31.65	37.25	
Wheat offal	5.95	3.75	1	-	
Palm oil	4.0	3.20	5.0	5.0	
Bone meal	1	1	1	1	
Cassava	2	2	2	2	
Fish meal	44	33	22	11	
Bauhinia	0	11	22	33	
Salt	0.3	0.3	0.3	0.3	
Premix	0.25	0.25	0.25	0.25	
DL-Methionine	2.0	2.2	2.4	2.6	
Lysine	3.5	4.0	4.4	4.8	
Total	100	100	100	100	
Calculated nutrient composition analysis					
Crude Protein %	40.00	40.00	40.00	40.00	
Metabolizable Energy (Kcal/Kg)	3212	3208	3209	3206	
Ether Extract %	4.69	4.50	4.88	5.01	
Crude Fibre %	2.52	2.59	2.81	3.11	
Calcium %	1.82	1.68	1.77	1.80	
Phosphorus %	1.27	1.17	1.01	1.12	
Lysine %	5.09	5.01	5.11	5.09	
DL-Methionine %	3.05	3.04	3.04	3.02	
Feed Cost ₦/Kg	119.51	114.23	110.08	105.18	320

Composition per 25kg of Bio premix is Vitamin A 4,000iu; Vitamin D 800,000iu; Vitamin E1 500mg; Niacin 10,000mg; Panthotenic acid 3,500mg; Biotin 15mg; Vitamin B 10mg; Folic acid 200mg; Chloric chloride 130,000mg; Manganese 60,000mg; Iron 15,000mg; Zinc 15,000mg; Copper 800mg; Iodine 400mg; Cobalt, 80mg; Selenium 400mg; Antioxidant 40,000mg.
All vitamins and minerals included in the composition met the NRC (1993) recommendations.

The second experiment comprised of locust meal at 0%, 25%, 50% and 75% graded levels of inclusion in replacement of fish meal, on an isonitrogenous (40% CP) and isocaloric (3212Kcal/Kg) basis. The graded levels of inclusion corresponded to 4 treatments and there was also a fifth treatment which comprised only the commercially formulated feed which served as control. The diet with exclusively fish meal (100%) along with 0% soaked *Bauhinia* Seed Meal ($SBSM_{96}$) and 0% Locust Meal (LM) also served as control. The gross compositions of the experimental diets are presented in tables 3.1 and 3.2.

Table 3.2: Gross Composition of Experimental Diets (%)

	Dietary treatments				
Ingredients **Replacement levels** **Of Sun-dried Locust Meal**	**Diet 1** **Fishmeal 100%** **SLM - 0%**	**Diet 2** **75%** **25%**	**Diet 3** **50%** **50%**	**Diet 4** **25%** **75%**	**Diet 5** **(Copens)** **100%**
Maize	25	17.45	12.00	6.00	
Soyacake	12	19.00	26.45	35.45	
Wheat offal	5.95	5.0	2.0	-	
Palm oil	4.0	5.0	4.0	3.0	
Bone meal	1	1	1	1	
Cassava	2	2	2	2	
Fish meal	44	33	22	11	
Locust	0	11	22	33	
Salt	0.3	0.3	0.3	0.3	
Premix	0.25	0.25	0.25	0.25	
DL-Methionine	2.0	2.0	2.5	2.5	
Lysine	3.5	4.0	4.5	4.5	
Total	100	100	100	100	
Calculated nutrient composition analysis					
Crude Protein %	40.00	40.00	40.00	40.00	
Metabolizable Energy (Kcal/Kg)	3212	3235	3225	3226	
Ether Extract %	4.69	3.60	3.55	3.45	
Crude Fibre %	2.52	2.97	3.21	3.40	
Calcium %	1.87	1.52	1.31	1.28	
Phosphorus %	1.27	1.06	0.96	0.85	
Lysine %	5.09	3.05	3.05	3.01	
DL-Methionine %	3.05	5.05	5.00	5.02	
Feed Cost ₦/Kg	119.51	114.11	116.88	112.46	320

Composition per 25kg of Bio premix is Vitamin A 4,000iu; Vitamin D 800.000iu; Vitamin E1 500mg; Niacin 10,000mg; Panthotenic acid 3,500mg; Biotin 15mg; Vitamin B 10mg; Folic acid 200mg; Chloric chloride 130,000mg; Manganese 60,000mg; Iron 15,000mg; Zinc 15,000mg; Copper 800mg; Iodine 400mg; Cobalt, 80mg; Selenium 400mg; Antioxidant 40,000mg.
All vitamins and minerals included in the composition met the NRC (1993) recommendations

3.8 Feeding of Experimental Fish

The fish were starved for 24 hours prior to the onset of the feeding trial experiment. On commencement of the feeding trial the fish were fed 5% of their biweekly body weight. The fish was fed equal rations twice daily at 08.00 – 09.00am and 17.00 – 18.00pm throughout the 84 days feeding trial experiment. Faecal matter and left-over feed were evacuated while replacing water every two (2) days prior to feeding in the morning. Fish mortality was monitored daily.

3.9 Weighing of Experimental Fish

The individual weight of each fish was determined immediately after acclimatization using an electronic top-loading Mettler balance (model PB 3002). The mean weights of fish per tank were recorded. The weighing continued biweekly until the experiment was terminated. Length measurement in form of standard length (SL) and total length (TL) of fish were recorded throughout the duration of the experiment using a metre rule measuring board on a biweekly basis. The standard length was derived from the tip of the snout to the base of the caudal fin and total length from the tip of the snout to the tip of the caudal fin (Gupta and Gupta, 2006). The biweekly weighing allowed for adjustment of feeding levels for the subsequent weeks.

3.10 Water Quality Assessment

Water temperature and pH were measured weekly using HANNA instruments Model: HI-98129 and HI-987130 respectively. Dissolved oxygen (DO) was measured using dissolved oxygen test kit (HANNA instruments model: HI-3810). Water in each tank was continuosly aerated by use of aerators with air stones. At the end of the feeding trial the water in each tank was drained off and the total number of fish was counted.

3.11 Feed Stuffs, Feed Processing and Feed Formulation and Least Cost Analysis

3.11.1 Feed stuffs

The best processed *Bauhinia monandra* seeds in terms of quality of nutrient and least amount of antinutrient, was utilized as the main protein source in the experimental feeding trial (Experiment II). The best processed seed was the seed soaked for 96 hours ($SPMS_{96}$); (Table 4.5). The locust was obtained from a market in Sokoto State, Nigeria. Other ingredients such as yellow maize, fish meal, palm oil, cassava starch (binder), vitamin and mineral premix, salt, soya bean cake and bone meal were obtained locally from a commercial feed store in Samaru, Zaria, Kaduna State, Nigeria. The soaked *Bauhinia* seed meal ($SBSM_{96}$) and the locust meal (LM) served as the main protein sources while the yellow maize and cassava starch served as the main energy sources as well as binder (coagulant). The palm oil served as a source of fatty acid. Fish meal (FM) was supplementary for the plant protein (Soaked *Bauhinia* seed meal $SBSM_{96}$) and locust meal (LM).

3.11.2 Processing of *Bauhinia* seed and other feed stuffs

Twelve (12kg) of dried *Bauhinia* seed was soaked in 120l of water for 96 hours; thereafter, the water was decanted, and the seed was air-dried for 72hrs; the dried seed, bone meal, maize and sorted fish (Clupeids) were ground using a hammer mill grinder. Salt, vitamin and mineral premix were then added to the ground feed stuffs then packed in clean polythene bags and sealed prior to use in feed preparation.

3.11.3 Feed preparation

The feed preparation was carried out using the method described by Adepaurusi and Eleyimi (2004), Eyo (2003) and Banyigyi (2001). The ground ingredients were sieved using a 0.2mm diameter sieve to obtain the powdery form. The ingredients were weighed

in the correct proportions as formulated in the diet (table 3.1 and 3.2) and then mixed thoroughly before using gelatinized cassava starch as a binder. The obtained homogenous mass of dough was passed through a locally improvised pelletizer to produce 2mm diets which were immediately sun dried for 72hrs, then packaged in cellophane bags and stored in a cool place.

3.11.4 Feed formulation

The feed formulation was based on 40% crude protein. The quantity of the feed ingredients required to formulate the experimental diets on an absolute basis of 100kg (100%) were obtained through computer formulation (Table 3.1 and 3.2) Three (3) experimental diets were prepared for the experimental fish in each of the experiment (Experiment II and III).

The information required for the computer formulation of least-cost feed as described by Lovell (1981) was adopted. These included: cost of feed ingredients, nutrient components of the feed, availability of feed materials, Essential Amino Acids (EAA), Essential Fatty Acids (EFA), phosphorus, calcium and digestible energy and protein ratio.

The standard procedures by Talabi (1986) using the linear programming techniques was adopted to obtain the correct feed formulation for production of cost effective feeds.

3.12 Biochemical Analysis of Feed and Fish Samples

Prior to onset of the experiment and on completion of the experiment samples of the prepared feeds and eviscerated whole carcasses of the fish in each treatment were collected and ground into fine powder and subjected to chemical analysis to obtain their proximate composition [moisture content, ash, lipids (crude fat), crude fibre (CF), crude protein (CP) and Nitrogen Free Extract (NFE)]; as described in section 3.2. The AOAC

method (1980) was used in the determination of moisture content, ash content, lipid content and crude fibre content while the method of Pearson's (1976) was used to determine nitrogen/crude protein content of the samples. The analytical procedures followed the same pattern as described in experiment I.

3.13 Growth Performance and Feed Utilization Parameters

3.13.1 Mean body weight gain

This was calculated as the difference between the initial and final body weights for fish

$= W_2 - W_1$

Where W_2 = Final body weight

W_1 = Initial body weight

3.13.2 Mean increase in standard length (CM)

$L_2 - L_1$

Where L_2 = Finial standard length

L_1 = Initial standard length

3.13.3 Percentage live weight gain (PLWG)

The PLWG was computed as the difference between the initial and final fish weight divided by the initial weight expressed as percentage

$$\frac{W_2 - W_1}{W1} \times 100$$ (Wannigama *et al*., 1985)

3.13.4 Specific growth rate (SGR) in percentage body weight per day

$$SGR = 100 \frac{(LnW_2 - LnW_1)}{t}$$ (Heper, 1988)

Where W_2 = Final Weight

W_1 = Initial wight

t = Period of experiment in days

Ln = base of natural logarithm

3.14 Feed Utilization (Total Unit of Feed Consumed)

3.14.1 The feed conversion ratio (FCR)

$$FCR = \frac{\text{Amount of Feed Fed}}{\text{Weight gain (g)}}$$ (Arunletaree and Moolthongnoi, 2008)

3.14.2 Protein efficiency ratio (PER)

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

Where protein Fed =

$$\frac{\text{\% protein in the diet}}{100} \times \text{total diet consumed}$$ (Olvera-Novoa, 1990)

3.14.3 Net protein utilization (NPU)

$$= \frac{\text{Fish protein gain}}{\text{Protein Fed}} \times 100$$(Dabrowski and Kozak, 1979)

Where Protein gain = Final body Protein – Initial body protein

Protein consumed = Total Dietary protein fed

3.14.4 Economy of feed and weight gain (cost effectiveness of diets)

The total financial costs of the various diets treatnments were estimated against fish weight gained so as to compare the economy of the various diets. The economy of weight gain (EWG) was calculated as follows:

$$EWG = \frac{\text{Financial cost of feed ₦}}{\text{Weight gain (g)}}$$ (Igbinosun, 1982)

Least cost analysis:

i. Cost of feeding/kg

ii. Cost of feeding/kg weight gain

iii. Cost of feed / kg protein produced

3.14.5 Condition factor

$$K = \frac{100W}{L^3}$$ (Madu and Akilo, 2001)

Where W = Final Body Weight (g)

L = Final Standard Length (cm)

3.15 Essential Amino Acid Index

The essential amino acid index is the geometric mean of the egg ratios of the acids, calculated as:

$$EAAI = \sqrt[n]{\frac{a}{a_e} \times \frac{b}{b_e} \times \frac{c}{c_e} \times \ldots \times \frac{j}{j_e}}$$ (McDonald *et al*., 1987)

Where a, b, c, … j = concentrations (g/kg) of the indispensable amino acids in the food protein, a_e, b_e, c_e … j_e = concentrations of the same amino acids in egg protein, and n = the number of amino acids entering into the calculation.

3.16 Statistical Analysis

Data for each parameter were subjected to analysis of variance (ANOVA) to determine the significance of the variations between parameters examined at ($P<0.05$). Least Square of Difference (LSD) was used to compare the differences among means of parameters evaluated.

CHAPTER FOUR

4.0 RESULTS

4.1 Physico Chemical Parameters of Water

The temperature values in the indoor semi-flow through non recirculatory system (Table 4.1a and 4.1b) ranged between 19.6°C – 27.8°C ($\overline{X}$ 23.7). There was no significant differences (P>0.05) in temperature between dietary treatments. The lower temperature ranges were recorded at the onset of the experiments, August, which coincided with the peak of rainy season

The hydrogen ion concentration (pH) values (Table 4.1a and 4.1b) of water ranged between 7.3 – 8.7 ($\overline{X}$ 7.65). The Dissolved Oxygen (DO) ranged between 3.4 – 6.8mg/l ($\overline{X}$=5.1mg/l).

All the physico chemical parameters of water in Tables 4.1a and 4.1b were within the range for culturing most tropical fishes the African cat fish, *Clarias gariepinus* inclusive (Viveen *et al.,* 1985; Adesulu, 2001).

Table 4.1a: The mean water parameter values in experimental tanks during the feeding trial of *Clarias gariepinus* fed bauhinia diet.

Bauhinia Diet	Temperature (T°C)	pH value	Dissolved oxygen (mg/l)
T_1 100% fishmeal 0SBSM$_{96}$	19.6 – 27.8 ($\overline{X}$ = 23.7)	7.4 – 8.4 ($\overline{X}$ = 7.9)	3.5 – 6.3($\overline{X}$ = 4.9)
T_2 75% fishmeal 25%SBSM$_{96}$	19.6 – 27.8($\overline{X}$ = 23.7)	7.3 – 8.5($\overline{X}$ = 7.9)	3.6 – 6.6 ($\overline{X}$ = 5.1)
T_3 50% fishmeal 50%SBSM$_{96}$	19.6 – 27.8($\overline{X}$ = 23.7)	7.3 – 8.5($\overline{X}$ = 7.9)	3.6 – 6.8 ($\overline{X}$ = 5.2)
T_4 25% fishmeal 75%SBSM$_{96}$	19.6 – 27.8($\overline{X}$ = 23.7)	7.4 – 8.6($\overline{X}$ = 8.0)	3.7 – 6.7 ($\overline{X}$ = 5.2)
T_5 100% Copens	19.6 – 27.8($\overline{X}$ = 23.7)	7.4 – 8.7($\overline{X}$ =8.1)	3.4 – 6.6 ($\overline{X}$ = 5.0)

Table 4.1b: The mean water parameter values in experimental tanks during the feeding trial of *Clarias gariepinus* fed locust diet.

Locust Diet	Temperature (T°C)	pH value	Dissolved oxygen (mg/l)
T_1 100% fishmeal	19.6 – 27.8 ($\overline{X}$ = 23.7)	7.4 – 8.4 ($\overline{X}$ = 7.9)	3.5 – 6.3($\overline{X}$ = 4.9)
T_2 75% fishmeal 25%locust	19.6 – 27.8($\overline{X}$ = 23.7)	7.5 – 8.5($\overline{X}$ = 8.0)	3.6 – 6.7 ($\overline{X}$ = 5.2)
T_3 50% fishmeal 50%locust	19.6 – 27.8($\overline{X}$ = 23.7)	7.4 – 8.6($\overline{X}$ = 8.0)	3.6 – 6.4 ($\overline{X}$ = 5.0)
T_4 25% fishmeal 75%locust	19.6 – 27.8($\overline{X}$ = 23.7)	7.3 – 8.7($\overline{X}$ = 8.0)	3.8 – 6.5 ($\overline{X}$ = 5.2)
T_5 100% Copens	19.6 – 27.8($\overline{X}$ = 23.7)	7.4 – 8.7($\overline{X}$ =8.1)	3.4 – 6.6 ($\overline{X}$ = 5.0)

4.2 Proximate Composition of Boiled *Bauhinia* Seed at Different Time Interval

The results clearly show that the value of Ash, CP, CF, EE, NFE and EAAI reduces with increase in toasting time. There was significant difference at $P < 0.05$ of the raw seed with the toasted seed. However, among the boiled seeds, there was no significant difference in the values of CF eventhough there was significant difference in the values of DM, CP, EE, Ash, NFE and EAAI at $P < 0.05$ (Table 4.2).

Table 4.2: Percentage proximate composition of boiled *Bauhinia* seed at different time intervals

Minutes Trts	10	20	30	40	Raw seed	SED ±	LSD(P<0.05)
DM	96.19 ^{c}	96.14 ^{b}	96.02 ^{a}	96.25 ^{d}	96.39 ^{e}	0.012	0.034
ASH	4.67 ^{c}	4.65 ^{c}	4.63 ^{c}	4.57 ^{b}	3.81 ^{a}	0.014	0.039
CP	30.48 ^{b}	30.47 ^{ab}	30.32 ^{a}	30.23 ^{a}	30.52 ^{b}	0.086	0.239
CF	7.37 ^{b}	7.36 ^{b}	7.35 ^{b}	7.33 ^{b}	6.41 ^{a}	0.014	0.038
EE	27.43 ^{b}	27.41 ^{b}	27.37 ^{a}	27.35 ^{a}	27.87 ^{c}	0.012	0.034
NFE	26.92 ^{d}	26.25 ^{a}	26.36 ^{b}	26.79 ^{c}	27.45 ^{e}	0.029	0.081
EAAI	0.61 ^{b}	0.57 ^{a}	0.55 ^{a}	0.55 ^{a}	0.64 ^{b}	0.013	0.036

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

4.3 Proximate Composition of Toasted *Bauhinia* Seed at Different Time Intervals

The results clearly show that the value of Ash, CP, CF, EE, NFE and EAAI reduces with increase in toastig time. There was significant difference at $P < 0.05$ of the raw seed with the toasted seed. There was significant difference in the values of DM, CP, EE, Ash, NFE and EAAI at $P < 0.05$ among the toasted seeds (Table 4.3).

Table 4.3: Percentage proximate analysis of toasted *Bauhinia* seed at different time intervals

Minutes Trts	10	20	30	40	Raw seed	SED ±	LSD(P<0.05)
DM	96.29 [b]	96.34 [c]	96.19 [a]	96.33 [bc]	96.39 [d]	0.015	0.042
ASH	3.89 [d]	3.54 [a]	3.54 [a]	3.61 [b]	3.81 [c]	0.028	0.057
CP	26.77 [d]	26.41 [c]	26.39 [b]	25.01 [a]	30.65 [e]	0.013	0.037
CF	9.07 [d]	9.05 [cd]	9.01 [c]	8.83 [b]	6.41 [a]	0.014	0.039
EE	27.21 [b]	27.06 [a]	27.04 [a]	27.01 [a]	27.87 [c]	0.017	0.048
NFE	29.36 [b]	30.29 [c]	30.22 [c]	31.87 [d]	27.45 [a]	0.047	0.131
EAAI	0.51 [c]	0.47 [b]	0.46 [ab]	0.43 [a]	0.64 [d]	0.010	0.028

Values with the same superscripts in the same row are not significantly different ($P > 0.05$) LSD.

4.4 Proximate Composition of Soaked *Bauhinia* Seed Meal

The results indicated that the values of Ash, CF and NFE reduces with increase in soaking time while the values of DM, CP, EE and EAAI increases with increase in soaking time. There was significant difference at $P < 0.05$ of the raw seed with the soaked seed, however the EAAI did not show any significant difference with the raw seed at $P < 0.05$. Among the soaked seeds, there was no significant difference in the values of EE and EAAI eventhough there was significant difference in the values of DM, CP, CF, Ash and NFE at $P < 0.05$ (Table 4.4)

Table 4.4: Percentage proximate composition of soaked *Bauhinia* seed meal

Hours Trts	24	48	72	96	Raw seed	SED ±	LSD(P<0.05)
DM	95.39 ^{c}	95.37 ^{c}	94.39 ^{b}	94.06 ^{a}	96.39 ^{d}	0.049	0.137
ASH	3.17 ^{b}	3.17 ^{b}	3.09 ^{a}	3.06 ^{a}	3.81 ^{c}	0.017	0.047
CP	30.81 ^{b}	30.85 ^{b}	32.38 ^{c}	32.45 ^{c}	30.65 ^{a}	0.028	0.079
CF	6.17 ^{b}	6.17 ^{b}	6.07 ^{a}	6.04 ^{a}	6.41 ^{c}	0.014	0.039
EE	27.37 ^{a}	27.38 ^{a}	27.39 ^{a}	27.41 ^{a}	27.87 ^{b}	0.130	0.036
NFE	27.87 ^{c}	27.80 ^{c}	25.47 ^{b}	25.11 ^{a}	27.45 ^{b}	0.057	0.160
EAAI	0.63	0.62	0.66	0.66	0.64	0.020	0.056NS

Values with the same superscripts in the same row are not significantly different ($P > 0.05$) LSD.

4.5 Amino Acid Profile (g/100g protein)

The amino acid profile of all the processed seed and presented in Table 4.5. The seed contained all the essential amino acids with the exception of tryptophan. The least lysine value was recorded in the Roasted seeds ($RBSM_{30}$ and $RBSM_{40}$). However the highest value was recorded in the soaked seed ($SBSM_{24}$, $SBSM_{48}$, $SBSM_{72}$ and $SBSM_{96}$). The least methionine value was recorded in roasted seed ($RBSM_{30}$ and $RBSM_{40}$) and soaked seed ($SBSM_{72}$) and ($SBSM_{96}$). The highest methionine value was recorded in Roasted Bauhinia ($RBSM_{10}$) and Boiled Bauhinia ($BBSM_{20}$). The amino acid profile of the raw seeds was generally high in comparison to some of the values in the processed seeds (Table 4.5).

TABLE 4.5: Amino Acid Composition (g/100g Protein) of Raw and Differently Processed *Bauhinia* Seed Meals (Amino Acid Profile)

	Raw	**Boiled**				**Roasted**				**Soaked**				**Locust**	**Fish meal**
EAA	BSM	$BBSM_{10}$	$BBSM_{20}$	$BBSM_{30}$	$BBSM_{40}$	$RBSM_{10}$	$RBSM_{20}$	$RBSM_{30}$	$RBSM_{40}$	$SBSM_{24}$	$SBSM_{48}$	$SBSM_{72}$	$SBSM_{96}$	LM	FM
Arginine	6.38	6.04	5.18	6.25	6.78	6.05	5.39	4.90	4.30	5.70	6.52	6.92	6.30	7.42	6.13
Histidine	1.96	2.05	2.00	1.54	2.30	2.05	1.86	1.80	1.64	2.25	1.80	1.30	1.16	4.15	2.65
Isoleucine	3.97	3.81	3.28	3.06	1.79	4.15	4.03	3.04	2.39	0.76	0.67	0.86	0.92	4.11	3.68
Leucine	6.50	7.05	5.51	6.36	5.02	6.58	5.60	6.90	4.40	6.02	4.65	6.43	6.93	5.91	7.02
Lysine	4.31	4.56	4.05	3.81	4.13	4.05	3.02	2.61	2.21	4.85	4.53	5.03	5.30	5.90	7.83
Methionine	1.40	1.40	1.50	1.37	1.40	1.56	1.38	0.96	0.73	1.36	1.18	0.90	1.00	2.31	2.87
Phenylalanine	4.31	4.46	3.43	3.09	3.27	4.30	3.96	4.00	3.19	3.85	4.00	4.20	4.50	4.46	4.54
Threonine	3.27	3.38	3.02	3.52	2.57	3.28	3.35	3.41	2.16	2.94	3.50	1.80	1.71	4.02	4.58
Tryptophan															
Valine	5.05	5.25	4.58	4.50	5.05	5.34	4.90	3.91	4.11	5.25	5.20	5.40	5.73	3.77	5.05
Aspartic acid	8.69	8.97	9.03	7.65	10.02	8.03	7.90	7.00	6.67	10.88	11.63	10.97	10.55	9.37	9.49
Serine	2.66	2.45	2.61	3.02	2.76	2.60	2.35	2.15	1.95	2.33	2.04	2.65	2.54	5.00	4.67
Glutamic acid	13.68	11.69	12.21	12.25	13.69	13.71	13.85	14.38	11.25	16.40	16.84	17.36	18.80	15.36	14.50
Proline	2.19	2.22	2.23	1.80	2.57	2.20	1.55	1.72	1.65	1.70	2.00	0.90	0.64	3.78	4.37
Glycine	3.91	4.03	3.57	3.79	4.08	3.99	3.60	4.21	2.03	4.00	3.94	3.73	3.66	4.80	7.05
Alanine	4.33	5.05	4.36	4.38	3.61	4.90	5.60	5.10	2.48	3.56	5.40	3.90	3.80	5.11	6.58
Cystine	1.26	1.25	0.92	0.85	1.19	1.20	0.99	0.95	0.63	1.61	1.00	1.80	1.76	1.66	0.92
Tyrosine	1.71	2.06	1.93	2.30	2.25	2.25	2.61	2.44	1.23	1.79	1.40	2.00	1.80	3.06	3.49

4.6 Anti-Nutritional Composition (mg/100g) of Boiled *Bauhinia* at Different Time Intervals

The results showed that boiling reduced the values of HCN, Tannin, Oxalate, Phytic Acid and Saponins. The values of the antinutritional composition of Boiled Bauhinia Seed reduced with increase in boiling time. There was significant difference ($P<0.05$) between processed and raw seeds only oxalate shows significant difference ($P<0.05$) in the boiled seeds in all the four treatments (10, 20, 30 and 40 minutes) (Table 4.6).

Table 4.6: Anti-nutritional composition (mg/100g) of boiled *Bauhinia* at different time intervals

Minutes Trts	10	20	30	40	Raw seed	SED ±	LSD(P<0.05)
HCN	0.64[c]	0.53 [b]	0.23 [a]	0.22 [a]	0.68 [d]	0.014	0.039
Tanin	6.24 [b]	6.23 [b]	6.23 [b]	4.35[a]	8.75 [c]	0.008	0.024
Oxalate	8.95 [d]	7.63 [c]	6.73 [b]	2.67 [a]	12.08 [e]	0.019	0.053
Phytic acid	10.02 [c]	4.01 [b]	3.03 [a]	3.01 [a]	11.39 [d]	0.025	0.070
Saponins	0.55 [a]	0.54 [a]	0.51 [a]	0.44 [c]	2.74 [b]	0.070	0.195

Values with the same superscripts in the same row are not significantly different ($P > 0.05$) LSD.

4.7 Anti-Nutritional Composition (Mg/100g) of Toasted *Bauhinia* Seed at Different Time Intervals

The results indicated that toasting reduced antinutritional factors. There was significant difference ($P< 0.05$) between the toasted and raw seeds. In toasted seeds there was no significant difference in the values of phytic acid and saponins in the four treatments (Table 4.7).

Table 4.7: Anti-nutritional composition (mg/100g) of toasted *Bauhinia* seed at different time intervals

Minutes Trts	10	20	30	40	Raw seed	SED ±	LSD(P<0.05)
HCN	0.64 [c]	0.53 [b]	0.52 [b]	0.42 [a]	0.68 [d]	0.007	0.020
Tanin	8.71 [b]	8.75 [c]	8.73 [bc]	6.23 [a]	8.75 [c]	0.008	0.023
Oxalate	11.21 [c]	10.02 [b]	10.01 [b]	8.74 [a]	12.08 [d]	0.018	0.050
Phytic acid	10.01 [a]	10.00 [a]	10.01 [a]	10.00 [a]	11.39 [b]	0.011	0.031
Saponins	0.61 [a]	0.58 [a]	0.55 [a]	0.53 [a]	2.74 [b]	0.075	0.209

Values with the same superscripts in the same row are not significantly different ($P > 0.05$) LSD.

4.8 Anti-Nutritional Composition (mg/100g) of Soaked *Bauhinia* Seed

The result showed that soaking reduced value of antinutritional composition. There was significant difference (P< 0.05) between soaked and raw seeds. The soaked seeds Tannin, Oxalate and Phytic acid showed significant difference in all the four treatments. There was no significant difference (P< 0.05) in Saponins in soaked seeds in the four treatments (Table 4.8)

Table 4.8: Anti-nutritional composition (mg/100g) of soaked *Bauhinia* seed

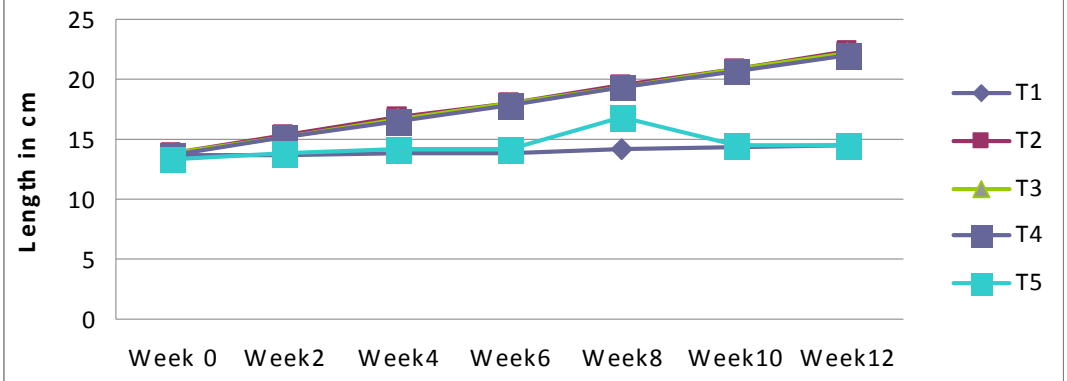
Hours Trts	24	48	72	96	Raw seed	SED ±	LSD(P<0.05)
HCN	0.42 [c]	0.21 [b]	0.16 [b]	0.03 [a]	0.68 [d]	0.037	0.103
Tanin	6.25 [d]	3.82 [c]	3.21 [b]	2.88 [a]	8.75 [e]	0.013	0.037
Oxalate	6.71 [d]	2.35 [c]	2.18 [b]	1.94 [a]	12.08 [e]	0.015	0.042
Phytic acid	3.55 [d]	3.44 [c]	3.13 [b]	0.75 [a]	11.39 [e]	0.005	0.015
Saponins	0.51 [a]	0.43 [a]	0.40 [a]	0.31 [a]	2.74 [b]	0.075	0.208

Values with the same superscripts in the same column are not significantly different (P > 0.05) LSD.

4.9 Mean Length (Cm) of *Clarias gariepinus* Fed Graded Levels of Soaked *Bauhinia* Seed Meal

The results indicate significant difference ($P < 0.05$) in mean length from week 2 to week 12 in all the diets. There was no significant differences ($P > 0.05$) in the initial stocking rate. In weeks 2, 4, 6, 8, 10 and 12 there was no significant difference in length for fish fed diets 2, 3 and 4. However fish fed diets 1 and 5 differed significantly ($P < 0.05$) from the rest in length. (Figure 4.1)

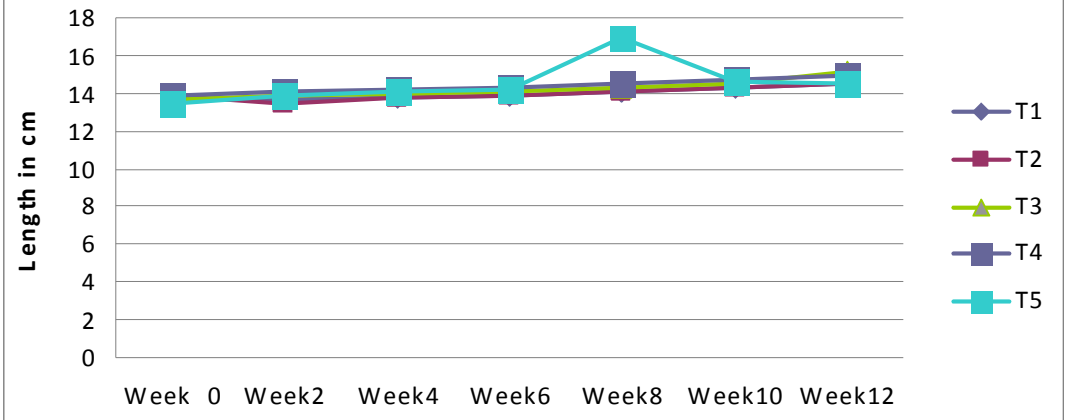
Fig. 4.1: Mean length (cm) of *Clarias gariepinus* fed graded levels of soaked *Bauhinia* seed meal



4.10 Mean Length (cm) of *Clarias Gariepinus* Fed Graded Levels of Locust Meal

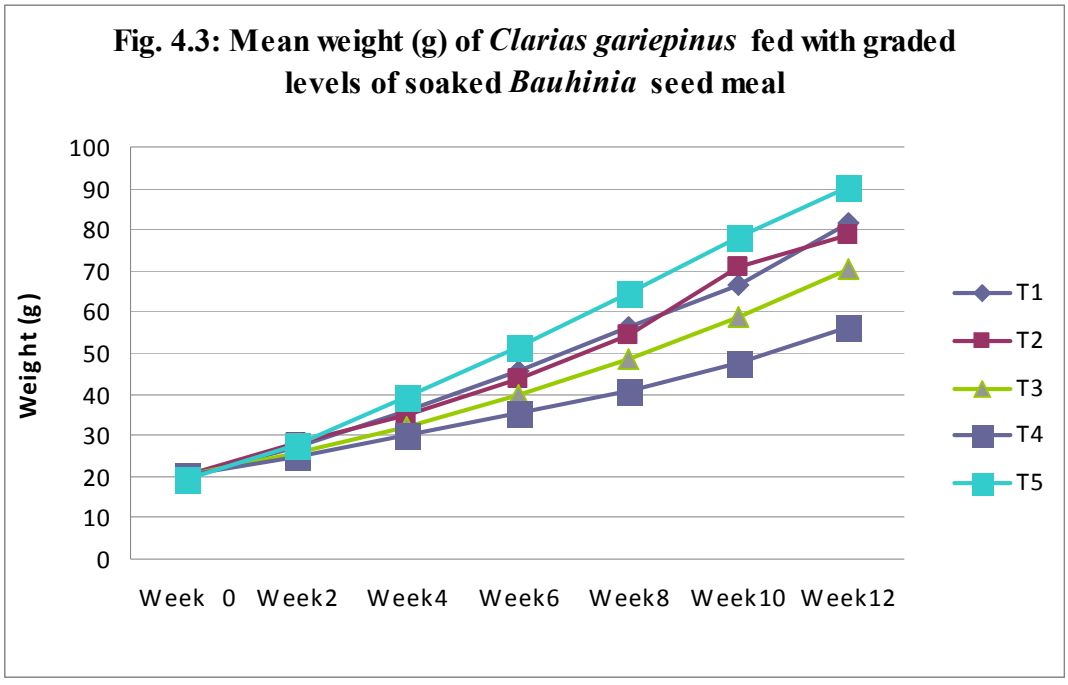
The mean length of *Clarias gariepinus* showed no significant differences (P>0.05) within weeks and among diets over 12 weeks. This result shows that at these stages (biweekly) of *Clarias gariepinus* the rate of increase in length was slow and consistent. Observation at week 2 indicated that fish fed diet 2 had reduced tail length. Again in diet 5 week 10 and 12 similar observations were made. Eventhough there were no significant differences (P>0.05)amongst the treatments, in week 2 diet 3 and 4 gave the highest mean length of 13.9cm each but in week 4 diet 4 gave the highest mean length of 14.2cm. Similar observation was also made in diet 5 in week 8 giving the highest length of 16.9cm which reduced subsequently at 10 and 12 weeks. However, in week 10 diet 4 gave the highest length of 14.7cm while diet 3 gave the highest of 15.16cm in week 12. (Figure 4.2)

Fig. 4.2: Mean length (cm) of *Clarias gariepinus* fed graded levels of locust meal



4.11 Mean Weight (g) of *Clarias gariepinus* Fed with Graded Levels of Soaked *Bauhinia* Seed Meal

There was no significant difference in the initial weight of *C. gariepinus*. The weight of fish increased significantly from week 2 to week 12. The mean length of fish fed diets 1, 2, and 5 were significantly different ($P < 0.05$) with diet 4 which gave the least mean weight of 25.0g. However in week 4 diet 5 (39.1g) gave the highest weight which differed significantly ($P<0.05$) with diets 1, 2, 3 and 4. Comparatively at 4 weeks diets 1, 2 and 5 enhanced weight gain better than diet 3 and 4. At 6, 8, 10 and 12 weeks diet 4 significantly gave the least weight while diet 5 significantly gave the highest mean weight of 51.4g, 64.6g; 78.2g and 90.4g. Generally, diet 1 and 2 showed consistent increase in all the weeks compared to diet 3 and 4. This result shows that diet 5 (90.4g) in the 12 weeks period gave the best weight followed by 1 and 2 particularly at the 12th week where diet 1 showed a weight gain of 81.5 followed by diet 2 which gave 78.5g (Figure 4.3).

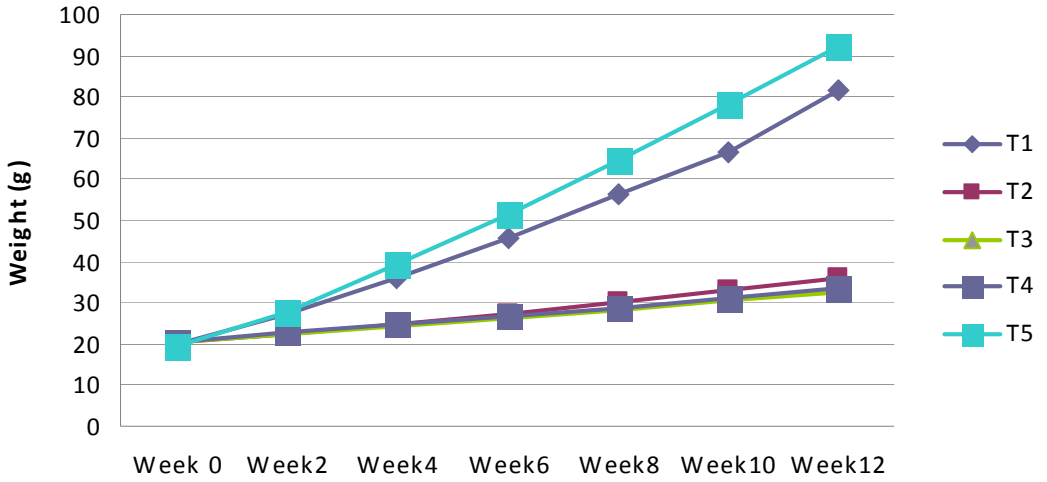


4.12 Mean Weight (g) of *Clarias gariepinus* Fed with Graded Levels of Locust Meal

Diet 5 significantly and consistently increased the weight of Clarias gariepinus from the second week 27.7g to the 12th week which gave 92.4g weight gain. Similar result was observed in mean length of fish fed diet 1.

There was no significant different (P > 0.05) in mean weight of fish for diets 2, 3 and 4 over 12 weeks period. However, the diets showed gradual increase in mean weight from week 2 to week 12 (Figure 4.4).

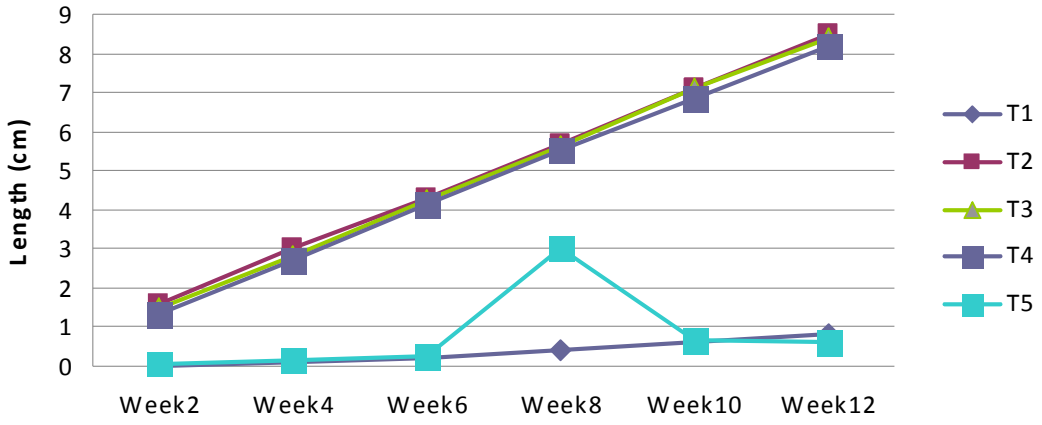
Fig. 4.4: Mean weight (g) of *Clarias gariepinus* fed with graded levels of Locust meal



4.13 Mean Increase in Length (cm) of *Clarias gariepinus* Fed with Graded Levels of *Bauhinia* Seed Meal

Fish fed diets 1 and 5 consistently increase and significantly gave lesser increase in length compared to diets 2, 3 and 4 which consistently gave higher increase in length (Figure 4.5). There was no significant difference (P>0.05) in diets 1, 2 and 3 over the 12 weeks period. However, diets 2, 3 and 4 performed better compared to diets 1 and 5 in terms of increase in length using graded levels of soaked *Bauhinia* seed meal for feeding *Clarias gariepinus*.

Fig. 4.5: Mean increase in length (cm) of *Clarias gariepinus* fed with graded levels of soaked *Bauhinia* seed meal



4.14 Mean Increase in Length (cm) of *Clarias gariepinus* Fed with Graded Levels of Locust Meal

Figure 4.6 indicated that there is no significant difference (P>0.05) in mean length increase from weeks 2 to 10. However, in week 12 significantly slight increase in mean length was observed with diet 3 (1.43cm) which had the highest value for increase in length. The least result was observed in diet 5 (0.63cm). Comparatively in terms of mean length increase soaked *Bauhinia* seed meal gave a better result.

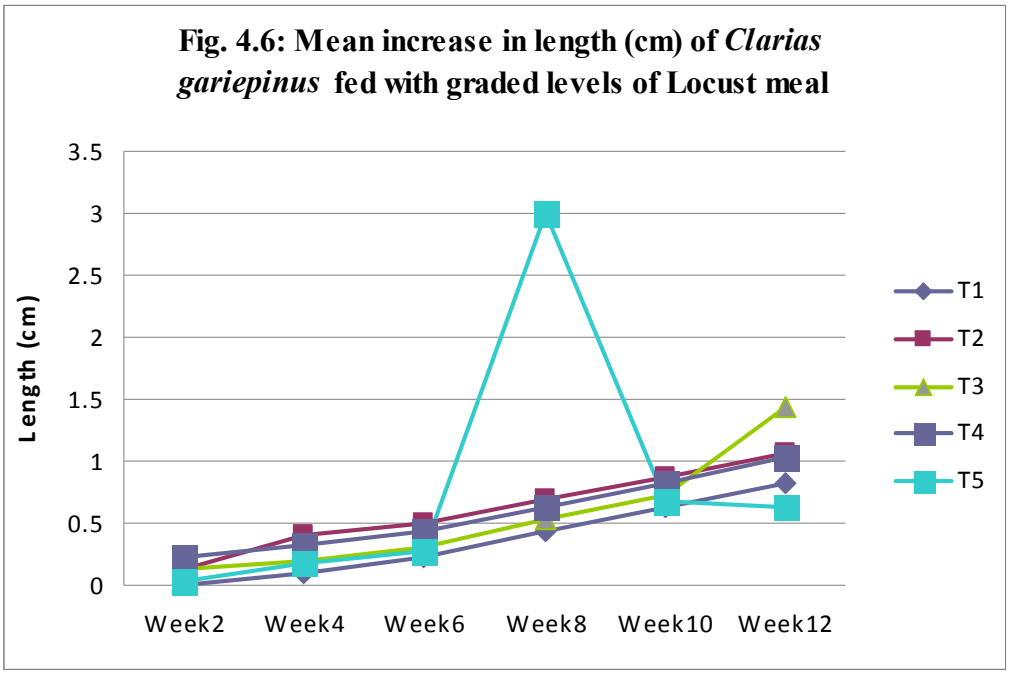
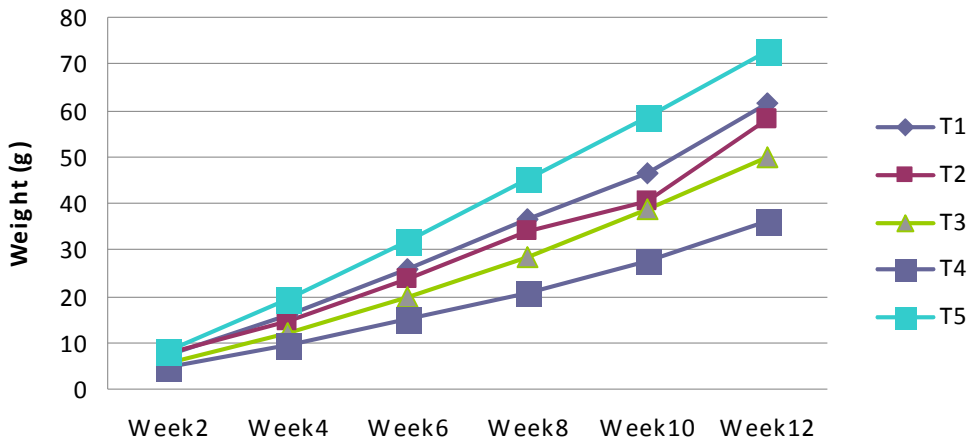


Fig. 4.6: Mean increase in length (cm) of *Clarias gariepinus* fed with graded levels of Locust meal

4.15 Mean Increase in Weight (g) of *Clarias gariepinus* Fed with Graded Levels of Soaked *Bauhinia* Seed Meal

Weight increased in all the diets over 12 weeks in all the diets over 12 weeks. There was significant difference in mean increase in weight of *Clarias gariepinus* fed diets 1, 2, 3, 4 and 5. However, the level of significance varied with diet and time. For instance there was no significant difference (P>0.05) in the mean increase in weight in week 2 diet 3 (5.40g) and diet 4 (4.73g). The same trend was observed in week 6 and week 8. In week 10 there was no significant difference (P>0.05) in mean increase in length in diet 1 (46.28g), diet 2 (40.43g), diet 3 (38.73g), however, fish fed diet 5 that gave the best result (58.60g). Subsequently in week 12 a similar trend was observed in diet 5 which gave a significantly higher (P<0.05) mean increase in length (72.8g) than fish fed diet 1 (61.43g), diet 2 (58.2g) and diet 3 (50.1g) (Figure 4.7).

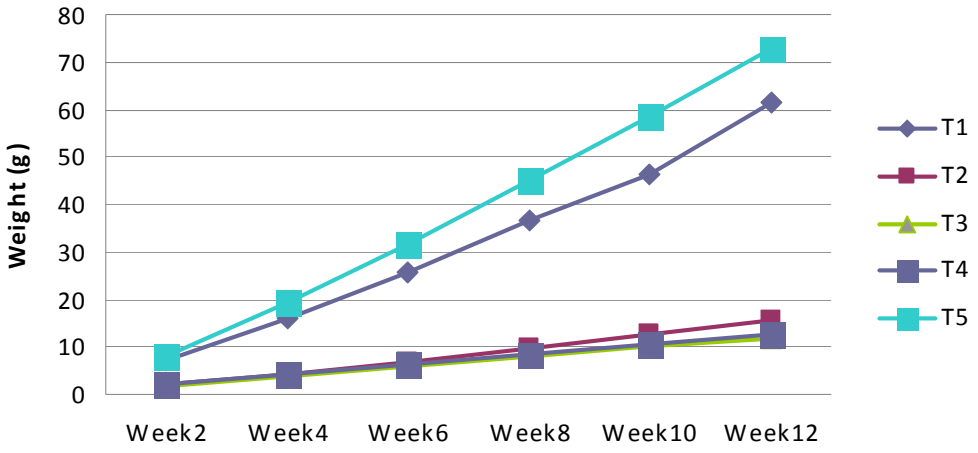
Fig. 4.7: Mean increase in weight (g) of *Clarias gariepinus* fed with graded levels of soaked *Bauhinia* seed meal.



4.16 Mean Increase in Weight (g) Of *Clarias gariepinus* Fed with Graded Levels of Locust Meal Over 12 Weeks

There was significant difference (P<0.05) in mean increase in weight of fish over the 12 weeks period. However diet 5 consistently gave the best increase in weight over the 12 weeks period. In week 12 diet 5 gave weight increase of 72.8g which was significantly higher (P<0.05) than fish feed diets 1, 2, 3 and 4. Diet 2, 3, and 4 showed no significant differences (P>0.05) in mean increase in length from week 2 to week 12. However, there gradual increase in weight forthnightly (Figure 4.8).

Fig. 4.8: Mean increase in weight (g) of *Clarias gariepinus* fed with graded levels of Locust meal over 12 weeks



4.17 Mean Proximate Carcass Composition of *Clarias gariepinus* Fed Graded Levels of Soaked *Bauhinia* Seed Meal and Locust Meal

4.17.1 Mean proximate carcass composition of *Clarias gariepinus* fed graded levels of soaked *Bauhinia* seed meal

The initial percentage composition of fish carcass was determined in relation to CP, EE and Ash. The initial carcass composition for fish fed soaked Bauhinia seed meal ($SBSM_{96}$) were recorded as CP (56.91%), lipid (12.77%), Ash (17.08) respectively. The final fish composition indicated a general trend of increase in CP and lipid which increased significantly ($P < 0.05$). These trends were similar to that obtained in fish fed locust meal with the exception of Ash content which had no particular trend in fish fed Bauhinia seed meal and Locust meal diets. It decreased in all the diets with the exception of diet 5 in both experiments. (Table 4.9)

The final fish carcass composition indicated that CP increased in all the diets compared to the initial carcass value (56.91%) of all the treatments. There were significant differences ($P<0.05$) in the protein contents of the fish fed the five diets. Diet 1 was highest 69.02% followed by diet 5 (67.88%), diet 2 (67.60%), diet 3 (66.74%) and diet 4 (62.16%) respectively.

The percentage lipid content of all the treatment groups were significantly higher ($P< 0.05$) than the initial lipid content (12.77%). There were significant differences ($P<0.05$) in the lipid contents of the fish fed experimental diets. The highest lipid content was recorded in diet 5 (15.01%) followed by diet 4 (14.65%), diet 3 (14.58%), diet 1 (13.89%) respectively. The least was in diet 2 (13.62%).

The percentage Ash content of the initial carcass was higher (17.08%) than the final carcass contents in all the experimental diets. There were significant differences ($P<0.05$) in percentage ash content of the fish fed the five diets. The highest Ash content was recorded in diet

5 (16.50%), diet 1 (16.39%), diet 2 (16.01%), 3 (15.07%) the least ash content was obtained in diet 4 (14.83%).

Table 4.9: Mean carcass composition (%) of *Clarias gariepinus* fed graded levels of soaked *Bauhinia* seed meal

Trts	Ash	Crude Protein (CP)	Ether Extract (EE)
Initial composition	17.08 [f]	56.91 [a]	12.77 [a]
T_1 100% fishmeal 0SBSM$_{96}$	16.39 [d]	69.02 [f]	13.89 [c]
T_2 75% fishmeal 25%SBSM$_{96}$	16.01 [c]	67.60 [d]	13.62 [b]
T_3 50% fishmeal 50%SBSM$_{96}$	15.07 [b]	66.74 [c]	14.58 [d]
T_4 25% fishmeal 75%SBSM$_{96}$	14.83 [a]	62.16 [b]	14.65 [e]
T_5 100% Copens	16.50 [e]	67.88 [e]	15.01 [f]
SED ±	0.016	0.019	0.014
LSD(P<0.05)	0.039	0.049	0.035

Values with the same superscripts in the same column are not significantly different (P > 0.05) LSD.

4.17.2 Mean carcass composition (%) of *Clarias gariepinus* fed graded levels of locust meal

At the end of the experiment the carcasss composition of fish fed diets 1, 2, 3, 4 and 5 were composed of crude protein: 69.02%, 63.68%, 61.54%, 59.11% and 67.88% respectively (Table 4.10); the Lipid values of fish fed diets 1 to 5 were; 13.89%, 12.03%, 12.52%, 13.39% and 15.01% respectively. Ash values were; 16.39%, 16.16%, 15.32%, 13.54% and 16.50% respectively. The crude protein and ether extract (lipid) had a similar trend; lower values were obtained in the initial carcass than at the end of the experiment.

The protein gains of each group of the experimental fish were significantly higher ($P<0.05$) than the initial protein level of 56.91%. There were significant differences ($P<0.05$) in the protein contents of fish fed the five diets. Fish fed diet 1 had the highest protein gain (69.02%) followed by diet 5 (67.88%), diet 2 (63.68%), diet 3 (61.54%) and diet 4 (59.11%) respectively. (Table 4.10)

The percentage lipid contents of all the groups of fish were higher in the final than the initial lipid contents of 12.77%. There were significant differences ($P<0.05$) in the lipid content of the fish fed the experimental diets. The highest lipid content was recorded in fish fed diet 5 (15.01%) followed by diet 1 (13.89%), diet 4 (13.39%) and diet 3 (12.52%) respectively. The least was in diet 2 (12.03%).

The percentage Ash of the initial carcass was (17.08%), however at the end of the experiment, there was significant difference ($P<0.05$) in percentage ash content of the fish fed the five diets. The highest value was recorded in diet 5 (16.50%) followed by diet 1 (16.39%), diet 2 (16.16%), diet 3 (15.32%) and the least was diet 4 (13.54%).

The crude fibre of fish in all treatments were higher at the end of the experiments than the initial crude fibre of the fish sample (3.34%). There were differences ($P<0.05$) in the CF content

of the fish fed the different experimental diets. The highest crude fibre content was in diet 2 (5.16%) followed by diet 3 (4.84%), diet 1 (4.69%) and diet 5 (4.35%) respectively. The least crude fibre content was recorded in diet 4 (3.37%) (Table 4.10)

Table 4.10: Mean carcass composition of *Clarias gariepinus* fed graded levels of locust meal

Trts	Ash	Crude Protein (CP)	Ether Extract (EE)
Initial composition	17.08 [f]	56.91 [a]	12.03 [a]
T_1 100% fishmeal	16.39 [d]	69.02 [f]	13.89 [e]
T_2 75% fishmeal 25%locust	16.16 [c]	63.68 [d]	12.77 [c]
T_3 50% fishmeal 50%locust	15.32 [b]	61.54 [c]	12.52 [b]
T_4 25% fishmeal 75%locust	13.54 [a]	59.11 [b]	13.39 [d]
T_5 100% Copens	16.50 [e]	67.88 [e]	15.01 [f]
SED ±	0.014	0.014	0.014
LSD(P<0.05)	0.037	0.035	0.035

Values with the same superscripts in the same column are not significantly different (P > 0.05) LSD.

4.18 Mean Proximate Composition of Soaked *Bauhinia* Seed Meal

There was significant difference in the mean crude protein values in soaked *Bauhinia* seed meal diets. The crude protein content of diet 5 (42.09%) was significantly higher ($P<0.05$) than diets 1, 2, 3 and 4 which gave 40.00%, 40.15%, 39.60%, and 39.57% respectively. (Table 4.11)

Table 4.11: Mean proximate composition of soaked *Bauhinia* seed meal diet

Proximate Composition	Diets				
	1	**2**	**3**	**4**	**5**
DM	93.89	93.04	93.77	93.94	93.79
Ash	4.90	4.08	4.38	3.89	5.85
CP	40.00^{b}	40.15^{c}	39.60^{a}	39.57^{a}	42.09^{d}
EE	8.67	8.11	8.51	8.72	8.99
CF	2.16	4.09	4.14	4.15	2.50
NFE	38.16	36.61	37.14	37.61	34.36
SED±	0.010	0.010	0.010	0.010	0.010
LSD ($P<0.05$)	0.028	0.028	0.028	0.028	0.028

Values with the same superscripts in the same column are not significantly different ($P > 0.05$) LSD.

4.19 Mean Proximate Composition of Locust Meal

There was significant difference (P<0.05) in the crude protein content in diets 1 to 5. The mean crude protein content of the locust meal diet indicated that diet 5 had the highest crude protein content while diet 4 had the least crude protein content. (Table 4.12)

Table 4.12: Mean proximate composition of locust meal diet

Proximate Composition	Diets				
	1	2	3	4	5
DM	93.89	94.20	93.71	92.37	93.79
Ash	4.90	4.45	4.17	4.99	5.85
CP	40.00[c]	41.26[d]	39.70[a]	39.58[a]	42.09[e]
EE	8.67	8.64	7.39	7.54	8.99
CF	2.16	3.91	3.98	4.25	2.50
NFE	38.16	35.94	38.47	36.01	34.36
SED±	0.017	0.017	0.017	0.017	0.017
LSD (P<0.05)	0.048	0.048	0.048	0.048	0.048

Values with the same superscripts in the same column are not significantly different (P > 0.05) LSD.

4.20 Growth Performance and Feed Utilization of *Clarias gariepinus* Fed Soaked *Bauhinia* Seed Meal for 84 Days.

4.20.1 Growth performance

4.20.1.1 Mean initial body weight gain (MIBW)

There was no significant difference (P>0.05) in the mean initial body weight (MIBW) of *Clarias gariepinus*. The initial weights of the Clarias gariepinus were relative and averaged 20.00g.

4.20.1.2 Mean final body weight gain (MFBW)

There was significant difference (P<0.05) in the mean final body weight (MFBW) of fish fed the various diets. Diet 5 gave the highest mean weight gain (92.43g), followed by diet 1 while diet 4 gave the least weight of 56.5g.

The results indicated that fish fed the control diets 1 and 5 (100% fish meal and 100% copens) respectively attained better weight gain than fish on diet 2, 3 and 4. (Table 4.13)

4.20.1.3 Percentage live weight gain (PLWG)

There was significant difference (P<0.05) in the percentage live weight gain values between the diets, and PLWG followed a similar trend with the mean weight gain (MWG). Diet 4 gave the least percentage PLWG (179.7%) and least MWG (36.30%). The highest PLWG values was obtained in diet 1 and 5 (the control diets, 100% fishmeal and 100% copens) respectively with a corresponding trend in MWG with values of 306.69g and 370.08g. (Table 4.13)

4.20.1.4 Mean initial standard length (MISL)

Results (table 4.13) with respect to standard length showed that the initial length of Clarias gariepinus were relative in mean (14.0cm) though they differed significantly ($P<0.05$). However by 84days, there was no significant difference ($P<0.05$) in the final standard length between diet 1 (14.53cm) and diet 5 (14.47cm). Fish fed the experimental formulated diets had significantly higher ($P<0.05$) values of mean final standard length, which was highest in diet 2 (22.30cm) followed by diet 3 (22.1cm) amd diet 4 (21.9cm). This result was also confirmed in mean length gain which implied that the formulated feed meals enhanced length gain.

4.20.1.5 Mean length gain (MLG)

The percentage length gain indicated that diets 2, 3 and 4 gave 61.59%, 60.14% and 59.85% respectively which was relatively higher compared to those of diet 5 (7.89%) and 100% fish meal diet (6.06%). (Table 4.13)

4.20.1.6 Specific growth rate (SGR)

There was significant difference ($P<0.05$) among fish fed the various diets with respect to specific growth rate. The best SGR results was obtained in fish fed the control diet (diet 5, 100% copens) which had the highest value (1.84), followed by diet 1 (1.67), diet 2 (1.61), diet 3 (1.48) respectively, while diet 4 had the least SGR value. (Table 4.13)

4.21 Feed Utilization

4.21.1 Feed Conversion Ratio (FCR)

The Feed conversion ratio (FCR) values of the control diets (1, 100% fish mean and 5, 100% copens) significantly differed ($P<0.05$) from those of diets 2, 3 and 4. Diets 2, 3 and 4 had the least FCR values (0.56) respectively. Therefore, the best result in terms of FCR was achieved in diets 2, 3 and 4. (Table 4.13)

4.21.2 Feed Efficiency Ratio (FER)

Feed efficiency ratio (FER) indicated that there was significant difference ($P<0.05$) amongst the diets. The FER values of fish fed the control diets (1 and 5) differed significantly ($P<0.05$) from diets 2, 3 and 4. This followed a similar pattern with the feed conversion ratio (FCR) since the feed efficiency ratio (FER) is an inverse proportion of the feed conversion ratio (FCR). The best results were achieved in fish fed diets 2, 3 and 4 with the highest values of FER (1.78) respectively, while diets 1 and 5 had the least FER values of 1.62 and 1.63 respectively. (Table 4.13)

4.21.3 Apparent Net Protein Utilization (ANPU)

Apparent net protein utilization (ANPU) results (table 4.13) indicated that there was significant difference ($P<0.05$) amongst the diets. Diets 1 and 5 (control diets) had the highest values 79.67 and 58.17 respectively, followed by diets 2 and 3 while diet 4 had the least ANPU value. This implies that diets 1 and 5 gave the best results in terms of ANPU (Table 4.13)

4.21.4 Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) results indicated that there was significant difference ($P<0.05$) among diets. Diets 3 and 4 differed significantly from the other diets. Diet 5 had the least PER value 3.88 followed by diet 1 (4.04) and diet 2 (4.44) respectively. Diet 3 and diet 4 gave the highest values of 4.51 and 4.50 respectively. Diets 3 and 4 gave the best PER results. (Table 4.13)

4.21.5 Gross Feed Conversion Efficiency (GFCE)

Gross feed conversion efficiency (GFCE) results showed that there was significant difference ($P<0.05$) amongst the diets. Diets 2, 3 and 4 had the highest values 178.57 respectively while diets 1 and 5 had the least values of 161.30 respectively. The GFCE result

followed a similar trend with FCR results since it is the reciprocal of the FCR expressed as a percentage. The best GFCE result was achieved in diets 2, 3 and 4.

4.21.6 Condition Factor (K)

Robustness and general well-being of the *Clarias gariepinus* (Teugels) expressed as the condition factor (K) was significantly different ($P < 0.05$) in diet 5 (100% copens) with a value of 3.05 compared to the rest of the diets (table 4.13). It was followed by diet 1 (100% fish, 0% *Bauhinia* seed meal) with 2.66; diet 2 (75% fishmeal, 25% soaked *Bauhinia* seed meal) with 0.70 diet 3 (50% fishmeal, 50% soaked *Bauhinia* seed meal) with 0.65 respectively. Diet 4 had the least condition factor (K value) of 0.35. The implication of this is that fish fed 100% copens (the commercially formulated fish diet) gave the best result.

4.21.7 Percentage Survival Rate (PSR)

Mortality was highest in diet 4 (25% fish meal, 75%, soaked *Bauhinia* seed meal, with 70% percentage survival rate) followed by diet 3 (50% fish meal, 50% *Bauhinia* seed meal, with 80% PSR value. The least PSR values of 96.67 and 93.33 were recorded in the control diets (diet 5 and diet 1).

Table 4.13: Growth Performance and Feed Utilization of Juvenile *Clarias gariepinus* Fed Soaked *Bauhinia* Seed Meal For 84 Days

Trts	M.I.B.W	M.F.B.W	M.W.G	P.L.W.G	M.I.S.L	M.F.S.L	M.L.G	P.L.G	SGR	FCR	FER	ANPU	PER	GFCE	K	PSR
T_1 100% fishmeal 0SBSM$_{96}$	20.03	81.46^{d}	61.34^{d}	306.69^{d}	13.70^{b}	14.53^{a}	0.83^{a}	6.06^{a}	1.67^{d}	0.62^{b}	1.62^{a}	79.67^{e}	4.04^{b}	161.30^{a}	2.66^{d}	93.33
T_2 75% fishmeal 25%SBSM$_{96}$	20.30	78.50^{c}	58.20^{c}	286.69^{c}	13.80^{c}	22.30^{d}	8.50^{e}	61.59^{e}	1.61^{c}	0.56^{a}	1.78^{b}	51.6^{c}	4.44^{c}	178.57^{b}	0.70^{c}	83.33
T_3 50% fishmeal 50%SBSM$_{96}$	20.20	70.30^{d}	50.10^{b}	248.02^{b}	13.80^{c}	22.10^{c}	8.30^{d}	60.14^{d}	1.48^{b}	0.56^{a}	1.78^{b}	41.64^{b}	4.51^{d}	178.57^{b}	0.65^{b}	80.00
T_4 25% fishmeal 75%SBSM$_{96}$	20.20	56.50^{a}	36.30^{a}	179.70^{a}	13.70^{b}	21.90^{b}	8.20^{c}	59.85^{c}	1.22^{a}	0.56^{a}	1.78^{b}	27.29^{a}	4.50^{d}	178.57^{b}	0.35^{a}	70.00
T_5 100% Copens	19.63	92.43^{e}	72.80^{e}	370.08^{e}	13.40^{a}	14.47^{a}	1.07^{b}	7.89^{b}	1.84^{e}	0.62^{b}	1.63^{a}	58.17^{d}	3.88^{a}	161.30^{a}	3.05^{e}	96.67
SED ±	0	0.0136	0.00316	0.0032	0.0154	0.01549	0.0154	0.0126	0.0126	0.0154	0.0154	0.158	0.0154	0.01516	0.01549	
LSD(P<0.05)	0NS	0.0377	0.0087	0.0089	0.0430	0.0430	0.0430	0.0351	0.0351	0.0430	0.043	0.0488	0.0430	0.04208	0.04300	

Values with the same superscripts in the same column are not significantly different (P > 0.05) LSD. Superscript ^{a} stands for the lowest value, while superscript ^{e} stands for the highest value.

Values are means of three separate determinations.

4.22 Growth Performance and Feed Utilization of *Clarias gariepinus* Fed Locust Meal for 84 Days.

4.22.1 Growth performance

4.22.1.1 Mean Initial Body Weight (MIBW)

There was no significant difference (P>0.05) in the mean initial body weight of *Clarias gariepinus*. The initial weight of *Clarias gariepinus* fish were relative and averaged 20.0g (Table 4.14).

4.22.1.2 Mean Final Body Weight (MFWB)

There was significant difference (P<0.05) in the mean final body weight of fish fed the various diets. Fish fed diet 5 had the highest weight gain (92.43g), then diet 1 (81.46g) while diet 4 gave the least mean final body weight gain (32.37g) (Table 4.14).

4.22.1.3 Mean Weight Gain (MWG)

Fish fed the control diets 1 and 5 (100% fish meal and 100% copens) attained better weight gain compared to the rest of the diets, there was significant difference (P<0.05) in mean weight gain (MWG) between diets 1 and 5. Similar observations were recorded for diets 2 (21.20g) and diets 3 (12.53g). Diet 4 had the least mean weight values (12.17g).

4.22.1.4 Percentage Live Weight Gain (PLWG)

Diet 1 and 5 had the highest PLWG 306.69% and 370.08% respectively. these diets were significantly difference (P<0.05) from diets 2, 3 and 4 (Table 4.14).

4.22.1.5 Mean Initial Standard Length (MISL)

The initial length of *Clarias gariepinus* differed significantly (P<0.05) however, the lengths were relative in mean (13.7cm). (Table 4.14)

4.22.1.6 Mean Final Standard Length (MFSL)

The result indicated that at the end of the 84 days feeding trial, there were no significant differences ($P>0.05$) in mean final standard length of diets 1 and 2. The least MFSL value was obtained in diet 5 (14.47) while diet 3 had the highest MFSL value (15.17cm) followed by diet 4 (14.90cm). There was significant difference ($P<0.05$) in MFSL among the fish fed various diets. The values of MFSL in the experimental compounded diets significantly differed ($P<0.05$) from the commercially formulated diet (100% copens) (Table 4.14)

4.22.1.7 Mean Length Gain (MLG)

Results (table 4.14) indicated that mean length gain (MLG) significantly differed ($P<0.05$) amongst the diets. Diets 3 and 5 significantly differed ($P<0.05$) from diet 1 (0.83), deit 2 (0.63) and diet 4 (1.03). The least MLG value was in diet 2 (0.63) the highest value was obtained in diet 3 (1.44) (Table 4.14)

4.22.1.8 Percentage Length Gain (PLG)

The highest percentage length gain was obtained in diet 3 (10.48) followed by diet 5 7.98cm), diet 4 (7.42cm) and 1 (6.06cm) respectively. Diet 2 had the least PLG value (4.53). Thus the best PLG was obtained in diet 3. (Table 4.14)

4.22.1.9 Specific Growth Rate (SGR)

The specific growth rate (SGR) of all the experimental diets were not significantly different ($P>0.05$). However, based on the mean values the highest SGR values were obtained in the control diets, diets 1 and 5 (100% fish meal and 100% copens) respectively. Diet 1 had the SGR value of 1.67 while diet 5 had 1.84. The least SGR value was obtained in diet 4 (0.56) followed by diet 3 (0.57). (Table 4.14)

4.22.1.10 Feed Conversion Ratio (FCR)

Diet 3 had the highest FCR value of 3.48, followed by diets 4 (3.24) and diet 2 (1.45) respectively (Table 4.14). The best FCR results were obtained in the control diets 1 and 5 (100% fish meal and 100% copens) with mean values of 0.62 each which represented the least FCR values in this experiment.

4.22.1.11 Feed Efficiency Ratio (FER)

Diets 1 and 5 gave the highest FER values of 1.62 and 1.63 respectively followed by diet 2 (0.69). The least FER value was obtained in diet 3 (0.29) (Table 4.14). The best diet in terms of FER was the control diet, diet 5 (100% copens) followed by diet 1 (100% fish meal). This had a similar trend with the FCR (Table 4.14).

4.22.1.12 Apparent Net Protein Utilization (ANPU)

Diets 1 and 2 had the highest ANPU values of 79.67% and 84.04% respectively, then diet 5 (58.17%) and diet 3 (56.72%). The least ANPU value was obtained in diet 4 (33.65%). The best diet in terms of ANPU is diet 1 (100% fish meal) followed by diet 2 (75% fish meal and 25% locust meal). The best ANPU result was obtained in diet 2 (84.04%) followed by diet 1 (79.67%) respectively (Table 4.14).

4.22.1.13 Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) values of fish fed diets 1 and 5 are significantly different ($P<0.05$) compared to fish fed diets 2, 3 and 4. Diets 1 and 5 (control diets) had the highest PER values of 4.04 and 3.88 respectively, followed by diet 2 (1.67) while the least PER value was obtained in diets 3 (0.72) and 4 (0.78) (Table 4.14). The best diet in terms of PER was diet 1 followed by diet 5 (Table 4.14).

4.22.1.14 Gross Feed Conversion Efficiency (GFCE)

Both diets 1 and 5 (the control diets) had the highest GFCE values of 161.3 respectively followed by diet 2 (75% fish meal and 25% locust meal) with 68.17, then diet 4 (25% fish meal and 75% locust meal) with a GFCE value of 30.86. Diet 3 had the least GFCE value (28.74). The best diets in terms of GFCE were diets 1 and 5. The trend is similar to that of the feed conversion ratio (FCR) (Table 4.14).

4.22.1.15 Condition Factor (K)

There was significant diference ($P<0.05$) in the condition factors (K) among the diets (table 4.14). The K values of diet 1 (2.66) and diet 5 (3.06) differentiated significantly from diet 2 (1.36), diet 3 (0.94) and diet 4 (0.98). There was no significant difference at ($P>0.05$) between the condition factor (K) values of diet 3 (0.94) and diet 4 (0.98). The best condition factor (K) was obtained in fish fed the control diet, diet 5 (100% copens) followed by diet 1 (100% fish meal).

4.22.1.16 Percentage Survival Rate (PSR)

The percentage survival rate (PSR) in Table 4.14 indicated that mortality was highest in fish fed diets 4 (25% fish meal, 75% locust) with 80% PSR value and 20% mortality, followed by diet 2 (75% fish meal and 25% locust meal). The control diets, diet 1 (100% fishmeal) and diet 5 (100% copens) had the highest PSR values of 93.33% (6.67% mortality) and 96.67% (3.33% mortality) respectively followed by diet 2 (86.76) with 13.33% mortality. The best diet in terms of PSR is diet 5 followed diets 1 and 2 respectively (Table 4.14).

Table 4.14: Growth Performance and Feed Utilization of Juvenile *Clarias Gariepinus* Fed Locust Meal For 84 Days

Trts	M.I.B.W	M.F.B.W	M.W.G	P.L.W.G	M.I.S.L	M.F.S.L	M.L.G	P.L.G	SGR	FCR	FER	ANPU	PER	GFCE	K	PSR
T_1 100% fishmeal	20.03	81.46 ^{d}	61.43 ^{d}	306.69^{d}	13.70 ^{b}	14.53^{b}	0.83 ^{b}	6.06 ^{b}	1.67	0.62 ^{a}	1.62 ^{c}	79.67 ^{d}	4.04 ^{e}	161.3 ^{d}	2.66 ^{c}	93.33
T_2 75% fishmeal 25%locust	20.40	41.60 ^{c}	21.20 ^{c}	103.92^{c}	13.90 ^{e}	14.53^{b}	0.63 ^{a}	4.53 ^{a}	0.85	1.45 ^{b}	0.69 ^{b}	84.04 ^{e}	1.67 ^{c}	68.17 ^{c}	1.36 ^{b}	86.67
T_3 50% fishmeal 50%locust	20.40	32.93 ^{b}	12.53 ^{b}	61.42 ^{b}	13.73 ^{c}	15.17^{d}	1.44 ^{e}	10.48^{e}	0.57	3.48 ^{d}	0.29 ^{a}	56.72 ^{b}	0.72 ^{a}	28.74 ^{a}	0.94 ^{a}	90.00
T_4 25% fishmeal 75%locust	20.20	32.37 ^{a}	12.17 ^{a}	60.25 ^{a}	13.87 ^{d}	14.90^{c}	1.03 ^{c}	7.42 ^{c}	0.56	3.24 ^{c}	0.31 ^{a}	33.65 ^{a}	0.78 ^{b}	30.86 ^{b}	0.98 ^{a}	80.00
T_5 100% Copens	19.63	92.43 ^{e}	72.8 ^{e}	370.08^{e}	13.40 ^{a}	14.47^{a}	1.07 ^{d}	7.98 ^{d}	1.84	0.62 ^{a}	1.63 ^{d}	58.17 ^{c}	3.88 ^{d}	161.30^{d}	3.05 ^{d}	96.67
SED ±	0	0.01549	0.0126	0.0173	0.0038	0.015	0.0126	0.013	0.015	0.0126	0.0154	0.1549	0.0154	0.0036	0.154	
LSD(P=0.05)	0NS	0.04300	0.0351	0.0480	0.0107	0.043	0.035	0.036	0.043NS	0.0351	0.043	0.0430	0.0430	0.0100	0.0430	

Values with the same superscripts in the same column are not significantly different ($p > 0.05$) LSD.

4.23 Least Cost Analysis, Economy of Weight Gain (EWG) and Economy of Protein Gain (EPG) of Juvenile *Clarias gariepinus* (Teugels) Fed *Bauhinia* Seed Meal Diets for 84 Days

4.23.1 Computerised least cost analysis [cost of feed (₦)/kg of experimental diet]

Diet 4 (25% fish meal and 75% soaked Bauhnia seed meal) had the least production cost of ₦105.18 per kg followed by diet 3 (50% fish meal and 50% *Bauhinia* seed meal) with ₦110.08 and diet 2 (75% fish meal and 25% *Bauhinia* seed meal) with ₦114.23. The most expensive diets were the control diets (diet 5, 100% copens and diet 1, 100% fish meal) which had ₦320.00 and ₦119.51 per kg as production costs respectively (Table 4.15).

4.23.2 Economy of weight gain (EWG)

Diet 1 (100% fish meal, control diet) gave the least and best EWG value of ₦1.91 followed by diet 5 the control diet of 100% copens (₦4.40) and diet 2 containing 75% fish meal and 25% soaked *Bauhinia* seed meal (₦5.39) respectively. The most uneconomical diets were diet 3 containing 50% fish meal and 50% soaked *Bauhinia* seed meal (₦8.79) and diet 4 containing 25% fish meal and 75% soaked *Bauhinia* seed meal (₦8.64) (Table 4.15).

4.23.3 Economy of protein gain (EPG)

In terms of economy of protein gain diet 1 (₦9.87) and diet 3 (₦9.90) were the most economical followed by diet 2 which had EPG value of ₦16.87 (table 4.15). The most uneconomical diet was diet 4 containing 25% fish meal and 75% soaked *Bauhinia* seed meal followed by diet 5, the control diet (100% commercially formulated diet, copens).

Table 4.15: Economy of Weight Gain and Protein Gain

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Cost/ ₦/kg of experimental diet	119.51	114.23	110.08	105.18	320.00
Cost of feed ₦/unit weight gain (g)	1.91	5.39	8.79	8.64	4.40
Cost of feed ₦/unit protein gain (g)	9.87	16.87	9.90	47.81	29.30

4.24 Least Cost Analysis, Economy of Weight Gain (Ewg) and Economy of Protein Gain (EPG) of Juvenile *Clarias gariepinus* (Teugels) Fed Locust Meal Diets for 84 Days

4.24.1 Least cost analysis [cost of feed/(₦)/kg of experimental diet]

The cheapest computerized least cost diet was diet 4 (25% fish meal and 75% locust meal) which was ₦112.46 per kg followed by diet 2 (75% fish meal and 25% locust meal) with ₦114.11 as production cost. This was followed by diet 3 (50% fish meal and 50% Locust meal) with ₦116.88 as production cost. The most expensive diets were the control diets (diet 5 and diet 1, containing 100% copens and 100% fish meal respectively). Diet 5 gave a production cost of ₦320.00 while diet 1 gave the production cost of ₦119.51 (Table 4.16).

4.24.2 Economy of weight gain (EWG)

Results indicated that the most economical and best diets were diet 1 (100% fish meal), 2 (75% fish meal and 25% locust meal) and 3 (50% fish meal and 50% Locust meal) with economy of weight gain (EWG) values of ₦1.91, ₦1.96 and ₦2.35 respectively. These values were followed by diet 4 (25% fish meal and 75% Locust meal) with EWG value of ₦3.09. The

most uneconomical diet was diet 5 (the control diet, 100% copens) with EWG value of ₦4.40 (Table 4.16).

4.24.3 Economy of protein gain (EWG)

The cost of feed per unit protein gain was more economical in diet 2 (75% fish meal and 25% Locust meal) followed by diet 1 (100% fish meal) and diet 3 (50% fish meal and 50% Locust meal) with EPG values of ₦8.97, ₦9.87 and ₦11.89 respectively. The most uneconomical diet was diet 5 (control diet, 100% copens) with EPG value of ₦29.30 followed by diet 4 (25% fish meal and 57% Locust meal) with EPG value of ₦21.40 (Table 4.16).

Table 4.16: Economy of Weight Gain and Protein Gain

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Cost/ (₦)/kg of experimental diet	119.51	114.11	116.88	112.46	320.00
Cost of feed (₦)/kg weight gain (g)	1.91	1.96	2.33	3.09	4.40
Cost of feed (₦)/kg protein gain (g)	9.87	8.97	11.89	21.42	29.30

CHAPTER 5

5.0 DISCUSSION

5.1 Physicochemical Parameters of Culture Tanks

The physico chemical parameters of water were within the acceptable range recommended for rearing and culture of most tropical fishes, including the African catfish. *Clarias gariepinus* like any other fish species require optimum levels of these water parameters for optimum survival, growth and reproduction. Boyd and Lichotkopler (1979) reported that pH of 6.5 – 9.0 and temperature of 22 – 27°C give the best growth for cultured tropical fishes. Adesulu (2001) indicated that any dissolved oxygen value below 4mg/litre begins to stress fishes and pH 4 kills fish due to corrosive effect, such acidic water diminishes the appetites of fish and thus reduces their growth rate, at pH of 9 water becomes unproductive because carbon dioxide becomes unavailable in such water and at pH 11, fish dies. The hydrogen ion concentration (pH) in this study ranged between 7.3 – 8.7.

5.2 Proximate Composition of Processed Seeds

Among the processed seeds, the soaked seeds gave the highest mean value (32.45%) of crude protein at 96hours duration of soaking, while boiling for 10 minutes gave the least value (30.48%) of crude protein. This trend may be due to the effect of hydrolysis during the soaking which increased the content of the Crude Protein (CP), a process often associated with microbial activities. Yashim *et al.* (2009) and Tamburawa (2010) reported that crude protein increased progressively with increasing duration of soaking *Crotalaria retusa* L. and Parkia seed respectively. The lower Crude Protein value in the heat treatments may be related to denaturation of protein on exposure to high temperature. For instance lower protein values were

obtained when *Mucuna, Parkia* and *Afezelia* seeds were roasted (Vadivel and Pungalenthi, 2007; Tamburawa, 2010 and Yusuf *et al.,* 2011). Unintended adverse effect may take place as result of denaturing of proteins during heat processing which alters the chemical nature and decreases the nutritional quality of proteins (Liener, 1980; Van der Poel, 1989; Norton, 1991).

The crude fibre values of all the processed seeds were higher in comparison to that of the soaked, but lower in the raw. Crude fibre decreased progressively in soaked locust bean seed meal. The crude fibre for the boiling and soaking were low because the fibre is broken or softened. The digestibility of nutrients and dry matter are significantly affected by high dietary fibre (Bridge *et al.*, 1982; Fair and Wright, 1990 and Safari *et al.*, 2005), therefore fiber renders such nutrients unavailable or reduces their utilization (Omoregie and Ogbemudia, 1993). Furthermore dietary fibre has a reductive effect on enzyme activity (Patridge *et al*., 1982), and interferes with nutrient digestion by decreasing the mean retention time of food in the digestive tract (Cherbut *et al.,* 1998). High fibre has also been associated with poor pellet durability (Tacon *et al.,* 1984).

The concentration of lipid reduced progressively from raw to boiled and toasted, but increased with soaking. Similarly Tamburawa (2010) reported a decline in lipids with increasing duration of toasting Parkia seed and a general increase with increased duration of soaking

The lowest value (25.01%) of fat obtained in the toasted seed meal at 40 minutes could be attributed to denaturing effect of heat and loss of volatile essential fatty acids. Feedstuffs with high fat content are prone to oxidative rancidity due to the effect of long time storage (UNDP, 1983; Sena, 1995; Effiong and Eyo, 1997). Rancid fats reduce palatability and therefore reduce availability of nutrients to fish (Rumsey, 1980) and can contain toxic compounds which inhibit growth. Carbohydrate present in feed could also ferment. Chemicals produced in the

degenerating feeds may reduce amino acids and vitamins available, vitamin C being particularly susceptible (Cockerell *et al.*, 1971).

However, the use of formulated feed within the stipulated maximum permissible period of storage of feedstuffs (New, 1987 and Effiong and Eyo, 1997) does not subject feedstuff to problems of chemical changes. The use of the formulated feed in this study was within the stipulated 1-3months (90days) maximum permissible period of storage of feed stuffs asserted by New (1987) and Effiong and Eyo (1997). Thus the issue of oxidative rancidity which is expected to hinder the use of the soaked seeds ($SBSM_{96}$) due to its high EE value (fat content) is ruled out in this study since the experiment was scheduled to be terminated at 84days, since the stability of the lipid content of the seed was still intact.

The least Ash value (3.06%) was obtained in soaked seed sample ($SBSM_{96}$) while the highest value (4.67%) was obtained in boiled seed sample at 10 minutes. The substantial reduction of the Ash content in the soaked seed meal sample when compared to the raw seed sample and other processed seed samples might be due to leaching of both micro and macro element into the soaking medium through the enhanced permeability of the seed coats during the soaking treatment. Wee and Wang (1987) reported the loss of certain nutrients in water during soaking of *Leucaena leucocephala* leaf. Vadivel and Pungalenthi (2007) made similar observations with *Mucuna pruriens* var.utilis. The least Ash value of the soaked seeds makes it a better energy source among other processing methods since Ash does not contribute to the TDN (Akinmutimi, 2004).

The NFE of seed meals had the highest values in the raw (27.45%) and roasted (31.87%) and the least value was in the soaked meal. However the high NFE value in roasted seeds indicated that it will be a better seed meal because it will enhance a high value of total digestible

nutrients, TDN (Akinmutimi, 2004). However based on its low CP value the roasted seed meal was not chosen.

The moisture content was not uniform, however the variations could still be due to experimental error and processing techniques with higher moisture content in the soaked seeds (5.94%), followed by boiled (3.75%), while the roasted and raw seeds had the least (3.67%) and (3.60%) respectively. The moisture contents of the processed *Bauhinia* seed meals were generally low and the values fall below 15% moisture content required as safe storage limit for plant food materials (Sena, 1998).

Eventhough the results of this study indicated that there were significant differences in essential amino acid index (EAAI) among the roasted *Bauhinia* seed meal and the boiled *Bauhinia* seed meal with the roasted seed for 10minutes and the raw seeds being the best (the boiled seed also followed the same pattern) while the soaked seed did not show any significant difference ($P>0.05$) implying that any of the treatment levels can be used for this study. However, based on the mean values the soaked *Bauhinia* seed meal ($SBSM_{96}$) had the highest EAAI value and thus it was choosen as the best in terms of EAAI.

The results of this finding indicated minimal loss of available amino acids in all the treatments with particular reference to methionine and lysine, the values were within the needed values and the minimum recommended nutrient levels in fish feed (NRC, 1993, Appendix 4) and also for normal growth of *Clarias gariepinus* (Eyo *et al.*, 1994). However, the acid hydrolysis involved in the amino acid profile determination through the use of Technicon Sequential Multi-sample Amino Acid Analyzer (TSM)-1model DNA 0209 resulted in the destruction of all tryptophan and considerable amount of cysteine and methionine present in the processed seed samples. However, the amino acid profile of the soaked seeds was chosen for use in this study

because the soaked seeds had the least anti-nutrient content and it is well established that the nutritional quality and efficient utilization of feeds is dependent on the maximum reduction of anti-nutrients.

Based on the results of this study the soaked *Bauhinia* seed meal gave the best results in terms of maximum levels of proximate components compared to boiling and roasting. The high crude protein value recorded in the soaked seed $SBSM_{96}$ in this study makes the seeds a potential source and better supplement for fish feed formulation. Hence, soaking was choosen as the best processing techniques for the subsequent feeding trial.

5.4 Anti-Nutrients

Results showed that there were significant differences in the antinutritional factors of the raw and differently processed *Bauhinia monandra* Linn seed meals, for all the parameters measured which implies that there was a general and progressive reduction in the contents of anti-nutritional factors as a result of processing, though this varied in degree with the different processing times. The presence of tannin, HCN, phytate and saponins confirms the reports of earlier workers that *Bauhinia monandra* seeds generally contain such anti-nutritional factors (Anhwange *et al.*, 2004; 2005, Vijayakumari *et al.,* 2007).

Amongst the various processing methods employed, the soaking method was found to reduce the levels of various anti-nutritional substances. Similar significant reduction of various antinutritional compounds during soaking treatment was reported for several under-utilized leguminuous materials such as Leucaena leaf meal (Wee and Wang, 1987); *Bauhinia purpurea* (Vijayakumari *et al.*, 1997a); rattle box seed, *Crotolaria retusa* L. (Yashim *et al.,* 2009); Locust bean meal seed (Tamburawa, 2010) and *Lablab purpureus* (Rongai) beans (Abeke *et al.*, 2011).

The levels of anti-nutrients in soaked seeds obtained in this study were within the tolerable limits/permissible levels. According to Francis (2001), the tolerable HCN limits for fish is yet to be established; however, the HCN levels of 0.03mg/100g obtained in this study is within tolerance limit of 5 – 50mg/kg which has been established for man (Balogun, 1997). The tannin levels of 2.88mg/100g obtained in the soaked seed is not within the tolerable or permissible levels for man which has been established as 15mg/kg. The tolerable limit for fish is not yet established (Francis, 2001). However, it has been indicated that fish are sensitive to tannins and that caution should be exercised in incorporating seeds and agroindustrial by-products containing high levels of tannins in fish feed (Francis, 2001).

The oxalate level is also within the tolerable limit for man 10 – 20 ppm/kg; phytic acid (0.75mg/100g) is within the recommended tolerable limits of below 5g/kg in fish feeds (Francis *et al.*, 2001). The saponin levels (0.31mg/100g) for this study is also within the established tolerance limits of below 1g/kg of diet in commonly cultured fish (Francis, 2001).

The reduction of phytic acid (mg/100g) was generally poor for all the processing methods with the exception of the soaked Bauhinia seed meal ($SBSM_{96}$). Alonos *et al.* (1998) reported that phytic acid content of faba bean seed was significantly reduced during soaking in water. Reduction in phytic acid during soaking could be attributed to leaching out in soaking water (Kataria *et al.,* 1989). High phytate contents have been found to retard growth and cause abnormalities in the intestinal histology of various commonly cultured fish species due to damage to the pyloric cecal region of the intestine with consequent impared nutrient absorption (Francis, 2001). Phytate also reduce the bioavailability of dietary phosphorus in fish (Francis *et al.*, 2002). Phytates inhibit dietary proteins (Satterlee and Abdulkadeer, 1983). Phytate also strongly inhibit the activity of trypsin and pepsin (Panda, 2006) and reduces the solubility of

starch by binding it; reducing the absorption and hence lowering glucose utilization (Akinmutimi, 2009). Phytates chelate with divalent and trivalent metal ions such as Fe^{2+}, Zn^{2+} (Khan *et al.*, 1986) thereby decreasing their absorption in the intestinal mucosa of the fish (Francis, 2002); thus they are rendered unavailable.

The trend observed in phytate levels in this study implies that the feed formulated may have bitter tastes particularly in the roasted seed meal sample ($RBSM_{40}$) which had relatively high and significant phytate content ($P \leq 0.05$) thus the feed could be rendered unacceptable due to the associated bitter taste.

Phytic acid content of *Bauhinia* seed reduced from 11.39mg/100g in raw seed to 0.75mg/100g in soaked seed; boiled seeed (3.01 mg/100g) and toasted seed (10.00 mg/100g) respectively. According to Abu (2005) fermentation reduced phytate in Locust bean seed (*Parkia filicoidea*). Kumar *et al.* (1978) reported that cooking decreased both water and extractable phytate phosphorus in legumes. Tamburawa (2010) indicated that boiling best reduced the amount of phytate in locust bean seed meal from 0.71 mg/100g (raw) to the bearest minimum with increased duration of boiling followed by soaking. However based on the result of this study a reverse trend was obtained which indicated that the results are at variance with each other probably due to the time interval employed for processing and the processing method.

The tannin and oxalate content reduction in this study were also very poor for all the processing methods particularly in the toasted meal which had the least tannin and oxalate reduction. None of the processing techniques employed was able to detoxify tannins and oxalates to the bearest minimum. Similarly, Tamburawa (2010) reported that soaking reduced the level of tannins to the bearest minimum with increased duration of processing time, from 1.08 in raw locust bean seed meal to 0.28 when soaked for 1day, 0.25 (2days), 0.17 (3days)

Francis (2002) indicated that limited information abounds on the effects of tannin on fish; tannin has been reported to assert a significant effect on protein digestibility (Bressani and Elias, 1980).

Francis (2001) also indicated that tannins can interfere with digestive processes by binding to feed proteins, vitamins, minerals and digestive enzymes. Dietary hydrolysable tannins was also reported to retard growth. The presence of high concentration levels of tannins therefore implies possibilities of poor protein digestibility caused by formation of protein – tannin complexes which irreversibly bind digestive enzymes, thus inhibiting the activities of the enzymes making them unavailable for breaking down proteins with the resultant effect that proteins and other nutrients are liable to escape digestion. The presence of high concentration of tannins also indicate possibilities of poor palatability of feed due to bitter tastes hence reduction in feed intake (Akimutimi, 2004). Patridge *et al.* (1982); Cherbut *et al*. (1998) and Akimutimi (2009) also reported that high tannin contents depresses cellulase activity by binding fibre, thus affecting bio degradability (digestibility). Thus the high values of tannin in the raw (RBSM meal) toasted and boiled samples ($RBSM_{40}$ and $BBSM_{40}$) renders them unsuitable for use in feed formulation.

In all the processes the levels of HCN and saponins were reduced to the bearest minimum levels with the least in the soaked seeds $SBSM_{96}$ (0.03mg/100g and 0.31mg/100g respectively). Fish fed cyanogen containing feed materials have generally shown reduced growth when compared to their respective controls (Ufodike and Matty, 1983 and Hossain and Jauncey, 1989b). Hydrogen cyanides the hydrolysed toxic products of cyanogens suppress natural respiration and cause cardiac arrest (Davies, 1991). The presence of cyanide inhibits action of porhyrin enzymes (cytochrome oxidase in tissues and rapidly leads to suffocation (Umar, 2006).

Francis (2002) indicated that low dietary levels of saponins promote fish growth thus considering the least minimum value for saponins in the processing methods applied in this study soaked *Bauhinia* seed may confer that advantage to the fish for the subsequent study; thus the soaked seed with the minimum level of saponin (0.30mg/100g at 96 hours) was chosen for feed formulation.

There is tendency of loosing organic sulphur – amino acids in form of methionine and cystein (for cyanide detoxification) if raw and toasted *Bauhinia* seed meal ($TBSM_{40}$) which contain 0.68 mg/100g and 0.42 mg/100g of HCN respectively are used in feed formulation. Thus the soaked seed ($SMSM_{96}$) with the least amount of HCN (0.03) was most desirable for feed formulation. The presence of HCN also reduces bioavailability of oxygen and thus reduces the oxygen binding capacity of haemoglobin.

The oxalate level of *Bauhinia* seed was substantially and significantly reduced from 12.08mg/100g in raw to 1.94mg/100g in the soaked seed sample $SBSM_{96}$ followed by the boiled seed $BBSM_{40}$ (2.67mg/100g) with least reduction in toasted seed sample $TBSM_{40}$ (8.74mg/100g). Oxalate impacts its effects by attaching to some divalent minerals like iron, copper and calcium thus reducing their bioavailabilities by impairing their absorption (Umar, 2006). The oxalate composition in all processed seeds with exception of soaked seeds sample in this study was higher than 2.5% oxalate level capable of exerting toxicological symptoms in man (Oke, 1969). This implies that $BBSM_{40}$ and $TBSM_{40}$ could be deleterious to fish production. Similarly, the findings of Tamburawa (2010) indicated that processing reduced the levels of oxalate to a substantial level from raw locust bean seed meal to the bearest minimum in soaked seeds with increased duration of soaking time. The results of Tamburawa (2010) indicated that oxalate reduced from 1.78mg/100g in the raw locust bean seed meal when subjected to soaking

for 1day to 1.25, 2days (0.67mg/100g) and 3days (0.46mg/100g) while soaking and subsequent fermentation for 3days reduced the oxalate level to 0.43mg/100g. However this result is at variance with the finding of Tamburawa (2010) which indicated that toasting reduced the level of oxalate to 0.41mg/100g. According to Tamburawa (2010) boiling also progressively and substantially reduced the level of oxalate to the bearest minimum with increased duration of processing time; in 1hour (0.22mg/100g), 2hours (0.21mg/100g), 3hours (0.24mg/100g), 4hours 0.22mg/100g, this is at variance with the findings of this study which indicated that oxalate was not reduced to the bearest minimum in boiled *Bauhinia* seed samples (10 minutes: 8.95mg/100g; 20 minutes: 7.63mg/100g; 30 minutes: 6.73mg/100g and 40 minutes: 2.67mg/100g). The variance could probably be due to increased duration of boiling used by Tamburawa (2010).

The reduced level of undesirable anti-nutritional components is essential in order to improve the nutritional quality of the seed meals and to effectively utilize their full potentials as feed.

The high levels of antinutritional factors (tannin, oxalate and phytic acid) in the raw *Bauhinia* seed meal (raw BSM), toasted *Bauhinia* seed meal ($TBSM_{40}$) rendered them unsuitable for feed formulation since high levels ultimately indicates retardation of growth. The levels of anti-nutrients in the boiled sample were more reduced to a considerable level than in the toasted. However, this was also unacceptable because the trend of reduction indicated that they still had higher values than the soaked seed samples. Since the soaking method of processing was able to reduce the anti-nutritional compounds to the bearest minimum without affecting the nutritional quality of *Bauhinia* seeds it follows therefore that it was the best processing method in this study. Hence soaking of *Bauhinia* seed for 96hours was choosen for the subsequent feeding trial.

On the basis of retention of maximum protein components compared to other processing methods, reduced cost of processing (appendix 5) and minimum anti-nutrients as well as relatively high amino acid index and profile (appendix 4) the soaked *Bauhinia* seed $SMSM_{96}$ was chosen to be utilized as a potential source and better supplement for replacement of fish meal at graded levels in the diet of Juvenile *Clarias gariepinus* (Teugels).

The result of the carcass composition after feeding trial experiment indicated that crude protein values of all the diet treatments were significantly higher than the initial CP ($P<0.05$). Nwanna and Bolarinwa (2001) indicated that the crude protein and lipid contents of the African catfish increased after feeding trials. It also agrees with the findings of Arunlertaree and Moolthongnoi (2008) who reported that final protein in experimental fish carcass was related to the percentage of lipid content in carcass. The fish carcass protein of all dietary treatments were higher than the initial carcass protein, indicating that there was synthesis and increased tissue protein production, thus the growth of fish was not due to increase in weight only as reported by Fuller (1969); Ipinjolu and Faturoti (1999) and Banyigyi *et al*. (2001). However fish fed the control diets (diet 1, 100% fish meal and diet 5, 100% copens) had significantly ($P<0.05$) higher carcass protein (69.02% and 67.88% respectively) than those fed the experimental diets containing graded levels of soaked *Bauhinia* seed meal which indicated that the control diets were superior in terms of crude protein due to their relatively high amino acid profile.

The body fat in fish fed diets 4 (25% fish meal and 75% soaked *Bauhinia* seed meal) and 5 (100% copens) were significantly higher ($P<0.05$) than all other diets indicating enhanced production of lipids in these groups of fish. Lipid has been associated with increased efficiency of metabolism (Ovie *et al.*, 1999).

The results obtained from fish fed on Soaked *Bauhinia* seed meal diets showed that fish carcass ash content decreased with increase in crude protein and lipid. Nwanna and Bolarinwa (2001) ascerted that ash and moisture contents of African catfish decreased after feeding trials while the crude protein and lipid contents increased. However, the ash content of the control diets which were still significantly higher than the experimental diets implies higher retention of ash which could be attributed to high efficiency and high nutrient contents associated with fish meal. Since the commercially formulated diets also have been noted for established high nutritive composition (ash inclusive) the high ash retention values obtained in the groups of fish fed the control diets is expected.

The fish carcass protein as well as the lipid in fish fed sundried Locust meal diets followed the same trend as the fish fed soaked Bauhinia meal diets. At the end of the experiment there was significant difference between the various diets ($P<0.05$) in the crude protein composition. The carcass proximate composition at the end of the feeding trial indicated that crude protein (CP) values of all the diets were higher than the initial carcass value (Table 4.18). Nwanna and Bolarinwa (2001) similarly reported that crude protein and lipid contents of African catfish increased after feeding trials with the implication that there was protein synthesis and that the growth of fish was not only due to weight increament but also due to protein synthesis as reported by Fuller (1969) and Banyigyi *et al*. (2001). The fish fed the control diets (diet 1, 100% fish meal and diet 5, 100% copens) had significantly ($P<0.05$) higher final carcass protein (69.02% and 67.88% respectively) than those fed the experimental formulated diets containing sun dried locust meal which implies there was better protein synthesis in fish fed the control diets. Fish meal has been noted for attribute of high efficiency due to high nutrient content.

Similarly the commercially formulated diet (copens) has been noted for high nutritive composition and efficiency (Agboola, 2004; Umar, 2006).

The body fat followed a similar pattern to that of fish fed Bauhinia seed meal. The body fat in fish fed diets 1 and 5 were higher than in other treatments, indicating enhanced production of lipids in these groups of fish. Lipid has been associated with increased efficiency of metabolism (Ovie *et al.*, 1999).

The final carcass ash composition did not follow the same trend as the crude protein (CP) and ether extract (EE) which increased. The final ash content of the fish decreased in all dietary treatments. Nwanna and Bolarinwa (2001) asserted that ash and moisture content of African catfish decreased after feeding trials while the crude protein and lipid contents increased.

The mean proximate composition (CP) of the experimental diets obtained from the laboratory analysis is at variance with that of the computer calculated report of this same study which gave a 40% CP for all the diets as desired implying that there was no significant difference among the diets ($P>0.05$). However, between treatments there was significant difference ($P<0.05$) in mean proximate composition of experimental diets in relation to the 40% CP, the experimental diets of this study ranged between 39.57% and 41.26% which is within the range of ±2, standard variation of such types which existed in the findings of Agboola (2004) while working with *Clarias gariepinus* (the proximate composition of the experimental diets g/100g in that report indicated that the five diets used had CP values of 43.35%, 44.44%, 43.69%, 43.88% and 43.88% respectively. According to Lall (1991) and Fasakin (1998) linear programming offers considerable potential in the development of "least cost formulation" of fish feed. However, wide fluctuations occur in the composition of feeds ingredients due to seasonal and geographic variations, thus formulation should be modified accordingly since physical

characteristics, milling and composition of the feed ingredients may have significant effect on the processing and quality of finished feed.

The crude lipid content of the diets ranged between 8.11 and 8.99 % (for *Bauhinia* seed meal) and 7.39 and 8.99% (for locust meal) this was in accord with the ascertations of Adikwu (2003) who reported that standard lipid requirement of most tropical fishes ranged between 8 and 10%.

Weight gain and standard length increases are known to be the most important indices for measuring fish responses to experimental diets and very reliable indicators of growth (Balogun, *et al*., 2004). The growth performance of the fish fed Bauhinia seed diets in terms of the mean weight gain (MWG) and percentage live weight gain (PLWG) indicated that there were positive and significant growth in fish fed the control diets 5 (72.80g) and diet 1 (61.38g) respectively and this was a reflection of the superiority of these diets over the experimental formulated diets. The most superior diet in terms of MWG and PLWG among the Bauhinia experimental diets was diet 2 (58.2g). The gradual trend of reduced values of MWG and PLWG observed with increased inclusion levels of the soaked *Bauhinia* seed meal diet 3 (50.10g) and diet 4 (36.3g) in *Clarias gariepinus* fed graded levels of soaked *Bauhinia* seed meal were below expectation, in spite of the conducive physico-chemical parameters of water recorded. This could be attributed to some conspicuous/remote reasons as reported by Lim and Dominy (1989) which included improper balance of essential nutrients such as amino acids and minerals, presence of toxic substances (anti-nutritional factor) or decrease in palatability and pellet water stability value in fish diets which invariably lead to reduced growth and poor feed efficiency. Generally high dietary levels of legumes have been found to be deleterious to the performance of the cultured fish (Liener, 1980). De Silva and Ganasekara (1989) also indicated that poor performance of fish could be due

to nutrient inbalance associated with plant protein source. Olvera *et al.* (1988) reported the presence of toxins could be devastating to cultured fish (*Oreochromis niloticus*) fed different levels of *Serbania grandiflora*. The quality of pelletised feed might also have contributed to the low weight recorded in this study among the fish fed the experimental formulated diets since nutrients present in feeds are prone to leaching in water due to poor stability and degradation of feed to the bottom water during feeding with the implication of significant losses in agricultural management (Falayi *et al.*, 2003). The fibre content of *Bauhinia* seed meal was within the limits of 4 – 6% recommended by Annune and Oniye (1993). However, the values of the ether extract content were high and exceeded the 6% level recommended by Annune and Oniye (1993). Eyo (2001) reported that the use of inferior feedstuffs affect the nutritive value and digestibility of compounded feeds. Pullin (1983) also reported that apart from nutrition, other factors such as anti-nutrients could be responsible for low values growth indices. The gradual decrease in values as inclusion level increased may be attributed to a reduction in palatability of diet which is in agreement with reports of Jauncey and Ross (1982) who reported that feed intake reduced with reduction in palatability. The results of this study on the basis of MWG and PLWG is similar to the findings of Hossain *et al. (*2001) and Balogun and Ologhobo (1989) who indicated that high inclusion of plant protein (*Bauhinia* seed meal) in the diet have been noted to suppress growth significantly in fish species like carp and *Clarias gariepinus* respectively.

The high SGR values, high FER, high ANPU, high GFCE, high PER, high K value, high PSR and low FCR values of fish fed diet 2 (25% *Bauhinia* and 75% fish meal) confers it with better advantages for growth and efficiency of feed utilization over the rest of the experimental formulated diets. This result agrees with the assertation of Olaniyi (2009a&b) who stated that the higher the specific growth (SGR) and the smaller the feed conversion ratio (FCR), the better the

feed quality. Adikwu (2003) also reported that a lower FCR value implies efficient feed utilization by fish.

The results further indicated poor and inconsistent growth indices in terms of length in the fish fed the experimental formulated diets as reflected in the MFSL, MLG and PLG. This could be attributed to microbial infection at the onset of this study which led to development of frayed tails in some fish. Papena (1980) and NAERLS (1999) reports on fish diseases indicated that fish with bacterial attack normally have frayed fins and tails which are invariably a reflection of stunted growth and poor water quality.

The Percentage Survival Rate (PSR) indicated that there was significant difference ($P<0.05$) among the PSR values of the fish fed different *Bauhinia* seed meal diets. The PSR values among the experimental formulated diets decreased with higher inclusion levels of Bauhinia, diet 2 (83.33%) followed by diet 3 (80.00%). The least was in diet 4 (70.00%), this could be attributed to the percentage of anti-nutrients present in the feed samples that might have resulted in low palatability and reduced feed consumption and utilization and this might as well resulted in deaths. Similarly, Pompa (1982) reported that high anti-nutrient and low palatability could result in low feed consumption and utilization. Some mortality that occurred could also be attributed to change of environment since the fish were transferred from outdoor to indoor environment. NAERLS (1999) indicated that change in environment could lead to sudden deaths. The earlier bacterial infection reported at the onset of this study to be responsible for mortality due to poor water quality (inadequate aeration).

Final mean weight gain and PLWG increased significantly ($P<0.05$) as replacement levels of soaked *Bauhinia* seed meal ($SBSM_{96}$) increased up to 25% level; which means that

Bauhinia monandra could replace up to 25% of the fish meal component in the diets for the African cat fish.

The fish fed diet 2 (25% *Bauhinia* seed meal) showed overall good performance in terms of MFBW, MWG, PLWG, MFSL, MLG, PLG, SGR, FCR, FER ANPU, PER, Condition factor (K) and PSR. Eventhough the FCR (0.56) was very low, showing good performance in terms of feed conversion and utilization of diets by *Clarias gariepinus* (Teugels) juveniles amongst all the fish fed the experimental diets, the performance in terms of growth and other feed utilization indices of *Clarias juveniles* fed 50% *Bauhinia* meal and 75% *Bauhinia* meal were observed to be poor compared with the juveniles fed 25% *Bauhinia* meal. The result therefore indicated that up to 25% *Bauhinia* seed meal could replace fish meal in the diet of African catfish juveniles without any adverse effect on the growth performance.

The fish fed diet 2 (25% locust meal) showed overall good performance in terms of Mean Final Body Weight (MFBW), Mean Weight Gain (MWG), Percentage Live Weight Gain (PLWG), Mean Final Standard Length (MFSL), Mean Length Gain (MLG), Percentage Length Gain (PLG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Feed Efficiency Ratio (FER), Apparent Net Protein Utilization (ANPU), Protein Efficiency Ratio (PER), Condition factor (K) and Percetage Survival Rate (PSR) indicating that up to 25% sundried locust meal could replace fish meal in the diet of *Clarias gariepinus* juveniles without any devastating effect on the growth performance.

The Mean Final Body Weight (MFBW), Mean Weight Gain (MWG), Percentage Live Weight Gain (PLWG) and Mean Length Gain (MLG) in fish fed locust meal diets decreased significantly ($P>0.05$) with increase in locust meal implying that fish fed the locust diets generally had poor growth. This could probably be attributed to the presence of chitin in the

insect (locust). Deforliat (1992) indicated that removal of chitin increases the quality of insect protein to a level comparable to that of products from vertebrate animals such that they can, to a substantial degree supplement predominantly cereal diets with many of their initially unavailable nutrients. Similarly, Deforliart (1992) reported that following alkali extraction, the true digestibility of protein concentrate obtained from whole dried adult honey bees (*Apis mellifera* L) was increased from 71.5% to 94.3%, the protein efficiency ratio (PER) from 1.50 to 2.47, and the net protein utilization (NPU) from 42.5 to 62.0. These values compared favorably with values of 96.8%, 2.50% and 70.0% respectively for casein (Ozimek *et al*., 1985).

CHAPTER 6

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, diet 2 (25% *Bauhinia* seed meal diet and 75% fish meal diet) had highest Mean Final Body Weight (MFBW), Mean Weight Gain (MWG), Percentage Live Weight Gain (PLWG), Mean Final Standard Length (MFSL), Mean Live Weight Gain (MLG), Percentage Live Weight Gain (PLWG), Specific Growth Rate (SGR), Feed Efficiency Ratio (FER), Apparent Net Protein Utilization (ANPU), Protein Efficiency Ratio (PER), Condition Factor (K), Percentage Survival Rate (PSR) with lowest Feed Convertion Ratio (FCR) values and Gross Feed Convertion Efficiency (GFCE). The result indicated that diet 2 (25% *Bauhinia* seed) was the best diet that supported optimum growth of *Clarias gariepinus* juveniles. In terms of relative cost of feed per unit of biomass yield diet 2 (25% *Bauhinia* seed meal) was the most economical. However, in terms of economy of protein gain diet 3 (50% *Bauhinia* seed meal) was the most economical followed by diet 2 implying the diet 2 was the cheapest, though diet 3 was more economical in terms of protein gain.

The growth indices for the locust meal diets indicated that diet 2 (25% locust meal diet) had highest Mean Final Body Weight (MFBW), Mean Weight Gain (MWG), Percentage Live Weight Gain (PLWG), Mean Final Standard Length (MFSL), Mean Live Weight Gain (MLG), Percentage Live Weight Gain (PLG), Specific Growth Rate (SGR), Feed Efficiency Ratio (FER), Apparent Net Protein Utilization (ANPU), Gross Feed Convertion Efficiency (GFCE), Protein Efficiency Ratio (PER), Condition Factor (K), Percentage Survival Rate (PSR) and lowest Feed Convertion Ratio (FCR) values. Similarly in comparison to the *Bauhinia* diet meal, diet 2 (25% locust meal diet) was the best diet that supported the growth of *Clarias gariepinus* and also had

the highest carcass crude protein. The most economical diet in terms of relative cost of feed per unit weight gain and the best diet in terms of feed per unit protein gain was also diet 2 (25% locust meal). The overall result implies that the locust meal diet was the best and most economical in terms of relative cost of feed per unit protein gain and weight gain compared to *Bauhinia* seed meal. However in terms of growth indices *Bauhinia* seed meal diet was the best.

The decreased trend in growth performance and feed utilization of the experimental diets with increased inclusion levels of *Bauhinia* and locust meals suggests that soaked *Bauhinia* seed meal ($SBSM_{96}$) at 25% level of inclusion and sundried locust meal at 25% level of inclusion have the potential of replacing fish meal in fish feed formulations provided they are utilized at low levels of inclusion. The increased carcass protein in all the treatment diets indicated that both *Bauhinia* seed meal and locust meal have the potential of replacing fish meal.

6.2 Recommendations

- Further investigation is recommended in the use of processing techniques such as fermentation and autoclaving which may reduce anti-nutrient levels and enhance efficient utilization of feed stuff
- The use of other culturable fish species such as *Cyprinus carpio* (Carp) and *Oreochromis niloticus* fed with Bauhinia and locust meal is recommended to determine the best performance level and the most suitable fish species for the aforementioned diets.
- Other culture systems such as concrete tanks, earthen ponds, tanks, race way and complete flow through systems should be explored to evaluate the effect of soaked *Bauhinia* seed meal and locust meal on growth and feed utilization.

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APPENDICES

Appendix 1: **Composition of essential mineral elements in the seed of *Bauhinia monandra (Linn.)***

Elements	Concentration (mg/g)
Potassium	74.20 ± 2.46
Calcium	77.9 ± 2.98
Magnesium	2.8 ± 0.02
Sodium	2.319 ± 0.07
Sulphur	4.166 ± 0.005
Phosphorus	1.594 ± 0.09
Iron	1.710 ± 0.11

Source: Anhwange, *et al.* 2005.

Appendix 2: **Moisture, ash, organic matter and protein contents of *Bauhinia monandra***

Nutritional contents of *B. monandra* seeds	
Moisture content (%)	7.55 ± 0.3
Ash content (%)	4.44 ± 0.07
Organic matter content (%)	95.56 ± 0.07
Protein content (%)	33.09 ± 2.30

Source: Anhwange, *et al.* 2004.

Appendix 3: **Nutritional and anti-nutritional content of seed of *Bauhinia monandra* (Linn.)**

Nutrients and anti-nutrients contents of *B. monandra*	
Lipids (%)	28.70 ± 0.20
Protein (%)	33.09 ± 1.33
Carbohydrates (%)	21.45 ± 0.12
Fibre (%)	3.25 ± 0.83
Phytate (mg/100g)	11.5 ± 0.47
Hydrogen cyanide (mg/100)	0.32 ± 0.00
Tannins (%)	6.0 ± 0.09
Saponins (%)	2.052 ± 0.0

Source: Anhwange, *et al.* 2005.

Appendix 4: Amino acid content (g/100g protein) of the seed of *Bauhinia monandra (Linn.)*

Amino acid	Concentration (g/100g protein)
Lysine	2.86
Phenylalanine	3.77
Leucine	2.13
Isoleucine	2.31
Methionine	1.54
Valine	3.54
Cystine	1.11
Threonine	2.70
Glutamic acid	11.75
Arginine	6.75
Aspartic acid	6.02
Serine	4.58
Glycine	3.09
Alanine	2.99
Histidine	2.39
Proline	2.37
Tyrosine	3.18

Source: Anhwange, *et al.* 2004.

Appendix 5: **Recommended nutrient levels in fish feed (amino acids)**

Nutrients **Amino acid**	**Minimum Recommended levels** **% of diet**
Arginine	1.5
Histidine	0.7
Isoleucine	0.9
Leucine	1.4
Lycine	1.8
Methionine + Cystine	1.0
Phenylalamine + Tyrosine	1.8
Threonine	0.8
Trptophane	0.2
Valine	1.2

Source: NRC (1993)

Appendix 6: **Cost of processing *Bauhinia* seeds (cost / kg)**

Processing method	**Average cost / kg (₦)**
Raw BSM	10
BBSM	30
RBSM	35
SBSM	15

Appendix 7: Prices of ingredients used in determination of Gross Composition of Experimental Diets at time of Experiment (August, 2009)

Calculations based on market price of the ingredients (₦/kg) at the time of the experiment (August, 2009). Starch binder (₦100/kg); palmoil slush (₦125/kg); salt (₦42/kg); bone meal (₦30/kg); wheat offal (₦30/kg); yellow maize (₦46/kg); soya bean cake (₦80/kg); local fishmeal (₦50/kg); copens feed (₦320/kg); locust (₦45/kg); cost of processed *Bauhinia* seed meal, $SBSM_{96}$ (₦15/kg); synthetic DL methionine (₦1,200/kg); lysine (₦1,000/kg); premix (₦480/kg).

Appendix 8: ANOVA FOR BOILED DRY MATTER (DM) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	0.1508	0.0377	251.33
Repss (r – 1)	1	0.0001	0.0001	
Ess (t – 1) (r – 1)	4	0.0006	0.00015	
Total ss(tr – 1)	9	0.1515		

Appendix 9: ANOVA FOR BOILED CRUDE PROTEIN CONTENT (CP)

Source	df	ss	ms	F
Trtss (t – 1)	4	0.1218	0.03045	4.0763
Repss (r – 1)	1	0.00252	0.00252	
Ess (t – 1) (r – 1)	4	0.02988	0.00747	
Total ss(tr – 1)	9	0.1542		

Appendix 10: ANOVA FOR BOILED CRUDE FIBRE CONTENT (CF)

Source	df	ss	ms	F
Trtss (t – 1)	4	1.4231	0.35578	1872.53
Repss (r – 1)	1	0.00006	0.00006	
Ess (t – 1) (r – 1)	4	0.00074	0.00019	
Total ss(tr – 1)	9	1.4239		

Appendix 11: ANOVA FOR BOILED ETHER EXTRACT CONTENT (EE)

Source	df	ss	ms	F
Trtss (t – 1)	4	0.3767	0.09418	627.87
Repss (r – 1)	1	0.00042	0.00042	
Ess (t – 1) (r – 1)	4	0.00058	0.00015	
Total ss(tr – 1)	9	0.3777		

Appendix 12: ANOVA FOR BOILED ASH CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	1.087	0.27175	1358.75
Repss (r – 1)	1	0.00032	0.00032	
Ess (t – 1) (r – 1)	4	0.00068	0.0002	
Total ss(tr – 1)	9	1.088		

Appendix 13: ANOVA FOR BOILED NITROGEN FREE EXTRACT CONENT (NFE)

Source	df	ss	ms	F
Trtss (t – 1)	4	1.8523	0.463075	544.79
Repss (r – 1)	1	0.0015	0.0015	
Ess (t – 1) (r – 1)	4	0.0034	0.00085	
Total ss(tr – 1)	9	1.8572		

Appendix 14: ANOVA FOR BOILED ESSENTIAL AMINO ACID INDEX (EAAI)

Source	df	ss	ms	F
Trtss (t – 1)	4	0.01106	0.00278	16.353
Repss (r – 1)	1	0.00004	0.00004	
Ess (t – 1) (r – 1)	4	0.00066	0.00017	
Total ss(tr – 1)	9	0.01176		

Appendix 15: ANOVA FOR SOAKED DRY MATTER (DM)CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	6.8351	1.70878	697.46
Repss (r – 1)	1	0.00362	0.00362	
Ess (t – 1) (r – 1)	4	0.00978	0.00245	
Total ss(tr – 1)	9	6.8485		

Appendix 16: ANOVA FOR SOAKED CRUDE PROTEIN (CP) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	6.5442	1.6361	2045.13
Repss (r – 1)	1	0.0002	0.0002	
Ess (t – 1) (r – 1)	4	0.003	0.0008	
Total ss(tr – 1)	9	6.5474		

Appendix 17: ANOVA FOR SOAKED CRUDE FIBRE (CF) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	0.169	0.0423	211.5
Repss (r – 1)	1	0.0002	0.0002	
Ess (t – 1) (r – 1)	4	0.0006	0.0002	
Total ss(tr – 1)	9	0.1698		

Appendix 18: ANOVA FOR SOAKED ETHER EXTRACT (EE) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	0.3742	0.09355	550.29
Repss (r – 1)	1	0.00012	0.00012	
Ess (t – 1) (r – 1)	4	0.00068	0.00017	
Total ss(tr – 1)	9	0.375		

Appendix 19: ANOVA FOR SOAKED ASH CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	0.7787	0.19468	671.31
Repss (r – 1)	1	0.00026	0.00026	
Ess (t – 1) (r – 1)	4	0.00114	0.00029	
Total ss(tr – 1)	9	0.7801		

Appendix 20: ANOVA FOR SOAKED NITROGEN FREE EXTRACT (NFE) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	14.3743	3.59358	1079.15
Repss (r – 1)	1	0.0073	0.0073	
Ess (t – 1) (r – 1)	4	0.0133	0.00333	
Total ss(tr – 1)	9	14.3949		

Appendix 21: ANOVA FOR SOAKED ESSENTIAL AMINO ACID INDEX (EAAI)

Source	df	ss	ms	F
Trtss (t – 1)	4	0.00264	0.00066	1.65
Repss (r – 1)	1	0.00025	0.00025	
Ess (t – 1) (r – 1)	4	0.0016	0.0004	
Total ss(tr – 1)	9			

Appendix 22: ANOVA FOR ROASTED DRY MATTER (DM) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	0.0467	0.01168	50.7826
Repss (r – 1)	1	0.0005	0.00005	
Ess (t – 1) (r – 1)	4	0.0009	0.00023	
Total ss(tr – 1)	9	0.0481		

Appendix 23: ANOVA FOR ROASTED CRUDE PROTEIN (CP) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	36.1035	9.02588	50143.8
Repss (r – 1)	1	0.0	*****	
Ess (t – 1) (r – 1)	4	0.0007	0.00018	
Total ss(tr – 1)	9	36.1042		

Appendix 24: ANOVA FOR ROASTED CRUDE FIBRE (CF) CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	10.7104	2.6776	13388
Repss (r – 1)	1	0.0002	0.0002	
Ess (t – 1) (r – 1)	4	0.0006	0.0002	
Total ss(tr – 1)	9	10.7112		

Appendix 25: ANOVA FOR ROASTED ETHER EXTRACT (EE)

Source	df	ss	ms	F
Trtss (t – 1)	4	1.0462	0.2616	872
Repss (r – 1)	1	0.0004	0.0004	
Ess (t – 1) (r – 1)	4	0.001	0.0003	
Total ss(tr – 1)	9			

Appendix 26: ANOVA FOR ROASTED ASH CONTENT

Source	df	ss	ms	F
Trtss (t – 1)	4	0.2087	0.05218	65.23
Repss (r – 1)	1	0.0014	0.0014	
Ess (t – 1) (r – 1)	4	0.0027	0.0008	
Total ss(tr – 1)	9	0.2128		

Appendix 27: ANOVA FOR ROASTED NITROGEN FREE EXTRACT (NFE)

Source	df	ss	ms	F
Trtss (t – 1)	4	20.8135	5.20338	23.334
Repss (r – 1)	1	0.0072	0.0072	
Ess (t – 1) (r – 1)	4	0.0089	0.00223	
Total ss(tr – 1)	9			

Appendix 28: ANOVA FOR ROASTED ESSENTIAL AMINO ACID INDEX (EAAI)

Source	df	ss	ms	F
Trtss (t – 1)	4	0.0515	0.01288	128.8
Repss (r – 1)	1	0.0002	0.0002	
Ess (t – 1) (r – 1)	4	0.0004	0.0001	
Total ss(tr – 1)	9	0.0521		

ANOVA FOR ANTINUTRIENTS

Appendix 29: ANOVA FOR BOILED HCN

Source	df	ss	ms	F
Trtss (t – 1)	4	0.38884	0.09721	486.05
Repss (r – 1)	1	0.00025	0.00025	
Ess (t – 1) (r – 1)	4	0.0008	0.0002	
Total ss(tr – 1)	9	0.38989		

Appendix 30: ANOVA FOR BOILED TANIN

Source	df	ss	ms	F
Trtss (t – 1)	4	19.5581	4.88953	61119.13
Repss (r – 1)	1	0.0	0	
Ess (t – 1) (r – 1)	4	0.0003	0.00008	
Total ss(tr – 1)	9	19.5584		

Appendix 31: ANOVA FOR BOILED OXALATE

Source	df	ss	ms	F
Trtss (t – 1)	4	93.8201	23.45513	63391.97
Repss (r – 1)	1	0.00004	0.00004	
Ess (t – 1) (r – 1)	4	0.00146	0.00037	
Total ss(tr – 1)	9	93.8216		

Appendix 32: ANOVA FOR BOILED PHYTIC ACID

Source	df	ss	ms	F
Trtss (t – 1)	4	133.01456	33.25364	51958.81
Repss (r – 1)	1	0.00064	0.00064	
Ess (t – 1) (r – 1)	4	0.00256	0.00064	
Total ss(tr – 1)	9	133.01776		

Appendix 33: ANOVA FOR BOILED SAPONIN

Source	df	ss	ms	F
Trtss (t – 1)	4	7.97144	1.99286	403.413
Repss (r – 1)	1	0.01024	0.01024	
Ess (t – 1) (r – 1)	4	0.01976	0.00494	
Total ss(tr – 1)	9	8.00144		

Appendix 34: ANOVA FOR ROASTED HCN

Source	df	ss	ms	F
Trtss (t – 1)	4	0.0869	0.02173	434.5
Repss (r – 1)	1	0.0019	0.0019	
Ess (t – 1) (r – 1)	4	0.0002	0.00005	
Total ss(tr – 1)	9	0.089		

Appendix 35: ANOVA FOR ROASTED TANIN

Source	df	ss	ms	F
Trtss (t – 1)	4	10.0013	2.500325	35718.93
Repss (r – 1)	1	0.00004	0.00004	
Ess (t – 1) (r – 1)	4	0.00026	0.00007	
Total ss(tr – 1)	9	10.0016		

Appendix 36: ANOVA FOR ROASTED OXALATE

Source	df	ss	ms	F
Trtss (t – 1)	4	13.0599	3.2649	9893.6
Repss (r – 1)	1	0.00004	0.0004	
Ess (t – 1) (r – 1)	4	0.0013	0.00033	
Total ss(tr – 1)	9	13.0616		

Appendix 37: ANOVA FOR ROASTED PHYTIC ACID

Source	df	ss	ms	F
Trtss (t – 1)	4	3.0694	0.7674	5903.08
Repss (r – 1)	1	0.0001	0.0001	
Ess (t – 1) (r – 1)	4	0.0005	0.00013	
Total ss(tr – 1)	9	3.07		

Appendix 38: ANOVA FOR ROASTED SAPONIN

Source	df	ss	ms	F
Trtss (t – 1)	4	7.5590	1.8898	331.54
Repss (r – 1)	1	0.0068	0.0068	
Ess (t – 1) (r – 1)	4	0.0226	0.0057	
Total ss(tr – 1)	9	7.5884		

Appendix 39: ANOVA FOR SOAKED HCN

Source	df	ss	ms	F
Trtss (t – 1)	4	0.520	0.13	94.203
Repss (r – 1)	1	0.00009	0.00009	
Ess (t – 1) (r – 1)	4	0.00552	0.00138	
Total ss(tr – 1)	9	0.52561		

Appendix 40: ANOVA FOR SOAKED TANIN

Source	df	ss	ms	F
Trtss (t – 1)	4	49.3491	12.3373	68540.56
Repss (r – 1)	1	0.0004	0.0004	
Ess (t – 1) (r – 1)	4	0.0007	0.00018	
Total ss(tr – 1)	9	49.3502		

Appendix 41: ANOVA FOR SOAKED OXALATE

Source	df	ss	ms	F
Trtss (t – 1)	4	154.7842	38.6961	168243.9
Repss (r – 1)	1	0.0007	0.0007	
Ess (t – 1) (r – 1)	4	0.0009	0.00023	
Total ss(tr – 1)	9	154.7858		

Appendix 42: ANOVA FOR SOAKED PHYTIC ACID

Source	df	ss	ms	F
Trtss (t – 1)	4	129.8702	32.4676	1082253
Repss (r – 1)	1	0.0008	0.0008	
Ess (t – 1) (r – 1)	4	0.0001	0.00003	
Total ss(tr – 1)	9	129.8711		

Appendix 43: ANOVA FOR SOAKED SAPONINS

Source	df	ss	ms	F
Trtss (t – 1)	4	8.70856	2.17714	384.654
Repss (r – 1)	1	0.00676	0.00676	
Ess (t – 1) (r – 1)	4	0.02264	0.00566	
Total ss(tr – 1)	9	8.73796		

EXPERIMENT I

Appendix 44: ANOVA FOR INITIAL STOCKING WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	13.57	3.39	13.6
Repss (r – 1)	2	2.42	1.21	
Ess (t – 1) (r – 1)	8	2.03	0.25	
Total ss(tr – 1)	14	18.04		

Appendix 45: ANOVA FOR WEEK 2 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	21.82	5.46	6.19
Repss (r – 1)	2	1.44	0.72	
Ess (t – 1) (r – 1)	8	7.0	0.88	
Total ss(tr – 1)	14	30.26		

Appendix 46: ANOVA FOR WEEK 4 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	152.34	38.1	20.48
Repss (r – 1)	2	8.21	4.11	
Ess (t – 1) (r – 1)	8	14.91	1.86	
Total ss(tr – 1)	14	175.46		

Appendix 47: ANOVA FOR WEEK 6 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	445.85	111.5	13.45
Repss (r – 1)	2	27.82	13.91	
Ess (t – 1) (r – 1)	8	66.33	8.29	
Total ss(tr – 1)	14	540		

Appendix 48: ANOVA FOR WEEK 8 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	940.9	235.2	9.42
Repss (r – 1)	2	77.0	38.5	
Ess (t – 1) (r – 1)	8	199.73	24.97	
Total ss(tr – 1)	14	1217.63		

Appendix 49: ANOVA FOR WEEK 10 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	1490.36	372.6	5.06
Repss (r – 1)	2	96.68	48.34	
Ess (t – 1) (r – 1)	8	589.34	23.7	
Total ss(tr – 1)	14	2176.38		

Appendix 50: ANOVA FOR WEEK 12 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	2152.54	538.1	7.02
Repss (r – 1)	2	227.27	113.6	
Ess (t – 1) (r – 1)	8	612.59	76.6	
Total ss(tr – 1)	14	2992.4		

EXPERIMENT II

Appendix 51: ANOVA FOR INITIAL STOCKING WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	13.59	3.39	13.6
Repss (r – 1)	2	2.42	1.21	
Ess (t – 1) (r – 1)	8	2.03	0.25	
Total ss(tr – 1)	14	18.04		

Appendix 52: ANOVA FOR WEEK 2 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	92.93	23.23	30.9
Repss (r – 1)	2	0.3	0.15	
Ess (t – 1) (r – 1)	8	6	0.75	
Total ss(tr – 1)	14	99.23		

Appendix 53: ANOVA FOR WEEK 4 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	622	155.5	100.3
Repss (r – 1)	2	0.45	0.23	
Ess (t – 1) (r – 1)	8	12.41	1.55	
Total ss(tr – 1)	14	634.86		

Appendix 54: ANOVA FOR WEEK 6 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	1758.09	439.5	152.1
Repss (r – 1)	2	0.37	0.19	
Ess (t – 1) (r – 1)	8	23.09	2.89	
Total ss(tr – 1)	14	1781.55		

Appendix 55: ANOVA FOR WEEK 8 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	3669.59	917.4	169.8
Repss (r – 1)	2	0.29	0.15	
Ess (t – 1) (r – 1)	8	43.2	5.4	
Total ss(tr – 1)	14	3713.08		

Appendix 56: ANOVA FOR WEEK 10 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	6230.49	1557.6	134.6
Repss (r – 1)	2	3.18	1.59	
Ess (t – 1) (r – 1)	8	92.55	11.57	
Total ss(tr – 1)	14	6326.22		

Appendix 57: ANOVA FOR WEEK 12 MEAN WEIGHT

Source	df	ss	ms	F
Trtss (t – 1)	4	10362.96	25.90.74	179.04
Repss (r – 1)	2	1.37	0.685	
Ess (t – 1) (r – 1)	8	115.78	14.47	
Total ss(tr – 1)	14	10480.11		

EXPERIMENT I

Appendix 58: ANOVA FOR INITIAL STOCKING MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	0.31	0.08	0.48
Repss (r – 1)	2	0.07	0.04	
Ess (t – 1) (r – 1)	8	1.28	0.16	
Total ss(tr – 1)	14	1.66		

Appendix 59: ANOVA FOR WEEK 2 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	7.34	1.835	61.2
Repss (r – 1)	2	0.15	0.08	
Ess (t – 1) (r – 1)	8	0.22	0.03	
Total ss(tr – 1)	14	7.71		

Appendix 60: ANOVA FOR WEEK 4 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	26.54	6.64	165.8
Repss (r – 1)	2	0.14	0.07	
Ess (t – 1) (r – 1)	8	0.3	0.04	
Total ss(tr – 1)	14	26.98		

Appendix 61: ANOVA FOR WEEK 6 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	56	14	368.4
Repss (r – 1)	2	0.07	0.035	
Ess (t – 1) (r – 1)	8	0.31	0.038	
Total ss(tr – 1)	14	56.38		

Appendix 62: ANOVA FOR WEEK 8 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	64.59	16.15	4.25
Repss (r – 1)	2	6.07	3.04	
Ess (t – 1) (r – 1)	8	30.42	3.80	
Total ss(tr – 1)	14	101.08		

Appendix 63: ANOVA FOR WEEK 10 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	145.38	36.345	519.2
Repss (r – 1)	2	0.16	0.08	
Ess (t – 1) (r – 1)	8	0.53	0.07	
Total ss(tr – 1)	14	146.07		

Appendix 64: ANOVA FOR WEEK 12 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	206.86	51.72	430.9
Repss (r – 1)	2	0.09	0.045	
Ess (t – 1) (r – 1)	8	0.94	0.12	
Total ss(tr – 1)	14	207.89		

EXPERIMENT II

Appendix 65: ANOVA FOR WEEK INITIAL STOCKING MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	0.47	0.12	0.59
Repss (r – 1)	2	0.0	0.0	
Ess (t – 1) (r – 1)	8	1.59	0.2	
Total ss(tr – 1)	14	2.06		

Appendix 66: ANOVA FOR WEEK 2 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	0.57	0.14	0.8
Repss (r – 1)	2	0.07	0.04	
Ess (t – 1) (r – 1)	8	1.53	0.19	
Total ss(tr – 1)	14	2.17		

Appendix 67: ANOVA FOR WEEK 4 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	0.36	0.09	0.83
Repss (r – 1)	2	0.01	0.005	
Ess (t – 1) (r – 1)	8	0.9	0.11	
Total ss(tr – 1)	14	1.27		

Appendix 68: ANOVA FOR WEEK 6 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	0.33	0.08	0.75
Repss (r – 1)	2	0.01	0.005	
Ess (t – 1) (r – 1)	8	0.89	0.11	
Total ss(tr – 1)	14	1.23		

Appendix 69: ANOVA FOR WEEK 8 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	17.15	4.29	1.17
Repss (r – 1)	2	7.81	3.91	
Ess (t – 1) (r – 1)	8	29.42	3.68	
Total ss(tr – 1)	14	54.38		

Appendix 70: ANOVA FOR WEEK 10 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	0.36	0.09	0.53
Repss (r – 1)	2	0.01	0.005	
Ess (t – 1) (r – 1)	8	1.38	0.17	
Total ss(tr – 1)	14	1.75		

Appendix 71: ANOVA FOR WEEK 12 MEAN LENGTH

Source	df	ss	ms	F
Trtss (t – 1)	4	1.09	0.27	0.59
Repss (r – 1)	2	0.33	0.17	
Ess (t – 1) (r – 1)	8	3.68	0.46	
Total ss(tr – 1)	14	5.1		

EXPERIMENT I: MEAN INCREASE IN WEIGHT

Appendix 72: ANOVA FOR WEEK 2

Source	df	ss	ms	F
Trtss (t – 1)	4	26.156	6.539	12.4316
Repss (r – 1)	2	0.912	0.456	
Ess (t – 1) (r – 1)	8	4.208	0.526	
Total ss(tr – 1)	14	31.276		

Appendix 73: ANOVA FOR WEEK 4

Source	df	ss	ms	F
Trtss (t – 1)	4	171.4707	42.8677	34.86
Repss (r – 1)	2	6.076	3.038	
Ess (t – 1) (r – 1)	8	9.8373	1.2297	
Total ss(tr – 1)	14	187.384		

Appendix 74: ANOVA FOR WEEK 6

Source	df	ss	ms	F
Trtss (t – 1)	4	478.24	119.5	16.017
Repss (r – 1)	2	24.0173	12.0087	
Ess (t – 1) (r – 1)	8	59.716	7.4645	
Total ss(tr – 1)	14	561.9733		

Appendix 75: ANOVA FOR WEEK 8

Source	df	ss	ms	F
Trtss (t – 1)	4	986.5573	246.6393	10.2847
Repss (r – 1)	2	70.6893	35.3447	
Ess (t – 1) (r – 1)	8	191.8507	23.9813	
Total ss(tr – 1)	14	1249.0973		

Appendix 76: ANOVA FOR WEEK 10

Source	df	ss	ms	F
Trtss (t – 1)	4	1552.973	388.2433	5.3234
Repss (r – 1)	2	93.172	46.556	
Ess (t – 1) (r – 1)	8	583.455	72.9319	
Total ss(tr – 1)	14	2229.6		

Appendix 77: ANOVA FOR WEEK 12

Source	df	ss	ms	F
Trtss (t – 1)	4	2217.686	554.4215	7.3793
Repss (r – 1)	2	216.389	108.194	
Ess (t – 1) (r – 1)	8	601.058	75.1324	
Total ss(tr – 1)	14	3035.133		

EXPERIMENT II: MEAN INCREASE IN WEIGHT

Appendix 78: ANOVA FOR WEEK 2

Source	df	ss	ms	F
Trtss (t – 1)	4	116.3373	29.08433	21176
Repss (r – 1)	2	0.7213	0.36965	
Ess (t – 1) (r – 1)	8	1.0987	0.13734	
Total ss(tr – 1)	14	118.1573		

Appendix 79: ANOVA FOR WEEK 4

Source	df	ss	ms	F
Trtss (t – 1)	4	682.3267	170.5817	355.6
Repss (r – 1)	2	0.916	0.458	
Ess (t – 1) (r – 1)	8	3.8373	0.4797	
Total ss(tr – 1)	14	687.08		

Appendix 80: ANOVA FOR WEEK 6

Source	df	ss	ms	F
Trtss (t – 1)	4	1858.777	464.69	344.5
Repss (r – 1)	2	0.796	0.398	
Ess (t – 1) (r – 1)	8	10.791	1.34888	
Total ss(tr – 1)	14	1870.364		

Appendix 81: ANOVA FOR WEEK 8

Source	df	ss	ms	F
Trtss (t – 1)	4	3817.2093	954.302	295.88
Repss (r – 1)	2	0.5973	0.2987	
Ess (t – 1) (r – 1)	8	25.8027	3.2253	
Total ss(tr – 1)	14	3843.6093		

Appendix 82: ANOVA FOR WEEK 10

Source	df	ss	ms	F
Trtss (t – 1)	4	6419.782	1604.95	184.62
Repss (r – 1)	2	2.321	1.1605	
Ess (t – 1) (r – 1)	8	69.546	8.6933	
Total ss(tr – 1)	14	6491.649		

Appendix 83: ANOVA FOR WEEK 12

Source	df	ss	ms	F
Trtss (t – 1)	4	10647.668	2661.917	238.59
Repss (r – 1)	2	0.94	0.47	
Ess (t – 1) (r – 1)	8	89.252	11.1565	
Total ss(tr – 1)	14	10737.86		

EXPERIMENT I: MEAN INCREASE IN LENGTH

Appendix 84: ANOVA FOR WEEK 2

Source	df	ss	ms	F
Trtss (t – 1)	4	7.537	1.88425	57.3069
Repss (r – 1)	2	0.064	0.032	
Ess (t – 1) (r – 1)	8	0.263	0.03288	
Total ss(tr – 1)	14	7.864		

Appendix 85: ANOVA FOR WEEK 4

Source	df	ss	ms	F
Trtss (t – 1)	4	27.0426	6.76065	206.87
Repss (r – 1)	2	0.1453	0.07265	
Ess (t – 1) (r – 1)	8	0.2614	0.03268	
Total ss(tr – 1)	14	27.4493		

Appendix 86: ANOVA FOR WEEK 6

Source	df	ss	ms	F
Trtss (t – 1)	4	56.8466	14.21165	332.98
Repss (r – 1)	2	0.1053	0.5265	
Ess (t – 1) (r – 1)	8	0.3414	0.04368	
Total ss(tr – 1)	14	57.2933		

Appendix 87: ANOVA FOR WEEK 8

Source	df	ss	ms	F
Trtss (t – 1)	4	63.9027	15.97568	4.0433
Repss (r – 1)	2	7.924	3.962	
Ess (t – 1) (r – 1)	8	31.6093	3.95116	
Total ss(tr – 1)	14	103.436		

Appendix 88: ANOVA FOR WEEK 10

Source	df	ss	ms	F
Trtss (t – 1)	4	146.817	36.7043	607.88
Repss (r – 1)	2	0.084	0.042	
Ess (t – 1) (r – 1)	8	0.483	0.06038	
Total ss(tr – 1)	14	147.384		

Appendix 89: ANOVA FOR WEEK 12

Source	df	ss	ms	F
Trtss (t – 1)	4	208.7133	52.17833	497.69
Repss (r – 1)	2	0.028	0.014	
Ess (t – 1) (r – 1)	8	0.8377	0.10484	
Total ss(tr – 1)	14	209.580		

EXPERIMENT II: MEAN INCREASE IN LENGTH

Appendix 90: ANOVA FOR WEEK 2

Source	df	ss	ms	F
Trtss (t – 1)	4	0.10266	0.02567	2.298
Repss (r – 1)	2	0.03733	0.01867	
Ess (t – 1) (r – 1)	8	0.08934	0.011168	
Total ss(tr – 1)	14	0.22933		

Appendix 91: ANOVA FOR WEEK 4

Source	df	ss	ms	F
Trtss (t – 1)	4	0.18267	0.045668	0.384
Repss (r – 1)	2	0.004	0.002	
Ess (t – 1) (r – 1)	8	0.95066	0.11883	
Total ss(tr – 1)	14	1.13733		

Appendix 92: ANOVA FOR WEEK 6

Source	df	ss	ms	F
Trtss (t – 1)	4	0.15733	0.039333	0.0973
Repss (r – 1)	2	0.00533	*****	
Ess (t – 1) (r – 1)	8	3.23467	0.4044	
Total ss(tr – 1)	14	3.39733		

Appendix 93: ANOVA FOR WEEK 8

Source	df	ss	ms	F
Trtss (t – 1)	4	14.236	3.559	0.897
Repss (r – 1)	2	7.504	3.752	
Ess (t – 1) (r – 1)	8	31.736	3.967	
Total ss(tr – 1)	14	53.476		

Appendix 94: ANOVA FOR WEEK 10

Source	df	ss	ms	F
Trtss (t – 1)	4	0.124	0.031	1.3478
Repss (r – 1)	2	0.00933	0.0046	
Ess (t – 1) (r – 1)	8	0.184	0.023	
Total ss(tr – 1)	14	0.31733		

Appendix 95: ANOVA FOR WEEK 12

Source	df	ss	ms	F
Trtss (t – 1)	4	1.07	0.2675	1.8105
Repss (r – 1)	2	0.388	0.194	
Ess (t – 1) (r – 1)	8	1.182	0.14775	
Total ss(tr – 1)	14	2.64		

EXPERIMENT I: CARCASS COMPOSITION

Appendix 96: ANOVA FOR CRUDE PROTEIN (CP)

Source	df	ss	ms	F
Trtss (t – 1)	5	45.4802	43.09616	119711.56
Repss (r – 1)	1	0.0006	0.0006	
Ess (t – 1) (r – 1)	5	0.0018	0.00036	
Total ss(tr – 1)	11	215.4826		

Appendix 97: ANOVA FOR ETHER EXTRACT (EE)

Source	df	ss	ms	F
Trtss (t – 1)	5	6.8067	1.36134	7319.0323
Repss (r – 1)	1	0.00087	0.00087	
Ess (t – 1) (r – 1)	5	0.00093	0.000186	
Total ss(tr – 1)	11	6.8085		

Appendix 98: ANOVA FOR ASH

Source	df	ss	ms	F
Trtss (t – 1)	5	7.6	1.52	6333.33
Repss (r – 1)	1	0	0	
Ess (t – 1) (r – 1)	5	0.0012	0.00024	
Total ss(tr – 1)	11	7.6012		

EXPERIMENT II: CARCASS COMPOSITION

Appendix 99: ANOVA FOR CRUDE PROTEIN (CP)

Source	df	ss	ms	F
Trtss (t – 1)	5	229.7315	45.9463	241822.6
Repss (r – 1)	1	0.00087	0.00087	
Ess (t – 1) (r – 1)	5	0.00093	0.00019	
Total ss(tr – 1)	11	229.7333		

Appendix 100: ANOVA FOR ETHER EXTRACT (EE)

Source	df	ss	ms	F
Trtss (t – 1)	5	11.553	2.3106	12161.05
Repss (r – 1)	1	0.00087	0.00087	
Ess (t – 1) (r – 1)	5	0.00093	0.00019	
Total ss(tr – 1)	11	11.5548		

Appendix 101: ANOVA FOR ASH

Source	df	ss	ms	F
Trtss (t – 1)	5	15.8762	3.17524	15120.9
Repss (r – 1)	1	0.00017	0.00017	
Ess (t – 1) (r – 1)	5	0.00103	0.00021	
Total ss(tr – 1)	11	15.8774		

EXPERIMENT I: PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS

Appendix 102: ANOVA FOR CRUDE PROTEIN (CP)

Source	df	ss	ms	F
Trtss (t – 1)	4	8.676	2.169	21690
Repss (r – 1)	1	0.0006	0.0006	
Ess (t – 1) (r – 1)	4	0.0004	0.0001	
Total ss(tr – 1)	9	8.677		

EXPERIMENT II: PROXIMATE COMPOSITION OF EXPERIMENTAL DIET

Appendix 103: ANOVA FOR CRUDE PROTEIN (CP)

Source	df	ss	ms	F
Trtss (t – 1)	4	9.6774	2.41935	8064.5
Repss (r – 1)	1	0	0	
Ess (t – 1) (r – 1)	4	0.001	0.0003	
Total ss(tr – 1)	9	0.6784		

Appendix 104: Mean length (cm) of *Clarias gariepinus* fed graded levels of soaked *Bauhinia* seed meal

Trts	Initial stocking	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal 0SBSM$_{96}$	13.7	13.7 [a]	13.8 [a]	13.9 [a]	14.1 [a]	14.3 [a]	14.5 [a]
T_2 75% fishmeal 25%SBSM$_{96}$	13.8	15.4 [b]	16.8 [b]	18.0 [b]	19.5 [b]	20.9 [b]	22.3 [b]
T_3 50% fishmeal 50%SBSM$_{96}$	13.8	15.2 [b]	16.6 [b]	18.0 [b]	19.3 [b]	20.9 [b]	22.1 [b]
T_4 25% fishmeal 75%SBSM$_{96}$	13.7	15.1 [b]	16.5 [b]	17.9 [b]	19.3 [b]	20.6 [b]	22.0 [b]
T_5 100% Copens	13.4	13.9 [a]	14.1 [a]	14.2 [a]	16.9 [ab]	14.5 [a]	14.5 [a]
SED ±	0.330	0.140	0.160	0.160	1.590	0.220	0.280
LSD(P<0.05)	0.760 NS	0.320	0.370	0.370	3.670	0.510	0.650

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

Appendix 105: Mean length (cm) of *Clarias gariepinus* fed graded levels of locust meal

Trts	Initial stocking	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal	13.7	13.7	13.8	13.9	14.1	14.3	14.53
T_2 75% fishmeal 25%locust	13.9	13.5	13.8	13.9	14.1	14.3	14.46
T_3 50% fishmeal 50%locust	13.7	13.9	14.0	14.1	14.3	14.5	15.16
T_4 25% fishmeal 75%locust	13.9	14.1	14.2	14.3	14.5	14.7	14.9
T_5 100% Copens	13.4	13.9	14.1	14.2	16.9	14.6	14.53
SED ±	0.360	0.360	0.270	0.270	1.570	0.340	0.550
LSD(P<0.05)	0.830NS	0.830NS	0.620NS	0.620NS	3.620NS	0.780NS	1.270NS

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

Appendix 106: Mean weight (g) of *Clarias gariepinus* fed with graded levels of soaked *Bauhinia* seed meal

Trts	Initial stocking	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal 0SBSM$_{96}$	20.03	27.4 [b]	36.1 [b]	45.8 [c]	56.5 [bc]	66.3 [bc]	81.5 [b]
T_2 75% fishmeal 25%SBSM$_{96}$	20.30	28.0 [b]	34.9 [b]	43.9 [bc]	54.2 [b]	70.7 [bc]	78.5 [b]
T_3 50% fishmeal 50%SBSM$_{96}$	20.20	25.6 [a]	32.1 [a]	39.8 [ab]	48.7 [ab]	58.9 [ab]	70.3 [a]
T_4 25% fishmeal 75%SBSM$_{96}$	20.20	25.0 [a]	29.9 [a]	35.3 [a]	40.9 [a]	47.7 [a]	56.5 [a]
T_5 100% Copens	19.63	27.8 [b]	39.1 [c]	51.4 [d]	64.6 [c]	78.2 [c]	90.4 [b]
SED ±	0.410	0.770	1.110	2.350	4.080	7.010	7.150
LSD(P<0.05)	1.940NS	1.770	2.560	5.420	8.310	16.170	16.490

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

Appendix 107: Mean weight (g) of *Clarias gariepinus* fed with graded levels of Locust meal

Trts	Initial stocking	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal	20.03	27.4 [b]	36.1 [b]	45.8 [b]	56.5 [b]	66.3 [b]	81.5 [b]
T_2 75% fishmeal 25%locust	20.40	22.5 [a]	24.8 [a]	27.3 [a]	30.2 [a]	32.9 [a]	35.8 [a]
T_3 50% fishmeal 50%locust	20.40	22.2 [a]	24.3 [a]	26.3 [a]	28.3 [a]	30.4 [a]	32.4 [a]
T_4 25% fishmeal 75%locust	20.20	22.6 [a]	24.7 [a]	26.7 [a]	28.8 [a]	30.9 [a]	33.3 [a]
T_5 100% Copens	19.63	27.7 [b]	39.1 [c]	51.4 [c]	64.6 [c]	78.2 [c]	92.4 [c]
SED ±	0.610	0.710	1.020	1.390	1.890	2.780	3.110
LSD(P<0.05)	1.580NS	1.640	2.350	3.200	4.360	6.410	7.170

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

Appendix 108: Mean increase in length (cm) of *Clarias gariepinus* fed with graded levels of soaked *Bauhinia* seed meal

Trts	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal 0SBSM$_{96}$	0.00 [a]	0.10 [a]	0.23 [a]	0.43 [a]	0.63 [a]	0.83 [a]
T_2 75% fishmeal 25%SBSM$_{96}$	1.57 [c]	3.03 [b]	4.30 [b]	5.67 [b]	7.10 [b]	8.47 [b]
T_3 50% fishmeal 50%SBSM$_{96}$	1.47 [bc]	2.83 [b]	4.23 [b]	5.60 [b]	7.13 [b]	8.37 [b]
T_4 25% fishmeal 75%SBSM$_{96}$	1.33 [b]	2.73 [b]	4.13 [b]	5.50 [b]	6.87 [b]	8.20 [b]
T_5 100% Copens	0.03 [c]	0.17 [a]	0.27 [a]	3.00 [ab]	0.67 [a]	0.63 [a]
SED ±	0.066	0.148	0.169	1.623	0.201	0.264
LSD(P<0.05)	0.152	0.341	0.389	3.743	0.463	0.610

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

Appendix 109: Mean increase in length (cm) of *Clarias gariepinus* fed with graded levels of Locust meal

Trts	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal	0.00	0.10	0.23	0.43	0.63	0.83 [ab]
T_2 75% fishmeal 25%locust	0.13	0.40	0.50	0.70	0.87	1.07 [ab]
T_3 50% fishmeal 50%locust	0.13	0.20	0.30	0.53	0.73	1.43 [b]
T_4 25% fishmeal 75%locust	0.23	0.33	0.43	0.63	0.83	1.03 [ab]
T_5 100% Copens	0.03	0.17	0.27	3.00	0.67	0.63 [a]
SED ±	0.086	0.281	0.519	1.626	0.124	0.314
LSD(P<0.05)	0.199NS	0.649NS	1.197NS	3.750NS	0.286NS	0.724

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

Appendix 110: Mean increase in weight (g) of *Clarias gariepinus* fed with graded levels of soaked *Bauhinia* seed meal.

Trts	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal 0SBSM$_{96}$	7.33 ^{b}	16.03 ^{c}	25.77 ^{c}	36.47 ^{bc}	46.28 ^{bc}	61.43 ^{bc}
T_2 75% fishmeal 25%SBSM$_{96}$	7.70 ^{b}	14.57 ^{c}	23.57 ^{bc}	33.90 ^{b}	40.43 ^{ab}	58.20 ^{bc}
T_3 50% fishmeal 50%SBSM$_{96}$	5.40 ^{a}	11.87 ^{b}	19.57 ^{ab}	28.53 ^{ab}	38.73 ^{ab}	50.10 ^{ab}
T_4 25% fishmeal 75%SBSM$_{96}$	4.73 ^{a}	9.67 ^{a}	15.03 ^{a}	20.67 ^{a}	27.47 ^{a}	36.30 ^{a}
T_5 100% Copens	8.03 ^{b}	19.47 ^{d}	31.73 ^{d}	45.0 ^{c}	58.6 ^{c}	72.80 ^{c}
SED ±	0.592	0.905	2.231	3.998	6.973	7.077
LSD(P<0.05)	1.365	2.087	5.144	9.220	16.079	16.320

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

Appendix 111: Mean increase in weight (g) of *Clarias gariepinus* fed with graded levels of Locust meal over 12 weeks

Trts	Week2	Week4	Week6	Week8	Week10	Week12
T_1 100% fishmeal	7.33 ^{b}	16.03 ^{b}	25.77 ^{b}	36.47 ^{b}	46.28 ^{b}	61.43 ^{b}
T_2 75% fishmeal 25%locust	2.10 ^{a}	4.37 ^{a}	6.93 ^{a}	9.80 ^{a}	12.57 ^{a}	15.42 ^{a}
T_3 50% fishmeal 50%locust	1.80 ^{a}	3.87 ^{a}	5.87 ^{a}	7.83 ^{a}	9.97 ^{a}	11.93 ^{a}
T_4 25% fishmeal 75%locust	2.17 ^{a}	4.27 ^{a}	6.30 ^{a}	8.37 ^{a}	10.47 ^{a}	12.50 ^{a}
T_5 100% Copens	8.03 ^{b}	19.47 ^{c}	31.73 ^{c}	45.0 ^{c}	58.60 ^{c}	72.80 ^{c}
SED ±	0.303	0.320	0.948	1.466	2.407	2.727
LSD(P<0.05)	0.698	0.738	2.187	3.382	5.552	6.289

Values with the same superscripts in the same row are not significantly different (P > 0.05) LSD.

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